

**ETHNOPHARMACOLOGY, BIOACTIVITY AND ANTHELMINTIC EFFICACY OF
MEDICINAL PLANTS TRADITIONALLY USED IN LOITOKTOK DISTRICT, KENYA**

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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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Dedication

This thesis is dedicated to my dear family for their immense support and commitment.

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List of Abbreviations

AU	African Union
CHCL ₃	Chloroform
epg	Eggs per gram of faeces
FEC	Feecal egg count
FECR	Faecal egg count reduction
g	grams
GABA	Gamma-aminobutyric acid
GIT	Gastrointestinal tract
HIV	Human immunodeficiency virus
IPR	Intellectual property rights
KAH	Kenya Association of Herbalists
Kg	Kilogram
LC ₅₀	Median lethal concentration
MeOH	Methanol
mg	Milligrams
ml	Millilitres
°C	Degrees Celsius
PCV	Packed cell volume
PRA	Participatory rural appraisal
Spp	Species
ST & I	Science, technology and innovations
µg	Micrograms
USD	US dollars
TWC	Total worm count
TWCR	Total worm count reduction
TMP	Traditional Medicinal Practitioners
WHO	World Health Hrganization

ABSTRACT

The practice of traditional medicine is as old as the human race and plants are an important source of research and development of new drugs. Anthelmintic resistance in human and animal pathogenic helminths has been spreading in prevalence and severity to a point where there is multi-drug resistance against the three major classes of anthelmintics. It has become a global phenomenon in gastrointestinal nematodes of farm animals, and hence the need for novel anthelmintic products.

The objectives of this study were to document plants which are commonly used in the treatment and control of helminthosis in Loitokitok District of Kenya and to determine the bioactivity, anthelmintic efficacy and preliminary phytochemistry of herbal remedies from selected plants from the study area. An ethnopharmacological study was done through gathering information from 23 traditional health practitioners from across the district. Plants used traditionally as anthelmintics were identified by the traditional healers and samples collected for botanical identification. Sheep belonging to local herders and naturally infected with mixed gastrointestinal nematodes were recruited for the evaluation of anthelmintic efficacy of three herbal remedies *Albizia anthelmintica*, *Embelia schimperi* and *Myrsine africana* remedies were prepared and administered by the methods prescribed by the traditional practitioners. Their efficacy was determined using percentage faecal egg count reduction test (FECRT%). Brine shrimp lethality and the presence of phytochemicals in aqueous and organic extracts were determined. A controlled anthelmintic efficacy study, with sheep artificially infected with mixed gastrointestinal nematodes, was carried out at the Faculty of Veterinary Medicine, University of

Nairobi. *Myrsine africana*, *Rapanea melanophloeos*, *Embelia schimperi*, *Albizia anthelmintica* and a combination of *A. anthelmintica* and *R. melanophloeos* (1:1) were prepared in versions slightly modified from the traditional ones and their efficacy determined using FECRT, percentage total and differential worm count reduction.

Eighty one medicinal plants were collected and identified as belonging to 46 families. The six most important families by their medicinal use values in decreasing order were Rhamnaceae, Myrsinaceae, Oleaceae, Liliaceae, Usenaceae and Rutaceae. Helminthosis in both livestock and humans was recognized as a major disease managed using medicinal in the study area. The most frequently used plant anthelmintics were *Albizia anthelmintica* (Fabaceae), *Myrsine africana* (Myrsinaceae), *Rapanea melanophloeos* (Myrsinaceae), *Embelia schimperi* (Myrsinaceae), *Clausena anisata* (Rutaceae) and *Olea africana* (Oleaceae) used by 70, 70, 17, 17, 13 and 9 percent of the respondents respectively. The efficacy against gastrointestinal (GI) nematodes in naturally infected sheep was 59, -11, -31 and 87 percent for *Myrsine africana*, *A. anthelmintica*, *E. schimperi* and albendazole respectively. Some of the phytochemicals detected in the extracts were, anthraquinones, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids. Organic extracts were generally more bioactive than the aqueous extracts with LC₅₀ of 11 to 581 µg/ml. and 149 to 1000 µg/ml respectively. The FECR values were 83, 34, 8, -55, 26 and 69 percent for *M. africana*, *R. melanophloeos*, *E. schimperi*, *A. anthelmintica*, combination of *A. anthelmintica* and *R. melanophloeos*, and albendazole respectively. The percentage total worm count reduction was 66, 70, 45, 61 and 55 for *M. africana*, *R. melanophloeos*, *E. schimperi*, *A.*

anthelmintica and the combination of *A. anthelmintica* and *R. melanophloeos* respectively, while albendazole had 35% reduction.

It was concluded that some of the plants used as anthelmintic remedies in Loitoktok contain many types of phytochemicals which could be responsible for their observed bioactivities and anthelmintic properties. However, it is recommended that some of the plants be used cautiously because adverse effects were observed in sheep that were treated with high doses of *A. anthelmintica*. The *Myrsine africana* remedy had particularly high efficacy in safe doses against GI nematodes of sheep, and hence merit further study to determine the most optimum dosage, toxicity profiles and determination of the active compound(s) with view of coming up with a novel anthelmintic product. The *H. contortus* used to artificially infect the sheep in this study were resistant to albendazole and that the *M. africana* remedy could be further evaluated against such resistant strains of gastrointestinal parasites.

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

In the tropics and subtropics, diseases caused by helminth parasites in livestock are a major constraint to productivity, especially in small ruminants (Perry *et al.*, 2002). The total animal loss due to helminthosis worldwide was estimated to be equivalent to the value of 30 million sheep and goats (Herlich, 1978). The cost of treatment against helminth parasites in ruminants alone worldwide was estimated at USD 1.7 billion annually (Lanusse and Prichard, 1993). The greatest losses associated with gastrointestinal (GI) nematode infections are sub-clinical, and economic assessments show that financial costs of internal parasitism are enormous (McLeod, 1995). One exception to this is the highly pathogenic nematode parasite of small ruminants, *Haemonchus contortus*, which is also capable of causing acute disease with detectable clinical signs and high mortality in all classes of stock. Haemonchosis has been identified as one of the top ten constraints to sheep and goat rearing in East Africa (Over *et al.*, 1992; Perry *et al.*, 2002; Mugambi *et al.*, 2005).

