

**PHARMACOLOGICAL EVALUATION OF ANTIMICROBIAL AND
BIOACTIVITY OF PLANT USED IN ETHNOMEDICINE AND
ETHNOVETERINARY MEDICINE IN MACHAKOS AND KITUI AREAS,
KENYA.**

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A thesis submitted in partial fulfillment of requirements for the degree of Master of Science
(MSc) of University of Nairobi

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August 2008

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DECLARATION

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

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Date 28/7/08

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DEDICATION

This work is dedicated to my Parents; Zabedee Wagate Wambugu and Anne Wanjiru Wagate, my siblings Wanjiku, Wambugu, Muthoni and Kamau, for their support

“Let the food be your medicine and medicine your food”

Hippocrates (460-377 BC)

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ACKNOWLEDGEMENT

Special acknowledgement to my supervisors Dr. Mbaria and Dr. Gakuya for availing their time, encouragement and guidance throughout the course of the project.

Special mention and thanks to the Traditional Medical Practitioners (TMP) of Ukamba Herbalist Association who shared their information with enthusiasm and who taught me a lot. Their zest to have the herbal products validated was encouraging. I also wish to thank traditional practitioners from Samburu, who I was privileged to spend time, interact with and share ideas with. My association with them helped to enrich my understanding on herbal medicine in Kenya and way forward.

I express gratitude to Commission for Higher Education for the considerable financial support which enabled successful completion of this study.

I wish to extend my heartfelt gratitude to the Staff, Department of Public Health, Pharmacology and Toxicology; Anne, Karanja, Gitau and Rahab Munenge for their support. I'm grateful to Anne, for offering knowledge on processing and extraction procedures.

I'm grateful to Nduhiu and Macharia, Senior Technologists in the department PHPT for availing their time and being on hand to give guidance, support and encouragement. Sincere gratitude to Ochung, Herbarium Curator for identification and characterization of the medicinal plants under study. I'm grateful to Mark, Koros and Charity for their input, advice and encouragement. To my friends Stanley, Carol, Sam, Eunice, Sarah, Kate, Amadeep and Fred for the precious moments.

To my family for their support. God bless you.

LIST OF ACRONYMS AND ABBREVIATIONS

ATCC:	American type culture collection
BA:	Blood agar
CAM:	Complementary and Alternative Medicine
Cfu:	Colony forming unit
EMEA:	European Medicines Licensing Agency
GACP:	Good Agricultural and Collection Practices
GoK:	Government of Kenya
GTZ:	Germany Technical Corporation
HIV/AIDS:	Human immunodeficiency virus/acquired Immunodeficiency Syndrome
IDRC:	International Development Research Centre
KEMRI:	Kenya Medical Research Institute
MHA:	Mueller Hinton agar
MHB:	Mueller Hinton broth
PBS:	Physiological buffer saline
TAM:	Traditional African Medicine
TH/TMP:	Traditional healers/traditional medical practitioners
UN:	United Nations
WHO:	World Health Organization

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ABSTRACT

The use of plants to cure diseases and relieve physical sufferings started from the earliest times of mankind's history. In Africa, 80% of the population uses traditional medicine for primary health care. Due to antimicrobial resistance becoming a global problem with far reaching implications for the survival of human race, efforts are continuously made to overcome this. Current efforts include research in finding new and innovative antimicrobials from plants.

Participatory Rural Appraisal (PRA) method was used to identify plants used in management of diseases by 110 traditional medical practitioners in Machakos and Kitui districts. Voucher specimens were deposited at Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi for identification. The two districts have a low doctor/patient ratio compounded with most hospitals being located in urban areas thus most of population seeks medical attention from TMP. The minimum inhibitory concentration (MIC) of twelve (12) methanolic plant extracts were determined using standard cultures of *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *Micrococcus lutea*. The data was stored in Microsoft Excel 2003[©], and then analyzed using Genstat® (Version 9.0). Comparison was computed for the susceptibility of different bacterial species and considered statistically different at $P < 0.05$.

Extracts of *Harrisonia abyssinica* and *Terminalia kilimandscharica* showed activity against all bacterial strains. *H. abyssinica* showed inhibitory concentration against *Pseudomonas aeruginosa* at 37.5mg/ml. *Ps. aeruginosa* was the least sensitive to the plant extracts with only 45% of plant extracts having inhibitory activity. *Bacillus cereus* was the most sensitive to the extracts with 83% of plant showing inhibitory activity and 64% had

MIC of less than 20mg/ml. The plant extracts were more active against Gram positive bacteria than Gram negatives. The positive controls showed significant inhibitory activity at concentration of less than 1mg/ml.

The bioactivity of the methanolic plant extracts was determined using the brine shrimp lethality test. The serial dilutions of 1000, 100 and 10 μ g/ml were made respectively. The percentage mortality was determined for each dilution and controls after 24 hours. The lethal concentration 50 (LC₅₀) and 95% confidence intervals were determined using probit analysis.

The plant extract with the lowest LC₅₀ value was the most toxic to brine shrimp nauplii while the one with highest LC₅₀ value was the least toxic. All plant extracts showed significant bioactivity at LC₅₀ <1000 μ g/ml except *Ziziphus abyssinica* which was the least toxic to brine shrimp with LC₅₀ >1000 μ g/ml. *Ajuga remota* was lethal to brine shrimp nauplii at 61.6 μ g/ml. Twenty five percent (25%) of methanolic plant extracts under investigation showed lethality to brine shrimp at LC₅₀ <200 μ g/ml.

The results of the present study support the continued use and further scientific validation of the selected medicinal plants by the herbalists in the management of infectious conditions. The plant extracts screened, *A. remota* showed the inhibitory concentration at less than 35mg/ml and also had the lowest LC₅₀ less than 100 μ g/ml, these results lend further support its traditional use.

CHAPTER ONE: INTRODUCTION

1.0 Background

Traditional healers have for long used plants to prevent or cure infectious conditions and conventional medicine has heavily relied on knowledge from traditional healers. Archeological evidence dating back to 2,800 years on the use of medicinal plants has been unearthed. This included seed clumps of *Capparis spinosa* L. and *Cannabis sativa* L. shoots, leaves and fruits in the Yanghai Tombs, Turpan District in Xinjiang, China. Based on the joint occurrence of *Capparis spinosa* and *Cannabis sativa*, and the pharmacological value of the seeds of *Capparis spinosa*, it was deduced that capper was utilized for medicinal purposes. *Capparis* has ferulic and sinapic acid which have medicinal value. The root bark is used for different illness like cough, asthma, paralysis, scrofula, toothache and spleen disease (Jiang *et al.*, 2007)

Plants are rich in a wide variety of phytochemicals that have antimicrobial properties (Baratta *et al.*, 1998a, b). Plants form the biggest source of products used in folk Veterinary and Human practice (Dery *et al.*, 1999). Most of the existing pharmaceutical multi-nationals have their roots in the use of crude plant extracts centuries ago (Mez-Mengold, 1971).

Medicine, in several developing countries, using local traditions and beliefs, is still the mainstay of health care. Eighty percent of population in Sub-Saharan Africa relies on traditional remedies (WHO, 2002). Most of these traditional remedies involve use of plants or their active principles. It is estimated that about a quarter of all prescription drugs offered for sale in the developed world still use active ingredients derived from plants (Cox and Balick, 1994).

The Kenyan population is estimated at 35 million with an annual birth rate of 2.4 percent and many natural habitats have been cleared for human settlement and agriculture. It is estimated that up to 75 percent of people use traditional therapies with a very low doctor/patient ratio often reaching 1:22,000 for outpatients (Wanyama, 1997a; GoK, 2000). Currently the Kenya forest cover is estimated to be 1.7% as compared to recommended acreage of 10% of total land mass. The usefulness of indigenous forest and shrubs as source of medicines will lead to their protection and propagation of this indispensable natural resource which supports 80 percent of population that relies on herbal medicine for their primary health care (WHO, 2002).

In the last two decades, research and development experts have promoted indigenous knowledge as a key to sustainable development (Chambers *et al.*, 1989; Warren *et al.*, 1995). Nevertheless, examples of the integration of indigenous knowledge into the research process and its application within the development context remain scarce. One reason for this may be the geographical and economic dominance of scientific knowledge making it difficult for Western scientists and development experts to deal with a different knowledge system (Antweiler, 1995).

There is also a fundamental difference between indigenous and scientific knowledge in that science is searching for information of universal significance, which is not context-related ('immutable mobiles'). Indigenous knowledge, by contrast, is a social product that is closely linked or even restricted to a cultural and environmental context ('mutable immobiles') (Antweiler, 1995). This means that it is dynamic, developing as the collective experience of specific social groups in interaction with their environment. (Kievelitz, 1995).

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. While 25 to 50 percent of current pharmaceuticals are derived from plants, only a few are used as antimicrobials (Cowan, 1999; Raskin *et al.*, 2002).

Many medicinal plants are currently used as unregulated crude preparations and their use by the public is increasing rapidly. There is need to consider the consequences of self-medicating with these preparations. There is need to investigate pharmacological and toxicological properties of herbal preparation in order to determine their safety and efficacy. It is incontrovertible that Traditional African Medicine (TAM) exhibits far more merits than demerits and its values can be exploited and be beneficial if scientifically validated. The Lusaka Declaration in 2001 (2001-2010) as the Decade for African Traditional Medicine has resulted in creation in East Africa, the Network on Medicinal Plants and Traditional Medicine launched in 2003. Its main objectives are to strengthen collaborative activities and to share information on medicinal plants (IDRC, 2003).

The antimicrobial activities of some herbs against important human pathogenic bacteria have been examined in details (Dorman and Deans, 2000; Ozcan and Boyraz, 2000; Ozcan and Erkmen, 2001; Sagdic, 2003; Baydar *et al.*, 2004). Test for the bioassay of medicinal plants have been done using brine shrimp lethality test (BST) (Meyer *et al.*, 1982). This was later modified by McLaughlin *et al.*, (1991). It is rapid, reliable, inexpensive and convenient tool to detect general bioactivity. The method has been

applied in screening plant extracts used across the world (Alkofahi *et al.*, 1997; Mwangi *et al.*, 1999; Gakuya, 2001; Wanyoike *et al.*, 2004; Moshi *et al.*, 2004, 2006, 2007a).

In order to utilize plants as medicines, man has had to discriminate between poisonous and non-poisonous materials in his environment. Several toxicity studies on medicinal plant extracts have documented and include *in-vivo*, *in-vitro* and herbal-drug interactions (Gathumbi, 1995; Gathumbi *et al.*, 2000; Wojcikowski *et al.*, 2004). World Health Organization African Regional Director has outlined a few guidelines on the responsibilities of all African nations for the realistic development of Tradition African Medicine, in order to sustain our health agenda and perpetuate our culture. This strategy provides for the institutionalization of traditional medicine in health care systems of the member states of the WHO African Region (WHO, 2003).

The gradual extinction of the forests and the inevitable disappearance of the aged Traditional Medical Practitioners should pose an impending deadline for us to learn, acquire and document our medical cultural endowment for the benefit of all Africans and indeed the entire humanity. For this reason, there is a need to involve TMPs in national healthcare systems through training and evaluation of effective remedies, as they are a large and influential group in primary healthcare (Akerele, 1987; Anyinam, 1987; Good, 1987).

1.1 Research Problem and Justification

Although Traditional Healers in Kenya and around the world use a variety of plant to treat different kinds of infectious and non-infectious diseases, their claim to the efficacy of these plants needs to be investigated and verified in order to elucidate the actual efficacy and their usefulness in treatment. In Kenya, traditional medicine continues to

play a major role in Primary Health Care (PHC). In Machakos and Kitui districts, there is a low doctor/patient ratio, disappearance of local traditional herbal use and erosion of the environment; all these factors call for concerted effort to collect, document and scientifically validate medicinal plant use. More than 70% of the Kenyan population relies on traditional medicine as its primary source of health care, while more than 90% use medicinal plants at one time or another. The advent of HIV/AIDS pandemic has over-stretched the Health Care System and Economy as a whole. The use of plants to cure diseases and relieve physical sufferings started from the earliest times of mankind's history. Due to antimicrobial resistance becoming a global problem with far reaching implications for the survival of human race, efforts are continuously made to overcome this. Current efforts include research in finding new and innovative antimicrobials from plants.

1.2 Null hypothesis

It was hypothesized that the crude plants extract had significant *in-vitro* antimicrobial activity on test bacteria and *in-vitro* bioactivity on brine shrimp nauplii.

1.3 General objectives

To assess the antimicrobial activity and bioactivity of methanol extract of selected plants.

1.3.1 Specific objectives

1. To collect and identify plants used by members of Ukamba Herbalist Society traditional practitioners for management of infections.
2. To investigate the antimicrobial activity of the medicinal plant extracts using microbial culture method by determining the minimum inhibitory concentration.
3. To determine the bioactivity of the plant extracts by use of brine shrimp lethality test.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

Since the discovery of the first antimicrobial in 1928, there has been widespread use of antimicrobial in treatment of infectious diseases in both man and animals. However, in recent years there is concern regarding the emergence of antimicrobial resistance in previous susceptible microbial populations. Infections caused by resistant pathogens represent an important source of morbidity and mortality and increase in cost of medical care. They are mainly based on selection pressure due to extensive use of antimicrobial drugs and presence of resistance genes. Overuse, inappropriate or inadequate therapy in medical clinics and hospitals has enhanced selection of resistant bacterial strains (Kapil, 2005).

Plants have an almost limitless ability to synthesize phytochemicals and tapping of this indispensable resource is the way forward to beat the challenges of 21st century and beyond. Therefore there is need to identify, collect, test and validate the medicinal plants used by traditional healers.

2.0.1 Herbal remedies versus conventional medicine

World Health Organization defines Traditional Medicine as the sum total of knowledge or practices; whether explicable or inexplicable, used in diagnosing, preventing or eliminating physical, mental or social diseases. This may rely on past experiences or observations passed from generation to generation, verbally or in writing. It comprises therapeutic practices that may have been in existence often for hundreds of years before development of modern scientific medicine and are still in use today without any documented evidence of adverse effects (WHO, 1978a). Herbal medicine is regarded by WHO as finished and labeled medicinal products that contain, as active ingredients,

aerial or underground parts of identified and proven plant materials, or combination whether in crude form or as plant preparations. They also include plant juices, gums, fatty oils and essential oils (WHO, 1978a). The explicable form of Traditional Medicine can be described as the simplified, scientific and the direct application of plant, animal or mineral materials for healing purposes and which can be investigated, rationalized and explained scientifically. The inexplicable form of traditional medicine include the spiritual, supernatural, magical, occultic, mystical or metaphysical forms that are not easily investigated, rationalized or explained scientifically.

According to WHO (1978b) Traditional Healer (TH) or Medical Practitioner (TMP) is described as a person who is recognized by the community in which s/he lives as competent to provide health care by use of vegetables, animal and mineral substances and certain other methods. They may be serving as herbalists, bonesetters, traditional psychiatrists, traditional pediatricians and general practitioners. In most parts of remote areas and urban areas of developing countries, they tend to be more readily available, accessible and approachable than conventional physicians. Their services are much more affordable than modern medical facilities.

The various herbal preparations include concoctions, decoctions, infusions, dried powders, ointments, tinctures and macerates. They are much more recognizable than the orthodox doctors who are mainly found in urban healthcare locations. The Traditional Healers administer these medications through various routes such as: oral, rectal, intrauterine and topical applications. Several official modern drugs have their origin in plants e.g. aspirin, morphine, digoxin, quinine, ergometrine, reserpine and atropine (Samuelsson, 2004).

