EFFICACY OF MEDICINAL PLANTS USED BY COMMUNITIES AROUND LAKE VICTORIA REGION AND THE SAMBURU AGAINST MYCOBACTERIA, SELECTED BACTERIA AND *CANDIDA ALBICANS*

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DECLARATION

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

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DEDICATION

This work is dedicated to you my lovely late mum, Mary Nyanchama, and to all mortals on whose shoulders I stood after you went home.

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Although this thesis is my original work, it could not have been completed without the assistance and support of so many people. First, I would like to thank my supervisors, Prof. Paul Okemo, Prof. Paul Mbugua, both of Kenyatta University, Kenya. Prof. Paul Okemo has been like a father to me and a role model of wise counsel since my second year of undergraduate studies and deserves special mention. Science aside, I thank him for sharing with me Beverly Sills' and Gary Player's wisdom; that there are no shortcuts to anywhere worth going, and that the harder I work, the luckier I will get. I will always remember this- "This is for the eggs".

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ACRONYMS AND ABBREVIATIONS

AIDS	-	Acquired Immune Deficiency Syndrome
ANOVA	-	Analysis of Variance
DD	-	Disc Diffusion
HIV	-	Human Immuno-Deficiency Virus
LJ	-	Lowenstein Jensen
MBC	-	Minimum Bactericidal Concentrations
MDR	-	Multi-Drug Resistance
MGIT	-	Mycobacterial Growth Indicator Tube
MIC	-	Minimum Inhibitory Concentrations
TB	-	Tuberculosis
WHO	-	World Health Organization
XDR	-	Extensively Drug Resistant

ABSTRACT

In Africa more than 70% of the people refer to ethnomedicine for their health issues. With the emergence of new diseases and drug resistance to infections, traditional medicine should be given more attention in modern research and development. Tuberculosis (TB), a deadly infectious disease that annually kills about 3 million people worldwide is complicated. This is due to significant toxicity, emergence of multidrug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) and lengthy therapy which creates poor patient compliance. There is also a major therapeutic problem due to emergence of *Escherichia coli, Klebsiella pneumoniae and* other ß-lactamase producers. Diarrhoeal diseases are responsible for 4.6 million deaths every year. These highlight the need to develop novel drugs. Natural products provide unlimited opportunities for new drug leads because of the unmatched chemical diversity. This study evaluated the antimicrobial potential of 34 medicinal plants used by communities living around the Lake Victoria region and the Samburu Community of northern Kenya, following an ethnobotanical survey. Plants were collected and identified at the Department of Pharmacy and Complimentary Alternative Medicine, Kenyatta University, Nairobi, Kenya; in whose herbarium voucher specimens were deposited. Methanolic extracts from plants were tested against four strains of Mycobacteria (*Mycobacteria tuberculosis, M. kansasii, M. fortuitum, and M. smegmatis*) obtained from Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. BACTEC MGIT 960 system was used. *Salmonella typhi* (clinical isolate), *Klebsiella pneumoniae* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 25852), *Escherichia coli* (ATCC 25922) *Staphylococcus aureus* (ATCC 20591) and *Candida albicans* (ATCC EK138), obtained from Kenyatta National Hospital in Nairobi, Kenya, were also screened against using standard procedures. The crude extracts were analyzed for presence of phytochemicals. *Croton macrostachyus, Vernonia amygdalina, Toddalia asiatica, Aloe secundiflora, Cordia sinensis,* and *Euphorbia scarlatina* gave strong antimycobacterial activity (zero GUs) against *M. kansasii,* at all concentrations used. *Entada abysinnica, T. asiatica, Salvadora persica, C. sinensis, Scadoxus multiflorus* and *E. scarlatina* extracts were active (zero GUs) against *M. tuberculosis*. Extracts from *Carissa edulis, V. amygdalina, A. secundiflora, Pistacia aethiopica, S. persica, S. multiflorus, E. scarlatina,* and *Acacia nilotica* were active (zero GUs) against *M. fortuitum.* Against *M. smegmatis, Carissa edulis, V. amygdalina, A. secundiflora, S. persica, S. multiflorus, E. scarlatina* and *A. nilotica* were active (zero GUs). *Eurphobia scarlatina* was active (Zero GUs) against all the strains of mycobacteria. There was significant difference of the means of the zones of inhibition of the *S. typhi, K. pneumoniae, P. aeruginosa, E. coli, S. aureus* and *C. albicans* at $P \leq 0.05$. The MICs and the MBCs of the extracts were determined by use of microtitre plate method with *E. abysinnica, T. asiatica, Thylachium africanum, A. secundiflora, A. nilotica* and *Momordica charantia* extracts showing good activity with MICS and MBCS of 4.687- 18.75 mg/ml in some test cultures. *Klebsiella pneumoniae* and C. *albicans* were mostly insensitive to extracts. Preliminary phytochemistry identified six phytochemicals to which tannins were common in most plant extracts. The data suggests that plant extracts could be a rich source of antimicrobial agents. Results also provide an indication of merit in their ethnomedicinal use.

CHAPTER ONE

INTRODUCTION

1.1 Background information

From prehistoric times man has used plants to alleviate and treat diseases (Potterat and Hamburger, 2008). The use of medicinal plants has always been part of human culture and it began from the era of early civilization as evidenced by the earliest recorded uses found in Babylon (1770 BC) and in ancient Egypt (1550 BC) (Akintonwa *et al.*, 2009). The origin of pharmaceutical natural products research can be traced to 1805, when the German Pharmacist Sertürner isolated morphine from Opium latex and soon recognized the superior therapeutic properties of the pure compound (Potterat and Hamburger, 2008). The isolation of morphine spurred the discovery of numerous important drug substances. For instance, emetine (1817), atropine (1819), quinine (1820), caffeine (1820), and digitoxin (1841) (Potterat and Hamburger, 2008). The French pharmacists Pelletier and Caventou were particularly prolific in isolating numerous important alkaloids, and Caventou established in the mid 1820s a factory for production of quinine which was to become the first commercial natural product and pure drug substance (Potterat and Hamburger, 2008).

Soon, factories in other European countries were established. For example, E. Merck Company in Germany (1827), and early pharmaceutical industry developed along with the discovery of an increasing number of plant alkaloids (Potterat and Hamburger, 2008). In the 19th and well into the 20th Century, ethnopharmacology provided a number of compounds with unique pharmacological properties and provided significant

advances in pharmacotherapy, like quinine (1820) as an antimalarial, cocaine (1860), the first local anesthetic, tubocurarine (1935), a muscle relaxant enabling modern surgical procedures, and reserpine (1951) as a first effective antihypertensive (Soltan and Zaki, 2009; Potterat and Hamburger, 2008). Towards the end of the 19th Century, rapidly growing understanding of organic synthesis and chemical structures led to first derivatives of natural products. Diacetylmorphin (1898) and acetylsalicylic acid (1899) were among the first compounds to be commercialized as pharmaceuticals (Soltan and Zaki, 2009). The contribution of plant-derived natural products to modern pharmacotherapy is considerable. Out of 243 structures which Sneader identified as the starting point for the development of our modern drugs, some 60 compounds are of plant origin (Potterat and Hamburger, 2008; Akintonwa, *et al.*, 2009). During the last decade, the uses of traditional medicine has expanded globally and gained popularity (Soltan and Zaki, 2009). The World Health Organization (WHO) estimated in 2001 that up to 80% of the world's population relies on traditional medicinal practices for some aspect of primary health care even for some complicated and disturbing infections like tuberculosis (Akintonwa, *et al.*, 2009; Soltan and Zaki, 2009).

Tuberculosis (TB) is a disease caused by several species of Mycobacteria often described as *Mycobacterium tuberculosis* complex which include; *M. tuberculosis*, *M. microti*, *M. africanum,* and *M. bovis* (Khan *et al.*, 2009; Okunade *et al.*, 2004). These are members of the slow growing Mycobacteria (Susan *et al.*, 2002). Out of them *Mycobacterium tuberculosis* (MTB) is the most frequent cause of the disease in humans. Central to the pathogenic success of MTB, is the ability of the disease to persist within

humans for long periods in a clinically latent state without causing any overt disease symptoms (Sherman *et al.,* 2001)**.** Mycobacteria other than *Mycobacterium tuberculosis* (MTB) complex, referred to as nontuberculous mycobacteria (NTM), have also been associated with human disease (Huang *et al.*, 2009).

With the emergence of the Human Immunodeficiency Virus (HIV) epidemic and the increased use of immunosuppressants, the incidence of NTM disease has increased substantially and NTM have accordingly been regarded as important pathogens (Huang *et al.,* 2009). The most commonly involved site of NTM disease is the lung, accounting for 75-94% of all NTM isolates. Over the last decade, the number of isolates of NTM from respiratory specimens has greatly increased and even surpassed the number of MTB isolates in many mycobacteriology laboratories (Barkan *et al.,* 2009; WHO, 2006). Although pulmonary tuberculosis (PTB) remains the most important mycobacterial disease from a public health standpoint, clinicians are encountering more and more patients with pulmonary disease caused by NTM (Huang *et al.,* 2009) and the disease has continued to claim more and more lives.

About two million people a year die from tuberculosis and researchers say new ways of tackling the disease are urgently needed as drug-resistant strains of the bacteria develop (Barkan *et al.*, 2009; Ogden and Porter, 2000; WHO 2006). Most people killed by tuberculosis live in South East Asia and sub-Saharan Africa, but WHO says new outbreaks have begun to occur in Eastern Europe after almost 40 years of steady falls in the number of infections (WHO, 2006).

Tuberculosis usually affects the lungs, but it can also affect other parts of the body, such as the brain, the kidneys, or the spine (WHO, 2005; WHO, 2006). According to the World Health Organization (WHO, 2006; WHO 2005), approximately one third of the world's population is infected with *M. tuberculosis*, and TB is the leading cause of death from a single infectious disease agent in adults worldwide.

The TB burden follows a strong socio-economic gradient between countries, within countries, and within communities, and the poorest have the highest risk (Lonnroth *et al.,* 2009). It has been used as a prime example of a ''social disease'', the control of which requires social, economic and environmental interventions (Lonnroth *et al.,* 2009). It is transmitted by exposure to tubercle bacilli in airborne droplet nuclei produced by a person with infectious TB during expiratory efforts, such as coughing, sneezing or singing (WHO, 2006; Lonnroth *et al.,* 2009). A necessary risk factor for TB infection is contact with a person with an active disease. The likelihood of having such a contact is determined by the underlying disease burden in the community. People living or working in environments where TB prevalence is particularly high are obviously at a higher risk of infection. For example there is evidence that prison staff, inmates and certain health care workers often get infected (Lonnroth *et al.,* 2009). The risk of exposure is also determined by the physical environment in which the contact takes place, including aspects of crowding, air flow and humidity.

Tuberculosis is an important health problem, and the issue has become even more so as a result of increasing drug resistant strains, which complicate efforts to control the disease (Shamaei *et al.,* 2009). The World Health Organization (WHO, 2006) has revealed details of a 'super strain' of tuberculosis (XDR), which is not only resistant to first line treatment drugs, but also three or more of the six classes of second-line drugs. Resistance to anti-TB drugs in populations is a phenomenon that occurs primarily due to poorly managed TB care. Problems include incorrect drug prescribing practices by service providers, poor quality drugs or erratic supply of drugs, and also patient non-adherence to prescriptions (Singh *et al.,* 2006; WHO, 2006). The rates of new cases of Multidrug-resistant tuberculosis (MDR-TB) and now Extensively Drug-Resistant Tuberculosis (XDRTB) continue to increase and this is now a major health concern (Zhang *et al.,* 2003). The management of MDR-TB and XDR-TB requires extended treatment and expensive and potentially toxic drug regimens, and often results in higher rates of treatment failure and death compared to drug sensitive disease (Park *et al.,* 2009).

The current global tuberculosis (TB) control paradigm mainly focuses on cutting transmission through early case detection and effective treatment (Lonnroth *et al.,* 2009). The medium-term goal of global TB control is to halve TB prevalence and death rates by 2015, and to achieve a decline in incidence, as established in the Millennium Development Goal (MDG) six (6) (Lonnroth *et al.,* 2009). A longer-term target is to 'eliminate' TB by reducing the incidence to less than 1 new case per million population per year by 2050.

Tuberculosis being among the leading causes of death in the world is a priority for investigators to find new targets for chemotherapy of increasingly drug- resistant strains (Cerda-Maira and Darwin, 2009). It has continued to be a major health problem in developing countries throughout the past century, principally because of poverty (Ogden and Porter, 2000), with countries like India reporting between 2.5 and 3 million new cases each year with 0.3 to 0.5 million deaths.

HIV/AIDS is the biggest risk factor for tuberculosis, increasing risks of TB infection resulting in disease by at least 100-fold. In the countries of sub-Saharan Africa, case rates have risen by up to 10-fold in a decade as HIV/AIDS has spread through the communities. A higher incidence of disseminated and Extrapulmonary TB is now found (Marjorie and Holenarasipur, 2005). Extrapulmonary sites of infection commonly include lymph nodes, pleura, and osteoarticular areas, although any organ can be involved (Sharma and Mohan, 2004). As tuberculosis spreads through populations, there is also increased incidence of other infections.

Currently there is a growing disease burden worldwide from water, sanitation and hygiene which account for 4% of all deaths and 5.7% of the total disease capacity (Omwenga *et al.,* 2009). Amongst the many known water borne diseases, diarrhoeal diseases (including typhoid) kill more than 1.8 million people every year, mostly children from developing countries. Enteric fever also continues to be a major health problem in developing countries with approximately 10 million cases which result in 700,000 deaths annually (Quave *et al.,* 2008). Chloramphenicol has been employed

successfully in the management of typhoid fever, but the efficacy of this antibiotic and other first line antibiotics such as Ampicillin, cotrimoxazole and tetracycline is becoming doubtful with the upsurge of enteric fever (Quave *et al.,* 2008). Resistance strains of *Salmonella typhi* to these antibiotics have emerged and continue to be of clinical significance.

Besides diarrhoeal diseases *Staphylococcus aureus* is considered a main pathogen of causing nosocomial infections (Quave *et al.,* 2008). The emerging antibiotic resistance of *S. aureus* with infection outbreaks among hospitalized patients is a serious problem worldwide (Heffernan *et al.,* 2009; Quave *et al.,* 2008). Extended-spectrum β-lactamase producers (ESBLs) such as *Escherichia coli* and *Klebsiella pneumoniae* are being increasingly identified in many parts of the world and are already prevalent in several countries (Heffernan *et al.,* 2009). β-lactamase is one of the major causes of drug resistance and *Klebsiella pneumoniae* which produces β-lactamase is the second most common cause (behind *Escherichia coli*) of community- and hospital-acquired Gram-negative bloodstream infections. *K. pneumoniae* bloodstream infections usually arise as a complication of focal urinary, gastrointestinal, or respiratory tract infections, although occasionally they can arise without a definable source (Drapeau *et al.,* 2010).

Other infective agents such as *Pseudomonas aeruginosa* are respiratory and skin pathogens seen in healthcare settings especially in immunosuppressed individuals and other highly vulnerable patients (Ho *et al.,* 2009). *P. aeruginosa* infects patients in the intensive care unit (ICU) and is now associated with significant morbidity and mortality

and therapeutic options are becoming increasingly limited especially with the continued emergence and spread of antimicrobial resistant strains (Kerr and Snelling, 2009). Given the potential severity of specific *P. aeruginosa* infections and problems in selecting optimal therapy for Multidrug resistant (MDR) isolates, identification and implementation of effective strategies to prevent these infections are urgent priorities. The urgency on skin infections of *P. aeruginosa* should also address *Candida albicans* which also infects skin and mucous membranes (Kerr and Snelling, 2009).

In recent years, severe fungal infections have significantly contributed to the increasing morbidity and mortality of immunocompromised patients who need intensive treatment including broad-spectrum antibiotic therapy. *Candida albicans* represents one of the most common pathogens which are responsible for fungal infections often causing hospital-acquired sepsis with an associated mortality rate of up to 40% (Panacek *et al.,* 2009). Currently most of the available effective antifungal agents are based on polyenes (amphotericin B), triazoles (fluconazole, itraconazole, voriconazole, posaconazole) or echinocandins (caspofungin, micafungin and anidulafungin) (Reboutier *et al.,* 2009). However, administration of these antifungals is often accompanied by various complications such as amphotericin B toxicity and adverse effects of some azoles including toxicity and drug interactions and yeast resistance to antifungal therapy (Panacek *et al.,* 2009). Due to toxic and adverse effects, other options for effective antifungal therapy must be sought to avoid the pain and danger the azole drugs pose to patients.

In Africa, traditional medicine is of great value and more than 70% of the people refer to traditional healers concerning health issues (Tijjani *et al.,* 2009). Plants produce a variety of secondary metabolites that have long been of interest to man. In recent years these are used, either directly as precursors or as lead compounds, in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens (Shokeena *et al.,* 2009).

1.2 Problem statement and justification

Diseases like tuberculosis are a major global health problem complicated by escalating rates of antibiotic resistance with an estimated 8-9 million new cases occurring worldwide annually (Barkan *et al.,* 2009). The issue has become even more so as a result of increasing drug resistant strains; and manifestations of HIV/AIDS which complicate efforts to control the disease (Shamaei *et al.,* 2009). As tuberculosis spreads through the populations, there is also increased incidence of other infections. Currently, there is a growing disease burden worldwide from water, sanitation and hygiene which account for 4% of all deaths (Tetali *et al.,* 2009). Diarrhoeal diseases are a major problem in all African countries especially in the tropical areas (Meite *et al.,* 2009; Tetali *et al.,* 2009). Other infective agents such as *Pseudomonas aeruginosa*, *Candida albicans* and *Staphylococcus aureus* are now common in healthcare settings especially in immunosuppressed individuals and other highly vulnerable patients. There is therefore an urgent need to develop more effective drugs to handle the expanding problem of drug- and multi-drug resistance.

In laboratory settings, plant extracts have been shown to have a variety of pharmacological effects, including anti-inflammatory, vasodilatory, antimicrobial, anticonvulsant, sedative, and antipyretic effects (Khan *et al.*, 2009).

The human practice of plant traditional medicines in communities around the Lake Victoria region and Samburu communities is based on the knowledge that has been passed orally from generation to generation and only a very few written documents are available Testing for the efficacy of the active compounds isolated from locally available plants is therefore a viable proposal.

1.3 Hypotheses

$H0_1$: The indigenous communities in the Lake Victoria region and the Samburu community do not have appropriate information on the medicinal plants used to treat tuberculosis and other common bacterial and antifungal diseases.

$H0_2$: The medicinal plant extracts do not have active ingredients (extracts) that can be tested against tuberculosis and other common bacterial and antifungal disease causing microbes.

$H0_3$: The active plant extracts used for treating tuberculosis and other common bacterial and antifungal disease causing agents cannot be compared to known drugs.

1.4 Objectives of the study

1.4.1 General objective

To determine ethnobotanical practices, identify and determine bioactivity of medicinal plants used for treatment of tuberculosis and other common bacterial and fungal diseases around Lake Victoria region and Samburu.

1.4.2 Specific objectives

i. To carry out an Ethnobotanical survey on the antimycobacterial, antibacterial and antifungal plants used by the communities living around the Lake Victoria region and the Samburu community.

ii. To screen crude extracts of selected plants for bioactivity against the tuberculosis causing bacteria using.

iii. To screen crude extracts against selected bacteria.

iv. To screen crude extracts against *Candida albicans*.

v. To determine the MICs and MBCs of the medicinal plants against selected bacteria and fungi.

vi. To determine the phytochemicals present in the extracts.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The discovery of the TB *bacillus* in 1882 was an outstanding advance in the understanding of transmissible diseases, and marked the beginning of the germ theory era (Lonnroth *et al.,* 2009). As a result of this discovery, a TB control paradigm based mostly on the biological understanding of the disease gradually emerged. It received a final boost with the discovery in the 1940s and 1950s of drugs that could cure the disease (Lonnroth *et al.,* 2009). With the introduction of chemotherapeutic measures, tuberculosis (TB) prevalence witnessed a significant decline in this second half of the 20th century (Shamaei *et al.,* 2009). But this was not only a period of rapid medical and health care advances, it was also a time of both rapid economic growth and accelerated welfare reforms in many industrialized countries (Lonnroth *et al.,* 2009). The current global tuberculosis (TB) control paradigm mainly focuses on cutting transmission through early case detection and effective treatment, making medical, social and economic interventions to be at the core of the global strategy against TB since it is seen as a "social disease" (Lonnroth *et al.,* 2009).

With all that effort, *Mycobacterium tuberculosis* infection still remains a major global health problem causing almost nine million illnesses and two million deaths each year (Kingkaew *et al.,* 2009). This is complicated by escalating rates of antibiotic resistance as well as the escalating HIV infection (Shamaei *et al.,* 2009; Pardini *et al.,* 2009;

Barkan *et al.*, 2009). In adults with normal immune systems, TB is usually confined to the lungs, but in persons with immune suppression, such as HIV infection, *Mycobacterium tuberculosis* (MTB) bacilli frequently disseminate beyond the lungs and cause disease in other organ systems (Kingkaew *et al.*, 2009; Shamaei *et al.*, 2009). Although most TB programs focus on the control of infectious pulmonary TB, about one in five TB cases worldwide is considered extrapulmonary, which is defined as TB occurring outside the lung parenchyma (Kingkaew *et al.*, 2009).

2.2 Tuberculosis epidemiology

Epidemiological TB data before the 20th century is imprecise (Lonnroth *et al.*, 2009). However, some broad trends have been identified with a reasonable level of certainty. The conventional wisdom is that the incidence of TB increased in industrialized countries in the 17th to 18th centuries, peaking at different times in different places from the middle of the 1700s in Great Britain to the beginning of the 1900s in Japan (Shamaei *et al.*, 2009; Lonnroth *et al.*, 2009). From these trends, a temporal association has been suggested between increased TB incidence and rapid industrialization and urbanization. Thailand is one of the 22 World Health Organization (WHO) designated high burden TB countries, with an estimated annual TB incidence of 142 per 100 000 population. The TB mortality rate in Thailand is 19 per 100 000 population (Kingkaew *et al.*, 2009). An estimated 15-20% of all TB cases in Thailand occur in HIV-infected persons.