Consequently, there is an urgent and ever-present need to control infections caused by *H. contortus* and other helminthes in small ruminants in the tropics. Treatment and control is generally achieved by treatment with synthetic anthelmintics in combination with prophylaxis through good husbandry and grazing management. However, misuse, overuse and poor formulations of these products have led to the development of anthelmintic resistance (AR) (Waller, 1997; Lans and Brown, 1998; Monteiro *et al.*, 1998; Gakuya *et al.*, 2007). Resistance in

human and animal pathogenic helminths has been spreading in prevalence and severity to a point where multidrug resistance against all the three major classes of anthelmintics has become a global phenomenon in gastrointestinal nematodes of farm animals (Kaminsky *et al.*, 2008). Furthermore, adulteration of anthelmintics has been found to be a common practice in Kenya (Monteiro *et al.*, 1998). Moreover, these drugs are relatively expensive and often unavailable to resource poor farmers in the rural areas, and especially so in the relatively fragile arid and semi-arid ecosystems. This means that novel approaches that are sustainable are needed for the control of helminthosis in both human and veterinary practice. This may entail an integrated approach, including biological control, targeted treatment, alternation and combination of anthelmintic treatments, copper oxide boluses, parasite vaccines, livestock breeds that are resistant and resilient to parasites, and the use of plants with anti-parasitic properties as well as the use of traditional herbal remedies (Waller, 2003).

The use of plants, or their extracts, for the treatment of human and animal ailments, including helminthosis is steeped in antiquity. The Greek physician Claudius Galenus (AD 130-200) became famous for using plant medicines prepared from vegetable substances by infusion or decoction. These became known generically as "galenical" drugs or preparations, and established the foundation for modern veterinary pharmacology (Paulsen, 2010).

The World Health Organization has recently estimated that 80% of the populations of developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs (WHO, 2008). On a global context, modern pharmacopoeia still contains in the order of 25% of

drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. There has been a resurgence of interest in traditional health practices throughout the world, which mainly encompasses ethnobotany and the use of herbal remedies. The forces responsible for this momentum include the perception that "natural is nice", concerns of synthetic drug residues in the environment and the food chain, and particularly the spectre of rapid emergence of multiple resistant pest organisms through misuse and overuse of conventional drugs. Kenya is endowed with a variety of indigenous medicinal plants which are used by the local herbalists for the treatment of various diseases and among them is helminthosis. However, most of these herbal remedies have not yet been scientifically validated or developed into viable products for the market. There are various methods that could be used for scientific proof and clinical validation such as chemical standardization, biological assays, animal models and clinical trials.

The main drawbacks to using traditional medicines (alternative medicines) in Kenya include the lack of properly formulated products, deficiency in dosage standardization and information on shelf-life. Data on efficacy, safety and other pharmacological and toxicological parameters of the herbal remedies are lacking despite their wide spread use in Kenya and other parts of Africa. Furthermore, there is real danger of erosion and disappearance of traditional medicinal knowledge due to modernity, environmental degradation as a result of increasing population pressure, and lack of clear policies to guide and regulate its utilization.

This study aimed at promoting the use of traditional medicine in the treatment and control of helminthosis by documenting and validating anthelmintic herbal remedies from selected plants commonly used in Loitoktok District, Kajiado County.

1.2 Objectives

The overall objective of this study was to document anthelmintic herbal remedies, which are commonly used in Loitoktok District of Kajiado County in Kenya, and determine their phytochemistry, bioactivity and efficacy against mixed gastrointestinal nematodes of sheep.

Specific objectives are:

1. Document medicinal plants with emphasis on those used in the treatment and control of helminthosis in Loitoktok District.
2. Determine the *in vivo* anthelmintic efficacy of herbal remedies in naturally parasitized sheep under normal grazing conditions in Loitoktok District.
3. Determine the phytochemicals in the extracts of medicinal plants commonly used in the treatment and control of helminthosis in Loitoktok District.
4. Establish brine shrimp lethality and LC₅₀ of extracts from medicinal plants commonly used in the treatment and control of helminthosis in Loitoktok District.
5. Determine the *in vivo* anthelmintic efficacy of medicinal plants, commonly used in the treatment and control of helminthosis in Loitoktok District, in sheep artificially infected with mixed GI nematodes under controlled conditions.
6. Establish the the clinico-pathological.effects in sheep treated with anthelmintic medicinal plants from Loitoktok District

1.3 Hypotheses

1. Medicinal plants form an important component in health care and in the treatment and control of helminthosis, in Loitoktok District
2. Extracts from plants used in remedies for the treatment and control of helminthosis in Loitoktok District contain several phytochemicals that could be responsible for their bioactivity.
3. Medicinal plants used in remedies for the treatment and control of helminthosis in Loitoktok District have sufficient *in vivo* anthelmintic efficacy.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

This literature review gives an account of helminthosis in livestock and the practice of traditional medicine with a bias on the African situation. The pros and cons in the two areas are discussed while highlighting gaps where they exist. The classes of parasites causing helminthosis in livestock, strategies and limitations for their control, including anthelmintic resistance, are described in detail. The review gives a historical background, documentation and trends on the use of traditional medicine with emphasis on medicinal plants. It also attempts to describe the institutional, policy and regulatory frameworks in Africa with emphasis on the Kenyan situation. Methods used in ethnopharmacology and ethnobotany, domestication and sustainability of medicinal plants are discussed. Moreover, it describes the Kenyan biodiversity and its potential for bioprospecting and drug discovery. The literature review further gives an insight on the phytochemicals, bioactivity, toxicity and safety of medicinal plants.

2.2 Helminthosis in Livestock

Helminthoses refers to a complex of conditions caused by parasites of the phyla platyhelminthes (flatworms) and Nematoda (roundworms). The two important classes of flatworms are cestoda (tapeworms) and trematoda (flukes). Some of the superfamilies of veterinary importance in Nematoda are Ancylostomatoidea, Ascaridoidea, Oxyuridoidea, Rhabditoidea, Strongyloidea, and especially Trichostrongyloidea (Anderson, 1992; Tibbo *et al.*, 2011). *Monezia* species is the commonest cestode of ruminants (Soulsby, 1982). The most important trematode of livestock in

Kenya is *Fasciola gigantica*, which is most endemic in marshy areas with poor drainage and high rainfall (Wamae *et al.*, 1990). In sheep and goats in Kenya, GI parasites are prevalent and especially the nematode *H. contortus* and to a lesser extent *Trichostrongylus columbriformis* and *Oesophagostomum* spp, with occasional infections with *strongyloides* and *Trichuris* spp. (Preston and Allonby, 1979; Carles, 1993; Gatongi *et al.* 1997; Maingi *et al.*, 2002).

The economic losses due to GI nematode infection are estimated to be enormous and therefore control of these parasites is considered to be important (Preston and Allonby, 1979; Perry *et al.*, 2002). For instance, the cost of treatment against helminth parasites in ruminants alone world wide was estimated at USD 1.7 billion annually (Lanusse and Prichard, 1993).