Traditional herbal remedies play a major role in primary and secondary health care for many Kenyan communities. Many Kenyan communities rely on wide range of indigenous practices to keep themselves and their livestock healthy (Gathuma *et al.*, 2004). Rural communities in Kenya are heavily dependent on plant resources that they utilize for various purposes including for medicinal purposes (Mugabe *et al.*, 1998). The use of the medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into the African continent. According to WHO (2002) herbal medicine has been widely used and integrated into primary health care in China, Ethiopia, Argentina, and Papua New Guinea.

While many conventional drugs or their precursors were/are derived from plants, there is fundamental difference between administering a pure chemical and the same chemical in plant matrix. Synergy is an important aspect in medicinal plant use, where pharmacological action of the chemical mixture is greater than the arithmetic of the actions of individual components. Example is in the use of insecticidal pyrethrins. Piperonyl butoxide has little insecticidal activity but interferes with insects' ability to break down the pyrethrins, thus increasing their toxicity (Kakko *et al.*, 2000). Eder and Mehnert (1998) described the basic advantages from chemical complexity leading to enhanced solubility or bioavailability.

2.1 Indigenous systems of medicine

The development of traditional medicine has been influenced by different cultural and historical conditions giving rise to various types of systems. Their common basis is a holistic approach to life, equilibrium between the mind, body, environment and an emphasis on health rather than on disease (Anyinam, 1987).

2.1.1 African traditional medicine

In contrast with conventional medicine, traditional African medicine takes a holistic approach. Good health, disease, social occurrences are not seen as chance occurrences but are believed to arise from actions of an individual(s) and spirits (Anyinam, 1987). The gathering and practice of herbal medicine was restricted to TMP's and their apprentice and in southern Africa, the use of alternative names of some herbs was only known to this group. The sustainable use of medicinal plants was facilitated through taboos, seasonal and social restrictions, and nature of plant gathering tools (Good, 1987). Indigenous knowledge among the Maasai of Kenya was acquired through observation and real life experiences (Miaron *et al.*, 2004).

2.1.2 Indian traditional systems

India has six recognized systems of medicine some with origin in India or introduced and assimilated (Prasad, 2002). They include ayurveda, siddha, unani and yoga, naturopathy and homeopathy. Homeopathy was introduced to India in 18th century and enriched by incorporating other systems of medicine (Prasad, 2002). Alongside these recognized systems there are healers in the folklore stream who are not recognized under any category (Ravishankar and Shukla, 2007).

2.1.2.1 Ayurveda

Ayurveda simply means "Science of life" and was organized around 1500 BC with its roots in folk medicine. Unlike other medical systems, it first provided philosophical framework that determined the therapeutic practice with good effects. Thus it evolved into rational system detached from religious influence, laying emphasis on the value of evidence of senses and human reasoning (Ramachandra, 1987).

It is considered not just as ethnomedicine but as complete medical system taking into account physical, psychological, philosophical, spiritual and ethical well being of humanity. It lays great importance on living in harmony with the Universe and harmony of nature and science (Ravishankar and Shukla, 2007). The WHO's concept closely resembles the concept of health defined in Ayurveda (Kurup, 2004). The concept of pathogenesis, diagnosis, treatment aspects and types, and dietics in Ayurveda are well defined (Ravishankar and Shukla, 2007).

2.1.2.2 Siddha system of medicine

The term "siddha" comes from "siddhi" meaning achievement. It has close affinity to Ayurveda but it maintains a distinctive identity of its own. Siddhars achieved supreme knowledge in the field of medicine, yoga or *tapa* (meditation) (Narayanaswamy, 1975). It has its roots in south India and over the years interacted with other forms of medicine. Its main emphasis is on three branches *Bala vahatam* (pediatrics), *Nanjunool* (toxicology) and *Nayana vidhi* (ophthalmology) (Narayanaswamy, 1975).

The therapeutics in both the Siddha and Ayurveda systems can be broadly categorized into *samana* and *sodhana* therapies. The latter consist well-known procedures categorized under panchakarma therapy. This therapy is not that well developed in Siddha system, only the vamana therapy has received attention of the Siddha physicians (Narayanaswamy, 1975). From the animal kingdom thirty five products have been included in the *materia medica* similar to preparations used in Ayurveda. A number of plant based preparations used are similar in profile to ones in Ayurveda and have been reviewed by Ravishankar and Shukla (2007).

2.1.2.3 Unani system of medicine

Unani medicine has its origin in Greece. It is believed to have been established by the great physician and philosopher- Hippocrates (460-377 BC). Galen (130-201 AD) contributed for its further development. Aristotle (384-322 BC) laid down foundation of anatomy and physiology. Dioscorides, the renowned physician of the 1st Century AD, made significant contribution to the development of pharmacology, especially of drugs of plant origin. The next phase of development took place in Egypt and Persia (the present day Iran). The Egyptians had well evolved pharmacy; they were adept in the preparation of different dosage forms like oils, powder, ointment and alcohol etc. (Samuelsson, 2004).

The Arabs were instrumental in the introduction of Unani medicine in India around 1350 AD. The basic principle is that the body is made up of four basic elements: earth, air, water and fire. The body is made up of simple and complex organs. They obtain their nourishment from four humors namely- blood, phlegm, black bile and yellow bile. These humors also have their specific temperaments. In the healthy state of the body there is equilibrium among the humors and the body functions in normal manner as per its own temperament and environment. Disease occurs whenever the balance of humors is disturbed. In this system importance is given for the preservation of health. It is conceptualized those six essentials namely: air, food and drink, bodily movements and response, psychic movement and response, sleep and wakefulness and evacuation and retention are required for maintenance of healthy state (Syed, 2002).

2.1.3 Homeopathy

This method was developed by German Physician Samuel Hahnemann (1755–1843) and is the therapeutic method using preparations of substances whose effects when administered to healthy subjects correspond to the manifestations of the disorder (symptoms, clinical signs, pathological states) in the individual patient. It is now widely practiced in the world (Ernst, 1997). Homeopathy is based on two main principals: the first principle; *Similia similibus curentur* ('like cures like') principle, patients with particular signs and symptoms can be helped by a homeopathic remedy that produces these signs and symptoms in healthy individuals. The second principle, *doses minimate* (potentiation through dilution) homeopathic remedies retain biological activity after repeated dilution and secession even when diluted beyond Avogadro's number (Ernst, 1997).

2.1.4 Chiropractic medicine

This is the system of therapy that utilizes the recuperative powers of the body and the relationship between the musculoskeletal structures and the functions of the body, particularly of the spinal column in the restoration and maintenance of health. Research into this form of therapy and effectiveness of spinal manipulation has led to it being incorporated into mainstream health care delivery system in several countries (Aker *et al.*, 1996; Hurwitz *et al.*, 1996).

2.1.5 Naturopathy

This describes a wide range use of therapies that are considered as "natural medicines". Lee and Kemper (2005) reviewed the beliefs of naturopathic practitioners; and noted that the body has a strong and innate power to heal itself; that the symptoms of disease

reveal the body's attempt to reach natural balance, and that the entire person (mental, social and social health) must be considered during treatment.

2.1.6 Osteopathy

This is system of health care that is based on the theory that disturbances in the musculoskeletal system affect other body parts, causing many disorders that can be corrected by various manipulative techniques in conjunction with conventional medical, surgical, pharmaceutical and other therapeutic procedures (Hruby, 1995). Osteopathic and chiropractic medicine are different in terms of training and education and in their view of the musculoskeletal system. The focus of osteopathic medicine has been the need to optimize blood circulation to maintain or restore health. The chiropractic approach is focused more on the nervous system and advocates adjustments of the spinal vertebrae to improve neurotransmission (Ross and Wood, 1995).

2.2 Ethno-veterinary medicine (EVM).

Traditional veterinary medicine forms a crucial link in animal health care especially in pastoral communities in Kenya. Disease control and treatment has been widely documented in these communities (Wanyama, 1997a, 2000; Miaron, 2003; Busmann, 2006). Due to non-existence of modern veterinary services, high cost of the services and/or lack of knowledge of the same, herbal medicine forms integral part of animal husbandry (Wanyama, 1997a, b; Dano and Bogh, 1999). Ethnopractices are a preserve of few people passed on from one generation to next (Wanyama, 2000).

The use of ethnoveterinary medicine is limited by the seasonal availability of certain plants, scarcity of treatment of infectious diseases, ineffectiveness of some treatments and often mis-diagnosis (Martin *et al.*, 2001). Treatment mostly is aimed at alleviation

of the clinical signs that are observed (Wanyama, 1997a; Miaron, 2003). In many parts of East Africa EVM is widely used, but it is largely unacceptable to the scientist and veterinarians as they regard it to be associated with superstition, and to be domain of the “quacks” (Dano and Bogh, 1999). In many instances, remedies have been identified for treatment of different conditions without validation. In many studies, plants identified by traditional healers are listed, often without proper documentation of preparation methods (Dano and Bogh, 1999; Guarrera, 1999).

Most of the research so far done in Kenya on the ethnoveterinary medicine is mostly anthelmintic effects. These studies have supported the use of some medicinal plants by various communities in Kenya. The studies have cut across the board involving identification of medicinal plants, laboratory and field analysis of the plant extracts and whole plants as used by traditional practitioners (Mbaria *et al.*, 1998; Gakuya, 2001; Gathuma *et al.*, 2004; Githiori, 2004).

2.3 Drug discovery from plants.

The use of plants for medicinal purposes has long been in existence and is widely documented in records kept in ancient China, India and Egypt (Samuelsson, 2004). Discoveries of ancient indigenous practices were by series of “trial and error” which then could not be substantiated by proven scientific theories. However, these practices have produced results of proven efficacies compared to conventional modern medicine. In Africa, traditional medicine has always existed and practised since time immemorial. Plants have been in use since the dawn of life in planet earth and were instrumental to survival of early man. Hence, the history of drug discovery and even drug chemistry is inexorably bound to the plant kingdom and the process of deriving drugs from plant sources is certainly not new (Parfitt, 1978).

Drug discoveries from plants lead to isolation of digoxin (*Digitalis purpurea*), quinine (*Cinchona officinalis*), codeine and morphine (*Papaver somniferum*), ephedrine (*Ephedra sinica*) during the 18th and 19th century (Samuelsson, 2004). The first *British Pharmacopoeia* of 1863 contained descriptions of 187 crude drugs including *Digitalis*, *Datura*, *Belladonna* and *Hyoscyamus* and the Families of plants with digitalis-like glycosides have been reviewed (Melero *et al.*, 2000).

In Kenya, ethnobotanical information and healing methods for both animals and man among local communities has not been exhausted. Nevertheless, several authors have documented the medicinal plants and herbs used by different communities in Kenya. These studies have advanced forward the need to undertake scientific investigation of medicinal plants since they have documented the extensive use of ethnobotanicals by different communities in Kenya (Kokwaro, 1993; Kaendi, 1997; Miaron *et al.*, 2004; Bussmann, 2006; Bussman *et al.*, 2006; Kareru *et al.*, 2007)

According to Tuley de Silva, (1997) some of the constraints associated with the processing of medicinal plants that may result in reducing their competitiveness in global markets which must be addressed are: indiscriminate harvesting and poor post-harvest treatment, poor agriculture and propagation methods, inefficient processing techniques, poor quality control procedures, high-energy losses during processing and lack of current good manufacturing practices, lack of trained personnel, facilities and access to markets, research and development on product.

The approaches taken to identify potential medicinal plants are varied and have been reviewed by Fabricant and Farnsworth, (2001).

- i) Random Phytochemical screening.
- ii) Random selection followed by more than one or more bioassay.
- iii) Follow-up of biologic activity reports.
- iv) Follow-up of ethnomedical uses of plants.

It must be emphasized that clinical studies with human subjects represent the only assessment of effectiveness and safety that can translate into medical practice and National Policy. There should be more patient observation and follow up, this is of importance for further ethnopharmacological investigation; secondly, this could be translated to recommendations for use in populations using assessed local treatments.

Graz. *et al* (2007) noted that clinical trials involving ethnopharmaceuticals need not be expensive. They noted that the best way is to create interdisciplinary research group involving traditional practitioners, research institute(s), physician, pharmacologist, epidemiologist/statistician and patients. In all prospective studies informed patient consent must be obtained and patients should have free-will to leave the study when they want to. The relevance of research question, rigor of data collection and analysis, and importance of the observed effects is uppermost determinant of success of clinical study. Observational studies including follow-up study (observing patients progress with no experimental intervention)/ case control study can be conducted better in real situations than in randomized controlled studies (Concarto *et al.*, 2000).

Concerto *et al* (2000) suggested strict principles for follow-up studies. These include: first, choose inclusion and exclusion criteria similar to those in experimental trials; secondly, adjust for differences in base-line susceptibility to the outcome; thirdly, use statistical methods similar to randomized controlled trials, including “intention to treat” analysis.

Graz *et al* (2007) noted that it’s important to choose the appropriate study design. The research question and objectives need to be well stipulated and understood. The study designs, depending on research question include:

- i) What is the most effective among traditional treatments used in one area for a given ailment? Retrospective Treatment Outcome (RTO) study will analyze records of patients’ progress for given disease or syndrome and all the different treatments used. It makes it possible to perform correlation tests between treatment and outcome, providing indices of effectiveness and outcome. The RTO study is conducted with questionnaires.
- ii) How to assess the overall results of traditional health care practices? Comparison of Prognosis and Outcome (CPO) is an observational clinical study exploring whether health care by traditional healers reaches minimum requirement. The prognosis of traditional healer is compared to actual patient progress (outcome).
- iii) How to assess the effectiveness of a traditional treatment? Prospective Dose Escalating (PDE) quasi-experimental clinical trial is possible when herbal preparation is given in highly variable doses and preliminary observations indicate that the therapeutic range is large with probability of toxicity at very low doses.

2.3.1 Classification of natural drugs

According to Samuelsson, (2004) the usage forms of phytomedicines is very diverse and includes: pure compounds which are often isolated from botanical drugs, traditionally used medicinal plants/herbs (Loose or in teabags to form infusions, including instant teas and tinctures, dried extracts, ethanolic extracts, essential oils, fatty acids), cut or powdered crude drugs that are used in unprocessed form and/or standardized extracts with relatively well established clinical and pharmacological profiles.

