

**ANTIBACTERIAL EFFICACY AND SAFETY OF SELECTED
KENYAN MEDICINAL PLANTS**

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DECLARATION

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

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DEDICATION

I dedicate this work to my late father **Michael Abiba Omodo**, my Mother **Margaret Dwera Abiba**, my late wife **Josephine Ngute Ogao** who continually loved, encouraged and prayed for me to complete this research work and also to my late brother **Patrick Lubondi Abiba**, for sacrificing all they had to facilitate my education.

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ABBREVIATIONS AND ACRONYMS

Ache	Acetylcholinesterase Enzyme
AMC	Augmentin
AMF	Amoxil
ALP	Alanine Phosphatase
ALT	Alanine Aminotransferase
AMP	2-Amino-2-Methyl-1-Propanol
AST	Aspartate Aminotransferase
ATP	Adenosine-5'-Triphosphate
ATCC	American Type Culture Collection
AZT	Azidothymidine
Br	Bromine
BUN	Blood Urea
Ca	Calcium
Cu	Copper
CK	Creatine Kinase
CNS	Central Nervous System
CMR	Centre for Microbiological Research
CFU	Colony Forming Units
COPD	Chronic Obstructive Pulmonary Disease
DLC	Differential Lymphocytes Count
DST	Diagnostic Sensitivity Test Agar
DMF	Dimethyl Formamide
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
FPM	Fundamental Parameters Method
Fe	Iron
GDH	Glutamate Dehydrogenase
G6PD	Glucose-6-Phosphate Dehydrogenase
GR	Growth Rate
HPPD	Hydroxyphenylpyruvate Dehydrogenase
Hb	Haemoglobin
HK	Hexokinase

K	Potassium
KNH	Kenyatta National Hospital
KEMRI	Kenya Medical Research Institute
LDH	Lactate Dehydrogenase
Ly	Lymphocytes
MM	Millimeter
Mn	Manganese
Mo	Monocytes
NAD	Nicotinamide Adenine Dinucleotide
N	Neutrophil
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
NACHR	Nicotinic Acetylcholine Receptor
NCES	New Chemical Entities
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
OAU	Organization of African Unity
MBC	Minimum Bactericidal Concentrations
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCA	Multi-Channel Analyzer
MDH	Malate Dehydrogenase
MI	Myocardial Infarction
MIC	Minimum Inhibitory Concentrations
MHA	Muller Hinton Agar
MHB	Muller Hinton Broth
MRSA	Methicillin-Resistant <i>Staphylococcus Aureus</i>
Nb	Niobium
NCI	National Cancer Institute
NCTC	National <i>Collection Of Type Cultures</i>
PPM	Parts per Minute
Pb	Lead
PCV	Packed Cell Volume
PRSP	Penicillin-Resistant <i>Streptococcus Pneumoniae</i>
RBC	Red Blood Cells

Rb	Rubidium
Rf	Retention Factor
Sr	Strontium
Stds	Sexually Transmitted Diseases
TCM	Traditional Chines Medicine
TLC	Thin Layer Chromatography
Ti	Titanium
TXRF	Total Reflection X-Ray Fluorescence System
UK	United Kingdom
USSR	United Soviet State of Russia
VRE	Vancomycin-Resistant Enterococci
V/V	Volume by Volume
WBC	White Blood Cells
WHO	World Health Organization
WM	Wash Medium
W/V	Weight By Volume
Y	Yttrium
Zn	Zinc
Zr	Zirconium

ABSTRACT

Many disease-causing organisms have become resistant to conventional antibiotics. The toxicity and side effects of conventional drugs have also become increasingly unmanageable, while their costs are prohibitive. It has therefore become necessary to continue searching for alternative medicines to treat such diseases. The possibility of developing pharmaceutical industries using local raw materials should be looked into critically so that the cost of drugs can be affordable to the local people. This study involved screening of various medicinal plants commonly used by herbal medicine practitioners for bioactive compounds against bacteria. Standard bioassay methods were employed throughout this study. They included preliminary screening bioassay against human pathogenic bacteria strains using Agar Disc Diffusion method, Minimum Inhibitory Concentration (MICs), Minimum Bactericidal Concentration (MBCs), Time kill kinetics, phytochemical determination methods and one dose *in vivo* toxicity tests using mice. Hexane, dichloromethane, acetone and methanol extracts of ten (10) medicinal plants: *Dichrostachys cinerea*, *Combretum molle*, *Euclea divinorum*, *Ficus sur*, and other five plants were screened by preliminary bioassay against nine (9) human pathogenic bacteria strains (*E. coli*, ATCC 25922 *S. aureus* ATCC 85923, *Pseudomonas aeruginosa* NCTC 10662, *Escherichia coli*, *Shigella flexinery*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Klebsiella* hospital isolates) using agar disc diffusion method. Acetone, methanol extracts of *D. cinerea* and *C. molle* showed remarkable inhibitory activity against all nine human pathogenic bacteria. Acetone and dichloromethane of *E. divinorum* and *F. sur* showed activity against all human pathogenic bacteria except *P. aeruginosa* and *klebsiella*. All extracts of six (6) plants were inactive against the test human pathogenic bacteria. MICs of acetone and methanol extracts of the four (4) plants *D. cinerea*, *C. molle*, and dichloromethane and acetone extracts of *E. divinorum* and *F. sur* were active against the test bacteria. MIC results showed that acetone and methanol extracts of *D. cinerea*, and *C. molle* gave very good low MICs ranges (0.3-2.5mg/ml), dichloromethane and acetone extracts of *E. divinorum* and *F. sur* gave MICs ranging between 7.5-100mg/ml. MBCs of acetone and methanol extracts of *D. cinerea*, and *C. molle* were as low as 0.3mg/ml while the highest being 5mg/ml. Phytochemical determination showed that both methanolic and acetone extracts of *D. cinerea* and *C. molle* contained alkaloids, tannins, steroids and triterpenoids, flavone glycones, anthrocyanins and reducing sugar. Kill kinetics studies of the bioactive organic extracts on the tested bacteria indicated that acetone extracts of *D. cinerea*, acetone extracts of *C. molle*, methanol extracts of *C. molle*, methanol extracts of *D. cinerea* at 4, 6 and 8mg/ml against *Staphylococcus aureus* ATCC 85923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* NCTC 10662, displayed dose and time dependent rapid kinetics of the bacterial killing. Because the toxicity of a drug to the bystander host cells could render it unsuitable for therapeutic purposes, the toxicity of the active plant extracts against the tested bacteria was assessed. The reduced growth rate, organ weights, white and red blood cells counts, plus increased levels of biochemicals in the serum in plant extract treated animals relative to that of the controls, indicate that these extracts contained constituents with toxic effects on them. Such constituents include some of the phytochemicals present in these extracts. The mineral content may also cause toxicity but this was not the case with the measured minerals since their levels were below the recommended daily allowance except for potassium which was 67 to 141 times the recommended daily allowance.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics (Cohen *et al.*, 1994; Poole, 2001; WHO, 2001) has led to the search for new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages (Herrera *et al.*, 1996; De Smet, 1997; Sokmen *et al.*, 1999; Kelmanson *et al.*, 2000; Meng *et al.*, 2000). It is important to investigate how the development of traditional medicine has developed into what it is now.

Medicines are chemical substances that are used for treatment, alleviation, control, prevention of diseases, and maintenance of health. The existence of man on earth has been accompanied by diseases, and with them, suffering and death. Man therefore, started searching for causes, prevention and treatment of diseases. Ancient management of these diseases comprised of magic, superstition and annual sacrifices.

Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Solecki, 1975). From that point the development of traditional medical systems incorporating plants as a means of therapy can be traced back only as far as recorded documents. However, the value of these systems is much more than a significant anthropologic or archeological fact. Their value is as a methodology of medicinal agents, which, according to the World Health Organization (WHO), almost 65% of the world's population has incorporated into their primary modality of health care

(Farnsworth *et al.*, 1985). Some plant-derived products are so effective and dependable that the medical fraternity cannot manage some diseases without them.

There are many examples of plant-derived products that have been made into medicines and used in treatment of diseases (Baladrin *et al.*, 1985). Thirty percent of the pharmaceutical agents used at present are plant derived (Kaye *et al.*, 2000). Traditional methods of drug discovery and development have been influenced by the need to prevail over illness and people's experience in witnessing and realizing the beneficial potentials of a plant to cure ailments, perhaps by trial and error (Sumner, 2000).

Plants have long provided mankind with herbal remedies for many infectious diseases and even today, they continue to play a major role in primary health care as therapeutic remedies in developing countries (Sokmen *et al.*, 1999). The search for biologically active extracts based on traditionally used plants is still relevant due to the appearance of microbial resistance of many antibiotics and the occurrence of fatal opportunistic infections. It is known that traditional healers use indigenous medicinal plants to treat many illnesses including sexually transmitted diseases (STD's) (Ndubani, 1997; 1999). In many African countries STD's are a major public health problem and there is a high prevalence rate of diseases, such as gonorrhoea and syphilis. It is widely believed that STD's are treated ineffectively by Western medicine. Some of those who go to a hospital may even come back to the traditional healers to "cleanse" the system after treatment by Western medicine (Moss *et al.*, 1999).

Plant materials remain an important resource to combat serious diseases in the world. Pharmacognostic investigations of plants are carried out to find novel drugs or templates

for the development of new therapeutic agents (Ko'nig, 1992). Among the more than 250 000 species of higher plants, only about 5–10% are chemically investigated (Nahrstedt, 1996). Since many drugs, like quinine and artemisinin (Wright and Phillipson, 1990), taxol and camptothecin (Debernardis *et al.*, 1996) were isolated from plants, and because of increased resistance of many microorganisms (like malaria parasites), towards established drugs, investigation of the chemical compounds within traditional plants is necessary (Phillipson, 1991). Malaria is still one of the most important parasitic diseases of mankind. The yearly statistics of the World Health Organization (WHO), concerning mortality caused by tropical diseases, particularly from malaria, is frightening. It is estimated that there are more than a million deaths and up to 500 million clinical cases of malaria each year (WHO, 1999).

From *Papaver somniferum* Keshi (*Opium poppy*), opium alkaloids such as, codeine and morphine are obtained (Pryde *et al.*, 1981). These drugs are useful and effective as analgesics, especially in general anaesthesia and as psychotropic agents. From the alkaloids of *Erythroxylon coca* cocaine was isolated and is used as a local anaesthesia and pschotropic agent (Farnsworth, 1983).

The willow tree (*Salix*sp.) has achieved a kind of symbolic significance associated with the discovery of aspirin, more than a century ago (Barrett *et al.*, 1999; Lévesque and Lafont, 2000; Cragg and Newman, 2001; Jones, 2001), although herbalists and patients have recognized the pharmacological and the clinical potential of not only the willow, but also a variety of other plants (Mayer *et al.*, 1949; Lévesque and Lafont, 2000; Sumner, 2000; Dewick, 2002). This comes as no surprise, because medical conditions are as old as

our species, and some animals are known to preferentially consume plants with medicinal properties (Sumner, 2000).

Aspirin therefore is another example of a simple derivative of the naturally occurring salicylic acid originally derived from *Willows salix spp.* Perhaps no analgesic drug has had such great impact on humans worldwide like aspirin. Lately, there have been reports that aspirin may help in the management of cardiac diseases.

Recently, a number of new drugs were discovered from ethno botanical leads such as benzoin isolated from *Styrax tonkinensis* used for oral disinfectant and emetine from *Psychotria ipecacuanha* for amoebic dysentery (Cox, 1994). Treatment failures arising from antibiotic-resistant bacteria (Boyce, 1992; Berns, 2003), stimulation of toxin production (Yoh *et al.*, 1999), together with the recent upturn in consumer mistrust of synthetic additives, have made it necessary to search for natural compounds from plants to replace antibiotics or artificial antimicrobials.

Drug discovery from medicinal plants led to the isolation of early drugs such as cocaine, codeine, digitoxin, and quinine, in addition to morphine, of which some are still in use (Newman *et al.*, 2000; Butler, 2004; Samuelsson, 2004). Isolation and characterization of pharmacologically active compounds from medicinal plants continue today. More recently, drug discovery techniques have been applied to the standardization of herbal medicines, to elucidate analytical marker compounds.

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist,

ethnobotanist, ethnopharmacologist, or plant ecologist who collects and identifies the plant(s) of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (traditionally used herbal remedies) or may involve taxa collected randomly for a large screening program. It is necessary to respect the intellectual property rights of a given country where plant(s) of interest are collected (Baker *et al.*, 1995). Phytochemists (natural product chemists) prepare extracts from the plant materials, subject these extracts to biological screening in pharmacologically relevant assays, and commence the process of isolation and characterization of the active compound(s) through bioassay-guided fractionation. Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets (Baker *et al.*, 1995). Pharmacognosy encapsulates all of these fields into a distinct interdisciplinary science.

Some of the earliest curative medicines to be discovered were antimalarial and antibiotics in the 18th century (Everyman's Encyclopedia, 1976). Malaria remains one of the world's most deadly diseases. It is estimated that more than two million people die from the disease each year (Prozesky *et al.*, 2001). Although many drugs are available, some have become ineffective as the *Plasmodium* parasites responsible for malaria have developed drug resistance (Peters, 1998). Traditional remedies are continually being investigated as plant derived antimalarial drugs become more sought after. Plants have invariably been a rich source for new drugs and some antimalarial drugs in use today (quinine and artemisinin) were either obtained from plants or developed using their chemical structures as templates (Gessler *et al.*, 1994). Scientific evaluation of medicinal plants

used in the preparation of traditional medicine has in the past provided modern medicine with effective drugs for the treatment of parasitic diseases (Iwu *et al.*, 1994).

With the emergence of chloroquine resistant *P. falciparum* malaria and reports of parasites resistant to alternative drugs such as the 4-aminoquinolines (chloroquine), antifolates and even amino alcohols (quinine, mefloquine, halofantrin) there has been renewed interest in antimalarial activity of the acridine (Figgitt *et al.*, 1992; Basco *et al.*, 1995).

Some examples of drugs from plants that served as models for the next generation of drugs are exemplified as follows: Khellin (from *Ammi visnaga* (L.) Lamk.), and is used as a bronchodilator in the United States until it was shown to produce nausea and vomiting after prolonged use. In 1955, a group of chemists in England set about to synthesize khellin analogs as potential bronchodilators with fewer side effects. This eventually led to the discovery of chromolyn (used as sodium chromoglycate), which stabilized cell membranes in the lungs to prevent the allergen-induced release of the substance ultimately causing broncho-constriction in allergic asthma patients (Sneader, 1985). Further studies elsewhere led to the synthesis of amiodarone, a useful antiarrhythmia agent (Sneader, 1985). Papaverine, useful as a smooth muscle relaxant, provided the basic structure for verapamil, a drug used to treat hypertension (Sneader, 1985).

Galegine was isolated as an active antihyperglycemic agent from the plant *Galega officinalis* L. This plant was used ethnomedically for the treatment of diabetes. Galegine provided the template for the synthesis of metformin and opened up interest in the synthesis of other biguanidine-type antidiabetic drugs (Sneader, 1985). In both 2001 and

2002, approximately one quarter of the best selling drugs worldwide were natural products or derived from natural products (Butler, 2004).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman *et al.*, 2000; 2003; Butler, 2004). Of all available anticancer drugs between 1940 and 2002, 40% were natural products per se or natural product-derived with another 8% considered natural product mimics (Newman *et al.*, 2003).

Some secondary plant metabolites are used though in limited quantities, as pharmacological tools to study various biochemical processes (Baladrin *et al.*, 1985). Diterpene esters (among which are phorbol derivatives) from the latex of various *Euphorbia* species are examples of potent irritants and cocarcinogens, which are useful in studies of chemical carcinogenesis (Pryde *et al.*, 1981). Higher plants have produced active compounds such as digitoxin which is an antihypertensive (Baladrin *et al.*, 1985).

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics (Cohen *et al.*, 1994; Poole, 2001; WHO, 2001) has led to the search for new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages (Herrera *et al.*, 1996; De Smet, 1997; Sokmen *et al.*, 1999; Kelmanson *et al.*, 2000; Meng *et al.*, 2000), and inhibit bacteria through different mechanisms than conventional antibiotics, and could therefore be of clinical value in the treatment of resistant microbes.

Renewed interest in traditional pharmacopoeias has meant that researchers are concerned not only with determining the scientific rationale for the plant's usage, but also with the discovery of novel compounds of pharmaceutical value. Instead of relying on trial and error, as in random screening procedures, traditional knowledge helps scientists to target plants that may be medicinally useful (Cox and Balick, 1994). Already an estimated 122 drugs from 94 plant species have been discovered through ethnobotanical leads (Fabricant and Farnsworth, 2001).

Atropa belladonna and *Datura stramonium* provides Belladonna alkaloids from which atropine, hyoscyamine and scopolamine are synthesized (Farnsworth, 1966). These drugs are used in medicine as parasympatholytic agents, acting in alleviating disorders in central nervous systems (CNS). *Physostigma venenosum* (calabar bean) and *Pilocarpus* *ssp.* are the source of physostigmine and pilocarpine which are used as parasympathomimetic agents (Baladrin *et al.*, 1985).

When swallowed, Khat *Catha edulis* stimulates the central nervous system (CNS) and causes other autonomic and toxic responses (El-Shoura *et al.*, 1995). Although the use of Khat is widespread, it has, until recently, remained confined to the regions where the plant is grown since only fresh leaves are potent enough to produce the desired effects.

In both 2001 and 2002, approximately one quarter of the best-selling drugs worldwide were natural products or derived from natural products (Butler, 2004). Galantamine (also known as galanthamine, trade name Reminyl) is a natural product discovered through an ethnobotanical lead and first isolated from *Galanthus worono-wii* Losinsk (Amaryllidaceae) in Russia in the early 1950s (Heinrich and Teoh, 2004; Pirttila *et*

al.,2004). Galantamine is approved for the treatment of Alzheimer's disease, slowing the process of neurological degeneration by inhibiting acetylcholinesterase (AChE) as well as binding to and modulating the nicotinic acetylcholine receptor (nAChR) (Heinrich and Teoh, 2004; Pirttila *et al.*, 2004).

Plants also provide useful active compounds used for making insecticides, fungicides (pyrethrum plant) and industrial raw materials (WHO, 1987). Despite this, plants are grossly under-studied and under-used as a source of novel drugs, especially in the developed world (Norman *et al.*, 1985). The reason for this could be due to over-reliance on chemically synthesized drugs as well as lack of well-documented literature or catalogue of medicinal plants.

1.2 Statement of the Problem

Due to resistance of microorganisms to conventional antibiotics, high prices for synthetic medicines and the long period required completing prescription doses of conventional medicines, there is need to search for alternative medicines especially from medicinal plants. Many disease-causing organisms have become resistant to conventional antibiotics. The toxicity and side effects of the conventional drugs have also become increasingly unmanageable, while their costs are prohibitive. New diseases keep on emerging and causing unbearable human suffering and death.

1.3 Research Questions

The study sought to answer the following questions:

- i. Are there some medicinal plants in Kenya that can be used to manage some of the diseases caused by pathogenic bacteria?

- ii. What are the antibacterial compounds in medicinal plants that are responsible for their action?
- iii. Are the active medicinal plant extracts safe?

1.4 Research Hypotheses

To achieve the objectives of the study, the following hypotheses were postulated:-

- i. The nine selected medicinal plant extracts are inactive towards the selected bacteria.
- ii. The phytoconstituents present in the selected medicinal plant extracts are unknown
- iii. The safety of the extracts from the selected medicinal plants is unknown

1.5 Study Objectives

1.5.1 General Objective

The general objective of this research was to develop novel antibacterial compounds from Kenyan plants used in ethno medicine. To achieve this, the study sought to address the following specific objectives:

1.5.2 Specific Objectives

- i. To evaluate the *in vitro* antibacterial activities of selected plant extracts using standard bioassay methods
- ii. To determine the phytochemicals in the active medicinal plant extracts
- iii. To evaluate the *in vivo* toxicological parameters of medicinal plant extracts

1.6 Justification

Plant-derived products have revolutionized the medical profession, as they have commonly been used in alleviation, control, treatment and prevention of diseases in addition to improved health and life expectancy. Natural products discovered from medicinal plants (and derivatives thereof) have provided numerous clinically used medicines. Even with all the challenges facing drug discovery from medicinal plants, natural products isolated from medicinal plants can be predicted to remain an essential component in the search for new medicines.

Although herbal medicines have been used by traditional healers from time immemorial, chemical constituents of most of them are unknown (Chhabra *et al.*, 1982). In addition, not much information is available on the antibacterial screening bioassays in much of Kenyan medicinal plants. It is because of the numerous medicinal uses and the many chemotherapeutic compounds identified in plants that this project was conceptualized.

1.7 Significance of the Study and the Anticipated Outcome

Active extracts and pure compounds from medicinal plants screened will be proposed for capsulation or tableting to be exploited on commercial basis by a possible institute of alternative medicine. The knowledge on methodology and phytochemical properties of the medicinal plants studied is expected to stimulate research on their potential application in curative medicines. In addition, knowledge gained from this research will be used in promoting the propagation of studied medicinal plants in order to exploit them for their medicinal values. Herbal medicine practitioners will be able to utilize the methods used in this research to screen various medicinal plants they use to confirm their efficacy and safety. The Kenya Government development plan of 1989 strongly

recommended that medicinal plants should be urgently studied, preserved and developed as alternative medicines and possible elucidation of new therapeutic preparations (Kenya National Development Plan, 1989). This research study was aimed at fulfilling some of the recommendations of the WHO and the Kenyan National Policy on traditional drug research.

CHAPTER TWO

LITERATURE REVIEW

2.1 WHO Policy on Medicinal Plant Research

Right from the beginning of the WHO's program, the organization recognized the peculiar circumstances that obtain in less industrialized countries with respect to traditional medicine and health care delivery. Both WHO and UNICEF recognized that in view of the widespread use and acceptability of traditional medical practice, no impact will be made on Africa's overall health care status without due recognition, development and integration of traditional medicine into the primary health care delivery system of each country or region. This recognition led to the WHO/UNICEF 1978 conference in Alma Ata, USSR, at which the participants resolved and specifically urged member states to:

- (i) initiate comprehensive programs for the identification, evaluation, cultivation and conservation of medicinal plants used in traditional medicine;
- (ii) ensure quality control of drugs developed from traditional plant remedies by using modern techniques and applying suitable standards and good manufacturing practices.

This conference was followed in 1988 by another in Thailand, at which the Chiang Mai declaration to 'save plants that save lives' was made. This declaration formally brought the rational and sustainable use and conservation of medicinal plants into the arena of public health policy (SEA/RC55/18 Rev.1).

The Alma Ata declaration of 1978 was followed subsequently by various policy decisions of the Inter-African Committee on Medicinal Plants and African Traditional Medicines of the Organization of African Unity (OAU). These policy decisions were aimed at implementing WHO's recommendations. Several African countries including Ghana have

initiated programs aimed at maximizing the contribution of herbal medicine to their primary health care delivery nationwide. These programs have raised some very pertinent questions and problems of considerable importance for Africa particularly, and for the 'Third World' in general. These include questions that are socioeconomic, ethical, legal and environmental in nature (AF/RC50/R3,).

In order to meet the growing demand, WHO issued a strategic paper on traditional medicine in 2002 (Resolutions AF/RC50/R3, SEA/RC55/18 Rev.1, EM/RC49/R.9 (D)). This strategy describes the commonly used traditional therapies and therapeutic techniques, including Ayurveda, Chinese, Arabic, Unani and indigenous medicine. A number of WHO Member States and partners in traditional medicine (organizations of the United Nations system, international organizations, nongovernmental organizations, and global and national professional associations) contributed to preparation of the strategy and have expressed their willingness to participate in its implementation.

WHO's role is to broaden the recognition of traditional medicine; to support its integration into national health systems depending on the circumstances of its use in countries; to provide technical guidance and information for the safe and effective use of such medicine; and to preserve and protect medicinal plant resources and knowledge of traditional medicine with a view to its sustainable use (SEA/RC55/18 Rev.1.). In recent years regional committees for Africa, South-East Asia, the Eastern Mediterranean and the Western Pacific discussed traditional medicine and adopted resolutions on the use of traditional medicine. The strategy has four main objectives, in line with those of WHO's medicines strategy:

- (i) To integrate relevant aspects of traditional medicine within national health care systems by framing national traditional medicine policies and implementing programmes;
- (ii) To promote the safety, efficacy and quality of traditional medicine practices by providing guidance on regulatory and quality assurance standards;
- (iii) To increase access to, and affordability of, traditional medicine;
- (iv) To promote rational use of traditional medicine.

It is estimated that the numbers of medicinal plants in the world vary between 30,000 and 75,000 (Norman *et al.*, 1985). Following this realization, a world conference was held in Rio de Janeiro in South America to review the issue in 1992. This followed another conference in Chiang mai, Thailand (Olayiwola, 1988). World Health Organization (WHO) has recommended that medicinal plants be scientifically investigated with a view to validating their efficacy and formulating their active ingredients into capsules and tablets for treatment of various diseases (Olayiwola, 1988).

2.2 Kenya National Policy on Medicinal Plants

The Kenya Government Development Plan of (1989) strongly recommended that medicinal plants should be urgently studied, preserved and developed as alternative medicines and possible elucidation of new therapeutic preparations.

Kenya has developed a national strategy for both promoting and regulating the use of traditional medicine, and providing alternative forms of treatment to the country's poor (Kenya Government Policy on Herbal Medicine Plan 2003). The new arrangement will boost research into the use of both traditional knowledge and modern medicines to curb

major diseases such as HIV/AIDS and malaria. It will also encourage the conservation of biological resources from which traditional medicines are drawn. Representatives of the ministries of health, agriculture, environment and national planning met in February 1989 in Nairobi with their counterparts from other countries in the eastern Africa region to discuss ways of incorporating traditional medicine into national health programmes. The meeting was organised by the National Council of Population Development and the US National Institutes of Health, and included participants from Uganda, Tanzania, Ethiopia, and Zambia. Kenya's move to regulate traditional medicine coincides with a draft bill on regulating traditional knowledge currently awaiting debate in parliament (Kenya Government Policy on Herbal Medicine Plan, 2003).

2.3 Kenyan Medicinal Plant Research

Research work done in Kenya on *Albizia gummifera* (J.F.Gmel.) C.A.Sm.), stem - bark which is used in Kenyan traditional medicine as treatment for coughs, gonorrhoea, fever, skin diseases, malaria and stomach pains (Kokwaro, 1976), revealed constituents (alkaloids) with anti-bacterial activity (Rukunga *et al.*, 1996). Some medicinal plants rich in alkaloids like *Vallesia antillana* Woodson, *Catharanthus roseus* (L.) G.Don, *Tabernaemontana coronaria* (L.) Willd and others have been screened and found to have anti-microbial activity, especially against Gram positive and Gram negative bacteria (Hernandez *et al.*, 1977; Hernandez, 1979). The active compounds in a *Strychnos spp.*, used as a chewing stick, were found to be dimeric tertiary toxiferine type alkaloids: bisnordihydrotoxiferine and caracurine V (Verpoorte *et al.*, 1978), which exhibited activity against both Gram positive and Gram negative bacteria, against some *Streptococcus* species associated with the occurrence of caries. Canthinone, an alkaloid from *Cartha edulis* and some derivatives has strong anti-microbial activity (Mitscher *et*

al., 1972). It has also been isolated from *Zanthoxylum elephantiasis*, extract which exhibited strong anti-microbial activity (Mitscher *et al.*, 1972). Fifty-five organic and aqueous extracts of 11 plants used in malaria therapy in Kisii District, Kenya were tested *invitro* against chloroquine (CQ)-sensitive and resistant strains of *Plasmodium falciparum* (Chhabra *et al.*, 2003).

In Kenya, Khat (*Catha edulis* (Vahl) Forssk. ex Endl.) is grown, and when swallowed, stimulates the central nervous system (CNS) (Nencini and Ahmed, 1989) and causes other autonomic and toxic responses (Duke, 1985; Iwu, 1993; El-Shoura *et al.*, 1995; Yousef *et al.*, 1995). Although the use of Khat is widespread, it has, until recently, remained confined to the Northern Kenya regions and Somali land.

It has been estimated that 74% of the pharmacologically active, plant-derived components were discovered after the ethno medical uses of the plants started to be investigated (Farnsworth and Soejarto, 1991; Sheldon *et al.*, 1997).

