

**ANTIMICROBIAL ACTIVITY, CYTOTOXICITY AND PHYTOCHEMISTRY OF
SELECTED MEDICINAL PLANTS IN MERU CENTRAL DISTRICT, KENYA**

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A thesis submitted in partial fulfillment of requirements for the degree of Master of Science in
Pharmacology and Toxicology of University of Nairobi

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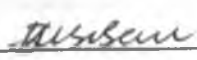
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DECLARATION

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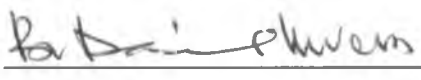
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DEDICATION

This thesis is dedicated to my wife Caroline Kirema-Musau and my children, son Edgar Allan Musau and daughter Sheila Muthoni Musau

On each bank of the stream all kinds of trees will grow to provide food.The trees will provide food and their leaves will be used for healing people.

(Ezekiel 47:12 Good News Bible)

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LIST OF ACRONYMS AND ABBREVIATIONS

ATCC	American type culture collection
AU	African Union
BA	Blood Agar
Cfu	Colony forming units
GoK	Government of Kenya
HIV/AIDS	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
ICRAF	International Centre for Research in Agro Forestry
MBC	Minimum Bactericidal Concentration
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum Inhibitory Concentration
THP	Traditional Health Practitioner
TMP	Traditional Medical Practitioner
TSA	Tryptone Soya Agar
WHO	World Health Organization

ABSTRACT

In Kenya, over 70% of the population relies on traditional medicine as source of primary healthcare due to its accessibility, affordability and acceptance. Traditional medicine and western medicine are used side by side depending on the cost of conventional medicine. The cost, side effects, toxicity and emerging resistance to antimicrobials has led to natural products being investigated as promising agents for the treatment of diseases.

Meru Central District has inequitable distribution of health facilities and people have to walk at least 7 kilometers to these facilities. High HIV/AIDS prevalence and low doctor / patient ratio means many people seek medical services from TMP. This study aimed at identifying and screening medicinal plants from this area for their pharmacological, toxicological and phytochemical properties.

Twenty three (23) herbal practitioners identified 86 plants from 37 plant families of ethnomedical interest. The plants were botanically identified by a plant taxonomist at the Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi. Selection of plants for this study was based on the informant consensus among the identified herbal medicine practitioners on their use for treating microbial infections in human.

Five (5) plants and seven (7) plant parts were selected and evaluated for their antimicrobial activity, cytotoxicity and phytochemical properties. They are *Piliostigma thonningii* (leaves and stem bark), *Ajuga remota* (leaves), *Ocimum suave* (leaves), *Erythrina abyssinica* (root bark and stem bark) and *Harissonia abyssinica* (whole plant).

Standard bacteria cultures of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* were used to determine the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of the plants' methanolic and water extracts through standard microbial techniques at the Department of Public Health, Pharmacology and Toxicology University of Nairobi. Comparison was computed for susceptibility of bacterial species and considered significant at $p < 0.05$.

Cytotoxicity of the methanolic extracts was determined using brine shrimp lethality test with serial dilutions of 1000 μ g/ml, 100 μ g/ml and 10 μ g/ml. Lethal concentration 50 (LC₅₀) at 95% confidence intervals was determined using Probit analysis. Established phytochemical tests were performed to show the presence or absence of secondary metabolites.

All the plants showed activity against the test bacteria except *Erythrina abyssinica* stem bark. *Piliostigma thonningii* stem bark was most active against the test bacteria. There was no difference in antibacterial activity between the methanolic and water extracts. Gram positive bacteria were more susceptible to the extracts than gram negative bacteria. *Bacillus cereus* was most susceptible while *Escherichia coli* most resistant. All the extracts had significant bioactivity at LC₅₀ < 1000 μ g/ml. *Ajuga remota* was most toxic with LC₅₀ of 69.24 μ g/ml. Tannins and saponins were present in all extracts. Flavones and anthraquinones were absent.

The plants had appreciable antibacterial activity. The results of this study support the use of these plants by the herbalists. *Piliostigma thonningii* stem bark exhibited the most antibacterial activity. Further studies should be carried out on this plant regarding its antimicrobial potential. More research is needed to document and scientifically validate the plants used by the Meru community.

CHAPTER ONE: INTRODUCTION

1.0 Background

Plants have a long history of use in treatment and management of different disease conditions. They have been used for treatment of many diseases all over the world since ancient times and about 25% of current drugs are derived from plants (Wanyoike *et al.*, 2004). In certain African countries up to 90% of the population relies exclusively on plants as sources of medicines (Hostetmann *et al.*, 2000). This ethnobotanical utilization of plants for treatment of various diseases, antidote against magic and for religious ceremonies forms the basis of traditional medicine (Kokwaro, 1993).

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health as well as to prevent, diagnose, improve or treat physical and mental health (WHO, 2009). It is the aggregate of beliefs, practices, measures, activities, pursuits and procedures of all kind which from prehistoric times enabled man to alleviate suffering prevent and cure diseases. Traditional healers have for centuries been the main providers of primary health care in Africa (Kala *et al.*, 2004).

Traditional medicine is an integral part of every culture in Kenya. It is affordable, socially acceptable and easily accessible. It is efficacious and holistic in its approach and caters for among other things the spiritual, emotional and physical care of the clients (Kareru *et al.*, 2007; Miaron *et al.*, 2004; Munguti, 1997; Njoroge and Busman, 2007). Like any other community in Kenya, the Meru community has a large traditional medicine base. A survey in Meru Central District identified 86 plants in 37 families, as having ethnomedical and ethnoveterinary uses (Itonga, personal communication).

Plants contain pharmacologically active compounds. Knowledge of the chemical constituents of plants is desirable for the discovery of therapeutic agents and other substances of economic value such as tannins, gums and oils (Chetri *et al.*, 2008). Identifying the chemical constituents of plants and understanding the probable actions of such constituents on humans and animals provides the rationale for traditional use of such plants in treating and managing disease conditions (Kisangau *et al.*, 2007; Njoroge and Busman, 2007).

The appearance of multidrug resistant pathogens and others with reduced susceptibility to available antimicrobials adds to the urgency for the search of new infection fighting strategies.

Due to the increase in HIV/AIDS prevalence and re-emergence of diseases like tuberculosis, the spectra of untreatable or difficult to treat microbial infections is increasing. The side effects and toxicity of available antimicrobials and their high cost further complicates the current methods of fighting infections (Bii, 2001; Janovska *et al.*, 2003; Runyoro *et al.*, 2006).

Efforts are now directed towards finding new and innovative antimicrobial agents with novel mechanisms of actions. Natural products of higher plants are probable sources of antimicrobial agents which have added advantages of being safe and biodegradable (Adenisa *et al.*, 2000).

Plants may serve as natural blue prints in the development of new drugs or as phytomedicines to be used to treat disease (Abubakar *et al.*, 2008). Infections associated with bacterial pathogens are among some of the indications for treatment with traditional remedies that include plant products (Njoroge and Busman, 2007).

Many drugs owe their origin to plants and Wanyoike *et al* (2004), estimated that about 25% of drugs are derived from plants. In some cases, natural materials continue to be the only viable

commercial source of the active compound. Glaxo Wellcome harvests 10000 metric tons dry weight of poppy capsule per year to provide a source of opiate alkaloids and about 170 metric tons per year of *Digitalis lantana* leaf as the source of digoxin (Evans, 2005).

Chemical studies of medicinal plants provide a valuable base for the discovery and development of new drugs of natural origin. This is because the medicinal value of plants lies in the chemical substances that produce a definite action on the body (Musyimi *et al.*, 2008). The use of medicinal plants by herbalists must be validated, regulated and evaluated to avoid the administration of dangerous concoctions and to provide scientific proof of their usage. Such validation includes antimicrobial assays, antiprotozal activity, anthelmintic activity and cytotoxicity (Alluri *et al.*, 2005; Camacho *et al.*, 2003; Moses *et al.*, 2006; Wasswa and Olila, 2006).

1.1 Research problem and justification

The substantial contribution to human health and well being made by medicinal plant species is now widely appreciated and understood. The demand for medicinal plant species is increasing and more people are interested in use of medicinal plants to treat and manage diseases. There is increase in knowledge among the general population regarding the side effects and toxicity of conventional drugs and the perceived safety of natural products.

More than 70% of Kenyan population relies on traditional medicine as the primary source of healthcare and 90% of Kenyan people use medicinal plants at one time or another. Plant cures and other traditional herbal remedies are particularly suitable for use in rural communities because they are inexpensive, sustainable and culturally appropriate alternatives to conventional treatments which are expensive. Meru central district has a doctor/ patient ratio of 1:33,259 and

HIV/AIDS prevalence of 38%. The health facilities in the district are not equitably distributed and the road network is poor.

The average distance to the nearest health facility is 7 km and most of the people have to walk to these facilities (GOK, 2005; 2007). This low doctor/patient ratio, the high HIV/AIDS prevalence, the inequitable distribution of health facilities and the inaccessibility to basic health services to the populace forces many people in the district to seek medical attention from traditional medical practitioners.

Antimicrobial resistance has become a global problem. Plants and other natural products are now being thoroughly researched as possible sources of new biologically active compounds with novel mechanisms of action. The major starting point for this research is folklore medicine including traditional remedies. Many traditional remedies in Kenya have not been scientifically validated. Elaborative ethnobotanical research has not been carried out in Meru Central District and there is little documentation on antimicrobial, cytotoxicity and phytochemical properties of medicinal plants used by the community.

1.2 Null hypothesis

It was hypothesized that the selected plants had antimicrobial activity, were cytotoxic and contained important phytochemical groups.

1.3 Objectives

1.3.1 Overall objective

The overall objective was to study the Pharmacological, Toxicological and Phytochemical properties of medicinal plants in Meru Central District, Kenya.

1.3.2 Specific objectives

1. To analyze the antimicrobial activity of the selected plants.
2. To carry out the cytotoxicity study of the plants extracts.
3. To carry out a preliminary phytochemical analysis of the selected plants.

CHAPTER TWO: LITERATURE REVIEW

2.1 Plants as sources of medicine

Plant molecules, their semi-synthetic and synthetic derivatives are important sources of drugs. They form part of herbal medicines. According to WHO (2009), Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients. From time immemorial, plants have been used as sources of preventive and curative medicinal preparations (Dery *et al.*, 1999). They have been used for both healing and killing depending on the knowledge of their dosage and synergistic action (Barquar, 1995).

Presently, plants continue to be used where in certain developing countries up to 90% of the population relies exclusively on plants as sources of medicine (Hostettmann *et al.*, 2000., Odera 1997). The use of medicinal plants is not unique to the developing countries. It is estimated that 33% of drugs produced in developed countries are from higher plants (Mwangi, 2004). Over 50% of the drugs in clinical trials for cancer were isolated from natural sources, and include Vinblastine, Vincristine, Etoposide, Teniposide and Taxanes (De Mesquita *et al.*, 2009).

Plants have been used as medicine all over the world since before recorded history (Chetri *et al.*, 2008). Years of experimentation with plants enabled man to know useful and harmful plants as well as their healing properties. Plant use is universal and all major systems of medicine in the world include aspects of plants. Several well known species, including Licorice (*Glycyrrhiza glabra*), Myrrh (*Commiphora* species), and Poppy capsule latex (*Papaver somniferum*), were referred to by the first known written record on clay tablets from Mesopotamia in 2600BC.

These plants are still used today for various diseases and as ingredients of official drugs or herbal preparations used in systems of traditional medicine (Young-Won *et al.*, 2006).

References to the use of medicinal plants as cure for diseases is found in the manuscript of "Eber Papyrus" of about 16th century B.C, which recorded the use of *Papaver somniferum*, *Ricinus communis*, *Urginea sp.* and *Aloe sp.* Theophrastus (370-287 BC) described over 500 plants. Piny the elder (23 - 79 AD) in his book *Histroria Naturalis*, described 1000 plants with their medicinal, anatomical and horticultural properties. Dioscorides (1st century AD) described 600 plant species in the book *De Material Medica*. Systematic pharmacopoeias have been developed as early as 3000 BC by the Chinese. Chinese medicinal plant texts date from 4th century BC.

In India, the earliest description of curative properties of plants is in Rig-Veda (2500 – 1800BC). The first London Pharmacopoeia appeared in 1618 and the first British Pharmacopoeia in 1864 (Barquar, 1995; Evans, 2005; Kisangau 1999; Shukla *et al.*, 2000). This long history of medicinal plants is known to have resulted in the development of formal systems of medicine particularly in Egypt, Arabia, India, China and Europe. Adams *et al.*, (2009) in a survey of historical usage of herbal medicine in Europe between 16th and 17th century were able to identify 63 plants that had clear indications.

Use of plants as sources of medicine is a major African socio-cultural heritage where it has been the mainstay of healthcare system for centuries. This knowledge of plants as medicine was passed orally from one generation to the next and often within a family (Elujoba *et al.*, 2005; Miaron *et al* 2004; Munguti, 1997). Despite the long history of plant usage in treating and preventing illnesses, less than 5 % of the world's plants species have been examined for their activity and constituents (Goodwin and Mercer, 2003).

2.2 Plant derived drugs

Plants are major sources of drugs with various functions in the human body. Scrutiny of medical indications by source of compounds has demonstrated that natural products and related drugs are used to treat 87% of all categorized human diseases, including as antibacterial, anticancer, anticoagulant, antiparasitic, and immunosuppressant agents, among others (Young-Won *et al.* , 2006).

2.2.1 Drugs acting on the cardiovascular system

Diseases of the cardiovascular system are a major cause of mortality especially in the developed countries. This has led to intense interest in research on treatment and prevention of cardiovascular diseases. In addition there is increased awareness in the population concerning healthy foods and healthy living, exercise and medicinal plant products to complement the conventional drugs in management and prevention of these diseases.

The plant kingdom is being researched on actively with the aim of identifying compounds that may serve as lead compounds for the synthesis of new drugs. At present various products have been identified that have several activities on the cardiovascular system. Quinidine is derived from the *cinchona* species and is an example of antiarrhythmic drugs derived from plants. It acts on both supraventricular and ventricular arrhythmias. It is currently used in atrial fibrillation (Pengelly, 2004).

Cardiac glycosides increase the force and contractility of the heart. They do this by inhibiting the membrane bound Na^+K^+ -ATPase to cause increase in intracellular Ca^+ hence increased cardiac muscle contraction. Cardiac glycosides increase atrial and ventricular myocardial excitability and also decrease the rate of atrial ventricular contraction. They increase vagal tone and myocardial sensitivity to vagal impulses. Among the cardiac glycosides, the most commonly used are those

from *Digitalis* species. They are Digitoxin from *Digitalis purpurea* and Digoxin from *Digitalis lantana* both of Scrophulariaceae family.

Other cardioactive glycosides are Strophanthin from *Strophanthus gratus* of Apocynaceae family, Convallotoxin and Convaloside from *Convallaria majalis* of Liliaceae family; Odoroside and Oleandrin from *Nerium oleander* of Apocynaceae Family. Some plant derived drugs have antihypertensive activity. Reserpine from the roots of *Rauwolfia serpentina* is used as a hypotensive agent. Other plants with hypotensive activity are *Crataegus*, *Tilia*, *Fagopyrum* and *veratrum* species (Evans, 2005).

Drugs acting on the cellular elements of blood are the platelet activating factor antagonists and the oral anticoagulants. Platelet aggregation leads to adhesion of platelets on the walls of blood vessels and formation of thrombi. This causes narrowing of the blood vessels and when thrombi are dislodged from its attachment in to circulation, heart malfunction occurs. Platelet aggregation is triggered by platelet activating factor which is released from activated basophils. Aspirin is frequently used to prevent thrombi formation. Various plants have been found to have activity against platelet activating factor. Species of *Forsythia*, *Arctium*, *Centipeda*, *Tussilago*, *Pyrola*, *Populous* and *Peucedanum* and the neolignan kadsurenone from *Piper futokadsura* have anti-platelet activating factor activity (Evans, 2005; Pengelly, 2004).

Oral anticoagulants inhibit the clotting mechanism and don't have activity on platelet aggregation. They are of great value in prevention and management of arterial thrombosis. Coumarin derivatives antagonize the effects of Vitamin K therefore preventing coagulation. Plant derivatives of *Melilotus officinalis*, *Gallium aparine* and *Lavandula officinalis* are used in herbal medicine and have activity against Vitamin K (Evans, 2005)

2.2.2 Drugs acting on the nervous system

Drugs acting on the central nervous system are classified as stimulants or depressants. Further classification is based on specific action such as anticonvulsants and other psychopharmacological actions. Drugs acting on the central nervous system include narcotic analgesics such as Morphine and Codeine from *Papaver somniferum*; analeptics like Strychnine from *Strychnos nox-vomica* and Lobeline from *Lobelia inflata* and depressants of motor activity like Atropine and Hyoscine from *Atropa belladonna* (Evans, 2005).