1. Alphabetical classification

The first pharmacopoeias were issued by autonomous cities, and became legally binding documents on the composition, preparation and storage of pharmaceuticals. They were mainly intended to bring some order into the many forms of preparation available at the time. These included: *Ricettario Fiorentino* (Florence, Italy), 1498; *Pharmacopoeia of Nuremberg* or *pharmacorum omnium*, (Frankonia, Germany) 1546 and *Pharmacopoeia Londiniensis*, 1618. The pharmacopoeias existing today in most countries do not include herbal preparations although some like *British pharmacopoeia* has between 300 and 500 licensed herbal medicines but none is included in essential drug list. The post marketing surveillance system, which includes adverse-affect monitoring, was established in 1964 and expanded to cover unlicensed herbal medicines in 1966. The *Chinese pharmacopoeia* was first published in 1963 it contains 992 national herbal monographs. Regulatory requirements include adherence to information contained in pharmacopoeias and monographs, safety requirements as for convectional pharmaceuticals, market surveillance and monitoring for adverse drug reaction since 1984. By the end of 2002 there were more than 9,000 registered herbal medicines with 1,242 included on the national essential drug list in China (WHO, 2005)

In Africa, few countries have established national policies, laws and regulation. There are no national monographs that exist and research institutions are either non-existent or weak. Some countries like Central African Republic are in the process of developing national pharmacopoeia and national monographs. In Kenya, the national policy, laws and regulation on TM/CAM are being developed. KEMRI was established in 1984 and conducts research on traditional medicine, this is also carried by research Institutions and universities. Neither a national pharmacopoeia nor monographs exist and herbal medicines are not regulated. In countries like Mozambique, in place of a national pharmacopoeia, the *African pharmacopoeia* (1985) is used and is legally binding. The national monographs are contained in the series *Plantas Medicinase seu uso tradicional em Mocambique* (1983-1991, five volumes). The information in this series is legally binding (WHO, 2005)

The Centurion Lake Declaration 2005 was launched to prepare *African herbal pharmacopoeia*. This declaration was made under Association for African Medicinal Plant Standards (AAMPS) in South Africa to review the problems and prospects of developing internationally acceptable African medicinal plant standards and to select fifty three (53) of the most important African medicinal plants. Delegates at the meeting signed a declaration pledging Africa-wide support for the preparation of African quality assurance and trading standards and the establishment of an association to help promote these standards and to develop an *African Herbal Pharmacopoeia* (WHO, 2005)

In North America, *American Herbal Pharmacopoeias* and *Therapeutic Compendium* were established to address the issue of integrating herbal medicines into health care system. The *American Herbal Pharmacopoeia* began developing qualitative and

therapeutic monographs in 1994, and intends to produce 300 monographs on botanicals, including many of the Ayurvedic, Chinese and Western herbs most frequently used in the United States. Once completed, these monographs will represent the most comprehensive and critically reviewed body of information on herbal medicines in the English language, and will serve as a primary reference for academicians, health care providers, manufacturers, and regulators (WHO, 2005)

2. Morphological classification

Botanical drug is a product that is either: derived from plant and transformed into a drug by drying part or whole plant, but no longer retains its structure or organ, and contains a complex mixture of biogenic compounds (e.g. fatty and essential oils, resins, gums etc). Isolated pure natural products are thus not 'botanical drugs' but chemically defined drugs derived from nature. The mostly used plant parts are listed below, with the Latin name that is used internationally in brackets: leaf (folia), flower (flos), aerial parts or herb (herba), fruit (fructus), bark (cortex), root (radix), rhizome (rhizome) and bulb (bulbus) (Wondimu *et al.*, 2007).

3. Taxonomical classification

Taxonomy is the science of naming organisms and their correct integration into the existing system of nomenclature. The basic classification of the plant kingdom into divisions circumscribes the main groups of plants, including: Algae, includes green algae (*Chlorophyta*) and the red algae (*Rhodophyta*); Mosses (*Bryophyta*); Ferns (*Pteridophyta*) and seed bearing plants (*Spermatophyta*). Only few of the first three mentioned members of plant kingdom have yielded pharmaceutically important products. Botanical classifications using this mode include the binomial one (this is the

genus and species names, plus the authority). This binomial nomenclature was developed by a Swedish botanist Carl von Linnaeus (1707-1778).

2.3.2 Adulteration and evaluation of crude drugs

Medicinal plants collected from the wild population may be contaminated by other species or plant parts through misidentification, accidental contamination or intentional adulteration, all of which may have unsafe consequences. The belief that herbal remedies are safe is deep rooted among the population and is not based on any conclusive scientific basis. It is important to note that botanicals are complex mixtures of chemicals/drug. Ernst (2002) described presence of heavy metals and synthetic drugs in traditional Indian and Chinese medicines respectively. Most of these are not disclosed and they present potential hazard to users. The drugs mostly encountered were ephedrine, chlorpheniramine, methyltestosterone and phenacetin. For example, PC-SPES is a patented herbal preparation marketed to "enhance prostate health," but commonly used to treat prostate cancer. Reports of its effectiveness have appeared in major medical journals (Sovak *et al*; 2002, Marks *et al*; 2002). However, after chemical analysis of PC-SPES the presence of diethylstilbestrol, indomethacin, warfarin or a combination of these drugs was revealed; this led to the product being withdrawn from the market (Sovak *et al*; 2002). Marcus and Grollman (2002) noted that the medical community has been slow to respond to the public health and educational problems associated with botanical supplements.

Consistency in composition and biological activity are essential for safe and effective use of therapeutic agents. The use of chromatographic techniques and marker compounds to standardize herbal preparations promotes batch-batch consistency but does not ensure consistent pharmacologic activity or stability. Moreover, analyses of

purportedly standardized herbal preparations reveal that botanical products often do not contain the amount of the compound stated on the label (Goldman, 2001).

2.4 Scientific evidence of activity of plant remedies.

2.4.1 Antimicrobial activity

In Kenya, a number of plants used in traditional medicine have been found to have antimicrobial activity. The methanol extracts of *Tetracera boiviniana* (roots and aerial parts) showed antimicrobial activity against *in-vitro* cultures of *B. cereus*, *E. coli*, *Ps aeruginosa*, *S. typhimurium*, *P. vulgaris* and *C. pyogenes* (Mbaria *et al.*, 2005). Chloroform and ethanol extracts of *Myrica salicifolia*, *Erythrina abyssinica*, *Solanum aculeastrum* and *Croton megalocarpus* showed antimicrobial activity against *in-vitro* cultures of *E. coli*, *K. pneumonia* and *S. typhi*. Water extracts except those of *Solanum aculeastrum* and *Croton megalocarpus* showed wider sensitivity to the bacterial strains (Kariuki *et al.*, 2005).

In Tanzania, twenty-seven (48%) out of 56 plants tested for their anti-candidal effects were found to be active using bioautography agar overlay method. Aqueous methanolic extracts of the root barks of *Albizia anthelmintica* and *Balanites aegyptiaca*, and roots of *Plectranthus barbatus* showed strong anticandidal activity (Runyoro *et al.*, 2006). In a study of 100 plants used by traditional healers in Rwanda to treat infections, their extracts (267 plant extracts) were screened for antibacterial, antifungal and antiviral properties. It was shown that 45% were active against *S. aureus*, 2% against *E. coli*, 16% against *Ps. aeruginosa*, 7% against *C. albicans*, 80% against *Microsporium canis* and 60% against *Trichophyton mentagrophytes*. More than 27% of the plant species exhibited prominent antiviral properties against one or more test viruses, more

specifically 12% against poliomyelitis, 16% against coxsackie, 3% against Semliki forest, 2% against measles and 8% against herpes simplex virus (Vlietinck *et al.*, 1995).

Dichloromethane extract of *Warbugia ugandensis* had *in-vitro* antimycobacterial activity against *Mycobacterium aurum*, *M. fortuitum*, *M. phlei* and *M. Smegmati* (Wube *et al.*, 2005). Forty crude extracts of twenty Cameroonian medicinal plants screened for antibacterial activity showed bacteriostatic effect on gram-negative pathogenic bacteria. *Euphorbia hirta* had the lowest inhibitory concentration against *S. aureus* and *Ps. aeruginosa* (Ngemenya *et al.*, 2006). Antiviral effects of some plants and herbs are documented. The use of *Aloe secundiflora* in free range chicken in Tanzania was observed to reduce mortality and severity of clinical signs of Newcastle disease as compared to non-treated ones (Waihenya *et al.*, 2002, 2005).

Bioassay-guided fractionation of extracts of *Toddalia asiatica*, a plant used by the Pokot community of Kenya to treat fevers, yielded the alkaloid nitidine as the major antimalarial component. Fractions containing nitidine had *in-vitro* 50% inhibitory concentrations against *Plasmodium falciparum* in the range of 9 to 108ng/ml for a range of chloroquine-susceptible and resistant strains (Gakunju *et al.*, 1995). Study on cytotoxic sesquiterpine (muzigadial) isolated from *Warbugia ugandensis* was found to have *in-vitro* trypanocidal activity (Olila *et al.*, 2001a). Other studies in Uganda have shown plants that have antibacterial and antifungal activity, antiviral and trypanocidal activity (Olila *et al.*, 2001b; Olila *et al.*, 2002).

In Burkina Faso, extracts of three plants were screened for *in-vitro* activity against *Plasmodium spp.* The methanol extracts of *Swartzia madagascariensis* showed the highest antimalarial activity. The experiment showed that the extracts of *Swartzia madagascariensis*, *Combretum glutinosum* and *Tinospora bakis* possess some measure of antimalarial activity. Methanol and alkaloid extracts showed higher activity than the aqueous extracts which are used in traditional medicine (Ouattara *et al.*, 2006).

2.4.2 Anthelmintic activity

Anthelmintic effects of variety of plant species have been documented around the world to validate their use. In Kenya, Githiori (2004) evaluated 11 plants for their *in-vivo* efficacy on *Haemonchus contortus* in sheep and *Heligmosomoides polygyrus* in mice. Using the *priori* value of 70%, no significant reduction in fecal egg count or total worm count was observed. *Albizia anthelmintica* had highest reduction in fecal egg count (FEC). *In-vivo* activity of *Albizia anthelmintica* on ruminant alimentary nematodes have been reported (Gakuya, 2001; Gathuma *et al.*, 2004). Other documented plants having activity against ruminant nematodes include: *Chrysanthemum cinerariaefolium* (Mbaria *et al.*, 1998); *Maerua edulis* (Gakuya, 2001); *Myrsine Africana* and *Hildebrandtia sepalosa* (Gathuma *et al.*, 2004).

In the rest of Africa, validation of herbal remedies has been reported. In Uganda, Grade and Longok, (2000), Waswa and Olila, (2006) identified and collected twenty one plants. Of these, seven were assayed for *in-vitro* activity against *Ascaris suum*, five showing appreciable results. In South Africa McGaw *et al.*, (2000) investigated *in-vitro* activity of 72 plant species against *Caenorhabditis elegans*. Extracts of *Acorus calamus* showed high levels of anthelmintic activity.

2.4.3 Pesticides

Larvicidal activities of five *Meliaceae* spp plants are documented against *Anopheles gambiae* (Ndung'u *et al.*, 2004). Crude methanol extracts of *Turraea wakefieldii* and *Turraea floribunda* were more potent than azadiractin against larvae of *Anopheles gambiae* (Ndung'u *et al.*, 2004). Larvicidal effects of neem extracts have been reported elsewhere (Vatandoost and Vaziri, 2004).

In India, the alcoholic and hexane extracts of seventeen (17) plants were found to be toxic to the egg, larval and pupal stages of the external gregarious larval parasitoid *Bracon brevicornis* Wesm. (Srivastava *et al.*, 1997). In Kenya acaricidal effects and traditional knowledge is reported (Wanzala *et al.*, 2006). In Saudi Arabia, acaricidal effects of some plant extracts are documented against *Hyalomma dromedarii* (Al-Rajhy *et al.*, 2003).

2.4.4 Socio-economic uses

Kenya and the rest of Africa are rich sources of medicinal plants. Perhaps, the best-known species is *Phytolacca dodecandra*. Extracts of the plant, commonly known as endod, are used as an effective molluscicide to control schistosomiasis (Lemma *et al.*, 1991). *Prunus africana* bark is exploited and exported mostly to U.S, Belgium and France, for the treatment of benign prostatic hyperplasia, has led to an annual international trade worth approximately US\$220million in the final pharmaceutical product (Cunningham *et al.*, 1997).

Other notable examples are *Catharanthus roseus*, which yields anti-tumour agents such as vinblastine and vincristine; and *Ricinus communis*, which yields the laxative--castor oil. In Botswana, Lesotho, Namibia and South Africa, *Harpagophytum procumbens* is

produced as a crude drug for export. Similarly, *Hibiscus sabdariffa* is exported from Sudan and Egypt. Other exports are *Pausinystalia yohimbe* from Cameroon, Nigeria and Rwanda, which yields yohimbine, and *Rauwolfia vomitoria*, from Madagascar, Mozambique and Zaire and is exploited to yield reserpine and ajmaline. The markets for branded non-prescription herbal medicines have grown from \$1.5 billion in 1994 to \$4.0 billion in 2000 in U.S alone with the same trend being followed in European countries as well (De Smet *et al.*, 2000). According to WHO (2003), the trade in herbal products was estimated to be worth US\$60 billion and growing.

Plants have been an indispensable source of both preventive and curative medicinal preparations for human beings (Dery *et al.*, 1999). Medicinal plants in Africa and other developing countries frequently provide economically disadvantaged groups such as small holders and landless people with their only form of cash income (GTZ, 2001). Medicinal plants are also important sources of therapeutic agents in the industrial production of pharmaceuticals (Lambert *et al.*, 1997).

2.4.5 Other uses

Plant extracts have also been reported to have analgesic and anti-inflammatory effects (Shanmugasundaram and Venkataraman, 2005; Okoli *et al.*, 2006; Ndebia *et al.*, 2007); antioxidant effects (Masoko and Eloff, 2007), anticonvulsant effects (Bum *et al.*; 2004; Moshi *et al.*, 2007b) and antidiabetic (Okokon *et al.*, 2006)

2.5 Collection and preparation of crude drugs.

Identification of medicinal plants (involvement of traditional practitioners and their informed consent is crucial) is the step that should not be over-looked. Collection of medicinal plants should be done sustainably taking into account environmental protection. The rising demand of herbal products is estimated by WHO (2003) to be

US\$ 14 billion/year and this is putting a lot of strain on the environment hence the need for sustainable harvesting.

Labeling and pressing of collected samples is done by placing the sample plant between two pressing boards and papers for transport and subsequent confirmatory identification in a Herbarium. The drying of plant samples can be done under shade, direct sunlight or in an oven. The effect of each method of drying on chemical constituents should be taken into account (WHO, 2002). Garbling involves separation and cleaning of dried plant samples by picking of dirt, debris, foreign organic matters and other unwanted plant parts. Gloves and air mask should be worn in case one is dealing with poisonous plants. During pulverization, milling can be done using pestle and mortar or electric grinder and the powder collected in an air-tight container.

2.5.1 Extraction methods and isolation

Water is universal solvent used to extract active ingredients. Dried plants are ingested as teas, or rarely as tinctures or inhaled via steam from boiling suspensions. Dried plant/herbal parts can be added to petroleum jelly and used topically. Poultices can be made from concentrated teas or tinctures. In alcoholic extractions, plant parts are dried, ground to fine powder and soaked in methanol or ethanol for extended period. Techniques for further chemical analyses include chromatography, bioautography, radioimmunoassay, fast atom bombardment mass spectrometry, tandem mass spectrometry, high performance liquid chromatography, capillary zone electrophoresis, nuclear magnetic resonance spectroscopy and x-ray crystallography (Bhattaram *et al.*, 2000).

2.5.2 Active phytochemicals in herbal remedies and herbs

These are primarily secondary metabolites and include alkaloids, phenolic and polyphenols, steroids, quinones, tannins, saponins, terpenoids, glycosides and cardenolids. Basic structures are represented in figure 2.1.

2.5.2.1 Phenols

Flavonoids occur both in free-state and as glycosides and are the largest group of naturally occurring phenols. They have been used extensively as chemotaxonomic markers. Phenolic compounds are important plant secondary products that are non-nutrients, but are useful for the plant's defense against foreign bodies. They also contribute to the flavor, color and astringency of plants. These phenolic compounds also have antioxidant properties that enable them to quench free radicals in the body. It is probable that herbal remedies contain active flavonoids. Their capability to interact with protein phosphorylation and the antioxidant, iron chelating, and free radical scavenging activity may account for the wide pharmacological profile of flavonoids. These include vasoprotective, anticarcinogenic, antineoplastic, antiviral, anti-inflammatory, anti-allergic, antiproliferative activity on cancer cells, antimicrobial activity and hepatoprotective activity (Jain *et al.*, 2006; Li *et al.*, 2007).

2.5.2.2 Quinones

These are aromatic rings with two ketone substitutions. They are abundant and highly active. They are responsible for the brown coloration of cut vegetables and are an intermediate in melanin synthesis in human skin (Schmidt, 1988). Anthraquinone is known to be bacteriostatic (Kazmi *et al.*, 1994). Hypericin from St. John's wort has antidepressant activity.