In Iran, TB is an important health problem, and the issue has become even more so as a result of increasing drug resistant strains which complicate the efforts to control TB

(Shamaei *et al.,* 2009). In Kenya Tuberculosis has regained its footing at a time when people have all but abandoned hope because of the AIDS epidemic (USAID, 2005). The timing could not be worse; the need for urgent action could not be greater (Kokwaro, 1993).

Epidemiological models of TB control predict that countries that achieve high-levels of TB program performance will see substantial reductions in TB incidence, prevalence, and mortality (Jittimanee *et al.,* 2009). The main targets for TB program performance are case detection and treatment success. For example countries that detect 70% of all estimated TB cases and successfully treat 85% of detected, acid-fast bacilli smear-positive pulmonary TB cases should expect declines in incidence of 8-12% per year (Lonnroth *et al.,* 2009; Jittimanee *et al.,* 2009).

2.3 Current tuberculosis therapy and duration

Treatment for tuberculosis uses antibiotics that are also static to the bacteria (Kishore *et al.,* 2009). Standard anti-TB regimen consist of isoniazid, rifampicin, ethambutol, plus pyrazinamide (HREZ) (Fig. 1) which are taken daily in the first two months (intensive phase), followed by daily intake of rifampin and isoniazid four months (continuous phase) (Huang *et al.,* 2009; Kishore *et al.,* 2009; Kingkaew *et al.,* 2009).

New anti-tubercular drug regimens are clearly needed to reduce the therapy time required for a durable cure and to treat the expanding problem of drug- and multidrug resistant *M. tuberculosis* (MDR) strains (Kishore *et al.,* 2009).

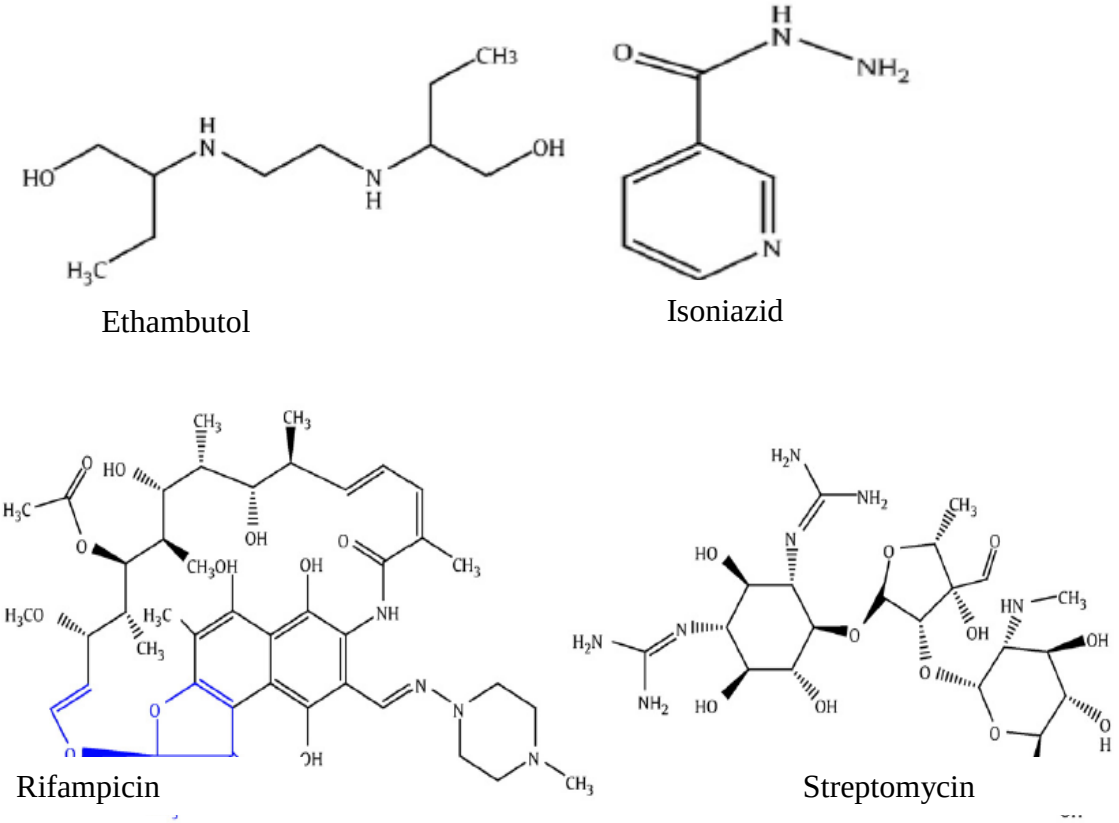


Figure 1. Molecular structures of the first line drugs used against tuberculosis (Kishore *et al.*, 2009).

2.4 Drug resistance in tuberculosis

Resistance has been defined as the temporary or permanent ability of an organism and its progeny to remain viable and or multiply under conditions that would destroy or inhibit other members of the strain (Cloete, 2003). Bacteria may be defined as resistant when they are not susceptible to a concentration of antibacterial agent used in practice. Identifying the trends in drug resistance is one of the important aspects in the assessment of TB epidemiologic trends and TB control planning (Shamaei *et al.*, 2009).

Multidrug-resistant tuberculosis (MDR-TB), defined as *in vitro* resistance to at least both isoniazid and rifampin, has become an emerging global public health crisis (Park *et*

al., 2009). Strains of multidrug resistant *Mycobacterium tuberculosis* are often resistant to other anti-tuberculous agents in addition to isoniazid and rifampin. When such an isolate is resistant to any second-line injectable agent (e.g., amikacin, capreomycin, or kanamycin) and any fluoroquinolone, the strain is termed extensively drug-resistant tuberculosis (XDR-TB) (Ani *et al.,* 2009; Shamaei *et al.,* 2009; Ruttoh *et al.,* 2009). This recently described extensively drug-resistant TB (XDR-TB) has been reported in South Africa and elsewhere where studies have been carried out. Despite the long and costly treatment involved, a considerable number die of the disease and many others have to endure the active and destructive form of TB (Shamaei *et al.,* 2009).

Whereas Tuberculosis (TB) came under significant control in the twentieth century following the discovery of effective drug combination therapy and interventions by global health programs, now the trend in drug resistance against all first-line drugs shows a significant increase (Ani *et al.,* 2009; Shamaei *et al.,* 2009; Park *et al.,* 2009). The changing pattern of global health events over the last two decades, particularly with the emergence and spread of HIV, with its characteristic immunosuppressive effects on the host, have aided the resurgence of infections caused by *Mycobacterium tuberculosis.* The pattern has necessitated the advancement of alternative strategies for effective combat of the emerging global problem of drug-resistant *M. tuberculosis*. Strains of *M. tuberculosis* resistant to anti-mycobacterial agents, including Multidrug-resistant TB (MDR-TB) and XDR strains have been reported globally (Ani *et al.,* 2009; Ruttoh *et al.,* 2009).

Non-adherence to treatment regimens remains a serious problem that not only leads to increasing drug resistance but also contributes to TB morbidity (Shamaei *et al.,* 2009; Drapeau *et al.*, 2010). Factors leading to non-adherence include distance to place of treatment; belief that TB has been cured once the symptoms disappear; lack of proper health education; poor drug availability (stock-outs); low-quality service from health providers, including bribes being sought; stigma resulting from the belief that people with TB also have AIDS; cost of transportation; weakness caused by AIDS; and fear of injections (Ani *et al.,* 2009; Shamaei *et al.,* 2009). A study carried out by Shamaei *et al.,* (2009) found that drug resistance to streptomycin (SM), isoniazid (INH) and ethambutol (EMB) in Iran was more frequent than to the other agents used against tuberculosis.

2.5 Drug resistance in other bacteria

2.5.1 *Escherichia coli*

Escherichia coli may be a commensal or pathogen (Zhang *et al.,* 2003). They are found in the digestive tract of warm blooded animals and humans and the pathogenic one is known to cause Urinary tract infections (UTIs) and diarrhoea. UTIs are one of the most common reasons for antibiotic therapy among adults, with more than 8 million physician visits per year (Johnson *et al.,* 2008). The management of UTIs is complicated by increasing rates of resistance in *Escherichia coli*, the most common cause of UTIs, to common oral antibiotics (Johnson *et al.,* 2008; Gagliotti *et al.,* 2008). Resistance genes commonly reside on transmissible plasmids, transposons, gene cassettes or other mobile genetic elements, allowing the horizontal spread of resistance genes between strains, species and even genera (Zhang *et al.,* 2003). *E. coli* can also be used to serve as an indicator for the acquisition of resistance to various antimicrobials by enteric organisms

(Lim *et al.,* 2007). The use of *E. coli* as the indicator bacteria is also appropriate because changes in the antibiotic resistance of this species may serve as an early warning of the development of resistance by related pathogenic bacteria (Gualco *et al.,* 2007; Lim *et al.,* 2007).

From the study on antibiotic sensitivity pattern some strains of *E. coli* are resistant to ampicillin, ciprofloxacin, co-trimoxazole, nitrofurantoin and tetracycline (Rani and Khullar, 2004). Ampicillin and sulfamethoxazole/trimethoprim (co-trimoxazole) resistance rates have reached 20-50% worldwide (Gualco *et al.,* 2007), whilst increasing percentages of ciprofloxacin- resistant mutants have been observed in Spain, Portugal and Italy. Also, prescription of fluoroquinolones is discouraged in uncomplicated UTIs in women due to the risk of increasing antimicrobial resistance to these agents, but is recommended in male UTIs and in complicated and upper UTIs in women (Gagliotti *et al.,* 2008).

2.5.2 *Klebsiella pneumoniae*

This is a capsulated Gram negative bacilli that is enteric in nature but can also be found in the respiratory tract of individuals (Talaro and Talaro, 2002). Since they were identified in the early 1980s, isolates of *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (ESBLs) have been a major cause of concern especially in developed countries, where numerous outbreaks and a variety of enzymes have been reported (Gould, 2008). Resistance of *Klebsiella pneumoniae* to extended spectrum β-lactam antibiotics is commonly mediated by β-lactamases (Talaro and Talaro, 2002).

Indeed, *K. pneumoniae* is a major host of plasmid-located extended-spectrum beta-lactamases (ESBL) producing strains that are resistant to aminoglycosides and other antimicrobial agents (Talaro and Talaro, 2002). This problem is particularly significant in developing countries where patients cannot afford carbapenems (Ho *et al.*, 2009).

Carbapenems are among the last treatment options for multidrug resistant Gram-negative infections (Maltezou *et al.,* 2009; Ho *et al.,* 2009). Carbapenems such as imipenem and meropenem are recommended as first-line therapy for severe infections caused by Enterobacteriaceae (Nordmann *et al.,* 2009). However, resistance to these agents is increasing worldwide with a considerable impact on clinical practice, restricting antimicrobial treatment options (Ho *et al.,* 2009). In Greece a high proportion of hospital acquired infections due to carbapenem nonsensitive Verona integron-encoded metallo-β-lactamase (VIM) metalloenzyme producing *Klebsiella pneumoniae* has been reported within the last 5 years (Maltezou *et al.,* 2009).

Quinolones and fluoroquinolones are broad-spectrum antimicrobial agents extensively used in both human and veterinary medicine, and therefore found as residues in the environment (Cremet *et al.,* 2009; Gould, 2008). This widespread use has been associated with increased levels of quinolone resistance as well, particularly in the last 10 years (Cremet *et al.,* 2009). This leaves us with few options, among them, searching for new drugs.

2.5.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa a Gram-negative aerobic bacterium with minimal nutritional requirements is common in moist environments (Ho *et al.,* 2009). It is a versatile pathogen associated with a broad spectrum of infections in humans. In healthcare settings the bacterium is an important cause of infections in vulnerable individuals including those with burns or neutropenia or receiving intensive care (Kerr and Snelling, 2009). *Pseudomonas aeruginosa* produces several virulence-associated factors and can cause a variety of disease manifestations. In addition to bacteraemia and endocarditis, infection of the urinary tract, respiratory tract, central nervous system, ear, eye, bone, joints and skin are most often reported (Ho *et al.*, 2009).

Mortality attributable to *P. aeruginosa* infection can be high because management of infections from the pathogen is difficult as *P. aeruginosa* is inherently resistant to many antimicrobials (Cloete, 2003). Furthermore, treatment is rendered increasingly problematic due to the emergence and spread of resistance to the few agents that remain as therapeutic options. Perhaps the most disturbing development in recent years has been the emergence of carbapenemases in MDR strains of *P. aeruginosa* (Kerr and Snelling, 2009). Loss of the carbapenems, which have been the mainstay of therapy for infections caused by MDR strains, severely limits therapeutic options, with colistin becoming the 'antibiotic of last resort'.

The emergence of colistin resistance in carbapenem-resistant *P. aeruginosa*, creating effectively pan-resistant strains, is a serious development (Kerr and Snelling, 2009).

Resistance of *P. aeruginosa* is ascribed to a change in the outer membrane protein prole, leading to improved exclusion of sodium dimethyldithiocarbamate (SMT) bactericide (Cloete, 2003; Hidron *et al.,* 2009). Given these challenges, it would seem reasonable to identify strategies that would prevent acquisition of the bacterium by hospitalized patients and also come up with alternative drugs that are affordable to the common man.

2.5.4 *Salmonella typhi*

The genus *Salmonella* is a Gram-negative rod shaped facultative anaerobic bacterium that can survive inside the host macrophages and cause persistent infection (Lahiri *et al.,* 2009). *Salmonella enterica* serovar Typhi (*Salmonella typhi*) causes typhoid fever and remains a major health problem, especially in developing countries (Yanagi *et al.,* 2009). Salmonella infection occurs via the orofecal route following which it invades the intestinal mucosa (Lahiri *et al.,* 2009). *Salmonella* survives in intestinal macrophages and gets disseminated to the liver and spleen via blood and lymph. A study carried out on the antibiotic sensitivity pattern of the various strains of *Salmonella typhi* showed that some strains are becoming resistant to ampicillin, ciprofloxacin, co-trimoxazole, nitrofurantoin and tetracycline (Rani and Khullar, 2004).

2.5.5 *Staphylococcus aureus*

Staphylococcus aureus, a Gram positive bacterium, causing disease in low-income countries is perceived as trivial in terms of morbidity and mortality compared with other infectious diseases such as malaria, tuberculosis, and HIV/AIDS and related infections (Nickerson *et al.,* 2009). On closer inspection, however, the neglected status of *S aureus*

as a developing world pathogen does not equate with its supposedly low rates of disease. This is due to methicillin resistant *Staphylococcus aureus* (MRSA) endocarditis that is increasing in frequency and has a high mortality (Rogers *et al.,* 2008). *S. aureus* has long been recognized as a cause, albeit an infrequent one, of community-acquired pneumonia, estimated to represent 1-10% of community-acquired pneumonia and 20-50% of nosocomial pneumonia (Hidron *et al.,* 2009).

2.6 Drug resistance in Fungi

2.6.1 *Candida albicans*

Candida spp. represent one of the most common pathogens which are responsible for fungal infections often causing hospital-acquired sepsis with an associated mortality rate of up to 40% (Panacek *et al.,* 2009). Candidiasis, caused by *Candida* species has increased dramatically in recent years. Among the various species, *Candida albicans* is the most common causative agent associated with serious fungal infection, accounting for more than 90% of cases (Naeini *et al.,* 2009). The emergence of resistance to azole derivatives and the underlying molecular mechanisms are well documented for *Candida albicans* isolates (Reboutier *et al.,* 2009; Panacek *et al.,* 2009).

Currently most of the available effective antifungal agents are based on polyenes (amphotericin B), triazoles (fluconazole, itraconazole, voriconazole, posaconazole) or echinocandins (caspofungin, micafungin and anidulafungin) (Panacek *et al.,* 2009). The management of *Candida* infection faces a number of problems including a limited number of effective antifungal therapies, toxicity of the available antifungal drugs, resistance of *Candida* to commonly used antifungal drugs, relapse of *Candida* infections

and the high cost of antifungal drugs (Naeini *et al.,* 2009). The difficulties associated with the management of *Candida* infections necessitate the discovery of new antifungal agents in order to increase the spectrum of activity against *Candid*a and combat strains expressing resistance to the available antifungal drugs (Panacek *et al.*, 2009).

2.7 Medicinal Plants

2.7.1 Historical background

Plants have always a great importance in many cultures (Vitalini *et al.,* 2009). Humans use them for their basic needs: feeding, clothing, sheltering, hunting and nursing. All over the world, people depended on herbs for the treatment of various ailments before the advent of modern medicine (Kuete *et al.*, 2009; Vitalini *et al.,* 2009). Medicinal plants constitute an arsenal of chemicals that could be exploited by human to prevent microbial invasion (Kuete *et al.*, 2009). As source of medicines, plants have formed the basis for sophisticated traditional systems and continue providing mankind with new remedies (Vitalini *et al.,* 2009). The importance of medicinal plants and traditional health systems in solving the health care problems of the world therefore should not be underestimated (Khan *et al.,* 2009). Traditional plants use is of tremendous importance in many societies, including most rural African communities (Bussmann, 2006). Because of the resurgence of interest, the research for plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin (Khan *et al.,* 2009).

In recent years, the interest in folk medicine has highly increased (Vitalini *et al.*, 2009). This discipline is gaining the scientific basis for its appropriate application within official medicine (Bussmann, 2006; Vitalini *et al.*, 2009). The knowledge of herbal remedies, developed through trial and error over the centuries, is being used as a guide to lead the chemists towards different classes of compounds. It is a fact that 25% of all medical prescriptions are based on substances derived from plants or plant-derived synthetic analogues (Vitalini *et al.*, 2009).

Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures (Bussmann, 2006). Some of these plants have been subjected to the isolation of the active ingredients (chemical compounds) (Bussmann, 2006). A large proportion of such medicinal compounds have been discovered with the aid of ethno-botanical knowledge of their traditional uses. In laboratory settings plant extracts have been shown to have a variety of pharmacological effects, including anti-inflammatory, vasodilatory, antimicrobial, anticonvulsant, sedative, and antipyretic effects (Khan *et al.*, 2009).

The rich knowledge base of countries like India and China in medicinal plants and health care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs (Tabi, 2006). India is a varietal emporium of medicinal plants and is one of the richest countries in the world with regard to genetic resources of

medicinal plants. At present, many people are relying on traditional medicine for their primary health care (Vitalini *et al.,* 2009; Tabi, 2006).

The World Health Organization (WHO) has acknowledged the need for inexpensive and effective treatment for common diseases in low-income countries (Tabi, 2006). Most traditional medical practitioners live and work at the community level, which can make such treatments available and affordable to most of the population. In fact, WHO estimates that approximately 80% of the world's population relies mainly on traditional remedies for their health care (Tijjani *et al.,* 2009; Shokeena *et al.,* 2009). In Ethiopia, about 800 species of plants are used in the traditional health care system to treat nearly 300 physical and mental disorders, and remains to be the main resource of treatment for a large majority (80%) of the people (Teklehaymanot, 2009).

It has been showed that a plant known as 'Noni' in Hawaii, or *Morinda citrofolia* L. can be used to fight various ailments (Vitalini *et al.,* 2009). Chemical compounds which appear to be remarkably effective at killing TB bacteria under laboratory conditions have been isolated from it. In Kenya, early accounts of Maasai plant use also indicate that a variety of plants (*Aloe secundiflora* Engl.; *Carissa edulis* Vahl.; *Secamone punctulata* Decne.; *Myrsine africana* L. and *Rhamnus staddo* A. Rich.) were used to treat infectious diseases like tuberculosis and polio (Bussmann *et al.,* 2006). In the Mediterranean area, several researches on plants traditionally used in different fields (human and veterinary sciences, food, minor nourishment, household and handicraft sectors), have been conducted (Vitalini *et al.,* 2009).

In West and Central Africa, traditional medicine *Cleistopholis patens* (Benth.) Engl. and Diels has a history of use to treat respiratory disease: in Zaire sap from pounded bark, or a bark decoction, is drunk for treating TB and simple bronchial afflictions, while in Nigeria the Yoruba use parts of the tree for curing coughs (Okunade *et al.,* 2004). Plant species of the family Moraceae have been reported for their antimicrobial potentials (Kuete *et al.,* 2009). Within this family, species of the genus *Morus* have also been reported to possess antimicrobial potency. There is still more work to be done locally and the present investigation will provide important baseline information for the use of medicinal plants and their constituents for the treatment of infections.

2.7.2 Phytochemicals found in medicinal plants

Despite progress in synthetic chemistry, plants still constitute an important source of pharmaceuticals and other compounds of economic importance (Zhang and Bjorn, 2009; Edeoga *et al.,* 2005). Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Zhang and Bjorn, 2009). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, phenolic compounds and others (Edeoga *et al.,* 2005).

2.7.2.1 Alkaloids

Alkaloids are low-molecular weight nitrogen-containing organic compounds (Fig. 2). They usually have heterocyclic structures and occur in approximately 20% of all plant

species (Zhang and Bjorn, 2009; Rao *et al.,* 2009). They function primarily as defensive compounds hence possess antibacterial, antifungal, phytotoxic, insecticidal, and hemolytic properties (Chen and Fadamiro, 2009).

According to Okunade *et al.,* (2004), 12 pure alkaloids were among the metabolites reported to have antimycobacterial activities. In another study (Lim *et al.,* 2009), 23 new and known naturally occurring alkaloids such as oxindole alkaloid and analogs have been assayed and found to possess antimycobacterial activities. The rhizomes and roots of *Clausena excavata* Burm. F. (Rutaceae) and the other indole alkaloid, all have been found to have modest activity against MTB.