The transmitters of autonomic nervous system are acetylcholine and noradrenaline together with its derivatives. The plant derived drugs acting on the autonomic nervous system either mimic the action of the neurotransmitters or antagonize them. Acetylcholine-like drugs are Pilocarpine from *Pilocarpus jaborandi*, Arecoline from *Areca catechu* and Muscarine from *Amanita muscaria*. Antagonists of acetylcholine include tropane ester alkaloids such as Atropine and Hyoscine from *Atropa belladonna* and neuromuscular blocking agents like Tubocurarine which is obtained from the bark of *Chondodendron tomentosum*. Tubocurarine is a long duration non-depolarizing neuromuscular blocking agent which is a competitive antagonist of nicotinic neuromuscular acetylcholine receptors (Evans, 2005; Pengelly, 2004).

Adrenaline like drugs includes Ephedrine from aerial parts of *Ephedra* species and antagonists of adrenaline are ergot alkaloids such as ergotamine. Ergot alkaloids are derived from the dried sclerotium of fungi *Claviceps purpurea* when it grows in the seeds of rye plant and other grass plants. Reserpine from the roots of *Rauwolfia serpentina* depletes noradrenaline (Evans, 2005; Pengelly, 2004)

2.2.3 Drugs acting on the gastro intestinal system

The bitters stimulate gustatory nerves in the mouth to give rise in the psychic secretion of gastric juice. Examples are the bitters from extracts of *Gentian*, *Quassia*, *Cinchona* and *Nox vomica*. Anticholinergics like Hyoscine and Hyoscyamine from *Atropa belladonna* regulate gastric motility and spasms. Ipecacuanha preparations are used as emetics while ginger is useful in prevention of motion sickness. Dill oil derived from *Anethum graveolens* is used to prevent flatulence in young babies.

Extracts from *Senna* species and castor oil from *Ricinus communis* are examples of laxatives and purgatives derived from plants. Arachis oil from the seeds of *Arachis hypogoea* and Esculine from the bark of *Aesculus californica* are used together with other drugs in suppositories. Morphine and codeine from *Papaver somniferum* reduce gastric motility in addition to their analgesic activity (Evans, 2005; Pengelly, 2004)

2.2.4 Drugs acting on the urinary and reproductive systems

Ergometrine which is derived from *Claviceps purpurea* is used as a uterine stimulant in child birth. Juniper is derived from *Juniperus* species and is a urinary antiseptic. It is also used in treatment of cystitis and urethritis. Yohimbine is derived from *Aspidosperma querbracho* is used in management of erectile dysfunction. *Prunus Africana* bark, *Serenoa repens* fruits and *Urtica dioica* roots are used for management of benign prostate hypertrophy (Evans, 2005; Pengelly, 2004).

2.2.5 Drugs used for treatment of malignancies

Plants have been used for the treatment of malignancies for centuries. Podophyllum was used over 2000 years ago by the Chinese as an antitumor drug. Etoposide and Teniposide are semi-synthetic derivatives of Podophyllotoxin which is derived from *Podophyllum hexadrum* and

Podophyllum emodi. Etoposide is used for small cell lung cancer and testicular cancer. Teniposide is used for pediatric cancers.

The alkaloids of *Cartharanthus roseus* yield Vinblastine and Vincristine which are currently used alone or in combination for treatment of various cancers such as Hodgkins Disease and child hood leukemia. Taxol from *Taxus brevifolia* is approved for treatment of breast cancer. Camptothecin derivatives like Topotecan and Irinotecan are used for ovarian cancer and colorectal cancer respectively. Camptothecin is from *Camptotheca acuminata* (Cragg and Newmann, 2006; Evans, 2005; Pengelly, 2004).

2.2.6 Drugs used on the endocrine system

Metformin is approved for management of non insulin dependent diabetes mellitus. It is derived from *Galega officinalis* that was historically used for treatment of diabetes in medieval Europe (Shukla *et al.*, 2000).

2.2.7 Drugs acting on the respiratory system

Ephedra and Ephedrine from *Ephedra* species and Xanthenes such as Theophilline from *Camellia sinensis* are used as nasal decongestants and bronchodilators. Codeine from *Papaver somniferum* is used in cough mixtures as antitussive. Menthol from *Mentha* spp and eucalyptus oil from *Eucalyptus* spp are used in inhalations and cough preparations for their soothing action (Pengelly, 2004). Tiotropium, a derivative of atropine from *Atropa belladonna* (Solanaceae) and related tropane alkaloids from other solanaceous plants, is a potent reversible nonselective inhibitor of muscarinic receptors.

Tiotropium bromide has been approved by the United States Food and Drug Administration (FDA) for the treatment of bronchospasm associated with chronic obstructive pulmonary disease

(COPD). Tiotropium is structurally analogous to ipratropium, a commonly prescribed drug for COPD, and has shown longer lasting effects (Evans, 2005; Pengelly, 2004; Young-Won, 2006)

2.2.8 Drugs acting on the skin and mucus membranes

Drugs affecting the skin may be emollients, absorbents, astringents, irritants or antiseptics.

Emollients and demulcents include fixed oils like *Arachis* oil fats. Waxes, gums such as acacia from *Acacia* species, and mucilage like psyllium from *Plantago ovata* are used as emollients and demulcents. They are the vehicles used in preparation of ointments, creams and lotions. Absorbents are mainly starch, alginates from sea weed, and charcoal. Astringents are mainly tannins. Antiseptics of plant origin are eucalyptus oil from *Eucalyptus* spp, thyme oil from *Thymus* spp and tars (Evans, 2005; Pengelly, 2004).

2.2.9 Drugs for treatment of infections

Quinine which is isolated from *Cinchona* spp has been one of the most effective agents for management of malaria. It is still used in some third world countries to combat malaria which is resistant to other drugs. Artemisinin and its derivatives from *Artemisia annua* (Asteraceae) are the drugs of choice for treatment of malaria. It is effective for treatment of chloroquine resistant malarial strains of *Plasmodium vivax* and *Plasmodium falciparum*.

Emetine which is an alkaloid from the root of *Cephaelis ipecacuanha* (Rubiaceae) is used as its hydrochloride or bismuthiodide for treatment of amoebic dysentery and Berberine from *Berberis vulgaris* is indicated for treatment of bacillary dysentery. *Echinacea* spp are used as adjuvant therapy in treatment and prophylaxis of upper respiratory tract infections. Thymol from *Thymus vulgaris* is used as a topical antifungal agent while Agrimophol from *Agrimonia eupatoria* is an anthelmintic (Evans, 2005; Pengelly, 2004). Some of the drugs derived from plants and their medical uses are summarized in table 2.1.

Table 2.1: Some of the drugs that have been developed from ethnobotanical leads

DRUG	PLANT SOURCE	MEDICAL USE
Aspirin	<i>Filipendula umaria</i>	Analgesic, anti-inflammatory, antipyretic.
Atropine	<i>Atropa belladonna</i>	Premedication in surgery, pupil dilator
Caffeine	<i>Camellia sinensis</i>	CNS Stimulant
Codeine	<i>Papaver somniferum</i>	Analgesic, antitussive
Colchicine	<i>Colchicum autumnale</i>	Gout management
Digoxin	<i>Digitalis lantana</i>	Atrial fibrillation
Ephedrine	<i>Ephedra sinica</i>	Bronchodilator
Ipecac	<i>Psychotria ipecacuanha</i>	Emetic
Physostigmine	<i>Physostigma venenosum</i>	Glaucoma
Pilocarpine	<i>Pilocarpus jaborandii</i>	Glaucoma
Psoralen	<i>Psoralea corylifolia</i>	Vitiligo
Quinine	<i>Cinchona pubescens</i>	Malaria
Reserpine	<i>Rauwolfia serpentina</i>	Antihypertensive
Scopolamine	<i>Datura stramonium</i>	motion sickness
Theophylline	<i>Camellia sinensis</i>	Management of asthma
Vinblastine	<i>Catharanthus roseus</i>	Hodgkin's disease
Vincristine	<i>Catharanthus roseus</i>	Leukemia

Sources: Evans, 2005; Kisangau, 1999; Pengelly, 2004; Sharma, 2007,

2.3 Classification of plant derived drugs

Classification of drugs derived from plants is by alphabetical, taxonomic, morphologic, pharmacologic/therapeutic and chemical/biogenetic (Evans, 2005)

2.3.1 Alphabetical

This is either Latin or vernacular names. It is used in dictionaries and pharmacopoeias. It is suitable for quick reference. Pharmacopoeias contain information on the composition, preparation and storage of pharmaceuticals. Pharmacopoeias were first formulated by autonomous cities and were intended to provide regulation on the various forms of medicinal preparations available at that time.

However many of pharmacopoeias in existence today do not include herbal preparations except few like the *British pharmacopoeia* and the *Chinese pharmacopoeia*. Many countries in Africa do not have national pharmacopoeias or national drug monographs. Mozambique has a national pharmacopoeia that is legally binding (Evans, 2005; Wagate, 2008). Few have research institutions dealing with herbal medicines. Kenya has not yet developed a national pharmacopoeia and herbal medicine is unregulated. However efforts are been made to regulate and control trade and profession of herbal medicine through an Act of Parliament.

2.3.2 Taxonomic

This is classification on basis of an accepted botanical system. The drugs are arranged according to the plants from which they are obtained. They are also arranged in classes, orders, families, genera and species of the plants (Evans, 2005; Wagate, 2008).

2.3.3 Morphologic

The drugs are divided into groups such depending on the part of plant. The most commonly used plant parts are leaf (folia), flower (flos), fruit (fructus), herb (herba), bark (cortex), rhizome

(rhizome) roots (radix), and bulb (bulbus). Others are latex, resins, gums, woods, seeds, oils, waxes and extracts (Evans, 2005; Wagate, 2008).

2.3.4 Pharmacologic or therapeutic classification

This is classification according to the action of the most important constituent or their therapeutic use. This type of classification is most common as more plants are being screened for their specific pharmacologic activity. (Evans, 2005)

2.3.5 Chemical or biogenetic

This is classification according to the important constituents such as alkaloids, glycosides, volatile oils or their biosynthetic pathways. This classification is biased towards phytochemistry (Evans, 2005).

2.4 Commerce in medicinal plants

Trade in medicinal plants is a major business. It is estimated to be worth US\$60 billion (WHO, 2004). This is because some of the constituents of these plants cannot be synthesized and therefore are only available in natural form. Cameroon is the major source of the bark of *Prunus africana* in the world market. The market value in Europe and America for *Prunus africana* is 150 million US dollars per year. Kenya exports 1923 tons of *Prunus africana* per year while Uganda and Madagascar 193 and 800 tons respectively (Evans, 2005). The bark is used for treating prostate gland hypertrophy (Evans, 2005; Hostetmann *et al.*, 2000).

Harpagophytum procumbens is used as a tonic, as a treatment for arthritis and rheumatism, to reduce fever, ease sore muscles, reduce cholesterol, and externally the ointment is used to treat sores, boils, and ulcers. It is also used to cleanse the lymph system and to remove toxins from the blood. It is produced in southern Africa, and Namibia is the biggest exporter in the region

(Hostetmann *et al.*, 2000; WHO, 2004). Between 10,000 and 15,000 harvesters rely on sales from its collection as their only source of cash (WHO, 2004).

The global demand for *Digitalis lantana* leaf is about 10000 tonnes per year. An analysis of the top selling pharmaceuticals in 1999 shows that 13 of the top selling 30 medicines with combined sales of over \$26 billion have active ingredients whose chemical structure is based on a compound found originally in nature (Evans, 2005).

2.5 Traditional herbal remedies and conventional modern medicine

Herbal remedies are well known natural sources for the treatment of various diseases since antiquity all over the world, and African traditional medicine is among the oldest medicinal system (Gurib-Fakim, 2006; Maregesi *et al.*, 2008; Wanyoike *et al.*, 2004). Many people use herbal remedies and it is estimated that over 80% of the people all over the world largely depend on traditional herbal medicine to meet their primary health care needs (Hamayun *et al.*, 2006). Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system of poor communities. It is the only means of medical treatment for such communities (Yinegar and Yewhalaw, 2007).

Herbal remedies play a major role in traditional medicine in rural areas of Kenya where they are often the therapeutic treatment of choice. Despite the large public and private health expenditures, the health services are still not close to people and healthcare has remained inaccessible to the majority of the population. The health facilities suffer from shortage of trained personnel and inadequate supplies of essential medicines. Besides, some services are not culturally and socially acceptable to some communities (Sindiga, 1995).

In the rural communities, traditional medicine offers a holistic approach to healthcare is socially acceptable, accessible, affordable and presumed to be efficacious. This is in contrast to conventional medicine which lacks all the above except efficacy. Traditional medicine has the widest spatial coverage compared to conventional medicine because each community has its own healers (Mutai *et al.*, 2009; Sindiga, 1995).

However traditional medicine does not keep with scientific and technological advancements. This means that some of the methods and techniques that previously worked have become obsolete. Its rationale is not well defined and some of its aspects like dosage including combinations are not known. Besides, there is no continuity of care since traditional medicine does not keep written records (Sindiga, 1995). It is often passed from generation to generation and often within a family (Miaron *et al.*, 2004). It therefore becomes a secret among the practitioners. This is the greatest impediment to the advancement of traditional medicine. Moreover, the subject of traditional medicine is not taught in health training institutions here in Kenya (Mwangi, 2004).

Traditional herbal medicine is a confluence of two factors: the belief in the efficaciousness of herbal medicine and the high cost and inaccessibility of healthcare (Munguti, 1997). In the developing countries, traditional herbal medicine is often used side by side with western medicine and it takes the upper hand when the cost of western medicine is beyond reach (Busia, 2005).

Plants contain many compounds. One of the most promising areas in the search for new biologically active compounds is the plants used in traditional medicine (Alonso, *et al.*, 1995). Antimicrobials of plant origin are not associated with many side effects and have an enormous

therapeutic potential to heal many infectious diseases since they are presumed to be safe. Even if they were toxic, they will not have great environmental impact since they are biodegradable (Iwu *et al.*, 1999).

For plants to be used alongside modern medicine, careful, phytochemical, pharmacological and toxicological standardization for the chosen plants must be instituted so that dosage can be described in an informed way (Midiwo *et al.*, 2005). The use of modern isolation techniques and pharmacological testing means that new plant drugs usually find their way into medicine as purified substances rather than galenical preparations (Evans, 2005).

2.6 Screening of herbal products

Medicinal plants have been the subject of human curiosity and need for a long time (Omino and Kokwaro, 1991). Every community in Kenya has a large traditional base of medicinal plants. Medicinal plants contain active principles which can be used for common infections. However, their use must be validated, regulated and evaluated to avoid the administration of dangerous concoctions by the herbalists (Kareru *et al.*, 2008, b). Despite the well documented ethnobotanical literature, very little scientific information such as efficacy or phytochemistry has become available on indigenous medicinally used plants (Midiwo *et al.*, 2005; Van Vuuren, 2008).

There is a need to systematically evaluate plants used in traditional medicine. Such research could lead to new drug discovery or advance the use of indigenous herbal medicines for orthodox treatments (Parekh and Chanda, 2006). It is necessary to carry out toxicity studies on medicinal plants, even where they have been used for decades, to determine acceptable and non-acceptable toxicity levels (Ogwal-Okeng *et al.*, 2003). Such validation and testing can be

achieved by various methods like chemical standardization, biological assays animal models and clinical trials (Kareru *et al.*, 2008, b).

Brine shrimp lethality test is a general bioassay that is capable of detecting a broad spectrum of bioactivity present in crude extracts of plants. It is efficient, rapid and inexpensive and requires small amounts of samples. This test has been used in bioassay fractionation of active cytotoxic and antitumour agents such as Trilobacin from the bark of *Asimia triloba*, *Annona muricata* and ent-kaur-16-en-19-oic acid from *Elaeoselinum foetidum* (Pisutthanan *et al.*, 2004). Antimicrobial assay of plants can offer a good source of new, safe and biodegradable antimicrobial drugs (Hamza *et al.*, 2006). Phytochemical determination of plant extracts forms basis of action of such extracts. Investigation of secondary plant metabolites has led to important breakthroughs in pharmacology (Farombi, 2003; Kisangau, 1999; Kokwaro, 1993; Nwaogu *et al.*, 2007; Runyoro *et al.*, 2006).