2.5.2.3 Terpenoids

This refers to compounds with basic skeletons derived from mevalonic acid or closely related precursor. The fragrance of plants is carried in the so-called quinta essential, or essential oil fraction. The oils are based on isoprene structure. When the compounds contain additional elements, usually oxygen, they are termed terpenoids. Based on their chemical structures, the following groups are identified:

a) Normal monoterpenes: these include all aliphatic and cyclic steam distillable monoterpenes. Non-steam distillable monoterpene glycosides have been found naturally. Based on their ring closure they are further divided into acyclic, monocyclic and bicyclic.

b) Iridoids (cyclopentanoid monoterpenes): these are characterized by a cyclopentanopyran ring nucleus. Most occur as β -D glucosides, but those of the nepetalactone type are without the sugar molecule and are volatile, occurring in essential oils. They are bitter in nature. They have medicinal properties (antimicrobial, hypotensive, antileukemic, laxative effect etc).

c) Tropolones: have seven-membered ring with double bond system conjugated with keto group and a hydroxyl group. They are restricted and identified in certain fungi and conifers. They structurally resemble phenols and are strongly fungicidal. The most common terpenoid is artemisin an antimalarial. Terpenoids have been documented to having antimicrobial activity (Ghoshal *et al.*, 1996).

2.5.2.4 Alkaloids

These are heterocyclic nitrogen compounds. They are basic in nature and contain one or more nitrogen atoms. They have pronounced pharmacological actions in animals and man. Morphine was first useful alkaloid to be isolated in 1805. They are distributed in

various parts of plants in different quantities including the leaves, bark, fruits, seeds, aerial parts, roots and rhizomes. They include diterpenoid alkaloid, glycoalkaloid and berberine. Alkaloids have antimicrobial activity and smooth muscle contractility (Rattmann *et al.*, 2005).

2.5.2.5 Tannins

These are complex substances containing mixture of polyphenols and are widely distributed in plant kingdom. They are protective and most tannins have molecular weight of 1000-5000. They are subdivided into hydrolysable and condensed (proanthocyanidins) tannins. Hydrolyzable tannins (HT) are polymers esterified to a core molecule, commonly glucose or a polyphenol such as catechin. Hydrolyzable tannins are potentially toxic to ruminants. Proanthocyanidins (condensed tannins) are relatively stable in the digestive tract of the animal, and rarely have toxic effects They have antiparasitic properties (Githiori, 2004).

2.5.2.6 Lectins and polypeptides

Peptides inhibitory to microorganisms were first reported in 1942. They are often positively charged and contain disulfide bonds (Zhang *et al.*, 1997). The mode of action may be formation of ion channels in microbial membrane or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors. Peptides include thionins and fabatin (Zhang *et al.*, 1997).

2.5.2.7 Glycosides

These are compounds that contain aglycone and glycone molecules. Widely present in plants where β -D glucose sugar moiety is present. On hydrolysis glycosides produce aglycone (genin) and glycone (sugar) molecules. They are classified on basis of

various parts of plants in different quantities including the leaves, bark, fruits, seeds, aerial parts, roots and rhizomes. They include diterpenoid alkaloid, glycoalkaloid and berberine. Alkaloids have antimicrobial activity and smooth muscle contractility (Rattmann *et al.*, 2005).

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linkages between glycone and aglycone moiety, and on basis of chemical nature of aglycone molecule. The most important in this group in medical circles are steroidal (cardiac) glycosides they contain cyclopentophenanthrene nucleus. About 50 cardenolides exist and are primarily found in: *Apocynaceae*, *Asclepiadiaceae*, *Brassicaceae*, *Celastraceae*, *Euphorbiaceae*, *Liliaceae*, *Moraceae*, *Ranunculaceae*, *Scrophulariaceae*, *Sterculiaceae* and *Tiliaceae* families. They affect the dynamics and rhythm of dysfunctional heart muscle. Important sources of the digitalis glycosides (digoxin, digitoxin) include: *Digitalis lanata* (white foxglove) and (red foxglove). Digitalis drugs are agents used for people in congestive heart failure. Cardenolides and bufenolides (occur in free-state or glycoside form) are triterpenoids which are highly poisonous and which come from 23- and 24-carbon steroids, with an isoprenoid substituent (Okunade *et al.*, 2004).

2.5.2.8 Essential oils/volatile oils

Essential oils are a group of natural organic compounds that are predominantly composed of terpenes (hydrocarbons) and terpenoids (oxygen containing hydrocarbons). Essential oils also contain simple phenols, sulphur containing mustard oils, methyl anthranilate and coumarins. These are mixtures of hydrocarbons terpenes, sesquiterpenes and polyterpenes and their oxygenated derivatives obtained from various plant parts. They evaporate at room temperature and thus are called etheral oils. They are generally insoluble in water and soluble in organic solvents. Volatile oils mainly contain terpene. Monoterpenes are major constituents of volatile oils. They may be acyclic, monocyclic or bicyclic, either as hydrocarbons or their oxygenated derivatives (terpenoids). The terpenoid are responsible for odor and taste. These have been

reported to have antibacterial, antifungal, antinoceptive, spasmolytic, antiplasmodial and insecticidal activity (Cimanga *et al.*, 2002, Abena *et al.*, 2007).

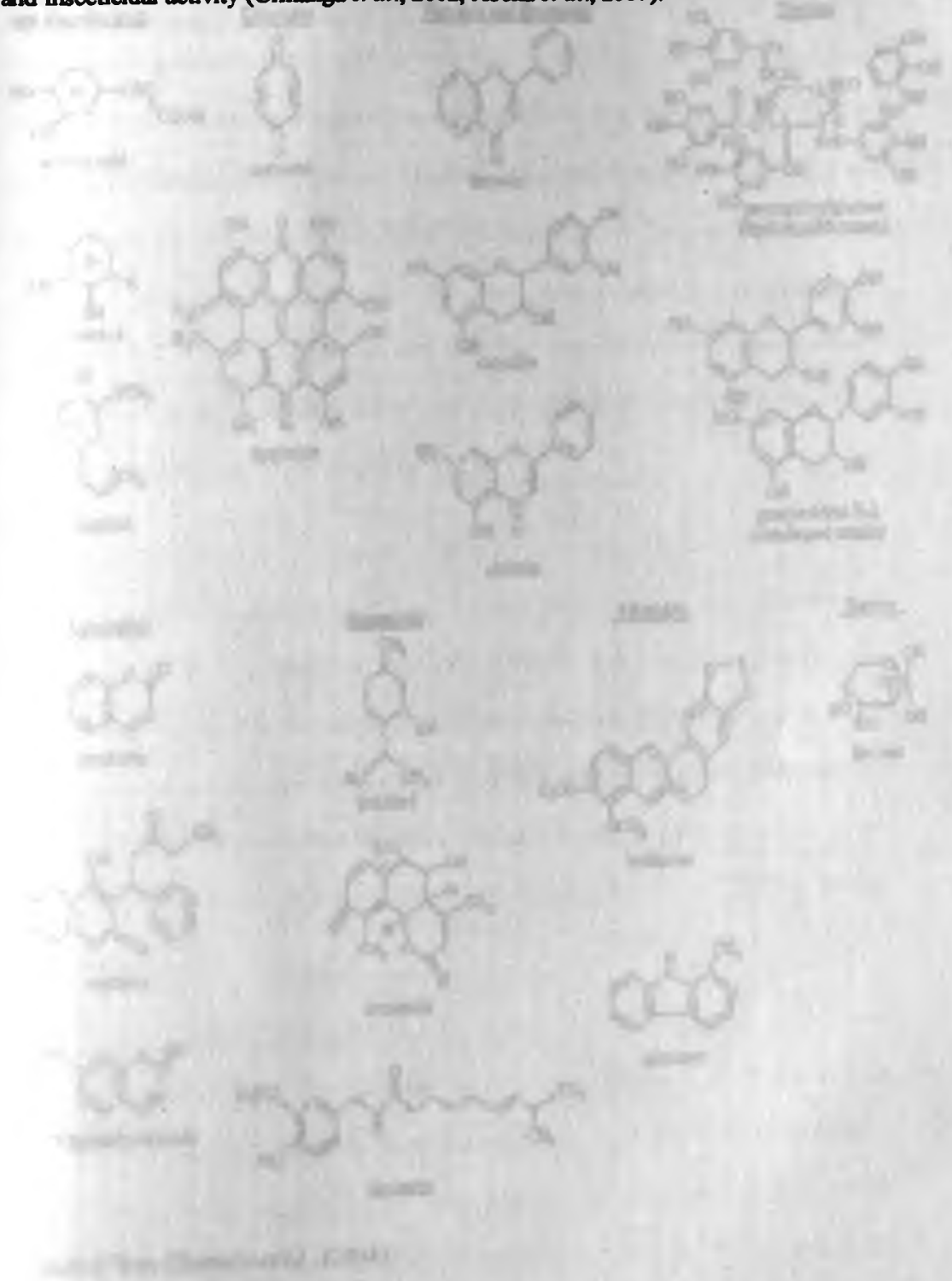
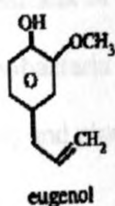
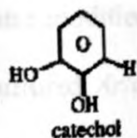
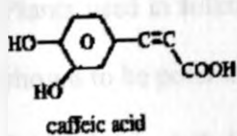
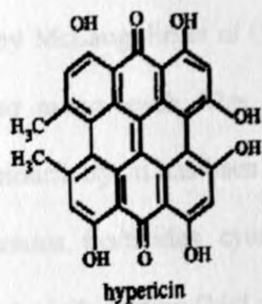


Fig. 2.1: diagram of different chemical structure isolated from plant extracts

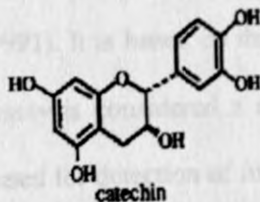
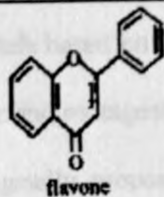
Simple phenols and phenolic acids



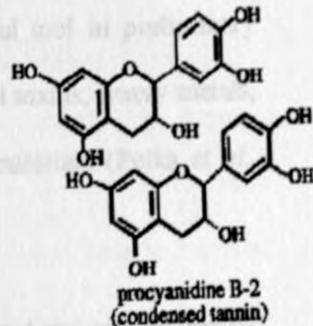
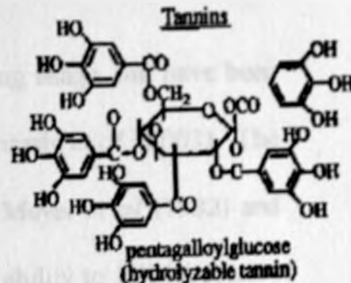
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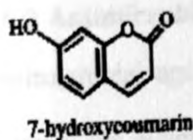
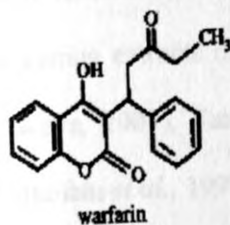
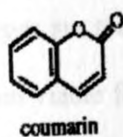
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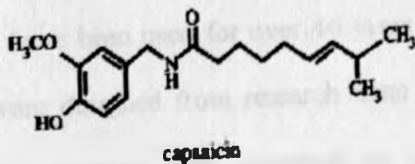
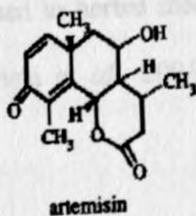
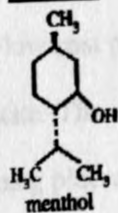
Tannins



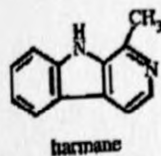
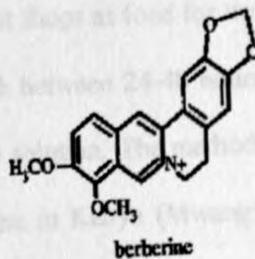
Coumarins



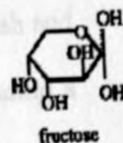
Terpenoids



Alkaloids



Sugars



Adapted from Okunade *et al.*, (2004)

2.6 In-Vitro bioassay techniques

2.6.1 Brine shrimp lethality test

Plants used in folkore are assumed to be safe based on their long usage, but have been shown to be potentially carcinogenic, toxic and mutagenic (Elgorashi *et al.*, 2003). The brine shrimp lethality test (BST) was originally proposed by Meyer *et al* (1982) and later modified by McLaughlin *et al* (1991). It is based on the ability to kill laboratory cultured *Artemia salina* leach. This assay is considered a useful tool in preliminary assessment of bioactivity. It has been used for detection of fungal toxins, heavy metals, cyanobacteria toxins, pesticides, cytotoxicity testing of dental materials (Pelka *et al.*, 2000) and plant toxicity testing (McLaughlin *et al.*, 1991).

The brine shrimp assay is a very useful tool since it is simple, rapid and an inexpensive bench top bioassay. It also allows the use of small quantities of extracts. The *Artemia spp* eggs are readily available at low cost price in pet shops as food for tropical fish and remain viable for years in dry state. The eggs hatch between 24-48 hours, providing a large number of nauplii upon being placed in brine solution. The method was used for screening extracts of plants used in herbal medicine in Kenya (Mwangi *et al.*, 1999; Gakuya, 2001), Tanzania (Moshi *et al.*, 2004), India (Alluri *et al.*, 2005), Jordan (Alkofahi *et al.*, 1997).

2.6.2 Antimicrobial testing techniques

Antimicrobial agents have been used for over 40 years (Zhanel *et al.*, 1991). Dosing regimes for agents were designed from research done on penicillin G (Eagle *et al.*, 1950). These dosing regiments are supposed to maintain antimicrobial serum concentrations above the minimum inhibitory concentration (Kunin, 1981). The activity of plant extract is defined and measured in terms of its ability to inhibit the growth of a

microbial population. According to Hostettmann, (1991) detection of antibacterial activity needs fulfillment of three conditions. First, the plant extract must be brought into contact with microbial cell wall. Secondly, optimal conditions for microbial growth must be present. Thirdly, selection of an appropriate means of judging the amount of growth.

In-vitro methods are divided into groups such as diffusion, dilutions, impedance and optical density methods (Koutsoumanis *et al.*, 1999; Tassou *et al.*, 2000). Among these, the dilution method provides more quantitative results (Manou *et al.*, 1998). Results obtained with other methods may not be comparable (Skandamis *et al.*, 2001; Tassou *et al.*, 2000). Bioautography combines thin layer chromatography with bioassay *in-situ* allowing for localization of active compounds within a sample. Bioautography is a useful method for the bioassay-guided fractionation of compounds with antimicrobial activity. However, some compounds show poor migration through the agar overlay and may not be detected (Gibbons and Gray, 1998).

2.6.2.1 Broth Dilution Methods

Broth of the antimicrobial agent(s) to be tested is usually prepared in serial two fold dilutions and placed in tubes of a broth medium that will support growth of test microorganisms. Antimicrobial agents are prepared in concentrated solutions and diluted to the appropriate concentrations in broth. Minimum inhibitory concentration is determined after overnight incubation; the tubes are examined for turbidity which indicates growth of microorganism. The microorganisms will grow in control tubes (negative) and any other tubes that do not contain enough antimicrobial agents to inhibit growth. The lowest concentration of the agent that inhibits growth of microorganisms as detected by lack of visual turbidity on marching the positive control is designated as

minimum inhibitory concentration. Minimum inhibitory concentration is used as 'gold' standards for determining the susceptibility of organisms and performance of other methods of susceptibility testing (William, 2001)

In broth techniques different methods exist for determining the MIC (Burt, 2004). Knowledge of MIC is important in order to apply the minimum essential concentration capable of preventing microbial growth (Lambert and Pearson, 2000).

2.6.2.2 Impedimetric analysis

This is an automated technique which is a combination of absorbance measurements with the common dilution method (Lambert and Pearson, 2000; Lambert *et al.*, 2001). Better results were obtained when optic density measurement was replaced with conductance measurements overcoming the inherent problems in Optical Density technique (Chorianopoulos *et al.*, 2006).