2.4 The History of Global Uses of Medicinal Plant

Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Solecki, 1975). From that point, the development of traditional medical systems incorporating plants as a means of therapy can be traced back only as far as recorded documents of their likeness. However, the value of these systems is much more than a significant anthropologic or archeological fact. Their value is as a methodology of medicinal agents, which, according to the World Health Organization (WHO), almost 65% of the world's population has incorporated into their primary modality of health care (Farnsworth *et al.*, 1985). The goals of using plants as sources of therapeutic agents are *a*) to isolate bioactive compounds for direct use as drugs, e.g.

digoxin, digitoxin, morphine, reserpine, taxol, vinblastine and vincristine; *b*) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g. metformin, nabilone, oxycodon (and other narcotic analgesics), taxotere, teniposide, verapamil, and amiodarone, which are based, respectively, on galegine, others are 9-tetrahydrocannabinol, morphine, taxol, podophyllotoxin, and khellin; *c*) to use agents as pharmacologic tools, like lysergic acid diethylamide, mescaline, yohimbine; and *d*) to use the whole plant or part of it as a herbal remedy, like cranberry, echinacea, feverfew, garlic, ginkgo biloba, St. John's wort, and saw palmetto.

Plants have been utilized as medicines for thousands of years (Samuelsson, 2004). These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Samuelsson, 2004). The specific plants to be used and the methods of application for particular ailments were passed down through oral history. Eventually information regarding medicinal plants was recorded in books and journals. Recently the use of plants as medicines has involved the isolation of active compounds that are formed into effective drugs. Examples of drugs developed and used from plant sources are many. The isolation of morphine from opium poppy in the early 19th century (Kinghorn, 2001; Samuelsson, 2004), cocaine, codeine, digitoxine and quinine are excellent examples.

Out of a total of 422, 000 flowering plants reported worldwide (Govaerts, 2001), more than 50,000 are used for medicinal purposes (Schippmann *et al.*, 2002). In India, more than 43% of the total flowering plants are reported to be of medicinal importance (Pushpangadan, 1995). Utilization of plants for medicinal purposes in India has been

documented long ago in ancient literature (Charak and Drdhabala, 1996). However, organized studies in this direction were initiated in 1956 (Rao, 1996) and of late such studies are gaining recognition and popularity due to loss of traditional knowledge and declining plant population.

Traditional methods of drug discovery and development have been influenced by the need to prevail over illness and people's experience in witnessing and realizing the beneficial potentials of a plant to cure ailments, perhaps by trial and error (Sumner, 2000). The clinical utilization of quinine isolated from the *cinchona tree* bark and the Chinese discovery of artemisinin from the herb *Artemisia annua* have stimulated much interest in plants as potential sources of new antimalarial drugs (Basco *et al.*, 1994). Quinine and its derivatives is a notable example and are used in the management of malaria. These cinchona alkaloid based drugs have been most effective and dependable in the management of malaria and as antiarrhythmic agents. For example, of the 104 new drugs developed over 37 year period, 60 originated from plants used in traditional medicine of China (Gen, 1986).

In India, the rhizomes of *Stephania glabra*, which has been known among the natives to be antiasthatic, anti-idysentric and antipyretic, has been popularized (Bhakumi and Gupa, 1982). In China, traditional medicine accounts for around 40% of all health care delivered. In Chile 71% of the population, and in Colombia 40% of the population, have used such medicines. In India, 65% of the populations in rural areas use Ayurveda and medicinal plants to help meet their primary health care needs. In developed countries, traditional, complementary and alternative medicines are becoming more popular. For example, the percentage of the population that has used such medicines at least once is

48% in Australia, 31% in Belgium, 70% in Canada, 49% in France and 42% in the United States of America.

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebanjo *et al.*, 1983). Proven medicinal plants are used in treatment of diseases either alone or in combination with other plants. They are used as anti-infectious agents, anti-malarial, anti-tumoral agents, laxatives, cardiovascular and neural remedies (Owonubi, 1988). Several laboratories have reported the effectiveness of common indigenous herbs against Gram-positive and Gram-negative microorganisms. Recently, *Morinda citrifolia* and *Alstonia boonei* have been demonstrated to be active against *Salmonella typhi* the causative organism of typhoid fever (Irobi *et al.*, 1995). Ikenebameh and Metitiri (1988) have also reported the efficacy of *Cassia alata* against a large spectrum of fungi and bacteria that are the causative organisms of several common diseases. Indeed indigenous medicinal plants have been the bedrock for modern medicine.

Thai native herbs are becoming more widely used at a commercial scale in the food industry, mainly for their flavoring properties, even though certain plants have demonstrated their effectiveness against pathogenic bacteria (Voravuthikunchai *et al.*, 2004). A number of them have not yet been investigated for their antibacterial activities. Hence, it is essential to establish the scientific basis for their therapeutic actions as these may serve as the source for the development of effective drugs to be used in the treatment of diseases.

Recent work on tannins from the fruit of *Terminalia citrine* (Gaertn.) Roxb.ex Fleming, used as traditional medicine for diarrhoea and some skin infection (Burapadaja *et al.*, 1995), revealed the antibacterial activity of crude methanol extract.

Alzheimer's disease is characterized by a progressive impairment of cognitive function including the loss of memory and the inability to perform basic daily life activities (Francis *et al.*, 1999). Recently, the Amaryllidaceae alkaloid galanthamine has been approved in a number of European countries for the treatment of Alzheimer's disease (Wilkinson and Murray, 2001). Although galanthamine was originally isolated from European amaryllids (Glish *et al.*, 2003) it is also found in several traditionally used African Amaryllidaceae (Viladomat *et al.*, 1997). Galanthamine and other acetylcholinesterase enzyme (AChE) inhibitors alleviate the symptoms of Alzheimer's disease by inhibiting the activity of AChE and hence maintain or elevate the levels of acetylcholine in the brain (Sramek *et al.*, 2000).

There are also four new medicinal plant-derived drugs that have been recently introduced in the U.S. market, Arteether (trade name Artemotil). Arteether is a potent anti-malarial drug and is derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* L. (Asteraceae), a plant used in Traditional Chinese medicine (TCM) (van Agtmael *et al.*, 1999; Graul, 2001). Other derivatives of artemisinin are in various stages of use or clinical trials as anti-malarial drugs in Europe (van Agtmael *et al.*, 1999). Tiotropium (trade name Spiriva) has recently been released to the United States market for treatment of chronic obstructive pulmonary disease (COPD) (Mundy and Kirkpatrick, 2004; Frantz, 2005).

Tiotropium, is an inhaled anticholinergic bronchodilator, based on ipratropium, a derivative of atropine that has been isolated from *Atropa belladonna* L. (Solanaceae) and other members of the Solanaceae family (Barnes *et al.*, 1995; Dewick, 2002; Mundy and Kirkpatrick, 2004). Tiotropium has shown increased efficacy and longer lasting effects when compared with other available medications (Barnes, 2002; Mundy and Kirkpatrick, 2004).

In Mexico, some steroids, which have proved to be of immense value, come from *Dioscorea spp.* (Mexican yams) (Baladrin *et al.*, 1985). From these steroids oral contraceptives, anabolics, corticosteroids, and mineral corticoids, which have proved to be very valuable medically, have been derived (Baladrin *et al.*, 1985).

The Greeks used willow tree as a form of medicine. The philosopher, Hippocrates (460–370 BC) recommended chewing willow bark to patients suffering from high temperature and pain (Riddle, 1999). He also prescribed a brew of willow leaves to ease the excruciating pains of childbirth. Around 500 years later (100 AD), another Greek physician, Dioscorides, prescribed willow bark also to reduce the symptoms of inflammation, and the use of willow bark has continued because of its analgesic and anti-inflammatory properties (Riddle, 1999). Yet another Greek physician, Celsius who treated women with prolapsed uterine with boiled willow leaves and vinegar, is the accepted author of the famed symptoms of inflammation ‘redness and swelling with heat and pain’. The Roman Pliny speaks of corns treated with a burned willow bark paste, and Galen treated bloody wounds and ulcers with willow leaves (Wells, 2003).

The modern era of aspirin discovery began in the 18th century, when more advanced scientific information had accumulated (MacLagan, 1876; Sneader, 1997). In 1763, the Reverend Edward Stone described the beneficial effects of willow bark, in a letter addressed to the Royal Society (MacLagan, 1876; Sneader, 1997). Here, Reverend Stone described his results of a clinical study, treating patients suffering from ague (fever, usually taken to be malaria) with powdered willow bark in a drum of water. One hundred and thirteen years later, Thomas MacLagan, a Scottish physician, in 1876 conducted a clinical investigation to test the therapeutic efficiency of willow powder (MacLagan, 1876; Sneader, 1997). He successfully treated himself with willow powder extract (salicin) before he applied it to a patient with acute rheumatism. His treatments resulted in a complete reduction of fever and joint inflammation.

In Europe Research into the willow tree became more focused, especially when supplies of Peruvian bark stopped as a result of the continental blockade imposed by Napoleon at the beginning of the 19th century (Jack, 1997). As publicity increased, more clinical and pharmaceutical research was conducted on aspirin, and by the 1920s, therapeutic applications became available for treating symptoms of pain related to rheumatism, lumbago and neuralgia (Jack, 1997).

Natural products have played an important role as new chemical entities (NCEs)- approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived worldwide (Newman *et al.*, 2003). Another 20% of NCEs during this time period were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural products (Newman *et al.*, 2003). Combining these categories, research on natural products accounts for approximately

48% of the NCEs reported from 1981–2002. Natural products provide a starting point for new synthetic compounds, with diverse structures and often with multiple stereo centers that can be challenging synthetically (Clardy and Walsh, 2004; Nicolaou and Snyder, 2004; Peterson and Overman, 2004; Koehn and Carter, 2005).

2.5 Current Global Uses of Medicinal Plants

In many parts of the world there is a rich tradition in the use of herbal medicine for the treatment of many infectious diseases. These infections may be local within the dermis and some can subsequently become generalized as a blood infection (Brantner and Grein, 1994). Because of the side effect and the resistance that pathogenic micro-organisms build against the antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine.

Medicinal plants may offer a new source of antibacterial agents for use. In many parts of the world medicinal plants are used for antibacterial, antifungal, and antiviral activities. These plant extracts were used as a source of medicinal agents to cure urinary tract infections, cervicitis vaginitis, and gastrointestinal disorders (Caceres *et al.*, 1990) and skin infections such as herpes simplex virus type 1 (Meyer *et al.*, 1996).

India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria and a horde of other indications (Perumal and Ignacimuthu, 1998; Dahanukar *et al.*, 2000). In many parts

of Russia, particularly in Siberia, there is a rich tradition in the use of herbal medicine for the treatment of various infectious diseases, inflammations and injuries (Gammerman and Grom, 1976).

Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Essawi and Srour, 2000). Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to be carried out. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al.*, 1999).

In Malaysia Calanolide A which is a dipyrano-coumarin natural product was isolated from *Calophyllum lanigerum* var. *austrocoriaceum* (Whitmore) Stevens (Clusiaceae), a Malaysian rainforest tree, (Yang *et al.*, 2001; Yu *et al.*, 2003). Calanolide A is an anti-HIV drug with a unique and specific mechanism of action as a non-nucleoside reverse transcriptase inhibitor (NNRTI) of type-1 HIV and is effective against AZT-resistant strains of HIV (Currens *et al.*, 1996; Yu *et al.*, 2003). Calanolide A is currently undergoing Phase II clinical trials (Creagh *et al.*, 2001). In the West Bank and Gaza Strip (Palestine) herbal medicine is used to treat various diseases including gastrointestinal diseases, urinary tract infections, infertility, and cutaneous abscesses (Roweha, 1983).

2.6 The Use of Medicinal Plants in Africa

In African society, medicinal and poisonous plants have always played an important role (Farnsworth, 1988; Balick *et al.*, 1994). More than 80% of the populations in developing countries depend on plants for their medical needs (Farnsworth, 1988; Balick *et al.*, 1994). Traditions of collecting, processing and applying plants and plant-based medications have been handed down from generation to generation (Von Maydell, 1996). Important components are sold in marketplaces or prescribed by traditional healers in their homes. Because of this strong dependence on plants as medicines, it would be important to study their safety and efficiency (Farnsworth, 1994). The value of ethno-medicine and traditional pharmacology is nowadays gaining increasing recognition in modern medicine because the search for new, potential medicinal plants is more successful if the plants are chosen on an ethno-medical basis. It has been estimated that 74% of the pharmacologically active, plant-derived components were discovered after the ethno medical uses of the plants started to be investigated (Farnsworth and Soejarto, 1991; Sheldon *et al.*, 1997).

In South Africa, as in most countries, mental illness is a costly problem and a great strain on the community as a whole (Swift and Asuni, 1975). African traditional healers treat mental illnesses and disorders of the central nervous system, including anxiety, fits, convulsions, epilepsy, hysteria, nightmares and mental disturbances, using a variety of indigenous plants (Watt and Breyer-Brandwijk, 1962; Gelfand *et al.*, 1985; Hutchings, 1992; Mander *et al.*, 1995; Hutchings *et al.*, 1996; Van Wyk *et al.*, 1997; Van Wyk and Gericke, 2000). A preliminary inventory of plants used for psychoactive purposes in southern African traditional healing has been compiled (Sobiecki, 2002). Many authors have also documented the effectiveness of traditional medicines in treating such disorders

(Bergman, 1973; Swift and Asuni, 1975; Holdstock, 1979; Gelfand *et al.*, 1985; Gumede, 1990).

Herbal medicines are an important part of different cultures and traditions of the African people (Mander, 1998). Today, most of the people in urban South Africa, as well as smaller rural communities, are reliant on herbal medicines for health care needs. Apart from their cultural significance, herbal medicines are generally more accessible and affordable to the poor (Mander, 1998). In southern Africa, 102 species are used in traditional healing practices for the treatment of convulsive conditions (Sobiecki, 2002).

The natives of the coast of West Africa, particularly, in Nigeria and Ghana, have long known *Occimum bacillium* to be an effective medicinal plant in the treatment of dysentery and diarrhea which are mainly caused by microorganisms (Chiori *et al.*, 1977; Omoregbe *et al.*, 1995). Of the nearly 4000 ethno-medicinal plant taxa used in South African traditional healthcare (Arnold *et al.*, 2000), relatively few are considered likely to give rise to serious toxicity. In Nigeria, as in many other countries, it is common among the natives to combine several herbs which they claim to have particular curative properties (Chiori *et al.*, 1977; Omoregbe *et al.*, 1995).

In Egypt's Nile Delta, diarrhoeal diseases are a potential cause of morbidity and mortality especially in children and young animals as in other developing countries (Maikere-Faniyo *et al.*, 1989; Almeida *et al.*, 1995). Medicinal plants are promising source of antidiarrhoeal drugs. For this reason, International Organizations, such as WHO, have encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medicinal practices (Anonymous, 1979; Lutterodt, 1989). Among these plants,

Mentha microphylla K. Koch (MM), *Zygophyllum album* L. F. Beier & Thulin. (ZA), and *Convolvulus arvensis* L. (CA) are common perennial herbaceous plants grown in the Nile region (Delta, valley and Faiyum), in the Oases of the Libyan desert and in Sinai. They are used as carminative, antiseptic and stimulant (Meng *et al.*, 2002). Moreover, *Convolvulus ravens* L. has anticancer effect. *Alhagi maurorum* Medik (AM) is another plant used for various types of diseases, and is a richly branched hairy shrub grown commonly in the Nile region, Mediterranean region, the oases, tropical Africa, Saudi Arabia and Palestine. Ibn El-Bitar reported the use of *Conyza dioscoridis* (A. Rich) in the treatment of epilepsy, and as remedy for cold, diarrhea, colic, and rheumatic pains (Ibn El-Bitar, 1890; Boulos and El-Hadidi, 1984). People customarily use the extracts of plants known to have antidiarrhoeal effects without any scientific base.

In Africa HIV/AIDS has become a devastating epidemic (Fan-Harvard *et al.*, 1991). *Candida albicans* is one of the earliest opportunistic infections in HIV patients (Fan-Harvard *et al.*, 1991). Several plants are used by traditional healers in the treatment of oral candidiasis.

African healers use a wide variety of plants in the preparation of treatments to eradicate gastrointestinal parasites such as nematodes and amoebae (McGaw *et al.*, 2000). The search for biologically active extracts based on traditionally used plants is still relevant due to the appearance of microbial resistance of many antibiotics and the occurrence of fatal opportunistic infections.

Malaria remains one of the world's most deadly diseases. It is estimated that more than two million people die from the disease each year (Prozesky *et al.*, 2001). Although many

drugs are available, some have become ineffective as the *Plasmodium* parasites responsible for malaria have developed drug resistance (Peters, 1998). Traditional remedies are continually being investigated as plant derived antimalarial drugs become more sought after. Of 14 South African plant species investigated for antimalarial activity (Prozesky *et al.*, 2001), most showed potential antiplasmodial activity against a chloroquine-resistant strain of *P. falciparum* using the flow cytometric method of Schulze *et al.* (1997).

In Mauritius, medicinal plants, for centuries, have been used for the treatment of a wide range of ailments, many of which are still in use today and hold favored positions among local traditional-practitioners. Plants are complex chemical storehouses of undiscovered biodynamic compounds with unrealized potential for use in modern medicine (Plotkin, 1988). It has long been established that naturally occurring substances in plants have anti-bacterial and anti-fungal activities. The use of medicinal plants towards certain types of illnesses has roots in the Mauritian traditional pharmacopoeia (Plotkin, 1988).

In Senegal, leaves of *Combretum glutinosum* Perr. (*Combretaceae*) have also been used as diuretic and for the treatment of jaundice (Akino *et al.*, 1994). The Republic of Congo is a country entirely situated in the high risk zone of endemic malaria. Many plants in Congo are used traditionally to heal different diseases (Wome, 1982; 1985) including malaria; they play an important role in the medical system of the country.

In C^ote-d'Ivoire many people still use traditional herbs to treat a variety of diseases including bacterial infections. According to Pousset (1994), more than 70% of the people in West African countries still use traditional medicine. The use of herbal remedies is

likely to be particularly important in places where modern medicines are too expensive for the local population. Thus, a large variety of traditional medicines is often found in remote areas. One such area is the Ferkessedougou region, in the north of C[^]ote-d'Ivoire where people have low incomes, mostly for subsistence. Plants that are eaten as foods in southern Africa were shown to also provide important health benefits in the form of antioxidant activity (Lindsey *et al.*, 2002). In the indigenous health care delivery system of Ethiopia, numerous plant species are used to treat diseases of infectious origin (Geyid *et al.*, 2005). Regardless of the number of species, if any of such claims could be verified scientifically, the potential significance for the improvement of the health care services would be substantial.

2.7 Antimicrobial Activities of Medicinal Plant Extracts

Several researchers have reported the effectiveness of common indigenous herbs against Gram-positive and Gram-negative microorganisms (Irobi *et al.*, 1995). Recently, *Momordica charantia* Descourt and *Alstonia boonei* (Gilg) Chiov) have been demonstrated to be active against *Salmonella typhi*, the causative organism of typhoid fever. Ikenebameh and Metitiri (1988) have also reported the efficacy of *Cassia alata* L. Roxburg against a large spectrum of fungi and bacterial that is the causative organisms of several common diseases. Indeed indigenous medicinal plants have been the bedrock for modern medicine.

Most plants so far tested have been found to have good activity against Gram-positive strains of bacteria (Rabe and van Staden, 1997; Kelmanson *et al.*, 2000). The fact that extracts are more active against Gram-positive bacteria can be attributed to the fact that

the cell wall of the Gram-positive bacteria is easier to penetrate than that of Gram-negative bacteria (Rang and Dale, 1987).

Fifty-four Chinese herbs were screened for *anti-H. pylori* activity, *Rheum palmatum*, *Rhus javanica*, (Krauss) and *Eugenia caryophyllata* (Engl.) F. White) have proved to have strong *anti-H. pylori* activity (Bae, 1998). Cranberry juice possesses modest *anti-H. pylori* activity (Burger *et al.*, 2000). The *anti-H. pylori* activities of *Aristolochia paucinervis* Pomel, black myrobalan and cinnamon were also examined (Gadhi, *et al.*, 2001; Malekzadeh *et al.*, 2001; Tabak *et al.*, 1999). *Anti-H. pylori* compounds from the Brazilian medicinal plant *Myroxylon peruiferum* (L. f.) have been successfully isolated (Ohsaki *et al.*, 1999). Extracts and fractions from seven Turkish plants were also demonstrated to elicit *anti-H. pylori* activity (Yesilada *et al.*, 1999). The leaves, roots and stems of Korean and Japanese *wasabi* exhibited bactericidal activities against *H. pylori*, and showed the leaves as having the highest bactericidal activity (Shin *et al.*, 2004).

2.8 Active Compounds Identified in Medicinal Plants

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebanjo *et al.*, 1983). Proven medicinal plants are used in treatment of diseases either alone or in combination with other plants. They are used as anti-microbial agents, anti-tumoral agents, laxatives, cardiovascular and nerval remedies (Owonubi, 1988).

Some biologically active plant-derived secondary metabolites have found application as drugs or as model compounds for drug synthesis and semisynthesis (Pryde *et al.*, 1981). They have also become natural models for the design and total synthesis of new drugs

(Farnsworth, 1977). Meperidine (Demeral), Pentazocine (Talwin), and Propoxyphene (Darvon) are examples of synthetic analgesic drugs based on opiates such as morphine and codeine as the models.

Tannins have been reported to possess cytotoxic and antineoplastic activity (Farnsworth, 1966). Tannins extracted from this tree have been found to inhibit hepatitis “B” virus surface antigen. Tannins have also been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Fluck, 1973).

Flavonoids possess antiviral, anti-inflammatory and cytotoxic activities. Coumarins are known to have anticoagulation, estrogenic, vasodilation, antibacterial, antiviral and antihelminthic properties while anthraquinones (emodins) are mainly used as cathartics. In addition, some flavonoids and isoflavonoids isolated from licorice such as licochalcone A, licoisoflavone B, and gancaonols have been reported to exhibit inhibitory activities against *H. pylori* (Fukai *et al.*, 2002).

Plant steroids have provided valuable medicines for various ailments (Pryde *et al.*, 1981). These include digitalis glycosides (digitoxin or digoxin), which are derived from *Digitalis purpurea* Carl Linnaeus and *Digitalis lanata* Ehrh. Digitoxin or digoxin is cardiotonic glycosides, which are very effective drugs in hypertension or heart disease management (Pryde *et al.*, 1981). *Rauvolfia serpentina* ((L.) Benth. ex Kurz) plant has produced antihypertensive, and psychotropic agents Reserpine, and methoserpidine which are of chemical importance (Baladrin *et al.*, 1985).

The taxanes, including paclitaxel and derivatives, act by binding tubulin without allowing depoly-merization or interfering with tubulin assembly (Schiff *et al.*, 1979; Horwitz, 2004). Camptothecin was isolated from *Camptotheca acuminata* Decne. (Nyssaceae) but originally showed unacceptable myelosuppression (Wall and Wani, 1996; Cragg and Newman, 2004). Interest in camptothecin was revived when it was found to act by selective inhibition of topoisomerase I, involved in cleavage and reassembly of DNA (Cragg and Newman, 2004). Together, the taxanes and the camptothecins accounted for approximately one-third of the global anticancer market in 2002, which brought in over 2.75 billion dollars (Oberlies and Kroll, 2004). Numerous derivatives of all four compound classes have been synthesized, some of which are currently in clinical use. All of these natural products have led to significant biological discoveries related to their unique mechanisms of action.

Vinflunine is a modification of vinblastine from *Catharanthus roseus* (L.) G. Don (Apocynaceae) for use as an anticancer agent with improved efficacy (Bonfil *et al.*, 2002; Okouneva *et al.*, 2003). Exatecan is analog of camptothecin from *Camptotheca acuminata* Decne. (Nyssa-ceae) and is being developed as an anticancer agent (Butler, 2004; Cragg and Newman, 2004).

Catharanthus alkaloids, obtained from *Catharanthus roseus* (L.) G. Don (Apocynaceae) (Baladrin *et al.*, 1985) have contributed vincristine and vinblastine, which are used chemically as anticancer (Leukemia) agents. Taxol from *Taxus spp* (yew) has only been recently licensed as a drug against vaginal, ovarian and breast cancers.

Some secondary plant metabolites are used though in limited quantities, as pharmacological tools to study various biochemical processes (Baladrin *et al.*, 1985). Diterpene esters (among which are phorbol derivatives) from the latex of various *Euphorbia* species are examples of potent irritants and cocarcinogens, which are useful in studies of chemical carcinogenesis (Pryde *et al.*, 1981). Saponins are a special class of glycosides which have soapy characteristics and facilitate the resorption of foods and medicine (Fluck, 1973). It has also been shown that saponins are active antifungal agents (Sodipo *et al.*, 1991).

Most spices, condiments, teas and other beverages such as coffee and cocoa owe their individual properties (flavours and aromas) to the pharmacologically active secondary metabolites that they contain such as vanillin, ephedrine and caffeine (Baladrin *et al.*, 1985). Plant derived enzymes of economic importance have also been isolated and purified (Pryde *et al.*, 1981). Papain and Chymopapain, which are derived from *Carica papaya*, L. are the best examples, and are economically used as meat tenderizers (Pryde *et al.*, 1981).

2.9 Resistance of Bacteria against Conventional Antibiotics

Pasteur developed rabies vaccine, in 1885, while Fleming discovered *Penicillium notatum* from which penicillin was isolated (Franklin and Snow, 1975). Later many more antibiotics such as sulphonamides, chloramphenicol and tetracyclin were discovered, and have been used to treat many diseases. Recently macro cyclic lactones, peptides, polynes and polyether antibiotics have been discovered (Franklin and Snow, 1975). Glycosides and aminoglycosides and nucleosides and nucleotides have also been used as powerful and effective antibiotics (Franklin and Snow, 1975).

Currently, there are many synthetic drugs for the treatment of various diseases (Franklin and Snow, 1975). Some of the disease causing organisms have become resistant to these drugs, rendering them ineffective. Pathogenic microbial organisms present a formidable array of defenses against growth-inhibiting drugs. The mechanisms of resistance development against therapeutic agents include:

- (i) Conversion of active drugs to inactive derivatives by enzyme(s) produced by resistant organisms, (Benveniste and Davis, 1973).
- (ii) Modification of the drug-binding site in the resistant organism through structural modification incorporated in strains.
- (iii) Loss of cell permeability to a drug.
- (iv) Increased levels of the enzyme inhibited by the drug.
- (v) Increased concentration of metabolites that antagonize the inhibiting substances (drugs).

In addition, there could be the enhancement of alternative metabolic pathways bypassing the inhibited pathway or decreased requirement for a product of the inhibited metabolic system. Resistance has affected most antibiotics (Sirot *et al.*, 1988), and is found against aminoglycosides, such as amikacin, gentamicin, netimicin and tombromycin. Due to their widespread and long period of usage, most microorganisms have developed resistance to β lactam (Mayhall *et al.*, 1977). Although many other classes of antibiotics (macrolides, lactone, polytone, polypeptides), have been developed research in new effective, non-toxic antimicrobials should be continued in preparation for resistance development against them. Due to competition for food and survival several microbes produce secondary metabolites that are toxic to other organisms but harmless to themselves. Consequently, early research development of antibiotics targeted micro-organisms as

sources of antimicrobial drugs (Baladrin *et al.*, 1985). Lately, research has shifted to marine life but little has been found. Relatively, very little has been done on arthropods including insects.

2.10 Mode of Action of Medicinal Plant-Derived Medicines

Nitisinone (trade name Orfadin) is a newly released medicinal plant-derived drug that works on the rare inherited disease, tyrosinaemia, demonstrating the usefulness of natural products as lead structures (Frantz and Smith, 2003). Nitisinone is a modification of mesotrione, an herbicide based on the natural product leptospermone, a constituent of *Callistemon citrinus* Stapf. (Myrtaceae) (Mitchell *et al.*, 2001). All three of these triketones inhibit the same enzyme, 4-hydroxyphenylpyruvate dehydrogenase (HPPD), in both humans and maize (Mitchell *et al.*, 2001). Inhibition of the HPPD enzyme in maize acts as an herbicide and results in reduction of plastoquinone and tocopherol biosynthesis, while in humans the HPPD enzyme inhibition prevents tyrosine catabolism and the accumulation of toxic bioproducts in the liver and kidneys.

Several known compounds isolated from traditionally used medicinal plants have already been shown to act on newly validated molecular targets, as exemplified by indirubin, which selectively inhibits cyclin-dependent kinases (Hoessel *et al.*, 1999; Eisenbrand *et al.*, 2004) and kamebakaurin, which has been shown to inhibit NF- κ B (Hwang *et al.*, 2001; Lee *et al.*, 2002). Other known compounds have also been shown to act on novel molecular targets, thus reviving interest in members of these frequently isolated plant compound classes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling Sites

3.1.1 Mwingi District of Kitui County

Mwingi District (fig. 13) is one of the thirteen districts in Eastern Province. It borders Kitui District to the south, Machakos District to the west, Mbeere and Meru South District to the north and Tana River District to the east. The District lies between $0^{\circ} 03'$ and $1^{\circ} 12'$ south and longitudes $37^{\circ} 47'$ degree $38^{\circ} 57'$ east. The District covers an area of $10,030.30 \text{ km}^2$.