2.2.1 Control strategies

Various methods of controlling helminths that are currently in use and some of which will be more useful and relevant in future have been proposed and can generally be divided into 2 major groups, that is, chemical and non-chemical (Gronvold *et al.*, 1993).

2.2.1.1 Chemical Methods

The major control method employed against helminth parasites all over the world is the use of chemotherapy through use of synthetic anthelmintics (Aragaw *et al.*, 2010; Sargison, 2011). The broad spectrum anthelmintics, which remove parasites of different stages and species, are the cornerstone of parasite control in gastrointestinal (GI) nematode infections. The major classes of synthetic anthelmintics used for the control of GI nematodes are: Group 1: Benzimidazoles and probenzimidazoles (BZs) whose mode of action is through polymerization of parasite microtubular proteins (Harder, 2002). Group 2: Tetrahydropyrimidines / Imidazothiazoles

(Levamisole & Morantel – Pyrantel group) whose mode of action is through interference with nicotinic receptors (Roos, 1997; Harder, 2002). Group 3: Macrocyclic lactones (MLs) or the avermectin / Milbemycin group whose mode of action is through interference with the chloride channels on helminth gamma-aminobutyric acid (GABA) receptor complexes and also inhibiting pharyngeal pumping, fecundity and motility resulting in paralysis and elimination from the host (Harder, 2002; Yates *et al.*, 2003). Other smaller groups of anthelmintics are referred to as narrow spectrum, because of limited activity against different stages and species of helminths, and include naphthalaphos, salicylinalides and substituted phenols (closantel, oxyclozanide and nitroxylnil), and triclabendazole. (Bowman *et al.*, 2003). Other forms of chemical control (use of poisons, repellants and pheromones) of worms have been practiced with varying results and levels of efficacy (Sargison, 2011).

2.2.1.2 Non-chemical methods

Apart from the use of drugs, there are husbandry practices and measures that are essential for the control of helminth parasites. Some of these practices include pasture spelling, avoiding early morning grazing, rotational and zero grazing – but these are to be carefully planned and carried out to maximize pasture consumption and productivity, and to encourage immunity of the animal (Bukhari and Sanyal, 2011).

Research towards biological control of helminths has tended to focus on predatory fungi such as *Duddingtonia flagrans*, *Arthobotrys oligosporum* and *Monacrosporium* species (Larsen 1999, 2000; Thomsborg *et al.*, 1999; De and Sanyal, 2009). However, the nematophagous fungi are

only effective against larvae in faecal pats but not those that have migrated or inside the host (Githigia *et al.*, 1997).

There are efforts to produce vaccines against worms in animals and humans. Research in this area is ongoing with some level of success (Harris, 2011). A vaccine for bovine lungworm using irradiated *Dictyocaulus viviparous* has been fairly successful but a similar approach using *H. contortus* was only able to protect lambs from 6 months of age (Gray, 1997; Bain, 1999). Current research on helminth vaccinology has focused on the production of synthetic or recombinant vaccines (Knox and Smith, 2001; Claerebout *et al.*, 2003; Newton and Meeusen, 2003).

Host resistance selection is another possibility in the control of helminthosis because certain breeds and species are more resistant or even more resilient than others. In Kenya, it has been found that the local red Maasai sheep (Rm) and the small East African goat are more resistant than the Dorper and the Galla goat respectively (Mugambi *et al.*, 1997, 2005; Baker *et al.*, 2003). However, it should be noted that the productivity of the resistant breeds may be much lower than that of the susceptible ones. Other non-chemical methods of helminth control include nutritional management, interspecific competition and sterile male technique (Bamaiyi, 2012). Considerable research has also shown that some plants not only affect the nutrition of animals but also have antiparasitic effects (Waghorn and McNabb, 2003).

2.2.1.3 Anthelmintic resistance and other limitations to helminth control

Resistance in human and animal pathogenic helminthes has been spreading in prevalence and severity to a point where multidrug resistance against the 3 major classes of anthelmintics (benzimidazoles, imidazothiazoles and macrocyclic lactones) has become a global phenomenon in GIN of farm animals (Kaminsky *et al.*, 2008). Many parasitic nematodes of medical and veterinary importance have the genetic features that favour the development of anthelmintic resistance (AR) (Papadopoulos, 2008). They possess the genetic potential to respond successfully to chemical attack and the means to assure dissemination of their resistant genes through host movement (Kaplan, 2004). The rate of development of AR depends on several factors. Within the most important of them stand the frequency of treatments and particularly when the same group of anthelmintic is used (Dorny *et al.*, 1994; Waller, 1997). Under dosing is considered another important factor for development of AR, because it allows the survival of heterozygous resistant worms and therefore, contributes to selection of resistant strains (Egerton *et al.*, 1988).

Different bioavailability among animal species for example between sheep and goats, results to an effectively lower dose. Goats require a dose rate 1.5 to 2 times higher than sheep, therefore when goats are treated with the same dose as the sheep, as it is often the case, they are practically under dosed. This may explain the fact that AR occurs more frequently in goats than in sheep (Hennessy, 1994). Goats develop resistant strains which can be passed onto sheep, easily when animals of these two species are kept together (Jackson, 1993). Cases have also been reported where the resistant nematode strains can be introduced into another farm or even another area by

animal transportation. In Kenya, resistance has mainly been reported in institutional farms which also happen to be the source of breeding stock for other smaller farms with potential danger of spreading this problem (Wanyangu *et al.*, 1996; Gakuya *et al.*, 2007).

The test most commonly used to detect AR is the faecal egg count reduction test (FECRT), which is suitable for all anthelmintics, including ones undergoing metabolism within the host (Coles, 2006). It is however, considered reliable only when more than 25% of the worm population is resistant (Martin *et al.*, 1989). Other impediments to the control of helminthosis include illiteracy/ignorance of animal keepers, nomadic practices, cost/poverty, unavailability, and inadequate quality extension services and regulation (Bamaiyi, 2012).

2.3 Traditional Medicine

Traditional medicine otherwise referred to as indigenous or folk medicine comprises knowledge systems that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as: "the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being (WHO, 2008).

When adopted outside of its traditional culture, traditional medicine is often called complementary and alternative medicine. The WHO also notes, though, that "inappropriate use of traditional medicines or practices can have negative or dangerous effects" and that "further

research is needed to ascertain the efficacy and safety" of several of the practices and medicinal plants used by traditional medicine systems (WHO, 2008). Core disciplines which study traditional medicine include herbalism, ethnomedicine/ethnoveterinary, ethnobotany and medical anthropology. Practices known as traditional medicines include Ayurveda, Islamic medicine, traditional Chinese medicine, acupuncture, Muti, traditional African medicine, and many other forms of healing practices.