2.6.2.3 Turbidometry

This technique detects only the upper part of microbial growth curves, and requires calibration in order to correlate the results with viable counts obtained on agar media (Daalgard and Koutsoumanis, 2001; Skandamis *et al.*, 2001).

2.7 Formulation and value addition of herbal remedies

Herbal remedies can be put to commercial use. Scientists are demanding that traditional knowledge should be validated to verify efficacy of treatment (Wanzala *et al.*, 2006). Preparation and dosages of the same remedy may often vary greatly. The required plant materials may not be available through the year and the pharmacological active

ingredients may vary according to season, site, harvest time, maturity and other factors (Martin *et al.*, 2001).

Indigenous knowledge particularly medicinal plants, is speeding up as firms and research groups seek to patent ingredients or preparations. Outsiders are by-passing the communities that developed them and commercializing the herbal remedies. Bioprospectors often apply for permits without indicating their commercial intent. Improving packaging of herbal products is one of the ways of value addition. To improve on value of herbal products, pharmacokinetics, bioavailability and interactions of some herbal remedies have been reviewed (Bhattaram *et al.*, 2002). Bhattaram *et al.*, (2002), notes that the determination of the pharmacokinetics and use of pharmacodynamics modeling can aid in more rational use of herbal products. Concerted research should be geared towards gathering data on toxicological and bioavailability of the products *in-vivo*.

2.7.1 Quality criteria and standardization

Phytopharmaceuticals are composed of many constituents and are thus capable of variation. The variability depends on ecological zone, season, and part of plant/shrub used, harvest, drying and growth conditions. The other conditions are polarity of the solvent, mode of extraction and instability of constituents which may influence quality and composition of plant extract (Bauer, 1998).

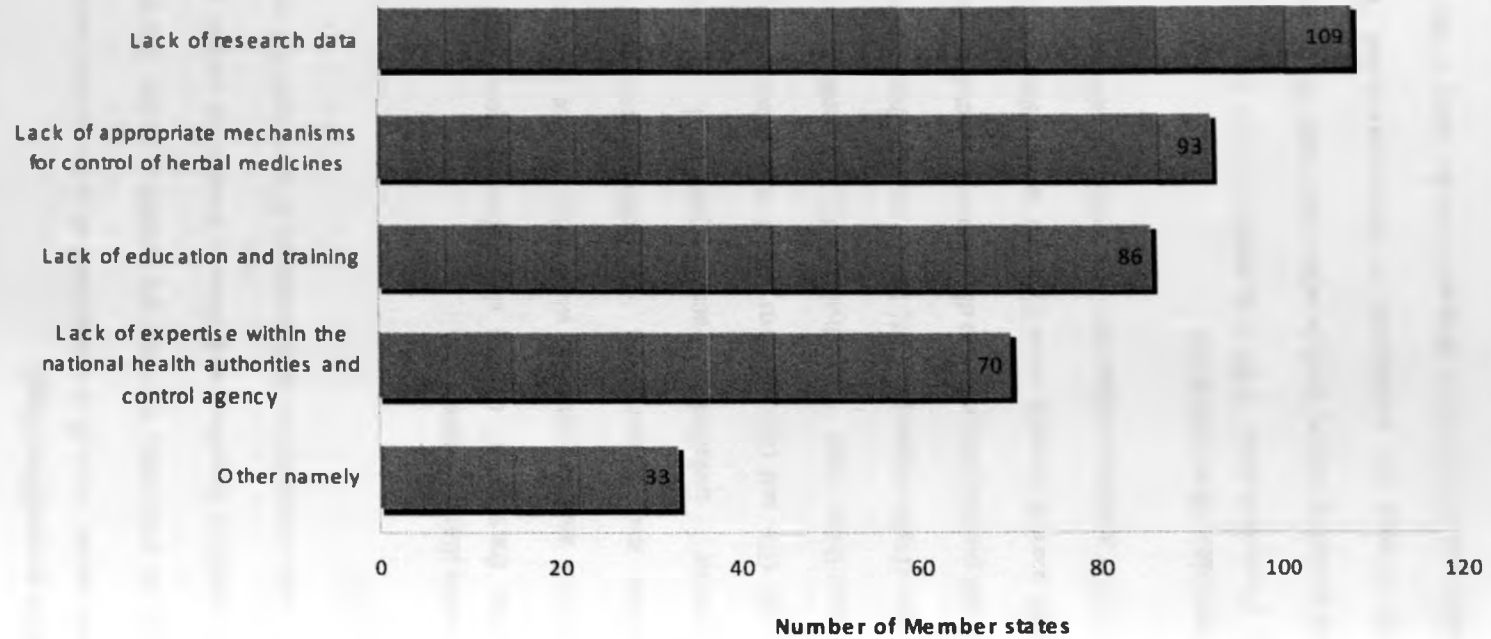
Products are pharmaceutically equivalent if they contain the same amount of same active substance(s) in same dosage forms that meet the same or comparative standards according to the Note for Guidance on the investigation of bioavailability and bioequivalence. This should apply for herbal drug preparation (HDP). Reproducible efficacy and safety of phytopharmaceuticals is based on reproducible quality. Thus in

order for them to be considered rational drugs, they need to be standardized and quality approved (Bauer, 1998). In addition to pharmacological, toxicological, and clinical studies of the herbal drugs, their composition needs clear documentation in order to obtain reproducible results (Bauer, 1998).

The WHO (2002) strategy for traditional medicinal plants had four main objectives of : farming policy, enhancing safety, efficacy and quality, ensuring access and promoting rational use. The WHO has recognized this problem and has published guidelines to ensure the reliability and repeatability of research on herbal medicines (WHO, 2000). A survey conducted by WHO, 2005 found that the main constraint in regulation of herbal products and medicine was lack of research data. Of the 129 countries polled, 109 reported this as a constraint among other constraints as represented in (Fig.2.2)

According to Di Stasi, (2005) standardization of phytomedicines could be best realized by considering three essential aspects: (i) selection of the plant species for use and studies of new phytomedicines is based on traditional knowledge(ethnopharmacological approach) and consequent benefit sharing; (ii) pharmacological, toxicological and phytochemical evaluation of the selected medicinal plants is appropriately done to ascertain their efficacy, safety and quality control; and (iii) strategic utilization of the biological material for the sustainable use of multiple forest resources and conservation of ecosystems.

Fig. 2.2: Graphical representation of main difficulties in regulation of herbal medicines in different countries. (Adapted from WHO, 2003)



2.8 Literature on selected medicinal plants under study

2.8.1 *Aloe secundiflora* Engl.

Common name: Aloe. It is categorized as a cactus and succulents. Plant has spines it does well in arid areas. Flowers are reddish in color. Has moderately curved leaves, flowers multiple branched and flowers themselves simple and spread out along the racemes. *Aloe secundiflora* is documented to have antiviral properties (Waihenya *et al.*, 2002; 2005).

2.8.2 *Cassia didymobotrya* Fres.

The ether fraction of *Cassia didymobotrya* leaves are documented to contain chrysophanol and aloe-emodin, while, ethyl acetate fraction contained kaempferol-3-rhamnoside and isoquercitrin. Metabolites from *in-vitro* cultures of *Cassia didymobotrya* included 7-acetylchrysophanol, chrysophanol-phycion-10,10'-bianthrone, (E)- and (Z)-3'-hydroxy-3,4,5'-trimethoxystilbene, (E)-4,3'-dihydroxy-3,5'-dimethoxystilbene and 7,4'-dihydroxy-3,5,3'-trimethoxyflavone and some known metabolites (Delle-Monache *et al.*, 1991). An antifungal thaumatin-like protein has been isolated and purified from *Cassia didymobotrya* (Fres.) cell cultures. The protein exerted antifungal activity towards some *Candida* species showing EC₅₀ values comparable to those of other antifungal thaumatin-like protein (Vitali *et al.*, 2006).

2.8.3 *Erythrina abyssinica* Lam

Family. *Pappilionaceae*. This is a medium sized tree 5-15m, deciduous with well rounded spreading crown. Bark is yellow-buff with trifoliolate, alternate; leaflets almost as broad as long. It's widespread in African savannahs. The crude extract and the flavonoids and isoflavonoids obtained from the roots of this plant have antiplasmodial activities (Yenesew *et al.*, 2003). The ethyl acetate extract of the stem bark of *Erythrina*

abyssinica showed anti-plasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 7.9 ± 1.1 and 5.3 ± 0.7 $\mu\text{g/ml}$, respectively (Yenesew *et al.*, 2004).

2.8.4 *Warbugia ugandensis* Sprague

Family: *Canellaceae*. Common names: East African greenheart, muthiga, Ol-msogoni. The tree is a native of Kenya occurring in lowland rainforest, upland dry evergreen forest and its relicts in secondary bushland and grassland. The bark smooth or scaly, pale green or brown. The leaves are alternate, simple and dotted. A cytotoxic sesquiterpine, characterized as muzigadial, was isolated from *W. ugandensis*. It was highly toxic in the brine shrimp assay, *in-vitro* trypanocidal, antifungal and antimycobacterial activity (Olila *et al.*, 2001a; Wube *et al.*, 2005).

2.8.5 *Harrisonia abyssinica* Oliv

This medicinal plant grows widely in Kenya where it's used for treatment of fever, bubonic plague, tuberculosis, hemorrhoids and snake bites. Crude extracts of root bark of the plant were shown to exhibit antimicrobial, cytotoxic and insect antifeedant activities. Six limonoids have been isolated including obacunone, harrisonin, 12 β -acetoxyharrisonin and pedonin. A tetranortriterpenoid, 11 β ,12 β -diacetoxyharrisonin, together with the known atalantolide, obacunone, harrisonin, 12 β -acetoxyharrisonin, pedonin, neorin R and perforaquassin A have been isolated from the root of *H. abyssinica* (Rajaba *et al.*, 1999).

2.8.6 *Schkuhria pinnata* O. Ktze

Schkuhria pinnata and its varieties have some use in popular medicine as insect repellents or insecticides particularly to kill fleas. *Schkuhria pinnata* chemical investigations on members of this genus have yielded several acetylenic compounds, hydrocarbons, sterols, triterpenes, sesquiterpenes, schkuhripinnatolides (Pacciaron *et al.*, 1994). The methanol and water extract have been shown to have *in-vitro* antiplasmodial effect (Muthaura *et al.*, 2007).

2.8.7 *Ajuga remota* Benth

This is from the family *Lamiaceae*. It is a short-lived perennial plant widely distributed in various parts of Kenya. It is an erect rhizomatous pubescent herb found growing in the grasslands of Kenya and other parts of East Africa. The herb is not eaten by animals, birds or insects. This is probably due to the very bitter taste of almost all its parts. The leaves of *Ajuga remota* are known to relieve tooth ache, while a decoction or infusion from leaves is prescribed by Kenyan herbalists for severe stomachache, treatments of malaria and oedema associated with protein-calorie malnutrition disorders in infants when breast-feeding is terminated. It is widely used in parts of Eastern and Central Kenya used in colds/flu, malaria, anthelmintic, anaplasmosis, stomach upsets and tonsils (cattle). It is commonly used as antibacterial and antimalarial in Kenya. Ergosterol-5, 8-endoperoxide has been found to be antimycobacterial. The defatted methanolic extracts of aerial plant were subjected to phytochemical analysis led to isolation of iridoids glycosides, flavonoid glycosides, 8-acetylharpagide, kaempferol 3-O-a-rhamnoside, quercetin 3-O-b-glucoside, quercetin 3-rutinoside, ajugarin I and ajugarin II (Manguro *et al.*, 2006).

2.8.8 *Terminalia kilimandscharica* Engl

This is deciduous plant about 3-13m found often on rocky outcrops, 300-1700m above sea level. The fruits are up to 11 cm long. It is commonly found in relatively dry lowlands of Eastern and Coast Provinces in Kenya. *Terminalia sp* have been found to have antimicrobial properties (Fyhrquist *et al.*, 2002). The leaves of *T. kilimandscharica* have been documented to have antimolluscicidal activity. Bioactive flavonoids and triterpenes from *Terminalia sp.* have been isolated (Garcez *et al.*, 2006).

2.8.9 *Entada leptostachya* Harms.

This is a climber and is most wide spread in Kenya found in drier parts of the country. It has a dense leafy canopy and large conspicuous pods. The bark is grey brown, rough or smooth. Phytochemical analyses of extracts have resulted in isolation saponins (Kareru *et al.*, 2008).

2.8.10 *Amaranthus hybridus* L.

It is commonly called Africa spinach, smooth pigweed or amaranth and is an important leafy vegetable in Kenya. Tepals gradually narrow into acute, often spinulose or bristle-like apex. This is a common weed growing wildly; it has a succulent reddish stem. The leafy vegetable is used traditionally in many African communities as a tapeworm repellent and relief of pulmonary problems. They contain antioxidants which have been found to be antiviral and also have other vital pharmacological effects (Chitindingu *et al.*, 2007)

2.8.11 *Ziziphus abyssinica* Hochst.

This is a thorny semi-evergreen shrub or small tree, 3-6m high. Flowers are green-yellow, in small star like heads. The bark is grey-black with the leaves that are alternate along the stems, oval and leathery. The pharmacologically active compounds in *Ziziphus* include cyclopeptides, triterpensaponins and flavonoids. They are used ethnomedically for antibiosis; wound healing, diabetes (II), anxiolytic and sedative properties. It has been reported to have antimicrobial properties (Runyoro *et al.*, 2006)

2.8.12 *Croton macrostachyus* Del.

This is deciduous tree with open and rounded crown, with large spreading branches. The leaves are soft and heart shaped. The flowers are creamy yellow and sweat scented. The fruits are pea-size capsules on drooping spikes. Mature capsules split open with a cracking noise to release shiny grey seeds. It is widely used for treatment of animal and human diseases. These include worm expulsion, gonorrhoea, stomach ache, anthrax, gum ailment and hemorrhage (Wondimu *et al.*, 2007).

CHAPTER THREE: MATERIALS AND METHODS

3.0 Introduction

Ethnobotanical surveys have been carried out on use of medicinal plants by diverse communities in Kenya (Miaron, 2003; Gathuma *et al.*, 2004; Kareru *et al.*, 2007). According to Natarajan and Iyer (2000), plants that are employed in traditional medicines worldwide are two to five times more likely to test out as pharmacologically active than those randomly sampled. Various methods have been used to derive the traditional knowledge on use of medicinal plants and identification of the plants. These include the use of structured questionnaires, participatory appraisal methods, consultative meetings, archeological findings and medicinal plants database. Participatory rural appraisal enables local people to share, enhance and analyse their knowledge of life ('knowledge for action') providing both qualitative and quantitative information (Cornwall and Jewkes, 1995). Leurs (1996) has reviewed the challenges that PRA faces at different levels. The changes in lifestyle have led to traditional knowledge rapidly eroding coupled with environmental degradation and loss of biodiversity. There is also lack of ethnobotanical surveys carried out in most parts of the country. For this purpose documentation on use of indigenous plant is important to preserve this knowledge. The aim of current study was to collect, identify and document the various medicinal plants, their parts and indications as used by traditional medicine practitioners in Machakos and Kitui Districts.

3.1 Study area, Selection and Identification of plants.

The plant samples were collected from Machakos and Kitui district, Eastern province (figure 3.1). Machakos district lies between latitudes $0^{\circ} 45'$ and $1^{\circ} 31'$ south. Longitudes $36^{\circ} 45'$ and $37^{\circ} 45'$ east. It covers an area of 6,281.4 Km². Most of it is semi-arid with average rainfall range of 500-1,300mm which is unreliable. It has a population of 906,644 with most health facilities located in main town centers. The doctor/population ratio is 1: 62,325 (GoK, 2000-2003). Kitui district is located between latitudes $0^{\circ} 37'$ and $3^{\circ} 0'$ south. Longitudes $37^{\circ}45'$ and $39^{\circ} 0'$ East. It borders Machakos and Makueni districts to the west, Mwingi District to the north, Tana River district to the east and Taita Taveta district to the south. The district covers an area of approximately 20,402 Km² including 6,290.3 Km² occupied by the uninhabited Tsavo National Park. It lies between 400-1800 above sea level. It is arid and semiarid with annual average rainfall ranging between 500-1050 mm (GoK, 2000-2003).