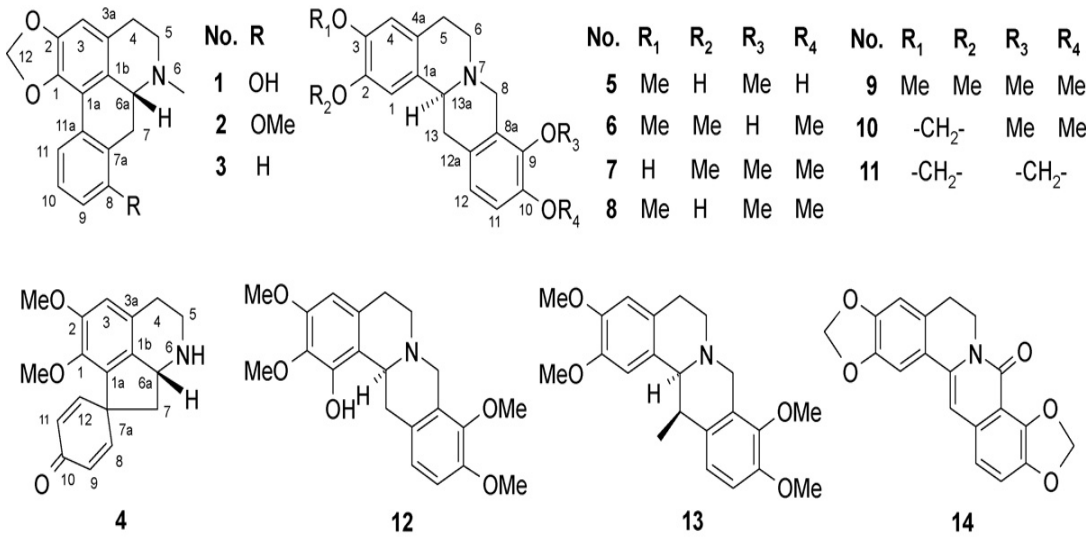


Figure 2. Chemical structures of alkaloids from the fresh rattan stem of *Fibraurea recisa* (Rao *et al.,* 2009).

2.7.2.2 Cardiac Glycosides

Cardiac glycosides are a class of natural products that are used to increase cardiac contractile force in patients with congestive heart failure and cardiac arrhythmias

(McConkey *et al.*, 2000) (Fig. 3). The most familiar are digoxin/ digitoxin and ouabain, which are derived from the plant genera *Digitalis* and *Strophanthus gratus* (Hook.) Franch. respectively. Cardiac glycosides have been used for pharmacological treatment of heart failure for more than 200 years (Rao *et al.*, 2009). Today they are still indicated for heart rate control in atrial fibrillation especially when concomitant heart failure is present. The current guidelines for the treatment of heart failure recommend the use of cardiac glycosides (at low concentrations) if serious heart failure symptoms remain even after optimal adjustment of all other therapeutic options.

Oleandrin and oleandrigenin are cardiac glycosides derived from oleander (*Nerium oleander* L.) that have been used in the treatment of cardiac abnormalities in Russia and China for years (McConkey *et al.*, 2000). Interestingly, anecdotal evidence has emerged from experience suggesting that they may produce beneficial side effects in patients with leiomyosarcoma, Ewing's sarcoma, prostate cancer and breast cancer.

The cardiac glycosides also represent an important class of useful, albeit somewhat dangerous steroids (McConkey *et al.*, 2000). These compounds are characterized by the steroidal cardenolide aglycone bonded at the C-3 position to a sugar moiety which can range from a monosaccharide to a trisaccharide.

Digoxin

ß-Acetyldigoxin

Methyldigoxin

Quabain/g-Strophantin

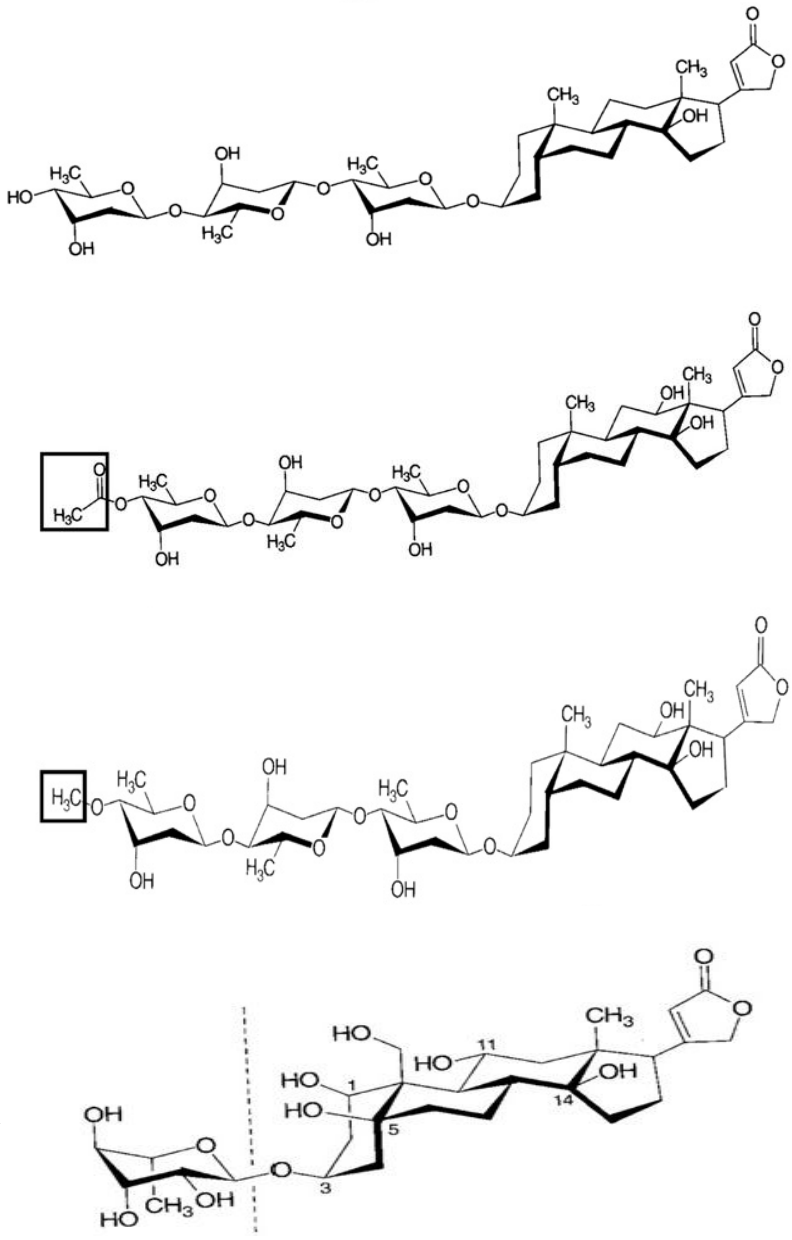


Figure 3. Molecular structures of cardiac glycosides (Hauck *et al.*, 2009).

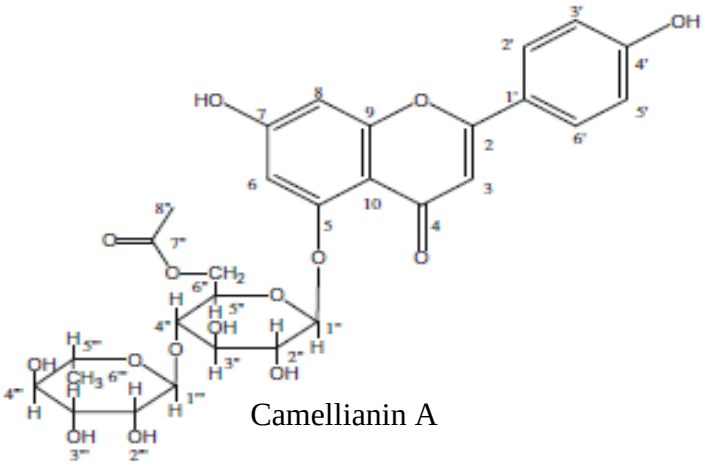
2.7.2.3 Flavonoids, Flavonols and Flavones

Flavonoids, widespread in fruits, vegetables, teas and medicinal plants, have received great attention and have been studied extensively, because they are a group of biologically active plant compounds (Yuan *et al.*, 2009) (Fig. 4). They are perhaps the

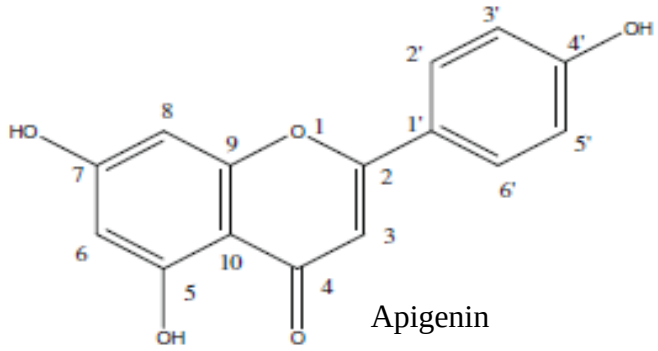
most important single group of phenolics comprising of a group of over 4000 aromatic plant compounds, which include anthocyanins, proanthocyanidins, flavonols and catechins (Carvalho *et al.,* 2010). In fact, anthocyanins have been reported as anti-inflammatory, vasotonic and anti-oxidant compounds, playing an important role in the prevention of degenerative illnesses such as cancer, Alzheimer's disease or cardiovascular illnesses (Diaz *et al.,* 2009).

Flavonols isolated from *Haplopappus sonoriensis* (A. Gray) S.F. Blake (Asteraceae), and flavone from *Lysionotus pauciflorus* Maxim. (Gesneriaceae) are believed to be the active principles of extracts from these plants (Onyilagha *et al.,* 2009; Okunade *et al.,* 2004). A leaf extract of *Haplopappus rigidus* Phil., is used locally to treat coughs in Chile (Morales *et al.,* 2003).

Adinandra nitida Merr. ex Li., a wild plant in south China, is a flavonoid-rich plant source. Its leaves have been consumed as health tea and herbal medicine for hundreds of years (Yuan *et al.,* 2009). It is reported to have many curative properties, such as reducing blood pressure, as well as antibacterial, antioxidant and analgesic effects. Studies have shown that the content of flavonoids in *A. nitida* leaves can be more than 20%, including camellianin A, camellianin B and apigenin. Camellianin A is however, the main flavonoid (Yuan *et al.,* 2009).



Camellianin A



Apigenin

Figure 4. Chemical structures of camellianin A and apigenin (Yuan *et al.*, 2009).

2.7.2.4 Tannins

Tannins are polymeric flavonoids that comprise a small part of the broad and diverse group of phenolic compounds that plants produce as secondary metabolites (Diaz *et al.*, 2009) (Fig. 5). They are subdivided into condensed and hydrolyzable compounds (Oszmianski *et al.*, 2007; Hargerman 2002). They are reported to exhibit antiviral, antibacterial and anti-tumor activities (Aiyelaagbe and Osamudiamen, 2009; Diaz *et al.*,

2009). It has been reported that certain tannins are able to inhibit HIV replication selectively (Aiyelaagbe and Osamudiamen, 2009).

Onwukaeme *et al.,* (2007) have found that exudates of *Anchomonas difformis*, and *Cyrtrospherma senegalense*, have been found to contain reducing sugars, whereas exudates of *Pycanthus angolensis* contain reducing sugars, flavonoids and tannins.

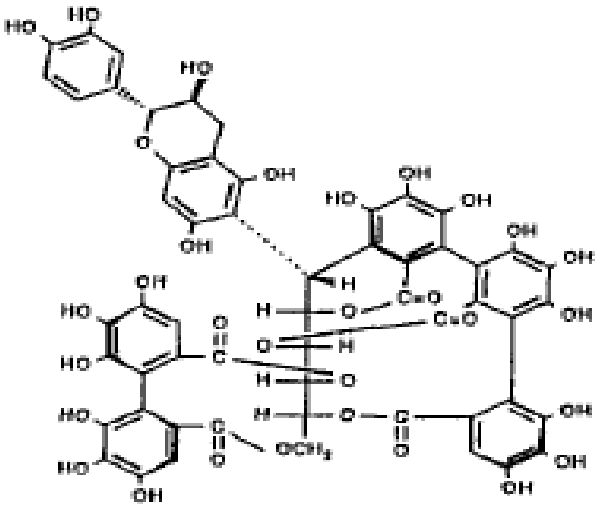


Figure 5. Chemical structures acutissimin B (Hargerman, 2002).

2.7.2.5 Terpenoids and essential oils

In plants, terpenoids represent a chemical defense against environmental stress and provide a repair mechanism for wounds and injuries (Salminen *et al.,* 2008) (Fig. 6). Interestingly, effective ingredients in several plant-derived medicinal extracts are also terpenoid compounds of monoterpenoid, sesquiterpenoid, diterpenoid, triterpenoid and carotenoid groups (Gordien *et al.,* 2009; Salminen *et al.,* 2008). The presence of antimycobacterial terpenoids in *Juniperus communis* L. aerial parts and roots justifies, to

some extent, the ethnomedicinal use of this species as a traditional anti-TB remedy (Gordien *et al.,* 2009). Whilst several terpenoids have been identified as responsible for the antimycobacterial activity of *Juniperus procera* L. (another species traditionally used as an anti-TB remedy), the antimycobacterial activity of common juniper has never been attributed to any pure active substance(s) (Gordien *et al.,* 2009).

Essential oils constitute a heterogeneous collection of chemical compounds (Zhang and Bjorn, 2009; Okunade *et al.,* 2004). They are synthesized by plants and are volatile and mostly soluble in ethanol (Zhang and Bjorn, 2009). They have traditionally been obtained from plants by extraction and distillation. Since the middle ages, they have been widely used for insecticidal, medicinal and cosmetic purposes.

Synthetic and natural plant terpenoids have also been reported to have moderate to high antimycobacterial activity against MTB (Okunade *et al.,* 2004) (Fig. 6). *Croton kongensis* Gagnep (Euphorbiaceae) has shown significant activity against MTB. Totarol from the outerbark of *Xanthocyparis nootkatensis* (D. Don) Farjon & D. K. Harder (synonym *Chamaecyparis nootkatensis*) (Cupressaceae) is reported to have activity against MTB also. Other compounds in this class that are very active and require further study are the hopane terpenoids and are obtained from the insect pathogenic fungus *Aschersonia tubulata.*

Juniperus communis L. a plant which has been reported as a traditional cure for tuberculosis (TB) and other respiratory diseases in crude form has been studied by

isolating and identifying the constituents responsible for the activity (Gordien *et al.*, 2009). The evaluation of the activity of the pure isolated compounds from its aerial parts against *Mycobacterium tuberculosis* indicated that it was inactive. Maybe this is because the labdane skeleton is required for activity (Gordien *et al.*, 2009).

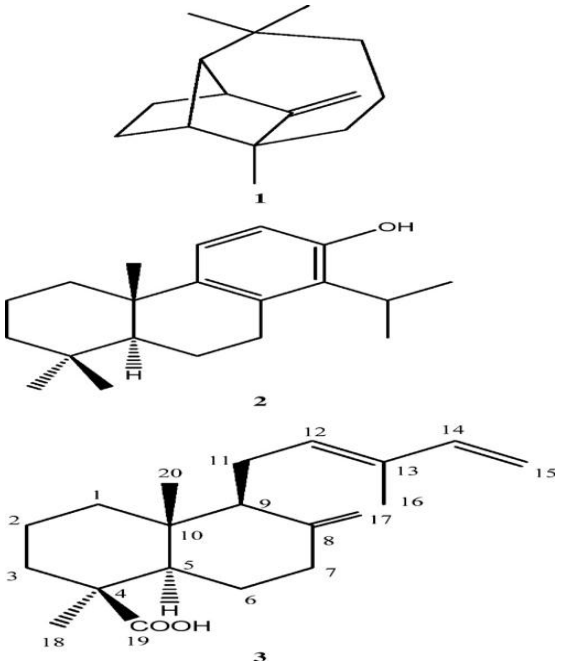


Figure 6. Structures of antimycobacterial terpenoids isolated from *Juniperus communis* (Gordien *et al.*, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study focused on various ecological zones of the Lake Victoria regions that represent diverse plant species and cultures of the region. The study concentrated on Siaya, Bondo, Teso and Kisii town which represented urban and peri-urban regions.

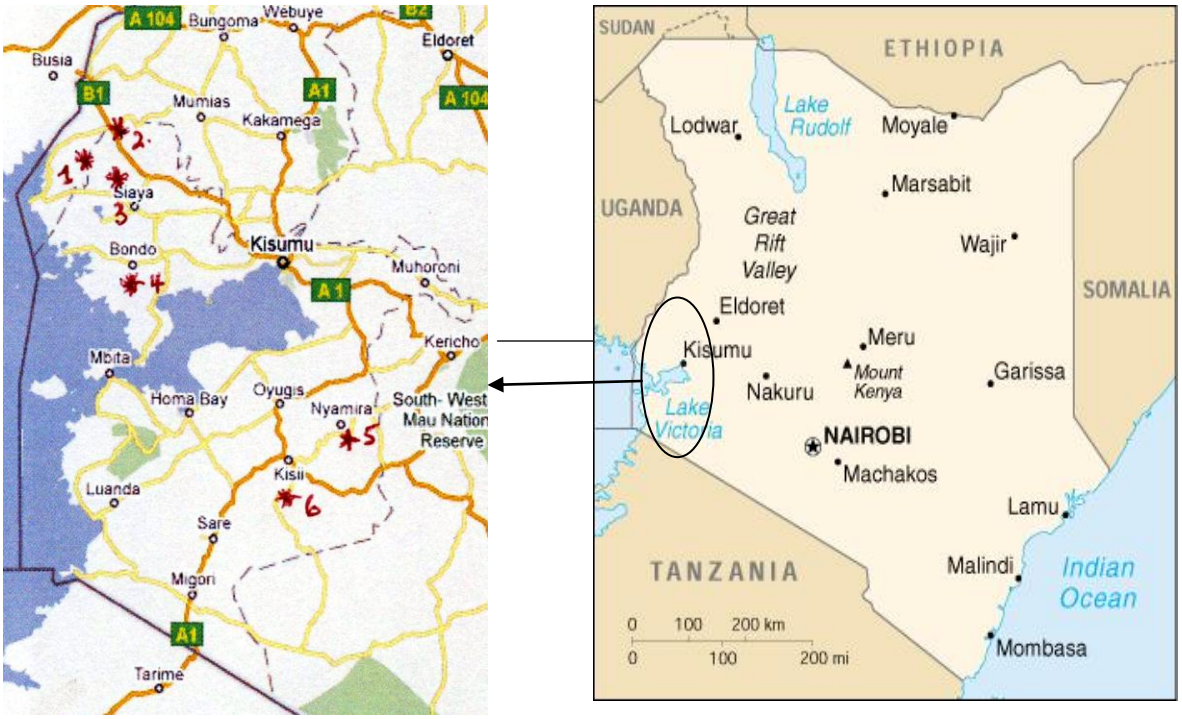


Figure 7. Map of Lake Victoria region (Source: Google earth, 2009)

This study was also carried out at Wamba Division, Samburu District, Rift Valley Province that is 0.98°N and 37.34°E whose main inhabitants are the Samburu community. This is an arid to semi-arid region with annual rainfall of between 250 -

500mm. The only semi-permanent river is the Ewaso Nyiro. For most of the year, the area experiences high temperatures which vary with altitude and are generally between 24ºC and 33 ºC during the day with no cloud cover. The soil is dry and sandy with poor vegetation cover (Bussmann, 2006).

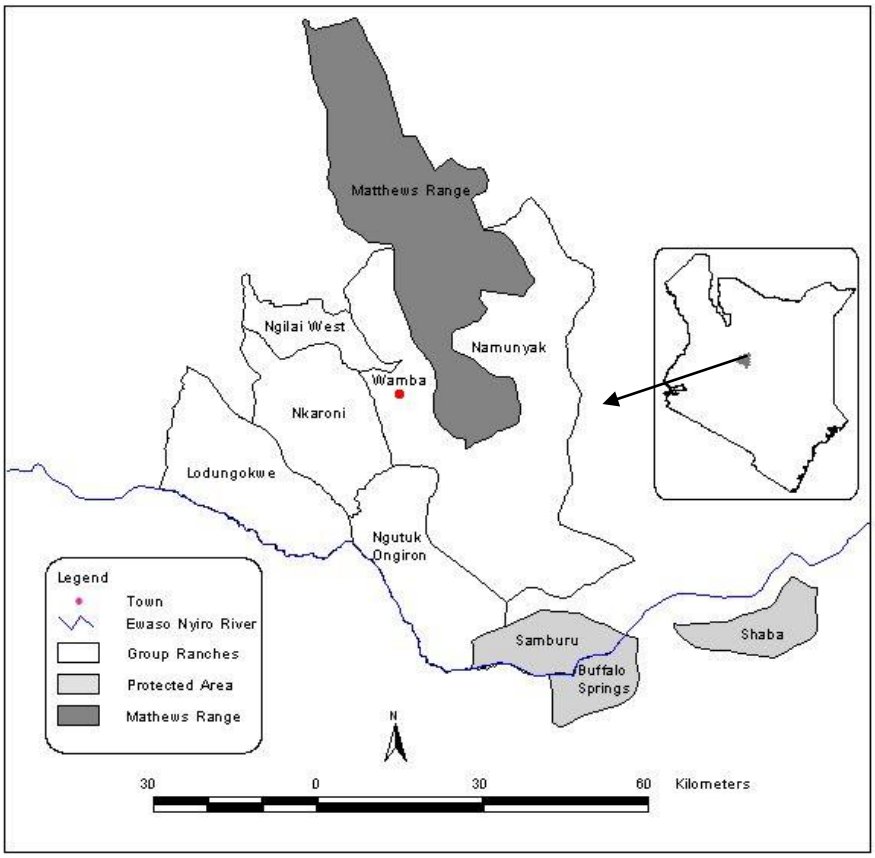


Figure 8. Map of Samburu region (Mariita *et al.*, 2010).