2.7 Quality control of herbal medicines

Currently, 33% of drugs produced in developed countries are derived from compounds that are originally isolated from plants. Twenty five percent (25%) of these owe their origin from tropical rain forests of Africa, Asia and South America. Their estimated value is about US\$65 billion (Mwangi *et al.*, 2005). People are getting more informed on the advantages of herbal medicines and supplements as natural sources for good nutrition and management of diseases. Many people irrespective of their social status are turning to herbal and complementary medicines because they perceive them as having few side effects and less toxic in comparison to conventional medicines (WHO, 2002).

The complementary medicine practitioner to patient ratio is better than doctor patient ratio meaning that there is better access to traditional/ complementary medicine. In the developed

countries like USA, Germany, France and Belgium, professional training on herbal medicines is offered. In these countries, up to 80% of prescriptions are phytomedicines and about 70% of the populations are regular users of complementary and alternative medicines. Herbal medicine is very advanced in countries like China where there are hospitals, universities and pharmaceutical companies dealing in herbal medicines (Mwangi *et al.*, 2005).

In Kenya, it is common to see hawking of herbal preparations in markets, buses and other public places. As more and more people use herbal medicines, there is a need for quality control especially in developing countries such as Kenya. Among issues that need to be addressed in determination of quality of herbal medicine is hygiene, during and after preparation of the herbal product; the presence of chemical residues such as fertilizers, pesticides and herbicides and the expected limits of heavy metals in the herbal preparations.

Assessment is needed for the level of contamination by bacteria or fungi found in the locality of growth of the plants and presence or absence of foreign matter on the herbal material. Justin-Temu *et al.*, (2009) identified dust, unspecified additives, and untreated water as possible sources of contamination of herbal medicines in Tanzania. Besides, it was not possible to determine the shelf life of these herbal preparations.

Quality control should also be carried out to ascertain the chemical constituents of the plant material. Plants contain chemical groups such as glycosides, alkaloids, sulphur groups, saponins, among others which occur in varying amounts. Their concentrations also vary on locality of the plant. When in high amounts, they may cause side effects and toxicity. Since the practice of herbal medicine is passed orally from generation to generation and often within a family, it is

important to begin the process of quality control with folklore concerning the medicinal plants.

Various tests can be done to assure quality of the herbal preparation. Some of the methods are:

2.7.1 Organoleptic, macroscopic and microscopic methods

These entail the use of sight, smell, taste and touch to assess the general characteristics of the herbal material. The shape, size, colour, surface, texture and characteristics of the cut surface are compared with an authentic sample. Visual evaluation of the plant is done to ensure that the plant is of the required species and that the right part is being used. Microscopic analysis through examination of microscopic structures like leaf stomata is done to identify the correct part, especially when different parts of plant have different indications (Evans, 2005; Kibwage *et al.*, 2005).

2.7.2 Ash values

The plant material is burnt and the residual ash is measured as total and insoluble ash. These are done to check the presence of undue extraneous matter such as soil, sand, metals and oxalates. Total ash value is important in substances which have little or no calcium oxalate such as ginger. Water soluble ash is also specified for ginger. Acid insoluble ash is obtained after boiling the total ash with hydrochloric acid and burning the remaining insoluble matter. It is useful in detecting excess soil and silica in plant material. Sulfated ash measures the extent of contamination by non-volatile inorganic material (Bandaranayake, 2006; Evans, 2005; Kibwage *et al.*, 2005).

2.7.3 Herbicide and pesticide residues

The most common are organochlorines, organophosphates, carbamates and pesticides of plant origin such as pyrethrum. They are due to agricultural practices like spraying, treatment of soils and fumigation during storage. These can be determined using appropriate methods such as gas

liquid chromatography, gas chromatography, gas chromatography coupled with mass spectrometry so that they do not exceed the expected limits (Evans, 2005; Kibwage *et al.*, 2005).

2.7.4 Foaming index and haemolytic activity

These two tests are important for plants that contain saponins. They are important to determine for aqueous decoctions because it is difficult to prepare and dose accurately in presence of foaming (Bandaranayake, 2006; Kibwage *et al.*, 2005).

2.7.5 Foreign matter

Material that is extraneous to the medicinal plant such as other plant parts, organisms, and mineral admixtures such as sand, stones and dust must be determined and removed. Herbal material should be free from moulds, insects and any other visible contaminant. Other ways to detect foreign matter include macroscopic and microscopic examinations and thin layer chromatography especially when the foreign matter is a chemical (Kibwage *et al.*, 2005).

2.7.6 Quantification of the active compounds

This is done by methods such as thin layer chromatography, liquid chromatography and gas chromatography. High performance liquid chromatography is essential for quantitative analysis of complex mixtures. High performance thin layer chromatography is useful to determine homogeneity of plant extract (Kibwage *et al.*, 2005).

2.7.7 Microbial load

This is done to determine presence of pathogens in the herbal material. Such microbial contaminants include fungi, bacteria and viruses. The source of microbial contamination is poor methods of harvesting, cleaning, drying, handling and storage of the herbal material. The total microbial count and total fungal count should be done especially because materials of vegetable origin tend to contain higher levels of microbial contamination than synthetic ones.

Fungal toxins like aflatoxin and other microbial toxins like bacterial endotoxins and mycotoxins are of important health concern. Their presence should be determined and thin layer chromatography employed for confirmation (Evans, 2005; Kibwage *et al.*, 2005).

2.7.8 Heavy metals

Contamination with heavy metals is either accidental or deliberate. High levels of lead are found in material collected by the roadside or downwind of factories using fossil fuels. Environmental pollution is the major cause of contamination of herbal products with heavy metals like lead, cadmium, copper, mercury and arsenic. Limit tests should be estimated against set standards and requirements. Atomic absorption spectrometry, inductively coupled plasma and neutron activation analysis are also used when the heavy metals are present in trace amounts (Bandaranayake, 2006; Evans, 2005; Kibwage *et al.*, 2005).

2.7.9 Moisture content

Moisture in combination with suitable temperature will lead to proliferation of microorganisms. Various methods for determining include loss on drying which may be done by spreading the material on glass plates and placing the plates on desiccators over phosphorus hydroxide. Vacuum drying over an absorbent material may also be used. Another way is to pass a dry inert gas through the heated sample to collect the water carried forward. Determination of water and volatile contents is also done through gravimetric methods such as the use of the Karl Fischer method. Spectroscopic methods such as nuclear magnetic resonance have been used to determine moisture in starch, cotton and other plant products. Other contamination, although rare, may be due to radioactive material during nuclear accidents (Bandaranayake, 2006; Evans, 2005; Kibwage *et al.*, 2005).

2.8 Regulation of traditional medicine and products

In 1991, the world health organization (WHO) established the traditional medicine programme whose objectives were to facilitate the integration of traditional medicine into national healthcare systems, to promote the rational use of traditional medicine through the development of technical guidelines and international standards in the field of herbal medicine and acupuncture and to act as clearing house for the dissemination of information on various forms of traditional medicine (Zhang, 1998). Back then, it was realized that developing countries have a great number of traditionally used herbal medicines and folklore knowledge about them but hardly any legislative criteria to establish these traditionally used herbal medicines as part of the drug legislation (DeJong, 1991). To date, these objectives of the WHO have not been achieved in many countries.

The potential of traditional medicine can be realized fully if traditional healthcare practitioners and the role of traditional medicine in health systems are officially recognized through the development of national policies. Regulatory and legal framework including a code of practice, training and continuing education to enhance skills and knowledge are some of needed interventions to empower THP's. The lack of regulation means that there are just as many fake remedies and false practitioners as there are genuine treatments (Kasilo and Trapsida, 2010).

The licensing TMP's is important because it serves as a mechanism for the traditional healers to increase their technical knowledge and will open official communication channels between the two parallel health systems and therefore provide a way of eliciting information on the types and extend of traditional healthcare. It will also encourage conventional healthcare practitioners to learn about the wider range of factors which should be considered in the diagnosis and treatment of patients (DeJong, 1991).

Most of the Americas, Europe, United States of America, Canada, Asia, the Mediterranean countries, the Pacific and Australia have established laws and regulations governing the trade and practice of herbal medicine. In these countries, there is no difference between herbal medicine and chemical drugs and all are treated the same. They undergo similar procedures before registration, marketing and importation (Zhang, 1998).

Only 21 countries in Africa have put in place a legal framework for the regulation of traditional medicine and products (Kasilo and Trapsida, 2010). Ghana, Guinea, Mozambique, Nigeria, South Africa, Togo, Central Africa Republic, Chad, Equatorial Guinea, Ethiopia, Gabon, Gambia, Tanzania and Uganda are among some of the countries in Africa that have a national policy and laws regarding traditional medicine (WHO, 2005).

In Kenya, a national policy, laws and regulations on traditional medicine, complementary and alternative medicine are being developed. There is no national programme that has been issued and no national office or expert committee has been established. Herbal medicines are not regulated and there is no herbal pharmacopoeia (WHO, 2005; AU, 2007). An act of parliament is underway to regulate and control herbal medicine practice here in Kenya. Meanwhile, the pharmacy and poisons board which is a statutory body established through an act of parliament seeks to control and regulate the trade in herbal medicine.

2.9 Some active principles in medicinal plants

The medicinal value of plants is due to chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavonoids, tannins, and phenolic compounds (Arwa *et al.*, 2008; Chetri *et al.*, 2008; Edeoga *et al.*, 2005).

2.9.1 Alkaloids

Alkaloids are generally white or colourless crystalline solids and exist as salts of organic acids. They are found in 15-30% of all flowering plants and are common in families such as *Fabaceae*, *Liliaceae*, *Apocynaceae*, *Solanaceae* and *Papaveraceae*. Most have a bitter taste. They are frequently stored in tissues other than their site of synthesis. In plants, alkaloids protect against predators, are nitrogen reserves and act as growth regulators especially germination inhibitors. They have been used because of their antispasmodic, antibacterial, decongestant, stimulant, antihypertensive and analgesic activities. Examples are Quinine, Morphine, Cocaine, Sparteine, Reserpine, Ergotamine, Pilocarpine, Ephedrine and Caffeine (Arwa *et al.*, 2008; Chetri *et al.*, 2008; Goodwin and Mercer, 2003; Kisangau, 1999; Kokwaro, 1993; Pengelly, 2004). Table 2.2 represents the major classes of alkaloids with their examples and pharmacological actions.

Table 2.2 Major alkaloid groups with their examples and pharmacological actions

Class	Example	Plant Species	Pharmacologic Action
Pyridine/Piperidine	Nicotine	<i>Nicotiana tabacum</i>	Adrenergic and CNS stimulant
	Lobeline	<i>Lobelia inflata</i>	Expectorant, bronchodilator
Tropane	Hyoscyamine	<i>Atropa belladonna</i>	Anticholinergic
	Scopolamine	<i>Datura metel</i>	Anticholinergic and CNS depressant
Quinoline	Quinine	<i>Cinchona spp</i>	Antimalarial
Isoquinoline	Morphine	<i>Papaver somniferum</i>	Analgesic, narcotic
	Emetine	<i>Cephaelis ipecacuanha</i>	Emetic
Quinolizidine	Sparteine	<i>Sarothamnus scoparius</i>	Cardiotonic, diuretic, oxytoxic
Pyrrrolizidine	Symphytine	<i>Symphytum spp</i>	Hepatotoxin
Indole	Reserpine	<i>Rauwolfia serpentina</i>	Antihypertensive
	Yohimbine	<i>Aspidosperma quebracho</i>	Aphrodisiac
Imidazole	Pilocarpine	<i>Pilocarpus jaborandi</i>	Miotic, cholinergic
Alkaloidal amines	Colchicine	<i>Colchicium autumnale</i>	Uric acid solvent
	Ephedrine	<i>Ephedra sinica</i>	Bronchodilator
Purine alkaloids	Caffeine	<i>Coffea arabica</i>	CNS Stimulant
Steroidal alkaloids	Veratrine	<i>Veratrum album</i>	Antihypertensive

Adapted from Pengelly, 2004

2.9.2 Anthraquinones

They are yellow-brown pigments, mostly occurring as O-glycosides or C-glycosides. They are found mostly in *Cassia*, *Senna* and *Aloe Species*. Most are used as dyes for textiles (Pengelly, 2004). Anthraquinones have many medicinal uses. They are used as laxatives and cathartics. The leaves and pods of *Senna spp* are indicated for gastric emptying before x-ray and abdominal operations. Anthraquinones are also used for dressing wounds, burns and other skin lesions. They are useful as purgatives, for treatment of diarrhea, typhoid, peptic ulcers and stomachaches because they regulate the flow of bile and promote the activity of entire digestive process (Kisangau *et al.*, 2007; Kokwaro, 1993; Pengelly, 2004).

2.9.3 Glycosides

They are a group of compounds having a sugar moiety attached to one or more non-sugar portions by a special bond. Most glycosides remain inactive until they are hydrolyzed in the large intestines to release the aglycone which is the active ingredient. The most common glycosides are cardiac glycosides and antihelminthic glycosides. Antihelminthic glycosides are taeniocides and are found in *Albizia*, *Hagenia*, *Maesa*, *Myrsine* and *Phytolaca* species.

Cardiac glycosides possess a lactone ring attached to the β position at C-17. They include Digoxin and Digitoxin from *Digitalis* spp. They are common in *Lilliaceae*, *Apocynaceae* and *Schrophularaceae* families. They affect the dynamics and rhythm of a dysfunctional heart because they increase the speed and force of systolic contractions of the heart. They have a positive inotropic effect and increase myocardial contractility. Cardiac glycosides cause an increase in atrial and ventricular myocardial excitability. They reduce the rate of atrioventricular conduction and cause an increase in vagal tone and myocardial sensitivity to vagal impulses (Chetri *et al.*, 2008; Kokwaro, 1993; Pengelly, 2004).

2.9.4 Tannins

They are the largest group of polyphenols. They are widely distributed in the bark of trees, insect galls, leaves, stems and fruit. They are glycosides of gallic acid or protocatechuic acid. They are classified as hydrolysable or condensed tannins. Hydrolysable tannins are derived from simple phenolic acids especially gallic acid, which is linked to a sugar by esterification. They break down on hydrolysis to give gallic acid and glucose or ellagic acid and glucose. They are readily soluble in water and alcohol. Condensed tannins are polymers of flavan-3-ols and flavan-3, 4-diols. On hydrolysis, they form insoluble red residues. They are partially soluble in water and alcohol.

Tannins are non crystalline compounds which produce a mild acid reaction with water. They precipitate proteins and this way, they have inhibitory effect on many enzymes. Tannins also precipitate mucus, caffeine and polysaccharides. They constrict blood vessels and this astringent action enables tannins to prevent diarrhea, stop bleeding and provide a protective coat to wounds. Tannins are used as vermifuge and are found majorly in *Acacia*, *Kigelia*, *Diospyros*, *Pterocarpus*, *Rhizophora* and *Spathoda* species (Kisangau, 1999; Kokwaro, 1993).

Some tannins act on arachdonic acid metabolism in leucocytes with important roles in reversing inflammations and are used in wound healing. There is a strong association between tannin content and the effects popularly attributed to wound healing and anti-inflammatory activity of the plants (Araujo *et al.*, 2008)

2.9.5 Triterpenoids

They are derived from a C₃₀ precursor, squalene which was first isolated from shark liver. They have a configuration similar to steroids found in plants and animals. They include phytosterols

found in *Withania somnifera*. They help in regulation of blood cholesterol (Kisangau, 1999; Pengelly, 2004).

2.9.6 Saponins

They are composed of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. They derive their name from soapwort plant (*Saponaria* of *Caryophyllaceae* family). They are common in *Sapindaceae*, *Aceraceae* and *Hippocastanaceae*. Their active portions form colloidal solutions in water. They produce lather in shaking and precipitate cholesterol. They serve as antifeedants and protect plants from microbes and fungi (Evans, 2005; Pengelly, 2004).