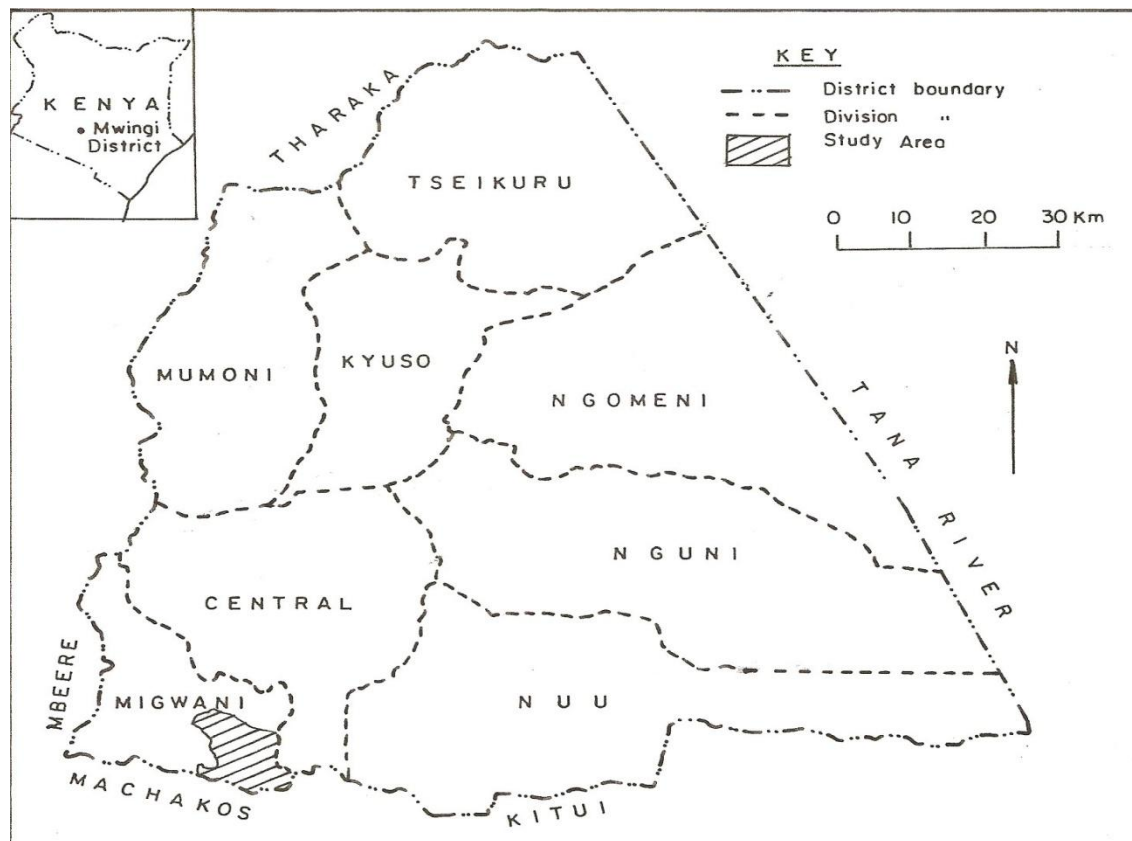


Figure 1: The Map of Mwingi District showing Migwani Division where some the medicinal plants were sampled

Administratively the District is divided into 9 divisions, namely Central, Migwani (where plants were sampled), Kyuso, Mumoni, Nguni, Ngomeni, Nuu, Mui and Tseikuru.

Mwingi District is generally plain with a few insurberga in Mumoni, Nuu and Mwingi Divisions. The highest point of the District is Mumoni Hill, with an altitude of 1,747m above sea level. The landscape is generally flat, with a plain that gently rolls down towards the east and northeast where altitudes are as low as 400m.

The highlands namely Migwani, Mumoni, Central and Mui Divisions receive more rainfall compared to the lowlands Nguni, Kyuso, and Tseikuru Divisions. The drier areas experience severe droughts, which have led to livestock deaths and food shortages. The District has red sandy soils, loamy sand soils and patches of black cotton soils. River valleys have saline alluvial soils of moderate to sometimes high fertility. Otherwise, soils are of low fertility and prone to erosion. Most hills are covered by shallow and stony soils unsuitable for crop farming.

Climate of the District is hot and dry for the greater part of the year. The maximum mean annual temperature ranges between 26⁰ C and 34⁰ C. The minimum mean annual temperatures in the district vary between 14⁰ C and 22⁰ C. Average annual temperature is 24⁰ C. The district has two rainy seasons, that is, March-May (long rains) and October-December (short rains). Rainfall ranges between 400mm and 800mm per year, but is erratic. The short rains are more reliable than the long rains.

3.1.2 Busia County

Busia District (fig. 14) is one of the districts that form Western Province. Butere/Mumias District borders it to the east, Bungoma District to the northeast, Teso District to the north, Siaya District to the southeast, Bondo District to the south and the Republic of Uganda to the west.

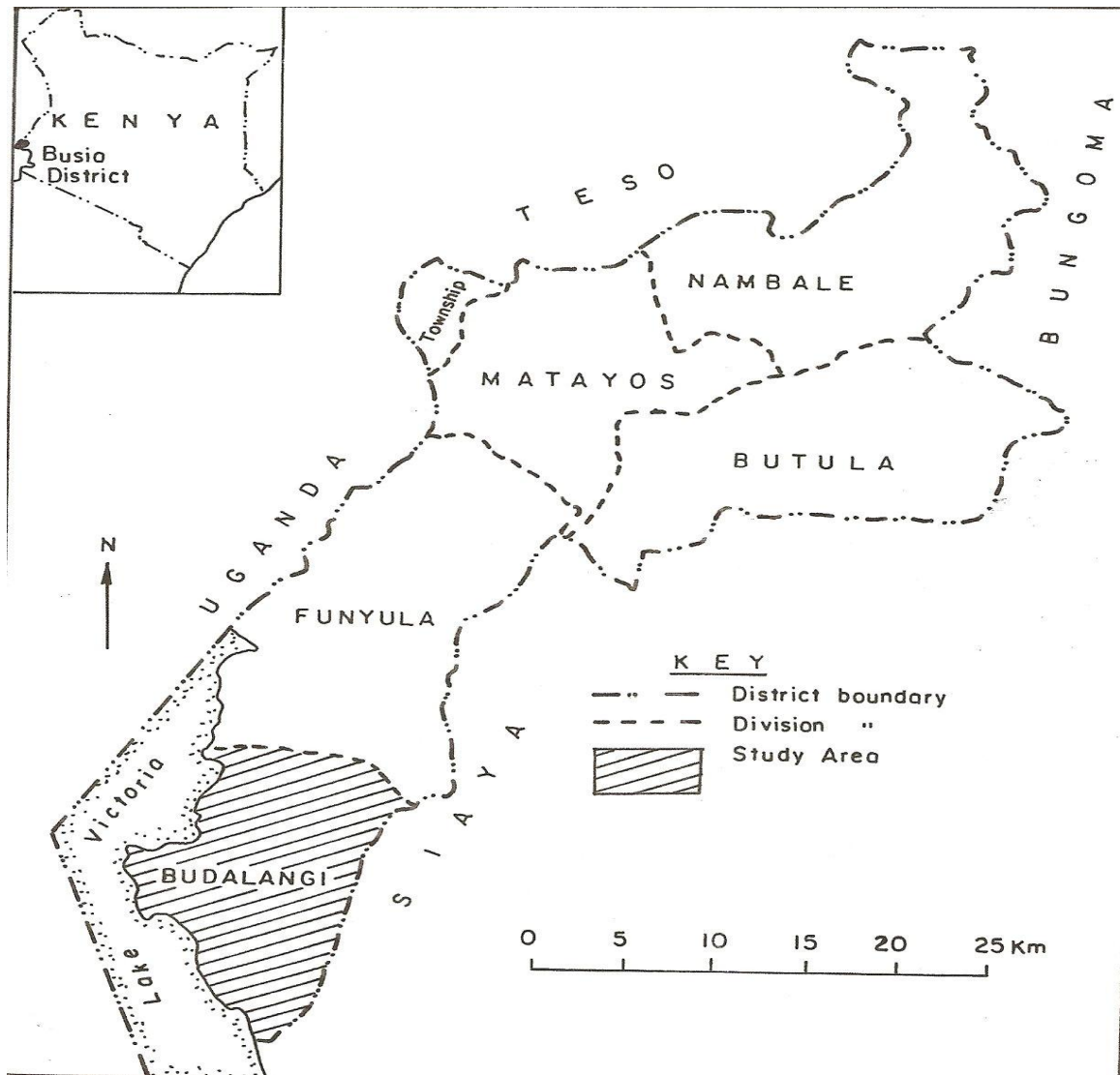


Figure 2: Map of Busia County showing Budalangi Division where some of the medicinal plants were actually sampled

It lies between latitudes $0^{\circ} 1' 36''$ South and $0^{\circ} 33'$ North and longitudes $33^{\circ} 54' 32''$ East and $34^{\circ} 25' 24''$ East. The District covers an area of $1,261.3 \text{ km}^2$. This includes an area of 137 km^2 , which is under permanent water surface. The permanent water surface is that of Lake Victoria. The District is divided into six administrative divisions namely Budalangi (where plant sampling was done), Butula, Funyula, Matayos, Nambale and Township.

Busia District falls within the Lake Victoria Basin. The altitude varies from 1,130m above sea level on the shores of Lake Victoria to 1,375 above sea level in the central part.

The southern part, which covers parts of Matayos Division, Funyula Division and the northern part of Budalangi Division is covered by a range of hills comprising the Samia Hills, which run from northeast to southwest culminating at Port Victoria. In the extreme south of the district is found the Yala Swamp. The area forms a colony of papyrus growth broken by irregular water channels and occasional small lakes with grassy islands.

There are two rain seasons in the district, the long rains and the short rains. The long rain season starts in March and continues into May, while the short rain season starts in late August and continues into October. The dry spells are from December through February and June/July. The mean annual rainfall for the district is 1,500mm with most parts of the district receiving between 1,270 and 1,290mm. The driest part of the district receives between 760 and 1,015mm of rainfall annually and is found along lakeshore. The mean annual rainfall in Budalangi and Funyula Divisions is between 1,020 and 1,270mm.

The annual mean maximum temperatures ranges from 26 and 30⁰C while the annual mean minimum temperatures vary between 14 and 18⁰C. Due to the proximity of district to Lake Victoria, the district records high rates of evaporation of between 1,800mm and 2,000mm per year. Thus, humidity is relatively high.

Most of the soils in the district are moderately deep, generally rocky and stony consisting of well-drained red clays which have a low natural fertility. In parts of Budalangi and Funyula divisions that adjoin Lake Victoria, soils are poorly drained and mainly of clay type due to frequent flooding. In the swamps, there are heavy clay types, which are very difficult to cultivate, both when it is dry and wet.

3.2 Plants of Study

3.2.1 Ethnobotanical Survey

An ethnobotanical investigation was carried out on the medicinal uses of the collected six (6) medicinal plants, namely *Dichrostachyus cinerea*, *Combretum molle*, *Ficus sur*, *Carissa edulis*, *Zanthoxylum usambarense* and *Securidaca Longipedunculata* collected from Migwani division of Mwingi District in Kitui County, in the Eastern province of the Republic of Kenya. The same ethnobotanical investigation was done on the other three (3) medicinal plants (*Euclea divinorum*, *Schkuhria pinnata* and *Crinum spp*) sampled from Bunyala (Budalang'i) District of Busia County in the Western Province of the Republic of Kenya. The divisions of Migwani and Budalang'i were visited. The interviews were mainly carried out among the Kamba (Mwingi District) and Luhyas of Busia County in Kikamba, Kiluhya the native language of the two areas respectively, and also in Kiswahili. Traditional healers from the divisions were chosen as sources of information. Six men and four women were interviewed. A questionnaire was used: one part consisting of more general questions, and one with more specific questions about the medicinal plants and their uses.

From the folklore in Mwingi District, Migwani Division, it was indicated that these plants were used medically to treat diarrhea, typhoid, cough, dysentery, and stomach ache by the local people. These plants are also shown in the literature to be used in the treatment of diarrhea, typhoid, cough, dysentery, and stomach ache and information by herbal practitioners (Kokwaro, 1976).

The folklore information obtained on the plants sampled from Busia District, Budalang'i Division, indicated that the three plants were medicinally used to treat constipation,

ulcers, malaria, and as tooth brush. Literature also indicates that these medicinal plants are used medically by the local people to treat coughs, liver and stomachache, chest pains and fever (Kokwaro, 1978).

In total nine medicinal plants were sampled and studied. There was need to scientifically authenticate those claims and to develop scientific and medical methods for using the medicinal plants safely.

3.2.1.1 *Dichrostachys cinerea*

Dichrostachys cinerea (L.) Wight & Arn. is in the family of Fabaceae (alt. Leguminosae), subfamily Mimosoideae, tribe Mimoseae. It is also placed in the Family of Mimosaceae, and its common names are “munoa mathoka” in Kamba, while it is known as Sickle Bush (English) (Beentje, 1994)). It is a shrub or small tree found in deciduous woodlands, ranging from southern and tropical Africa to India and Australia. With such a wide distribution, it is not surprising that it has a wide variety of forms. It forms thickets where overgrazing has prevented veldt fires. Resembling an acacia, *D. cinerea* can be distinguished by several characteristics. The thorns are modified branchlets (the thorns of Acacia species are modified stipules). When in flowers, the trees can be easily distinguished from acacias by their fluffy, bicolour inflorescences, which are composed of a basal, sterile, pinkish part and a fertile, yellow part. Flowering occurs from October-January. The pods are borne in clusters and are twisted in shape, another useful identifying characteristic (Beentje, 1994).

Ethno botanical information obtained from herbal practitioners in Mwingi district of Eastern province of Kenya at the time *D. cinerea* was chosen for this research was that it

is used to treat diarrhea and cough and women fertility. Various parts of the tree are used medicinally. The leaves, for instance, are used to treat snakebite, toothache and sore eyes, as they are believed to have anaesthetic properties (Kokwaro, 1976).

The bark of *D. cinerea* is also used to alleviate headache, toothache, dysentery, elephantiasis and root infusions are consumed to treat leprosy, syphilis, coughs, as an anthelmintic, purgative and strong diuretic (Turrill *et al.*, 1952). The leaves are particularly useful and can be mercerated and used to treat epilepsy and can also be taken as a diuretic and laxative, and its powder can be used in the massage of fractures

3.2.1.2 *Combretum molle*

Combretum molle R.Br. ex G.Don [family Combretaceae] is known as “Kiama or Muama” by Kamba community in Mwingi District of Eastern Province of Kenya, from where it was collected (Kokwaro, 1976). *C. molle* is a tree, less often shrub, 2-8 or (12) m and of the family Combretaceae. Its bark is grayish, rough fissured and often young leaves and flowers appearing at the same time.

C. molle is commonly found in wooded grassland, woodland, in the transition zone evergreen bushland/forest, and also in dry bushland but then mostly confined to shallow rocky soils, lava or granite. It is often also very common on rocky hillsides (Beentje, 1994). The Kamba community use *C. molle* medicinally as Tooth brush, stomach ache, and dysentery (Kokwaro, 1976). It is recorded in literature as also being used to treat hookworm, stomach pains, snake bite, leprosy, fever, dysentery and general body swellings, chest complaints and coughing by different communities in Kenya and Eastern Africa (Beentje, 1994). Research shows that the stem bark acetone extract of *C. molle* has

high antibacterial action against Gram negative organisms such as *E. coli* and *S. dysenteriae* with an MIC value of 50 mg/ml (Asres *et al.*, 2006).

3.2.1.3 *Euclea divinorum*

Euclea divinorum Hiern, Ebenaceae family is known as “Musinda bakhene”, in Luhya (Budalangi, Busia district) while in Dholuo it is called “Ochol” (Kokwaro, 1976). *E. divinorum* is a shrub or tree 1-9 (-15) m, evergreen with a dark grey bark and fissured. It is found in dry forests (margins), riverine in bushland or forest, in bushed grassland or pasture, evergreen bushland and in wooded grassland on anthills. It is often a weed of pastures, due to its phenomenal power of copping and root suckering.

The Luhya community of Budalangi, Busia County uses the roots of *E. divinorum* medically as tooth brush (tooth paste), for constipation and ulcers (Kokwaro, 1976). The Kamba community in Eastern Province of Kenya uses the roots of *E. divinorum* which they call Mukinyei medically to treat chest pains, pneumonia, or internal body swellings, as purgative, and as worm medicine.

3.2.1.4 *Ficus sur*

Ficus sur Forssk, Moraceae family, is known in Kiswahili as “Mukuyu”, while the Kamba community also calls it “Mukuyu”, as is the same in most Abantu tribes of East Africa, while in English it is called fig tree (Kokwaro, 1976).

F. sur is a tree 4-5-25m in height, sometimes epiphytic; buttresses may be present and its bark grey or whitish. *F. sur* is mostly found in riverine forest and bush, groundwater forest, but less often in forest away from water. Figs are produced from September to

March (South Africa) when they are borne in large clusters mostly low down on the trunk and can even appear at ground level arising from the roots (Arnold and De Wet, 1993). The Kamba community, where it was collected uses its stem bark medically in treatment of typhoid and diarrhea.

3.2.1.5 *Schkuhria pinata*

Schkuhria Pinata (Lam.) Kuntze ex Thell Fabris family is known as “Akech” in Tholuo and Luhya communities, and is used by the same communities to treat gastrointestinal problems, malaria, and stomachaches cough, chest, and liver (Kokwaro, 1976).

S. Pinata is a weedy annual herb that grows to about 70 cm high. It produces numerous small yellow flowers on numerous airy erect stems which grow more woody as it matures (Kokwaro, 1976). *S. Pinata* is traditionally used as a blood cleanser; used internally and externally for pimples, blackheads, and acne; as a diuretic and antimicrobial for urinary tract problems; for skin problems (eczema, dermatitis for malaria (Tanaguchi, 1978; Weimann *et al.*, 2002)

3.2.1.6 *Carisa edulis*

Carisa edulis (Forssk.) Vahl, Apocynaceae family is a spiny, much branched, small tree, shrub or scrambler, up to 5 m in height, with a milky sap. Bark grey, smooth, young branchlets with or without hairs; spines simple, straight, 2-5 cm long. Flowers white inside, pink red outside, in dense cymes. The plant is found in forest edges, bushland, thicket or bush grassland, especially in rocky places (Kokwaro, 1976).

The plant is known as “mukawa” in Kikamba, and is used by the community to treat malaria and fever (Kokwaro, 1976). In some other communities the plant is also used for indigestion, lower abdominal pains, and chest pains. The fruits are edible and very tasty, a bit astringent, and are sometimes taken against dysentery (Beentje, 1994). The decoction of its roots is used as a pain killer.

3.2.1.7 *Zanthoxylum usambarense*

Zanthoxylum usambarense (Engl.), Rutaceae family is a tree 2.5-15m height with barks furrowed, brown, and rocky bosses. It has branches with straight or slightly upcurved spines 6-12mm long. It has white yellow flowers in terminal panicles 10-15cm long with petals 2-4.5mm long. Its fruits are paired and reddish rounded follicles, 8-10mm (Beentje, 1994). *Z. usambarense* is found in dry forest or its remnants, such as clump thickets or secondary bushland. *Z. usambarense* is known as “Mvuu” by Kamba community, and used by the same community to treat malaria and fever, and its bark decoction also used as an emetic (Kokwaro, 1976). Its wood is used in housebuilding, for furniture and to make bows.

3.2.1.8 *Securidaca longipedunculata*

Securidaca longipedunculata (Fres) of Polygalaceae family is a shrub or small tree 2.8m height. It has a grey bark and smooth or flaking in rectangular patches. It has beautiful pink or violet flowers, with yellow leaves narrowly ovate or elliptic. *S. longipedunculata* is found in secondary bush and wooded grassland, and forest edges (Beentje, 1994).

S. longipedunculata is known as “Muguuka” by the Kamba community, and is a highly regarded medicinal and magical tree, by the community (Beentje, 1994). *S. longipe-*

dunculata is a threatened and protected species. The violet tree is used for medicinal purposes and other uses in rural areas (Wyk, 1998). What makes it so threatened is the fact that roots are the target for people using this plant, which makes it difficult for the plant to survive constant harvesting.

S. Longipedunculata is used by the Kamba community for treatment of rheumatic fever and as pain reliever and stiffness in muscular sprains, strains, stiffness and skin diseases (Kokwaro, 1976). The violet tree is the most popular of all the traditional medicinal plants in South Africa and is used for almost every conceivable ailment (Wyk and Gericke, 2000; Wyk *et al.*, 1997). The roots are extremely poisonous, smell like wintergreen oil and contain methyl salicylate which may partly indicate why they have a wide diversity of uses, such as arrow poison in some parts of Africa including West Africa. The roots and bark are taken orally either powdered or as infusions for treating chest complaints, headache, inflammation, abortion, ritual suicide, tuberculosis, infertility problems, venereal diseases and for constipation (Kokwaro, 1976). Toothache can also be relieved by chewing the roots. Mixed roots of the violet tree and dwarf custard apple are used to treat gonorrhoea. Powdered roots or wood scrapings are used to treat headache by rubbing them on the forehead, while infusions from the roots are used to wash tropical ulcers (Kokwaro, 1976).

In Limpopo, the vhaVenda people use roots for mental disorders and as protection against children's illness during breastfeeding (Wyk and Gericke, 2000;). It is believed that many African people use the powdered violet tree roots as a sexual boost for men. The vhaVenda people mix the powdered root with '*mageu*' (maize or sorghum beverage) and it is given to a man to drink if he is sexually weak (Wyk *et al.*, 1997).

3.2.1.9 *Crinum linn*

Crinum linn (Linn) is of the Amaryllidaceae family is a bulbous plant with leaves reaching 75 cm long by 6 cm wide, and scape 60–100 cm high bearing 4–6 white flowers with a broad purple band along the centre in a large umbel to 15 cm across (Beentje, 1994). The Luhya community in Bunyala district calls *Crinum* Linn. “amanyasi”, and it is used for treatment of fever, skin diseases and stomach ache (Kokwaro, 1976).

3.3.1 Plant Sample Collection

The plant samples were collected either as whole plants, stems, root barks, stem barks or even leaves depending on which part of the plant had been reported by herbal medical practitioners to be of medicinal value. The plant samples were identified by a taxonomist (Simon Mathenge from University of Nairobi, Botany Department), and Voucher specimen deposited at Kenyatta University herbarium, in the Department of Plant and Microbial Sciences and the National Museums of Kenya for verification.

3.3.2 Processing and Extraction of Plant Extracts

The plant parts (roots, leaves or barks) of plants were collected and dried under shade for 14 days to ensure that all moisture was completely removed from them, and also to avoid decomposition of active compounds. They were then ground into a powder using laboratory Warring Blender (for leaves) and electrical Laboratory mill number 8 (Christy and Norris, Christy Turner Ltd., Knightsdale Road, Ipswich, Suffolk, United Kingdom, IP1 4LE) (for roots and barks) and then sealed in polythene bags until the time of extraction. Percolation method of extraction of active compounds from medicinal plants was employed, with solvents of increasing polarity (from the least polar to the most polar), hexane, dichloromethane, acetone, and methanol.

3.3.2.1 Percolation Extraction

The nine plants collected for the study were processed and extracted using organic solvents of increasing polarity using percolation cold methods and their yields recorded.

The powdered plant part (1kg) was weighed and then transferred into 1 litre conical flask. Cold sequential extraction was done with distilled organic solvents of increasing polarity, which included n-hexane [$\text{CH}_3(\text{CH}_2)_4\text{CH}_3$], bp 69°C), Dichloromethane (CH_2Cl_2), Acetone ($\text{CH}_3)_2\text{CO}$, bp 56.5°C and Methanol (CH_3OH), bp 65.5°C .

The plant powders were extracted in hexane by soaking them in it for three days then filtered. The residues from step one were dried then soaked in dichloromethane and extracted for three days again. This was repeated for acetone and methanol. The filtrates of extracts obtained in each step were filtered then concentrated under vacuum using a rotary evaporator (Bibby RE 100, Bibby Sterlin Ltd. Staffordshire, UK) at the boiling points indicated for each organic solvent. They were dried using calcium chloride in a desiccator to remove water, and then weighed and kept ready for bioassay.

3.4 Bioassays

3.4.1 Bacterial Culture Used

The bacteria used for bioassay were collected from Kenyatta National Hospital (KNH), National Public Health Laboratories, Ministry of Health and Kenya Medical Research Institute (KEMRI) at the Centre for Microbiology Research (CMR) where they were maintained in a lyophilized state (Table 1).

Table 1: Bacteria Cultures for Bioassay

<i>Microorganisms</i>	Number or Type
<i>Escherichia coli</i>	ATCC 25922
<i>Staphylococcus aureus</i>	ATCC 85923
<i>Pseudomonas aeruginosa</i>	NCTC 10662
<i>Escherichia coli</i>	Isolate
<i>Shigella flexineri</i>	Isolate
<i>Pseudomonas aeruginosa</i>	Isolate
<i>Staphylococcus aureus</i>	Isolate
<i>Shigella dysenteriae</i>	Isolate
<i>Klebsiella pneumonie</i>	Isolate

3.4.1.1 *Escherichia coli*

Escherichia coli is a Gram negative bacterium, usually motile rod that is commonly found in the lower intestine of warm-blooded animals. *E. coli* organisms form part of the normal microbial flora on the intestinal tract of humans and animals. They can also be found in water, soil, and vegetation. *E. coli* causes diarrheal disease especially in infants but also in adults.

3.4.1.2 *Staphylococcus aureus*

Staphylococcus aureus literally the "golden cluster seed" and also known as *golden staphylococcus*) is the most common cause of staph infections. *S. aureus* are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. Bacteriological culture of the nose and skin of normal humans invariably yields staphylococci. It is frequently found in the nose of a person. About 20% of the populations are long-term carriers of *S. aureus* (Kluytmans *et al.*, 1997).

Skin infections are the most common type of disease produced by *Staphylococcus SPP* (Liu *et al.*, 2008). *Staphylococcus* infections of the skin can progress to impetigo (a

crusting of the skin) or cellulitis (inflammation of the connective tissue under the skin, leading to swelling and redness of the area). In rare cases, a serious complication known as scalded skin syndrome can develop (Liu *et al.*, 2008). In breastfeeding women, *Staphylococcus* can result in mastitis (inflammation of the breast) or in abscess of the breast. Staphylococcal breast abscesses can release bacteria into the mother's milk (Liu *et al.*, 2008).

3.4.1.3 *Pseudomonas aeruginosa*

Pseudomonas species can be found in water, soil, sewage and vegetation. They can also be found in the intestinal tract (Balcht *et al.*, 1994). It is a Gram negative rod. *P. aeruginosa* is frequently present in hospital environment, especially in moist places such as sinks, bowls, drains, cleaning buckets and humidifiers. It can also be found in rice paddy fields, the mud of river banks and surface stagnant water. The organism can infect cattle, pigs and other animals (Balcht *et al.*, 1994).

P. aeruginosa causes skin infections especially at burn sites, wounds, pressure sores and ulcers, often as a secondary invader; urinary infections, respiratory infections especially in patients with cystic fibrosis or conditions that cause immunosuppression; external ear infections (otitis externa), eye infections and septicaemia especially in persons already in poor health. *Pseudomonas* species are resistant to most of the commonly used antibiotics (Todar, 2007).

3.4.1.4 *Salmonella typhi*

Salmonella typhi is a Gram negative rod, responsible for causing enteric fever (typhoid) and occasionally meningitis, osteomyelitis and arthritis (Kim *et al.*, 2004). *S. typhi* is

mostly found in the intestines of humans, excreted in the faeces and urine of infected patients and is present in the gall bladders of long-term carrier. Infection is by ingesting the organism in contaminated food or water, or from contaminated hands. This organism is spread by water.

3.4.1.5 *Shigella dysenteriae*

Shigella dysenteriae is a facultative anaerobic, Gram-negative rod from the family Enterobacteriaceae (Ryan and Ray, 2004). *S. dysenteriae* is primarily a human pathogen, as are all Shigellae. *Shigella* species are found only in the human intestinal tract. Carriers of pathogenic strains can excrete the organisms up to two weeks after infection and occasionally for longer periods. *Shigellae* are transmitted by the faecal-oral (faeces to mouth) route (Hale *et al.*, 1996). *Shigella* species cause bacillary dysentery (shigellosis). Shigellosis has a high death rate especially among young children, with most deaths being caused by *S. dysenteriae* (Hale *et al.*, 1996).