3.2 Medicinal plants selection criteria

When screening for biologically active plant constituents, the selection of the plant species to be studied was a crucial factor for the ultimate success of the investigation (Potterat and Hamburger, 2008). In this study, the method of collecting ethnobotanical

information included: interviewing key informants using generative methodology, Focus Group Discussions (FGD), market visits, in-depth interviews, cross sectional surveys, checking various websites, examination of herbarium materials and use of questionnaires (Appendix I). Checklists of respondents were obtained from village government offices, and then respondents selected on the basis of known herbal medicinal practices.

Medicinal plants to be tested were selected based on their preference. Preference of the plant species was ranked using informant-indexing techniques. The model used was "species choice value". Species choice value is defined as the percentage of informants who cited a number of species mentioned for that category by all the informants (Kremen *et. al.,* 1998).

3.3 Plant collection and identification

Plants were collected from Bondo, Siaya Teso, Kisii south, Kisii Central (all in western Kenya) and Samburu in North Rift, based on the ethnobotanical survey that was carried out. The plants were authenticated by a plant taxonomist from the Department of Pharmacy and Contemporary Medicine, Kenyatta University, Nairobi, Kenya, and voucher specimens collected and deposited at the Kenyatta university herbarium to aid confirmation of plant identity.

3.4 Drying, grinding and methanolic extraction

The plant samples collected were chopped into small pieces, shade dried and ground using hammer type milling machine (Meecan, CM/L-1364548, India) at the Department of Pharmacy/CAM, Kenyatta University, Nairobi, Kenya. The powdered material was transferred into and extracted in the soxhlet extractor using methanol for 72 h (Aiyelaagbe and Osamudiamen, 2009). The extracts were filtered through a Whatmann filter paper No. 42 (125 mm) and concentrated using a rotary evaporator (Laborota 4000, SN 090816862, Germany) with the water bath set at 40 °C (Edeoga *et al.,* 2005), then dried in a dessicator over anhydrous $CuSO_4$. The powdered residues were transferred into vials and stored at 4 °C in airtight vials before analysis.

3.5 Test cultures

The four species of mycobacteria used for the assays were obtained from the Center for Respiratory Diseases Research (CRDR), Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. These included *Mycobacterium tuberculosis, M. kansasii, M. smegmatis* and *M. fortuitum*. Rejuvenation was done on the LJ slants for 14 days at 37 ^{0}C using standard procedures (Asres *et al.* 2001; Ani *et al.,* 2009).

The test cultures for general antibacterial activity were obtained from Kenyatta National Hospital in Nairobi and included *Salmonella typhi* (clinical isolate), *Klebsiella pneumoniae* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 25852), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 20591) and *Candida albicans*

(ATCC EK138), a yeast like fungus, were obtained from Kenyatta National Hospital in Nairobi, Kenya and used in the antibacterial and antifungal activity tests, respectively.

3.6 Antimycobacterial testing

3.6.1 Proportional method (use of Lowenstein Jensen (LJ) slants)

The extracts were dissolved in DMSO (to get the desired final concentrations of 2mg/ml, 1mg/ml and 0.5mg/ml) and added to the medium (till tube is half full) before heating at 85°C for 50 min. in a slanting position and prepared ready for use after storage at room temperature for 48 h to exclude contamination (Ani *et al.,* 2009; Asres *et al.,* 2001). The tubes containing the medium were inoculated with strains of mycobacteria. A stock solution of 2.0mg/ml of isoniazid was prepared. Isoniazid was used as the positive control, while DMSO vehicle was the negative control.

Isolates of the four species of Mycobacteria were prepared for antimicrobial susceptibility testing by the proportion method. Using a 3mm internal diameter (24 standard wire gauge) wire loop, about 4mg fresh culture was scraped from LJ medium into 500 µl sterile distilled water in a bijou with five glass beads and vortexed for about 30 sec to homogenize. The suspension was made up to 4ml volume by adding 3.5ml sterile distilled water and allowed to settle for about 30 min before gently aspirating the upper portion into a fresh bijou bottle to get the suspension. The suspension was further diluted to obtain the turbidity of 0.5 McFarland standard (Ani *et al.,* 2009). Bacterial suspension was inoculated into extract-free and extract-containing LJ slopes and incubated at 37° C. Growth was recorded weekly (for 6 weeks) as: +++ for confluent

growth, ++ moderate growth, + less growth and – for no growth. Cultured tubes were examined visually and sample tubes showing less growth than negative control tubes were considered to be inhibitory.

3.6.2 BACTEC MGIT™ 960

This was used in the antimycobacterial activity assessment of the plant extracts. The extracts were dissolved in 0.01% DMSO to final concentrations of 0.5, 1.0 and 2.0 mg ml^{-1}. A stock solution of 2.0 mg ml^{-1} of isoniazid was used as the positive and 0.01% DMSO as the negative control, respectively. Into the 7 ml BBL™ MGIT™ tubes, 0.8 ml of the mixture of growth OADC (containing Oleic acid, Bovine albumen, Dextrose and Catalase) supplement (added to provide essential substances for rapid growth of mycobacteria) and BBL™ MGIT™ PANTA (a mixture of antimicrobial agents) were added. Then 1 ml of the extract was added into the BBL™ MGIT™ tubes containing the supplement to attain appropriate concentrations of 0.5, 1.0 and 2 mg ml^{-1}. *Mycobacterium* suspension (adjusted to 0.5 McFarland standard) was introduced into the BBL™ MGIT™ tubes. The strains included *M. tuberculosis (*Mtb*), M. kansasii* (Mk), *M. fortuitum* (Mf) and *M. smegmatis* (Ms). The BACTEC MGIT™ 960 system was loaded using manufactures' instructions and incubated at 37° C. Culture vials which remained negative for a minimum of 42 days (maximum 56 days) were removed and recorded as negative, while growth units (GUs) for the positive ones were recorded appropriately (Becton, Dickinson and Company, 2007). The same was done for the controls. Results were provided as positive/negative and numerical Growth Units (GUs) using a non-radiometric evaluation technique (Becton, Dickinson and Company, 2007).

3.6.3 Smear microscopy and staining

The detection of acid-fast bacilli (AFB) in a stained smear was used as the quickest and easiest procedure to provide preliminary laboratory confirmation of the presence of mycobacteria in both the liquid and solid media, as well as to find out if there was any contamination (Harris *et al.,* 2000). The carbolfuchsin-based staining procedure, Ziehl-Neelsen was used as outlined by Harris *et al.,* (2000).

3.7 Disc Diffusion technique (DD)

The antibacterial and antifungal activities of extracts from the 8 plant species were assayed *in vitro* by agar disc diffusion (DD) method (Parekh and Chanda, 2007). Filter paper discs (6 mm) were impregnated with the plant extracts. Mueller Hinton agar and Potato Dextrose Agar (PDA) were prepared using manufactures' instructions for purposes of culturing the bacteria and fungi respectively. Normal saline solution was used to dilute a 24 h culture of the bacterial type culture or clinical isolate to attain a 0.5 McFarland standard. Spread plate method was used to culture 100 µl of the microbial suspension that was introduced into the Petri dishes (Meite *et al.*, 2009). Eighteen dry sterile discs (6 mm diameter) were soaked in the plant extract (made by dissolving 300 mg of the extracts in 1000 µl of methanol) air dried and placed on the spread plates at reasonable distances. Discs impregnated with methanol and air dried were used as negative controls and various standard conventional antibiotics (Amoxicillin (Hangzhou Ruijian chemical Co., Ltd., batch 490805241); Ciprofloxacin (Chengdu Ware Yuanheng Pharmaceutical Co., Ltd, batch 20070907); Fluconazole (Pfizer Ltd., UK batch 30) as positive controls. The plates were then incubated at 35 ºC for 24 h. This was replicated three times for each pathogen.

Candida albicans was cultured by taking 100 µl from the broth and spreading on PDA. The culture was incubated at 25° C for 72 h. The cork boarer was used to pick a section of the young mycelium which was placed at the centre of the PDA plate and the dry discs which were impregnated with 100 µl of the plant extracts placed at a distance around the inoculum mycelium. The inoculum was incubated at 25 ˚C for 72 h. Fluconazole and dry discs treated with methanol were also used as positive and negative controls respectively. All tests were performed in triplicate. Microbial growth inhibition was determined by measuring the zones of inhibition using a transparent ruler.

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

The Minimum Inhibitory Concentration (MIC) which is the least amount of antimicrobial agent that will inhibit visible growth of an organism after an overnight incubation was determined using the microtitre dilution broth method in 96–well micro plates. This was done only where the plant extract showed strong antibacterial activity by the disk diffusion method (≥9-15 mm) (Rani and Khullar, 2004). The wells were filled with 50 µl of the Nutrient broth for bacterial strains and Potato dextrose broth for *C. albicans*. The extract was then prepared by taking 300 mg of the plant extract and mixing it with 1000 µl of DMF (0.01% Dimethyl formanide) for complete dissolution of the extract. Then 50 µl of the plant extract was dispensed into the first well before serial dilutions were done by transferring 50 µl of nutrient or potato dextrose broth containing the extract from the first well to the second well, and from the second well to the third

well through the fourth well. Fifty microlitres (50 µl) of the test isolate was then dispensed into each well. One well (without extract or drug) was used as negative control of the growth of the microorganisms in the medium whereas another well with 50 µl of the antibiotic (Amoxicillin /Ciprofloxacin/fluconazole) was used as positive control. Incubation was done at 37° C for 24 h.

For the determination of MBC/MFC, wells where there was no growth were subcultured on nutrient agar and PDA. The lowest concentration of the plant extracts that did not yield any colony on the solid medium (Nutrient or PDA agar) after sub culturing and incubating for 24 h for bacterial strains and 72 h for *C. albicans* was taken as the MFC/MBC. All tests were performed in triplicates (Ruttoh, 2009).

3.9. Phytochemical screening

3.9.1 Test for alkaloids (Wagner's method)

Alkaloids presence was determined by dissolving and filtering 200 mg plant extract in 10 ml methanol followed by filtration using Whatmann filter paper No. 42 (125 mm) filters. One thousand microlitres (1 ml) of the filtrate was then mixed with 6 drops of Wagner's reagent (Obdoni and Ochuko, 2001). Creamish, brownish-red or 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.

3.9.2 Test for cardiac glycosides (Keller-Killani test):

Five milliliter (5 ml) of each extract were treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides. A (+) reaction was recorded when a faint green-blue colour was observed (indicating low concentrations of detectable cardiac glycosides); a (++) reaction was recorded when a medium green-blue colour was observed (indicating moderate concentrations of detectable cardiac glycosides); and a (+++) reaction was recorded when a deep green-blue colour was observed (indicating high concentrations of detectable cardiac glycosides) (Aiyelaagbe and Osamudiamen, 2009).

3.9.3 Test for flavonoids

Five milliliters (5 ml) of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. A (+) reaction was reported in pale yellow colour; (++) in moderate yellow and (+++) in strong yellow coloration, indicating low, moderate or high concentration of flavonoids respectively in the plant extract (Edeoga *et al.,* 2005).

3.9.4 Test for saponins

To 0.5 mg of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigourously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously, after which it was observed for the

formation of an emulsion (Aiyelaagbe and Osamudiamen, 2009). A (+) sign was recorded when the froth reached a height of 50 mm; a (++) sign with the height of 60-100 mm; and a (+++) sign with a height of more than 100 mm to indicate low, moderate and high concentration of saponins respectively in the plant extract.

3.9.5 Test for tannins

About 0.5 mg of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration (Edeoga *et al.,* 2005). 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. The reactions were used to indicate the presence of different concentrations of detectable tannins, with (+) representing low, (++), moderate and (+++) high levels of tannins.

3.9.6 Test for terpenoids (Salkowski test)

To 0.5 mg each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration at the interface indicated the presence of terpenoids (Aiyelaagbe and Osamudiamen, 2009; Edeoga *et al.,* 2005). 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.

3.10 Statistical analysis

The densities of growth in LJ were presented in tables and the growth units from the BACTEC MGIT 960 compared with the growth units of the controls and the data presented appropriately. For general antibacterial and antifungal assays, the average zones of inhibition values were expressed as means for each test cultures and data was analyzed using the Minitab Statistical Software 13.20 © 2000 Minitab Inc. PA 16801-9928, USA. Among the groups significance tests were performed using the one-way ANOVA at 5% significance level. P value < 0.05 was considered as significant. The significant means were separated by the Tukey's test (Appendix III).

CHAPTER FOUR

RESULTS

4.1 Ethnobotanical survey

The survey gathered information on 34 plant species reported by the informants for their medicinal use by the communities living around the Lake Victoria region and the Samburu community (Table 1). From the Lake Victoria region, a total of seventeen plants were collected and studied. Out of these, seven plants were collected from Kisii, five from Bondo, four from Siaya and one from Teso. The remaining seventeen plants were collected from various conservancies in Samburu. Eight plants were collected from Nkaroni Conservancy, six from Namunyak Conservancy, two from West Gate Conservancy and one from Lodungokwe.

The reported species were distributed among 23 botanical families (Table 1). Euphorbiaceae and Mimosaceae were most represented with four plant species each, followed by Verbenaceae which had three. Fabaceae, Rutaceae and Capparaceae were represented by two plant species each and of the remaining 17 families each had one plant species. All the medicinal plants were reported in their local (Vernacular) names (Samburu, Kisii, Dholuo, and Teso). The harvesting was done sustainably and depended on the parts of the plant the communities used in the treatment of various diseases. Most species were reported to be used as remedies against human gastrointestinal problems, several species against TB, asthma, malaria and chest problems.

Table 1: Medicinal plants used by the communities around the Lake Victoria region and Samburu Community of Kenya to treat mycobacterial, selected bacterial and fungal diseases.

Botanical Name	**Family name**	**Local name(s)**	**Where collected from**	**Part(s) used**	**Diseases treated**
Carissa edulis Vahl	Apocynaceae	Omonyangateti (Kisii)	Kisii South	Roots	Polio, TB, asthma, gonorrhoea, malaria
Entada abysinnica Steudel ex A. Rich.	Fabaceae	Laginaria (Luo)	Bondo (Sakwa)	Leaves	Pneumonia, stomach ache, bronchitis, coughs, arthritis
Croton macrostachyus Hochst. ex Ferret *et* Galinier	Euphorbiaceae	Odhwidho (Luo) Omosocho (Kisii)	Siaya (Alego)	Stem bark	stomacha ache, TB, asthma, coughs, rheumatism
Vernonia amygdalina Del.	Asteraceae	Okelo – okelo (Luo) Omosabakwa (Kisii)	Kisii south	Root barks	Gastrointestinal problems, TB, asthma
Momordica charantia L.	Cucurbitaceae	Echokilayiti (Teso)	Teso	Whole plant	Asthma, TB, pneumonia
Ocimum gratissimum L.	Labiatae	Obweny (Luo)	Siaya	Leaves	Ear problems, chest pains
Lantana camara L.	Verbenaceae	Nyamndhi (Nyabende/ Atek) (Luo)	Bondo (Sakwa)	Leaves	Ulcers, TB, pneumonia, chest pains, malaria
Clerodendrum myricoides (Hochst)Vatke	Verbenaceae	Okwerogweno (Luo) Omonyasese (Kisii),	Siaya (Alego)	Roots	Gonorrhea , mumps, TB, chest problems, hepatitis
Toddalia asiatica (L.) Lam.	Rutaceae	Nyalwet kwach (Luo) Ekenagu ekiegarori (Kisii)	Bondo (Alego)	Roots	Stomach ache, measles, TB,

Lantana trifolia L.	Verbenaceae	Nyabendwiny (Luo) Obori bwenyoni (Kisii)	Bondo (Sakwa)	Leaves	Heart problems, gonorrhea, TB, coughs
Erythrina abyssinica Lam ex DC	Fabaceae	Omotembe (Kisii)	Kisii Central	Root barks	TB, malaria, stomach ache
Euphorbia tirucalli L.	Euphorbiaceae	Ekerachwoki (Kisii)	Kisii Central	Stem	TB, asthma
Fuerstia africana T.C.E.Fr.	Lamiaceae	Mienya (Luo) Ekebunga baiseke (Kisii)	Bondo (Sakwa)	Leaves	Malaria, asthma, chest problems
Aloe secundiflora Engl.	Aloaceae	Omogaka (Kisii)	Kisii Central	Leaves	Polio, malaria, stomach ache, chest problems
Zanthoxylum gillettii De Wild.) Waterm	Rutaceae	Egekoma (Kisii)	Kisii Central	Stem bark	TB, asthma
Pistacia aethiopica Kokwaro	Anacardiaceae	Not Recorded	Siaya	Leaves	TB, oral infections
Croton megalocarpus Hutch.	Euphorbiaceae	Omosocho (Kisii)	Kisii south	Leaves	Stomachache, ear infection, TB
Salvadora persica L. var. persica	Salvadoraceae	Sokotei (Samburu)	Nkaroni	Roots/ branches	Chest problems, stomachache, teeth problems,
Acacia Senegal (L.) Willd.Var.	Mimosaceae	Lderekesi (Samburu)	Nkaroni	Bark	Stomachache
Acokanthera friesiorum Markgr.	Apocynaceae	Chipilikwa (Samburu)	Namunyak	leaves	Diarrhoea
Plumbago dawei Rolfe.	Plumbaginaceae	Lkiarianthus (Samburu)	Namunyak	Bark	Malaria, diarrhoea
Loranthus	Loranthaceae	Lardenyai	Nkaroni	Whole	Stomach ache

acaciae Zucc.		(Samburu)		plant	
Cordia sinensis Lam.	Boraginaceae	Silapani (Samburu)	Nkaroni	Bark	Diarrhoea
Acacia horrida (L.) Willd.	Mimosaceae	Lerai (Samburu)	Westgate	Bark	Diarrhoea
Albizia anthelmitica Brongn.	Mimosaceae	Lamurtana (Samburu)	Nkaroni	Bark	Deworming, diarrhoea
Thylachium africanum Lour.	Capparaceae	Loimugi (Samburu)	Namunyak	Bark	Diarrhoea
Boscia angustifolia Guill. and Perr	Capparaceae	Lororoi (Samburu)	Nkaroni	Bark	Diarrhoea, gonorrhea
Cissus quadrangularis L.	Vitaceae	Sukurtut (Samburu)	Namunyak	Stem	Stomach aches
Grewia simi K. schum.	Tiliaceae	Lngalayoi (Samburu)	Nkaroni	Roots	Diarrhoea
Acacia etbaica Schweinf.	Mimosaceae	Lchakwai (Samburu)	Namunyak	Leaves	Stomach ache
Scadoxus multiflorus (Martyn) Raf.	Amaryllidaceae	Loilei (Samburu)	Westgate	Leaves	Stomach ache
Commiphora africana (A. Rich) Engl.	Burseraceae	Lcheni-ngiro (Samburu)	Namunyak	Bark	Eye problems, Stomach ache
Euphorbia scarlatica (L) O. Ktze	Euphorbiaceae	Mpopongi (Samburu)	Lodungokwe	Stem	Stomach ache, common cold, TB
Acacia nilotica (L.) Del.	Mimosaceae	Lkiloriti (Samburu)	Namunyak	Stem bark	Stomach ache

Plates representing some of the medicinal plants

Plate 1: *Erythrina abyssinica*

Plate 2: *Carissa edulis*

Plate 3: *Boscia angustifolia*

Plate 4: *Clerodendrum myricoides*

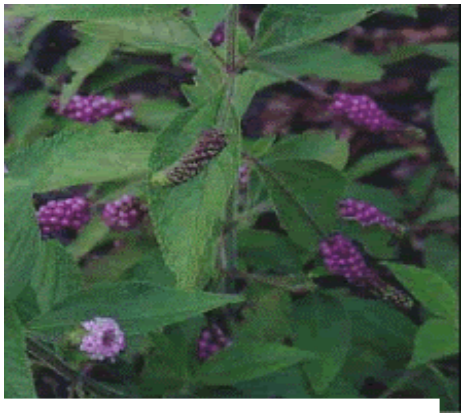


Plate 5: *Lantana trifolia*

Plate 6: *Vernonia amygdalina*

Plate 7: *Croton macrostachyus*

Plate 8: *Toddalia asiatica*

Plate 9: *Euphorbia tirucalli*

Plate 10: *Salvadora persica*

Plate 11: *Loranthus acacia*

Plate 12: *Acacia nilotica*

4.2 Antimycobacterial results

The methanol extracts of the medicinal plants studied gave various results against *M. tuberculosis, M. kansasii, M. fortuitum* and *M. smegmatis* (Table 2). *Carissa edulis, Entada abysinnica, Croton macrostachyus, Vernonia amygdalina, Toddalia asiatica, Aloe secundiflora, Salvadora persica* and *Euphorbia scarlatina* showed encouraging activity in most of the mycobacteria cultures tested using the automated BACTEC **M**ycobacterial **G**rowth **I**ndicator **T**ube (MGIT) 960TB system, which is a state of the art, *in-vitro* diagnostic instrument designed and optimized for the rapid detection of mycobacteria (Plate 13 and 14). They all showed activity against slow (*M. tuberculosis, M. kansasii*) and fast (*M. fortuitum* and *M. smegmatis*) growing mycobacteria. Results were provided as positive/negative and numerical Growth Units (GUs) using a non-radiometric evaluation technique. BBL^{TM} MGIT^{TM} 7ml indicator tubes were used (Plate 14).