2.3.1 The value and role of traditional medicine

Traditional medicine is generally available, affordable and commonly used in large parts of Africa, Asia and Latin America. WHO estimates that up to 80% of the population in developing countries still depends on traditional medicine for their primary health care needs (Hostettman *et al.*, 2000). Approximately one-half of all licensed drugs that were registered worldwide in the 25 year period prior to 2007 were natural products or their synthetic derivatives and more than 30% of modern medicines are derived directly or indirectly from medicinal plants. Examples of these medicines are analgesic (aspirin, belladonna); anticancer medicines (vincristine, vinablastine); antihypertensive agents (reserpine); antimalarials (quinine, artemesinine) and decongestants (ephedrine) (Newman and Cragg, 2007). In 2006, the WHO estimated the trade for medicinal plants to be approximately USD 14 billion annually, and the demand was growing at the rate of 15-20% per annum and that the demand would be around USD 5 trillion by the year 2050 (Booker *et al.*, 2012).

2.3.2 Documentation of African traditional medicinal knowledge

Most of the African cultures have a tradition of passing indigenous knowledge from generation to generation orally and therefore, it is not as well documented as compared to most other cultures of the world (Heldberg and Staugard, 1989). The earliest records on the African traditional medicine was that by the famous Arab doctor and philosopher, Avicenna, who lived 980 – 1037 A.D. There are several isolated ethnobotanical records in the herbaria from the colonial era but systematic written accounts on African traditional medicine are a much fairly recent occurrence (Daziell, 1956; Watt and Breyer-Brandwijk, 1962; Sofowora, 1993; Oliver-Brewer, 1986; Kokwaro, 2009; Gachathi, 2007; Dharani, 2010). The colonial era ethnographers viewed the practice of traditional healing just as witchcraft, magic and ritualism (Forster, 1976; Yoder, 1982). The Scientific Technical Commission of the organization of African Union (OAU, now AU) established the first volume of the African pharmacopoeia in 1985 (Hostettmann, *et al.*, 2000).

2.3.3 Institutional and Policy framework on traditional medicine in Africa

Traditional medicine and its practitioners were officially recognized by the Alma-Ata declaration in 1978 as important resources for achieving health for all. Since then the member states and WHO governing bodies have adopted a number of resolutions and declarations on traditional medicine. Notable among these are “Promoting the role of traditional medicine in health systems: A strategy for the African region” in 2000. This strategy provides for the institutionalization of traditional medicine in health care systems of the member states of the WHO African Region. The African Union (AU) summit of Heads of State and Government, in

2001, declared the period 2001 - 2010 as the Decade on African Traditional Medicine and in 2003 adopted an action plan for its implementation. In addition, the Director General of the World Health Organization also declared 31st August every year as the African Traditional Medicine Day. Further, the AU Conference of African ministers of health held in Windhoek from 17 to 21 April 2011 discussed the End-of-Decade Review report and renewed the Decade from 2011 to 2020. All these declarations signify the importance and the approval by Governments and international institutions on the need to institutionalize African traditional medicine in health care (Chatora, 2003).

Some of the relevant guidelines developed for adaption/adoption by the member states include the following: 1) Guidelines for the formulation, implementation, monitoring and evaluation of a National Traditional Medicine Policy 2) Model legal framework for the practice of traditional medicine: The Traditional Health Practitioners Bill; 3) Model Codes of Ethics for Traditional Health Practitioners 4) A Regional framework for the registration of traditional medicines in the WHO African Region; 5) A regulatory framework for the protection of intellectual property rights (IPR) and indigenous knowledge of traditional medicines in the WHO African Region. These guidelines and others provide a basis for the incorporation of African traditional medicine in a manner that is most suitable for individual member states. As more people use this traditional health care facility, there is an urgent need for the appropriate systems of quality control in the practice as well as in the production and use of the medicines. Such systems will protect the public and also ensure that the best practices and the most useful medicines are made available in the most affordable manner (Kofi – Tsekpo, 2004).

2.3.4 Regulation of Traditional Medicine Practice in Kenya

Regulation of Traditional Medicine Practice (TMP) in Africa still remains a big challenge (Mandiba, 2010). Several Asian countries have integrated TM into their national health care systems but this is yet to be done in most African countries and indeed Kenya (Xiaorui, 2000). TM was officially recognized in the 1990's and patent law revised to include it (WHO, 2001). Recently, a task force was formed to draft laws for regulation of TMP with a view of integrating it into the mainstream health care system but there are challenges to be overcome (Mwangi, 2004; NCAPD, 2008). Currently, the Kenyan Pharmacy and Poisons board (PPB) is registering complimentary products but they have to be formulated in a commercial manner (PPB, 2010). However, most of these are currently being imported from Asia and especially China and India.

In Kenya, the registration of TMPs is done by the Ministry of Culture and Social Services, but most of the TMPs are not even aware of it unless they are practicing in urban areas where the local authorities enforce the registration. It follows therefore, that there is a dearth of information as to the actual number of TMPs. In addition there are many fake ones especially in the urban areas and hence the need for state regulation to protect the citizenry (Kigen *et al.*, 2013). On their part progressive TMPs have come together to form an advocacy and self regulatory group known as the Kenya Association of Herbalists (KAH) through which to interact amongst themselves and engage the state authorities.

2.4 Ethnopharmacology and Ethnobotanical Methods

Ethnobotany is the scientific study of indigenous people's plant use and management of plant diversity as well as their perception and classification of nature. It stands at the interface of

several disciplines and relies on knowledge and research methods of botany, ecology and anthropology (Martin, 1995; Likhoba and Siboe, 2008). A distinction can be made between biological and anthropological ethnobotany (Ellen, 1996). The former being the narrowest as research is conducted from a bio-economic perspective while the later is more holistic and operates within a cultural-linguistic paradigm and directly investigates the relations between plants and people in their cultural context. This type of ethnobotany further recognizes the fact that knowledge is, highly variable between individuals, situational and dynamic. Ethnobotanical surveys can also be classified as ‘rapid’ or ‘in-depth’ (Martin, 1995). Rapid surveys are usually based on the methodology of participatory rapid appraisal (PRA) and the information gathered is usually qualitative rather than quantitative. A rapid survey should be the starting point of any in-depth study.

Ethnopharmacology deals with investigations on indigenous people and their drug use. Methodologies applied in ethnobotany/ethnopharmacology have advanced greatly over the past decades, with various analytical tools and statistical analyses augmenting traditional qualitative approaches, leading to more sophisticated ways of collecting data and more profound interpretations of research results (Martin, 1995).