3.4.1.6 *Klebsiella pneumoniae*

Klebsiella strains can be found in the intestinal tract of humans and animals, and also in plants, soils and water (Ryan and Ray, 2004). *K. pneumoniae* can be found as a commensal in the mouth and upper respiratory tract, and also in moist environments in hospitals and elsewhere. *Klebsiellae* are Gram negative, non-motile, capsulated rods. *K. pneumoniae* causes chest infections, and occasionally severe pneumonia resulting in coughing (Ryan and Ray, 2004).

3.5 Agar Disc Diffusion Screening of Medicinal Plant Extracts

Various bioassays like Agar disc diffusion, MIC and MBC can be used to test for biological activity, firstly *in vitro* and later, for promising natural products, *in vivo*. In this study screening of antimicrobial activity of ten (10) medicinal plant species against nine (9) Gram-positive and Gram-negative human pathogenic bacteria was carried out.

The preliminary screening of plant extracts was done using agar paper disc diffusion method (Bauer *et al.*, 1966) was used to evaluate the antimicrobial activity. The procedure commonly used is the Kirby-Bauer antimicrobial susceptibility test (Bauer *et al.*, 1966). The basic concept of Kirby-Bauer procedure is that the size of the zone of inhibition can be correlated with clinical susceptibility of an organism to an antimicrobial. The standard procedure provided for the use of Muller-Hinton agar, a standard inoculum applied in a standard manner, and a single disc of a pre-described potency for each antimicrobial tested. Each Plate was streaked with 0.1 ml of each bacterial culture (1.5×10^8 cells/ml) by McFarland nephelometer barium sulphate standard).

3.5.1 Preparation of Media

The media used was Muller Hinton Agar (MHA), (Oxoid Ltd, Solaar House, 19 Mercers Row, Cambridge, CB5 8BZ, UK) also known as Diagnostic Sensitivity Test (DST) Agar. The media was prepared according to the manufacturer's instructions. Fourty three grams of DST Agar was dispensed in 1L ml of distilled water, mixed thoroughly and boiled to dissolve. This was then sterilized by autoclaving at 15lPsi pressure (121°C) for 15 min. The media was cooled to about 55°C and then ascetically poured onto sterile Petri dishes and left to solidify at room temperature for a few minutes.

3.5.4 Preparation of the Inoculum

The bacteria cultures used were 24 h cultures, inoculated the previous day and incubated at 37⁰C.

3.5.3 Preparation of McFarland Standard

A 0.5 McFarland standard was prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), with 9.95 mL of 1% sulfuric acid (H_2SO_4) with constant stirring. This gave an approximate cell density (1.5×10^8 CFU/ml), giving optical density (absorbance) of 0.132 at 600 nm wavelength. The standard was distributed into screw cap tubes which were tightly sealed to prevent loss by evaporation. They were stored protected from light at room temperature. Before use the standards were vigorously shaken.

3.5.4 Inoculation of Bacteria

Using a sterile wire loop (sterilized on the flame), a small part of the 24 h bacteria colony of each test bacteria was taken and mixed well in sterile 0.89% saline. The turbidity of the bacterial was compared to match the McFarland standard. The McFarland bacteria standard prepared this way contained 1.5×10^8 CFU/ml. Fifty microlitres McFarland bacteria standard was introduced on each Plate, containing culture of 1.5×10^8 CFU cells/ml by McFarland nephelometer barium sulphate standard. Sterile L-shaped glass rods were then used to spread the prepared bacterial culture on to the dry Muller Hinton Agar media and left to dry completely.

3.5.5 Preparation of Paper Discs

The paper discs used for bioassay were prepared from Whatman number one filter paper. Using a paper punch whose diameter was ascertained to be 5 mm, the filter papers were punched to produce 5 mm paper discs. These discs were then sterilized by autoclaving at 15 Psi pressures (121⁰C) for 15 minutes in a well sealed universal bottle.

3.5.6 Impregnation of Extracts onto Paper Discs

A 100 mg of each extract was weighed and dissolved in 1ml of methanol. Five microlitres of each dissolved fraction was applied on to a sterile paper disc. The paper discs were left to dry completely at room temperature for a few minutes

3.5.7 Concentration of Extract on the Paper Disc

Each disc contained a concentration of 500 µg or 0.5mg of the crude extract as per the following calculation.

$$100 \text{ mg} / \text{milliliters} = 100,000\mu\text{g/ml}$$

$$5\mu\text{l} = 1,000,000 \mu\text{g}$$

$$\begin{aligned} \text{Therefore, } 100,000 \times 5 / 1000 &= \text{weight of extract in } 5\mu\text{l} = 500\mu\text{g} \\ &= 100,000 \times 5 / 1000 = 500\mu\text{g which is } = 0.5\text{mg} \end{aligned}$$

3.5.8 Placement of Extract Impregnated on Paper Discs on Petri Dish

Extract impregnated paper discs were picked by forceps and carefully placed on to the inoculated DST Agar plates in compartments already drawn on the plates. A 90 mm plate accommodated six discs in order to prevent unacceptable overlapping of zones. After all the discs were placed on the inoculated plates, the later were then incubated at 37⁰C for 24 h.

3.5.9 Measuring of Zones of Inhibitions

The diameters of zones (mm) of inhibitions (edge was taken as the point of inhibition as judged by the naked eye) of the test strain were determined with a ruler. Since the diameter of the paper disc was 5mm, it therefore, follows that 5mm represents no inhibition. Zone size of 9 mm and less was taken as resistant while sizes greater than 9 mm were considered sensitive (Bauer *et al.*, 1966). Each experiment was repeated five (5) times to ensure that results were reproducible.



Figure 3: Zones of inhibition around filter paper disks saturated with anti-microbial compounds. The diameter of the zone of inhibition is a measure of the effectiveness of an anti-microbial compound.

3.6.0 Determination of Minimum Inhibitory Concentrations (MICs)

A modified agar micro dilution method (Lorian, 1996) was used to determine the Minimum inhibitory Concentration (MICs) of extracts of the medicinal plants that produced significant inhibition zones with agar paper disc diffusion bioassay method. McFarland standard of each bacteria strain (1.5×10^8 Colony forming units (CFU) Cells/ml) were made as previously discussed. One micro litre of an overnight culture of

each bacterial strain was applied onto MHA supplemented with the medicinal plant extracts. Inoculation of bacteria on MHA plates was done using an automatic inoculator machine.

A 1000 mg of the extracts were weighed and dissolved completely in 1ml of methanol to give a concentration of 1000 mg/ml. One millilitre of sterile 0.89% saline was added to end up with 500 mg/ml. Serial double dilutions in sterile 0.89% saline of 500 mg/ml were made to obtain the following concentrations in milligrams per millilitre: 500, 250, 125, 62.5, 31.25 and 15.6. Then 2 mls of each of these concentrations were added to 18mls of sterilized MHA and cooled to 50⁰C to make the following final extract concentrations in milligrams per ml: 50, 25, 12.5, 6.25, 3.125, and 1.5 mg. The MHA mixed with extracts on Petri dish plates were left to solidify at room temperature.

McFarland standard of an overnight bacterial culture of each strain of 1.5×10^8 CFU cells/ml were made. One micro litre of the culture was inoculated onto MHA supplemented with the medicinal plant extracts. Inoculation of bacteria on MHA plates was done using automatic inoculator machine. This was done by taking 1ml of McFarland standard of each bacteria culture containing 1.5×10^8 CFU cells/ml and placing it in its relevant well on the automatic inoculator machine. The automatic inoculator was then adjusted and set to pick a volume from each well and inoculate on to the MHA plates.

On control plate labeled 'before' (without extract but before inoculation) to show that bacteria colonies were present and grew accordingly was inoculated and incubated. The second control plate labeled 'after' (without extracts but after inoculation) to confirm that bacteria colonies were present after inoculation was also inoculated and incubated.

The plates were incubated at 35⁰ C for 18 h. and observations recorded accordingly. The plates showing no bacterial growth were streaked and inoculated on fresh MHA plates and incubated for 18 h to confirm the bacteria were truly inhibited. This procedure was performed and repeated three times and results expressed as the mean values of the lowest concentration of plant extracts that produced complete suppression of colony growth, that is the MIC. Antimicrobial agents with low activity against an organism had a high MIC while a highly active antimicrobial agent gave a low MIC.

3.7. Determination of Minimum Bactericidal Concentrations (MBCs)

The broth dilution MBC method was used to quantitatively measure the *in vitro* activity of an antimicrobial agent against a bacterial isolate. The minimum bactericidal concentrations (MBC) of plant extracts bioassays were done according to the methods by Boaky-Yiadon (1979).

A 1000mgs of extracts were weighed and completely dissolved in 1ml methanol to give a concentration of 1000mg/ml. Doubling serial dilution of the extract was made in 0.89% saline to give the following concentrations in mg/ml:500, 250, 125, 62.5, 31.25, and 15.6. A ten times dilution was made of the extracts by adding 0.2 ml of the extract into 1.8ml of Muller Hinton Broth (MHB) to make the following final concentrations in milligrams per millilitre: 50, 25, 12.5, 6.25, 3.125, and 1.5mg/ml. Nine tubes for each test organism were set at every concentration.

A McFarland standard of each bacterial strain of 1.5×10^8 CFU was made. Fifty microlitres McFarland standard of each test organism was added to different extract concentrations. The tubes were then incubated at 37⁰ C for 18 h. At the end of incubation period, the

tubes were examined for turbidity to determine whether there was bacterial growth. To confirm growth, 50 µl from each tube was taken and plated onto nutrient agar and incubated at 37⁰ C for 18 h. Nutrient agar with no bacterial growth were indicated as negative, confirming that the extracts actually killed the bacteria at that concentration, while plates with bacterial growth were taken as positive, confirming that the extracts had merely inhibited bacteria at that concentration.

The lowest concentration of the plant extracts that did not yield any colony growth in MHB after the incubation period was taken as the MBC. The minimum bactericidal concentration was read as the lowest extract concentration which yielded no bacterial growth upon subculture. This procedure was performed and repeated three times and results expressed as the mean values of the lowest concentration of plant extracts that were completely showed bactericidal effect to the bacteria colony growth. Antimicrobial agents with low activity against an organism had a high MBC while a highly active antimicrobial agent gave a low MBC.

3.8 The Time-kill Kinetics

The objective of this study was to screen for antimicrobial activity in selected medicinal plants. The effects of some of the plants' extracts on test pathogenic bacteria have been determined, but just as important are the dosage and killing rates, which have to be determined. Kill kinetics is the system that indicates the rate and extent of bacterial killing, and provides a better and accurate description of antimicrobial activity than does MICs (Zhanel *et al.*, 1991)

In this section, acetone and methanol extracts of *D. cinerea* and *C. molle* were tested for their kill kinetics against *S. aureus* (Gram positive cocci), *E. coli* (a Gram negative rod) and *P. aeruginosa* (a potent β -lactamase producing Gram negative rod).

3.8.1 Test Cultures

The test pathogenic bacteria strains used were *S. aureus* ATCC 85923, *E. coli* ATCC 25922, and *P. aeruginosa* NCTC 10662, respectively. These pure bacterial cultures were used based on their MIC and MBC reactions.

In this section, acetone and methanol extracts of *D. cinerea* and *C. molle* were tested for their kill kinetics against *S. aureus* (Gram positive cocci), *E. coli* (a Gram negative rod) and *P. aeruginosa* (a potent β -lactamase producing Gram negative rod).

3.8.2 Time Kill Kinetics Bioassay Method

The method of Fabry *et al.* (1998) for determining extracts' concentrations derived from MICs and MBCs for kill kinetics was used. The Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentrations (MBCs) for these three bacteria were determined as detailed in sections 3.6 and 3.7. These MICs and MBCs were used as a guide for the series of concentrations that were tested. Three hundred and twenty milligram of each extract was dissolved in 1ml of DMF, and made to 10 ml using sterile MHB (stock solution). Five milliliter of the stock solution was added to 5 ml of MHB to form a final concentration of 16 mg/ml. Doubling dilutions of this concentration were made in MHB containing 2.5% DMF to form concentrations of 8, 4, 2, 1, 0.5 mg/ml.

The three test bacteria were grown on blood agar and incubated at 37⁰C for 24h. Log phase cultures were obtained by suspending about 2.6 x 10⁶cells/ml in MHB and incubated at 37⁰C for 24h. This was necessary in order to determine whether the extracts could eradicate cells known to be resistant to many antimicrobialagents. Twenty microlitres of the 24h culture of each bacterium were added to each of the 10ml of the corresponding concentrations of extract in MHB and incubated at 37⁰C. Initial control counts of bacteria were obtained by serial dilution and spread plating 0.1ml of this inoculum on MHA just before incubation. Subsequent 0.1ml of each extract concentration were serially diluted and spread plated at intervals of 2h, 4h, 6h, 8h, and 24h. All spread plates on MHA were allowed to stand for 30min at 4⁰C before spreading. The plates were incubated at 37⁰C for 24h. Plate counts were made after 24h of incubation and only plates containing between 30-300 counts for each series of dilutions were counted and recorded. Growth curves were plotted to show the log₁₀ of colony forming units (CFU) against time.

3.9.0 *In vivo* Single Dose Toxicity Test

3.9.1 Experimental Animals

The study used male Swiss albino mice (3-4 weeks old) that weighed 22-25g. These were bred in the animal house at the Biochemistry and Biotechnology Department of Kenyatta University. The mice were housed at a temperature of 25⁰C with 12h/12h darkness-photoperiod cycles and fed on rodent pellets and water *ad libitum*.

The mice were divided into five different groups of five mice each. One group served as the untreated control. The other groups were treated with 500mg/kg body weight of the extract. The 500 mg/kg body weight dose was selected on the basis that administration of an extract at higher dose than the therapeutic dose might be toxic. There were four active

plant extracts that were subjected to this *in vivo* toxicity tests. These four extracts were the ones that were active against the pathogenic bacteria in previous bioassays. The four extracts were administered orally to each of the four groups accordingly on a daily basis for a period of a month. Saline was administered to the fifth group as control. During this period, the mice were allowed free access to mice pellets and water and observed for any signs of general illness, change in behavior and mortality. At the end of one month the mice were sacrificed.

3.9.1.2 Determination of Body Weight

The body weight of each mouse was assessed during the acclimatization period, once before and immediately after commencement of dosing, once weekly during the dosing period and once on the day of sacrifice.

3.9.1.3 Absolute Organ Weight

On the day of sacrifice, all the animals were euthanized. Different organs namely the heart, liver, lungs, spleen, kidneys and testis were carefully dissected out and weighed in grams. Necropsy samples were collected and stored in 10% formalin. The tissues were processed using the standard protocols of histopathology.

3.9.2. Biochemical Assays

3.9.2.1 Preparation of Sera Samples

At the end of the experimental period (4 weeks), all animals were exsanguinated and blood samples were drawn from the heart of each sacrificed animal. The samples were collected in plastic test tubes and allowed to stand for 3 h to ensure complete clotting. The

clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen at -20°C .

Sample analysis was done using the Olympus 640 chemistry auto analyzer. This is a discrete, random access clinical analyzer capable of performing a wide range of chemistry tests in a single run. All reagents for the machine were commercially obtained to fit the required volumes and concentration. The reagents were in specific containers referred to as "reagent cartridges". The reagent cartridges were bar coded for the identification by the machine.

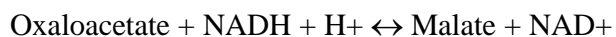
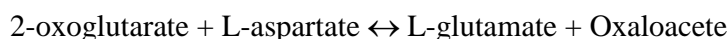
The machine was programmed for the selected tests for each sample. The sample sectors were then placed into the autoloader assembly. A number of events that occurred simultaneously were performed automatically under direct control of the instrument microprocessors.

3.9.3 Laboratory Determination of Enzyme Activities and Analytes

3.9.3.1 Determination of the Activity of Aspartate Aminotransferase (AST)

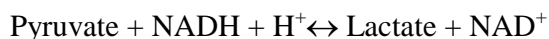
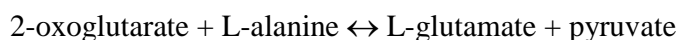
The method used is as described by Henry *et al.* (1960). AST reagent was used to measure AST activity by an enzymatic rate method. In the reaction, AST catalyzed the reversible transamination of L-aspartate and α -ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate was then reduced to malate in the presence of malate dehydrogenase (MDH) with concurrent oxidation of reduced β -nicotinamide adenine dinucleotide (NAD). The ratio of the sample to reagent was 1 part sample to 11 parts reagents (23 μL : 253 μL reagent). The absorbency was measured at 340 nm and its change was directly proportional to the activity of AST. The autoanalyser calculated and

expressed the activity in IU/L. The reaction took place at 37⁰C for three minutes. The principal of the reaction is as follows:



3.9.3.2 Determination of the Activity of Alanine Aminotransferase (ALT)

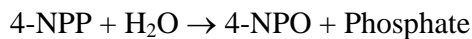
The method used is as described by Henry *et al.* (1960). The ALT reagent was used to measure ALT activity by an enzymatic rate method. In the reaction, the ALT catalyzes the reversible transamination of L-alanine and α -ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with concurrent oxidation of β -Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β -Nicotinamide Adenine Dinucleotide (oxidized form) (NAD⁺). Pyridoxal-5-phosphate was required in this reaction as a cofactor for the transaminase activity by binding to the enzyme using Schiff-base linkage. The ratio of the sample to reagent was 1 part sample to 11 parts reagents (23 μ L: 253 μ L reagent). The absorbance was measured at 340 nm and its change was directly proportional to the activity of ALT. The autoanalyser calculated and expressed the activity in IU/L. The reaction took place at 37⁰C for 3min. The principal of the reaction is as follows:



3.9.3.3 Determination of the Activity of Alkaline Phosphatase (ALP)

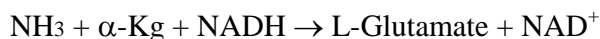
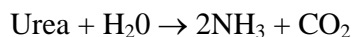
The ALP reagent was used to measure ALP activity by kinetic method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction, ALP catalyzed the hydrolysis of the colorless organic phosphate ester substrate (p-nitrophenylphosphate) to the yellow

colored product (p-nitrophenol and phosphate). This reaction occurred at alkaline pH of 10.3. The ratio of the sample to reagent was 1 part sample to 50 parts reagents (5 μ L: 250 μ L reagent). The absorbance was measured at 410 nm and this change was directly proportional to the activity of ALP. The autoanalyser calculated and expressed the activity in IU/L. The reaction took place at 37⁰C for three minutes (International Federation of Clinical Chemistry, 1983). The principal of the reaction is as follows:



3.9.3.4 Determination of the Blood Levels of Urea (BUN)

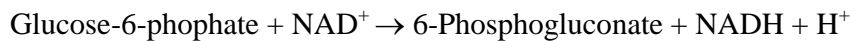
The method used is as described by Tiffany *et al.* (1972). The BUN reagent was used to measure the concentration of urea by an enzymatic rate method. In the reaction, urea was hydrolyzed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GIDH) catalyzed the condensation of ammonia and α -ketoglutarate to glutamate with concomitant oxidation of reduced β -Nicotinamide Adenine Dinucleotide (NADH) to β -Nicotinamide Adenine Dinucleotide (NAD⁺). The ratio of the sample to reagent was 1 part sample to 100 parts reagents (3 μ L: 300 μ L reagent). The absorbance was measured at 340 nm and this change was directly proportional to the activity of BUN. The autoanalyser calculated and expressed the activity in μ M. The reaction took place at 37⁰C for one minute. The principal of the reaction is as follows:



3.9.3.5 Determination of the Activity of Creatine Kinase (CK)

The method used is as described by Oliver and Rosalki (1967) method based on the primary reaction that is catalyzed by CK resulting in production of creatine and ATP. The ATP produced in the primary reaction is then employed in a coupled enzymatic glucose

assay employing hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD). The ratio of the sample to reagent was 1 part sample to 50 parts reagents (5 μ L: 250 μ L reagent). The autoanalyser calculated and expressed the activity in IU/L. The reaction took place at 37⁰C for three minutes. The production of NADPH in the indicator reaction is monitored at 340 nm and is related to CK activity within the patient specimen (Oliver and Rosalki, 1967).



3.9.4 Determination of Hematological Parameters

Blood was examined using standard protocols (Jain, 1986). Red blood cells, white blood cells, hemoglobin and mean hematocrit count were determined using the coulter count system Beckman Coulter® (ThermoFisher, UK). The machine automatically dilutes a whole-blood sample of 30 μ l, lyses and counts and gives a print out result of absolute numbers of white blood cells (WBC) (expressed as number of cells x 10⁹ per liter) and red blood cells (RBC) number of cells x 10¹² per liter). In addition Hb (in grams per liter) and MCHC (in grams per deciliter) were measured by the analyzer. The autoanalyser utilizes two independent methods. These methods are the independent method for determining the white blood cells (Coulter 1956), and the modified cyanomethaemoglobin method for determining the haemoglobin (International Committee for Standardization in Haematology 1978).

Differential leucocyte counts for neutrophils, lymphocytes and monocytes were examined from stained blood films (Jain, 1986). Air-dried thin blood films stained with giemsa stain

were examined microscopically using magnification 400 and 1000 for differential WBC counts and cell morphologies respectively. Neutrophil (N), lymphocyte (L) and monocytes (M) absolute counts (number of cells $\times 10^9$) per liter were obtained by expressing their percent differential counts against the total WBC absolute counts (Jain, 1986).

3.9.5 Determination of the Mineral Content of the Plant Extracts

3.9.5.1 Preparation of Plant Extracts

Each dried plant sample were filtered and weighed. At least three pellets weighing 300-1000mg/cm² were prepared for analysis using the press pellet machine and placed onto the sample tray. To enhance binding about 25 mg of cellulose was mixed with the ground plant material.

3.9.5.2 Elemental Analysis by Total Reflection X-Ray Fluorescence System (TXRF System)

TXRF system was used to determine the content of Potassium (K), Calcium (Ca), Titanium (Ti), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Lead (Pb), Bromine (Br), Rubidium (Rb), Strontium (Sr), Yttrium (Y), Niobium (Nb) Zirconium (Zr), in the plant samples.

The Total Reflection X-Ray Fluorescence system analysis consists of an x-ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source, Cd¹⁰⁹ (half-life, $T_{1/2} = 453$ days and activity = 10mCi) are incident on the sample that emits the characteristic X-rays. These X-rays are detected by Si (Li) detector (EG&G Ortec, 30mm²*10mm sensitive volume, 25 μ m Be window) with an energy resolution of 200 eV at 5.9 keV Mn K _{α} - line. The spectral data for analysis were collected using

personal computer based Canberra S-100 multi-channel analyzer (MCA). The acquisition time applied in the TXRF measurement was 1000 seconds. For data analysis, the X-ray spectrum analysis and quantification was done using IAEA QXAS software (QXAS, 1992) that is based on the fundamental parameters method (FPM). With this method, if the type and properties of all elements contained in a sample are known, then the intensity of each fluorescent X-ray is derived theoretically. By using this method, the composition of unknown sample is extrapolated by its fluorescence X-ray intensity of each element. The results are expressed in parts per million (ppm).

3.9.6 Histopathology

Autopsies samples were collected and stored in 10% formalin. The tissues were processed using the standard protocols of histopathology. The heart, lungs, liver, kidney and testes were observed for any histopathological changes.

3.10 Phytochemical Screening

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebanjo *et al.*, 1983). Proven medicinal plants are used in treatment of diseases either alone or in combination with other plants. They are used as anti-infectious agents, anti-malarial, anti-tumoral agents, laxatives, cardiovascular and nerval remedies (Owonubi, 1988). Some biologically active plant-derived secondary metabolites have found application as drugs or as model compounds for drug synthesis and semisynthesis (Pryde *et al.*, 1981). Some secondary plant metabolites are used though in limited quantities, as pharmacological tools to study various biochemical processes (Baladrin *et al.*, 1985).

Traditional medicinal plants have an almost maximum ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most of these are secondary metabolites, of which 12,000 plant-derived agents have been isolated in the recent past. Many of these substances serve as plant defense mechanisms against invasion by micro-organisms, insects and herbivores. Some of the plant substances such as terpenoids are responsible for odor (quinones and tannins) plus pigment of the plant (Geissman, 1963). Many compounds are responsible for plant flavor (like the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds (Geissman, 1963). The useful major groups of anti-microbial phytochemicals can be divided into several categories that include alkaloids, flavonoids, quinones, essential oils, lectins, polypeptides, phenolics, polyphenols, tannins and terpenoids (Geissman, 1963).

Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by Sofowora (1993) and Harborne (1973) method. The bioactive extracts were filtered, concentrated in vacuo using a rotary evaporator and dried. The extracts were tested for alkaloids, tannins, steroids, triterpenes, flavonoids and anthraquinones according to the method of Sofowora (1993) and Harborne (1973). The presence or absence of these secondary metabolites was confirmed by colour changes of solutions after the addition of the testing reagents. Results were read and indicated as (+ve), showing the presence and (-ve) showing the absence of the phytochemicals.

3.10.1 Testing for the Presence of Alkaloids

The crude extract (2 g) was hydrolyzed with 2 ml of 2% HCl solution by heating in a water bath for 10 min., then a few drops of Mayer's reagent was added to 5 ml of the

filtrate. The appearance of turbidity indicated the presence of alkaloids (Harborne, 1973; Trease and Evans, 1978).

3.10.2 Testing for Tannins

The methods of Sofowora (1993) and Harborne (1973) were employed in testing for these compounds. The test for tannins was carried out by dissolving 3g of each plant extract in 6 ml of distilled water. This was filtered and then ferric chloride reagent added to it. The occurrence of a blackish blue colour showed the presence of gallotannins and a green blackish colour indicated catechol tannins.

3.10.3 Testing for the Presence of Anthraquinones

A few drops of 0.5 M KOH was added to 1 ml of the extract followed by 1 ml of 3% hydrogen peroxide. The mixture was boiled for a few min and then cooled and filtered. Five millilitre of the solution was made acidic by adding acetic acid and then extracted with benzene. To the benzene layer, ammonium solution was added. Appearance of a reddish colour indicated the presence of anthraquinones.

3.10.4 Testing for the Presence of Triterpenes and Steroids

The extract was also tested for free glycoside bound anthraquinones (Wall, 1952; Sofowora, 1993). A crude (2 g) extract was defatted with n-hexane and the residue extracted with 40 ml of chloroform. To 0.5 ml portion of the chloroform extract, 0.5 ml of acetic acid was added followed by 2 drops of concentrated sulphuric acid. Gradual colour change to green-blue confirmed the presence of sterols and triterpenes.

3.10.5 Testing for Saponins

The method of Wall *et al.* (1952) and Sofowora (1993) was used to test for saponins. Saponins have the ability to produce frothing in aqueous solution and to haemolyse red blood cells, the property which was used as screening test for the compounds. The extract was subjected to frothing test for the identification of saponin. Haemolysis test was further performed on the frothed extracts in water to remove false positive results

3.10.6 Testing for the Presence of Flavonoids

The presence of flavonoids was determined using 1% aluminum chloride solution in methanol, concentrated HCl, magnesium turnings, and potassium hydroxide solution (Kapoor, 1969; Earnsworth *et al.*, 1974). A crude (1 g) extract was defatted by extracting with n-hexane. The residue was dissolved in 80% ethanol (30 ml) and treated as follows: (a) 4 ml of 1% aluminium chloride in methanol solution was added to 3 ml of the ethanol solution. The appearance of a pale yellow colour indicated the possible presence of flavonoids. (b) Ethanol solution (2 ml) was reacted with 0.5 ml of concentrated hydrochloric acid and a few magnesium turnings. Red colour appearance indicated the presence of flavonoids.

3.10.7 Coumarins

Coumarins were detected in plant material simply by placing a small amount of moistened sample in a test tube and covering the tube with filter paper moistened with dilute sodium hydroxide solution. The covered test tube was then placed in a boiling water bath for several minutes, and then the paper was removed and exposed to ultraviolet light. The presence of coumarins was detected by a yellow-green fluorescence appearance within a few minutes (Franklin *et al.*, 1961).

3.10.8 Reducing Sugar

Methanol extract (1 ml) was diluted with 2 ml of water, 1 ml of Feling's solution was added and the mixture heated. A brick-red precipitate indicated the presence of reducing sugar.

3.11 Data Analysis

Combination treatment data were analysed by completely randomized block design of ANOVA (SPSS version 12.0 program, Instat. GraphPad Software. Inc., San Diego. CA). Differences between means of the flow cytometry experiments and control groups were analysed by paired t-test.