Plate 13: Bactec MGIT 960 System

Plate 14: BBL^{TM} MGIT^{TM} tube

From the proportional method (using Lowenstein (LJ) slants), the culture tubes were examined visually and sample tubes showed no growth in all concentrations of 2.0 mg/ml. The results in other concentrations corresponded with those of BACTEC MGIT 960, albeit missing the quantification aspect. Where there was moderate growth, the extract could still be considered to be inhibitory because there was less growth than in the negative control tubes (Appendix II). The LJ slants were used as a gold standard method (Plate 15 and 16).

Sample plates of some Mycobacteria strains on LJ slants

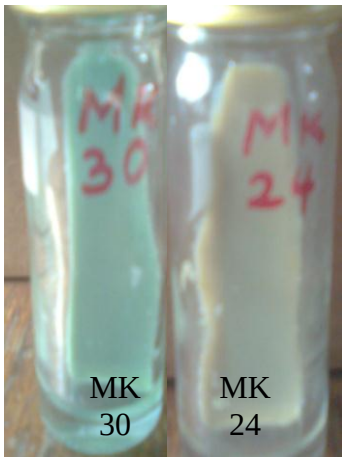


Plate 15: Mk 30 showing *Cissus Quadrangularis* (+) and Mk 24 *Ocimum gratissimum*

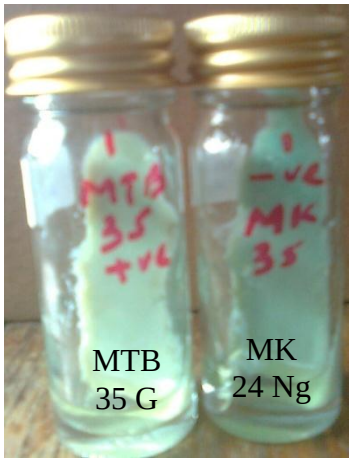


Plate 16: Mtb 35 showing growth (G) of Mtb on LJ Containing *Croton megalocarpus* extract, and no growth (Ng) for Mk

Table 2: Antimycobacterial activity (GUs) of medicinal plants using BACTEC MGIT™ 960 System

Botanical name of plant	**Activity on slow growers at various concentrations (mg/ml)**						**Activity on fast growers at various concentrations (mg/ml)**					
	2		1		0.5		2		1		0.5	
	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mf	Ms	Mf	Ms	Mf	Ms
C. edulis	0	0	0	206	770	294	0	0	0	0	0	0
E. abysinnica	0	0	0	0	1079	0	0	0	0	0	18	97
C. macrostachyus	0	0	0	160	0	398	0	0	0	0	76	48
V. amygdalina	0	0	0	67	0	451	0	0	0	0	0	0
M. charantia	0	0	3013	21408	ND	ND	0	0	113	5012	ND	ND
O. gratissimum	0	0	678	10990	ND	ND	0	0	56	371	ND	ND
L. camara	0	0	5775	0	9198	128	0	0	37	75	ND	ND
C. myricoides	0	0	0	2212	1974	6752	0	0	0	0	438	10561
T. asiatica	0	0	0	0	0	0	0	0	1	0	1205	138
L. trifolia	0	0	16648	6310	ND	ND	0	0	75	89	ND	ND
E. abyssinica	0	0	724	19741	ND	ND	0	0	174	4915	ND	ND
E. tirucalli	0	0	0	12511	673	16435	0	0	79	3120	ND	ND
F. africana	0	0	4883	15162	ND	ND	0	0	197	2091	ND	ND
A. secundiflora	0	0	0	95	0	157	0	0	0	0	0	0
Z. gillettii	0	0	27710	6643	ND	ND	0	0	120	654	ND	ND
P. aethiopica	0	0	0	577	897	1056	0	0	0	0	0	132
C. megalocarpus	0	0	2170	7404	ND	ND	0	0	178	88	ND	ND
S. persica	0	0	144	0	257	10	0	0	0	0	0	0
A. senegal	0	0	1208	0	ND	4569	0	0	0	0	653	12407
A. friesiorum	0	0	773	0	ND	15098	0	0	76	48	450	11608
P. dawei	0	0	15663	0	ND	3891	0	0	0	0	775	996

L. acaciae	0	0	147	0	5660	783	0	0	0	75	601	1871
C. sinensis	0	0	0	0	0	0	0	0	209	178	986	15651
A. horrida	0	0	4896	0	ND	198	0	0	0	0	209	178
A. anthelmitica	0	0	1702	0	3114	1603	0	0	0	0	701	14761
T. africanum	0	0	6432	2515	ND	ND	0	0	0	0	189	2477
B. angustifolia	0	0	0	0	501	183	0	9	0	120	618	13801
C. quadrangularis	0	0	256	900	ND	ND	0	0	0	0	1041	120
G. simi	0	0	247	0	9025	1848	0	0	99	67	406	125
A. etbaica	0	0	7404	2170	ND	ND	0	0	0	0	564	1507
S. multiflorus	0	0	468	0	10258	0	0	0	0	0	0	0
C. africana	0	0	958	2557	ND	ND	0	17	112	4660	870	12400
E. scarlatica	0	0	0	0	0	0	0	0	0	0	0	0
A. nilotica	0	0	3656	19613	ND	ND	0	0	0	0	0	0
Negative controls	745	2002	37611	3862	10597	18683	187	2957	212	5266	893	16017
Potisive control	0	0	0	0	0	0	0	0	0	0	0	0

Key:

Mk *Mycobacterium kansasii* Mf *M. fortuitum*

Mtb *M. tuberculosis* Ms *M. smegmatis*

*Note: The higher the growth index, indicated as growth units (GUs), the less inhibitory the extract is to *Mycobacteria* spp. (Compared to negative control)

4.2.1 Antimycobacterial results for *M. tuberculosis*

Some medicinal plant extracts showed strong antimycobacterial activity against *M. tuberculosis* when tested using BACTEC MGIT 960 system (Table2). There was complete inhibition (zero GUs) of the *M. tuberculosis* by *Entada abysinnica, Toddalia asiatica, Cordia sinensis, Scadoxus multiflorus and Euphorbia scarlatina* at all the extracts' concentrations used.

The performance of some of the medicinal plants was appreciable at the concentration of 0.5mg/ml, whilst completely inhibiting the growth of *M. tuberculosis* at 1.0mg/ml. They included *Salvadora persica* (10 GUs), *Boscia angustifolia* (183 GUs), *Aloe secundiflora* (157 GUs) and *Lantana camara* (128 GUs). There was no growth (zero GUs) in the positive control (Isoniazid), whereas there was growth in the negative control (DMSO) (187-2957 GUs). Resistance to a drug is detected when 1% or more of the bacterial population is resistant to the drug concentration under test (Becton, Dickinson and Company, 2007). Among the least bioactive plant extracts against *M. tuberculosis* were those from *Mormordica charantia, Ocimum gratissimum, Erythrina abyssinica, Fuerstia africana, Zanthoxylum gillettii* and *Acacia nilotica*.

4.2.2. Antimycobacterial results for *M. kansasii*

Six medicinal plant extracts showed strong antimycobacterial activity against *M. kansasii* when tested using BACTEC MGIT 960 system (Table2). There was complete inhibition (zero GUs) of the *M. kansasii* by *Croton macrostachyus*, *Vernonia*

amygdalina, Toddalia asiatica, Aloe secundiflora, Cordia sinensis, and *Euphorbia scarlatina* at all the extracts' concentrations used.

There was appreciable inhibition at the concentration of 1.0mg/ml in some extracts that included; *Carissa edulis, Clerodendrum myricoides, Euphorbia tirucalli,* and *Pistacia aethiopica,* all of which gave complete inhibition (Zero GUs) of *M. kansasii*. At the same concentration, other extracts like *Salvadora persica* (144 GUs), *Grewia simi* (247 GUs), and *Loranthus acaciae* (147) were just average. There was no growth (zero GUs) in the positive control (Isoniazid), whereas there was growth in the negative control (DMSO) (745-37611 GUs).

Among the least bioactive plant extracts were those from *Lantana camara, Lantana trifolia, Fuerstia africana, Zanthoxylum gillettii, Croton megalocarpus, Plumbago dawei, Acacia horrida, Acacia etbaica* and *Thylachium africana.*

4.2.3. Antimycobacterial results for *M. fortuitum*

Eight medicinal plant extracts showed strong antimycobacterial activity against *M. fortuitum* when tested using BACTEC MGIT 960 system (Table 2). There was complete inhibition (zero GUs) of the *M. fortuitum* by *Carissa edulis, Vernonia amygdalina, Aloe secundiflora, Pistacia aethiopica, Salvadora persica, Scadoxus multiflorus, Euphorbia scarlatina* and *Acacia nilotica* at all the extracts' concentrations used. There was moderate antimycobacterial activity by *Entada abysinnica* (18 GUs) and *Croton macrostachyus* (76 GUs) in the concentration of 0.5mg/ml. There was no growth (zero GUs) in the positive control (Isoniazid), whereas there was growth in the negative control (DMSO) (187-893 GUs).

Among the least bioactive plant extracts against *M. fortuitum* were those from *Croton megalocarpus, Mormordica charantia, Erythrina abyssinica, Fuerstia africana Zanthoxylum gillettii, Cordia sinensis* and *Commiphora africana*.

4.2.4. Antimycobacterial results for *M. smegmatis*

Five medicinal plant extracts showed strong antimycobacterial activity against *M. smegmatis* when tested using BACTEC MGIT 960 system (Table 2). There was complete inhibition (zero GUs) of the *M. smegmatis* by *Vernonia amygdalina, Aloe secundiflora, Salvadora persica, Scadoxus multiflorus* and *Euphorbia scarlatina* at all the extracts' concentrations used. There was moderate antimycobacterial activity by *Entada abysinnica* (97 GUs), *Croton macrostachyus* (48 GUs), *Toddalia asiatica* (138), *Pistacia aethiopica* (132 GUs), *Cissus quadrangularis* (120 GUs) and *Grewia simi* (125 GUs) in 0.5mg/ml concentration. There was no growth (zero GUs) in the positive control (Isoniazid), whereas there was growth in the negative control (DMSO) (2957-16017 GUs).

Among the least bioactive plant extracts against *M. smegmatis* were those from *Euphorbia tirucalli, Lantana trifolia, Clerodendrum myricoides, Croton megalocarpus, Erythrina abyssinica, Fuerstia, africana, Zanthoxylum gillettii, Cordia sinensis, Acacia senegal, Boscia angustifolia* and *Commiphora africana*.

4.3 Antibacterial and antifungal results

4.3.1 Zones of inhibition

Results obtained by disc diffusion method showed strong antimicrobial activity (size of zone of inhibition ≥9–15 mm) for *Entada abysinnica* against *Salmonella typhi* (10.33mm) and *Candida albicans* (14.10mm), *Momordica charantia* against *Staphylococcus aureus* (10.66mm), *Toddalia asiatica against Pseudomonas aeruginosa* (10.66mm), *Lantana trifolia* against *Staphylococcus aureus* (20.00mm), and *Aloe secundiflora* against *Pseudomonas aeruginosa* (18.00mm) (Table 3). There was also some moderate antimicrobial activity (size of zone of inhibition ≥5–9 mm) shown by some extracts like that of *Acacia nilotica* against *Klebsiella pneumoniae* (9.00mm).

Some of the zones of inhibition produced by the extracts are given in the Plates 17-21. The arrows are pointing at the area of the inhibition zones.

Plate 17: Zone of inhibition of Ciprofloxacin against *K. pneumoniae* (positive control)

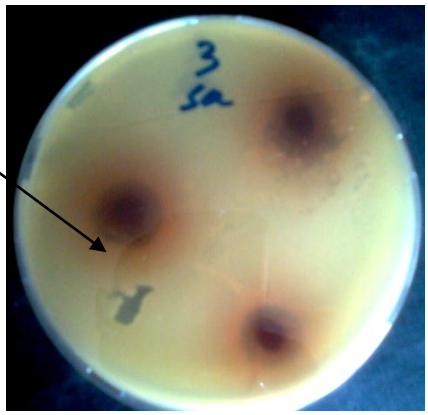


Plate 18: Zone of inhibition of *Croton macrostachyus* against *S. aureus*

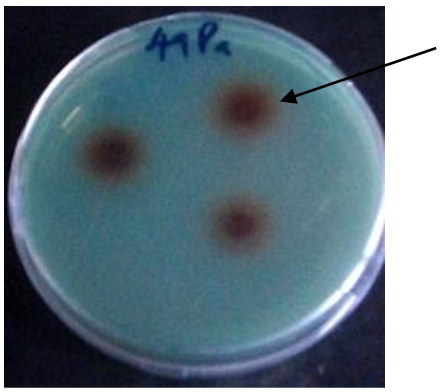


Plate 19: Zone of inhibition of *Acacia nilotica* against *P. aeruginosa*

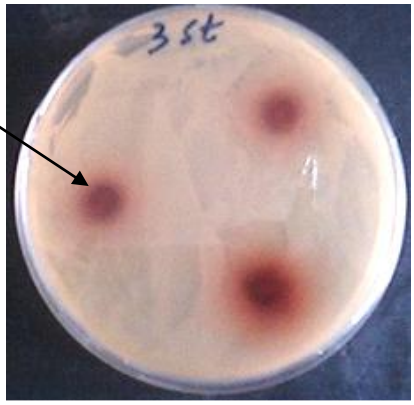


Plate 20: Zone of inhibition of *Croton macrostachyus* against *S. typhi*

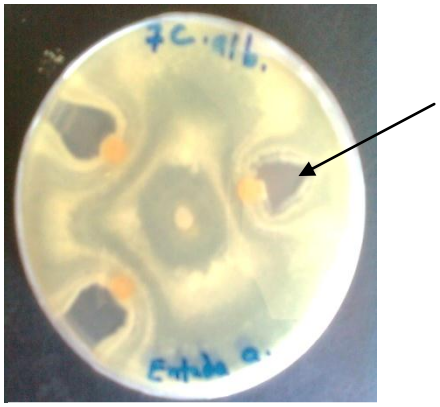


Plate 21: Zone of inhibition of *Entada abyssinica* against *C. albicans*

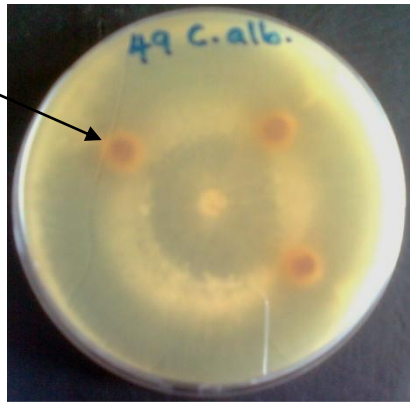


Plate 22: Zone of inhibition of *Acacia nilotica* against *C. albicans*

Table 3: Zones of inhibition (mm) produced by the extracts

Botanical name of plant	***S. typhi***	***S. aureus***	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	***C. albicans***
C. edulis	9.00	8.00	7.00	7.66	8.00	7.00
E. abysinnica	10.33	9.66	7.66	8.33	7.33	14.10
C. macrostachyus	8.00	7.66	7.00	7.33	6.66	8.00
V. amygdalina	7.33	8.66	6.66	8.33	8.00	7.66
M. charantia	8.66	10.66	6.00	9.33	8.00	7.66
O. gratissimum	8.66	8.66	7.00	7.66	6.66	8.00
L. camara	6.66	9.00	6.00	8.66	7.33	6.33
C. myricoides	7.66	8.00	7.00	7.66	6.66	7.66
T. asiatica	6.33	8.66	7.00	10.66	6.00	8.00
L. trifolia	8.66	20.00	8.66	10.00	6.66	6.66
E. abyssinica	8.00	8.66	7.33	10.33	8.00	8.00
E. tirucalli	6.33	6.33	7.66	6.33	7.00	7.00
F. africana	7.33	8.00	7.66	6.66	6.66	7.00
A. secundiflora	12.00	13.33	15.00	18.00	8.00	7.00
Z. gillettii	6.66	7.33	6.00	9.33	7.00	7.66
P. aethiopica	8.66	7.33	8.33	7.00	6.66	7.00
C. megalocarpus	7.66	8.00	6.66	8.00	7.66	7.00
T. africanum	7.67	10.66	8.00	10.00	7.33	6.00
B. angustifolia	8.00	9.00	7.33	6.66	7.00	6.00
G. simi	6.66	6.66	8.00	7.00	7.33	6.00
S. multiflorus	9.00	8.33	8.33	6.33	7.33	7.00
E. scarlatica	8.00	7.00	7.00	6.00	7.33	6.66
Acacia nilotica	8.66	8.00	12.00	11.66	9.00	10.33
Positive controls	16.00	21.33	20.22	17.33	17.66	13.00
Negative controls	6.00	6.00	6.00	6.00	6.00	6.00

Controls

The Negative controls were made from Methanol discs (evaporated).
The positive control for *Candida albicans* was Fluconazole
The positive control for *Salmonella typhi* and *Klebsiella Pneumoniae* was Zeftazidime and Ciprofloxacin respectively.
The positive control for *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* was Amoxicillin

4.3.1.1 Inhibition against *S. typhi*

Most of the extracts had a promising performance against the *S. typhi* ATCC 2202 (Table 3). *Aloe secundiflora* produced 12.00mm and *Entada abysinnica* a zone of inhibition of 10.33mm. *Carissa edulis* and *Scadoxus multiflorus* produced average zones of inhibition of 9.00mm. The remaining extracts produced zones of inhibition as represented in Table 3. None of the extracts was completely inactive against the test organism. The means of the zones of inhibition showed a significant difference at $P \leq 0.05$ (Appendix III a.).

4.3.1.2 Inhibition against *S. aureus*

Momordica charantia, Lantana camara, Lantana trifolia, Aloe secundiflora, Thylachium africanum and *Boscia angustifolia* all gave strong antimicrobial activity based on their zones of inhibition against *S. aureus* as shown in Table 3. None of the extracts was completely inactive against the test organism. The means of the zones of inhibition showed a significant difference at $P \leq 0.05$ (Appendix III b.).

4.3.1.3 Inhibition against *E. coli*

The performance of two of extracts was particularly good against *E. coli*. *Aloe secundiflora* gave a zone of inhibition of 15.00mm, whereas *Acacia nilotica* gave a zone of inhibition of 12.00mm. Some of the extracts like those from *Momordica charantia, Lantana camara* and *Zanthoxylum gillettii* possessed no inhibitory activity with zones of

inhibition of 6.00mm. From the means of zones of inhibition, the extracts produced no significant differences at P≤ 0.05 (Appendix III c.).

4.3.1.4 Inhibition against *P. aeruginosa*

Most of the plant extracts screened against *P. aeruginosa* ATCC 25852 produced appreciable results except *Euphorbia scarlatica*. *Aloe secundiflora* produced an average zone of inhibition 18.00mm. Among the plants that produced average zones of inhibition are *Toddalia asiatica, Lantana trifolia, Erythrina abyssinica, Thylachium africanum, Acacia nilotica* with zones of inhibition of 10.66mm, 10.00mm, 10.33, 10.00mm and 11.66mm respectively. The means of the zones of inhibition had a significant difference at P≤ 0.05 (Appendix III d.).

4.3.1.5 Inhibition against *K. pneumoniae*

For *K. pneumoniae,* most of the plant extracts gave just average or below average zones of inhibition. *Acacia nilotica* was the only plant extract that showed some activity against the test organism with a zone of inhibition of 9.00mm. The means of the zones of inhibition had a significant difference at P≤ 0.05 (Appendix III e.).

4.3.1.6 Inhibition against *Candida albicans*

The medicinal plants were also screened against *Candida albicans*. *Entada abysinnica* gave a tapering zone of inhibition of 14.10mm, bigger than that of fluconazole which

had a zone of inhibition of 13.00mm. *Acacia nilotica* also gave a good zone of inhibition of 10.33mm.There was significant difference of the means of the zones of inhibition at P≤ 0.05 (Appendix III f.).

4.3.2 The Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal/ Fungicidal Concentration (MBCs/ MFCs)

The Minimum Inhibitory Concentration (MIC) which is the least amount of antimicrobial that will inhibit visible growth of an organism after an overnight incubation was determined using the microtitre dilution broth method in 96–well micro plates. This was performed only where the plant extract showed strong antibacterial activity by the disk diffusion method (≥9-15mm) (Rani and Khullar, 2004). The various results obtained are as presented in Table 4.

Table 4: Minimum Inhibitory Concentrations (MICs) (mg/ml) and Minimum Bactericidal (MBCs) (mg/ml) produced by the medicinal plants against bacterial test cultures.

Plant	***S. typhi***		***S. aureus***		***E. coli***		***P. aeruginosa***		***K. pneumoniae***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. abysinnica	18.75	37.5	37.5	75						
L. camara			37.5	37.5						
T. asiatica							9.375	9.375		
L. trifolia			37.5	37.5			37.5	37.5		
T. africanum			18.75	37.5			4.69	4.69		
B. angustifolia			37.5	75						
A. secundiflora	37.5	37.5	37.5	37.5	18.75	18.75	9.375	18.75		
S. multiflorus	37.5	75								
A. nilotica					4.69	18.75	18.75	18.75	18.75	18.75
M. charantia			37.5	37.5			37.5	37.5		
E. abyssinica							37.5	37.5		
Positive controls	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69
Negative controls	Growth observed in all tubes									

Against *S. typhi* (ATCC 2202), only the extracts that gave good results (A zone of inhibition ≥9-15mm) had their MICs and MBCs determined. These were plants such as *Aloe secundiflora, Entada abysinnica* and *Scadoxus multiflorus* (Table 4). *Aloe secundiflora* had a MIC similar to the MBC (37.5 mg/ml). *Entada abysinnica* produced a MIC of 18.75 mg/ml, and a MBC of 37.5mg/ml, and *Scadoxus multiflorus* a MIC of 37.5mg/ml and a MBC of 75mg/ml. No extract had a MIC or MBC equal or greater than that of the control used against *S. typhi*.