2.4.1. Selection of informants

Two methods of selecting information are commonly used: random and targeted selections. Random selection is appropriate to obtain information on the distribution of knowledge in communities. Random selection of informants can be done by random sampling or by stratified random sampling. The later technique can be especially useful in obtaining information from

marginal groups and areas of the survey. Using such systematic sampling techniques reduces bias in the sampling process. Targeted selection is better at obtaining specialist information, as only local specialists will be sought. Key informants, local experts or “gatekeepers” have to be selected based on their expertise and they are usually identified by other local people during the PRA. Elderly people can be specifically targeted for historical information by in-depth collection of life histories (Martin, 1995).

2.4.2 Herbarium specimen collection

Plant collections are basic to ethnobotanical surveys. They allow for determination of the botanical identity of the plants studied, so that information collected can be referenced to botanical names. They also allow collecting use information from local informants if living plants are not available at the interview site. Voucher specimen collection for the herbarium involves harvesting and preserving plant parts that allow their scientific determination, mounting them on herbarium sheets and providing an accompanying label. The basic tools for collections are a field press and a field notebook. A good voucher specimen should have all the representative parts needed for accurate identification, which are branches, leaves, flowers and / or fruits (Earle Smith, 1971).

2.4.3 Bioprospecting

Bioprospecting involves intensive exploration of organisms for naturally occurring substances that could improve human life. Studies have shown that ethnobotanical surveys involving local specialists are more successful and cost effective in identifying plants with biological activity

than random collections, taxonomic and chemical relationships between plants (Farnsworth, *et al.*, 1985). In trials involving a traditional healer versus random collection conducted in Belize, ethnobotanical surveys had 25% success rate compared to 6% of random collections in selecting plants with activity against HIV (ethnobotanical surveys in the forests of Belize). The intermediate strategy used is to target families known to have high contents of biologically active compounds. While bioprospecting, researchers should always beware of the intellectual property rights (IPRs) of the communities from which knowledge has been obtained and ensure that they benefit in a fair manner from any economic returns accruing from the findings, if not legally required then from an ethical perspective.

2.4.4 Domestication and sustainability of medicinal plants

At about 1% annually, Africa has one of the highest deforestation rates in the world and there is real danger of desertification, loss of traditional community lives and cultural diversity, and the accompanying knowledge on medicinal plant species (Iwu, 1995; Rukangira, 2001a). Alam and Belt (2009) looked at the ecological threats to resources with respect to medicinal plant species and their depletion at a rapid pace due to over collection from their natural habitats. The collection and marketing of medicinal plants from the wild form an important source of livelihood for many of the poor in developing countries. A key outcome of their research has been the call for tightening restrictions on collection practices and secondly through advocating cultivation on a large scale. However, the same study (Alam and Belt, 2009) conclude that the cultivation of medicinal plants is more difficult than usually envisaged and recommends a thorough technical and economic feasibility study of the value chain, long-term involvement and

an understanding of the prevalent farming systems to ensure success. Other proposed solutions to sustainability include growing medicinal plants as crops in their natural habitats (Iwu, 1995; Van Staden, 1999). However, there are those who claim and believe that plant species grown under agricultural conditions will not have the same medicinal properties as their counterparts growing in the wild (Cunningham, 1993).

The information that is gathered through in-depth ethnobotanical surveys will help to decide on the most promising species (species priority setting) and on the objectives of domestication of the species, but more importantly involve the local communities at every stage of the process for ownership and sustainability (Guarino, 1995; Rukangira, 2001a).

2.5 Kenyan Biodiversity and Trends

Kenya has a large diversity of ecological zones and habitats including lowland and mountain forests, wooded and open grasslands, semi-arid scrubland, dry woodlands, and inland aquatic, coastal and marine ecosystems. In addition, a total of 467 lake and wetland habitats are estimated to cover 2.5% of the country. Forests are the backbone of Kenya's economy through agriculture and tourism. They also support livelihoods through provision of food, medicine, wood for construction and fuel, and serve as water catchment areas. Despite their importance, indigenous forests have been rapidly declining from 1,687,390 ha in 1994 to 1.2 million ha, and plantation forests have declined from 165,000 ha in 1988 to the current 120,000 ha. Most of this loss has occurred through forest excisions and encroachment for agriculture and settlements (FSK, 2008; KFWG, 1999). Other losses have occurred through forest fires and overexploitation of preferred forest species leading to the possibility of species extinction (Rukangira, 2001b; Shanley, 2003).

Moreover, the general populace is unable to make informed decisions on biodiversity management as they lack adequate information on the non-consumptive use values of the resources such as ecosystem functions, maintaining water cycles, climate regulation, and photosynthetic fixation of solar energy, production and protection of soil, storage and cycling of essential nutrients, absorption and breakdown of pollutants.

Globally, the value of biodiversity as a key component of the environment was recognized during the Rio Earth summit in Brazil in 1992. The summit came up with integrated strategies to halt and reverse the negative impact of human behaviour on the physical environment and promote environmentally sustainable economic development all over the world (<http://www.cbd.int/history/>)

2.6 Phytochemicals in medicinal plants

Plants have evolved secondary biochemical pathways that allow them to synthesize a raft of bioactive compounds, often in response to specific environmental stimuli, such as herbivore-induced damage, pathogen attacks, or nutrient deprivation (Reymond *et al.*, 2000; Hermsmeier *et al.*, 2001; Chitwood, 2002). Bioactive compounds in plants are defined as secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals (Bernhoft, 2010). Most plants, even common food and feed plants are capable of producing bioactive compounds. However, the typical medicinal or poisonous plants contain higher concentrations of more potent bioactive compounds than food and feed plants. Phytochemicals can be unique to specific species or genera and play protective roles (such as antioxidant, free radical-scavenging,

and UV light-absorbing and anti-proliferative agents) and defend the plant against parasites and microorganisms such as bacteria, fungi, and viruses. In this regard, phytochemicals often act as agonists or antagonists of neurotransmitter systems (Wink, 2000, 2003) or form structural analogs of endogenous hormones (Miller and Heyland, 2010). Well over 80,000 natural compounds have been described from plants and over 20,000 from microorganisms and fungi. The phytochemical investigation involves separation and isolation of the constituents of interest, characterization of the isolated constituents and quantitative evaluation (Evans, 2002). Some of the elucidation methods utilized include mass spectrometry, nuclear magnetic resonance and X-ray diffraction (Harbone, 1993; Wink, 1993a, b). The identified compounds can then be used as “markers” for the quality control of herbal preparations or as leads for drug discovery (Harvey, 2008).