Preliminary visits were done to identify and select herbalists who took part in this study. The Provincial Director, Ministry of Gender, Sports, Culture, and Social Services provided a list of authentic herbalist groups. These groups were selected to cover most of the area under our study. The initial selection was based on the willingness of herbalists to give voluntary information and interaction with researchers during consultative meetings. These meetings were participatory in nature, with researchers as facilitators.

Ethnobotanical data was collected during a 12-month period from 110 herbalists practicing in the study area. They were both men and women aged 40 to 80 years. All the herbalists interviewed were Christians. Non-Christian herbalists were said to combine herbal medicines with witchcraft and were therefore avoided.

The indigenous knowledge was collected using Participatory Rural Appraisal method (PRA). An expert in PRA from the National Museums of Kenya participated in this research. Formal interviews through questionnaires were avoided as it was found to be intimidating to the herbalists, majority of whom were semi- illiterate.

The samples were collected and deposited at the herbarium, department of Land Resource Management and Agricultural Technology (LARMAT), College of Agriculture and Veterinary Sciences for botanical identification and classification. A thorough literature search was done on the plants from electronic databases, putting into account the uses given by the herbalists. Results of this are represented in (table 3.1). Photographs of the plants *in-situ* were also taken (plates 3.1-3.8).

Fig 3.1: Map of Kenya illustrating the geographical locations of Machakos and Kitui District

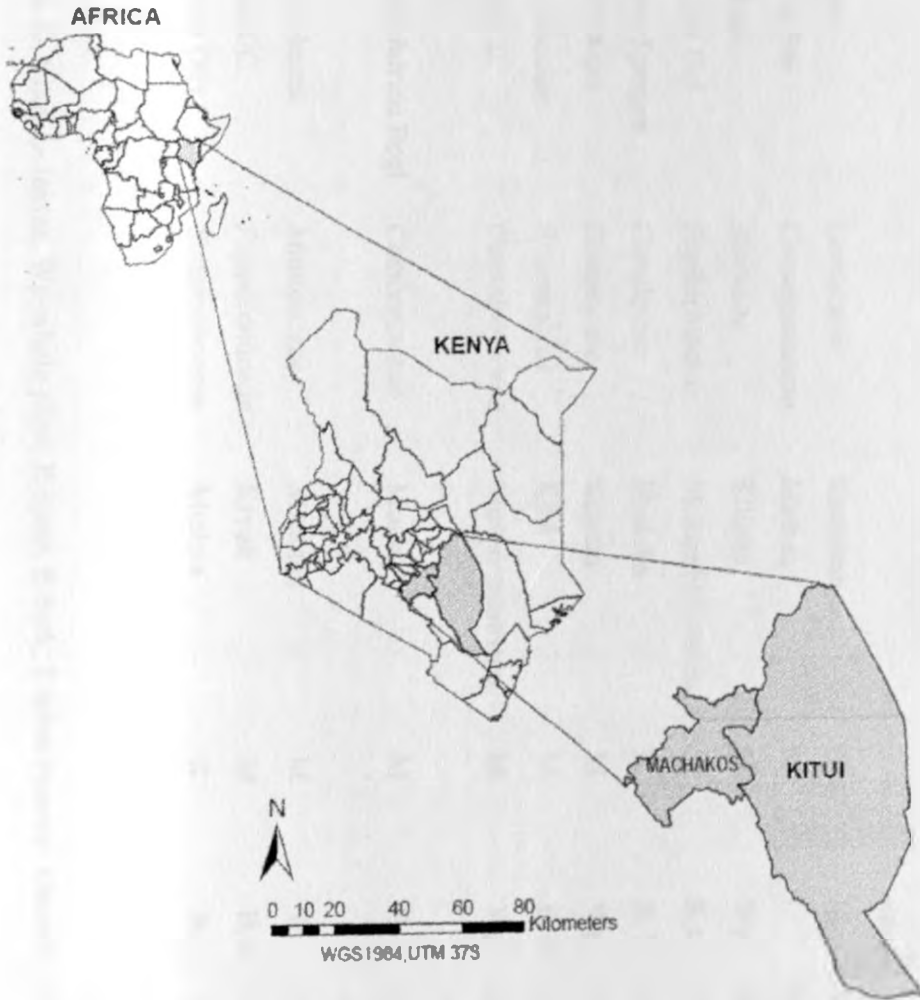


Table 3.1: Botanical identification uses and parts used of selected medicinal plants studied.

Botanical name	Family	Local name	Location	Part used	Uses
<i>Ajuga remota</i> Benth	<i>Lamiaceae</i>	Katetema	M	L	Malaria, boils
<i>Cassia didymobotrya</i> Fes	<i>Caesalpinaceae</i>	Muthaa	M	L	typhoid
<i>Aloe secundiflora</i> Engl.	<i>Aloaceae</i>	Kiluma	M	Wp	Malaria, pneumonia
<i>Croton macrostachyus</i> Del.	<i>Euphorbiaceae</i>	Mukambi/kitundu	M	R,L	Typhoid, measles
<i>Warbugia ugandensis</i> Sprague	<i>Canallacea</i>	Muthika	K	B, L	Cough
<i>Schkuhria pinnata</i> O. Ktze	<i>Compositae</i>	Kaututi	M	Wp	Malaria, joint pains, diabetes
<i>Ziziphus abyssinica</i> Hochst	<i>Rhamnaceae</i>	Kiae	M	B, R	Kidney, stomach
<i>Amaranthus hybridus</i> L.	<i>Caesalpinaceae</i>	Muvisi/musavula	M	Wp	Urinary tract infection (UTI), kidney and stomach ailments
<i>Terminalia kilimandscharica</i> Engl.	<i>Combretaceae</i>	Muuku	M	B	Cough, sexually transmitted diseases(STD)
<i>Entada leptostachya</i> Harms	<i>Mimosaceae</i>	Mwaita	M	T	Tuberculosis, cough
<i>Erythrina abyssinica</i> DC.	<i>Pappilionaceae</i>	Kivuti	M	B, R	Pneumonia, STD's, Prostate
<i>Harrisonia abyssinica</i> Oliv	<i>Simaroubaceae</i>	Muthiia	K	B, L	Pneumonia, syphilis, infertility, malaria, stomach ailments, eye ointment

Key: M- Machakos, K- Kitui, L- leaves, Wp-whole plant, R-roots, B-bark, T-tuber (source: Ukamba Herbalist Society)



Plate 3.1) *Amaranthus hybridus* L. (Muvisi/musavuli)



Plate 3.2) *Harrisonia abyssinica* Oliv. (Muthia)



Plate 3.3) *Croton macrostachyus* Del.(Mukambi/Kitundu)



Plate 3.4) *Erythrina abyssinica* DC (Kivuti)



Plate 3.5) *Schkuhria pinnata* O. Ktze (Kaututi)

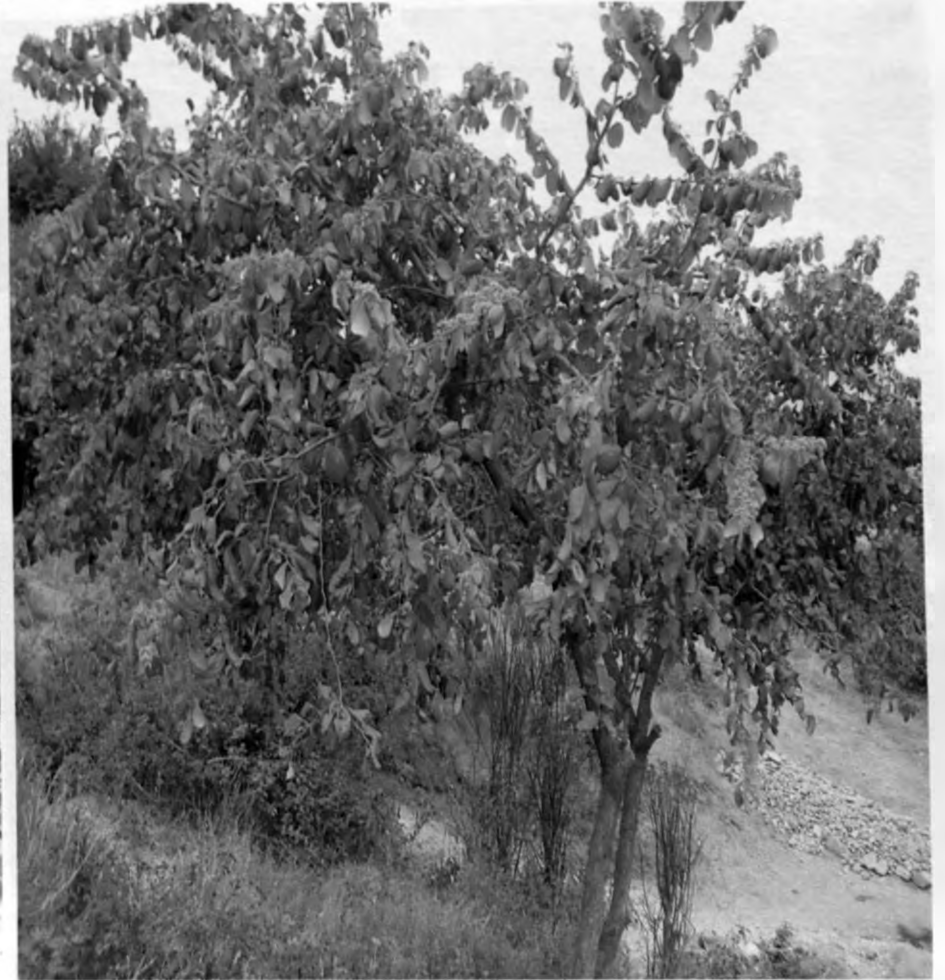


Plate 3.6) *Terminalia kilimandscharica* Engl. (Muuku)



Plate 3.7) *Ziziphus abyssinica* Hochst (Kiae)



Plate 3.8) *Cassia didymobotrya* Fres. (Muthaa)

3.1.2 Preparation of plant samples for extraction

The plant materials were dried at room temperature and then chopped into small pieces using a knife. The dried and chopped plant sample was placed in Cunningham grinder that had both high and low speed. The sample was ground using one level of high speed and two levels of low speed each lasting 15 seconds. This was repeated until all the dry sample was turned into powder. The grinding process was done in a fume chamber for protection from the fumes emitted. The powder obtained was packed in 500grams portions and was placed in clean airtight polythene paper (Gakuya, 2001).

3.1.3 Extraction procedure

Maceration method using 70% v/v methanol in water was used for extraction of active ingredient from plant materials as described by Gakuya, (2001). Four hundred grams of the plant powder were extracted separately. The powdered sample was placed in conical flask and methanol added until the powder was submerged. The conical flask was corked with appropriate stopper and shaken thoroughly. The process took 4 days at room temperature during which proper shaking was regularly done to allow proper percolation and extraction. On the fifth day, the extracts were filtered using Whatman No. 1 filter papers into another conical flask. Each of the extract was then evaporated to dryness under pressure in a rotary evaporator. Methanol that evaporated was collected into another flask and recycled.

The resultant viscous substance weighed ten grams and was put in sterilized beakers, which were covered tight using aluminum foil. The resultant viscous substance obtained was stored in a refrigerator at +4°C pending freeze drying, bioassay and antimicrobial activity testing. Freeze-drying was done using Edwards' lyophilizer courtesy of Department of zoology, Chiromo Campus (Edwards High Vacuum, Model M6B).

The samples in the beaker were placed on a cooled, temperature controlled shelf within the freeze dryer. After complete freezing, the pressure was lowered. Moisture from the methanol extract was lost by sublimation. The extracts were left in freeze dryer overnight. The following day the dried samples were removed from the freeze drier and the brim of the beakers sealed using paraffin film. About five grams of extract was obtained for each plant sample. Samples were stored in airtight test tubes in cool dry place pending further investigation.

3.2 TESTS FOR ANTIBACTERIAL ACTIVITY

3.2.1 Introduction

Houghton *et al.*, (2007) noted that the cost and ethical concerns of *in-vivo* tests have lead to wide spread use of *in-vitro* tests in ethnopharmacological studies. In recent times there has been a surge of antibiotic resistance of most clinically significant pathogens like *Mycobacterium tuberculosis* with emergence of multi drug resistant organisms (Aibinu *et al.*, 2007). The non availability, lack of access and high cost of new generation antibiotics have resulted in increase morbidity and mortality (Williams, 2000). Thus there have been renewed efforts towards searching for new antimicrobials from materials of plant origin. The objective of this study was to test the *in-vitro* antimicrobial activity of different plant extracts used by Traditional Herbalists in Eastern province for management of infection.

3.3 Test bacteria and drugs used as positive control

The reference strains used for the screening were *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Micrococcus lutea* (ATCC 9341) and *Bacillus cereus* (ATCC 11778). Bacteria were obtained from stock cultures from department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi. The bacterial stock cultures were maintained on glycerol stored at 4°C. The four microorganisms maintained on blood agar base were used to assess the antimicrobial activity of the methanol plant extract. Antibiotics Benzyl penicillin and streptomycin powder were used as reference standards for gram positive and gram-negative bacteria respectively, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

3.3.1 Antibacterial testing

A loopful of stock cultures of standard organisms that were stored in glycerol solution were sub inoculated on BA (Oxoid) and incubated for 24 hours at 37°C. The sub-cultured bacteria were used as stock cultures and were kept refrigerated at +4°C. The following day one single colony was picked using a sterile loop and put into 3 ml sterile physiological buffer saline (PBS). Serial dilutions were made to density equivalent Macfarland opacity No. 5 containing 0.5 ml of 10% barium chloride in 1% sulfuric acid and adjusted by the standard plate count method (Black, 1996). One tenth of ml of the bacterial suspension was spread on pre-prepared Mueller Hinton Agar (Oxoid) and incubated for 18 hours at 37°C. The number of colonies per ml was calculated. The number of colony forming units was 2.2×10^8 cfu/ml.

Broth dilution technique as described by Suffredini, (2006) was used to test for inhibitory activity of plant extracts. Pre-sterilized MHB was dispensed into sterilized 10 ml test tubes using sterile 10ml pipette. The test tubes were clearly labeled and put in test tube rack. For test with gram positive bacteria 200 mg of extract was dissolved in 2ml sterile MHB. For the inhibitory tests on gram negative bacteria 500mg of plant extract was dissolved in 2 ml sterile PBS. Serial two fold dilution of plant extracts was made. Using sterile 1 ml pipette 0.1 ml of bacterial suspension was dispensed into each of the test tubes. They were incubated at 37°C for 24 hours. All experiments were performed in triplicates. Another tube containing the bacterial inoculum without plant extract was used as negative control. After overnight incubation, visual turbidity was noted and 0.1 ml from non-turbid tubes was sub-cultured to MHA plates. The inocula were spread on agar using sterile glass rods. The plates were incubated for 24 hours at 37°C. For the positive controls, two-fold dilution of streptomycin powder at

concentration of 40 mg/ml and benzylpenicillin 10 mg/ml were made as above for gram negative and gram positive bacteria respectively.

The MIC was defined as the lowest concentration that inhibited any visible bacterial growth on the culture plates (Prescott *et al.*, 1999; Shahidi, 2004; Aibinu *et al.*, 2007).

This was determined from readings on the culture plates after incubation.