Entada abysinnica, Lantana camara, Lantana trifolia, Thylachium africanum, Boscia angustifolia, Aloe secundiflora and *Momordica charantia* all gave good zones of inhibition and so had their MICs and MBCs determined against *S. aureus* (ATCC 20591). Only *Thylachium africanum* gave a high activity with MIC of 18.75mg/ml compared to other extracts. *Acacia nilotica* was very active with MIC of 4.8765 mg/ml (same as of positive control) and MBC of 18.75 mg/ml against *E. coli* (ATCC 25922). *Aloe secundiflora* gave a high activity with both MIC and MBC of 18.75 mg/ml.

Most of the plant extracts screened for MICs and MBCs against *P. aeruginosa* (ATCC 25852) showed very good activity. *Thylachium africanum* gave very good results, with similar MIC and MBC of 4.8765 mg/ml (similar to the positive control). *Toddalia asiatica* showed good activity with similar MBC and MIC of 9.375mg/ml. Other results were also promising (Table 4). Only *Acacia nilotica* had its MIC and MBC determined

against *K. pneumoniae* (Clinical isolate). It produced a similar MIC and MBC of 18.75mg/ml. Other extracts showed poor activity against *K. pneumoniae*.

Entada abysinnica and *Acacia nilotica* showed high activity against *C. albicans* (ATCC EK138). *Entada abysinnica* produced similar MIC and MBC of 18.75mg/ml. *Acacia nilotica* gave a MIC of 9.375mg/ml, and MBC of 18.75mg/ml.

4.4 Preliminary phytochemical screening

The preliminary phytochemical screening of methanolic extracts of various medicinal plants is presented in Table 5. The phytochemicals that were screened for their presence include tannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids.

Table 5: Phytochemical screening of the plant extracts results.

Preliminary phytochemical screening test results of the medicinal plant samples						
Botanical name of plant	**Tannins**	**Saponins**	**Flavonoids**	**Terpenoids**	**Cardiac glycosides**	**Alkaloids (Wagner's test)**
Carissa edulis	++	+	+++	+	-	++
Entada abysinnica	+++	+++	+++	-	+	-
Croton macrostachyus	-	+	+	-	-	-
Vernonia amygdalina	+	+	+++	-	-	+
Momordica charantia	++	-	+++	+	+	+
Ocimum gratissimum	+++	+	+++	++	++	+++
Lantana camara	+++	+	+++	+	++	+
Clerodendrum myricoides	+++	+	+++	+	+	+++
Toddalia asiatica	+	-	++	-	-	+
Lantana trifolia	+++	++	++	-	+	+
Erythrina abyssinica	++	++	+++	+	-	+++
Euphorbia tirucalli	+++	-	+++	+++	+	+
Fuerstia africana	+	-	++	+++	+	+
Aloe secundiflora	+	-	++	++	-	-
Zanthoxylum gillettii	-	+	+	++	-	+
Pistacia aethiopica	+++	++	+++	++	-	++
Croton megalocarpus	++	++	++	-	-	-
Salvadora persica	-	-	+	+	+	+
Acacia senegal	+	++	+	++	+	-
Acokanthera	++	-	++	-	+	-

friesiorum						
Plumbago dawei	++	-	++	-	+	-
Loranthus acaciae	+++	+	+	-	+	+
Cordia sinensis	+++	+	+	+	-	-
Acacia horrida	++	++	-	++	++	+++
Albizia anthelmitica	+	++	+	-	-	++
Thylachium africanum	+++	+++	++	-	++	+++
Boscia angustifolia	+	-	-	-	-	+
Cissus quadrangularis	+++	+	+	+++	+	+
Grewia simi	+++	+++	+	-	+	-
Acacia etbaica	+	++	++	+++	++	+
Scadoxus multiflorus	+++	+++	+++	+	++	+++
Commiphora africana	++	+	-	-	+++	+++
Eurphorbia scarlatina	+	-	+++	+++	++	++
Acacia nilotica	+++	++	++	++	+	+++

Key: +++ = Present in high concentration, ++ = Moderately Present, + = Trace, - = Absent.

4.4.1 Tannins

Based on the investigation, tannins were found to be in all extracts except in *Zanthoxylum gillettii* and *Salvadora persica.* Compared to the other phytochemicals, tannins were mostly present in higher concentrations (Table 5). They were found to be of high concentration in *Entada abysinnica, Ocimum gratissimum, Lantana camara,* and *Clerodendrum myricoides*. The brownish green or blue-black colouration indicated the presence of tannins (Plate 23).

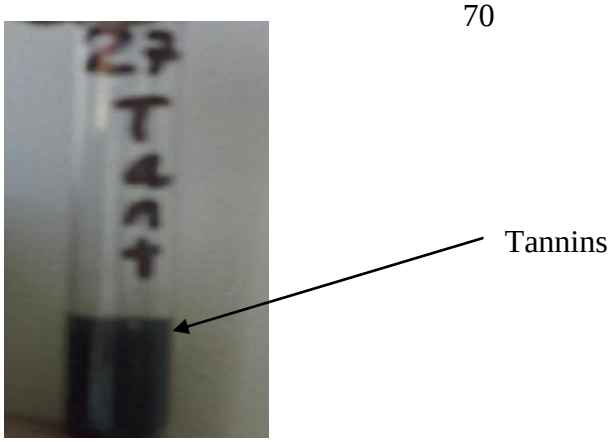


Plate 23: *Lantana trifolia* extract's blue-black colouration indicating presence of tannins

4.4.2 Saponins

Saponins were also present, mostly in moderate amounts. *Entada abysinnica, Thylachium africanum, Grewia simi* and *Scadoxus multiflorus* are the only extracts that had saponins in high concentrations. In some extracts like *Momordica charantia, Salvadora persica, Euphorbia tirucalli, Fuerstia africana* and *Boscia angustifolia,* the saponins were absent (Table 5). A stable persistent froth observed after shaking the solution vigorously indicated the presence of saponins (Plate 24).

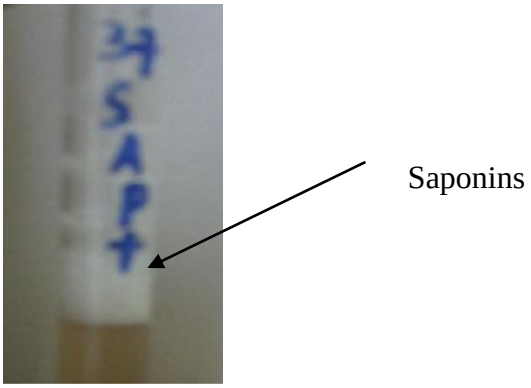


Plate 24: *Thylachium africanum* extract's persistent froth indicating presence of saponins

4.4.3 Flavonoids

Flavonoids were found mostly in moderate to high concentrations as presented in Table 5. They were however absent in *Acacia horrida, Boscia angustifolia* and *Commiphora africana*. Observation of a yellow colouration that disappeared on standing indicated the presence of flavonoids (Plate 25).

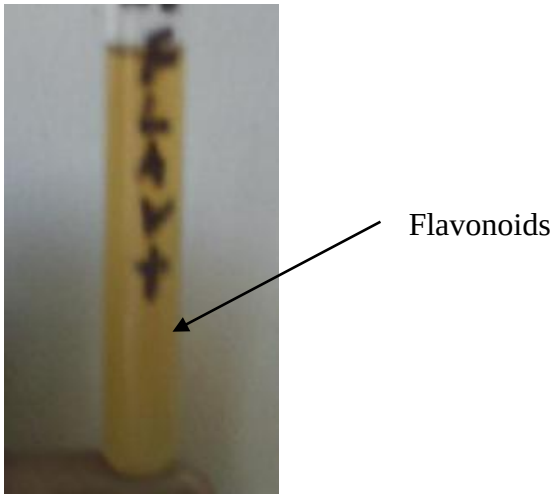


Plate 25: *Entada abysinnica* extract's yellow colouration indicating presence of flavonoids

4.4.4 Terpenoids

The terpenoids were also screened for and results presented as shown in Table 5. They were mostly found in moderate amounts except in *Euphorbia tirucalli, Fuerstia africana, Cissus quadrangularis, Acacia etbaica,* and *Euphorbia scarlatina* where they were found in high concentrations. The phytochemical was not present in some extracts like *Entada abysinnica, Grewia simi, Albizia anthelmitica, Croton macrostachyus* and *Vernonia amygdalina*. Observation of a reddish brown colouration at the interface indicated the presence of terpenoids (Plate 26).

Terpenoids

Plate 26: *Lantana trifolia* extract's reddish brown colouration at the interface indicating presence of terpenoids

4.4.5 Cardiac glycosides

Cardiac glycosides were found in a number of extracts in moderate or trace amounts (Table 5). They were found in high concentration only in *Commiphora africana.* This phytochemical was absent in extracts such as *Carissa edulis, Boscia angustifolia, Cordia sinensis, Vernonia amygdalina* and *Aloe secundiflora.* Observation of green blue colour indicated presence of cardiac glycosides (Plate 27).

Cardiac glycosides

Plate 27: *Lantana camara* extract's green blue colouration indicating presence of cardiac glycosides

4.4.6 Alkaloids (Wagner's test)

This was determined using Wagner's test. The phytochemical was found to be present in most extracts but was more abundant in a few extracts like *Commiphora africana, Acacia horrida, Cordia sinensis* and *Thylachium africanum* (Table 5). The phytochemical was absent in plant extracts such as those of *Cordia sinensis, Grewia simi, Acokanthera friesiorum* and *Plumbago dawei.* Creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of alkaloids.

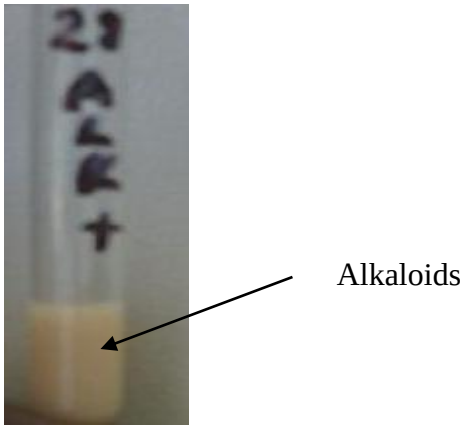


Plate 28: *Carissa edulis* extract's creamish precipitate indicating presence of alkaloids.

CHAPTER FIVE

DISCUSSIONS

5.1 Antimycobacterial activity

The positive and negative controls used in BACTEC MGIT 960 were isoniazid which was used as positive control, and 0.01% DMSO as negative control. For an extract to be said to be active against mycobacteria, it should have 99% inhibitory effect (Becton, Dickinson and Company, 2007).

5.1.1 *Mycobacterium kansasii*

The methanol extracts of the medicinal plants studied gave various results against *M. kansasii*. Those that showed strong activities with Zero GUs at 0.5mg/ml were; *Croton macrostachyus, Vernonia amygdalina, Toddalia asiatica, Cordia sinensis, Aloe secundiflora, Boscia angustifolia* and *Eurphobia scarlatina. Croton macrostachyus* had high activity against *M. kansasii* (Zero GUs at 0.5mg/ml). Flavonoids, though detected in this plant extract in trace amounts might be responsible for activity against *M. kansasii*. This is supported by a study carried out by Copp (2003) using *Erythrina gibbosa*; the flavonoids phaseollidin and erythrabyssin II were found to have antimycobacterial properties. Flavonoids from *C. macrostachyus* have also been found to be active against HIV/AIDS in a study done and patented by Chepkwony *et al.,* (2009), meaning that this plant should be analyzed further to identify active principles. *Vernonia amygdalina* extract had notable activity (Zero GUs at 0.5mg/ml) but the extract had Flavonoids as the only phytochemicals present in high concentration.

Flavonoids in *V. amygdalina* are reported to exhibit antioxidant activity and are effective scavengers of superoxide anions (Nwinyi *et al.,* 2009). Thus this can significantly affect the cell wall of the test culture which invariably may lead to the collapse of the cell wall and overall affect the entire mechanism of the organism as reported by Nwinyi *et al.*, (2009). In Uganda, *V. amygdalina* is used to treat a multiplicity of diseases including schistosomiasis, amoebic dysentery, and gastrointestinal problems (Masaba, 2000; Erasto *et al.,* 2006). The plant has been observed to be used by wild chimpanzees and gorillas for self-deparasitization (Alawa *et al.,* 2003). A study in Uganda (Masaba, 2000) demonstrated the presence of antimalarial activity in the acetone-water and aqueous extracts of *V. amygdalina*. A study by Taiwo *et al.,* (2009) showed that the phytochemicals in *V. amygdalina* include steroid glycosides and sesquiterpetene lactones like vernodalin, Vernolide, Hydroxyvernolide, vernomydin and vernodal which have antimalarial, anthelminthic and antitumorigenic properties (Arhoghro *et al.,* 2009; Alawa *et al.,* 2003). Pharmacological studies have also shown that the leaf extracts have both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing *Diabetes mellitus* (Arhoghro *et al.,* 2009). Almost all the pharmacological activities observed for this plant have been attributed to these bioactive compounds.

From this study, *Toddalia asiatica* was found to have high inhibitory activity against *M. kansasii* (Zero GUs at 0.5mg/ml). The extract was found to have moderate amounts of flavonoids, whereas tannins and alkaloids were found in trace amounts. More than 6400 flavonoids have been shown to have various interesting properties, including

antibacterial, antiprotozoal, anti-inflammatory, dietary antioxidant, vascular and oestrogenic activities, mainly mediated through inhibition of oxidases (Brown *et al.,* 2007). Some have been found to inhibit *de novo* fatty acid biosynthesis in mycobacteria as well as mycolic acid biosynthesis (Brown *et al.*, 2007).

There was high inhibitory activity (Zero GUs at 05.mg/ml) against *M. kansasii* by *Cordia sinensis* extract. Phytochemical investigation revealed that the extract had high concentrations of tannins, which could be responsible for observed activity, and trace amounts of saponins, flavonoids and terpenoids. This is supported by a study carried out by Okunade *et al.,* (2004) on *Combretum molle* (Combretaceae) which was evaluated using two strains of MTB, *Typus humanus* and an unidentified patient strain. They obtained punicalagin, the only tannin so far associated with antimycobacterial activity. The East Africa leaves of this species are chewed or pounded, soaked in water, and the juice drunk to treat chest complaints, or as an inhalant in hot steam baths (Okunade *et al.,* 2004). This could be the same case for *Cissus quadrangularis* which was active against *M. kansasii* with complete inhibition (Zero GUs) at 1.0mg/ml and 501 GUs at a concentration of 0.5mg/ml.

The *Euphorbia scarlatica* completely inhibited growth (Zero GUs) in all concentrations used against *M. kansasii.* It had high concentrations of terpenoids and flavonoids, which *Aloe secundiflora* only had in moderate concentrations, in trace amounts or absent. *Aloe secundiflora* extracts were active against both fast and slow growing mycobacteria. It gave complete inhibition against *M. kansasii* (Zero GUs) at 0.5mg/ml. Terpenoids have

been found to have the ability to inhibit the functions of fatty acid synthase (Copp, 2003). This is actually the same target site as that of pyrazinamide (PZA), a first-line drug in tuberculosis chemotherapy. Terpenoids were abundant in *E. scarlatina* and could possibly be responsible for the activity observed. Secondary metabolites of terpenoid origin dominate the number of natural products reported against antimycobacterial activity. This is because terpene derivatives are typically of moderate to high lipophilicity which would aid in their penetration of the mycobacterial cell wall. Activity could be attributed to the flavonoids which have been found to inhibit *de novo* fatty acid biosynthesis in mycobacteria as well as mycolic acid biosynthesis (Brown *et al.,* 2007).

Based on the current investigation, the following plant extracts were not active on *M. kansasii; Momordica charantia, Lantana trifolia, Acacia horrida, Albizia anthelmitica, Thylachium africanum, Boscia angustifolia, Cissus quadrangularis, Acacia etbaica, Grewia simi, Scadoxus multiflorus, Carissa edulis* and *Acacia nilotica.* Despite the presence of all or most of the phytochemicals in most of these plant extracts, they produced moderate to poor activity. This could be because the phytochemicals were acting in antagonism (Ruttoh, 2009).

5.1.2 *Mycobacterium tuberculosis*

The phytochemicals in plant extracts can act as leads in providing useful scaffolds or templates, for the development of new antitubercular drugs, as the genome sequence of *M. tuberculosis* has been established. Promising leads from plant sources may also act on newer targets and thus may play a crucial role in the development of new generation antitubercular drugs (Negi *et al.,* 2009).

Entada abyssinica extracts produced particularly strong activity against *M. tuberculosis* where it was potent at 0.5 mg/ml with Zero GUs. Antimycobacterial properties of the medicinal plant could be due to the abundant flavonoids, saponins and tannins that were found in it.

Flavonoids have been found to affect fatty acid and mycolic acid synthesis (Brown *et al.,* 2007). Chemically, the mycobacterial cell wall is composed of three covalently linked macromolecules: peptidoglycan, arabinogalactan, and mycolic acids. More than 60% of the Mycobacterium cell wall is lipid and this makes it impermeable to stains and dyes, resistance to many antibiotics, resistance to killing by acidic and alkaline compounds, resistance to osmotic lysis via complement deposition, resistance to lethal oxidations, and able to survive inside of macrophages (Negi *et al,* 2009). Thus, cell wall biosynthesis is a good target by some flavonoids which kill the bacterium as enzymes involved in the biosynthesis are pathogen specific and they do not have homologues in the mammalian system (Negi *et al*, 2009). But, the major problem with this type of antimycobacterial chemotherapy is the very long treatment time. Among the present anti-TB drugs, isoniazid and ethionamide inhibit mycolic acid biosynthesis, while ethambutol inhibits arabinogalactan biosynthesis (Negi *et al,* 2009). Activity of *E. abysinnica* against other respiratory problems like bronchitis and coughs has already been reported (Olajide and Alada, 2001).

Just like in *M. kansasii*, *Toddalia asiatica* and *Aloe secundiflora* were found to have high inhibitory activity (Zero GUs and 157 GUs respectively at 0.5mg/ml). Both plants were found to have moderate amounts of flavonoids and varying amounts of other phytochemicals. More than 6400 flavonoids have been shown to have various interesting properties, including antibacterial, antiprotozoal, anti-inflammatory, dietary antioxidant, vascular and oestrogenic activities, mainly mediated through inhibition of oxidases (Brown *et al.,* 2007). Some have been found to inhibit *de novo* fatty acid biosynthesis in mycobacteria as well as mycolic acid biosynthesis. The plant is used traditionally in Kenya by many communities for the treatment of malaria, fever, stomachache, toothache, influenza, rheumatism coughs as well as nasal and bronchial pains, and although all parts of the plant are claimed to have medicinal value, roots are believed to be more potent (Jain *et al.,* 2006; Duraipandiyan and Ignacimuthu, 2009; Orwa *et al.,* 2008).

There was high inhibitory activity against *M. tuberculosis* by *Cordia sinensis* and *Scadoxus multiflorus* extracts, with Zero GUs at 05.mg/ml for both. From the preliminary phytochemistry, *C. sinensis* had high concentrations of tannins. Other phytochemicals were either absent or only detected in trace amounts. Tannins have been known for acidic property (Fontoin *et al.,* 2008). It is these tannins in red wine that give it some acidic property (hydrolyzable tannins), with the major effect of tannins in red wine being the astringent or drying/puckery effect (Fontoin *et al.,* 2008). According to Zhang e*t al.,* (2003) *M. tuberculosis* is more susceptible to acidic pH and weak acids than *M. smegmatis.* The weak acids were more active against *M. tuberculosis* at higher

acid pH than close to neutral pH. *M. tuberculosis* was found to be less able to maintain its internal pH and membrane potential at acid pH than *M. smegmatis*. The antituberculous activity of weak acids correlated with their ability to disrupt the membrane potential but not the internal pH. The significance of these findings is discussed in relation to *M. tuberculosis* physiology and development of new antituberculous agents. The same could be said of *Boscia angustifolia* which had high activity against *M. tuberculosis* (183 GUs compared to the negative control which had 10597 GUs). It generally gave moderate activity. The extract of *B. angustifolia* tested positive to tannins and alkaloids only, with both being detected in trace amounts. All the other phytochemicals were absent.

Acacia horrida extract was active with GUs of 198 at 0.5mg/ml. The phytochemistry indicated presence of all phytochemicals except flavonoids. Activity may be due to the abundant presence of alkaloids. A pyrrole alkaloid solsodomine was isolated from the plant *Solanum sodomaeum*, collected in Libya (Kaur *et al.,* 2009). The alkaloid inhibited the growth of *M. intracellulare*, *M. tuberculosis*, *M. avium* and *M. smegmatis*. The activity of *A. horrida* against *M. tuberculosis* could possibly be due to the presence of alkaloids, the only phytochemical it possesses with the most abundant concentration. This is in agreement with a study carried out by Copp, (2003) where he found the alkaloids to have the ability to inhibit the function of mammalian fatty acid synthase that lead to recent synthetic studies that used the structure as a template for the discovery of new antitubercular compounds (Gibbons *et al.,* 2003; Copp, 2003; Kaur *et al.,* 2009). *Boscia angustifolia* which was active against *M. tuberculosis* with complete inhibition

(Zero GUs) at 1.0mg/ml and 183 GUs at a concentration of 0.5mg/ml could also be active due to the presence of alkaloids.

The *Euphorbia scarlatica* completely inhibited growth (Zero GUs) in all concentrations used against *M. tuberculosis.* It had terpenoids and flavonoids in high concentrations, whereas cardiac glycosides and alkaloids were present in moderate amounts. Terpenoids were abundant in *E. scarlatina* and could possibly be responsible for activity. They have been found to have the ability to inhibit the functions of fatty acid synthase (Copp, 2003). This is actually the same target site as that of pyrazinamide (PZA); a first-line drug in tuberculosis chemotherapy. Secondary metabolites of terpenoid origin dominate the number of natural products reported with antimycobacterial activity. This is because terpene derivatives are typically of moderate to high lipophilicity which would aid in the penetration of the mycobacterial cell wall.