Saponins are amphipathic in nature and are used as surfactants to enhance penetration of macromolecules such as proteins through cell membranes. They are used as adjuvant in vaccines production. Saponins are also used as detergents, oil emulsifiers, foam producers in beverages and fire extinguishers. Saponins complex with cholesterol and can therefore be used to lower blood cholesterol. They are precursors of important therapeutic drugs such as cortisone and contraceptive oestrogens. They have pharmacological activities such as cytotoxicity, antitumor, antimutagenic, anti-inflammatory, antiviral, antihelminthic, antibacterial and cardiac effects (Kareru *et al.*, 2008; Lacaille-Dubois and Wagner, 1996; Pengelly, 2004; Watt and Bray-bradwijk, 1962).

2.9.7 Flavonoids

They occur as yellow or white pigments in plants and are found in free state or as glycosides. Flavonoids are responsible for the shades of yellow, orange and red in flowers (Pengelly, 2004). The chemical structure of flavonoids is based on a C₁₅ skeleton consisting of two benzene rings connected by a three carbon chain. The three carbon chain is closed to form a heterocyclic ring.

Flavonoids are products of both the shikimic and acetate pathways. The most commonly occurring flavonoids are flavones and flavonols. Flavonoids are found in high quantities in tea, onions, citrus fruits and apples (Evans, 2005; Pengelly, 2004).

Flavonoids have a protective role in the heart and circulatory system. They strengthen capillaries through membrane stabilization and are used for peripheral vascular disorders. Flavonoids have antioxidant effects and have synergistic effects with ascorbic acid. Studies have shown that flavonoids have anti-inflammatory, hepatoprotective, antiviral, antiproliferative and anticarcinogenic effects. Flavonoids also have antiallergic, antiatheromatous and antihypertensive effects. Flavonoids also prevent platelet aggregation. Examples of flavonoids with medicinal value are Rutin, Quercetin, Kaempferol and Nomilin (Akaneme 2008; Arwa, *et al.*, 2008; Harbone, 1989; Jain *et al.*, 2006; Middleton and Kandaswami, 1993; Pengelly, 2004).

2.9.8 Oils and fats

Oils and fats may be fixed oils, essential oils, sulphur oils or resins. Fixed oils do not distill at the temperature of boiling water. They consist of molecules of fatty acids which form salt or ester with one molecule of glycerin. Fixed oils include those of *Balanites* and *Trichilia* species and are used as ointment bases and emollients. *Ximemia* species contain cathartic oils while fixed oils from *Euphorbia* species such as *Ricinus communis* and croton species are used as purgatives.

Essential oils are non nitrogenous compounds that are volatile and usually odourless liquids. They are responsible for the scents of flowers or other parts of plants. Most have pleasant smell. They regulate intestinal movements, prevent colic and are used as condiments with food. Some are poorly absorbed from gut and are used as vermifuge for round worms and hookworms for example the oil from *Chenopodium* species. They are also used to treat wounds because they hinder bacterial growth.

Sulphur oils are found in the seeds of *Boschia*, *Capparis*, *Cleome* and *Cruciferae* species. They are irritating and cause reddening and vesication of skin. They are used as carminatives in small doses and as emetics in large doses. Resins are solid fats. Gum resins are found in *Boswellia*, *Comiphora* and *Calotropis* species. Resins from *Albizia* and *Fagara* species are used for urinary tract infections, while those from *ipomea* as purgatives. Resins are used for wound dressing because of their adhesive quality. Some resins are poisonous and are used as arrow poison. Other resins are irritating and cause vomiting and purging if taken in large doses (Evans, 2005; Pengelly, 2004).

2.9.9 Toxalbumins

They are poisonous and irritant proteins that are found in seeds of some plants. They cause violent vomiting and inflammation of the eyes and nose. They are found in *Euphorbiaceae* species such as Castor oil, Croton oil and Jatropha oil (Pengelly, 2004).

2.9.10 Lignans

Lignans are derived from shikimic acid-cinamic acid pathways followed by a series of enzymatic reactions to form dimeric molecules (Heittiarachchi 2006). They are compounds formed essentially by union of two molecules of a phenylpropane derivative. About 300 Lignans have been isolated (Evans, 2005). Lignans have both antioxidant and antimicrobial activities. The simple lignan, nor-dihydro-guaiaretic acid (NDGA) from *Larrea tridentate* is a potent antioxidant (Hettiarachchi, 2006; Pengelly, 2004).

2.9.11 Lectins

They are high molecular weight polypeptides. They are carbohydrate binding proteins. They interact with glycoproteins bearing polysaccharides side chains on cell membranes of animals to cause agglutination and cytotoxicity. Lectins act as mitogens and stimulate production of

immunoglobulins. They are widespread in legumes. They may be toxic and cause impairment of tissue functions (Evans 2005; Pengelly, 2004).

2.10 Literature review of the selected plants

2.10.1 *Ajuga remota* Benth

Family: Labiatae

Ajuga remota is found growing in grasslands of Kenya and other parts of East Africa. It is an erect rhizomatous pubescent herb. It is not eaten by insects, birds or animals. *Ajuga remota* is a widely used plant in African folklore medicine. The leaves are used to treat toothache. The leaf decoction is used for stomachache, diarrhea, malaria, pneumonia, leishmaniasis, liver problems, and high blood pressure (Gachathi , 1993; Kokwaro , 1993; Kuria and Muriuki , 1984; Munguti, 1997).

Various principles have been isolated from genus *Ajuga*. Phytoecdysteroids, neo-Clerodane Diterpenes and Diterpenes, Anthrocyanidin-glucosides, Iridoid glycosides, Withanolides, Triterpenes, Flavonoids, Triglycerides, Ajugarin 1, 11,111 and an aglycone with a cardenolide structure; Ajugasterone C, Ajugalactone, Cyasterone. Others are β - Ecdysone, Ergosterol-5,8 Endopoxide and Essential oils (Coll and Tandron, 2005; Kubo *et al.*,1976,1980,1982,1983; Kuria and Muriuki,1984).

The isolates from *Ajuga remota* have been shown to act as anabolic, analgesic, antibacterial, antiestrogenic, antifungal, anti-inflammatory, antihypertensive, antileukemic, antimycobacterial, antioxidant, antipyretic, cardiogenic, hypoglycemic, vasorelaxant, antifeedant and insect growth inhibiting (Cantrell *et al.*,1999; Israili and Lyousi ,2009; Kubo *et al.*, 1976, 1981; Kuria and Muriuki, 1984).

2.10.2 *Harrisonia abyssinica*, Oliv

Family: Simaroubaceae

Harrisonia abyssinica grows widely in Kenya. It is a much branched prickly shrub, or small tree up to 6m, sometimes climbing. It is evergreen and the bark is armed. Prickles are short and hooked. Leaves are 2–3 inches long and leaflets are in about 3 pairs, obovate-elliptical or oval, obtuse, remotely dentate-serrate or occasionally lobed. Flowers are cream or yellow in colour.

Harrisonia abyssinica is used ethnobotanically in Africa for management of fever, oedema, pneumonia, arthritis and tuberculosis. It is also used to treat plague, nausea, vomiting, snakebite, abdominal pains, hemorrhoids, dysentery, gonorrhoea and skin infections (Balde *et al.*, 1995; Kokwaro, 1993).

Principles isolated from *Harrisonia abyssinica* are Harrisonin, Obacunone, Pedonin, 12 β -Acetoxyharrisoninneorin R, Perforaquassin A, Cycloabysinone and 3-friedelanone (Balde *et al.*, 1995; Rajab *et al.*, 1997; 1999). Extracts from *H. abyssinica* have shown antiviral, antifungal, and cytotoxic activity. They also have anticandida and antibacterial activity (Balde *et al.*, 1995; Kirira *et al.*, 2006; Maregesi *et al.*, 2008; Runyoro *et al.*, 2006).

2.10.3 *Erythrina abyssinica*, Lam

Family: Papilionaceae

Erythrina abyssinica is medium-sized tree, usually 5-15 m in height, deciduous, thickset, with a well-branched, rounded, spreading crown. The trunk is short and the bark is yellow-buff when fresh but otherwise grey-brown to creamy brown, deeply grooved, thickly corky and often spiny. When damaged the tree exudes a brown, gummy sap. The leaves are compound, trifoliolate and alternate. Flowers are orange-red, up to 5 cm long. The fruits are cylindrical, woody pods, 4-16 cm long and deeply constricted between the seeds. They are densely furry, light brown in colour

and open to set free 1-10 shiny, red seeds with a grey-black patch. *Erythrina abyssinica* is the most widespread species in Africa. It is found in savannahs throughout eastern and southern Africa (Beentje, 1994; ICRAF, 1992; Noad and Birnie, 1989).

Pounded parts of *Erythrina abyssinica* are used in steam form in Kenya to treat anthrax. The bark is boiled with goat meat for treating gonorrhoea. The bark of the green stem may also be pounded and then tied into a fine piece of cloth and the liquid from it squeezed into the eyes to cure inflammation of the lids. The bark may be roasted until black, powdered, and applied to burns and general body swellings. A decoction is taken orally as an antihelminthic and to relieve abdominal pains. The roots are used to treat syphilis and the leaves to cure skin diseases in cattle (Kokwaro, 1993).

Flavonoids from *Erythrina abyssinica* showed antibacterial activity while the ethanolic extracts of stem bark exhibited antiviral activity. The crude extract had antiplasmodial activities and its ethyl acetate extract of the stem bark showed antiplasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* ((Kamat, *et al.*, 1981; Vlietnick *et al.*, 1995; Yenesew *et al.*, 2003; Yenesew *et al.*, 2004).

2.10.4 *Ocimum suave*. Wild

Family: Labiatae

Ocimum suave is an erect annual, with much branched glabrous or slightly pubescent stems. The leaves are long, petiolate and ovate. The plant is about 1-2 ft long. Many of *Ocimum* species are highly aromatic and several are economically important as sources of essential oils, medicinal herbs and food flavorants (Paton *et al.*, 1999). *Ocimum suave* is found in tropical Asia and in West and Eastern Africa where its geographical distribution is limited to mountainous areas (Mekonen *et al.*, 2003; Tan *et al.*, 2002).

Ocimum suave has various traditional medicine uses. The Leaf decoction is used for croup, bronchopneumonia, stomachache and fever in children. It is used as a nasal decongestant. The leaves are used in treatment of skin diseases, fungal infections and tumors. Stem, twigs and roots are used for stomach complains and management of menstrual problems. The whole plant is used for treatment of gonorrhoea, dysentery and diarrhoea. It is also used for treatment of gout, piles and as a carminative and stimulant. In some parts of East Africa, *Ocimum suave* is used to treat ulcers and as a cathartic. In Ethiopia, *Ocimum suave* is traditionally used to relieve fever and pain (Ayensu, 1978; Kokwaro, 1993; Tan *et al.*, 2002; Watt and Brayer-bradwijk, 1962).

The extracts induced hypothermia at high doses in mice. A possible mechanism to its antipyretic activity could be through inhibition of prostaglandin synthesis (Mekonen *et al.*, 2003). Extracts of *Ocimum suave* inhibited lesion formation by HCL/Ethanol at the dose of 250mg/kg in rats. This was through increase of mucous production and physiochemical reinforcement of the gastric mucosa or effects similar to endogenous prostaglandins (Tan *et al.*, 2002). The components of *Ocimum* species have antifungal, antibacterial, insecticidal and insect repellent activities (Desta ,1993; Perez – Alonzo, *et al.* ,1995).

2.10.5 *Piliostigma thonningii* (Schum.) Milne – redhead.

Family: Caesalpiniaceae.

Piliostigma thonningii is a shrub or tree with a few scattered branches and a light crown that grows to a height of 20 ft. The bark is fissured grey. The buds are rusty hairy and the leaves are lobed (the shape of a camel's foot). The flowers are white to pink and the fruit is a large smooth flattened yellowish brown pod that turns dark brown and with fine cracks on drying (Jimoh and Oladiji, 2005; Maundu *et al.*, 1999). *Piliostigma thonningii* is widely distributed in Africa and Asia. In Kenya, it grows on western region, the central region and the wooded grasslands and

woodlands of the coast region. It is found in open and often disturbed sandy areas (Maundu *et al.*, 1999).

Piliostigma thonningii has several ethnopharmacological uses. The Leaves are used for coughs, chest complains and leprosy. They are also used for small pox, snake bite, dog bite, febrifuge and rheumatism. Stem, roots and twigs are chewed or their decoction drunk for stomach pains, dysentery, respiratory ailments, hook worm, skin diseases, gonorrhoea, fever and gum inflammation. The root decoction is drunk for pain in the area of spleen and for hookworms (Ayensu , 1978; Jimoh and Oladiji, 2005). Various preparations of parts of *Piliostigma thonningii* have been used to arrest bleeding, to treat fevers and bacterial infections, as a laxative, antihelminthic and as an anti-inflammatory agent (Ibewuke *et al.*, 1997; Igoli *et al.*, 2005).

2.11 Literature review on the selected bacteria species

2.11.1 *Bacillus cereus*

They are large cells, often in chains and form ellipsoidal spores. They are highly motile and hemolytic on blood agar. The spores can survive normal cooking. *Bacillus cereus* produce food poisoning syndrome which is characterized by nausea, vomiting, abdominal pain and diarrhea. Other infections caused by *Bacillus cereus* are endophthalmitis, bacteremia and septicemia, fulminant sepsis with hemolysis, meningitis, empyema, pleurisy, brain abscess, urinary tract infections and osteomyelitis. These infections are severe especially with the immunocompromised patients, alcoholics, drug abusers and those with neoplasias.

2.11.2 *Escherichia coli*

Escherichia coli are one of the five species of genus *Escherichia*. The others are *E. blattae*, *E. fergusonii*, *E. hermannii*, and *E. vulneris*. *E. coli* are part of normal flora of healthy individuals.

Escherichia coli cause bacteremia, meningitis, diarrhoeal diseases and infections of the urinary tract.

2.11.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa are aerobic, non spore forming, gram negative rods which are straight or slightly curved. They are 1.5 -5 μm in length and 0.5 – 1 μm in width. They are motile due to the presence of flagella. They produce a grape like or corn taco like odor. Colonies are usually spreading and flat. They have serrated edges and a metallic sheen associated with autolysis of the colonies. The colonies are bright green in colour which is formed when Pyoverdin combines with Pyocyanin. Identification is by a positive oxidase test and production of blue green, red or brown diffusible pigments on Mueller Hinton agar or other non dye containing agars.

Pseudomonas aeruginosa are able to survive in aqueous environments and are a problem in hospital environment. They cause nosocomial infections of the respiratory and urinary tracts, wounds and peritonitis. Other infections caused by *pseudomonas aeruginosa* are folliculitis, meningitis, community acquired pneumonia and bacteremia.

2.11.4 *Staphylococcus aureus*

Staphylococcus aureus is a member of genus staphylococcus. They are anaerobic gram positive cocci. They are large cells usually 6-8mm in diameter and are not motile. They do not form spores and are hemolytic on blood agar. The colonies are pigmented and have a cream-yellow colour. They are smooth, entire, slightly raised and translucent.

Staphylococcus aureus is a human opportunistic pathogen and is found on skin, skin glands, mouth, intestinal tract, genitor-urinary tract, upper respiratory tract, mammary glands and mucus membranes. As a nosocomial pathogen, it is a major cause of mortality and morbidity. Most of the infections due to *Staphylococcus aureus* are acute and pyogenic and include skin infections

such as furuncles, cellulitis, impetigo and post operative wound infections. Other infections are bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, cerebritis, meningitis, scalded skin syndrome, food Poisoning, toxic shock syndrome and chorioamnitis (Murray *et al.*, 2003).

2.12 Antimicrobial assays

Antimicrobial activity testing can be performed by any of the following methods:

2.12.1 Broth dilution method

Serial two fold dilutions of the antimicrobial agent to be tested are prepared and placed in sterile tubes with appropriate broth that will allow for growth of the test microorganisms. Control tubes which don't contain any antimicrobial agent are also set. After 24hour incubation, the tubes are observed for turbidity which is a show of microbial growth. The microorganisms will grow in the control tubes and any other tube without sufficient concentration of antimicrobial agent to inhibit growth.

A variation of the broth dilution is the micro broth dilution method which uses volumes in μl and test samples in μg . After incubation, microbial growth is indicated by turbidity or use of a suitable dye. In broth dilution methods, the lowest concentration of the antimicrobial agent that inhibits growth of the microorganisms is regarded as the minimum inhibitory concentration (Hettiarachhi, 2006; Moshi *et al.*, 2009; Suffredini *et al.*, 2006; Wagate, 2008).