CHAPTER FOUR

RESULTS

4.1 Yield of Plant Extract

Table 2 shows yields in milligrammes of extracts from all extracted plant materials. Differences in yields between polar and none polar organic solvents are not big. Methanol extracts of all plants had the highest yields examples being those of *Ficus sur* (195.3 mg) and *D. cinerea* (184 mg).

Acetone solvent had the highest yield of *C. molle* extract, compared to the rest of the solvents. Hexane and dicholoromethane (none polar) had the least yields of extracts for all plant extracts.

Table 2: Yield in milligrams of each plant extract per 50 g of plant material

Plant name	Hexane Extracts (mg)	DCM Extracts (mg)	Acetone extract (mg)	Methanol Extracts (mg)
<i>Dichrostachys cinerea</i>	142.5	65.4	172.0	184
<i>Combretum molle</i>	57.3	72.8	182.6	175.1
<i>Euclea divinorum</i>	125.6	67.3	121.1	147.8
<i>Fiscus sur</i>	76.4	69.3	154.4	195.3
<i>Schkuhria piñata</i>	35.8	54.2	78.0	74.3
<i>Carisa edulis</i>	46.3	64.6	78.4	87.9
<i>Zanthoxylum usambarense</i>	56.9	96.0	87.6	98.0
<i>Securidaca longipedaculata</i>	64.2	73.8	98.5	187.7
<i>Amarylidaeeae crinum</i>	45.0	64.2	88.7	94.2
<i>Launaca cornuta</i>	32.0	43.0	54.0	48.0

4.3 Agar Disc Diffusion Bioassays

4.2.1 Antibacterial Activity of *D. cinerea* Extract by Agar Disc Diffusion

The sensitivity of nine pathogenic bacteria to the stem extracts of *D. cinerea* was tested and compared to that of antibacterial antibiotic Augmentin. The results shown (Table 3) are the average of inhibition zones for individual extract. Hexane extracts of *D. cinerea* were not active against all pathogenic bacteria (Table 3). Dichloromethane extract of *D. cinerea* was only active against *S. aureus* ATCC 85923 (14 mm), while slightly active against *S. aureus* isolate (9.6 mm) and *E. coli* ATCC 25922 (7.8 mm). The same extract was not active against the rest of the test bacteria.

Table 3: Zones of inhibition of *D. cinerea* stem extracts using the disc diffusion method

<i>Micro-organisms</i>	Hexane extracts	Dichloro Methane extracts	Acetone extract	Methanol extracts	Augmentin (AMC) 20µg/ml
<i>E. coli</i> ATCC 25922	5.8±0.21	7.8±0.32	10.6±0.11*	7.0±0.23	16.0±0.03
<i>S. aureus</i> ATCC 85923	6.8±0.31	14.0±0.41*	11.4±0.32*	8.4±0.28	14.0±0.02
<i>P. aeruginosa</i> NCTC 10662	5.0±0.22	5.0±0.21	7.4±0.25	6.0±0.21	7.0±0.02
<i>E. coli</i> isolate	5.2±0.21	5.0±0.24	8.2±0.43	8.2±0.26	16.0±0.03
<i>S. typhi</i> isolate	5.6±0.23	5.6±0.21	13.8±0.54*	7.0±0.27	22.0±0.05
<i>S. flexinery</i> isolate	5.4±0.20	5.0±0.21	6.8±0.25	5.8±0.21	17.0±0.04
<i>P. aeruginosa</i> isolate	5.0±0.21	5.0±0.22	6.4±0.33	6.4±0.15	20.0±0.04
<i>S. aureus</i> isolate	5.0±0.21	9.6±0.31*	9.8±0.26*	9.8±0.32*	11.0±0.01
<i>S. dysenteriae</i> isolate	5.0±0.22	5.0±0.21	5.6±0.21	5.6±0.22	16.0±0.03
<i>Klebsiella</i> isolate	5.0±0.21	5.0±0.22	10.8±0.43*	5.8±0.21	12.0±0.02

.Values are means ± SD of five determinations; values followed by asterix are significant (p<0.05). Differences between means of the zones of inhibitions were analysed by student's t-test (SPSS version 12.0 program, Instat. GraphPad Software. Inc., San Diego. CA).

Acetone extracts of *D. cinerea* were also observed to be active against *S. typhi* isolate (13.8 mm), *S. aureus* ATCC 85923 (11.4 mm), *Klebsiella* isolate (10.8 mm), *E. coli*

ATCC 25922 (10.6 mm), *S. aureus* isolate (9.8 mm). The activity varied significantly among the test bacteria ($P < 0.05$), with a mean inhibition of 9.08 ± 0.45 . The same extract was not active against *S. flexinerry* isolate, *P. aeruginosa* isolate and *S. dysenteriae* isolate.

Methanol extracts of *D. cinerea* were only active against *S. aureus* isolate (9.8 mm), *S. aureus* ATCC 85923 (8.4 mm), *E. coli* isolate (8.2 mm) but not active against the rest of the test bacteria. The activity was not significant among test bacteria ($P < 0.05$) with a mean inhibition of 7.00 ± 0.23 compared to acetone extract.

4.2.2 Antibacterial Activity of *C. molle* Extracts by Agar Disc Diffusion

Hexane and Dichloromethane extracts of *C. molle* were not active against the test bacteria (Table 4). Acetone extracts of *C. molle* were active against *S. typhi* isolate (18.4 mm), *S. aureus* ATCC 85923 (11.4 mm), *S. aureus* isolate (10.8 mm), *E. coli* ATCC 25922 (10.8 mm), *Klebsiella* isolate (10.6 mm), *P. aeruginosa* NCTC 10662 (10.0 mm) in descending order. The inhibition activity of this extract varied significantly among the test bacteria ($P < 0.05$), with a mean inhibition at 10.00 ± 0.54 .

In a descending order, the activity of methanol extract of *C. molle* was *S. typhi* (19.6 mm), *E. coli* ATCC 25922 (15.4 mm), *E. coli* isolate (12.4 mm), *S. aureus* ATCC 85923 (12.2 mm), *Klebsiella* isolate (11.4 mm), *S. dysenteriae* (11 mm), *S. aureus* isolate (10.4 mm). The activity of this extract varied significantly among the test bacteria ($P < 0.05$), with a mean inhibition of 13.36 ± 0.65 . The methanol extract inhibited the growth of all the test bacteria

The sensitivity of nine pathogenic bacteria to the stem extracts of *C. molle* was tested and compared to that of antibacterial antibiotic Augmentin (AMC).

Table 4: Zones of inhibition of *C. molle* stem extracts using the disc diffusion method

Microorganisms	Hexane Extract	DCM Extract	Acetone Extract	Methanol Extract	Augmentin (AMC) 20 µg/ml
<i>E. coli</i> ATCC 25922	5.6±0.21	5.4±0.26	10.8±0.3*	15.4±0.3*	19.0±0.03
<i>S. aureus</i> ATCC 85923	5.0±0.23	5.0±0.21	11.4±0.4*	12.2±0.4*	17.0±0.02
<i>P. aeruginosa</i> NCTC 10662	5.2±0.22	5.0±0.21	10.0±0.35*	7.6±0.24	8.0±0.02
<i>E. coli</i> isolate	5.0±0.32	5.0±0.31	8.25±0.24*	12.4±0.33*	21.0±0.03
<i>S. typhi</i> isolate	6.0±0.29	6.2±0.21	18.4±0.45*	19.6±0.42*	20.0±0.05
<i>S. flexinery</i> isolate	5.0±0.31	5.0±0.27	5.0±0.25	5.0±0.21	15.0±0.04
<i>P. aeruginosa</i> isolate	5.0±0.24	5.0±0.24	7.6±0.23	8.5±0.23	23.0±0.04
<i>S. aureus</i> isolate	9.8±0.27	5.0±0.26	10.8±0.2*	10.4±0.2*	21.0±0.01
<i>S. dysenteriae</i> isolate	5.6±0.21	5.6±0.23	5.6±0.21	11.0±0.4*	16.0±0.03
<i>Klebsiella</i> isolate	5.8±0.31	5.4±0.22	10.6±0.4*	11.4±0.5*	12.0±0.02

Values are means ± SD of five determinations; values followed by asterisk are significant ($p < 0.05$). Differences between means of the zones of inhibitions were analysed by student's t-test (SPSS version 12.0 program, InStat. GraphPad Software. Inc., San Diego. CA).

4.2.3 Antibacterial Activity of *E. divinorum* by Agar Disc Diffusion

Table 5 shows that in descending order the hexane extracts of *E. divinorum* were active against *S. aureus*, ATCC 85923 and *Klebsiella* isolate, and *E. coli* ATCC 25922, and *S. flexinery*. The inhibition activity was significantly varied among the test bacteria ($t = 6.107$, $df 49$, $P = 0.0000$), with a mean inhibition value of 9.64 ± 0.46 .

Table 5: Zones of inhibition of *E.divinorum* extracts using disc diffusion method

Microorganisms	Hexane Extract	DCM Extract	Acetone Extract	Methanol Extract	Augmentin (AMC) 20µg/ml
<i>E. coli</i> ATCC 25922	9.4±0.24*	10.8±0.32*	6.8±0.22	5.0±0.21	22.0±0.03
<i>S. aureus</i> ATCC 85923	17.0±0.42*	16.2±0.45*	8.6±0.24	5.0±0.25	27.0±0.02
<i>P. aeruginosa</i> NCTC 10662	8.6±0.25	10.8±0.26*	5.0±0.31	5.0±0.45	10.0±0.02
<i>E. coli</i> isolate	7.8±0.26	8.0±0.24	7.8±0.32	5.0±0.21	25.0±0.03
<i>S. typhi</i> isolate	8.5±0.34	12.2±0.35*	10.6±0.27*	5.0±0.23	24.0±0.05
<i>S. flexinery</i> isolate	9.2±0.22*	11.2±0.34*	6.0±0.28	7.8±0.42	13.0±0.04
<i>P. aeruginosa</i> isolate	7.0±0.24	8.2±0.23	5.0±0.31	5.0±0.21	21.0±0.04
<i>S. aureus</i> isolate	8.4±0.31	11.8±0.25*	8.4±0.42	5.0±0.21	19.0±0.01
<i>S. dysenteriae</i> isolate	8.2±0.21	7.0±0.41	5.0±0.43	5.0±0.34	14.0±0.03
<i>Klebsiella</i> isolate	12.2±0.40*	11.0±0.34*	5.0±0.21	7.0±0.42	11.0±0.02

Values are means ± SD of five determinations; values followed by asterix are significant (p<0.05) Differences between means of the zones of inhibitions were analysed by student's t-test of (SPSS version 12.0 program, Instat. GraphPad Software. Inc., San Diego. CA).

In descending order, Dichloromethane extracts of *E. divinorum* were shown to be active against *S. aureus* ATCC 85923 (16.2 mm), *S. typhi* (12.2 mm), *S. aureus* isolate (11.8 mm), *S. flexinery* (11.2 mm), *Klebsiella* (11 mm), *E. coli* ATCC 25922 10.8 mm), and *P. aeruginosa* NCTC 10662 (10.8 mm). The activity of the extract did vary significantly among test bacteria (t = 7.788, df 49, P = 0.000), with a mean inhibition value of 10.76 ± 0.42.

Except for the *S. typhi* isolate, *S. aureus*, ATCC 85923, *S. aureus* isolate and *E. coli* isolate respectively, acetone extracts of *E. divinorum* were inactive against all the test bacteria. Methanol extracts of *E. divinorum* were inactive against all the test bacteria.

The sensitivity of nine pathogenic bacteria to the root stem extracts of *E. divinorum* was tested and compared to that of antibacterial antibiotic Augmentin (AMC).

4.2.4 Antibacterial Activities of *Ficus sur* Extracts by Agar Disc Diffusion

The sensitivity of nine pathogenic bacteria to the root stem extracts of *F. sur* was tested and compared to that of antibacterial antibiotic Augmentin (AMC).

Hexane and Dichloromethane extracts of *F. sur* were inactive against all test pathogenic bacteria except Dichloromethane extract that was active against *S. dysenteriae* (Table 6).

In descending order, acetone extracts of *F. sur* were active against *S. aureus* ATCC 85923, *P. aeruginosa* NCTC 10662, *P. aeruginosa* isolate, *S. aureus* isolate and *Klebsiella* isolate. The same extract had no inhibitory activity against *E. coli* ATCC 25922, *E. coli* isolate and *S. flexinery*. Statistical analysis shows that the activity of this extracts varied significantly among the test bacteria ($t = 6.175$, $df 49$, $P = 0.000$), with a mean inhibition value of 11.28 ± 0.81 .

Methanol extracts of *F. sur* were only active against *S. aureus* ATCC 85923 (10.4 mm) and inactive against the rest of pathogenic test bacteria, their activity varying significantly among test bacteria ($t = 2.580$, $df 49$, $P = 0.013$) with a mean value of 6.98 ± 0.37 .

Table 6: Zones of inhibition of *Fiscus sur* extracts using disc diffusion method

Microorganisms	Hexane Extract	Dichloromethane Extract	Acetone Extract	Methanol Extract	Augmentin (AMC) 20µg/ml
<i>E. coli</i> ATCC 25922	5.0±0.26	6.0±0.32	6.4±0.28	6.8±0.21	17.0±0.03
<i>S. aureus</i> ATCC 85923	5.0±0.21	5.0±0.22	18.4±0.45*	10.4±0.25*	24.0±0.02
<i>P. aeruginosa</i> NCTC 10662	5.0±0.25	5.0±0.27	18.4±0.42*	7.8±0.24	9.0±0.02
<i>E. coli</i> isolate	5.0±0.23	5.0±0.24	6.4±0.23	5.0±0.21	22.0±0.03
<i>S. typhi</i> isolate	5.0±0.25	5.0±0.22	8.4±0.26	5.2±0.28	19.0±0.05
<i>S. flexinery</i> isolate	5.0±0.26	5.0±0.21	5.6±0.24	5.0±0.25	17.0±0.04
<i>P. aeruginosa</i> isolate	7.0±0.22	6.4±0.24	17.6±0.42*	7.0±0.28	23.0±0.04
<i>S. aureus</i> isolate	5.0±0.23	5.4±0.27	13.2±0.23*	8.4±0.35	22.0±0.01
<i>S. dysenteriae</i> isolate	5.0±0.26	12.4±0.35*	8.2±0.21	6.2±0.27	18.0±0.03
<i>Klebsiella</i> isolate	5.0±0.21	5.0±0.24	9.2±0.36*	8.0±0.24	15.0±0.02

Values are means ± SD of five determinations; values followed by asterix are significant (p<0.05) Differences between means of the zones of inhibitions were analysed by student's t-test of (SPSS version 12.0 program, Instat. GraphPad Software. Inc., San Diego. CA).

All extracts of *Schkuhria pinata*, *Carisa edulis*, *Zanthoxylum chalybeum*, *Securidaca longipedaculata*, *Amarylidaceae crinum*, and *Launaca cornuta* showed no inhibitory activity against all test pathogenic bacteria. Extracts of these plants were therefore not, included in the subsequent bioassays stages. The acetone and methanol extracts of *D. cinerea* and *C. molle*, hexane, dichloromethane and acetone extracts of *E. divinorum* and methanol extract of *F. sur* were subjected to minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs), and Time Kill Kinetics.

4.3.1 Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for Acetone and Methanol Extracts *D. cinerea*

Table 7, shows the MICs and MBCs of acetone and methanol root extracts of *Dichrostachys cinerea*. The MICs and MBCs of the acetone extract for the reference bacteria were 7.25 and 7.5 mg/mL for *E. coli* ATCC25922, 0.85 and 1.9 mg/mL, for *S. aureus* ATCC 85923, and 0.85 and 1.9 mg/mL for *P. aeruginosa* NCTC 10662. For the bacterial isolates, the MICs and MBCs of the acetone root extract were 1.75 and 7.5 mg/mL, for *E. coli*, 0.85 and 1.9 mg/mL, for each of *S. typhi*, *S. flexineri*, *S. dysenteriae*, *S. aureus* and *P. aeruginosa*, and 0.85 and 7.5 mg/mL for *Klebsiella* species, respectively.

The MICs and MBCs of the methanol extract for the reference bacteria were 3.75 and 4.0 mg/mL for *E. coli* ATCC 25922 and *P. aeruginosa* NCTC 10662, 1.75 and 2.0 mg/mL for *S. aureus* ATCC 85923. The same extracts recorded MICs and MBCs of 3.75 and 4.0 mg/mL for *E. coli*, *S. flexineri*, *S. dysenteriae*, *S. aureus* and *P. aeruginosa* and *Klebsiella* bacterial isolates. The same extract gave MICs and MBCs of 7.5 and 7.5 mg/mL for *S. typhi* isolate.

Table 7: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for *D. cinerea* acetone and methanol extracts

Micro- Organisms	Acetone		Methanol	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>E. coli</i> ATCC 25922	7.25±0.12	7.5±0.04	3.75±0.02	4.0±0.06*
<i>S. aureus</i> ATCC 85923	0.85±0.01	1.9±0.05*	1.75±0.03	2.0±0.02*
<i>P. Aeruginosa</i> NCTC 10662	0.85±0.01	1.9±0.13*	3.75±0.21	4.0±0.05*
<i>E. coli</i> Isolate	1.75±0.14	7.5±0.13	3.75±0.02	4.0±0.06*
<i>S. typhi</i> isolate	0.85±0.02	1.9±0.13*	7.5±0.02	7.5±0.02
<i>S. flexineri</i> isolate	0.85±0.02	1.9±0.13*	3.75±0.02	4.0±0.06*
<i>P. aeruginosa</i> isolate	0.85±0.02	1.9±0.13*	3.75±0.02	4.0±0.06*
<i>S. aureus</i> isolate	0.85±0.02	1.9±0.13*	3.75±0.02	4.0±0.06*
<i>S. dysenteriae</i> isolate	0.85±0.02	1.9±0.13*	3.75±0.02	4.0±0.06*
<i>Klebsiella</i> isolate	0.85±0.02	7.5±0.13	3.75±0.02	4.0±0.06*

Values are means \pm SD of five determinations; values followed by asterix are significant ($p < 0.05$). Differences between means of the MICs and MBCs were analysed by student's t-test of (SPSS version 12.0 program, Instat. GraphPad Software. Inc., San Diego. CA).

4.3.2 Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for acetone and methanol extract of *C. molle*

Table 8 shows MICs and MBCs of acetone and methanol root extracts of *Combretum molle*. The MICs and MBCs of the acetone extract for the reference bacteria were 5.0±0.07 mg/mL and 5.5±0.14 mg/mL for *E. coli* ATCC 25922 and *P. aeruginosa* NCTC 1062, and 0.25±0.03 mg/mL and 2.0±0.11 mg/mL for *S. aureus* ATCC85923. Acetone root extract gave MICs and MBCs of 5.0±0.07 Mg/mL and 5.5±0.14 mg/mL for *E. coli*, *S. typhi*, *S. flexineri*, *S. dysenteriae*, *S. aureus*, *P. aeruginosa* and *Klebsiella* bacterial isolates.

Methanol extract of *C. molle* recorded MICs and MBCs for the reference bacteria of 0.25 and 2.2 mg/mL for *S. aureus* ATCC85923, 2.5 and 3.0 mg/mL for *P. aeruginosa* NCTC 10662 and 5.0 and 5.0 mg/mL, for *E. coli* ATCC25922. MICs and MBCs of the same extract for bacterial isolates were 5.0 and 5.0 mg/mL for *E.coli*, *S. typhi* and *Klebsiella* species (Table 8). It can also be seen that for isolates the MICs and MBCs of 0.25 and 0.6 mg/mL were recorded for *S. flexineri* and *S. aureus*, 0.25 and 2.5 mg/mL for *P. aeruginosa* and 1.25 and 2.0 mg/mL for *S. dysenteriae*.

Table 8: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for *C. molle* acetone and methanol extracts

Microorganisms	Acetone		Methanol	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>E. coli</i> ATCC 25922	5.0±0.07	5.5±0.14	5.0±0.06	5.0±0.06
<i>S. aureus</i> ATCC 85923	0.25±0.03	2.0±0.11*	0.25±0.03	2.2±0.12*
<i>P. Aeruginosa</i> NCTC 10662	5.0±0.07	5.5±0.14	2.5±0.11	3.0±0.13*
<i>E. coli</i> isolate	5.0±0.07	5.5±0.14	5.0±0.06	5.0±0.07
<i>S. typhi</i> isolate	5.0±0.07	5.5±0.14	5.0±0.06	5.0±0.07
<i>S. flexineri</i> isolate	5.0±0.07	5.5±0.14	0.25±0.03	0.6±0.04*
<i>P. aeruginosa</i> isolate	5.0±0.07	5.5±0.14	0.25±0.03	2.5±0.14*
<i>S. aureus</i> isolate	5.0±0.07	5.5±0.14	0.25±0.03	0.6±0.03*
<i>S. dysenteriae</i> isolate	5.0±0.07	5.5±0.14	1.25±0.13	2.0±0.12
<i>Klebsiella</i> isolate	5.0±0.07	5.5±0.14	5.0±0.07	5.0±0.07

Values are means ± SD of five determinations; values followed by asterix are significant (p<0.05). Differences between means of the MICs and MBCs were analysed by student's t-test of (SPSS version 12.0 program, Instat. GraphPad Software. Inc., San Diego. CA).

4.3.3 Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for Acetone and Methanol Extracts of *E. divinorum*

As shown in Table 9, the MICs and MBCs for the acetone extracts of *E. divinorum* for the reference bacteria were 6.25 and 8.5 mg/mL for *E. coli* ATCC 25922, 0.04 and 1.5 mg/mL, for *S. aureus* ATCC 85923, and 50.0 and 55 mg/mL for *P. aeruginosa* NCTC 10662. For the bacterial isolates, the MICs and MBCs of the acetone extract were 6.25 and 8.5 mg/mL for *E.coli*, 2.5 and 2.5 mg/mL for *S. typhi*, 6.5 and 8.0 mg/mL for *S.*

flexineri, 50.0 and 55.5 mg/mL for *P. aeruginosa*, 2.5 and 3.0 mg/mL for *S. aureus*, 6.25 and 7.5 mg/mL for *S. dysenteriae* and 50.0 and 55.5 mg/mL for *Klebsiella* species.

The MICs and MBCs of the dichloromethane extract for the reference bacteria were 7.5 and 6.25 mg/mL for *S. aureus* ATCC 85923, 25.0 and 25.0 mg/mL for *E. coli* ATCC 25922, and 50.0 and 50.0 mg/mL for *P. aeruginosa* NCTC 10662. For the bacterial isolates, the MICs and MBCs of the dichloromethane extract were 2.5 and 3.5 mg/mL for *S. aureus*, 12.5 and 12.5 mg/mL for *S. flexineri*, *S. dysenteriae* and *Klebsiella* species, while 50.0 and 50.0 mg/mL was recorded for *E. coli*, *S. typhi*, and *P. aeruginosa*.

The MICs and MBCs of the hexane extract for the reference bacteria were 12.5 and 15 mg/mL for *S. aureus* ATCC 85923, 25.0 and 30.0 mg/mL for *E. coli* ATCC 25922 and *P. aeruginosa* NCTC 10662. Bacterial isolates recorded MICs and MBCs for the hexane extract at 12.5 and 14.5 mg/mL for *P. aeruginosa*, 25.5 and 30.0 mg/mL for *E. coli*, *S. typhi*, *S. flexineri*, *S. aureus*, *S. dysenteriae* and *Klebsiella* species (Table 9).

Table 9: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for *E. divinorum* hexane, dichloromethane and acetone extracts

Micro-Organisms	Hexane		Dichloromethane		Acetone	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>E. coli</i> ATCC 25922	25.0±0.15	30.0±0.17	25.0±0.15	25.0±0.15	6.25±0.13	8.5±0.14
<i>S. aureus</i> ATCC 85923	12.5±0.14	15.0±0.16	7.5±0.12	6.25±0.11	0.04±0.02	1.5±0.03
<i>P. aeruginosa</i> NCTC 10662	25.0±0.16	30.0±0.17	50.0±0.36	50.0±0.56	50.0±0.46	55.0±0.36
<i>E. coli</i> isolate	25.0±0.15	30.0±0.15	50.0±0.56	50.0±0.26	6.25±0.11	8.5±0.14
<i>S. typhi</i> isolate	25.0±0.14	30.0±0.17	50.0±0.26	50.0±0.76	2.5±0.03	2.5±0.03
<i>S. flexineri</i> isolate	25.0±0.15	30.0±0.18	12.5±0.14	12.5±0.14	6.5±0.13	8.0±0.15
<i>P. aeruginosa</i> iso	12.5±0.14	14.5±0.09	50.0±0.26	50.0±0.28	50.0±0.36	55.5±0.76
<i>S. aureus</i> isolate	25.0±0.15	30.0±0.17	2.5±0.03	3.5±0.02	2.5±0.03	3.0±0.04
<i>S. dysenteriae</i> isolate	25.0±0.13	30.0±0.19	12.5±0.14	12.5±0.14	6.25±0.12	7.5±0.13
<i>Klebsiella</i> isolate	25.0±0.15	30±0.17	12.5±0.14	12.5±0.14	50.0±0.26	55.0±0.66

Values are means ± SD of five determinations; values followed by asterisk are significant (p<0.05). Differences between means of the MICs and MBCs were analysed by student's t-test of (SPSS version 12.0 program, Instat. GraphPad Software. Inc., San Diego. CA).

4.3.4 Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for Acetone and Methanol Extract of *Ficus sur*

Results of MICs and MBCs for the acetone extracts of *Ficus sur* are shown in Table 10.

The reference bacteria recorded MICs and MBCs at 5.0 and 7.5 mg/mL for *S. aureus* ATCC 85923, 50.0 and 65.0 mg/mL for *E. coli* ATCC 25922 and 50 and 60 mg/mL for *P. aeruginosa* NCTC 10662. For the bacterial isolates, the MICs and MBCs of the acetone

extract were 50 and 65 mg/mL for *E.coli*, 50 and 60 mg/mL for *S. typhi*, 25 and 30 mg/mL for *S. flexineri*, 25 and 30 mg/mL for *P. aeruginosa*, 12.5 and 14.5 mg/mL for *S.aureus*, 25 and 31 mg/mL for *S. dysenteriae* and 50.0 and 55 mg/mL for *Klebsiella* species.

The MICs and MBCs of the methanol extract for the reference bacteria were 6.5 and 8.5 mg/mL for *S. aureus* ATCC 85923, 100.0 and 125.0 mg/mL for *E. coli* ATCC 25922, and 50.0 and 75.0 mg/mL for *P. aeruginosa* NCTC 10662. For the bacterial isolates, the MICs and MBCs of the methanol extract were 100 and 125 mg/mL for *E.coli*, 100 and 120 mg/mL for *S. typhi*, 12.5 and 13.5 mg/mL for *S. flexineri*, 12.5 and 14 mg/mL for *P. aeruginosa*, 5 and 7.5 mg/mL for *S. aureus*, 12.5 and 13.5 mg/mL for *S. dysenteriae* and 100 and 125 mg/mL for *Klebsiella* species.

Table 10: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for *F. sur* acetone and methanol extract

Micro- Organisms	Acetone		Methanol	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>E. coli</i>				
ATCC 25922	50.0±0.26	65.0±0.36	100.0±1.56	125.0±1.76
<i>S. aureus</i>				
ATCC 85923	5.0±0.02	7.5±0.04	6.25±0.03	8.5±0.04
<i>P. Aeruginosa</i>				
NCTC 10662	50.0±0.28	60.0±0.27	50.0±0.26	75.0±0.46
<i>E. coli</i> isolate	50.0±0.27	65.0±0.36	100.0±1.56	125.0±3.56
<i>S. typhi</i> isolate	50.0±0.26	60.0±0.86	100.0±1.58	120.0±2.03
<i>S. flexineri</i> isolate	25.0±0.13	30.0±0.13	12.5±0.02	13.5±0.04
<i>P. aeruginosa</i> isolate	25.0±0.15	30.0±0.14	12.5±0.03	14.0±0.04
<i>S. aureus</i> isolate	12.5±0.03	14.5.0±0.06	5.0±0.02	7.5±0.02
<i>S. dysenteriae</i> isolate	25.0±0.04	31.0±0.07	12.5±0.03	13.5±0.04
<i>Klebsiella</i> isolate	50.0±0.26	55.0±0.36	100.0±1.56	125.0±2.76

Values are means ± SD of five determinations; values followed by asterix are significant (p<0.05). Differences between means of the MICs and MBCs were analysed by student's t-test of (SPSS version 12.0 program, Instat. GraphPad Software. Inc., San Diego. CA).