The phytochemicals can be subdivided into a number of distinct groups on the basis of their chemical structure and synthetic pathways, and these groups can, in turn, be broadly differentiated in terms of the nature of their ecological roles and therefore their ultimate effects and comparative bioactivity in animals. The largest and most prevalent of phytochemical groups are the alkaloids, terpenes, and phenolic compounds (Handa *et al.*, 2008).

2.6.1 Phenols and Polyphenols

Phenols, sometimes referred to as phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) attached to an aromatic hydrocarbon group. They have relatively higher acidities than aliphatic alcohols and some are germicidal while others like estradiol are estrogenic. The simplest of the class is phenol (C₆H₅OH). Polyphenols have more than one

phenol groups per molecule and are therefore classified on this basis. The subdivision of polyphenols into tannins, lignins and flavonoids is based on the variety of simple polyphenolic units derived from plant metabolism of the shikimate pathway (Dewick, 1995). Polyphenols are further subdivided into hydrolysable tannins, which are gallic and ellagic acid esters of sugars; and phenylpropanoids such as lignins, flavonoids, and condensed tannins (Reed, 1995). Of these, the flavonoids represent the largest, most diverse group, encompassing over 6000 compounds. Flavonoids can then be subdivided according to modifications of the basic skeleton into chalcones, flavones, flavonols, flavanones, isoflavones, flavan-3-ols, and anthocyanins (Handa *et al.*, 2008; Bowsher and Tobin, 2008). Tannins are astringent, non crystalline and form colloidal and acidic solution with water. Condensed tannins are the most abundant polyphenols found in virtually all plant families comprising up to 50% of the dry weight of leaves.

Tannins have been shown to precipitate gelatin and proteins which inhibits, in some ruminants, the absorption of nutrients from high tannin grains like sorghum. There is a correlation between esophageal and nasal cancer in humans and regular consumption of certain herbs with high tannin concentration (Lewis and Elvin-Lewis, 1977). Condensed tannins have been shown to reduce gastrointestinal parasite loads in goats by reducing worm fertility, eliminating adult worms, and retarding the establishment of incoming larvae. It is important to note that the results vary depending on the plant species. For example, condensed tannins from some plants appear to only be effective against parasites that affect the small intestine and not those that dwell in the abomasum like *Hemonchus contortus*. Research continues on these plants in order to find out the

factors that affect the effectiveness of the compounds in addition to their effect on host nutrition (Waller and Thamsborg, 2004; Waller, 2006; Knox *et al.*, 2006).

2.6.2 Alkaloids

Alkaloids are a structurally diverse group of over 12,000 cyclic nitrogen-containing compounds that are found in over 20% of plant species (Zulak *et al.*, 2006). Alkaloids are complex nitrogen-containing compounds generally produced from plants. Alkaloids are basic in nature and mostly colourless solid crystalline substances with a bitter taste. They are soluble in organic solvents but insoluble in water whereas their salts are soluble in water and insoluble in organic solvents (Singh and Bhandari, 2000). Alkaloids are distributed in the various tissues depending on the plant species. They occur in plants as salts and are therefore extracted with water or mild acid and then recovered as crystalline material with a base. Alkaloids were earlier on classified according to known products or plants and animals of their origin. However, currently they are being classified according to the biologically important amine that stands out in their synthetic process and therefore, resulting in the following groups: pyridine, pyrrolidine, tropane, quinoline, isoquinoline, phenethylamine, indole, purine, pyrazole and terpenoid (Carey, 1987). Most are heterocyclic with nitrogen atom(s) located in the heterocyclic ring and could be mono or poly depending on the number of rings. Most alkaloids are physiologically active or toxic to animals and man, for instance pyrrolizidine alkaloids have been known to be toxic to animals for a very long time (Bull *et al.*, 1968). Quinine, isolated from the cinchona tree, is an alkaloid that has been used to treat malaria in Europe since 1633 and even earlier on in South America.

2.6.3 Terpenoids

Terpenoids also called isoprenoids are polymers derived from two or more isoprene (2 methyl-1, 3-butadiene or C_5H_8) molecules. There are over 10,000 types (over 30,000 compounds) of terpenoids and they are the largest class of naturally occurring organic compounds (Fahy *et al.*, 2005). They are classified on the basis of the number of the isoprene molecules thus, hemiterpenes (2), monoterpenes (3) or diterpenes (4) and so on. They are further sub classified according to the number of rings present in the molecule. Monoterpenoids are volatile essential oils for example camphor, menthol and pinene. Diterpenoids are widely distributed in latex and resins and can be quite toxic. Steroids and sterols are produced from terpenoid precursors including vitamin D, glycosides and saponins (which lyse red blood cells). Artemesinine or qinghaosu the recent antimalarial drug is a sesquiterpene isolated from the Chinese plant *Artemesia annua*. Their extraction and isolation depends on the chemical groups present such as alkaloid or saponin.

2.6.4 Glycosides

Glycosides are compounds that contain a sugar (glycone) and a non sugar (aglycone) molecule. Most glycosides are crystalline, colourless compounds and optically levorotatory. They are soluble in water and alcohol but insoluble in other organic solvents like ether and chloroform. Glycosides are classified on the basis of linkage between the glycone and aglycone moieties like O, C, N or S and also on the basis of the chemical nature of the aglycone molecule such as alcohol, aldehyde or steroid. Glycosides are generally extracted with a mixture of water and methanol or ethanol. Enzyme inactivation by boiling or acidification is essential before or during

extraction from fresh plant material. Volatile oils are extracted by use of organic solvents like ether or benzene at 50°C or by distillation (Singh and Bhandari, 2000).

2.6.5 Lectins and polypeptides

Lectins are sugar binding proteins (glycoproteins) that are highly specific for their sugar moieties. They have molecular weights of 60,000-100,000 (Etzler, 1983) and are known for their ability to agglutinate certain animal cells especially erythrocytes and/or precipitate glycoconjugates. Lectins occur ubiquitously in nature and are found in most plants especially in seeds and tubers but also other tissues. For example, ricin from castor beans is famous for its toxicity and is one of the earliest lectins to be isolated. Most lectins are toxic, resistant to cooking and digestive enzymes (Van Damme *et al.*, 1998). Lectins are classified according to the source, such as plant, animal or microbial. Animal lectins are further sub classified based on their amino acid sequence homology and evolutionary relatedness, while those of plants are grouped according to the plant family. Microbial lectins tend to be classified according to function like adhesions, toxins or hemagglutinins (Etzler, 1983). Because of their precise carbohydrate specificities, lectins can be blocked by simple sugars and oligosaccharides. Wheat lectin for example, is blocked by the sugar N-acetyl glucosamine and its polymers (Goldstein and Poretz, 1986). These natural compounds are therefore, potentially exploited as drugs for lectin induced diseases.