Based on the current investigation, the following plant extracts were found to be inactive against *M. tuberculosis; Momordica charantia, Lantana trifolia, Albizia anthelmitica, Thylachium africanum, Cissus quadrangularis, Carissa edulis, Acacia etbaica, Grewia simi* and *Acacia nilotica.*

5.1.3 *Mycobacterium fortuitum*

Carissa edulis and *Aloe secundiflora* had strong activity of Zero GUs at 0.5mg/ml against *M. fortuitum.* Flavonoids, which were detected in high and moderate concentrations respectively, could be responsible for the inhibitory effect of the plant.

Different mechanisms have been linked to flavonoid-mediated cytotoxicity, including proteasome inhibition, inhibition of fatty acid synthesis, topoisomerase inhibition, inhibition of phosphatidyl-inositol 3-kinase, induction of cell cycle arrests, accumulation of p53 or enhanced expression of c-fos and c-myc (Yuan *et al.*, 2009). The 4-carbonyl group of flavonoids has been reported to correlate with inhibition of fatty acids. The presence of the 2–3 double bonds has been linked to efficient binding and inhibition of the Pglycoprotein (P-gp). Cheng and Pieters (2010) have reported that novel proteasome inhibitors could be used as potential drugs to combat tuberculosis. In a study carried out by Tolo *et al.*, (2006), *C. edulis* aqueous root bark extract was found to contain potential agents with activity against *Herpes simplex* virus. No acute toxicity was observed in mice at the oral therapeutic dose of 250 mg/kg.

The results for *Salvadora persica* extract showed strong antimycobacterial activity against *M. smegmatis* (Zero GUs at 0.5mg/ml). The results were comparable to those of the standard drug (Isoniazid). The extract contained bioactive constituents like alkaloids, terpenoids and flavonoids. The findings were in agreement with those of Edeoga *et al.*, (2005) and Ahmed *et al.*, (2008). The activity of *Scadoxus multiflorus* was the same as that of *S. persica* against *M. fortuitum*, albeit in *S. multiflorus* all the phytochemicals tested for were present. The results too were comparable to those of the standard drug (Isoniazid) in BACTEC MGIT 960 system. The presence of a major porin gene, MspA in *M. fortuitum* which is absent in slow-growers could be the reason for low inhibitory activity against the fast growers (Sharbati-Tehran *et al.*, 2005). This gene helps in the diffusion of molecules directly through the porin channels and in the

possibility of altered diffusion rates of molecules through the mycolic acid layer (Sharbati-Tehran *et al.,* 2005). They could be helping in the uptake of phytochemicals.

The *Euphorbia scarlatica* completely inhibited growth (Zero GUs) in all concentrations used against *M. fortuitum.* The detectable phytochemicals were flavonoids, and terpenoids which were present in high concentration, as well as alkaloids and cardiac glycosides which were present in moderate amounts. Terpenoids in *Eurphobia scarlatina* could possibly be responsible for activity. They have been found to have the ability to inhibit the functions of fatty acid synthase (Copp, 2003). This is actually the same target site as that of pyrazinamide (PZA); a first-line drug in tuberculosis chemotherapy. Secondary metabolites of terpenoid origin dominate the number of natural products reported with antimycobacterial activity. This is because terpene derivatives are typically of moderate to high lipophilicity which would aid in their penetration of the mycobacterial cell wall. *Acacia nilotica* extract appeared particularly good against *M. fortuitum* where it was potent at 0.5mg/ml. The flavonoids, found in moderate amounts could be responsible for high activity against *M. fortuitum.* Flavonoids from the same plant have been found to be active against HIV/AIDS in the work patented by Chepkwony *et al.,* (2009).

Based on the current investigation, the following plant extracts were inactive against *M. fortuitum; Momordica charantia, Lantana trifolia, Cordia sinensis, Albizia anthelmitica, Thylachium africanum, Toddalia asiatica, Croton macrostachyus, Boscia angustifolia, Cissus quadrangularis, Acacia etbaica* and *Grewia simi.*

5.1.4 *Mycobacterium smegmatis*

Mycobacterium smegmatis was used as a test model organism in the initial screening process due to its genetic similarity to *M. tuberculosis* but lack of virulence as an infectious organism (Mativandlela *et al.*, 2008).

In this investigation, *Carissa edulis* had strong activity of Zero GUs at 0.5mg/ml against *M. smegmatis.* Like in *M. fortuitum* the presence of flavonoids could also be responsible for the inhibitory effect of the plant, especially against the current mycobacterium strain. Flavonoids have been found to inhibit fatty acid and mycolic acid biosynthesis (Brown *et al.*, 2007), using a similar mode of action that a number of flavonoids use to inhibit the growth of *E. coli* and *P. falciparum in vivo* by targeting specific enzymes of fatty acid biosynthesis.

The results for *Salvadora persica* and *Aloe secundiflora* extracts showed strong antimycobacterial activity against *M. smegmatis* (Zero GUs at 0.5mg/ml). The results were comparable to those of the standard drug (Isoniazid). This indicates that the extracts contained bioactive constituents like alkaloids, tannins and flavonoids. The targets could include flavoprotein oxidoreductases such as thioredoxin (Mativandlela *et al.*, 2008; Ahmed *et al.*, 2008). Thioredoxin molecules have been found to enable novel antioxidant defense in mycobacteria (Bryk *et al.*, 2002). *Acacia horrida* extract was also found to be active with 178 GUs at 0.5mg/ml when compared to the negative control which had 16017 GUs hence the test culture was 99% susceptible. Activity may be due

to the abundant presence of alkaloids (Kaur *et al.,* 2009; Ahmed *et al.,* 2008; Akor *et al.,* 2009).

Cissus quadrangularis was active too against *M. smegmatis* with complete inhibition (Zero GUs at 1mg/ml) and 120 GUs at 0.5mg/ml, and inactive against all the other Mycobacteria. Phytochemical studies of *C. quadrangularis* found several phytochemical constituents such as terpenoids, tannins (both in high concentration) and flavonoids. This is in agreement with other studies carried out, that found it to have the same phytochemicals in addition to sterols, steroids, triterpenoids, carotenes, ascorbic acid, linoleic acid and stilbene derivatives (Panthong *et al.,* 2007; Bum *et al.,* 2008).

Acacia nilotica extract appeared particularly promising against *M. smegmatis* where it was potent at 0.5mg/ml. This activity could be attributed to the fact that all mycobacteria are characterized by a thick hydrophobic cell wall, which is penetrated by porins mediating diffusion of small hydrophilic nutrients into the cell. MspA (an extremely stable octameric protein composed of 20 kDa monomers) from *M. smegmatis* belongs to a novel class of porins present in many fast-growing mycobacteria (including *M. fortuitum*) but apparently absent in slow-growers (Sharbati-Tehran *et al.,* 2005). Hence the fact that the extract had moderate activity against fast growers and not slow growers could be because of low diffusion rates of the phytochemicals. This plant has been found by Kaur *et al.,* (2005) as a multipurpose plant as it is extensively used for the treatment of various diseases, e.g. colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma It also serves as a source of various products, including

polyphenols. This plant extract tested positive for all the phytochemicals including flavonoids, contrary to a study carried out by Banso (2009), who found the stem bark of *A. nilotica* not to have flavonoids.

The activity of *S. multiflorus* extracts against *M. smegmatis* strain showed that the plants contain pharmacologically active substances because the plant extracts gave complete inhibition at 0.5mg/ml. The results were comparable to those of the standard drug (Isoniazid) in BACTEC MGIT 960 system. From the study, with 125 GUs at 0.5mg/ml, *Grewia simi* was also active against *M. smegmatis.* Like in *A. nilotica*, the activity could be attributed to porins mediated diffusion of small hydrophilic nutrients into the cell (Sharbati-Tehran *et al.,* 2005).

The *Euphorbia scarlatica* completely inhibited growth (Zero GUs) in all concentrations used against *M. smegmatis.* The extract had high concentrations of terpenoids and flavonoids. Terpenoids have been found to have the ability to inhibit the functions of fatty acid synthase (Copp, 2003; Akor *et al.,* 2009). This is actually the same target site as that of pyrazinamide (PZA); a first-line drug in tuberculosis chemotherapy. Terpenoids were abundant in *E. scarlatina* and could possibly be responsible for the activity. Secondary metabolites of terpenoid origin dominate the number of natural products reported with antimycobacterial activity. This is because terpene derivatives are typically of moderate to high lipophilicity which would aid in their penetration of the mycobacterial cell wall.

Based on the current investigation, the following plants were inactive against *M. smegmatis; Momordica charantia, Lantana trifolia, Cordia sinensis, Albizia anthelmitica, Thylachium africanum, Boscia angustifolia* and *Acacia etbaica*.

5.2 Antibacterial and antifungal

5.2.1 Inhibition zones

General antibacterial and antifungal results indicated that most plants had moderate to strong activity (zone of inhibition were between 7mm-9mm). A zone of inhibition ≥9–15 mm is an indication of strong antimicrobial activity (Rani and Khullar, 2004). Against *S. typhi*, *Carissa edulis* had strong antimicrobial activity with a zone of inhibition of 9.00mm. It could be possible that the alkaloids found in the roots played an important role in the medicinal value of the plant (Nedi *et al.*, 2004). *Entada abysinnica* also gave strong antibacterial and antifungal results. It gave zones of inhibition of 10.33mm, 9.66mm and 14.10mm against *S. typhi, S. aureus* and *C. albicans* respectively.

Entada abysinnica extract was most active against *C. albicans* with an inhibition zone (14.10mm) greater than that of fluconazole (inhibition zone 13.00mm) (positive control). Phytochemical screening of *E. abysinnica* revealed that flavonoids, saponins and tannins were most abundant while cardiac glycosides were less abundant. The extract tested negative for alkaloids and terpenoids. Some of these phytochemicals like flavonoids have been known to be antidiarrhoeal from other studies (Meite *et al.*, 2009). The demonstration of antimicrobial activity against Gram-positive and Gram-negative

bacteria and *C. albicans* could also be indicative of the presence of broad spectrum antibiotic compounds (Salama and Marraiki, 2008). Lack of appreciable activity of the plant extract against *E.coli* and *K. pneumoniae* could be due to the fact that they are extended-spectrum β-lactamase producers (ESBLs) that are being increasingly identified in many parts of the world and are already prevalent in several countries (Heffernan *et al.*, 2009). β-lactamase is one of the major causes of drug resistance.

The activity of *E. abysinnica* could also be attributed to the presence of saponins. Previous investigations on the saponins isolated mainly from *Medicago sativa* L. have shown that, depending on their structure, they possess antimicrobial activity, principally against plant pathogens and some yeasts pathogenic to humans (Avato *et al.,* 2009). Tannins have also been reported to prevent the development of micro-organisms by precipitating microbial protein and making nutritional protein unavailable for them (Banso, 2009). A number of flavonoids have been shown to inhibit antifungal properties mainly mediated through inhibition of oxidases and NADH usage (Brown *et al.,* 2007). For *S. aureus*, the phenomena is in accordance with the previous findings from other plant samples that revealed that antimicrobial activity could be ascribed to different mechanisms of the action other than inhibition against β-lactamase (Zuo *et al.,* 2008); such as the inhibition against bacterial topoisomerase and efflux pump. Antibacterial activity of saponins against Gram positive bacteria from different plant sources has already been reported (Avato *et al.,* 2009).

Croton macrostachyus, Vernonia amygdalina had only moderate to poor activity (zones of inhibition of 6.66mm-8.0mm) against the test cultures used for general antibacterial and antifungal screening, possibly due to the antagonistic nature of the phytochemicals (Ruttoh, 2009).

Momordica charantia was active only against *S. aureus* (10.66mm) and *P. aeruginosa* (9.33mm) with zones of inhibition that indicated strong antimicrobial nature. Activity against the two test cultures could be attributed to the presence of the flavonoids. Flavonoids are reported to exhibit antioxidant activity and are effective scavengers of superoxide anions. Thus this can significantly affect the cell wall of *S. aureus* which invariably may lead to the collapse of the cell wall and overall, affect the entire mechanism of the organism (Nwinyi *et al.,* 2009). They could have also interfered with the integrity of lipopolysaccharide in *P. aeruginosa* (Mdluli *et al.,* 2006). In Mexico *M. charantia* is used for diabetes and dysentery; the root is a reputed aphrodisia. In Peruvian herbal medicine, the leaf or aerial parts of the plant are used to treat measles, malaria, and all types of inflammations. In Nicaragua, the leaf is commonly used for stomach pain, diabetes, fevers, colds, coughs, headaches, malaria, skin complaints, menstrual disorders, aches and pains, hypertension, infections, and as an aid in childbirth (Sage Press, 2002).

Against the common bacteria and fungi, *Toddalia asiatica* extract was active only against *P. aeruginosa* with a zone of inhibition of 10.66mm, whereas *Lantana trifolia* extract, which was inactive against the mycobacteria strains, had strong antimicrobial

activity against *S. aureus* and *P. aeruginosa* (zones of inhibition of 20mm and 10mm respectively). *Aloe secundiflora* extract showed significant inhibition to *E. coli and P. aeruginosa* (zone of inhibition of 15mm and 18.00mm respectively).

In the previous studies by Al-bayati and Sulaiman (2008) and Omwenga *et al.,* (2009), methanol extracts of *Salvadora persica* had been found to inhibit most bacteria like *S. aureus, P. aeruginosa*, and fungi such as *C. albicans*; and therefore further bioassays were done. This was attributed to various chemicals contained in its extracts, such as sodium chloride and potassium chloride, as well as salvadourea and salvadorine, saponins, tannins vitamin C, silica, and resin, in addition to cyanogenic or lignan glycosides, alkaloids, terpenoids, and oleic, as well as stearic acids (Al-bayati and Sulaiman, 2008). Phytoalexins which tend to fall into several classes including terpenoids, glycosteroids and alkaloids have been found to be antibacterial from previous studies (Camacho-Corona *et al.,* 2008). They may be responsible for observed activity of the medicinal plants studied.

Thylachium africanum extract was active against *S. aureus* (10.66mm) and *P. aeruginosa* (10.00mm). It contained tannins, saponins and alkaloids in high concentrations. The presence of tannins could be responsible for antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds there by killing the bacteria by directly damaging its cell membrane (Aliero *et al.,* 2008). *Scadoxus multiflorus* was only active against *S. typhi* with a zone of inhibition of 9.00mm. It contained all phytochemicals tested. The antibacterial

activity exhibited by its extract may be attributed to the presence of alkaloids and flavonoids in high concentrations as observed in the phytochemical screening (Aliero *et al.,* 2008). In the same study, *S. multiflorus* bulbs were found to contain alkaloids, flavonoids, tannins, saponin and cardiac glycosides. The presence of these phytochemical components in this species is an indication that they may perhaps have some medicinal potential.

Despite the presence of all phytochemicals some of the plant extracts like *B. angustifolia* produced moderate to poor activity against the common bacteria and *C. albicans,* which could be due to phytochemicals antagonism (Ruttoh, 2009). *Euphorbia scarlatica* gave moderate to mild activity against the bacteria strains and fungi; hence no MIC or MBC/MFC was determined. The reason for that kind of activity on the fungi (*C. albicans*) despite the presence of most phytochemicals can be attributed to the possibility of antagonistic activity of the phytochemicals (Ruttoh, 2009). *Pseudomonas aeruginosa* exhibits intrinsic resistance to several antimicrobial agents *(*Moniri *et al.,* 2006*)*. It produces some broadly specific multi-drug efflux systems. The anti-*Pseudomonas* β-lactams represent an effective solution against *Pseudomonas. spp.* infections *(*Moniri *et al.,* 2006*)*. That is possibly why the test culture was totally resistant against *E. scarlatina* (with a zone of inhibition of 6.00mm) plant extract.

General antibacterial and antifungal results for *A. nilotica* were good with the extract showing strong antimicrobial activity against *E. coli, P. aeruginosa, K. pneumoniae,* and *C. albicans* (Zones of inhibition of 12.00m, 11.66mm, 9.00 and 10.33 respectively). It

was the only plant extract that was active against most test cultures, conforming to the study carried out by Banso (2009) who found it to be active against *E. coli, P. vulgaris, K. pneumoniae* and *Aspergillus niger*. It had all the tested phytochemicals, mostly in high and moderate concentration. Their activity may thus be attributed to the high concentration of phytochemicals detected in their extracts, particularly alkaloids and flavonoids, as in *E. abysinnica* and *C. edulis*. The presence of these phytochemical components in the two plant species is an indication that they may have medicinal properties as used by the Samburu herbalists.

5.2.2 Minimum Inhibitory Concentrations and the Minimum Bactericidal/ Fungicidal concentrations

Toddalia asiatica was bactericidal with both MIC and MBC of 9.375mg/ml against *P. aeruginosa*. *Momordica charantia* extract was bactericidal, producing similar MICS and MBCs of 37.5mg/ml in both *S. aureus* and *P. aeruginosa.*

Lantana trifolia extract which was not active against the mycobacteria strains, had strong antimicrobial activity against *S. aureus* and *P. aeruginosa*. The extract was bactericidal against the two test cultures with both MICs and MBCs being 37.5mg/ml in both cases. For *Aloe secundiflora,* it was bactericidal against *E. coli,* with an MIC and MBC of 18.75mg/ml. It was bacteriostatic against *P. aeruginosa* with an MIC of 9.375 and MBC of 18.75mg/ml. This activity is possibly due to high content of essential oils such as monoterpenes and sesquiterpene. The extract was bactericidal since the MIC and MBC were the same. This result agrees to Nwinyi *et al.,* (2009) where it is pointed out

that sesquiterpene was tested and found to be active against *E.coli* but had minimal effect on *S. aureus*.

Thylachium africanum was bactericidal against *P. aeruginosa*, with MIC and MBC of 4.687mg/ml, whereas against *S. aureus* had the MIC of 18.75mg/ml, and MBC of 37.5mg/ml.

Acacia nilotica extract produced good MICs ranging from 4.6875mg/ml to 18.75mg/ml. The extract was bactericidal against *P. aeruginosa* and *K. pneumoniae* (MICs and MBCs at the same level), and bacteriostatic against *E.coli* and *C. albicans* (had MICs and MBCs/MFCs at different levels). This activity is probably due to the fact that each of the phytochemicals identified has a record of one therapeutic usage or another. Some of these phytochemicals like flavonoids have been known to be antidiarrhoeal from other studies (Meite *et al.,* 2009). Phytochemical screening of the stem bark of *A. nilotica* done in Nigeria revealed that the plant contains terpenoids, alkaloids, saponins and glycosides (Banso, 2009). Negative results were recorded for steroids and flavonoids which confirm the absence of these active principles. The active principles identified in this study exhibited antimicrobial activity against *Streptococcus viridans*, *Staphylococcus aureus*, *Shigella sonnei, Bacillus subtilis*, and *Escherichia coli*.

5.2.3. Plants without antimycobacterial, antibacterial and antifungal activity

The plants without inhibitory activity could still be useful as immune boosters or of nutritional value (Omwenga *et al.,* 2009). There is a possibility that some could be active because of synergism as a number of plants are boiled together before use. The plants

without inhibitory activity included: *Clerodendrum myricoides, Commiphora africana, Pistacia aethiopica, Loranthus acaciae, Acacia senegal, Acokanthera friesiorum, Plumbago dawei, Croton megalocarpus, Zanthoxylum gillettii, Mormordica charantia, Ocimum gratissimum, Lantana camara, Erythrina abyssinica, Euphorbia tirucalli,* and *Fuerstia africana.* All these have one thing in common; the presence of flavonoids except in *Commiphora africana*.

This is not to say that flavonoids have no role in bioactivity of extracts. It is possible that they have strong synergistic effect (Lechner *et al.,* 2008). Indeed work has been done to identify plant natural products which modulate the susceptibility of different strains of fast-growing mycobacteria to the first-line antituberculotic isoniazid (INH), whereby several flavonoids without significant antimycobacterial activities at the tested concentrations were screened for their ability to decrease the minimum inhibitory concentrations (MICs) of INH (Lechner *et al.,* 2008; Omwenga *et al.,* 2009). Flavonoids with different substitution patterns, namely epicatechin, isorhamnetin, kaempferol, luteolin, myricetin, quercetin, rutin and taxifolin were tested to examine structure-activity relationships (SARs) of these compounds. The strongest synergistic effects were observed in *M. smegmatis* followed by *M. phlei*. This clearly indicates the tendency of INH potentiation by certain flavonoids which can be exploited to get antimycobacterial drugs (Lechner *et al.,* 2008).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The aim of the current study was to find out whether the medicinal plants used by the communities in the Lake Victoria region and the Samburu community in the North Rift Valley are active against the tuberculosis causing agents and other pathogens. From the results most of the herbal plants selected showed activity against either the mycobacteria, the other bacteria test cultures, or the fungus *C. albicans*, hence the reason as to why they are commonly used by herbalists for treatment of various diseases. Among the conclusions observed from the study are:

i.) The results from the investigation revealed that most of the medicinal plants contain pharmacologically active substances with antimycobacterial, antibacterial and antifungal properties. It also points out that there is a possibility of getting effective compounds from natural sources, which can be of value in the fight against tuberculosis and other infectious diseases. The study also provides support for the use of these plants in the management of infectious diseases in the communities living around the Lake Victoria region and the Samburu community of eastern Samburu District in northern Kenya.

ii.) Some of the extracts screened had antimycobacterial activity on the test cultures used. Among the most active plants is *E. scarlatina*, whose extract was able to completely inhibit the growth of all the mycobacteria strains used, at all the tested concentrations. *Entada abysinnica* was also active against the slow growing mycobacteria, *S. typhi* and a fungus-*C. albicans.*

iii.) The extracts were found to be generally more active against mycobacteria culture strains than the common bacteria and fungi.

iv.) The presence of several phytochemical compounds known to be of medicinal importance points out a possible source for anti- mycobacterial agents to address the problem of MDR-TB. It also points out that there is a possibility of getting effective compounds from natural sources, which can be of value in the fight against tuberculosis and other infectious diseases. The study also provides support for the use of these plants in the management of infectious diseases in the communities living in the Lake Victoria region and Samburu community of Kenya.