4.6.0 Results of Time kill kinetics

4.4.1 Kill Kinetics of Acetone Stem Extract of *D. cinerea* against *E. coli*

As observed in Figure 16, the population of *E. coli* increased in the control from 2.6×10^6 CFU/ml at the point of inoculation to 8.5×10^7 CFU/ml after 8h. There after the population decreased to 6.8×10^6 in 24h. In the 1mg/ml extract concentration, the population decreased from the initial inoculum to 4.8×10^5 CFU/ml after 8h. There after this increased to 6.8×10^5 in 24h. At 2 mg/ml extract concentration there was a decrease in population from the initial inoculum to 2.4×10^5 CFU/ml after 8h, which kept on reducing to 1.7×10^5 after 24h. In the 4mg/ml extract concentration; there was a sharp decrease in the bacteria population for the first 6h to 3.0×10^8 CFU/ml. This was followed by a steady killing of the entire population in 24h. At 8mg/ml extract concentration the bacteria were all killed in 8h.

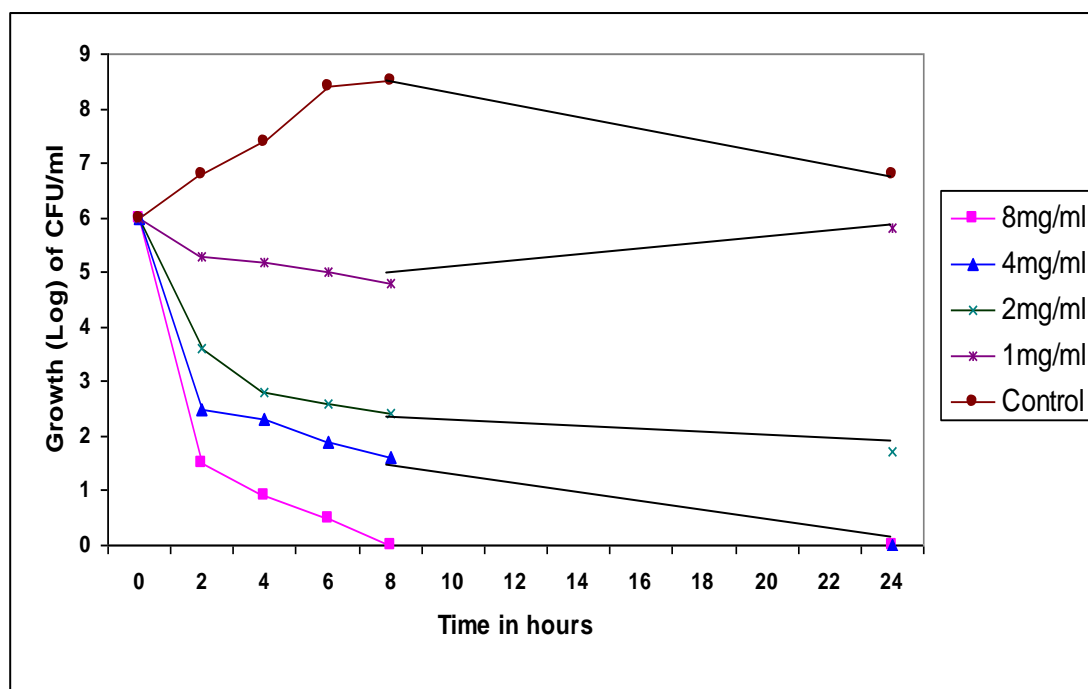


Figure 4: Time kill kinetics activities of acetone stem extract of *D. cinerea* at various concentrations against *E. coli*

4.4.13 Time Kill Kinetics of Acetone Stem Extract of *D. cinerea* against *S. aureus*

As shown in Figure 17, the population of *S. aureus* decreased in all concentrations of *D. cinerea* extracts. In the control, however, the population steadily increased up to 8.7×10^6 CFU/ml within 8h. There after the population decreased and by 24h it was at 6.5×10^6 CFU/ml. In both concentrations of 0.5mg/ml and 1mg/ml the bacteria population decreased to 4.7×10^5 CFU/ml and 2.5×10^5 CFU/ml at 8h, and reduced to 3.3×10^5 CFU/ml and 2.1×10^5 CFU/ml after 24h, respectively. The population of *S. aureus* decreased steadily from the initial inoculum to 1.7×10^5 after 8h in the concentration of 2mg/ml, and got completely killed within 24h. In the extract with a concentration of 4mg/ml, the population of *S. aureus* rapidly decreased within 2h to 2.0×10^5 CFU/ml then the whole population was killed in 8h.

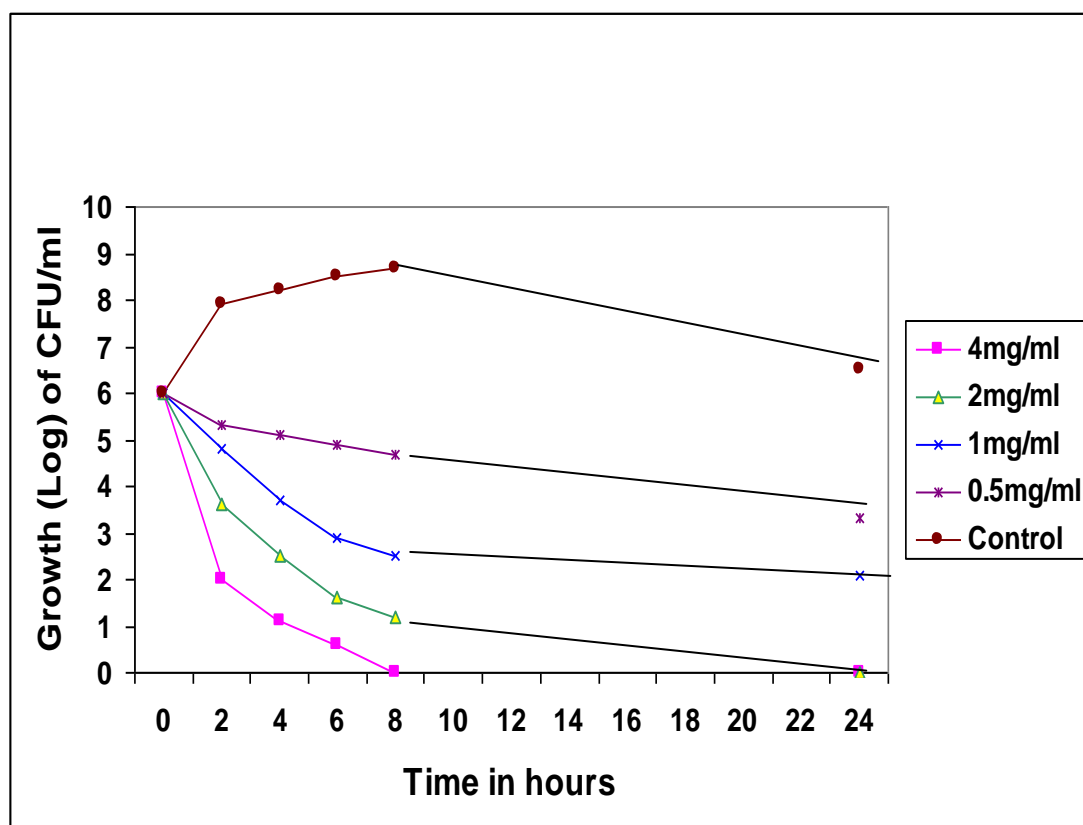


Figure 5: Time Kill kinetics of acetone stem extract of *D. cinerea* at various concentrations against *S. aureus*

4.4.14 Time Kill Kinetics of Acetone Stem Extract of *D. cinerea* against *P. aeruginosa*

Fig. 18 shows the effect of different concentrations of acetone extract of *D. cinerea* on *Ps. aeruginosa*. In the control, the population of bacteria increased from the initial inoculum to 6.7×10^6 CFU/ml within 8h. This decreased slightly to 6.1×10^6 CFU/ml in 24h. When 0.5mg/ml of acetone extract of *D. cinerea* was applied to the population of *P. aeruginosa*, the later remained almost unchanged for 8h, but slightly decreased to 5.3×10^6 CFU/ml in 24h. At 1mg/ml concentration, the population decreased to 3.1×10^5 CFU in 8h from the initial inoculum of 2.6×10^6 . Concentration of 2mg/ml of acetone extract of *D. cinerea* reduced the population of bacteria in 8h to 2.4×10^5 CFU/ml, and then gradually killed the whole population in 24h. When 4mg/ml concentration was used, the *P. aeruginosa* population was completely killed in 8h from the initial inoculum of 2.6×10^6 CFU/ml.

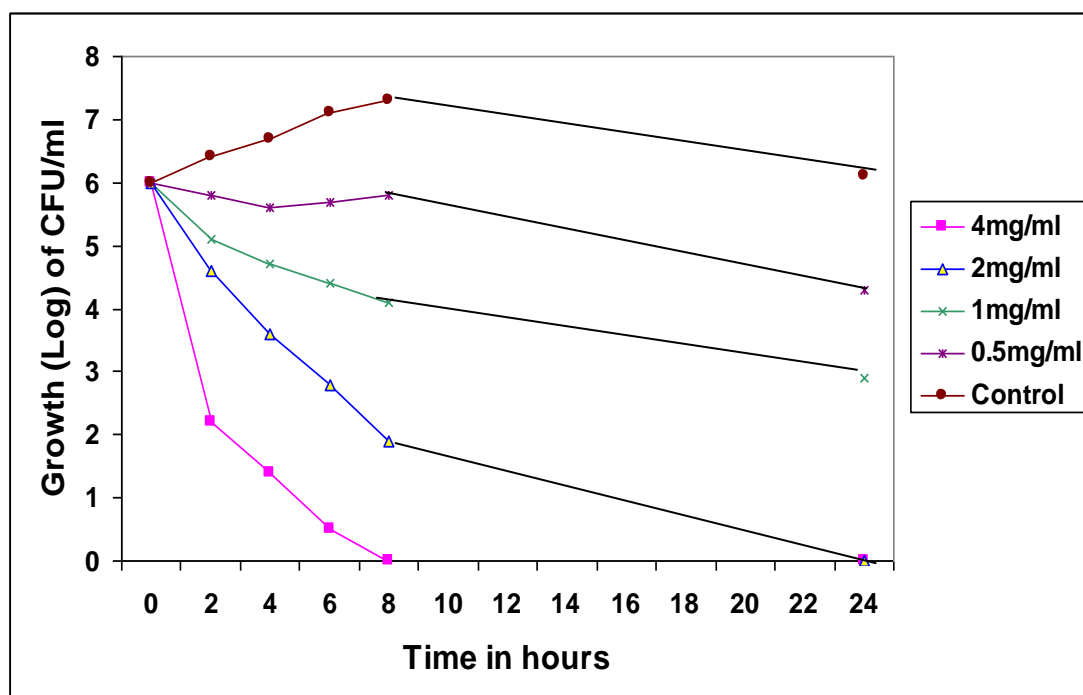


Figure 6: Time kill kinetics of acetone stem extract of *D. cinerea* at various concentrations against *P. aeruginosa*

4.4.15 Time Kill Kinetics of Methanol Stem Extract of *D. cinerea* against *E. coli*

As shown in Figure 19, different concentrations of methanol extract of *D. cinerea* affected the population of *E. coli* differently. In the control, the population increased sharply in 8h to 9.6×10^7 CFU/ml, and then dropped to 5.2×10^7 CFU/ml in 24h. At 0.5mg/ml concentration the population slightly decreased and then increased to 6.2×10^7 CFU/ml in 8h, after which it dropped to 4.2×10^7 CFU/ml in 24h. When *E. coli* population was subjected to 1mg/ml concentration of methanol extract of *D. cinerea*, its population decreased slowly to stand at 4.3×10^5 CFU/ml at 8h.

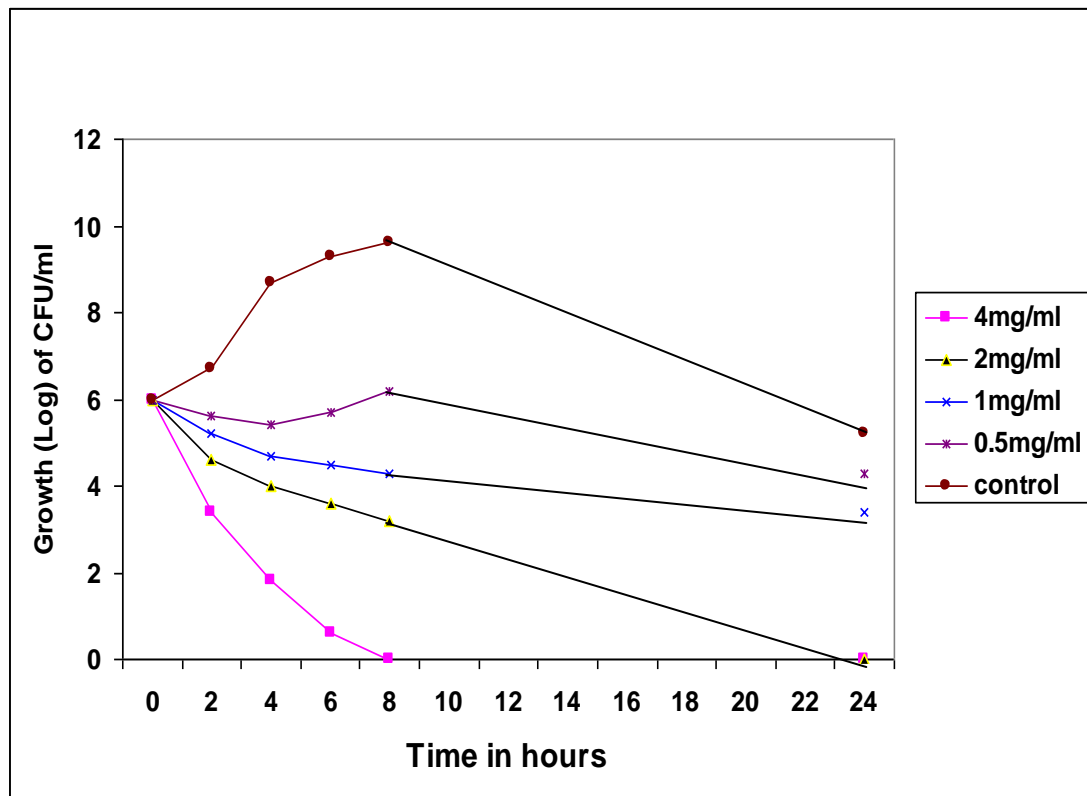


Figure 7: Time kill kinetics of methanol stem extract of *D. cinerea* in various concentrations against *E. coli*

This trend of slow decrease continued on to 3.4×10^5 CFU/ml at 24h. However, the effects of 2mg/ml and 4mg/ml when introduced on to the population of this *E. coli* population were dramatic. The 2mg/ml concentration decreased the population in 8h to 3.2×10^5 CFU/ml, and completely killing the entire population in 24h. The sharp

reduction in the population of the bacteria was witnessed in 4mg/ml where it was completely killed by the 8th h from the initial inoculum of 2.6×10^6 CFU/ml.

4.4.16 Time Kill Kinetics of Methanol Extract of *D. cinerea* against *S. aureus*

As observed in Figure 20, the population of *S. aureus* was affected differently by various concentrations of methanol stem extract of *D. cinerea*. In the control, the population increased from the initial inoculum of 2.6×10^6 CFU/ml to 9.2×10^7 CFU/ml at 8h mark, but then decreased to 6.5×10^6 CFU/ml in 24h. 0.5mg/ml concentration of *D. cinerea* extract killed the population of *S. aureus* completely within 24h from the initial inoculum. The concentration of 2mg/ml sharply killed the population of this bacterium in 8h, while 4mg/ml concentration rapidly killed the population of this organism completely in just 4h.

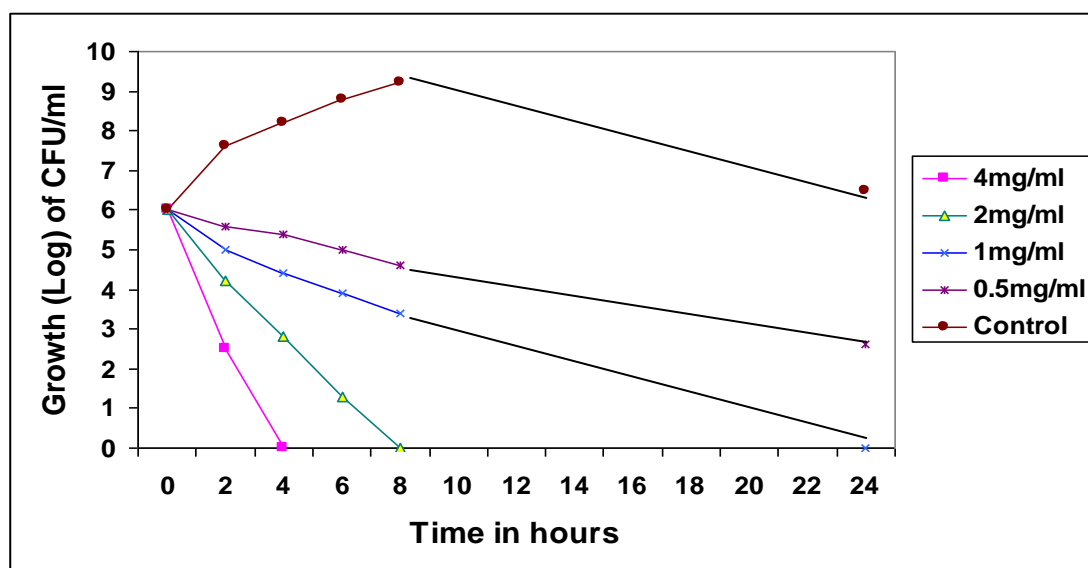


Figure 8: Time kinetics of methanol stem extract of *D. cinerea* at various concentrations against *S. aureus*

4.4.17 Time Kill Kinetics of Methanol Extract of *D. cinerea* against *P. aeruginosa*

The killing rate of methanol stem extract of *D. cinerea* against *P. aeruginosa* is shown in Fig. 21. The results show that the control bacteria population increased from the initial inoculum of 2.6×10^6 CFU/ml to 9.2×10^6 CFU/ml in 6h and 9.9×10^6 CFU/ml in 8h. The population reduced slightly in 24h to 8.2×10^6 CFU/ml. The 0.5mg/ml concentration did not decrease the population of *P. aeruginosa*, so that in 24h, it only reduced it to 2.4×10^6 CFU/ml. 1mg/ml concentration reduced the population of *P. aeruginosa* to 3.2×10^5 CFU/ml, while 2mg/ml reduced it to 1.4×10^5 CFU/ml after 24h respectively. The 4mg/ml extract completely killed the population of *P. aeruginosa* within 8h.

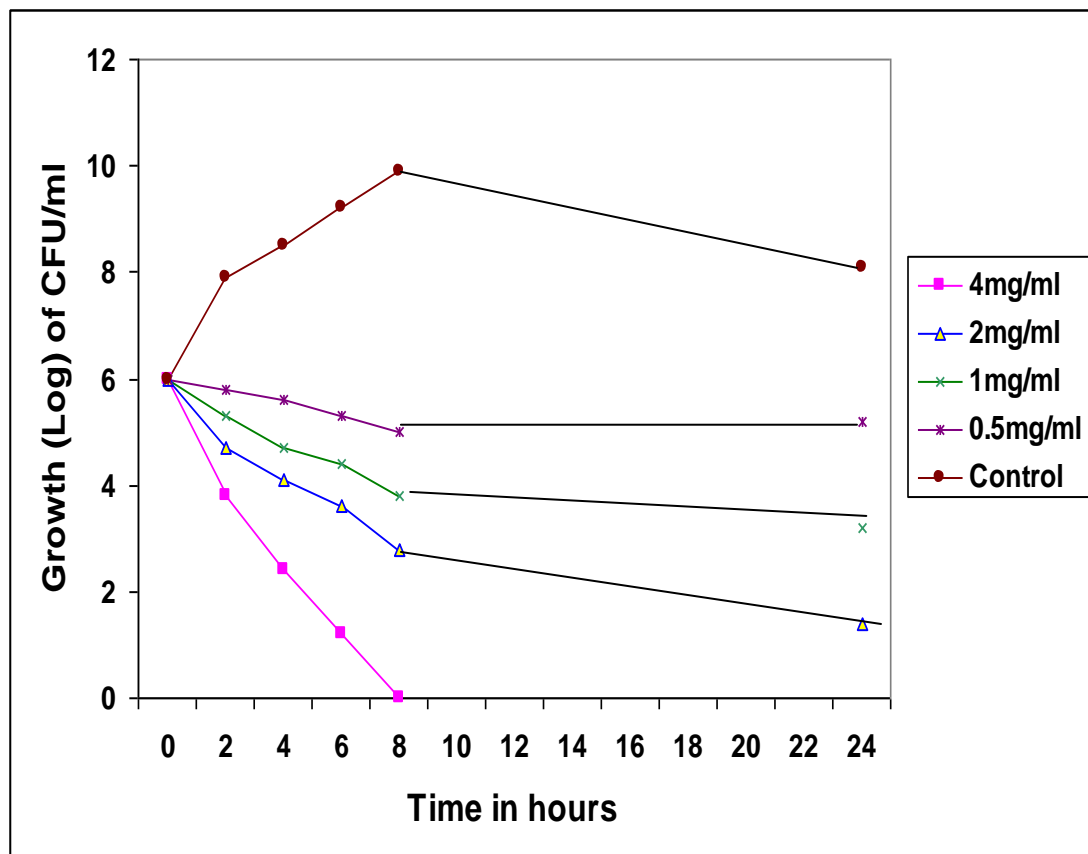


Figure 9: Time kill kinetics of methanol stem extract of *D. cinerea* at various concentrations against *P. aeruginosa*

4.4.18 Time Kill Kinetics of Acetone Extract of *C. molle* against *E. coli*

Fig. 22 shows the death response of a population of *E. coli* to acetone stem extract of *C. molle*. The population in the control culture increased from the initial inoculum of 2.6×10^6 CFU/ml to 9.6×10^6 CFU/ml in 24h. When subjected to acetone extract of *C. molle* at a concentration of 1mg/ml, *E. coli* population decreased for the first 6h and then increased to 6.6×10^6 CFU/ml at 24h. Extract concentrations of 2mg/ml and 4mg/ml reduced the *E. coli* population gradually until it reached 1.2×10^6 CFU and 2.4×10^6 CFU in 24h, respectively. At a concentration of 8mg/ml, the *E. coli* population decreased from the initial inoculum of 2.6×10^6 CFU/ml to zero in 6h.

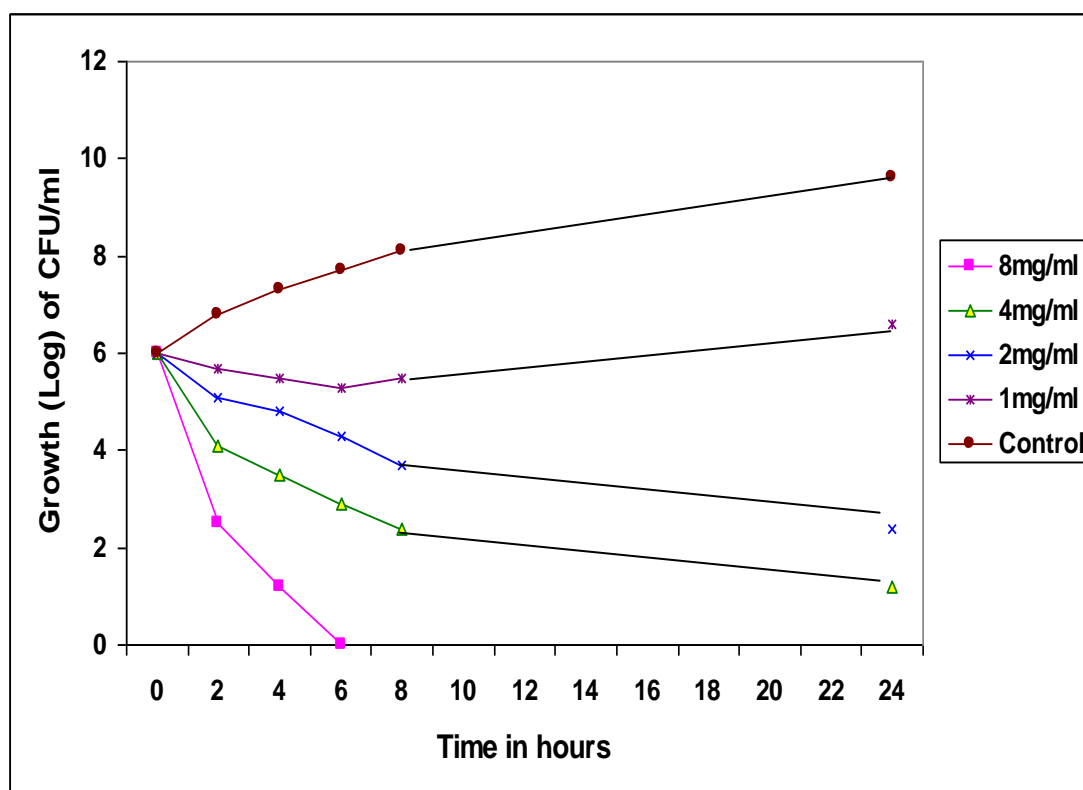


Figure 10: Time kill kinetics of acetone stem extract of *C. molle* at various concentrations against *E. coli*

4.4.19 Time Kill Kinetics of Acetone Extract of *C. molle* against *S. aureus*

The effects of the killing patterns of various concentrations of acetone stem extracts of *C. molle* on *S. aureus* are shown in Figure 23. In the control culture, the population of *S. aureus* increased to 9.2×10^7 CFU/ml in the first 8h and then decreased to 8.1×10^7 CFU/ml. This decrease in *E. coli* population was higher than that of initial inoculum of 2.6×10^6 CFU/ml. At 0.5mg/ml concentration, the population decreased slightly and by 24h it was at 4.7×10^5 CFU/ml. The concentrations of 1mg/ml, 2mg/ml and 4mg/ml had remarkable killing rates on *S. aureus*, by completely killing the population at 24, 8, and 4h, respectively.

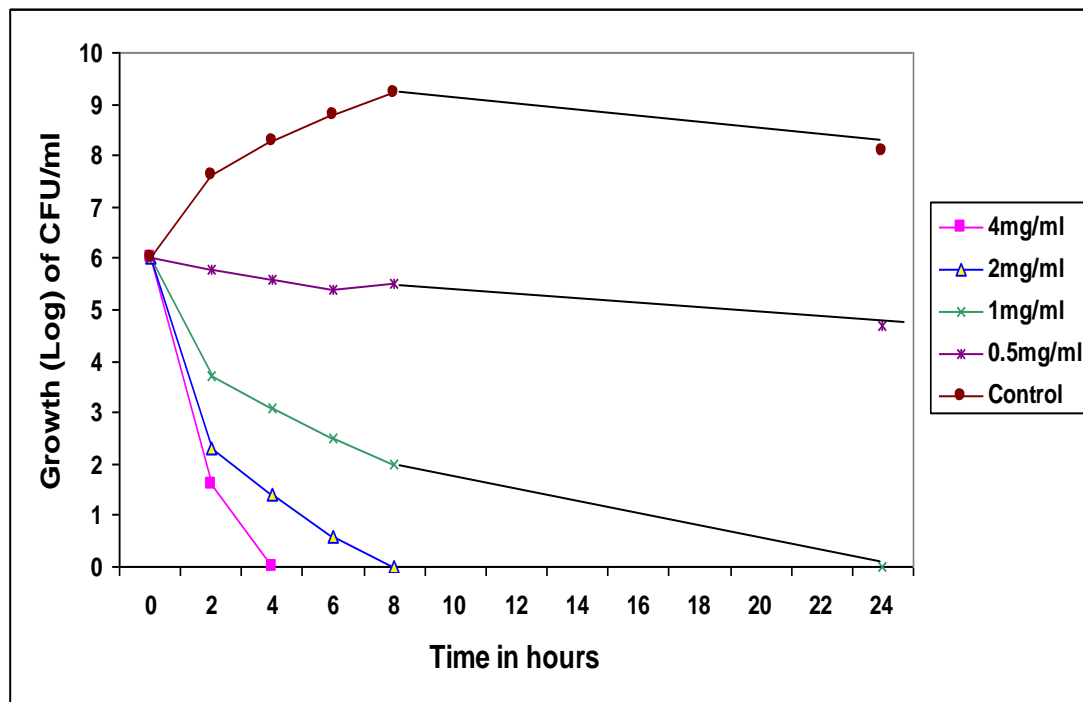


Figure 11: Time kill kinetics of various concentrations of acetone stem extract of *C. molle* against *S. aureus*

4.4.20 Time Kill Kinetics of Acetone Extract of *C. molle* against *P. aeruginosa*

Figure 24 shows the killing effects of various concentrations of acetone stem extracts of *C. molle* on *P. aeruginosa* population. The population in the control steadily increased to 9.7×10^6 in 24h, while in 1mg/ml concentration the population decreased then increased steadily to 7.2×10^6 CFU/ml in 24h from the initial inoculum. 2mg/ml concentration decreased the population in 24h to 2.0×10^5 CFU/ml, but 4mg/ml concentration completely killed the population of the bacteria in 24h, while 8 mg/ml concentration killed the same population in 6h, respectively.