2.6.6 Essential/volatile oils

Essential or volatile oils are a mixture of hydrocarbon terpenes, sesquiterpenes and polyterpenes and their oxygenated derivatives obtained from the various parts of plants. At ordinary

temperatures they evaporate and therefore are called volatile or ethereal oils. They contain odorous or flavouring compounds hence known as essential oils because they produce essence. Fresh volatile oils are colourless but oxidize and resinify on exposure to the atmosphere for long. In conifers they occur in all tissues but in others only in particular tissues such as eucalyptus leaves, cinnamon bark and rose petals. The hemolytic factor in onions (*Allium cepa*) for instance is the pungent essential oil, N-propyl disulphide (Hutchinson, 1977; Lincoln *et al.*, 1992). The monoterpenes are the major constituents of volatile oils and they may be acyclic, monocyclic or bicyclic, either as hydrocarbons (terpene) or their oxygenated derivatives (terpenoids).

2.7 Plants with anthelmintic activity

There are many plants reported to have anthelmintic activity both in animals and humans around the world (Akhtar *et al.*, 2000; Waller *et al.*, 2001; Athanasiadou *et al.*, 2000; Gathuma *et al.*, 2004; Fajmi and Taiwo, 2005; Githiori *et al.*, 2006; Hussain, 2008; Iqbal and Jabbar, 2010). Some anthelmintic plants and remedies in the British Veterinary codex (1965) include the oil of chenopodium, derived from *Chenopodium ambrosioides*, used against nematodes of animals and humans (de Bairacli Levy, 1991). The active ingredient is believed to be a monoterpene, Ascaridole (Ketzis *et al.*, 2002). The other plant is the male fern *Dryopteris filix-mas* used against cestodes and nematodes of ruminants. Also in the codex were plants of the genus *Artemesia* used against ascarids of swine and cestodes of poultry. In Nigeria, Nwude and Ibrahim (1980) reported 18 plants used for their anthelmintic activity while Kasonia *et al* (1991) reported 11 for the same purpose in Zaire. A review by Tagboto and Townson (2001) listed 39 plants against cestodes, 16 against trematodes and 45 against nematodes in humans worldwide.

ITDG and IIRR (1996) published an ethnoveterinary manual in Kenya listing 18 plants used traditionally by various communities as anthelmintics in livestock. Kokwaro (1993) listed 79 plants used as general anthelmintics across East Africa.

Although the majority of the evidence on the antiparasitic activity of these plants is based on anecdotal observations, there is growing number of controlled studies that aim to scientifically verify, validate and even quantify such bioactivity. About 150 plants used as anthelmintics in ethnoveterinary around the world have been validated using standard parasitological techniques in animals (Iqbal and Jabbar, 2010). Here in Kenya, some plants used in traditional anthelmintic remedies have been evaluated as follows: *Chrysanthemum cinerariifolium* (Mbaria, 1999), *Albizia anthelmintica* (Gakuya, 2001; Gathuma *et al.*, 2004; Githiori, 2004), *Maerua edulis* and *subcordata* (Gakuya, 2001), *Myrsine africana* (Gathuma *et al.*, 2004; Githiori, 2004), *Hildebrandtia sepalosa* (Gathuma *et al.*, 2004; Githiori, 2004), *Embelia schimperi* (Bøgh *et al.*, 1996), *Jasminum abyssinicum* (Komen *et al.*, 2005), *Tephrosia vogelli* and *Vernonia amygdalina* (Siamba *et al.*, 2007). However, some of these studies have been inconsistent with the reports of traditional practitioners and even among different researchers. Such differences might be as a result of the variations in the locality, season, harvesting and storage of the plant materials evaluated. In some cases it might be due to experimental models used and other methodological limitations (Ignacio *et al.*, 2001). Two main approaches have been used in efficacy studies against helminths. The first one is through feeding plants or their parts to naturally or experimentally infected animals. The second one is by testing extracts and concoctions from medicinal plants via *in vivo* and *in vitro* systems.

Some *in vitro* evaluations have utilized the nematode parasite *H. contortus*, or in the other instances *in vivo* monoculture or mixed GI nematode infections (Table 2.1). Several studies have also used the non-parasitic nematodes such as *Caenorhabditis elegans* and *Rhabditis pseudoelongata* (McGaw *et al.*, 2004; Okpekon *et al.*, 2004).

Table 2.1: *In vivo* evaluation of some plant preparations against Gastro intestinal nematodes of ruminants

Plant species	Parts used ^a	Active principles ^b	Host ^c Infection	References
<i>Albizia anthelmintica</i>	S, RB	sesquiterpenes, kasotoxin	Sh, Sm	Grade & Langok (2000), Gakuya (2001), Gathuma <i>et al</i> (2004), Githiori (2006)
<i>Ananas comosus</i>	L	bromelain	Sh, Sm, Bm	Baldo (2001), Hordegen <i>et al</i> (2003), Githiori (2006)
<i>Azadrachta indica</i>	S, L	azadrachtin	Sh, Sm, Bm	Chandrawathani <i>et al</i> (2002), Hordegen <i>et al</i> (2003), Githiori (2006)
<i>Caesalpinia crista</i>	S		Sm	Hordegen <i>et al</i> (2003)
<i>Chenopodium ambrosioides</i>	L, S,O	ascaridole	Sm	Ketzis <i>et al</i> (2002)
<i>Chrysanthemum cinerariaefolium</i>	Fl	pyrethrins	Sm	Mbaria <i>et al</i> (1998)
<i>Embelia ribes</i>	Fr		Sm	Hordegen <i>et al</i> (2003)
<i>Hageni abyssinica</i>	Fr	kosotoxin	Gm	Abebe <i>et al</i> (2000), Githiori (2006)
<i>Hildebrandtia sepalosa</i>	RB		Sm	Gathuma <i>et al</i> (2004), Githiori (2006)
<i>Jasminum abyssinicum</i>	L		Sm	Komen <i>et al</i> (2005)
<i>Maerua edulis</i>	T		Sm	Gakuya (2001)
<i>Myrsine africana</i>	Fr	benzoquinones		Gathuma <i>et al</i> (2004), Githiori (2006)
<i>Nauclea latifolia</i>	B	resin, tannins, alkaloids	Sm	Onyeyili <i>et al</i> (2001)
<i>Rapanea melanophloeos</i>	Fr	benzoquinones		Githiori (2006)
<i>Terminalia glauscens</i>	B	anthraquinone	Bm	Nfi <i>et al</i> (1999)
<i>Vernonia anthelmintica</i>	S, L		Sm, Bm	Nfi <i>et al</i> (1999), Hordegen <i>et al</i> (2003)

Key: ^aParts used: B = bark, Fl = flowers, Fr = fruits, L = leaves, O = oil, R = roots, RB = root bark, S = seeds, T = tuber; ^bwhere specified.