2.12.2 Disc diffusion assay

This uses cellulose discs. The discs are impregnated with the antimicrobial agent and then placed equidistantly onto the inoculated plates. The plates are incubated for a stipulated time and antimicrobial activity is determined by measuring the diameters of zones of inhibition

(Hettiarachchi, 2006; Kisangau *et al.*, 2007; Moshi *et al.*, 2009; Mothana *et al.*, 2008; Parekh and Chanda, 2006).

2.12.3 Well diffusion assay

Small circular wells are made in the solidified inoculated media. These wells are then filled with the antimicrobial agent to be tested. The solution is allowed to diffuse into the media and then incubated. The antimicrobial activity is determined as a measure of the zone of inhibition around each well (Hettiarachchi, 2006; Kisangau *et al.* 2007; Mbaria *et al.*, 2006).

2.13 Ethnopharmacological documentation of Meru central district

Ethnomedical information for Meru central district is scanty. There has been no elaborative ethnobotanical research done on the area. Studies carried out earlier provide information on the general Meru region. The Meru people have preferences for certain medicinal, fruit and ornamental plants such as *Fragoropsis angolensis* and *Prunus africana* (Barr and McGrew, 2004). Githinji (1990) recorded 37 medicinal Labiateae species in Kenya. Of these, 8 were from the Meru community. Kokwaro (1993) recorded 46 plant species of ethnomedical interest among the Meru community.

Gathirwa *et al.* (2008) demonstrated antiplasmodial activity and safety of five medicinal plants commonly used by the Meru community for the treatment of malaria, and recommended that the two plants that had good antiplasmodial and anti-malarial properties be subjected to further chemical and biochemical investigations. A study in the area identified 86 plants in 37 families of ethnomedical and ethnoveterinary uses (Itonga, personal communication).

CHAPTER THREE: MATERIALS AND METHODS

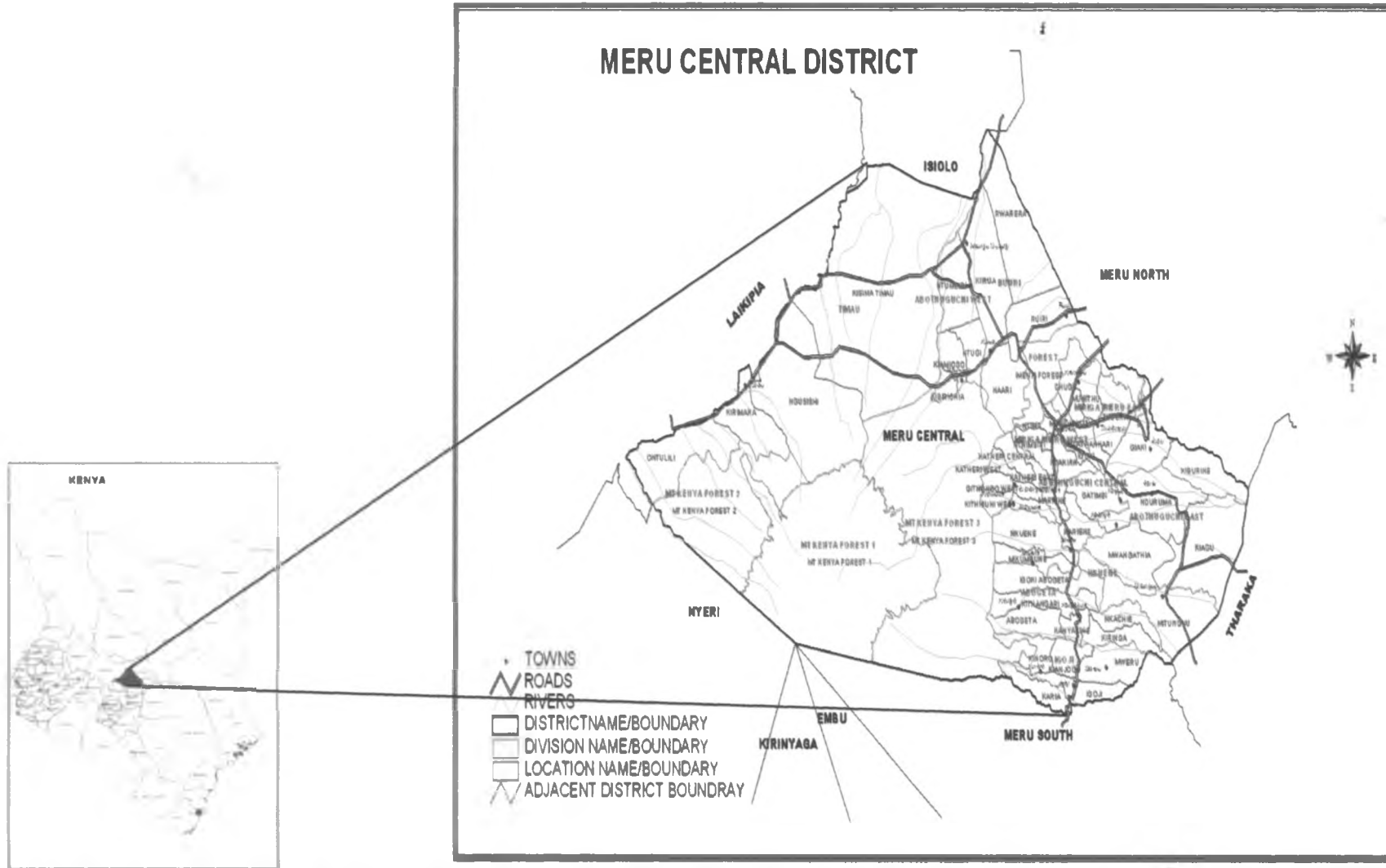
3.1 The study area

The study was carried out in Meru central district of Eastern province, Kenya. The district is an important agricultural centre for production of food and cash crop for the nation of Kenya. The average annual rainfall range is 381-2032mm. The district has forest reserves like the Imenti forest (Barr and McGrew, 2004).

Meru central district is one of the 13 districts of Eastern Province. It is on the east of Mount Kenya. It borders Laikipia district to the West. To the south, it borders Nyeri, Kirinyaga, Embu and Meru South districts, while to the east is Tharaka district. Meru North and Isiolo districts border to the North. It is on the Latitude 0⁰3'45" North and 0⁰2'30" south and lies on Longitude 370 and 380 east. The total area covered by the district is 2982 km². The estimated population as of 2008 was 569,992 people. Figure 3.1 shows the location of Meru central district in Kenya.

The district has 160 health facilities. The doctor/patient ratio is 1:33,259. This means that most of the health services are provided by other cadres of health personnel. Most of the facilities lack qualified health personnel. The HIV/AIDS prevalence is 38%. The distribution of health facilities is not equitable and there is inaccessibility of basic health services to the populace. The road network in the district is poor and the average distance to the nearest health facility is 7 km. Most of the people have to walk to these facilities (GoK, 2005; 2007). The low doctor/patient ratio, the high HIV/AIDS prevalence and the inaccessibility of the health facilities to the general population forces many people to rely on traditional medical practitioners for their health care needs.

Figure 3.1 Map of Kenya showing the position of Meru central district



3.1.1 Selection and collection of plant materials

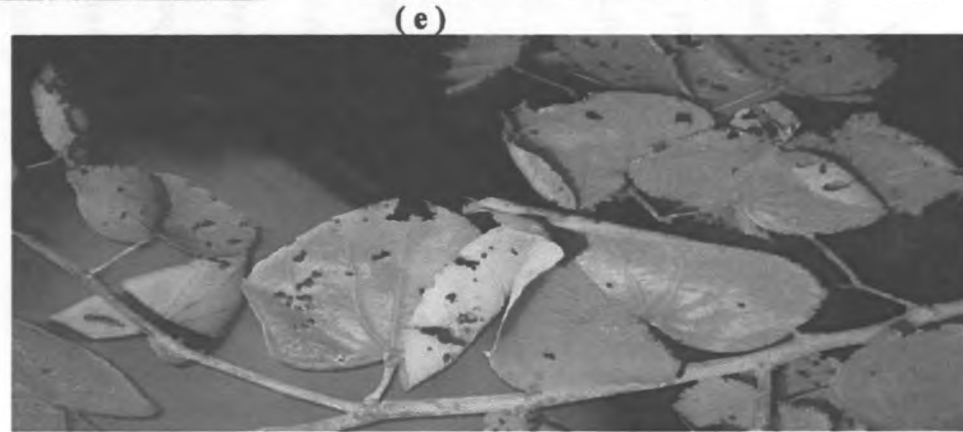
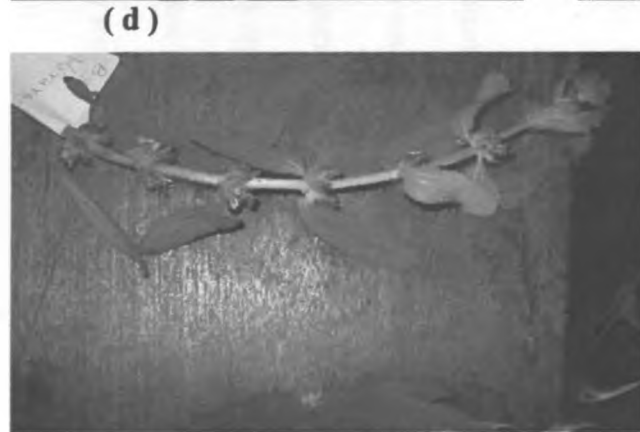
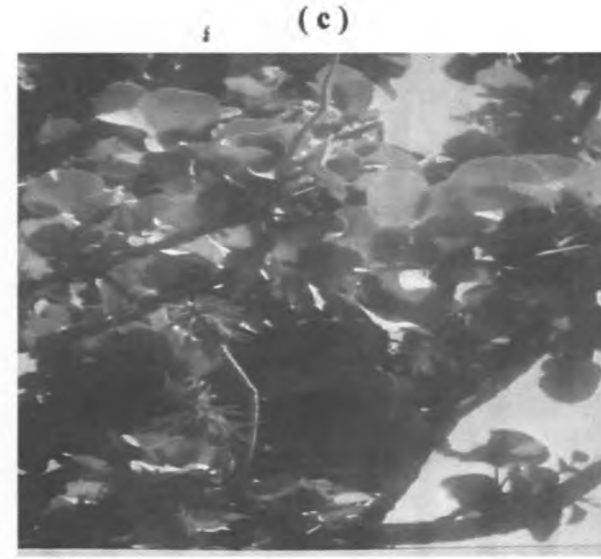
Ethnomedical information was obtained from authentic herbal practitioners who were identified with the help of the District Cultural and Social Officer and the Local administration. Authentic herbal medical practitioners were interviewed using an open ended semi structured questionnaire according to Martin (1995). The herbal practitioners identified the plants by their local names and gave the uses of each plant and parts including the modes of administration and dosages. Voucher specimens were collected, identified scientifically by a plant taxonomist and deposited at the Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi.

The information provided by the herbal practitioners was compared with available literature on the plants. This was done through a thorough search on the plants in both print and electronic databases. Further visits to the herbalist were done to determine the plants that had greater acceptance and usage. Selection of the plant materials for this study was based on the informant consensus among the identified herbal practitioners on their traditional uses in treating microbial infections in human. The plant material for this study was collected between the months of September and November 2009. The selected plants are listed in table 3.1 and shown on plates 3.1-3.5. Their ethnobotanical uses, the parts used and the local names are included in the table.

Table 3.1: The plants from Meru Central District selected for study on their antimicrobial activity, cytotoxicity and phytochemical properties

Family	Plant Species	Local Name	Life form	Part used	Ethnomedical uses
CAESALPINIACEAE	<i>Piliostigma thonningii</i>	Mukuura	Tree	Stem bark, Leaves	Cough, colds, chest pains, stomachache, wounds, Menorrhagia
LABIATEAE	<i>Ajuga remota</i>	Kirurage	Herb	Leaves	Pneumonia, fever, toothache, dysentery, stomachache, eye infection, tongue infection
	<i>Ocimum suave</i>	Makuri	Tree	Leaves	Blocked nostrils, abdominal pains, sore eyes, ear infections, cough, disinfectant, insecticide
PAPILIONACEAE	<i>Erythrina abyssinica</i>	Muuti	Tree	Root bark, Stem bark	Anthrax, syphilis, gonorrhea, burns, body swellings, colic, antidote for poisoning
<u>SIMAROUBACEAE</u>	<i>Harrisonia abyssinica</i>	Mutagataga	Shrub	Whole plant	Stomachache, abdominal pains, fever, nausea, vomiting, plague, swollen testicles, dysentery, gonorrhea, tuberculosis

Plate 3.1 : Photographs of aerial parts of *Harrisonia abyssinica* (a), *Ocimum suave* (b), *Erythrina abyssinica* (c), *Ajuga remota* (d) and *Piliostigma thonningii* (e)



3.2 Preparation of plant extracts

The plants' parts (whole plant, stem bark, root bark, and leaves) were scrutinized for any foreign matter or moulds and cleaned with distilled water. They were chopped into small pieces and air dried under shade at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. The dried plant material was ground using a laboratory mill to fine powders. This was done in a fume chamber to protect from fumes and dust during the grinding. The powdered plant material obtained was packed in 500gram portions and placed in clean air tight polythene papers (Gakuya, 2001; Wagate, 2008).

3.2.1 Extraction

3.2.1.1 Water extraction

Five hundred grams (500 grams) of the plant powder was extracted separately by placing in conical flasks and distilled water was added until the powder was fully submerged. Stirring and shaking was done to ensure proper mixing. The conical flasks were corked tightly with stoppers. Shaking was done regularly to allow for percolation for four days. On the fifth day filtration was done using Whatman No.1 filter paper and the resultant liquid was collected in sterilized beakers which were covered tightly in aluminium foil and stored in a refrigerator at $+4^{\circ}\text{C}$ pending freeze-drying, bioassay and antimicrobial assay.

3.2.1.2 Methanol extraction

Five hundred grams (500 grams) of the plant powder was extracted separately by placing the powder in conical flasks and 70% methanol added until the powder was fully submerged. Thorough stirring was done to ensure proper mixing and then the conical flask was corked with appropriate stopper. Thereafter, shaking was done regularly to allow percolation and extraction.

On the fifth day, the extracts were filtered using Whatman No.1 filter paper into another conical flask.

Methanol was removed in a rotary evaporator and collected for recycling. The resultant viscous substance was put in sterilized beakers which were covered in aluminium foil and then stored in a refrigerator at $+4^{\circ}\text{C}$ pending freeze drying, bioassay and antimicrobial assay. Freeze drying was done by use of Virtis Bench Top 3[®] Model freeze drier (The Virtis Company, Newyork), courtesy of Department of Veterinary Anatomy and Physiology, University of Nairobi.

3.3 Antibacterial Activity Testing

The methanolic and aqueous extracts were screened for their antibacterial activity. Broth dilution assay was used to screen the extracts for antibacterial activity. The gram positive bacterial strains used were *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 25922). The gram negative bacterial strains were *Escherichia coli* (ATCC 11778) and *Pseudomonas aeruginosa* (ATCC 27853). They were obtained from stock cultures at the Department of Public Health, Pharmacology, and Toxicology, University of Nairobi. The positive controls were antibiotics Ampicillin sodium powder (Flamingo Pharmaceuticals, India) for gram positive bacteria and Gentamycin sulphate (Shangdong Kangtai, China) for gram negative bacteria (Kisangau *et al.*, 2007; Koshy *et al.*, 2009; Pavithra *et al.*, 2010). DMSO 2% was used as negative control (Botelho *et al.*, 2007).

3.3.1 Bacteria quantification

Standard microorganisms, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus* were inoculated on blood agar plates from stock cultures and incubated at 37°C for 24 hours. A colony from each BA plate of the standard microorganisms was emulsified

in 3ml physiological saline solution in sterile tubes. Four rows of 10 tubes each with 9 ml of sterile physiological saline solution were made and each row labeled with the standard microorganism to be quantified. Ten fold serial dilutions of the standard microorganisms were made. TSA plates were inoculated with 1 ml from each tube and incubated at 37⁰c for 24 hours. Colonies on the TSA plates were counted and quantified. The numbers of colony forming units were 2.2×10^8 cfu/ml (Akinyemi *et al.*, 2005; Koshy *et al.*, 2007).

3.3.2 Determination of MIC and MBC

Test tube method was used in the procedure for determining the MIC and MBC. Standard microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus* were inoculated on BA plates and incubated at 37⁰C for 24 hours. A colony of each standard microorganism was emulsified in 3ml of sterile physiological saline.