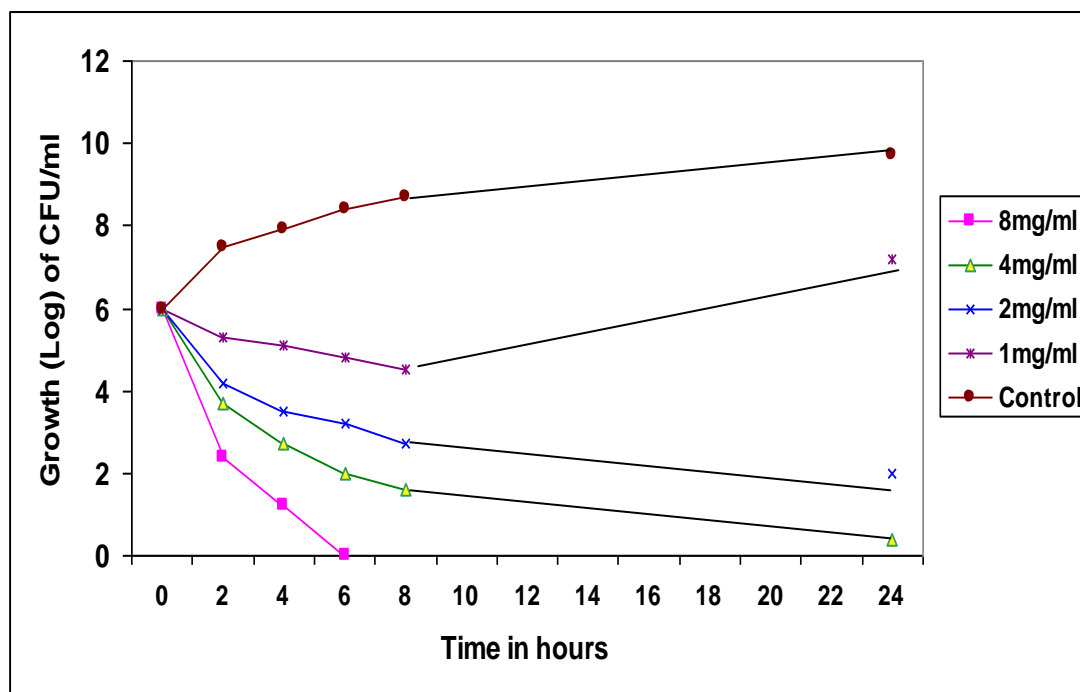


Figure 12: Time kill kinetics of acetone stem extract of *C. molle* at various concentrations against *P. aeruginosa*

4.4.21 Time Kill Kinetics of Methanol Extract of *C. molle* against *E. coli*

Figure 25 shows the killing rate of *E. coli* population by the methanol stem extract of *C. molle*. In the control, the population of *E. coli* increased almost linearly up to 7.4×10^6 CFU/ml in 8h and then decreased to 5.7×10^6 in 24h. When the population of *E. coli* was exposed to 1mg/ml, 2mg/ml and 4mg/ml concentrations it was reduced to 4.1×10^5

CFU/ml, 2.1×10^5 , CFU/ml and 1.2×10^5 CFU/ml, respectively in 24h, from the initial inoculum of 2.6×10^6 CFU/ml. But the concentration of 8mg/ml sharply and completely killed the entire population of *E. coli* within 4h, from the initial inoculum.

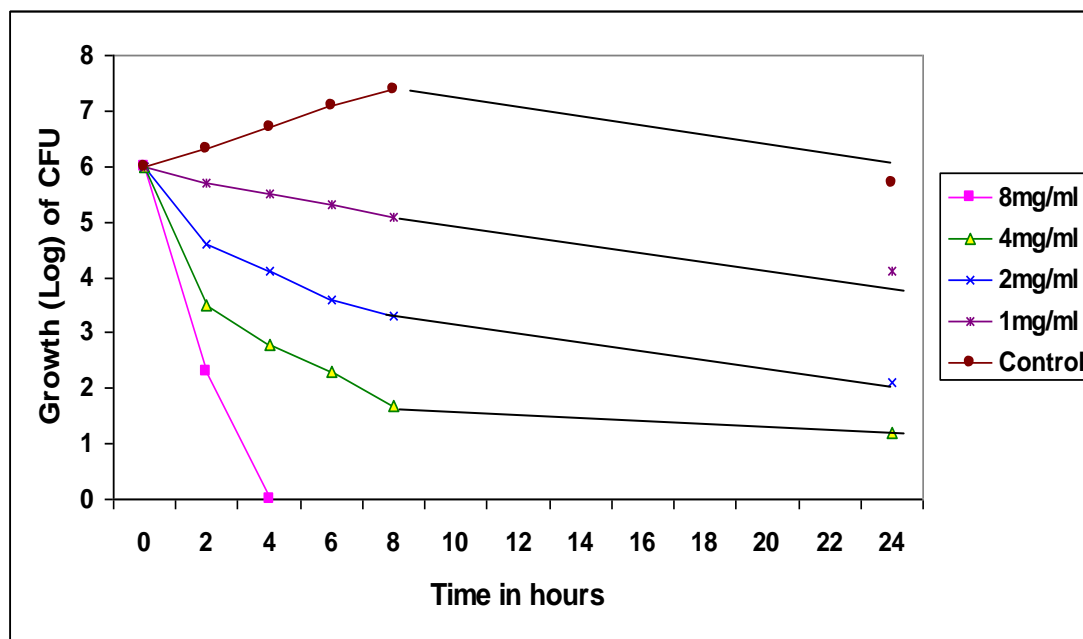


Figure 13: Time kill kinetics of methanol stem extract of *C. molle* at various concentrations against *E. coli*

4.4.22 Time Kill Kinetics of Methanol Extract of *C. molle* against *S. aureus*

When the population of *S. aureus* was exposed to various concentrations of methanol stem extract of *C. molle* it responded as shown in Figure 26. The control bacteria population increased from the initial inoculum of 2.6×10^6 CFU/ml to 9.1×10^7 CFU/ml in 8h, after which it slightly decreased to 8.3×10^7 in 24h. Except for bacteria population in the control, various concentrations of methanol extract of *C. molle* killed *S. aureus* population in varying degrees. At an extract concentration of 0.5mg/ml, the population reduced to 4.2×10^5 CFU/ml in 8h, and then increased slightly to 4.7×10^5 after 24h. 1mg/ml concentration also reduced the population to 2.7×10^5 CFU in 8h, and then again increased marginally to 3.1×10^5 CFU/ml in 24h. The concentrations of 2mg/ml and

4mg/ml completely killed *S. aureus* population in 24h and 6h respectively from the initial inoculum of 2.6×10^6 CFU/ml.

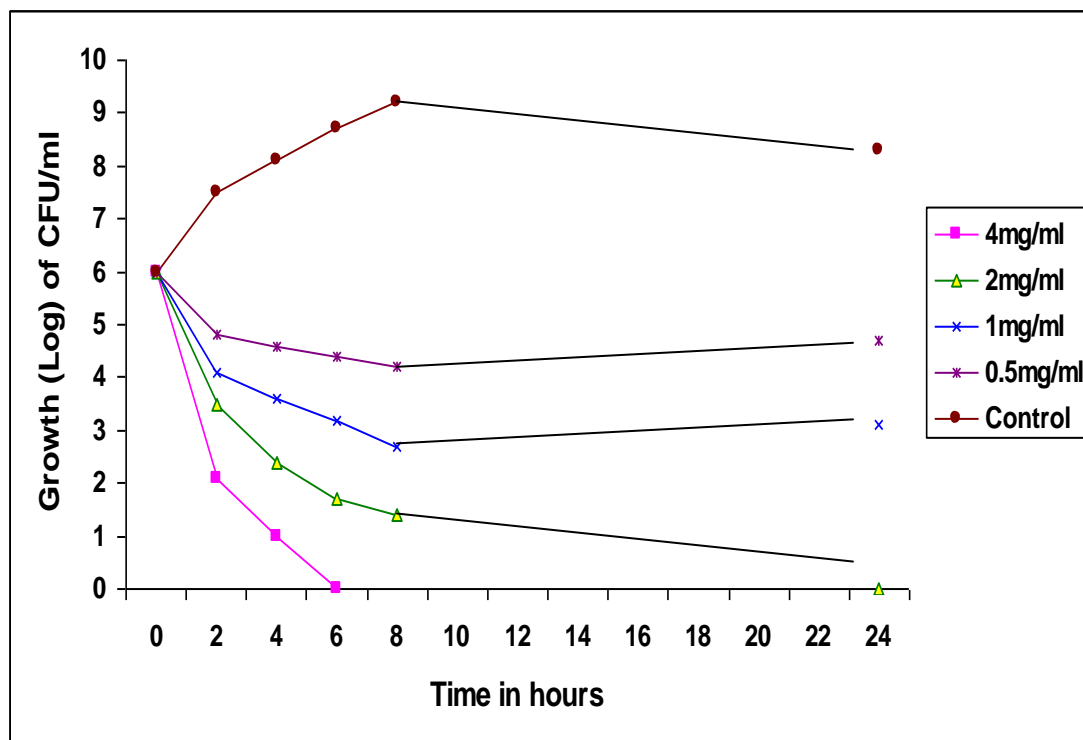


Figure 14: Activities of methanol stem extract of *C. molle* at various concentrations against *S. aureus*

4.4.23 Time Kill Kinetics of Methanol Extract of *C. molle* against *P. aeruginosa*

The killing rates of methanol stem extract of *C. molle* on the population of *P. aeruginosa* are shown in Figure 27. The population in the control increased to 8.2×10^6 CFU/ml within 8hrs, but slightly decreased to 7.8×10^6 CFU in 24h. The concentration of 0.5mg/ml only marginally reduced the population slightly to 5.3×10^5 CFU/ml in 24h. The effect of 1mg/ml concentration on *P. aeruginosa* was similar in pattern to the 0.5mg/ml concentration, only that the former had lower CFU/ml compared to the later. However, the concentrations of 2 mg/ml and 4 mg/ml dramatically reduced and killed *P. aeruginosa* population. 2 mg/ml concentration completely killed the population of *P. aeruginosa* in 24h, while 4 mg/ml concentration killed it in 8h.

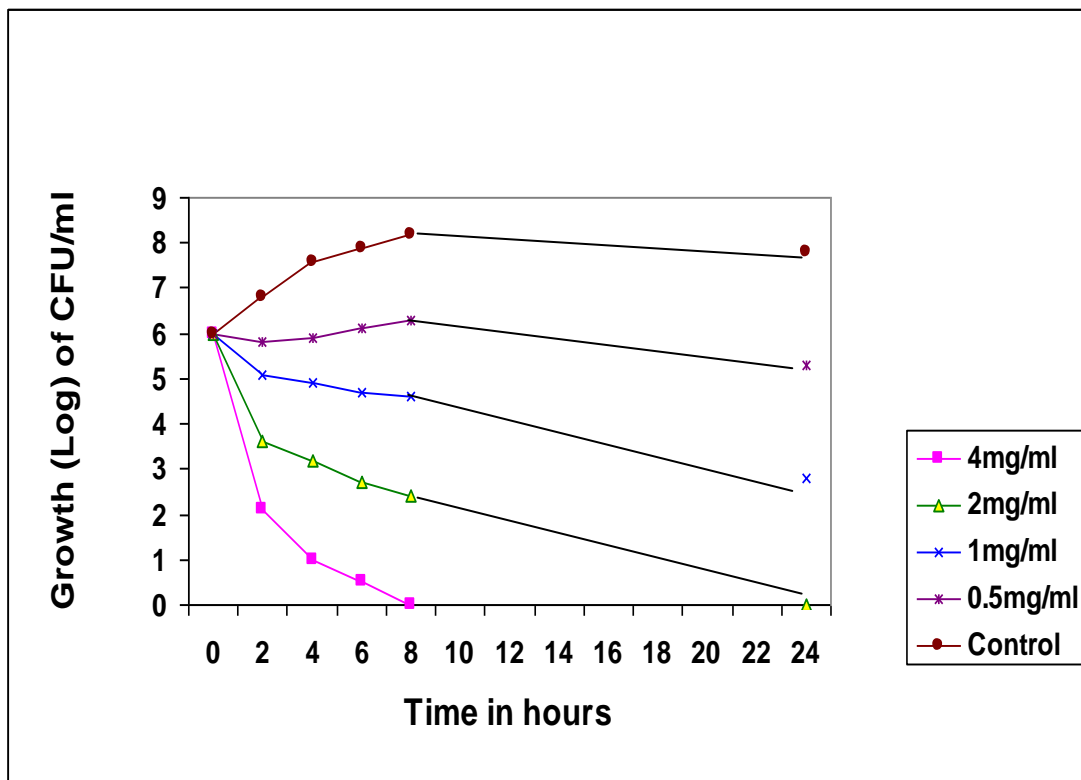


Figure 15: Activities of methanol stem extract of *C. molle* at various concentrations against *P. aeruginosa*

4.7.0 Phytochemicals Determination Tests

4.7.1 Phytochemical Screening of Selected Plant Extracts

Phytochemical screening was done on crude extracts of *C. molle* (acetone and methanol extracts), *D. cinerea* (acetone and methanol extracts), *E. divinorum* (dichloromethane (DCM) extract) and *F. sur* (methanol extract).

The phytochemical compounds present in the plant extracts are shown in Table 11. Acetone and methanol extracts of *D. cinerea*, *C. molle* and *E. divinorum* showed the presence of alkaloids, tannins, anthraquinones, triterpenes and steroids, saponins, flavonoids, coumarins and reducing sugars at varying intensities (Table 11). Methanol extract of *F. sur* had all the tested phytochemicals except alkaloids.

Table 11: Phytochemical determination of selected plants

Phytochemicals	<i>C. molle</i> Methano I	<i>C. molle</i> Acetone	<i>D. cinerea</i> Acetone	<i>D. cinerea</i> Methanol	<i>E. divin.</i> DCM	<i>F. sur</i> Methanol
Alkaloids	+++	+++	+++	+++	++	-
Tannins	+++	+++	+++	+++	+	+
Anthocyanins	+	++	+	+	+	+
Triterpenes and steroids	++	+	+	++	+	+
Saponins	++	+	+	++	++	++
Flavonoids	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Reducing Sugars	+	+	+	+	+	+

Key:+++ the reaction is strongly positive; ++ reaction is moderately positive; + reaction is slightly positive; - reaction is negative.

4.8 In Vivo Toxicity Single Dose Studies

4.8.1 Effect of Oral Administration of 0.5g/Kg Body Weight of the Plant Extracts on Body Weight of Mice for One Month

Table 12 shows the effect of oral administration of acetone and methanol stem extracts of both *C. molle* and *D. cinerea* at 0.5g/kg body weight in mice. Results show that oral administration of acetone and methanol extracts of *D. cinerea* and *C. molle* at 0.5g/kg body weight dose in mice reduced their body weights in the second, third and fourth weeks relative to the control mice.

Table 12: The effect of oral administration of 0.5g of different plant extracts/kg body weight mouse for one month

Treatment (500mg/kg/day)	WEEKS				
	0	1	2	3	4
Control	20.5±0.4	27.6±0.3	29.1±0.3	31.1±0.6	32.1±0.5
<i>Dichrostachys cinerea</i> (Acetone stem extract)	27.6±0.6	29.1±0.5	22.6±1.6*	27.6±0.8*	26.1±0.5*
<i>Dichrostachys cinerea</i> (Methanol stem extract)	26.1±1.3	26.5±0.9	25.8±1.4*	24.9±1.8*	27.4±1.1*
<i>Combretum molle</i> (Acetone stem extract)	25.4±0.3	26.6±0.9	24.9±0.9*	26.3±0.8*	25.9±0.8*
<i>Combretum molle</i> (Methanol stem extract)	26.1±1.7	25.4±1.9	24.7±1.7*	26.8±1.3*	27.8±0.5*

Results are expressed as Mean±SD (SEM) for five animals per group. Differences between mean body weight of the control mice and mice treated with each of the plant extracts was compared using the Student's t test. *P< 0.05 was considered statistically significant.

4.8.2 Effect of Oral Administration of 0.5g/Kg Body Weight Dose of Different Plant Extracts for One Month on Some Organ Weights in Mice

Table 13 shows the effect of oral administration of acetone and methanol stem extracts of both *C. molle* and *D. cinerea* to mice at 0.5g/kg body weight dose for one month on some organ weights. Results show that oral administration of acetone extract of *C. molle* significantly reduced the weight of the heart, liver, kidney and eyes, while *C. molle* methanol extract significantly increased the weight of lungs, and kidneys compared to those of the control mice. Results also show that *D. cinerea* acetone extract significantly decreased the weight of the liver and kidneys while *D. cinerea* methanol extracts significantly reduced the weight of the heart, liver and kidneys compared to those of the control mice.

Table 13: The effect of oral administration of 0.5g/kg body weight of different plant extracts for one month on the mean organ weight (g) in mice

Treatment (mg/kg/day)	Heart Mean±SEM	Lungs Mean±SEM	Liver Mean±SEM	Spleen Mean±SEM	Kidneys Mean±SEM	Testes Mean±SEM	Brain Mean±SEM	Eyes Mean±SEM
<i>C.molle</i> acetone stem extract	0.12±0.01*	0.29±0.02	1.62±0.07*	0.24±0.01	0.35±0.02*	0.20±0.02	0.36±0.01	0.02±0.00*
<i>C.molle</i> methanol stem extract	0.15±0.01	0.40±0.05*	1.86±0.015	0.21±0.02	0.41±0.03*	0.22±0.01	0.36±0.02	0.03±0.00
<i>D.cinerea</i> acetone stem extract	0.13±0.01	0.32±0.02	1.68±0.014*	0.24±0.04	0.40±0.02*	0.19±0.02	0.38±0.03	0.04±0.00
<i>D.cinerea</i> methanol stem extract	0.12±0.01*	0.24±0.01	1.05±0.016*	0.26±0.02	0.37±0.01*	0.16±0.02	0.39±0.01	0.03±0.00
Control	0.15±0.003	0.28±0.02	2.34±0.10	0.28±0.14	0.53±0.02	0.19±0.01	0.41±0.03	0.04±0.00

Results are expressed as Mean±SD (SEM) for five animals per group. Differences between mean of the weight of individual organs of mice treated with each of the plant extracts and the control mice was compared using the student's t test *p < 0.05 was considered statistically significant.

4.8.3 Effect of Oral Administration of 0.5g/Kg Body Weight of Different Plant Extracts for One Month on Some End Point Hematological Parameters in Mice

Table 14 shows the effect of oral administration of different plant extracts at 0.5g/kg body weight dose of different plant extracts for one month on some end point hematological parameters in mice. Results show that oral administration of acetone and methanol stem extracts of *D. cinerea* to mice at 0.5g/kg body for one month significantly decreased WBC compared to the normal control; while oral administration of the same dose of stem acetone stem extracts of *C. molle* for one month to mice significantly increased WBC while the methanol extract of the same plant significantly decreased WBC compared to the control mice.

Results also indicate that the red blood cells (RBC), hemoglobin levels (Hb), packed cell volume (PCV), platelets (PLT), were significantly decreased by oral administration of 0.5g/kg body weight dose to mice daily for one month by *C. molle* and *D. cinerea* methanol and acetone extracts, while the mean cell volume (MCV), mean cell hemoglobin levels (MCH), lymphocytes count (Lym), monocyte count (Mo), and GR count were significantly increased by oral administration of 0.5g/kg body weight dose daily to mice for one month by *C. molle* and *D. cinerea* methanol and acetone extracts.

Table 14: The effect of oral administration of 0.5g/kg body weight of different plant extracts on some end point hematological parameters in mice

<i>Hematological parameters</i>	<i>Control</i>	<i>C. molleacetone stem extract</i>	<i>C. mollemethanol stem extract</i>	<i>D. cinereaacetone stem extract</i>	<i>D. cinereamethanol stem extract</i>
WBC (x10 ³ /μL)	4.06±0.50	8.44±4.84*	2.42±0.48*	2.46±0.38*	2.30±0.22*
RBC (x10 ⁶ /μL)	5.03±0.33	2.92±0.56*	2.72±0.29*	2.34±0.37*	3.11±0.35*
Hb (g/dL)	8.80±0.60	5.34±0.62*	4.64±0.49*	4.0±0.49*	5.34±0.57*
PCV (%)	40.24±1.24	17.98±0.39*	15.96±1.34*	14.72±0.65*	18.24±1.56*
MCV (fL)	48.58±0.87	58.18±1.71*	56.48±0.47*	60.20±1.65*	59.86±1.27*
MCH (pg)	14.22±0.25	16.72±0.27*	16.76±0.12*	17.18±0.61*	16.70±0.42*
MCHC (g/dL)	34.12±0.46	36.16±7.74	29.7±0.23	27.82±0.70	28.16±0.68
PLT (x10 ³ /μL)	339.5±52.9	262.4±77.3	247.2±40.4*	174.4±29.2*	229.4±18.0*
RDW (%)	17.64±0.43	17.10±0.23	16.58±0.20	17.28±0.48	18.02±0.46*
PCT (%)	0.07±0.00	0.08±0.01	0.09±0.01	0.08±0.01	0.12±0.01*
MPV (fL)	3.76±0.05	4.54±0.44	3.84±0.20	5.00±0.37	4.36±0.31
PDW (%)	16.26±0.29	17.32±0.31	17.34±0.29	18.08±0.23	17.26±0.51

Results are expressed as Mean±SD (SEM) for five animals per group. Differences between mean hematological parameters of the control mice and mice treated with each of the plant extracts was compared using the Student's t test. *P< 0.05 was considered statistically significant.

4.8.4 Effect of Oral Administration of 0.5g/Kg Body Weight of Different Plant Extracts on Some End Point Differential Leucocytes Count (DLC) in Mice

Table 15 shows the effect of oral administration of acetone and methanol stem extracts of both *C. molle* and *D. cinerea* to mice at 0.5g/kg body weight dose for one month on some differential lymphocytes count (DLC) in mice. Results show that oral administration of acetone *C. molle* stem extract at 0.5g/kg body weight dose to mice for one month significantly increased the neutrophils and lymphocytes counts compared to those in the control mice; *C. molle* and *D. cinerea* acetone extracts at the same dose for one month significantly decreased the levels of monocytes compared to those in the control mice while the acetone and methanol stem extracts of *C. molle* and the acetone extracts of *D. cinerea* at the same dose for one month in mice significantly decreased eosinophils levels compared to those in the control mice.

Table 15: The effect of oral administration of 0.5g/kg body weight of different plant extracts on some end point hematological parameters [(Differential Lymphocytes Count (DLC)] in mice

Treatment (mg/kg/day)	Differential white blood cell count			
	Neutrophils Mean±SEM	Lymphocytes Mean±SEM	Monocytes Mean±SEM	Eosinophils Mean±SEM
<i>C. molle</i> Acetone stem extract	6.59±0.41*	1.5±0.11*	0.03±0.01*	0.26±0.03*
<i>C. molle</i> Methanol stem extract	1.90±0.37*	0.35±0.11*	0.08±0.03	0.10±0.12*
<i>D. cinerea</i> Acetone stem extract	1.97±0.41*	0.37±0.07*	0.04±0.02*	0.08±0.02*
<i>D. cinerea</i> Methanol stem extract	1.79±0.42*	0.35±0.10*	0.06±0.02	0.11±0.02
Control	2.51±0.31	1.17±0.07	0.15±0.02	0.22±0.04

Differential lymphocytes count (DLC %) is calculated by multiplying the absolute number of each white cell type by the number of each cell counted (expressed as a decimal fraction) by the total WBC count.

4.8.5 Effect of Oral Administration of 0.5g/Kg Body Weight of Different Plant Extracts for One Month on Some End Point Biochemical Parameters in Mice

Table 16 shows the effect of oral administration of acetone and methanol stem extracts of both *C. molle* and *D. cinerea* to mice at 0.5g/kg body weight dose for one month on some end point biochemical parameters. Results show that oral administration of *C. molle* acetone and methanol stem extracts significantly decreased the levels of blood urea nitrogen (BUN), aspartate aminotransferase activity (AST), creatine kinase activity (CK) while the methanol extract of *C. molle* significantly decreased the ALT activity compared to the values in the control mice; Oral administration of *D. cinerea* acetone stem extracts significantly decreased the level of blood urea nitrogen (BUN) and significantly increased the activities of AST and CK, while *D. cinerea* methanol stem extracts significantly decreased the activity of AST and CK, and increased the activity of ALT compared to those of the control mice.

Table 16: The effect of oral administration of 0.5g/kg body weight of different organic plant extracts for one month on some end point biochemical parameters in mice

Treatment (mg/kg/day)	BUN (mM) Mean±SEM	AST (IU/L) Mean±SEM	ALT (IU/L) Mean±SEM	ALP (IU/L) Mean±SEM	CK (IU/L) Mean±SEM
<i>C. molle</i> Acetone stem extract	3.9±0.7*	115.4±12.4*	122.0±18.2	301.6±35.1	374.4±54.4*
<i>C. molle</i> Methanol stem extract	2.8±0.5*	76.2±8.03*	68.0±14.8*	235.6±19.01	269.0±27.5*
<i>D. cinerea</i> Acetone stem extract	2.3±0.1*	187.2±83.7	181.0±67.8*	304.6±50.8	438.6±92.4*
<i>D. cinerea</i> Methanol stem extract	4.6±0.8	111.8±37.1*	152.2±33.4*	233.8±34.4	333.4±61.5*
Control	5.4±0.7	145.0±15.2	127.8±13.1	225.8±32.84	166.2±24.3

Results are expressed as Mean±SD (SEM) for five animals per group. Differences between mean of the measured biochemical parameters of the control mice and mice treated with each of the plant extracts was compared using the Student's t test. *P< 0.05 was considered statistically significant.

4.8.6 Mineral Composition of Acetone and Methanol Stem Bark Extracts of *C. molle* and *D. cinerea* (in $\mu\text{g}/100\text{g}$)

Table 17 shows the trace element composition of the organic plant extracts used in this study. Results show that acetone and methanol stem bark extracts of *C. molle* and *D. cinerea* contain the minerals potassium (K), calcium (Ca), iron (Fe), copper (Cu), Zinc (Zn), bromine (Br), rubidium and the toxic heavy metal lead (Pb) at varying concentrations.

Table 17: Mineral composition of organic stem bark extracts of *C. molle* and *D. cinerea* (in $\mu\text{g}/100\text{g}$)

Daily trace element administered ($\mu\text{g}/\text{day}$)				
Element	<i>C. molle</i> acetone stem extract	<i>C. molle</i> methanol stem extract	<i>D. cinerea</i> acetone stem extract	<i>D. cinerea</i> methanol stem extract
K	BDL	362000 \pm 9292	723333 \pm 32641	201333 \pm 6333
Ca	BDL	8445 \pm 15	BDL	BDL
Mn	BDL	BDL	BDL	BDL
Fe	2196 \pm 79	1029 \pm 24	47200 \pm 462	35366 \pm 491
Cu	1170 \pm 10	BDL	1300 \pm 10	BDL
Zn	BDL	BDL	2960 \pm 21	1733 \pm 32
Pb	799 \pm 57	918 \pm 24	BDL	BDL
Br	472 \pm 46	629 \pm 28	1980 \pm 36	856 \pm 3

Results are expressed as Mean \pm SD for three replicates for each plant extract. BDL indicates that the minerals levels in these plant extracts are below the limit of detection by TRXF.

4.8.7 Quantity of Minerals in 0.5g/Kg Body Weight Plant Extracts Administered to Each Mouse per Day (Mg/Day)

Table 18 shows the amount of minerals present in acetone and methanol stem extracts of both *C. molle* and *D. cinerea* orally administered to mice at 0.5g/kg body weight dose per day ($\mu\text{g}/\text{g}/\text{day}$ (μl)) for one month. Results show that the daily administration of element Potassium (K) in *C. molle* methanol and *D. Cinerea* stem acetone and methanol and bromine

(Br) in *C. molle* methanol and *D. cinerea* methanol extracts were above the daily requirements, while the mineral Iron (Fe) in both *C. molle* and *D. cinerea* acetone and methanol stem extracts were below the daily requirements.