6.2 Recommendation for future work

i. To isolate, identify, characterize and elucidate the structure of the bioactive compounds and screen them against mycobacterial strains.

ii. Because only one part of the plant was used, studies should be carried out to study different parts of the same plant and compare their activities.

iii. Pharmacological and toxicological studies of active plants should be done to investigate the antimicrobial mechanisms of action of the studied samples.

iv. It could also be interesting to study the activity of the combined drugs such as isoniazid and active crude extracts.

v. The extracts that exhibited good *in vitro* anti-mycobacterial activity could be subjected to *in vivo* studies in animal models so as to extrapolate in *vitro/in vivo* activities of the extracts.

vi. The communities to be sensitized on the safe use of medicinal plants and conservation of the medicinal plants be given a priority.

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APPENDICES

Appendix I - Traditional Medical Practioners (TMP Questionnaire

Kenyatta University researchers in collaboration with VicRes are conducting research on the Use of medicinal plants to treat tuberculosis and other infections. We are soliciting information from you to determine the efficacy of the medicinal plants against the said infections. We appreciate your cooperation and the information given will be treated with utmost confidentiality. Thank you in advance.

Improving the management of TB in the Lake Victoria Basin and Samburu communities

Recorder……. Date……

District…… Location…….

Sub-location…… Village……

A. Biodata

Name		Sex	
Age		Religion	
Main occupation		Other occupation	

B. Knowledge acquisition

1. Do you treat respiratory infections?

Yes	No

2. Which respiratory infections do you treat? *Please tick against what you treat*
3.

TB	
Asthma	
Ordinary cough	
Other (name)	

4. Please list the signs by which you recognize TB

5. What are the local names of TB?

6. What are the causes of TB?

7. How did you learn how to treat TB?

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

C. Treatment practices and knowledge

1. Do you belong to a healers association?

Yes	No

2. How many patients do you treat per day?

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

4. How many people work at your practice?

5. How many people gather plants for you?

6. What materials do you use in your treatments

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

7. Are there any rituals involved?

Yes	No

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

Respiratory infection	Plant spp	Part used	Time of collection	Formula	Dose	Route administration	Preservation methods

Codes:

a. Respiratory infection (1 TB; 2 Asthma; 3 ordinary cough; 4 other)

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

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

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

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

D. Patients

1. Are patients comfortable about seeking TB treatment from you?

Yes	No

2. Where do most of your customers come from?

From this village	
From other districts	
From other countries	
Do not know	

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

4. How did you assess that they had recovered?

THANK YOU VERY MUCH

APPENDIX II: LOWENSTEIN JENSEN (LJ) SLANTS

a. Slow growers

i. Concentration of extracts 2mg/ml

Plant Specimen	Time in Weeks															
	1		2		3		4		5		6		7		8	
	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb
C. edulis	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
E. abysinnica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. macrostachyus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. amygdalina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. charantia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O. gratissimum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. camara	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. myricoides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. asiatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. trifolia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. abyssinica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. tirucalli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. africana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. secundiflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z. gillettii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aethiopica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. megalocarpus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. persica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. senegal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. friesiorum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. dawei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

L. acaciae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. sinensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. horrida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. anthelmitica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. africanum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. angustifolia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. quadrangularis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G. simi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. etbaica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. multiflorus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. africana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. scarlatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nilotica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Negative controls	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potisive control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ii. Concentration of extracts 1mg/ml

Plant Specimen	Time in Weeks															
	1		2		3		4		5		6		7		8	
	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mk	Mtb
C. edulis	-	-	-	-	-	+	-	+	+	++	+	++	++	++	+++	-+++
E. abysinnica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. macrostachyus	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
V. amygdalina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. charantia	-	-	-	-	+	+	+	+	+	+	+	+++	++	+++	+++	+++
O. gratissimum	-	-	-	-	-	+++	++	+++	+++	+++	+++	++	+++	+++	+++	+++
L. camara	-	-	-	-	-	+	+	+	+	+	++	+	++	+++	+++	+++
C. myricoides	-	-	-	-	-	-	-	-	-	++	++	++	++	++	++	++
T. asiatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. trifolia	-	-	-	-	++	++	++	++	++	++	++	++	++	++	++	++
E. abyssinica	-	-	-	-	-	-	-	++	++	++	++	++	++	++	+++	+++
E. tirucalli	-	-	-	-	-	-	+	+	+	+	++	++	++	++	++	++
F. africana	-	-	-	-	+	+	++	++	++	+++	+++	+++	+++	+++	+++	+++
A. secundiflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z. gillettii	-	-	-	-	+	+	++	+	++	++	+++	+++	+++	+++	+++	+++
P. aethiopica	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++	++
C. megalocarpus	-	-	-	-	-	-	+	++	++	++	++	++	+++	+++	+++	+++
S. persica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
A. senegal	-	-	-	-	-	-	++	++	++	+++	++	+++	+++	+++	+++	+++
A. friesiorum	-	-	-	-	+	+	+	+	++	++	++	++	+++	+++	+++	+++
P. dawei	-	-	+	+	+	+	+	+	+	++	++	++	+++	+++	+++	+++
L. acaciae	-	-	-	+	+	+	+	+	+	+	+	+	++	++	+++	+++
C. sinensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. horrida	-	-	-	-	+	+	+	+	++	++	++	++	++	++	++	++

A. anthelmitica	-	-	-	+	+	+	+	+	+	+	++	++	+++	+++	+++	+++
T. africanum	-	-	-	-	++	++	++	++	++	++	++	++	++	++	++	++
B. angustifolia	-	-	-	-	-	-	-	-	-	-	+	+	++	++	++	++
C. quadrangularis	-	-	+	+	+	+	++	++	++	++	++	++	++	+++	+++	+++
G. simi	-	-	-	-	+	+	+	++	++	++	++	+++	+++	+++	+++	+++
A. etbaica	-	-	+	+	+	+	+	+	+	+	++	++	++	++	+++	+++
S. multiflorus	-	-	-	-	-	-	-	-	-	-	-	-	+++	++	++	++
C. africana	-	-	-	-	-	+	+	++	++	++	++	++	++	++	++	++
E. scarlatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nilotica	-	-	-	-	-	-	-	-	++	++	++	++	++	++	+++	+++
Negative controls	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potisive control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a. Fast growers

i. Concentration of extracts 2mg/ml

Plant Specimen	Time in Weeks															
	1		2		3		4		5		6		7		8	
	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms
C. edulis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. abysinnica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. macrostachyus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. amygdalina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. charantia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O. gratissimum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. camara	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. myricoides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. asiatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. trifolia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. abyssinica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. tirucalli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. africana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. secundiflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z. gillettii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aethiopica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. megalocarpus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. persica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. senegal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. friesiorum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. dawei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. acaciae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

C. sinensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. horrida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. anthelmitica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. africanum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. angustifolia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. quadrangularis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G. simi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. etbaica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. multiflorus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. africana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. scarlatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nilotica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Negative controls	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potisive control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ii. Concentration of extracts 1mg/ml

Plant Specimen	Time in Weeks															
	1		2		3		4		5		6		7		8	
	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms	Mf	Ms
C. edulis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. abysinnica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. macrostachyus	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
V. amygdalina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. charantia	-	-	-	-	+	+	+	+	+	+	+	+++	++	+++	+++	+++
O. gratissimum	-	-	-	-	-	-	-	-	-	-	-	++	+++	+++	+++	+++
L. camara	-	-	-	-	-	+	+	+	+	++	++	++	++	++	++	+++
C. myricoides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. asiatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
L. trifolia	-	-	-	-	+	+	+	+	+	++	++	++	++	++	++	++
E. abyssinica	-	-	-	-	-	-	-	+	+	+	+	+	++	++	++	++
E. tirucalli	-	-	-	-	-	-	+	+	+	+	++	++	++	++	++	++
F. africana	-	-	-	-	+	+	+	+	++	+++	+++	++	+++	++	+++	+++
A. secundiflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z. gillettii	-	-	-	-	+	+	++	+	++	++	++	++	++	++	+++	+++
P. aethiopica	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++	++
C. megalocarpus	-	-	-	-	-	-	+	+	+	+	+	+	++	++	++	++
S. persica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
A. senegal	-	-	-	-	-	-	+	+	+	+	+	+	+	+	++	+
A. friesiorum	-	-	-	-	+	+	+	+	++	++	++	++	+++	+++	+++	+++
P. dawei	-	-	+	+	+	+	+	+	+	++	++	++	++	++	++	++
L. acaciae	-	-	-	+	+	+	+	+	+	+	+	+	++	++	+++	++
C. sinensis	-	-	-	-	-	-	-	-	-	-	-	-	+	+	++	++
A. horrida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A. anthelmitica	-	-	-	-	-		-	-	-	-	-	+	+	+	+	++
T. africanum	-	-	-	-	++	++	++	++	++	++	++	++	++	++	++	++
B. angustifolia	-	-	-	-	-	-	-	-	-	-	+	+	+	++	++	++
C. quadrangularis	-	-	-	-	-	-	-	-	-		-	-	-	+	++	++
G. simi	-	-	-	-	-	-	-	-	-	-	-	+	++	++	++	++
A. etbaica	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-
S. multiflorus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. africana	-	-	-	-	-	+	+	+	+	+	+	+	++	++	++	+++
E. scarlatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nilotica	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
Negative controls	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	++
Potisive control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX III: TABLES REPRESENTING DEGREES OF SIGNIFICANCE OF MEANS OF ZONES OF INHIBITION IN VARIOUS TEST CULTURES

a. S. typhi

Analysis of Variance for *S. typhi*

Source	DF	SS	MS	F	P
botanica	24	309.889	12.912	107.60	0.000
Error	50	6.000	0.120		
Total	74	315.889			

Level	N	Mean	StDev	Individual 95% CIs for Mean Based on Pooled StDev
Acacia n	3	8.660	0.000	(*)
Aloe sec	3	12.000	0.000	(*)
Boscia	3	8.000	0.000	(-*)
Carissa	3	9.000	1.000	(*)
Cleroden	3	7.660	0.000	(-*)
Croton ma	3	8.000	0.000	(-*)
Croton me	3	7.660	0.000	(-*)
Entada a	3	10.333	0.577	(*-)
Erythrin	3	8.000	0.000	(-*)
Euphorbi t	3	8.000	0.000	(-*)
Euphorbi s	3	6.330	0.000	(*)
Fuerstia	3	7.330	0.000	(*-)
Grewia s	3	6.660	0.000	(*-)
Lantana	3	6.667	0.577	(*-)
Lantana	3	8.660	0.000	(*)
Momordic	3	8.667	1.155	(*)
Ocimum g	3	8.660	0.000	(*)
Pistacia	3	8.660	0.000	(*)
Scadoxus	3	9.000	0.000	(*)
Thylachi	3	7.670	0.000	(-*)
Toddalia	3	6.330	0.000	(*)
Vernonia	3	7.330	0.000	(*-)
Zanthoxy	3	6.660	0.000	(*-)
Negative	3	6.000	0.000	(*)
Positive	3	16.000	0.000	(*-)

Pooled StDev = 0.346

6.0 9.0 12.0 15.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000318

Critical value = 5.47

b. S. aureaus

Analysis of Variance for *S. aureus*

Source	DF	SS	MS	F	P
botanica	24	1006.228	41.926	224.60	0.000
Error	50	9.333	0.187		
Total	74	1015.562			

Level	N	Mean	StDev	Individual 95% CIs for Mean Based on Pooled StDev
Acacia n	3	8.000	0.000	(*)
Aloe sec	3	13.330	0.000	(*)
Boscia	3	9.000	0.000	(*)
Carissa	3	8.000	1.000	(*)
Cleroden	3	8.000	0.000	(*)
Croton ma	3	7.000	0.000	(*)
Croton me	3	8.000	0.000	(*)
Entada a	3	9.667	0.577	(*)
Erythrin	3	8.660	0.000	(*)
Euphorbi t	3	7.000	0.000	(*)
Euphorbi s	3	6.330	0.000	(*)
Fuerstia	3	8.000	0.000	(*)
Grewia s	3	6.660	0.000	(*)
Lantana	3	9.000	1.732	(*)
Lantana	3	20.000	0.000	(*)
Momordic	3	10.667	0.577	(*)
Ocimum g	3	8.660	0.000	(*)
Pistacia	3	7.330	0.000	(*)
Scadoxus	3	8.330	0.000	(*)
Thylachi	3	10.660	0.000	(*)
Toddalia	3	8.660	0.000	(*)
Vernonia	3	8.660	0.000	(*)
Zanthoxy	3	7.330	0.000	(*)
Negative	3	6.000	0.000	(*)
Positive	3	21.330	0.000	(*)

Pooled StDev = 0.432

10.0 15.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000318

Critical value = 5.47

c. E.coli

Analysis of Variance for *E.coli*

Source	DF	SS	MS	F	P
botanica	24	721.8005	30.0750	2255.63	0.000
Error	50	0.6667	0.0133		
Total	74	722.4672			

Level	N	Mean	StDev	Individual 95% CIs for Mean Based on Pooled StDev
Acacia n	3	12.000	0.000	*
Aloe sec	3	15.000	0.000	*)
Boscia	3	7.330	0.000	*)
Carissa	3	7.000	0.000	*)
Cleroden	3	7.000	0.000	*)
Croton ma	3	7.000	0.000	*)
Croton me	3	6.660	0.000	(*
Entada a	3	7.667	0.577	*)
Erythrin	3	7.330	0.000	*)
Euphorbi t	3	7.000	0.000	*)
Euphorbi s	3	7.660	0.000	*
Fuerstia	3	7.660	0.000	*
Grewia s	3	8.000	0.000	*
Lantana	3	6.000	0.000	*
Lantana	3	8.660	0.000	(*
Momordic	3	6.000	0.000	*
Ocimum g	3	7.000	0.000	*)
Pistacia	3	8.330	0.000	(*
Scadoxus	3	8.330	0.000	(*
Thylachi	3	8.000	0.000	*
Toddalia	3	7.000	0.000	*)
Vernonia	3	6.660	0.000	(*
Zanthoxy	3	6.000	0.000	*
Negative	3	6.000	0.000	*
Positive	3	20.220	0.000	(*

Pooled StDev = 0.115

8.0 12.0 16.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000318

Critical value = 5.47

d. P. aeruginosa

Analysis of Variance for *P. aeruginosa*

Source	DF	SS	MS	F	P
botanica	24	661.883	27.578	71.87	0.000
Error	50	19.186	0.384		
Total	74	681.069			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
Acacia n	3	11.667	0.577	(-*-)
Aloe sec	3	18.000	0.000	(-*-)
Boscia	3	6.667	0.577	(-*)
Carissa	3	7.667	0.577	(-*-)
Cleroden	3	7.667	0.577	(-*-)
Croton ma	3	7.000	1.000	(-*)
Croton me	3	8.000	0.000	(-*-)
Entada a	3	8.333	0.577	(-*-)
Erythrin	3	10.443	0.510	(-*-)
Euphorbi t	3	6.000	0.000	(-*-)
Euphorbi s	3	7.667	2.082	(-*-)
Fuerstia	3	6.667	0.577	(-*)
Grewia s	3	7.000	0.000	(-*)
Lantana	3	8.667	0.577	(-*)
Lantana	3	10.000	0.000	(-*-)
Momordic	3	9.333	0.577	(*-)
Ocimum g	3	7.660	0.000	(-*-)
Pistacia	3	7.000	0.000	(-*)
Scadoxus	3	6.333	0.577	(-*-)
Thylachi	3	10.000	0.000	(-*-)
Toddalia	3	10.333	0.577	(-*-)
Vernonia	3	8.330	0.000	(-*-)
Zanthoxy	3	9.333	0.577	(*-)
Negative	3	6.000	0.000	(-*-)
Positive	3	17.333	0.577	(*-)

Pooled StDev = 0.619

8.0 12.0 16.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000318

Critical value = 5.47

e. K. pneumoniae

Analysis of Variance for *K. pneumoniae*

Source	DF	SS	MS	F	P
botanica	24	376.794	15.700	27.38	0.000
Error	50	28.667	0.573		
Total	74	405.460			

Level	N	Mean	StDev	Individual 95% CIs for Mean Based on Pooled StDev
Acacia n	3	9.000	1.732	(--*-)
Aloe sec	3	8.000	0.000	(-*-)
Boscia	3	7.000	1.000	(--*-)
Carissa	3	8.000	1.000	(-*-)
Cleroden	3	6.667	0.577	(--*-)
Croton ma	3	11.000	0.000	(--*-)
Croton me	3	7.667	0.577	(-*-)
Entada a	3	7.333	0.577	(-*--)
Erythrin	3	8.333	0.577	(-*-)
Euphorbi t	3	7.333	0.577	(-*--)
Euphorbi s	3	7.000	0.000	(--*-)
Fuerstia	3	6.667	0.577	(--*-)
Grewia s	3	7.333	0.577	(-*--)
Lantana	3	7.333	0.577	(-*--)
Lantana	3	6.667	0.577	(--*-)
Momordic	3	8.000	1.732	(-*-)
Ocimum g	3	6.660	0.000	(--*-)
Pistacia	3	6.667	0.577	(--*-)
Scadoxus	3	7.333	0.577	(-*--)
Thylachi	3	7.333	1.155	(-*--)
Toddalia	3	6.000	0.000	(-*-)
Vernonia	3	8.000	0.000	(-*-)
Zanthoxy	3	7.000	1.000	(--*-)
Negative	3	6.000	0.000	(-*-)
Positive	3	17.667	0.577	(-*-)

Pooled StDev = 0.757

8.0 12.0 16.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000318

Critical value = 5.47

f. C. albicans

Analysis of Variance for *C. albicans*

Source	DF	SS	MS	F	P
botanica	24	283.044	11.793	33.06	0.000
Error	50	17.837	0.357		
Total	74	300.881			

Level	N	Mean	StDev	Individual 95% CIs for Mean Based on Pooled StDev
Acacia n	3	10.333	0.577	(-*--)
Aloe sec	3	7.000	0.000	(-*--)
Boscia	3	6.000	0.000	(-*-)
Carissa	3	7.000	1.000	(-*--)
Cleroden	3	7.667	0.577	(--*-)
Croton ma	3	8.333	1.528	(--*-)
Croton me	3	7.000	0.000	(-*--)
Entada a	3	14.107	0.958	(-*-)
Erythrin	3	8.000	0.000	(--*-)
Euphorbi t	3	6.667	0.577	(-*--)
Euphorbi s	3	7.000	1.000	(-*--)
Fuerstia	3	7.000	0.000	(-*--)
Grewia s	3	6.000	0.000	(-*-)
Lantana	3	6.333	0.577	(-*-)
Lantana	3	7.667	0.577	(--*-)
Momordic	3	7.667	0.577	(--*-)
Ocimum g	3	8.000	0.000	(--*-)
Pistacia	3	7.000	0.000	(-*--)
Scadoxus	3	7.333	0.577	(-*--)
Thylachi	3	6.000	0.000	(-*-)
Toddalia	3	8.000	1.000	(--*-)
Vernonia	3	7.660	0.000	(--*-)
Zanthoxy	3	7.333	0.577	(-*--)
Negative	3	6.000	0.000	(-*-)
Positive	3	13.000	0.000	(-*--)
Pooled StDev =		0.597		6.0 9.0 12.0 15.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000318

Critical value = 5.47

APPENDIX IV: PUBLICATIONS

I. Published articles

1. **R. M. Mariita**, C.K.P.O. Ogol, N.O. Oguge and P. O. Okemo* (2011). Methanol extract of three medicinal plants from Samburu in northern Kenya show significant antimycobacterial, antibacterial and antifungal properties. ***Research Journal of medicinal plant*** **5**(1): 54-64.
   Availablefrom:http://scialert.net/qredirect.php?doi=rjmp.0000.18809.18809&linkid=pdf

2. **Richard M. Mariita,** John A.Orodho1, Paul O. Okemo*, Paul K. Mbugua (2010). Antifungal, antibacterial and antimycobacterial activity of *Entada abysinnica* Steudel ex A. Rich. (Fabaceae) methanol extract. ***Pharmacognosy Research.*** **2**(3): 163-168.
   Available from: http://www.phcogres.com/text.asp?2010/2/3/163/65511

3. **Richard M. Mariita**, Callistus K.P.O. Ogol, Nick O. Oguge and Paul O. Okemo*(2010). Antitubercular activity and phytochemical investigation of methanol extracts of medicinal plants used by the Samburu community of Kenya. ***Tropical Journal of Pharmaceutical Research*****. 9**(4). 379-385.
   Available from: http://www.tjpr.org/vol9_no4/2010_9_4_8_Mariita.pdf

4. **Richard M. Mariita,** Paul O. Okemo*, John A. Orodho, Claude Kirimuhuzya, Joseph N. Otieno, Magadula J. Joseph (2010). Efficacy of 13 selected medicinal plants from the Lake Victoria region of Kenya against tuberculosis, diarrhea causing bacteria and *Candida albicans*. ***International Journal of pharmacy and Technology.*** **2**(3)771-791.
   Available from: http://www.ijptonline.com/wp-content/uploads/2009/10/771-791.pdf

II. Article(s) under review

1. **Mariita M.R.,** Orodho J. A., Okemo P.O.*, Tabuti J., Kirimuhuzyia C., Otieno J., and Magadula J. (2010). Methanol extracts of *Aloe secundiflora* Engl., a local *spp.* used by indigenous communities around Lake Victoria, Kenya, inhibits tuberculosis and diarrheal causing bacteria. ***Pharmacognosy Research***.