Plant extracts were weighed and mixed in 4ml of sterile Mueller Hinton broth to make a master dilution of 100mg/ml for gram positive bacteria and 250mg/ml for gram negative bacteria. Five (5) culture tubes containing 2ml Mueller Hinton broth each were arranged in four rows for each plant extract. Two fold serial dilutions were made from the master dilution. For gram positive bacteria, the concentrations were made as 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. For gram negative bacteria, the concentrations were made as 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml and 7.8125mg/ml.

100 μ l of the test bacteria was inoculated into tubes in the respective rows. The tubes were incubated at 37⁰C for 24hours. The culture tubes with the lowest dilution that showed no turbidity were recorded as MIC. This is the lowest concentration that inhibited any visible growth of bacteria. For determination of MBC, 100 μ l of broth from all tubes that showed

turbidity was removed aseptically and then plated on TSA plates using pour plate method. The plates were incubated at 37⁰C for 24 hours.

After incubation the lowest concentration of the plant extracts showing no bacteria growth was recorded as the MBC. The MBC is the lowest concentration at which 99.9% or more of the initial bacteria inoculums was killed. All the tests were performed in triplicate (Akinyemi *et al.*, 2005; Botelho *et al.*, 2007; Dewanjee *et al.*, 2007; Pavithra *et al.*, 2010; Wagate, 2008)

3.4 Cytotoxicity Study

In vitro assay of *Artemia salina* was used to detect cell toxicity of the plant extracts (McLaughlin and Rogers 1998; McLaughlin *et al.*, 1991; Meyer *et al.*, 1982).

3.4.1 Preparing the marine salt solution

An electric weighing machine (Sartorius-Werke Gmbh type 2472) was used to weigh thirty three grams (33g) of marine salt. This was then put in a 1 L conical flask. Distilled water was added gradually and shaking done until all the marine salt was dissolved. Thereafter, distilled water was added to 1 L mark to make the final marine salt solution (Gakuya, 2001; Wagate, 2008).

3.4.2 Hatching the brine shrimp

An improvised shallow tank made up of a shallow rectangular plastic container having two unequal chambers and several holes on the divider was used to hatch the brine shrimp. It was filled with marine salt solution (33gm of marine salt in 1 L of distilled water). A spatula (provided with the brine shrimp eggs) was used to scoop about 50mg of the eggs which were sprinkled on the larger compartment of the container.

About 5 mg of yeast was added to the chamber with the eggs to serve as food for the nauplii. The larger compartment with the eggs was darkened by covering it. The smaller compartment was left uncovered and illuminated by a 40 watt electric bulb. After 48 hours of exposure, the nauplii were collected on the illuminated side by use of a Pasteur pipette. These nauplii were used in the brine shrimp lethality test (Pisutthanan *et al.*, 2004).

3.4.3 Preparing the plant extracts for the test

Methanol extracts of the plants were used for the study. One hundred milligrams (100mg) of each extract were weighed using an analytical balance (Sartorius-Werke Gmbh type 2472). This was transferred into universal bottle and ten milliliters of marine salt solution added to dissolve.

An electric mixer (Vortex Reamix 2789[®]) at 2800rpm was used to stir the mixture in order to dissolve completely. This gave a final stock solution of 10,000 μ g/ml from which serial dilutions were made (Gakuya, 2001; Wagate, 2008).

3.4.4 Cytotoxicity test

From the final stock solution of each plant extract, 500 μ l, 50 μ l and 5 μ l were transferred to sets of graduated tubes. Ten shrimps were transferred to each tube using Pasteur pipette and marine salt solution added to the 5ml mark. This formed three sets of dilutions of 1000 μ g/ml, 100 μ g/ml and 10 μ g/ml respectively. Each dilution was set in five tubes.

DMSO in concentrations of 0.02%, 0.2% and 2% was used as the negative control. Vincristine sulphate (BIOCRISTIN-AQ[®], Biochem, India) in concentrations of 1000 μ g/ml, 100 μ g/ml, 10 μ g/ml and 1 μ g/ml was used as the positive control (Bastos *et al.*, 2009; Khan *et al.*, 2008; Moshi *et al.*, 2009; Ripa *et al.*, 2009). After 24 hours, the numbers of live nauplii were counted and percentage mortality calculated for the plant extracts and controls. Probit analysis was used to determine the lethal concentration (LC₅₀) at 95% confidence interval (Finney, 1971).

3.5 Preliminary Phytochemical Screening

A sample of 100g of powdered plant extract of each species was extracted in 70% methanol for 48 hours. The extract was then filtered under vacuum. The filtrate was evaporated to dryness in a rotor evaporator at 45°C and further dried to a constant weight at 45°C in a hot air oven.

Preliminary tests were done as follows:

3.5.1 Test for alkaloids

Half a gram (0.5g) of each plant extract was stirred in 2 ml of 1% aqueous hydrochloric acid and heated in a boiling water bath for 10 minutes. The mixture was filtered while hot and treated with Dragendorff's reagent. Turbidity or precipitation indicated presence of alkaloids.

3.5.2 Test for sterols and triterpenes

Half a gram (0.5g) of the extract was defatted with hexane. The residue was then extracted in dichloromethane and the solution dehydrated with magnesium sulphate anhydride. The mixture was treated with 0.5ml acetic anhydride followed by 2 drops of concentrated sulphuric acid. A gradual appearance of green blue colour was indicative of sterols while colour change from pink to purple indicated triterpenes.

3.5.3 Test for saponins

Half a gram (0.5g) of the plant extract was shaken in 5ml of distilled water. Frothing that persisted for at least half an hour was indicative of saponins.

3.5.4 Test for flavonoids and flavones

Two hundred milligrams (200mg) of the extract was dissolved in 4 ml of 50% methanol. The solution was warmed and metal magnesium added. Five drops of concentrated sulphuric acid was added. A red colour was observed for flavonoids and orange colour for flavones.

3.5.5 Test for tannins

Half a gram (0.5g) of the extract was dissolved in 2ml of distilled water and filtered. Two drops of ferric chloride added to the filtrate. A blue black precipitate developed when tannins were present.

3.5.6 Test for cardiac glycosides

(Keller Killian test): Hundred milligrams (100mg) of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under-layered with 1 ml of concentrated sulphuric acid. The appearance of a brown ring at the interface of the two layers with the lower acidic layer turning blue green upon standing indicated the presence of cardiac glycosides.

3.5.7 Test for anthraquinones

One gram (1gm) of the extract was dissolved in 70% acetone to a final concentration of 50mg/ml. The **Bonträger Test** was used to test for anthraquinones. Two milliliters (2 ml) of the test sample was shaken with 4 ml of hexane to defat. The upper lipophilic layer was separated and treated with 4ml of dilute ammonia. The lower layer changed to violet, then pink and indicated the presence of anthraquinones.

Sources: Chetri *et al.*, 2008; Fawole *et al.*, 2009; Hettiarachchi, 2006; Kareru *et al.*, 2008,a; Kisangau, 1999; Kisangau *et al.*, 2007; Ngbede, *et al.*, 2008; Orech *et al.*, 2005; Parekh and Chanda, 2007; Siddiqui and Ali 1997.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Ethnobotanical survey

This study was conducted during an ongoing project in Meru Central District where ethnobotanical survey in the area identified 86 plants in 37 families as having major ethnomedical uses. Major details of the ethnobotanical survey are published elsewhere. My discussion with the herbalists showed that they used most of the plants in combination when treating various infections. Based on this ethnobotanical survey and my discussion with the herbalists, the leaves of *Ajuga remota* and *Ocimum suave*, the stem bark and leaves of *Piliostigma thonningii*, the stem bark and root bark of *Erythrina abyssinica* and *Harrisonia abyssinica* (the whole plant) were identified for the current study on their antimicrobial activity, cytotoxicity and phytochemistry.

4.1.2 Antibacterial Testing

Table 4.1 and 4.2 show the activity of the bacteria species on the plant extracts. This activity was determined by the presence or absence of growth of the bacteria on the plates with various concentrations of the plant extracts after inoculation and incubation. Table 4.3 and figure 4.1 show the MIC and MBC of the plant extracts. The MIC and MBC of the test antibiotics were less than 1mg/ml. Overall the MIC and MBC of both the water and methanol plant extracts were not significantly different.

When the MIC and MBC of the extracts against the bacteria were compared, there was significant difference between the bacteria species. Gram negative bacteria were more resistant to the plant extracts than gram positive bacteria. There was no difference in activity between the different plants parts used. Methanol extracts of *Ajuga remota*, *Ocimum suave*, *Piliostigma*

thonningii, *Erythrina abyssinica* (root bark), and *Harrisonia abyssinica* showed activity against *Staphylococcus aureus* and *Bacillus cereus*. The water extracts of *Erythrina abyssinica* root bark were active against *Staphylococcus aureus* and *Bacillus cereus*.

However, *Erythrina abyssinica* stem bark did not have activity against *Staphylococcus aureus* and *Bacillus cereus* at the concentrations tested. Water extracts of *Ocimum suave*, *Ajuga remota* and *Erythrina abyssinica* roots were active against *Pseudomonas aeruginosa*. About 64.3% of the extracts had activity against *Pseudomonas aeruginosa*. *Escherichia coli* was the most resistant bacteria. The most active plant extract against the bacteria species was the stem bark of *Piliostigma thonningii*. *Erythrina abyssinica* stem bark showed no activity against the test bacteria.

Table 4.1: The antibacterial activity of the aqueous and methanolic extracts of the five plants obtained from Meru Central District against gram positive bacteria

Plant	Part	Type of extract	100mg/ml		50 mg/ml		25 mg/ml		12.5mg/ml		6.25mg/ml		3.125mg/ml	
			SA	BC	SA	BC	SA	BC	SA	BC	SA	BC	SA	BC
<i>Ocimum suave</i>	Leaves	Water	G	G	G	G	G	G	G	G	G	G	G	G
<i>Ocimum suave</i>	Leaves	Methanol	NG	NG	NG	NG	NG	NG	G	G	G	G	G	G
<i>Piliostigma thonningii</i>	Leaves	Methanol	NG	NG	NG	G	G	G	G	G	G	G	G	G
<i>Ajuga remota</i>	Leaves	Methanol	NG	NG	G	NG	G	G	G	G	G	G	G	G
<i>Ajuga remota</i>	Leaves	Water	G	NG	G	NG	G	G	G	G	G	G	G	G
<i>Erythrina abyssinica</i>	Root bark	Methanol	NG	NG	NG	G	NG	G	NG	G	NG	G	G	G
<i>Erythrina abyssinica</i>	Root bark	Water	NG	NG	NG	NG	NG	NG	NG	G	NG	G	NG	G
<i>Piliostigma thonningii</i>	Leaves	Water	G	G	G	G	G	G	G	G	G	G	G	G
<i>Piliostigma thonningii</i>	Stem bark	Methanol	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
<i>Piliostigma thonningii</i>	Stem bark	Water	G	G	G	G	G	G	G	G	G	G	G	G
<i>Erythrina abyssinica</i>	Stem bark	Water	G	G	G	G	G	G	G	G	G	G	G	G
<i>Erythrina abyssinica</i>	Stem bark	Methanol	G	G	G	G	G	G	G	G	G	G	G	G
<i>Harrisonia abyssinica</i>	Whole plant	Methanol	NG	NG	NG	NG	NG	NG	NG	NG	NG	G	G	G
<i>Harrisonia abyssinica</i>	Whole plant	Water	G	G	G	G	G	G	G	G	G	G	G	G

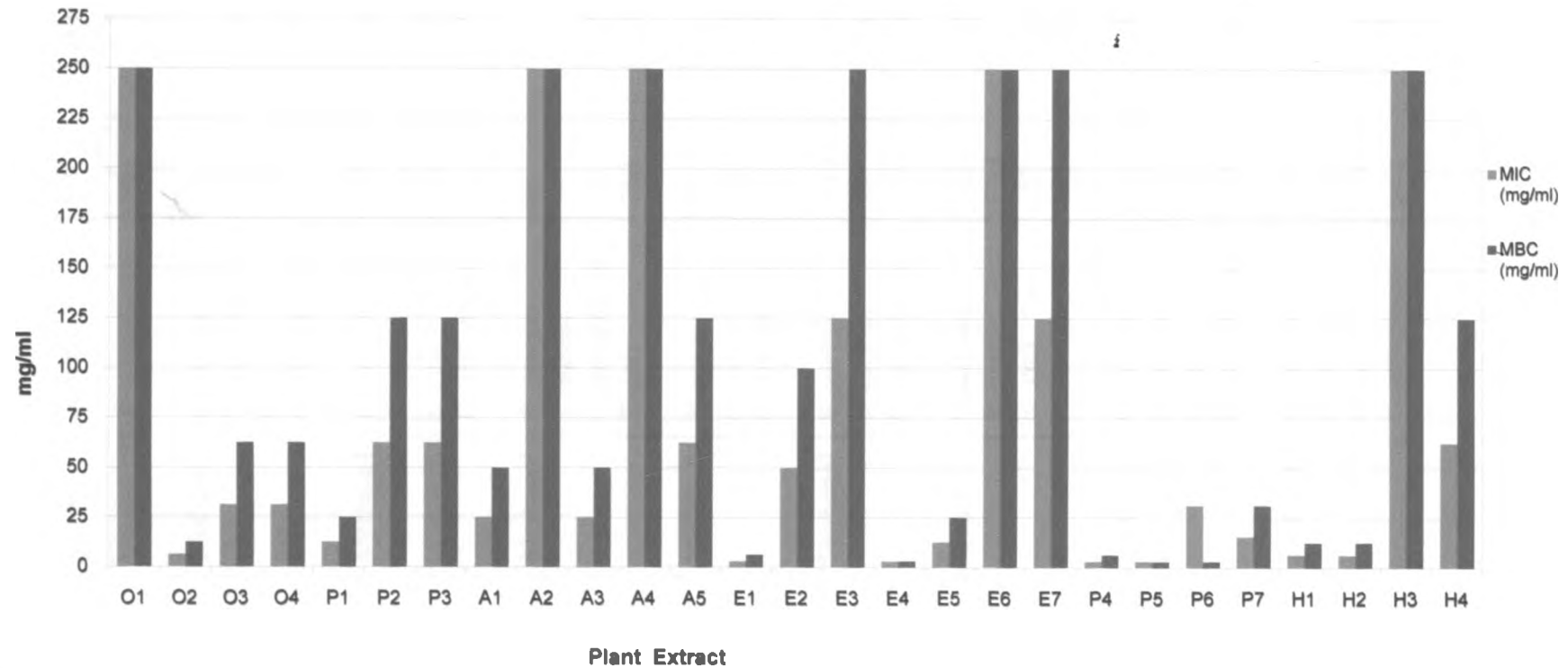
Key: BC - *Bacillus cereus*; G – Growth; NG – No Growth; SA – *Staphylococcus aureus*

Table 4.2: The antibacterial activity of the aqueous and methanolic extracts of the five plants obtained from Meru Central District against gram negative bacteria

Plant	part	Type of extract	250 mg/ml		125 mg/ml		62.5 mg/ml		31.25 mg/ml		15.625 mg/ml		7.8125 mg/ml	
			EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA
<i>Ocimum suave</i>	Leaves	Water	G	NG	G	G	G	G	G	G	G	G	G	G
<i>Ocimum suave</i>	Leaves	Methanol	NG	NG	NG	NG	NG	G	G	G	G	G	G	G
<i>Piliostigma thonningii</i>	Leaves	Methanol	NG	NG	NG	G	G	G	G	G	G	G	G	G
<i>Ajuga remota</i>	Leaves	Methanol	NG	NG	G	NG	G	G	G	G	G	G	G	G
<i>Ajuga remota</i>	Leaves	Water	NG	NG	G	NG	G	G	G	G	G	G	G	G
<i>Erythrina abyssinica</i>	Root bark	Methanol	G	NG	G	G	G	G	G	G	G	G	G	G
<i>Erythrina abyssinica</i>	Root bark	Water	NG	NG	G	G	G	G	G	G	G	G	G	G
<i>Piliostigma thonningii</i>	Leaves	Water	G	G	G	G	G	G	G	G	G	G	G	G
<i>Piliostigma thonningii</i>	Stem bark	Methanol	NG	NG	NG	NG	NG	NG	G	NG	G	G	G	G
<i>Piliostigma thonningii</i>	Stem bark	Water	G	G	G	G	G	G	G	G	G	G	G	G
<i>Erythrina abyssinica</i>	Stem bark	Water	G	G	G	G	G	G	G	G	G	G	G	G
<i>Erythrina abyssinica</i>	Stem bark	Methanol	G	G	G	G	G	G	G	G	G	G	G	G
<i>Harrisonia abyssinica</i>	Whole plant	Methanol	NG	NG	G	NG	G	G	G	G	G	G	G	G
<i>Harrisonia abyssinica</i>	Whole plant	Water	G	G	G	G	G	G	G	G	G	G	G	G