Table 18: The mineral composition of the organic stem bark extracts of *C. molle* and *D. cinerea* (in µg/500g)

Element	Daily mineral administration (µg/day)				RDA for mouse/day (µg/day)
	<i>C. molle</i> acetone stem extract	<i>C. molle</i> methanol stem extract	<i>D. cinerea</i> acetone stem extract	<i>D. cinerea</i> methanol stem extract	
K	-	9.05	18.08	5.03	0.075 ^m
Ca	-	0.21	-	-	1.3 ^m
Fe	0.05	0.03	1.18	0.884	10.71
Cu	0.029	-	0.033	-	0.32
Zn	-	-	0.074	0.043	6.79
Pb	0.02	0.023	-	-	None
Br	0.012	0.016	0.05	0.021	0.08 ^m

Results are expressed as Mean for three replicates for each plant extract. -indicates that the minerals levels in these plant extracts are below the limit of detection of TRXF.

4.8.9 Histopathology

Histological examination of liver, kidney and intestine specimens from normal mice and mice orally treated with methanol stem extract of *C. molle* (0.5g/kg/body weight/day) for 30 days are shown on Plates 1, 2, 3, 4, 5 and 6, respectively.

4.8.8.1 The Liver

The normal liver of a mouse observed shows intact hepatocytes (Plate 1). Normal liver hepatic cords are one cell thick and show a “streaming effect” towards the central vein. The heart showed no pathology. Liver specimens obtained from mice treated orally with a

methanol stem extract of *C. molle* (0.5g/kg/body weight/day) for 30 days revealed liver infiltrates, vacuolar degeneration, venous congestion and necrosis of liver cells (Plate 2). The same liver specimen exhibited liver fatty degeneration. The liver cells also showed swollen hepatocytes (arrows), distended cytoplasm and prominent congestion of blood vessels.

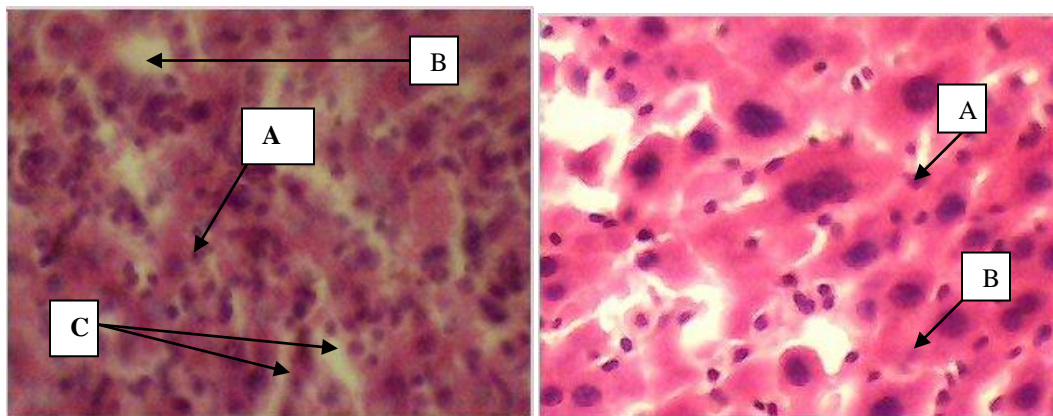


Plate 1: Histological section of a normal liver of a mouse. Note: intact hepatocytes (arrow A), normal unobstructed central vein (arrows B) and sinusoids (arrows C)
Magnification: Camera 640x480 pixels

Plate 2: Histological section of a liver of a mouse treated orally with a methanol stem extract of *C. molle* (0.5g/kgbw/day) for 30 days. Hypertrophy of sinusoidal liver cells and congestion of blood vessels is observable (arrows A). Mild steatosis with small fat droplets in the liver (arrow B)
Magnification: Camera 640x480 pixels

4.8.8.2 The Kidney

The observed kidney of a normal mouse showed intact blood vessels (Plate 3). The kidney, heart and spleen showed no pathology. Examination of kidney tissues from mice treated orally with a methanol stem extract of *C. molle* (0.5g/kg/body weight/day) for 30 days revealed infiltrates and tubular atrophy (arrows) (Plate 4). There was an intense infiltration of inflammatory cells causing necrosis and loss of cellular details (arrows). These features are consistent with an acute nephritis.

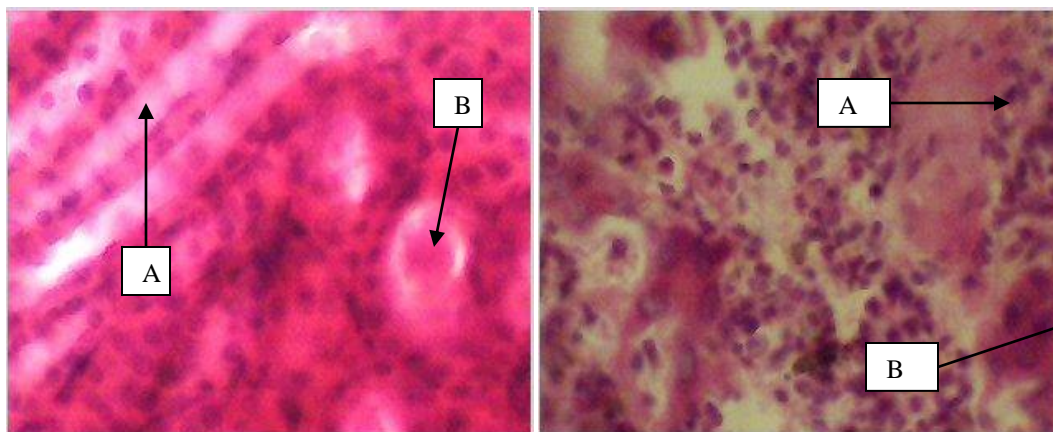


Plate 3: Histological section of a normal kidney from a mouse orally treated with normal food and water for 30 days. Note that the renal tubules are closely compressed (arrow A) and blood vessel (arrow B). Magnification: Camera 640x480 pixels

Plate 4: Histological section of a kidney from a mouse treated orally with methanol stem extract of *C. molle* for 30 days. Note that there is an intense infiltration of inflammatory cells causing necrosis and loss of cellular details (arrows A). Features are consistent with an acute nephritis (arrow B). Magnification: Camera 640x480 pixels

4.8.8.3 The Intestines

There is a moderate infiltration of the villus streama by more inflammatory cells and minimal oedema (arrow).

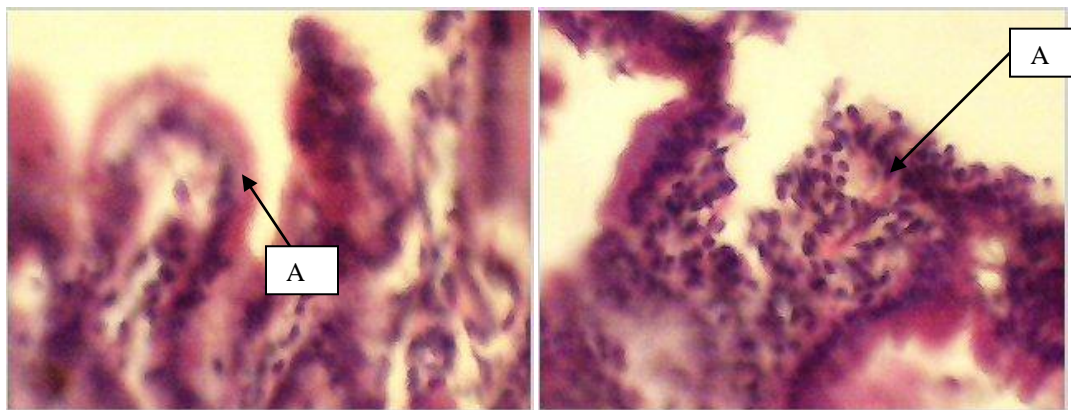


Plate 5: Histological section of intestines from a normal mouse treated with normal food and water for 30 days. Note the structure of the normal villi (arrow A). Magnification: Camera 640x480 pixels

Plate 6: Histological section of intestines from a mouse treated with methanol stem extract *C. molle* for 30 days. Note that there is a moderate infiltration of the villus streama by more inflammatory cells and minimal oedema (arrow A). Magnification: Camera 640x480 pixels

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The inhibition of growth of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella spp.* by acetone and methanol stem extracts of *D. cinerea*, *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *Klebsiella spp.* and *Shigella dysenteriae* by acetone and methanol extracts of *C. molle*, *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *Shigella flexineri*, *S. dysenteriae* and *Klebsiella spp.* by dichloromethane extract of *E. divinorum*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *S. dysenteriae* and *Klebsiella* isolates by acetone and methanol extracts of *F. surindicata* that these extracts contain antibacterial constituents. These inhibitory constituents could be contributed by the phytochemicals present in these plant extracts. Acetone and methanol extracts of *C. molle* and *D. cinerea*, and dichloromethane extracts of *E. divinorum* contain alkaloids, tannins, saponins, flavonoids, anthocyanins, triterpenoids, coumarins and reducing sugars, while the methanol extracts of *F. sur* contain the same phytochemicals as *C. molle*, *D. cinerea*, *E. divinorum* extracts except alkaloids. The phytochemicals present in these plant extracts have been reported to inhibit growth of both gram negative such as *E. coli*, *P. aeruginosa*, *S. typhi*, *S. flexineri*, *S. dysenteriae* and *Klebsiella spp.* and gram positive bacteria such as *S. aureus* (Chukwujekwu *et al.*, 2011; Souâd *et al.*, 2009; Selime *et al.*, 2010; Bylka *et al.*, 2004; Doss *et al.*, 2009; Funatogawa *et al.*, 2004; Karou *et al.*, 2005) and are therefore acting as broad spectrum antimicrobials.

Alkaloids such as ramiflorines A and B, cryptolepine, and quindoline have been reported to be active against both gram positive and gram negative bacteria such as *S. aureus*, *E. coli*, *S. flexneri* and *Shigella boydii* (Karou *et al.*, 2005).

Flavonoids such as luteolin-7-O-glycoside, luteolin-7,3'-O-diglycoside, apigenin, quercetin-3-O-glycoside, kaempferol-3-O-glycoside, abyssinone-V 4'-O-methyl ether, 6,8-diprenylgenistein, alpinumisoflavone, burttinone, baicalin, naringin, erylatissin A, erylatissin B, and erylatissin are reported to be active against both gram positive and gram negative bacteria such as *Bacillus subtilis*, *S. aureus*, *Bacillus cereus*, *E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa* (Akroum *et al.*, 2009; Chukwujekwu *et al.*, 2011). Coumarins such as cniforin A and edultin are reported to be active against *S. aureus* and *P. aeruginosa*.

Saponin extracts have been reported to be active against *B. cereus*, *B. subtilis*, *Enterococcus faecalis*, *E. coli*, *S. Typhi*, *S. aureus*, *P. aeruginosa*, (Abbasolu and Türköz, 1995; Kredy, 2010; Hassan *et al.*, 2010); Saponins such as medicagenic acid, hederagenin and 3-O- $\{\beta$ -D-glucopyranosyl-(1 \rightarrow 6)- $[\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranosyl-(1 \rightarrow 4)- $[\beta$ -D-glucopyranosyl-(1 \rightarrow 2)]- β -D-xylopyranosyl}-2,16-dihydroxy-23,29-dihydroxymethyl-olean-11,13(18)-diene-28-oic acid are active against *S. aureus*, *E. faecalis*, *B. subtilis*, *B. cereus* and *S. pneumoniae* (Avato *et al.*, 2006).

Crude tannin fraction from *Solanum trilobatum* Linn has been reported to inhibit the growth of *S. aureus*, *P. aeruginosa*, *S. typhi* and *E. coli*. Monomeric, dimeric and trimeric

hydrolyzable tannins have been reported to inhibit the growth of *Helicobacter pylori* (Funatogawa *et al.*, 2004).

Triterpenoids such as 1α , 23β -dihydroxy-12-oleanen-29-oic-acid- 23β -O- α -4-acetyl-rhamnopyranoside and 1, 22-dihydroxy-12-oleanen-30-oic acid are active against *S. aureus* and *E. coli* (Angeh *et al.*, 2007); α and β -amyrin are active against *S. aureus*. Camaric acid is active against *S. typhi*, *P. aeruginosa* and *S. aureus* (Innocent, 2011). The coumarins such as osthénol and edultin are active against *S. aureus* and *P. aeruginosa* (de Souza *et al.*, 2005; Ng *et al.*, 1996).

For organic extracts which exhibited similar values for MIC and MBC against the test bacteria, then the MIC of the extract indicated the bactericidal activity while for those extracts which exhibited an MBC greater than MIC, then the MIC of the extract indicated the bacteriostatic activity. Bactericidal agents prevent the emergence of resistant mutants by killing the microorganisms (Anantharaman *et al.*, 2010).

Acetone extracts of *D. cinerea* are generally more active to both the gram positive (which have a cell wall) and the gram negative bacteria (which do not have a cell wall) than the methanol extracts since they exhibit lower MICs and MBCs indicating that the antibacterial constituents were more concentrated in the acetone extracts and acted as broad spectrum antibiotics. Generally, plant extracts are more active towards gram positive bacteria than towards gram negative bacteria (Basri and Fan, 2005).

For *C. molle* extracts, methanol extracts were generally more active against both the gram positive and the gram negative bacteria than the acetone extracts since they exhibit lower MICs and MBCs indicating that the antibacterial constituents were more concentrated in the methanol extracts and again acted as broad spectrum antibiotics. The antibacterial action of these extracts occurs via several mechanisms such as inhibition of nucleic acid, protein, and cell wall synthesis and inhibition of transcription (Leonard *et al.*, 1988; Terao *et al.*, 1988; Morse *et al.*, 1986; Holten *et al.*, 2000).

Kill kinetics studies of the bioactive organic extracts on the tested bacteria indicated that some extracts such as the methanol extracts of *D. cinerea* at 4mg/ml against *S. aureus* ATCC 85923, acetone extracts of *C. molle* at 4mg/ml against *S. aureus* ATCC 85923 completely killed bacteria populations within four hours. Acetone extracts of *C. molle* at 8mg/ml against *E. coli* ATCC 25922, *P. aeruginosa* NCTC 10662 and methanol extracts of *C. molle* at 8mg/ml against *S. aureus* ATCC 85923 completely killed the test bacteria population within six hours, while acetone extracts of *D. cinerea* at 8mg/ml against *E. coli* ATCC 25922, at 4mg/ml against *S. aureus* ATCC 85923, at 4mg/ml against *P. aeruginosa* NCTC 10662 (8 hours), at 4mg/ml against *E. coli* ATCC 25922 and methanol extracts of *D. cinerea* at 2mg/ml against *S. aureus* ATCC 85923, at 4mg/ml against *P. aeruginosa* NCTC 10662 killed bacteria population within eight hours. Acetone extracts of *C. molle* at 2mg/ml against *P. aeruginosa* NCTC 10662, and methanol extracts of *C. molle* at 4mg/ml against *P. aeruginosa* NCTC 10662 also killed bacteria population within eight hours. All these extracts acetone and methanol extracts of *D. cinerea* and *C. molle* displayed dose and time dependent rapid kinetics of the bacterial killing.

Acetone extracts of *D. cinerea* at 4mg/ml against *E. coli* ATCC 25922, at 2mg/ml against *S. aureus* ATCC 85923 and *P. aeruginosa* NCTC 10662 and methanol extracts of *D. cinerea* at 2mg/ml against *E. coli* ATCC 25922, at 1mg/ml against *S. aureus* ATCC 85923 killed bacteria populations within 24h. Acetone extracts of *C. molle* at 1mg/ml against *S. aureus* ATCC 85923, at 4mg/ml against *P. aeruginosa* NCTC 10662 and methanol extracts of *C. molle* at 2mg/ml against *S. aureus* ATCC 85923 and *P. aeruginosa* NCTC 10662 killed bacteria populations at 24h. All these acetone and methanol extracts exhibited slow dose and time dependent kinetics of the bacterial killing for both Gram negative and Gram positive bacteria. Antibacterial agents that display rapid kinetics of bacterial killing can potentially contain infection with greater rapidity than those that exhibit slow killing kinetics (Anantharaman *et al.*, 2010).

A time-dependent bactericidal effect takes place when the concentration of the antibacterial exceeds the MIC for the microorganism while concentration-dependent bactericidal effect occurs when an antibiotic has a high concentration at the binding site for it to eliminate the microorganism (Anantharaman *et al.*, 2010).). In addition, kinetic studies of the bioactive organic extracts on the tested bacteria indicated that acetone extracts of *D. cinerea* at 2mg/ml against *E. coli* ATCC 25922 and at 0.5mg/ml and 1mg/ml against *S. aureus* ATCC 85923 and *P. aeruginosa* NCTC 10662, and methanol extracts of *D. cinerea* at 0.5mg/ml and 1mg/ml against *E. coli* ATCC 25922, at 1mg/ml against *S. aureus* ATCC 85923, at 1mg/ml and 2mg/ml against *P. aeruginosa* NCTC 10662 revealed existence of bacterial strains whose growth inhibition was bacteriostatic. Acetone extracts of *C. molle* at 2mg/ml and 4mg/ml against *E. coli* ATCC 25922, at 1mg/ml against *S. aureus* ATCC 85923, at 0.5mg/ml against

P. aeruginosa NCTC 10662, and methanol extracts of *C. molle* at 1mg/ml, 2mg/ml and 4mg/ml against *E. coli* ATCC 25922, at 0.5mg/ml and 1mg/ml against *P. aeruginosa* NCTC 10662) also indicates the existence of bacterial strains whose growth is inhibited (bacteriostatic) and therefore resisting antibacterial action.

This long-term survival of bacterial strains in the presence of an antibiotic even beyond 24 hours is due to the production of osmotically stable L-forms that remain viable and even multiply under the influence of the antibiotic and regrow to vegetative forms after the antibiotic is inactivated. The two types of L-forms include unstable L-forms, which can divide but can revert to the original morphology and stable L-forms, which are unable to revert to the original forms. L-forms of bacteria are strains of bacteria that lack cell walls (Leaver *et al.*, 2009). The L-forms surviving antibacterial clearance even after 24 hours in this study may be stable L-forms.

Further, kinetics studies of extracts at concentrations below the MIC (such as the methanol extracts of *D. cinerea* at 0.5mg/ml against *P. aeruginosa* NCTC 10662, acetone extracts of *C. molle* at 1mg/ml against *E. coli* ATCC 25922 and *P. aeruginosa* NCTC 10662, methanol extracts of *C. molle* at 0.5mg/ml and 1mg/ml against *S. aureus* ATCC 85923) initially inhibited bacterial growth up to 8hrs and thereafter promoted their growth, a phenomenon common to bacteria resistant to antibiotics. Such L-forms of bacteria are osmotically stable L-forms that remain viable and even multiply under the influence of the antibiotic and regrow to vegetative forms after the antibiotic is inactivated (Leaver *et al.*, 2009). L-forms can develop from Gram-positive as well as from Gram-negative bacteria. The L-forms are

generated in a culture medium that has the same osmolarity as the bacterial cytosol (an isotonic solution), which prevents cell lysis by osmotic shock (Leaver *et al.*, 2009). For *E. coli* the resistance may be due to genetic factors or due to cell membrane permeability. The resistance attributed to *Pseudomonas aeruginosa* could be capsule related (Woolfrey and Enright, 1990). *Pseudomonas aeruginosa* is very adaptable and tolerant to antibiotics (Morse *et al.*, 1986). In conclusion, the phytochemicals present in these plants contribute to their activity against gram positive and gram negative bacteria and thus justifies their continued use in traditional medicine.

Because the toxicity of a drug to the bystander host cells could render it unsuitable for therapeutic purposes, the toxicity of the active plant extracts against the tested bacteria was assessed. The reduced growth rate in plant extract treated animals in relation to that of the controls indicate that these extracts contained constituents which either retard growth. Such constituents include some of the phytochemicals present in these extracts. Such phytochemicals may include saponins, alkaloids, terpenoids and tannins. The mineral content may also cause toxicity but this was not the case with the measured minerals since their levels were below the recommended daily allowance except for potassium which was 67 to 141 times the recommended daily allowance. These phytochemicals include alkaloids, tannins, terpenoids, saponins, flavonoids, coumarins, anthocyanin's, and sugars which have been reported to be toxic. Retarded growth could have resulted from reduced feed intake of the animals due to either the feeds bitter taste or reduced appetite by the animals to the feed or the bad smell of the feed (Tucci, 2010). Parameters assessing feed intake, lack of appetite or the bad smell of the feed were however, not determined in this study.

Some of the toxic alkaloids include confine, solanine, methyllycaconitine, nudicauline, and geyerline, 2-pentylpiperidine (Radulović *et al.*, 2011), and pyrrolizidine alkaloids (Wiedenfled, 2011). Tannins reduce feed intake by decreasing palatability and by reducing feed digestion. Palatability is reduced because tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. Low palatability depresses feed intake and thus efficiency of production. Digestibility reduction negatively influences intake because of the filling effect associated with undigested feed stuff. Tannins are divided into two: hydrolyzable and condensed tannins. Hydrolyzable tannins are converted by microbial metabolism and gastric digestion into absorbable low molecular weight metabolites such as tannic acid which are toxic. The major lesions associated with hydrolyzable tannins poisoning are hemorrhagic gastroenteritis which decreases absorption of nutrients, necrosis of the liver, and kidney damage with proximal tubular necrosis. Hemorrhagic gastroenteritis is confirmed histologically by the infiltration of villus streama by more inflammatory cells and minimal oedema of intestines of animals administered with 0.5g *C. molle* methanol extract/kg body weight.

Protanthocyanidins (PAs) (condensed tannins) retard growth by inhibiting feed intake and digestibility (Click and Joslyn, 1969). Proanthocyanidins (PAs) which are not absorbed by the digestive tract, damage the mucosa of the gastrointestinal tract, decreasing the absorption of nutrients such as proteins and carbohydrates and essential amino acids such as methionine and lysine. They also increase excretion of proteins and essential amino acids and alter the excretion of certain cations. This may have contributed to reduced body weight in the extract treated animals could also be partly explained by the general reduction in the weight of the

liver, kidneys and heart; however, oral administration of 0.5g daily of *C. molle* methanol extract/kg body weight animal had no effect on the heart weight.

The changes in organ weight can partly be explained by the reduction in red blood cell count, hemoglobin concentration, packed cell volume, and an increase in mean cell hemoglobin and mean cell volume, (macrocytic anemia) induced by the daily administration of animals with 0.5g of plant extracts/kg body weight mouse. Reduction in red blood cell count could either result from increased hemolysis or reduced nutrient absorption leading to malnutrition. The nutrients requiring normal absorption for normal growth of mice include the vitamins, proteins, carbohydrates, lipids, and minerals.

Macrocytic anemia may be induced by a deficiency of either vitamin B₁₂ or folate; these vitamin deficiencies causes a reduction in DNA synthesis in all mitotic tissues with preserved RNA synthesis resulting in restricted cell division of the progenitor cells. However, it takes a few weeks of a folate free diet to induce deficiency while it takes several years of a vitamin B₁₂ free diet to induce deficiency. Folate absorption is largely through the jejunum while vitamin B₁₂ absorption is only through the terminal ileum, and intrinsic factor (IF) is an absolute requirement. Intrinsic factor (IF), a protein secreted by gastric parietal cells, binds to the vitamin B₁₂ and carries it to the terminal ileum, where there are IF receptors, and absorption occurs. The transport protein of vitamin B₁₂ in the blood is Transcobalamin-II (TC-II). Deficiency of folate or vitamin B₁₂ causes premature cell death which results in a reduced number of mature erythrocytes.

Macrocytic anemia results in tissue hypoxia. Tissue hypoxia causes most tissues to initially enlarge and as the swollen cells continue rupturing, the organ size reduces (organ atrophy) (Voet and Voet, 2004). During tissue hypoxia, cells which rely only on glycolysis for ATP production rapidly deplete the store of phosphocreatine (a source of rapid ATP production) and glycogen. As the rate of ATP production decreases below the level required by membrane ion pumps for the maintenance of proper intracellular ionic concentrations, the osmotic balance of the cell is disrupted so that the cell and its membrane enveloped organelles swell. The overstretched membrane becomes permeable thereby leaking their enclosed contents. The decreased intracellular pH that accompanies anaerobic glycolysis because of lactic acid production permits the released lysosomal enzymes which are only active at acidic pH to degrade the cell contents. Among the degraded components are the initially elevated serum enzymes which are later reduced to values below the control values. The reduced metabolic activity results in irreversible cell damage (Voet and Voet, 2004).

The decreased or increased levels of white blood cell count and increased neutrophil and decreased lymphocytes, eosinophils and monocytes observed in animals orally administered with 0.5g daily of *C. molle* methanol extract and *D. cinerea* methanol and acetone extracts/kg body weight could be associated with liver/tissue necrosis and bone marrow disorders caused by toxic constituents in the extracts. The reduced levels of white blood cell count imply a reduced ability of the body to respond to infection (Alberts, 2005). Reduced platelet counts in animals orally administered with 0.5g daily of plant extracts/kg body weight for one month could be associated with reduced production in the bone marrow caused by toxic constituents such as phytochemicals in the plant extracts.

Kidney, liver and heart atrophy resulting from necrosis induced by toxic constituents in the plant extracts could explain the decreased levels of blood urea nitrogen, a biomarker for kidney damage, aspartate and alanine aminotransferase biomarkers for liver damage and creatine kinase a biomarker of heart muscle damage. Liver damage was histologically confirmed by the observation that liver specimens from animals orally administered with 0.5g daily of *C. molle* methanol stem extract/kg body weight for one month revealed liver infiltrates, vacuolar degeneration, prominent congestion of blood vessels, swollen hepatocytes, distended cytoplasm, cell necrosis and fatty degeneration; kidney damage was histologically confirmed by the observation that kidney specimen from animals orally administered with 0.5g daily *C. molle* methanol stem extract/kg body weight for one month revealed infiltrates and tubular atrophy, intense infiltration of inflammatory cells, necrosis and loss of cellular detail-features consistent with acute nephritis. Saponins hemolyse red blood cells and cause cell death of many tissues (Al-Sultan *et al.*, 2003; Diwan, 2000).

Alkaloids have been reported to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia (Zeinsteger *et al.*, 2003). Terpenoids have been reported to increase membrane permeability to divalent and monovalent ions (Zeinsteger *et al.*, 2003). In conclusion, the phytochemicals present in the organic extracts of *C. molle* and *D. cinerea* contributes to their antibacterial activity and toxicity to the bystander cells of the host organism.

5.2 Conclusions

In conclusion, the findings of this study indicate that:

33.3% (4/12) of the plants used in this study demonstrated antibacterial activity using the agar paper disc diffusion method and the standard broth microdilution method. Acetone and methanol extracts of *C. molle* and *D. cinerea* demonstrated the best antibacterial activity against the tested gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexineri*, *Shigella dysenteriae*) and gram positive (*Staphylococcus aureus*) bacteria. The organic extracts contained triterpenoids, tannins, flavonoids, alkaloids, saponins, anthrocyanins and glycosides which have previously been reported to possess antibacterial activity.

Daily administration of 1g organic extracts/kg body weight of *C. molle* and *D. cinerea* orally to mice for one month demonstrated toxicological effects as evidenced by changes in the measured hematological and biochemical parameters, body and organ weights and histological indices. The causes of the observed toxicological effects could have been the saponins, alkaloids, terpenoids, anthrocyanins and tannins present in these plants which have previously been demonstrated to be toxic. These extract constituents caused malnutrition due to reduced nutrient absorption and macrocytic anemia which resulted in tissue hypoxia leading to the observed body, and tissue/organ abnormalities.

5.3 Recommendations

- (i) The mechanism behind antibacterial activity for the organic extracts of *C. molle* and *D. cinerea* should be established.
- (ii) Identification of phytochemical (bioactive) compound(s) directly associated with antibacterial activity of the two plants and assess whether the individual isolated compounds retain or lose their activity or are more active than their parent crude extracts. Comprehensive toxicity (long term studies on reproductive, toxicokinetic profiling and carcinogenic tests) of the compounds with antibacterial activity should be done.
- (iii) Investigation of combination dosages of *C. molle* and *D. cinerea* extracts should be done to create a rationale for a combination therapy in the management of bacterial diseases.
- (iv) After a thorough verification of bioactivity and safety of combination dosages of *C. molle* and *D. cinerea* extracts, potentially easy-to-consume bioactive extracts of these plants should be developed.
- (v) Isolation and Identification of the specific compounds of *C. molle* and *D. cinerea* plants responsible for the observed toxicological effects should be established.
- (vi) Since *C. molle* and *D. cinerea* exhibit both positive and negative biological effects, care should be taken in their use. However, in normal traditional medical practice, several plants are normally combined to give the drug used. This practice may improve efficacy and lower toxicity.

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Appendix A

A Sample of a questionnaire used to Gather Ethnopharmacological Information about Medicinal plants from Herbal Medicine Practitioners for this Research

Appendix B

Elements analysis Readings Showing Elements Present in the Plant Extracts