3.4 TEST FOR BIOACTIVITY USING BRINE SHRIMP LETHALITY TEST

3.4.1 Introduction

Test for the bioassay of medicinal plants have been done using brine shrimp lethality test (BST) (Meyer *et al.*, 1982). This was later modified by McLaughlin *et al.* (1991). It is rapid, reliable, inexpensive and convenient tool to detect general bioactivity. The method has been applied in screening plants extracts used across the world (Gakuya, 2001; Wanyoike *et al.*, 2004; Moshi *et al.*, 2004, 2006, 2007a). Plants used in folklore are assumed to be safe based on their long usage, but have been shown to be potentially carcinogenic, toxic and mutagenic (Elgorashi *et al.*, 2003). Few of the Kenyan medicinal plants have been evaluated for their biological activity. However many of the specific bioassays used to screen plant extracts are costly requiring special reagents and equipments. The brine shrimp lethality test (BST), originally proposed by Meyer *et al.* (1982) and later modified by McLaughlin *et al.* (1988). It is based on the ability to kill laboratory cultured *Artemia salina* leach. This assay is considered a useful tool in preliminary assessment of toxicity. It has been used for detection of fungal toxins, heavy metals, cyanobacteria toxins, pesticides, cytotoxicity testing of dental materials (Pelka *et al.*, 2000) and plant toxicity testing (McLaughlin *et al.*, 1991). The present study reports the brine shrimp bioassay of some Kenyan medicinal plants used in eastern province of Kenya.

3.5 Hatching the brine shrimp

The method described by Gakuya, (2001) was used. Thirty-three (33) grams marine salt were weighed on an electric weighing machine and transferred into 1 liter conical flask. Distilled water was added gradually concurrently stirring to dissolve marine salt. When

all marine salt had dissolved distilled water was added to 1 liter mark to constitute the marine salt solution.

Brine shrimp eggs (acquired from department of PHPT, Faculty of Vet. Medicine, U.o.N) were hatched in shallow rectangular plastic double chambered box with a dividing wall which had 1-2mm holes. The box was filled with marine salt solution (33g of marine salt in 1L distilled water). Using a spatula about 50mg of brine shrimp eggs was sprinkled and about 5mg of dry yeast which served as food for the nauplii was sprinkled in the dark compartment. The other compartment was illuminated through a hole in the lid of the box and kept under a light source using a 40 watts electric bulb. After 48 hours, the phototropic nauplii were collected by use of a Pasteur pipette from the lighted compartment and subjected to brine shrimp lethality test.

3.5.1 Preparation of plant extracts for bioassay

All the methanol extract under study were treated the same, 0.1 grams of methanol plant extract was weighed (Mettler PM 4600, Delta Range[®]) and transferred into a universal bottle. Ten milliliters of marine salt solution (33g of marine salt in one (1) liter distilled water) was added to dissolve and stirred using electrical mixer (Voltex Reamix 2789[®]) at 2800 rpm to make final stock concentration of 10,000 μ g/ml. Serial dilutions were prepared from this stock solution (Gakuya, 2001).

3.5.2 Cytotoxicity bioassay

Three dilutions were prepared by transferring 500 μ l, 50 μ l and 5 μ l of plant extract to the set of five graduated tubes. Ten shrimps were transferred into each of the vial using Pasteur pipette and marine salt was added to 5ml mark to make dilutions of 1000 μ g/ml.

100µg/ml and 10µg/ml. Five graduated vials were set for each dilution and a further five for the control. The tubes were left at room temperature and the number of live larvae counted after 24 hours. The percentage mortality was determined for each dilution and controls. Where control deaths occurred within 24 hours, the data was corrected using the equation: %death= $\frac{\text{test-control}}{\text{control}} \times 100$ (Gakuya, 2001).

The results were interpreted using probit method of Finney computer program acquired from Department of Pharmacology and Pharmacognosy, Faculty of Pharmacy, University of Nairobi. The program uses the number of dose level, number of brine shrimp for every concentration, percentage mortality for every concentration and the dose level. The lethal concentration 50 (LC₅₀) and 95% confidence intervals were determined using the computer program (McLaughlin *et al.*, 1991).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.0 ETHNOBOTANICAL SURVEY

Most of the medicinal plants are sourced from the wild, but some like the *Amaranthus hybridus* and *Ajuga remota* are more common in the surrounding. The practice of farming in medicinal plants is not well developed and this needs to be explored due the rapid destruction of the environment. Leaves were widely used, this was in agreement with what has been reported elsewhere (Wondimu *et al.*, 2007). There was preference for the use of combination of different plant parts, with the plants being for multiplicity of conditions. Here *A. hybridus* is reported to be used as remedy for different ailments. However, it is widely reported to be used as edible leafy vegetable (Orech *et al.*, 2005). During the field trips and discussion the herbalists called for concerted efforts to manage the environment, thereby need for positive mutually beneficial relationship with researchers and value addition for herbal products.

4.1 ANTIBACTERIAL TESTING

4.1.1 Data handling and analysis

The data was entered and handled in Microsoft Excel 2003 software. The data was then exported to GenStat for *Windows* 9th edition. When comparing different samples results, they were considered to be statistically different when $P < 0.05$ (student t-test for unpaired samples).

4.1.2 Results and discussion

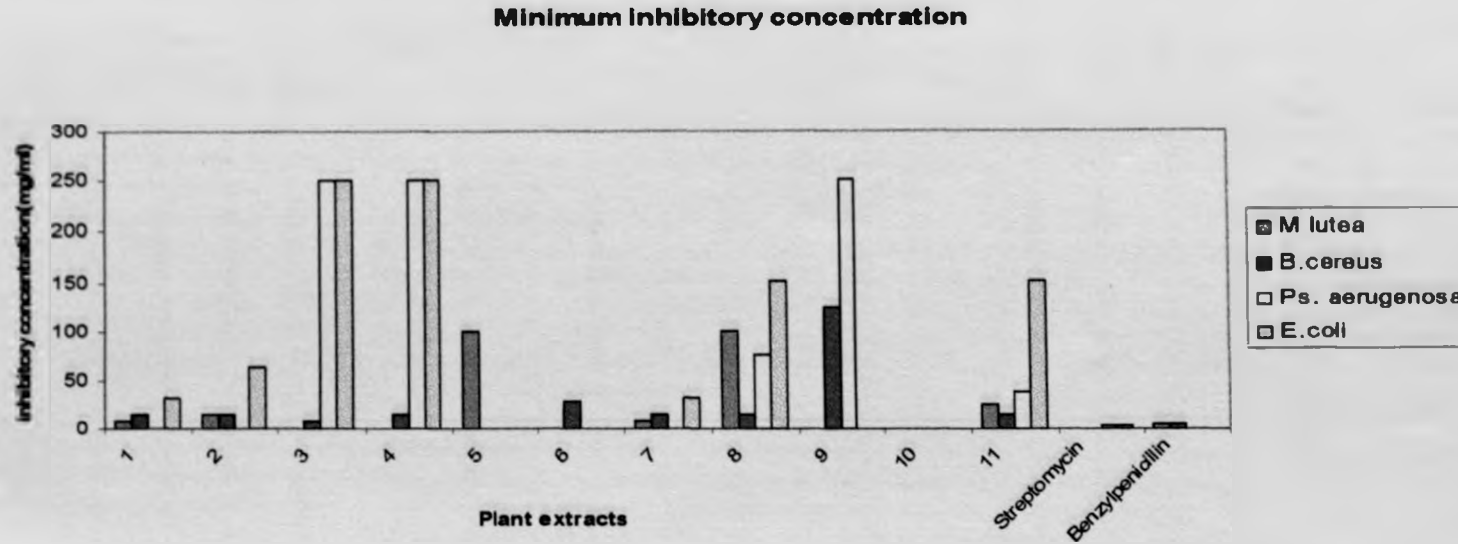
The *in-vitro* antibacterial activity (MIC) of the plant extracts are shown in Table 4.1 and Figure 4.1 for the inhibitory concentrations on each bacteria species.

Table 4.1: Minimum inhibitory concentrations (MIC mg/ml) for methanol extracts of selected medicinal plants

Botanical name	Part(s) used	<i>M. lutea</i>	<i>B. cereus</i>	<i>Ps. aeruginosa</i>	<i>E. coli</i>
<i>Ajuga remota</i>	L	7.8	15.6	-	31.25
<i>Cassia didymobotrya</i>	L	15.6	15.6	-	62.5
<i>Aloe secundiflora</i>	Wp	-	7.8	250	250
<i>Croton macrostachyus</i>	R, L	-	15.6	250	250
<i>Schkuhria pinnata</i>	WP	100	-	-	-
<i>Ziziphus abyssinica</i>	B,R	-	28.75	-	-
<i>Amaranthus hybridus</i>	Wp	7.8	15.6	-	31.25
<i>Terminalia kilimandscharica</i>	B	100	15.6	75	150
<i>Entada leptostachya</i>	T	-	125	250	-
<i>Erythrina abyssinica</i>	B,R	-	-	-	-
<i>Harrisonia abyssinica</i>	B,L	25	15.6	37.5	150
Streptomycin				0.25	0.25
Benzylpenicillin		0.6	0.6		

Key: L- Leaves, Wp-whole plant, R-roots, B-bark, T-tuber

Figure 4.1: Minimum inhibitory concentrations (MIC mg/ml) for methanol extracts of selected medicinal plants.



Key:

1. *Ajuga remota*

2. *Cassia didymobotrya*

3. *Aloe secundiflora*

4. *Croton macrostachyus*

5. *Schkuhria pinnata*

6. *Ziziphus abyssinica*

7. *Amaranthus hybridus*

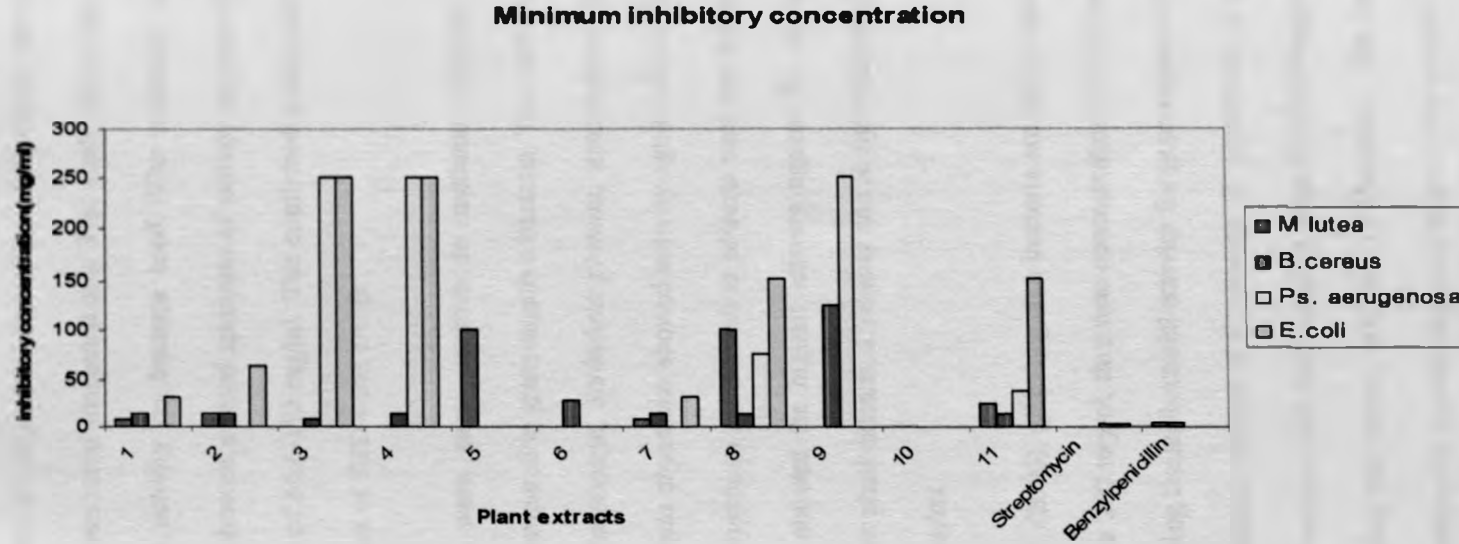
8. *Terminalia kilimandscharica*

9. *Entada leptostachya*

10. *Erythrina abyssinica*

11. *Harrisonia abyssinica*

Figure 4.1: Minimum inhibitory concentrations (MIC mg/ml) for methanol extracts of selected medicinal plants.



Key:

1. *Ajuga remota*
2. *Cassia didymobotrya*
3. *Aloe secundiflora*
4. *Croton macrostachyus*
5. *Schkuhria pinnata*

6. *Ziziphus abyssinica*
7. *Amaranthus hybridus*
8. *Terminalia kilimandscharica*
9. *Entada leptostachya*
10. *Erythrina abyssinica*

11. *Harrisonia abyssinica*

The methanolic plant extracts were found to have antibacterial activity except methanol extracts of *Erythrina abyssinica*. *E. abyssinica* showed no antibacterial activity on any of the test bacteria at the various serial concentrations. This study shows that the plant extract has no antimicrobial activity on bacteria used. The methanol extracts of *Terminalia kilimandscharica* showed a broad spectrum of activity against all bacterial strains at tested concentration of 15.6-150 mg/ml. The confirmed antibacterial activity validates the use in management of STD's and cough.

The gram positive bacteria were more sensitive to methanol extracts at lower concentrations of 7.8mg/ml compared to gram negative bacteria. The methanol extracts of *Ajuga remota*, *Cassia didymobotrya*, *Schkuhria pinnata*, *Amaranthus hybridus*, *T. kilimandscharica* and *Harrisonia abyssinica* showed activity against *M. lutea*. Extracts of *H. abyssinica*, *Erythrina abyssinica* and *Schkuhria pinnata* were not inhibitory to *B. cereus*. *Harrisonia hybridus* showed the highest activity against *Ps. aeruginosa* of 37.5mg/ml. Compared to other plant extracts *A. remota* and *A. hybridus* had the lowest MIC ranging from 7.8-31.25mg/ml.

According to Suffredini *et al.*, (2006), gram-negative bacteria are hardly susceptible to plant extract in doses as low as 200 mg/ml, thus neat concentrations of 250 mg/ml were made and this was used in testing the antibacterial activity for gram negative bacteria. *E. coli* was not sensitive to methanol extracts of *S. pinnata*, *Z. abyssinica*, *E. abyssinica* and *H. abyssinica*, the other extracts had MIC ranging from 31.25-250mg/ml with *A. remota* and *A. hybridus* having the lowest MIC of 31.25mg/ml. The antibacterial activity of *W. ugandensis* methanolic extracts was not done in the current study, but previous study showed the alcohol and water extracts had greater activity against *S. aureus* than *E. coli* (Olila *et al.*, 2001b).

B. cereus ATCC 11778 was sensitive to 81.8% of extracts with inhibitory concentration as low as 7.8mg/ml. *P. aeruginosa* was least sensitive with only 38.46% of plant extracts showing activity. There was no significant statistical difference between the MIC of different bacterial species. The action of the methanol extract was significant at higher concentration than that of commercial antimicrobial used as positive controls (0.6 mg/ml and 0.25 mg/ml for gram negative and positive bacteria respectively).

All methanol plant extracts studied showed antibacterial activity except *E. abyssinica* on one or more bacteria under study. This could justify the use of the plants for treatment of infections by the traditional healers in Ukambani. The use of *E. abyssinica* for management of pneumonia and sexually transmitted diseases may not be of value. The use of *A. hybridus* and *A. remota* in treatment of urinary tract infections caused by *E. coli* is supported by the current study. Chitindingu *et al.*, (2007) isolated antioxidant and phenolic from *A. hybridus* which are known to have antibacterial activity; this could explain the antibacterial activity of the methanolic extracts in the current study.