Key: EC – *Escherichia coli*; G – Growth; NG – No Growth; PA – *Pseudomonas aeruginosa*

Figure 4.1: MIC and MBC of the 7 methanolic plants' extracts used Ethnomedically in Meru Central district



Key:

O1 - <i>O. suave</i> Leaves Water extract on <i>P. aeruginosa</i>	A3 - <i>A. remota</i> Leaves Water extract on <i>B. cereus</i>	E7 - <i>E. abyssinica</i> Root bark Water extract on <i>P. aeruginosa</i>
O2 - <i>O. suave</i> Leaves Methanol extract on <i>S. aureus</i>	A4 - <i>A. remota</i> Leaves Water extract on <i>E. coli</i>	P4 - <i>P. thonningii</i> Stem bark Methanol extract on <i>S. aureus</i>
O3 - <i>O. suave</i> Leaves Methanol extract on <i>E. coli</i>	A5 - <i>A. remota</i> Leaves Water extract on <i>P. aeruginosa</i>	P5 - <i>P. thonningii</i> Stem bark Methanol extract on <i>B. cereus</i>
O4 - <i>O. suave</i> Leaves Methanol extract on <i>P. aeruginosa</i>	E1 - <i>E. abyssinica</i> Root bark Methanol extract on <i>S. aureus</i>	P6 - <i>P. thonningii</i> Stem bark Methanol extract on <i>E. coli</i>
P1 - <i>P. thonningii</i> Leaves Methanol extract on <i>S. aureus</i>	E2 - <i>E. abyssinica</i> Root bark Methanol extract on <i>B. cereus</i>	P7 - <i>P. thonningii</i> Stem bark Methanol extract on <i>P. aeruginosa</i>
P2 - <i>P. thonningii</i> Leaves Methanol extract on <i>E. coli</i>	E3 - <i>E. abyssinica</i> Root bark Methanol extract on <i>P. aeruginosa</i>	H1 - <i>H. abyssinica</i> Whole plant Methanol extract on <i>S. aureus</i>
P3 - <i>P. thonningii</i> Leaves Methanol extract on <i>P. aeruginosa</i>	E4 - <i>E. abyssinica</i> Root bark Water extract on <i>S. aureus</i>	H2 - <i>H. abyssinica</i> Whole plant Methanol extract on <i>B. cereus</i>
A1 - <i>A. remota</i> Leaves Methanol extract on <i>S. aureus</i>	E5 - <i>E. abyssinica</i> Root bark Water extract on <i>B. cereus</i>	H3 - <i>H. abyssinica</i> Whole plant Methanol extract on <i>E. coli</i>
A2 - <i>A. remota</i> Leaves Methanol extract on <i>E. coli</i>	E6 - <i>E. abyssinica</i> Root bark Water extract on <i>E. coli</i>	H4 - <i>H. abyssinica</i> Whole plant Methanol extract on <i>P. aeruginosa</i>

Table 4.3 MIC/MBC levels of aqueous and methanolic extracts of the selected plants obtained in Meru Central District

Plant	Part	Extract	Bacteria	MIC (mg/ml)	MBC (mg/ml)
<i>Ocimum suave</i>	Leaves	Water	<i>P. aeruginosa</i>	250	250
<i>Ocimum suave</i>	Leaves	Methanol	<i>B. cereus</i>	12.5	25
			<i>S. Aureus</i>	6.25	12.5
			<i>E. coli</i>	31.25	62.5
			<i>P. aeruginosa</i>	31.25	62.5
<i>Piliostigma thonningii</i>	Leaves	Methanol	<i>B. cereus</i>	100	100
			<i>S. aureus</i>	12.5	25
			<i>E. coli</i>	62.5	125
			<i>P. aeruginosa</i>	62.5	125
<i>Ajuga remota</i>	Leaves	Methanol	<i>B. cereus</i>	25	50
			<i>S. aureus</i>	25	50
			<i>P. aeruginosa</i>	62.5	125
			<i>E. coli</i>	250	250
<i>Ajuga remota</i>	Leaves	Water	<i>B. cereus</i>	25	50
			<i>E. coli</i>	250	250
			<i>P. aeruginosa</i>	62.5	125
<i>Erythrina abyssinica</i>	Root bark	Methanol	<i>S. aureus</i>	3.125	6.25
			<i>B. cereus</i>	50	100
			<i>P. aeruginosa</i>	125	250
<i>Erythrina abyssinica</i>	Root bark	Water	<i>S. aureus</i>	3.125	3.125
			<i>B. cereus</i>	12.5	25
			<i>E. coli</i>	250	250
			<i>P. aeruginosa</i>	125	250
<i>Piliostigma thonningii</i>	Stem bark	Methanol	<i>S. aureus</i>	3.125	6.25
			<i>B. cereus</i>	3.125	3.125
			<i>E. coli</i>	31.25	62.5
			<i>P. aeruginosa</i>	15.625	31.25
<i>Harissonia abyssinica</i>	Whole plant	methanol	<i>S. aureus</i>	6.25	12.5
			<i>B. cereus</i>	6.25	12.5
			<i>E. coli</i>	250	250
			<i>P. aeruginosa</i>	62.5	125

4.1.3 Brine Shrimp Lethality Test

The results are represented in the tables below. Table 4.4 represents the percentage mortality of the shrimps at the various concentrations. Table 4.5 shows the lethal concentration 50 (LC₅₀) of the plant extracts at 95% confidence intervals. The results of the LC₅₀ (µg/ml) are as follows: *Ajuga remota* (Leaves) 69.2, *Piliostigma thonningii* (Leaves) 186.45, *Erythrina abyssinica* (Stem Bark) 219.1, *Erythrina abyssinica* (Root Bark) 232.3, *Piliostigma thonningii* (Stem Bark) 264.8, *Harrisonia abyssinica* (Whole Plant) 299.5 and *Ocimum suave* (Leaves) 312.55. The LC₅₀ of vincristine sulphate was less than 1 µg/ml and DMSO did not kill the brine shrimps at the tested concentrations.

Figure 4.2 represents the percentage brine shrimp mortality due to serial dilutions of the extracts and positive control. All the plants in the study were toxic to the brine shrimp. *Ajuga remota* was most toxic to the shrimp. *Ajuga remota* leaves and *Piliostigma thonningii* leaves were toxic to the brine shrimp at low concentrations of less than 200 µg/ml

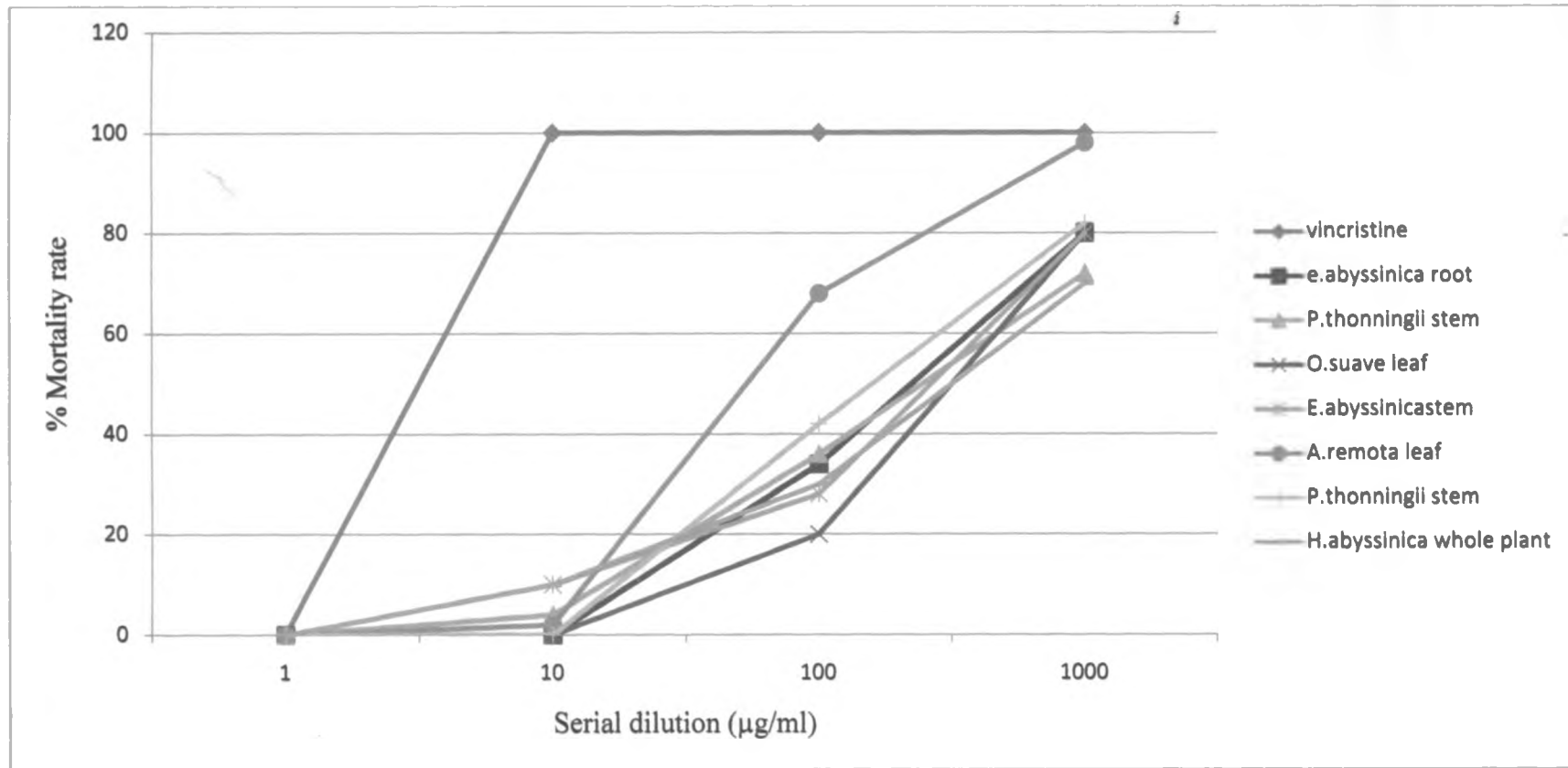
Table 4.4 Percentage brine shrimp mortality caused by serial dilution of methanolic extracts of the selected plants obtained in Meru Central District

Botanical Name	Local Name	Parts Used	Serial Dilution of the Methanol Extract ($\mu\text{g/ml}$)		
			10	100	1000
<i>Erythrina abyssinica</i>	MUUUTI	Stem bark	10	28	80
<i>Erythrina abyssinica</i>	MUUTI	Root bark	0	34	80
<i>Harrisonia abyssinica</i>	MUTAGATAGA	Whole plant	10	30	70
<i>Piliostigma thonningii</i>	MUKUURA	Stem bark	4	36	72
<i>Piliostigma thonningii</i>	MUKUURA	Leaves	0	42	82
<i>Ajuga remota</i>	KIRURAGE	Leaves	2	68	98
<i>Ocimum suave</i>	MAKURI	Leaves	0	20	80

Table 4.5 Lethal Concentration 50 (LC₅₀) in µg/ml, and 95% Confidence (CI) of the selected plants' extracts obtained in Meru Central District

BOTANICAL NAME	LOCAL NAME	PART TESTED	LC ₅₀	95% CI
<i>Erythrina abyssinica</i>	MUUTI	Stem Bark	219.1	61.3 – 1347.7
<i>Erythrina abyssinica</i>	MUUTI	Root Bark	232.3	85.4 – 818.5
<i>Harrisonia abyssinica</i>	MUTAGATAGA	Whole Plant	299.5	74.1 – 6008.2
<i>Piliostigma thonningii</i>	MUKUURA	Leaves	186.4	67 – 652
<i>Piliostigma thonningii</i>	MUKUURA	Stem Bark	264.8	80.7- 1737.5
<i>Ocimum suave</i>	MAKURI	Leaves	312.55	111.3 – 1075.3
<i>Ajuga remota</i>	KIRURAGE	Leaves	69.2	26.4 – 179.1

Figure 4.2 Comparison of the percent brine shrimp mortality due to the serial dilutions of plant extracts and that due to vincristine sulphate



4.1.4 Preliminary Phytochemical Analysis

Tannins and saponins were found in all the plant extracts. Alkaloids, flavonoids and cardiac glycosides were each found in 85.7% of the plant extracts. Sterols were present in 71.4% of the plant extracts and triterpenes were present in 28.6% of the plant extracts. Anthraquinones and flavones were absent in all the plant extracts. The results of the phytochemical testing are represented in table 4.6

4.1.4.1 *Erythrina abyssinica* root bark and stem bark

Alkaloids, tannins, saponins, triterpenes, flavonoids and cardiac glycosides were present in the root bark and stem bark of *Erythrina abyssinica*. Flavones, sterols and anthraquinones were absent in the root bark and stem bark of *Erythrina abyssinica*.

4.1.4.2 *Piliostigma thonningii* stem bark and leaves

This study showed the presence of alkaloids, tannins, saponins, sterols, flavonoids and cardiac glycosides in the stem bark and leaves of *Piliostigma thonningii*. Flavones, triterpenes and anthraquinones were absent in the stem bark and the leaves of *Piliostigma thonningii*.

4.1.4.3 *Ajuga remota* leaves

This study established that *Ajuga remota* leaves have alkaloids, tannins, saponins, sterols, flavonoids and cardiac glycosides. Flavones, triterpenes and anthraquinones were absent on the leaves of *Ajuga remota*.

4.1.4.4 *Ocimum suave* leaves

In this study, tannins, saponins, sterols, and flavonoids were detected in the leaves of *Ocimum suave*. Alkaloids, flavones, triterpenes, cardiac glycosides and anthraquinones were absent.

4.1.4.5 *Harrisonia abyssinica* whole plant

In this study the plant was found to contain alkaloids, tannins, saponins, sterols and cardiac glycosides. Triterpenes, flavones, flavonoids and anthraquinones were not detected in *Harissonia abyssinica*

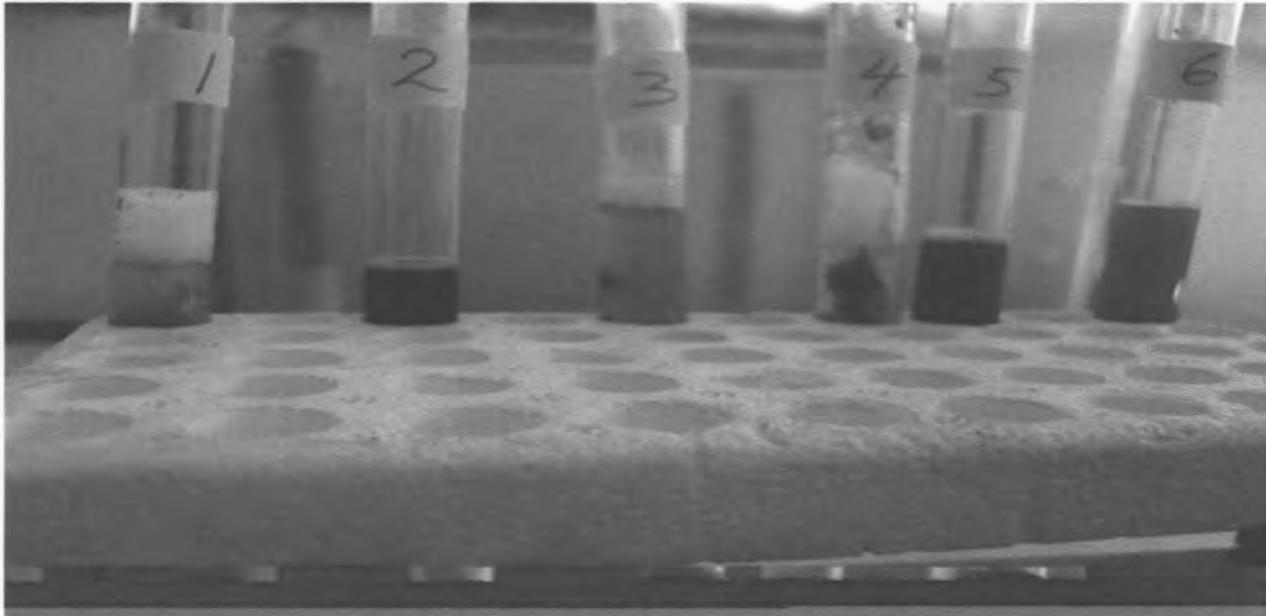
Plates 4.1 and 4.2 (a and b) show some of phytochemical the results.

Table 4.6 Phytochemical results of the selected plants obtained in Meru Central District

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Plant	Alkaloids	Tannins	Saponins	Sterols	Triterpenes	Flavones	Flavonoids	Anthraquinones	Cardiac glycosides
<i>Erythrina abyssinica</i> root bark	+	+	+	-	+	-	+	-	+
<i>Piliostigma thonningii</i> leaves	+	+	+	+	-	-	+	-	+
<i>Piliostigma thonningii</i> stem bark	+	+	+	+	-	-	+	-	+
<i>Harissonia abyssinica</i> whole plant	+	+	+	+	-	-	-	-	+
<i>Erythrina abyssinica</i> stem bark	+	+	+	-	+	-	+	-	+
<i>Ocimum suave</i> leaves	-	+	+	+	-	-	+	-	-
<i>Ajuga remota</i> leaves	+	+	+	+	-	-	+	-	+

Plate 4.1 Positive frothing test for saponins



1. *Erythrina abyssinica* root bark
2. *Erythrina abyssinica* stem bark
3. *Piliostigma thonningii* leaves
4. *Harrisonia abyssinica* whole plant
5. *Piliostigma thonningii* stem bark
6. *Ocimum suave* leaves

4.2 DISCUSSION

4.2.1 Ethnobotanical survey

This study was able to establish that the Meru have a large traditional medicine base like many communities in Kenya. There is great potential for use of these traditional remedies in treating infections due to bacteria. Knowledge on the use of the herbal medicine is passed from generation to generation among the Meru community. This concurs with what Miaron *et al* (2004) observed among the Maasai community of Kenya.

Few of the herbalists kept botanical gardens and most of the medicinal plants are sourced from the wild. The area receives sufficient amounts of rainfall and has good soils that make wide range of tree species and herbs grow. For the treatment of various infections most of the herbalists favoured combination of different plants. Leaves are still the most widely used among the plant parts. This is in agreement with previous reporting in other parts of Kenya by Wagate (2008).

4.2.2 Antibacterial testing

The MIC and MBC of the antibiotics were less than 1mg/ml. Overall the MIC and MBC of both the water and methanol plant extracts were not significantly different. This supports the use of the plants by the herbalists since many use water as solvent when needed. There was significant difference between the bacteria species. Gram positive bacteria were more susceptible to the plant extracts than gram negative bacteria. There was no difference in activity between the different plants parts used.

All the plants' parts tested had activity against the test bacteria except the stem bark of *Erythrina abyssinica*. This may imply that there is no basis for use of the stem bark of *Erythrina abyssinica* in treating infections. Wagate, (2008), was also not able to detect antibacterial activity of

Erythrina abyssinica. However further tests are needed to confirm whether the stem bark of *Erythrina abyssinica* has any antimicrobial activity.

Of significance is that 64.3% of the extracts had activity against *Pseudomonas aeruginosa* which is a major cause of opportunistic infections especially in hospitals. The activity of these plants towards *Pseudomonas aeruginosa* should be investigated further. The stem bark of *Piliostigma thonningii* was the most active against all the test bacteria. Ibewuke *et al* (1997) showed that the methanol extracts of *Piliostigma thonningii* inhibited the activity of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. This study supports those findings. Further research is needed to study the antibacterial spectrum of this plant.

4.2.3 Brine shrimp lethality test

Studies show that brine shrimp assay is an excellent method for preliminary investigations of toxicity and to screen medicinal plants. Besides it has been used to detect fungal toxins, pesticide and cytotoxicity of dental materials (Alluri, *et al.*, 2005). The technique is easily mastered, costs less and utilizes small amount of test material (McLaughlin and Rogers, 1998).

Meyer *et al* (1982) and Santos *et al* (2003) laid down the process of interpretation of the results of bioassay such that extracts are toxic where $LC_{50} < 1000\mu\text{g/ml}$ and non toxic when $LC_{50} > 1000\mu\text{g/ml}$. All the plants in the study were toxic to brine shrimp with *Ajuga remota* being most toxic. Previous studies have shown isolates of *Ajuga remota* are cytotoxic and have antileukemic action (Israili and Lyousi, 2009; Wagate, 2008).

Earlier studies have shown *Harissonia abyssinica* is cytotoxic (Balde *et al.*, 1995; Kirira *et al.*, 2006; Maregesi *et al.*, 2008). This study showed the LC_{50} *Harissonia abyssinica* to be $299.48\mu\text{g/ml}$. This value qualifies to be toxic (Meyer *et al.*, 1982; Santos *et al.*, 2003).

All the plants in this study were cytotoxic and this could be due to the secondary metabolites contained in these plants such as flavonoids and saponins (Akaneme, 2008; Arwa, *et al.*, 2008; Harbone, 1989; Jain *et al.*, 2006; Middleton and Kandaswami, 1993; Pengelly, 2004).

4.2.4 Preliminary Phytochemical analysis

The medicinal value of plants lies in chemical constituents that produce definite action on the human body. In this study, tannins and saponins were found in all the plant extracts. Alkaloids, flavonoids and cardiac glycosides were each found in 85.7% of the plant extracts. Sterols were present in 71.4% of the plant extracts and triterpenes were present in 28.6% of the plant extracts. Anthraquinones and flavones were absent in all the plant extracts.

4.2.4.1 *Erythrina abyssinica* root bark and stem bark

Alkaloids, tannins, saponins, triterpenes, flavonoids and cardiac glycosides were present in the root bark of *Erythrina abyssinica*. Kareru *et al* (2008, a) observed that *Erythrina abyssinica* had saponins and that about 76% of plants so far studied contain saponins. This study supports those findings. Saponins have various activities among them antibacterial, anti-inflammatory, antiviral and as surfactants. This may explain why *Erythrina abyssinica* root bark is used for treatment of bacterial infections such as anthrax, gonorrhoea and syphilis by the Meru community.

The anti-inflammatory activity of *Erythrina abyssinica* is the reason why it is used by the Meru community to manage pain and inflammation associated with burns, body swellings. Alkaloids exhibit activities such as analgesic, antispasmodic and bactericidal effects. This may be useful in relieving pain and explains why the plant is used in cases of body pains, burns and infections. Further, the presence of alkaloids in this plant may explain its uses in relieve of colic, healing of wounds and treatment of bacterial infections among the Meru community. Tannins have astringent action that enables them to constrict blood vessels, stop bleeding and provide a protective coat on wound. Presence of tannins in *Erythrina abyssinica* may support the use of this plant for burns and wounds.

Flavonoids have many activities in the human body such as anti-inflammatory, antiviral and prevention of allergies. They also have hepatoprotective activity, are antiproliferative, have anticarcinogenic properties and are useful antioxidants. Their presence in this plant explains why it may be useful in management of body swellings, antidote for poisoning and treatment of bacterial infections like gonorrhoea, syphilis and anthrax. The presence of cardiac glycosides in this plant may be indicative of its activity on the heart (Araujo *et al.*, 2008; Arwa *et al.*, 2008; Chetri *et al.*, 2008; Kareru *et al.*, 2008,a; Kisangau, 1999; Kokwaro, 1993; Jain *et al.*, 2006; Pengelly, 2004). However, there is no reported use of this plant for this purpose among the Meru community.

4.2.4.2 *Piliostigma thonningii* stem bark and leaves

Previous studies have shown that the seeds of *Piliostigma thonningii* have saponins, flavonoids, phenolics, anthraquinones, sterols and triterpenes, and cardiac glycosides (Jimoh and Oladji, 2005). The leaves had flavonols and exhibited antimicrobial activities (Ibewuke *et al.*, 1997). This study was able to show the presence of alkaloids, tannins, saponins, sterols, flavonoids and cardiac glycosides. Listed among the ethnomedical uses of *Piliostigma thonningii* is treatment of coughs, colds and chest pains.

The presence of flavonoids and alkaloids in this plant supports its ethnomedical uses. Flavonoids have anti allergy properties and may be useful for coughs and colds. They also have antimicrobial activities and together with alkaloids may explain why this plant is useful in management of infection due to bacteria. Since tannins have astringent action, their presence in this plant may explain its use for management of wounds and menorrhagia. However more studies are needed to establish this as a fact.

Sterols and triterpenes are precursors of other steroids in the body which include Vitamin D and sex hormones (Akaneme, 2008). Abnormal uterine bleeding may be due to hormonal imbalance and the presence of sterols can explain the use of this plant for management of menorrhagia by the Meru community. More studies are needed to determine the type of sterols present in this plant and whether they have any role in management of hormonal disorders. The presence of cardiac glycosides may suggest that this plant has activity in the heart. It is worth noting that this is not a listed indication of this plant among the Meru community.

4.2.4.3 *Ajuga remota* leaves

Ajuga remota has been ethnomedicinally used by many African communities for management of fever, toothache, malaria, dysentery, eye infections, stomachache and pneumonia (Gachathi, 1993; Kokwaro, 1993; Kuria and Muriuki, 1984). Studies done earlier have shown that this plant has glycosides, triterpenes, essential oils, flavonoids and sterols. This study established that *Ajuga remota* has alkaloids, tannins, saponins, sterols, flavonoids and cardiac glycosides. Sterols are precursors of steroids in the human body and their presence in this plant mean that it may have use in treating hormonal disorders (Akaneme, 2008).

The presence of cardiac glycosides in *Ajuga remota* suggests that the plant may be of use in management of heart conditions. Previous study has shown that this is a possible mechanism of action (Galiwango, 1989). The presence of alkaloids, tannins, and flavonoids in this plant may explain the reason for its use in bacterial infections, relieve of pain and fever among the community (Israili and Lyousi, 2009).

4.2.4.4 *Ocimum suave* leaves

Among the Meru community, *Ocimum suave* has been used for treatment of blocked nostrils, abdominal pains, sore eyes, ear infections and cough. It is also used as a disinfectant/insecticide

(Kokwaro, 1993). Earlier studies showed that the extracts of this plant had fever relieving properties and antiulcer activity (Desta, 1993; Perez – Alonzo, *et al.*, 1995; Tan *et al.*, 2002).

In this study, tannins, flavonoids, saponins, and sterols were present. The presence of saponins and flavonoids may explain the use of this plant for treatment of infections such as those of chest, eyes, stomach and nose among the Meru community. Flavonoids have antiallergic properties (Middleton and Kandaswami, 1993) and this may be the reason for use of *Ocimum suave* leaves in management of symptoms associated with allergies such as blocked nose and cough.

Tannins have astringent action and this is a possible explanation why this plant is used for treatment of abdominal pains (Kisangau, 1999; Kokwaro, 1993). The presence of saponins explains why this plant is used as a disinfectant/ insecticide (Kokwaro, 1993). Sterols are precursors of steroids in the body such as Vitamin D and sex hormones (Akaneme, 2008). However, there is no recorded use of this plant among the Meru community for treatment of hormonal disorders.

4.2.4.5 *Harissonia abyssinica* whole plant

Harissonia abyssinica is used ethnobotanically by the Meru community for management of stomach ache, abdominal pains, fever, nausea, vomiting, plague, dysentery, gonorrhoea and tuberculosis (Kokwaro, 1993). In this study *Harissonia abyssinica* was found to contain alkaloids, tannins, saponins, sterols and cardiac glycosides. The presence of alkaloids may be the reason why *Harissonia abyssinica* is used for treatment of infections due to bacteria because some alkaloids have been shown to have antibacterial activities. Alkaloids also have antispasmodic action and this may explain the use of this plant for management of abdominal pains, nausea and vomiting.

The action of alkaloids as decongestants and antibacterial lends some credence for use of this plant in management of tuberculosis (Arwa *et al.*, 2008; Chetri *et al.*, 2008; Pengelly, 2004). However, more research is needed concerning the use of this plant for management of TB. The presence of alkaloids and saponins may explain why this plant is used in management of infections such as plague among the Meru community.

Since tannins are known to exhibit astringent action, the use of this plant for abdominal pains, stomachache or diarrhea is supported by the findings of this study. Sterols are precursors of steroids in the human body and their presence in this plant mean that it may have use in management of hormonal disorders (Akaneme, 2008). Principles isolated from this plant have been shown to have antibacterial, antifungal, antiviral, cytotoxic activity (Balde *et al.*, 1995; Kirira *et al.*, 2006 Maregesi *et al.*, 2008). The results of this study support these previous studies.

CHAPTER FIVE: GENERAL CONCLUSIONS

Due to the increase in antimicrobial resistance to the available conventional drugs, researchers are now turning to plants and other natural products as sources of drugs. The new drugs are expected to have minimal side effects, be less toxic and provide novel mechanisms of action. Despite the fact that plants form components of every system of medicine in the world, less than 5% of over 250,000 species in the world have undergone any meaningful analysis.

Folklore medicine is a major starting point for this research as it provides a guide to the assumed properties of the plants and other natural products. However, in countries like Kenya, The subject of traditional herbal medicine is not taught in the institutions that train healthcare workers. In many instances the traditional medicine practitioners are illiterate or semi-illiterate. It is worth noting that in countries like Belgium, Germany and France, herbal medicine is included in training of healthcare providers. In China, herbal medicine is taught in institutions like universities and there are big pharmaceutical companies that deal with herbal medicine.

For research in herbal medicine to be effective, collaboration between the herbal practitioners and conventional health care givers is of paramount importance. There is need for mutual understanding and trust between the herbal practitioners and the researchers so that this research can take root. The greatest shortcoming of herbal medicine in Kenya is that very few natural products have undergone any scientific validation. Most of the natural products are not well understood with regard to their actions. Besides, the use of these products has not been evaluated in long term use.

Plants contain several constituents whose concentration is what determines the action of the plant. It is possible that toxicity can arise due to synergism of these constituents. Research ought

to be carried out to determine the accepted levels of the plants constituents that will not harm the users. This can be possible if studies of the plants are carried out in animal models to show the overall effect of the plant and that of each of its constituents. Further research can then be carried out to determine what aspects traditional herbal medicine can be incorporated in the national health care system to complement the conventional drugs.

All the plants in this study had activity against some or all the bacteria tested. The greatest activity was shown by the methanolic stem bark of *Piliostigma thonningii*. There were several active substances in the extracts such as alkaloids, tannins, saponins, flavonoids, sterols and cardiac glycosides. The combined action of these substances is what gives the plants their overall activity.

The fact that all the plants in this study were toxic to brine shrimp is evidence that these plants should be investigated further for their toxicity. Phytochemical screening of medicinal plants forms the basis of understanding the medicinal properties of plants. Although these plants were active against the test bacteria, their use against general bacterial infections should not be advocated for until further investigations. The herbalists should be guided by use of more scientific evaluations and studies on the plants that showed significant antibacterial activity.

For now, the study was able to show some proof that the plants have antibacterial activity and that the herbalists can use them for the specific infections caused by the bacteria that were shown to be susceptible.

Recommendations

1. The plants in this study showed good antibacterial activity therefore the herbalists should be encouraged to use them. Further pharmacologic, toxicological and phytochemical

analysis should be done on the other plants. The aim should be to identify which constituents are responsible for the actions and at what concentrations so that they can be isolated and made into medicine.

2. The herbalists should be encouraged to plant botanical gardens to conserve and propagate the medicinal plants.
3. More research should be carried out to study and document the medicinal plants used by the Meru community.
4. Policies should be put in place so that traditional medicine is taught in health training institutions and is incorporated in the national health care system.

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Appendix 1: Preparation of blood agar medium

Suspend 40g of blood agar in 1 liter of distilled water and boil to dissolve. Sterilize by autoclaving at 121⁰c for 15 minutes. Cool the base to 45-55⁰c. Add 7% sterile blood to the sterile media. The blood is warmed to 37⁰c before adding. Dispense the mixture into sterile Petri dishes.

Appendix 2: Preparation of Mueller Hinton broth medium

Typical formula (g/l)

Beef dehydrated infusion	300
Casein hydrosylate	17.5
Starch	1.5

Dissolve 21g in 1 liter of distilled water. Bring to boil to dissolve medium completely. Sterilize by autoclaving at 121⁰c for 15minutes

Appendix 3: Preparation of Tryptone Soya Agar.

Typical formula (g/l)

Tryptone	15
Soya peptone	5
Sodium chloride	5
Agar	15

Suspend 40g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121⁰c for 15minutes