The current study reports *Z. abyssinica* activity against *B. cereus*. Its mechanism of action in relieving stomach ailments may be other than antibacterial properties. In previous studies it was shown to have antifungal properties (Runyoro *et al.*, 2006). *C. macrostachyus* is reported to be used as anthelmintic, antimalarial, venereal diseases, cough, and skin rash. Matu and van Staden, (2003) reported the absence of antibacterial activity of methanolic leaves extracts of *C. macrostachyus*. However, this study showed inhibitory activity against *B. cereus*, *Ps. aeruginosa* and *E. coli*. Matu and van Staden, (2003) reported low activity for hexane extracts. This could be due to locality of plant species, parts used, time of collection, storage conditions and methods of analyzing as reasons for contrasting observations.

Previous study by Matu and van Staden (2003) reported that extracts of *A. remota* had no antibacterial activity on test bacteria. The current study shows otherwise, the family *Lamiaceae* contains terpenoids which are known to possess antifungal, antibacterial and anti-insect activities (Cole, 1992).

The current study supports the use of *C. didymobotrya* in management of typhoid. *S. pinnata* is reported to have antimalarial activity (Muthaura *et al.*, 2007). In the current study it only had inhibitory activity against *M. lutea*. The extract of *E. leptostachya* showed inhibitory activity high concentrations and its use for tuberculosis management as reported by herbalists should be studied in details using appropriate methodologies.

Previous studies have reported the antibacterial properties of *A. secundiflora* (Waihenya *et al.*, 2002) and the current study collaborates this. The *H. abyssinica* and *A. hybridus* showed broad antibacterial activity, this study supports the continued use for treatment of pneumonia, syphilis and other bacterial infections.

The lack of sensitivity of *Ps. aeruginosa* to 62% of plant extracts could be attributed to the fact that the bacteria is naturally resistant to many antibiotics due to permeability barrier offered by its outer membrane or to the fact that herbal preparations are subject to contamination and deterioration (WHO, 2000). The storage requires special conditions of humidity and temperature. The extracts which were inactive *in-vitro* may have properties similar to prodrugs with their metabolites being active *in-vivo*. The fact that extracts are more active against gram-positive bacteria can be attributed to the fact that the cell wall of the gram-positive bacteria is easier to penetrate than that of Gram-negative bacteria (Rang and Dale, 1987)

4.2 BRINE SHRIMP LETHALITY TEST

4.2.1 Data handling and analysis

The bioactivity data was analyzed using Finney's computer program (McLaughlin *et al.*, 1991).

4.2.2 Results and Discussion

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of selected methanol extracts medicinal plants. The results are represented in table 4.1, 4.2 and figure 4.2 below. Table 4.2 represents the lethal concentration 50 and 95% confidence intervals.

Table 4.1: Percentage brine shrimp mortality caused by serial dilution of methanol tracts

Botanical name	Local name	Parts used	Serial dilution of methanol extract (µg/ml)		
			10	100	1000
<i>Ajuga remota</i>	Katetema	L	0	74	100
<i>Cassia didymobotrya</i>	Muthaa	L	6	10	94
<i>Aloe secundiflora</i>	Kiluma	Wp	0	8	88
<i>Croton macrostachyus</i>	Kitundu	R, L	0	4	86
<i>Warbugia ugandensis</i>	Muthika	B, L	0	2	86
<i>Schkuhria pinnata</i>	Kaututi	Wp	0	8	68
<i>Ziziphus abyssinica</i>	Kiae	B, R	0	0	2
<i>Amaranthus hybridus</i>	Musavuli/muvisi	Wp	0	44	98
<i>Terminalia kilimandscharica</i>	Muuku	B	4	34	90
<i>Entada leptostachya</i>	Mwaitha	T	2	32	62
<i>Erythrina abyssinica</i>	Kivuti	B, R	0	4	88
<i>Harrisonia abyssinica</i>	Muthiia	B, L	0	46	56

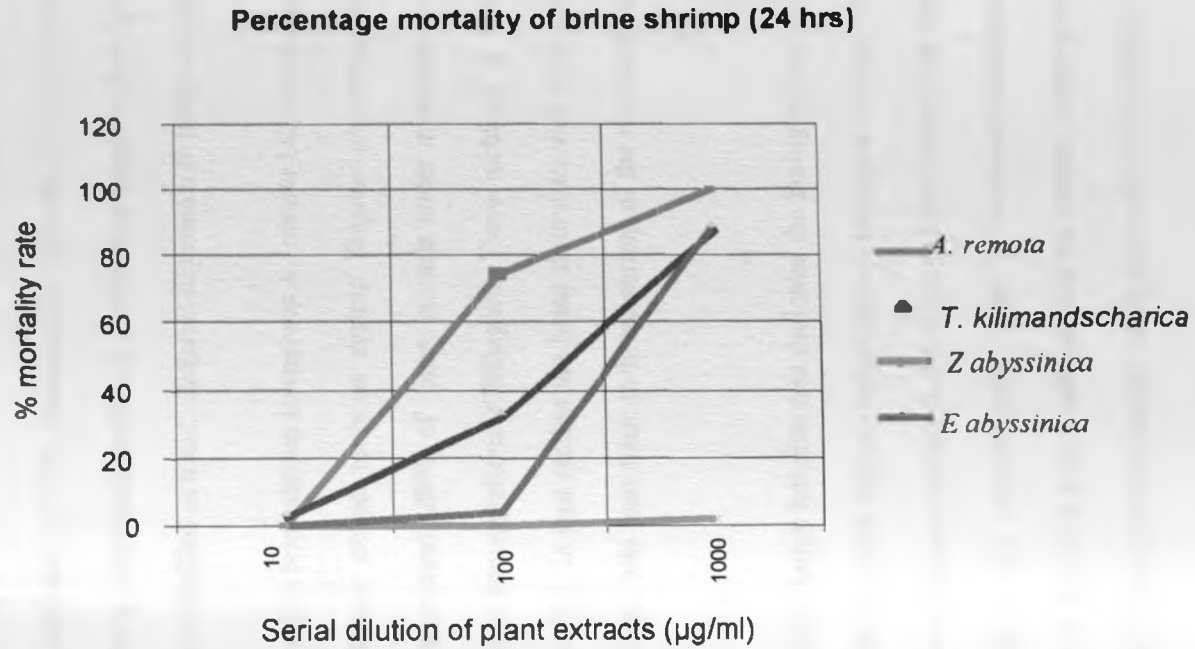
Key: L- leaves, Wp-whole plant, R-roots, B-bark, T-tuber

Table 4.2: Lethal Concentration 50 (LC₅₀) in µg/ml and 95% confidence intervals (CI) of selected plant extracts.

Botanical name	Local name	Part tested	LC₅₀ (µg/ml)	95% CI
<i>Ajuga remota</i>	Katetema	L	61.6	19.8-144.6
<i>Cassia didymobotrya</i>	Muthaa	L	230.9	91.3-650.9
<i>Aloe secundiflora</i>	Kiluma	Wp	332.9	133.9-829.7
<i>Croton macrostachyus</i>	Kitundu	R, L	387.1	160.2-936.9
<i>Warbugia ugandensis</i>	Muthika	B, L	397.4	172.1-940.8
<i>Schkuhria pinnata</i>	Kaututi	Wp	557.0	202.6-3158.4
<i>Ziziphus abyssinica</i>	Kiae	B, R	>1000	5.4-2497.4
<i>Amaranthus hybridus</i>	Musavuli/ muvisi	Wp	116.1	45.1-316.7
<i>Terminalia kilimandscharica</i>	Muuku	B	164.1	56.6-502.8
<i>Entada leptostachya</i>	Mwaitha	T	421.3	127.9-6301.3
<i>Erythrina abyssinica</i>	Kivuti	B, R	368.7	157.0-872.9
<i>Harrisonia abyssinica</i>	Muthiia	B, L	392.4	103.1-15928.6

Key: L- leaves, Wp-whole plant, R-roots, B-bark, T-tuber

Fig. 4.2: Brine shrimp mortality of serial dilutions of methanol extracts of selected medicinal plants



Results were interpreted according to Santos *et al.* (2003); Meyer *et al.* (1982), as toxic where LC_{50} was $<1000 \mu\text{g/ml}$ and non-toxic if LC_{50} was $>1000 \mu\text{g/ml}$. The results of LC_{50} ($\mu\text{g/ml}$) were as follows: *Ajuga remota* (61.6), *Cassia didymobotrya* (230.9), *Aloe secundiflora* (332.9), *Croton macrostachyus* (387.1), *Warbugia ugandensis* (397.4), *Schkuhria pinnata* (557.0), *Amaranthus hybridus* (116.1), *Terminalia kilimandscharica* (164.1), *Entada leptostachya* (421.3), *Erythrina abyssinica* (368.7) and *Harrisonia abyssinica* (392.8) were classified as toxic. *Ziziphus abyssinica* (>1000) was classified as non toxic.

The most toxic to brine shrimp larvae was *A. remota* LC_{50} of $61.6 \mu\text{g/ml}$ (9.8-144.6). The least lethal methanolic extract to brine shrimp *Ziziphus abyssinica* with LC_{50} of $>1000 \mu\text{g/ml}$. Twenty eight percent (28%) of plant extracts under investigation showed lethality to brine shrimp at low concentrations $<200 \mu\text{g/ml}$. These included *A. remota*, *A. hybridus* and *T. kilimandscharica*. In past studies the brine shrimp toxicity of *A. hybridus* was reported to be $>1000 \mu\text{g/ml}$ and was least toxic to brine shrimp of the nine leafy vegetables tested (Orech *et al.*, 2005).

Although brine shrimp lethality test indicates the biological activity in plant extracts, lack of lethality does not mean that the extract has no biological activity as reported by Mwangi *et al* (1999), *Prunus africana* extract is not toxic to brine shrimp but has pharmacological activity and is widely and commercially used in treatment/management of benign prostate hypertrophy. The results below indicate that the extracts under investigation have bioactivity, which should be assessed thoroughly using more effective bioassay.

Apart from efficacy, safety of herbal medicines is of paramount importance as there is not much that is known about many plants that are used in traditional medicine. Toxicity results from animals will be crucial as a way to definitively judge the safety of these plants, as and when they are found to have enough potential for development. The present results only suggest possibility of other hitherto unreported biological activities, of toxic nature or even anticancer activity. Plants of the Family *Combretaceae* are known to be sources for combretastins (Rogers and Verotta, 1996), which have potent anticancer activity. *T. kilimandscharica* extracts had one of highest LC_{50} of 164.1 $\mu\text{g/ml}$ (56.6-502.8). It has been speculated that combrestatins are responsible for this lethality (Meyer *et al.*, 1982).

CHAPTER FIVE: GENERAL CONCLUSIONS

The results on antibacterial tests indicate that plants used in traditional medicine have antimicrobial activity. The growth of bacterial organism on some crude methanol extracts can be due to natural resistant of the organism or lack of antimicrobial principle. The plants that were under study were those identified by traditional healers. They are used for management of infectious diseases. The extract of *A. secundiflora*, *C. didymobotrya*, *C. macrostachyus*, *A. hybridus*, *T. kilimandscharica*, *E. leptostachya*, *A. remota* and *H. abyssinica* had broad-spectrum antibacterial activity. Their use by traditional medical practitioners for treatment/management of infections or prevention of opportunistic infections is thus justifiable. The medicinal plant extracts showing narrow spectrums anti-bacterial activities were those of *Schkuhria pinnata*, *Ziziphus abyssinica* and were against gram-positive bacteria.

The indiscriminate use of antimicrobials has lead to development of resistance to drugs. Several researchers have reported the cost effective use of medicinal plants. Stermitz *et al.*, (2000) has reported synergy of herbal remedies with convectional antimicrobials. The current study supports the continued intensive study of traditional remedies including phytochemistry, drug interactions and value addition. Smith *et al.*, (1999) noted that the emergence of bacterial resistance threatens to return us to the era before the development of antibiotics due to increase in antimicrobial resistance in health care associated pathogens. The rapid development of resistance including the emergence of multi-drug resistant tuberculosis (MDR-TB) shows that the potency of prevalent antibiotics is decreasing steadily. This situation calls for urgent new and safe antimicrobials for replacement of invalidate antimicrobial or use antibiotic in a rotation programs (Quale and Atwood, 1996).

The use of bioassay of bioactivity using brine shrimp lethality test has been done for some medicinal plants in Kenya (Mwangi *et al.*, 1999, Gakuya, 2001). Mwangi *et al.*, 1999 indicated that lack of lethality to brine shrimp does not mean absence of biological activity. The current studies showed that most of extracts had biological activity and for those that showed low lethality it does not mean they lack biological activity.

There was association between lethality to brine shrimp (less than 250 µg/ml and antibacterial (ranging from 7.5-150mg/ml) activity of 36% plant extracts in the current study. *A. remota* was lethal to brine shrimp at LC₅₀ of 61.6µg/ml (9.8-144.6) and low inhibitory concentration range 7.8-31.25mg/ml; the same was found for the *A. hybridus* which had LC₅₀ of 116.1 (45.1-316.7) with MIC ranging from 7.8-31.25mg/ml.

Z. abyssinica with LC₅₀ of less than 1000µg/ml had a corresponding narrow spectrum of antibacterial activity. Only the drug sensitive *B. cereus* was sensitive at MIC of 28.75mg/ml. The same was observed for *S. pinnata* LC₅₀ of 557.0µg/ml (202.6-3158.4) which had MIC of 100mg/ml against *M. lutea*. Both methanol extracts were only active against gram positive bacteria.

Plants of the Family *Combretaceae* are known to be sources for combretastins that have potent anticancer activity; *T. kilimandscharica* had high bioactivity and broad antibacterial activity. Thus using the findings of this study and the study of the anticancer compound in previous studies this plant should be investigated further in more details.

In view of the complexity and heterogeneity of African traditional medicine, a system of incorporation in the current health care systems has to be developed. The WHO Regional Director for Africa has developed model guidelines that the Member States can adapt or adopt

as may be appropriate in the respective Member States. Some of the relevant guidelines touch on policy, legal framework, code of ethics and intellectual property rights.

Recommendations:

1. The plants showed appreciable antibacterial properties thus their continued use of the plants should be encouraged. Further phytochemical and toxicological studies need to be done. It is advisable that active principles are identified, toxicity evaluation and phytotherapeutic products taken to clinical trials, this may represent cheaper and affordable medicines to our population.
2. Some of medicinal plants under investigation require special mention like *Terminalia kilimandscharica*. This plant demonstrates enormous prospects and further study on it are worthwhile.
3. There is need to collect and document useful trees and shrubs used by different communities around the country. Sustainable harvest should be encouraged and enforced for posterity.
4. The use of phytotherapeutics should go beyond policy change to policy implementation. Research, development and intellectual property rights should go in tandem. Traditional medical practitioners and communities should be involved.

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Appendix 1: preparation of Blood Agar medium

Suspend 40g of blood agar base in 1 liter of distilled water and boil to dissolve. Sterilize by autoclaving at 121°C for 15 minutes. Cool the base to 45-55°C. Add 7% sterile blood to the sterile media. The blood is warmed to 37°C before adding. Dispense the mixture into sterile Petri dishes.

Appendix 2: Preparation of Mueller Hinton Agar (Oxoid®) medium

Typical formula (g/l):

Beef dehydrated infusion	300
Casein hydrolysate	17.5
Starch	1.5
Agar	17.7

Suspend 38g in 1L of distilled water. Bring to boil to dissolve medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

Appendix 3: preparation of Mueller Hinton Broth medium (Oxoid®)

Typical formula (g/l):

Beef dehydrated infusion	300
Casein hydrolysate	17.5
Starch	1.5

Dissolve 21g in 1L of distilled water. Bring to boil to dissolve medium completely. Sterilize by autoclaving at 121°C for 15 minutes.