^cHost infection: Bm = bovinds mixed infection, Gm = goats mixed infection, Sm = sheep mixed infection; Sh = sheep infected with *Hemonchus contortus* only.

Others used the rodent nematode *Heligmosomoides polygyrus*, trematode *Schistosoma mansoni* and the cestode *Hymenolepis diminuta* (Molgaard *et al.*, 2001). However, it may be unrealistic to directly extrapolate *in vitro* findings into the live animal due to several factors, the major among which is the bioavailability of the active ingredients at the target point. Furthermore, the use of free-living non-parasitic nematodes (such as *C. elegans*) as models for parasitic ones may not be very appropriate (Geary and Thompson, 2001).

2.8 Bioassay and isolation of active compounds from medicinal plants

2.8.1 Sample preparation and extraction of active compounds

Sample preparation and extraction is the first crucial step in the preparation of quality medicinal plant formulations. A typical extraction process may contain the following: collection and authentication of the plant material and drying, size reduction, extraction, filtration, concentration, and drying and reconstitution (Handa *et al.*, 2008). The main object of research in this area has been to standardize and optimize the extraction procedures in order to improve efficiency and yields of bioactive constituents by varying solvents and applications (Kothari, 2010). During the extraction process solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Ncube *et al.*, 2008).

The purpose of standardizing extraction procedures for crude drugs is to attain therapeutically desired portions and to eliminate the unwanted materials by treatment with a selective solvent (menstrum). The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, soxhlet extraction, aqueous-alcoholic extraction, counter current extraction, microwave-assisted extraction, ultrasound extraction (sonication),

supercritical fluid extraction and phytonic extraction (with hydrofluorocarbon solvents). The composition and quality of the extracts will depend on the plant material used, solvent and extraction procedure (Ncube *et al.*, 2008).

2.8.2 Bioassay of medicinal plant extracts

Bioassays offer special advantage in the standardization and quality of heterogeneous herbal products. Physical analytical methods, such as chromatography, are by themselves not be very useful for this purpose as they are usually insensitive to the chemical complexities found in crude botanical extracts (McLaughlin *et al.*, 1998). Most often a desired biological response is due to not one but a mixture of bioactive plant components and the relative proportion of single components can vary from batch to batch while the bioactivity still remains within tolerable levels. Thus, physical or chemical analysis of a single component in such a mixture may not be adequate. Unfortunately, many phytochemists have simply been engaged in isolating, characterizing and publishing lots of novel chemicals of plant origin without regard to bioactivities. For practical application in health care, today's work in medicinal plant chemistry should include bioassays (McLaughlin *et al.*, 1998). The extracts are screened for biological activity, the "active" ones selected, fractionations directed with bioassays and bioactive compounds identified using a combination of other readily available technologies like separation techniques (chromatography) and structural elucidation methods (spectrometry and x-ray crystallography). When the chemical structure is known, total or partial synthesis and preparation of derivatives/analogues is then possible, and modulation of the biological activity and definition of the structure-activity relationship could then be investigated (Verpoorte, 1989).

Bioassays can be performed *in vitro* using isolated organs or whole live organisms (bacteria, protozoa, fungi, helminths, mollusks, and insects), *in vivo* (mammals, birds, amphibians), and using cell cultures (plant and animal) and cellular systems (enzymes, receptors etc.) (Hamburger and Hostettmann, 1991; Souza Brito, 1996).

2.8.3 Brine Shrimp Lethality Test

The brine shrimp lethality test (BST) is a rapid general bioassay described by Meyer *et al* (1982) and refined by McLaughlin *et al.* (1991). This *in vivo* lethality test in a simple zoologic organism can be used as a convenient tool for screening and fractionation in the discovery and monitoring of natural products (McLaughlin *et al.*, 1998). The eggs of brine shrimp, *Artemia salina* (Leach), are readily available in pet shops at low cost and remain viable for years in a dry state. Upon being placed in sea water, the eggs hatch within 48 hours to provide large numbers of larvae (nauplii) for experimental use.

Brine shrimp larvae have been used in bioassay previously but the authors have developed a method whereby natural product extracts, fractions or pure compounds are tested at initial concentrations of 10, 100 1000 ppm (or $\mu\text{g/ml}$) in vials containing 10 shrimp in each of three replicates (Meyer *et al.*, 1982; McLaughlin *et al.*, 1991). Survivors are counted after 24 hours. The data collected are processed in a simple computer program for probit analysis to estimate the LC_{50} values with 95% confidence interval for significant comparison of potencies (Finney, 1971).

2.9 Toxicology and safety of medicinal plants

Bioactive compounds are almost always toxic in high doses. The basic premise here is that, pharmacology is toxicology at a higher dose and toxicology is pharmacology at a lower dose. However, plants used in traditional medicine are assumed to be safe based on their long usage according to knowledge accumulated over centuries (Fennell *et al.*, 2004). Scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic (Schimmer *et al.*, 1994; Kassie *et al.*, 1996; De Sa Ferrira and Ferrao, 1999). Poisoning from traditional medicine is usually a consequence of misidentification, incorrect preparation or inappropriate administration and dosage (Stewart and Steenkamp, 2000), and frequently due to self-administration (Popat *et al.*, 2001). Furthermore, some interactions between herbal and conventional drugs when taken together could alter their pharmacokinetics and result in short or long term undesirable outcomes (Chen *et al.*, 2011; Hu *et al.*, 2005; Izzo *et al.*; 2009; Tsai *et al.* 2012).

Highly trained traditional medicine practitioners possess considerable knowledge of medicinal plants and how to avoid acute poisoning (Savage and Hutchings, 1987). However, a growing number of healers do not possess formal training or sufficient knowledge, skill and experience to practice successfully (Bodenstein, 1973). In addition, many potentially toxic plants remedies are available over the counter from herbalist retailers and medicinal plant traders without regulation (Bodenstein, 1973; Cunningham, 1988; Popat *et al.*, 2001). Substitution of plants from the same family is common and especially so with bark products (Grace *et al.*, 2002). Purposeful

adulteration by unscrupulous healers and traders of traditional medicine is also not uncommon (Cunningham, 1988; Manana and Eloff, 2001; Monteiro, 2008).

Acute toxicity test is the single most important test carried out on chemicals of biological interest (Loomis, 1978). The test involves the single dose administration of the chemical in question with a purpose of determining the consequent symptomatology and lethality. Median lethal dose (LD_{50}) is the least dosage of any substance that is expected to kill half of the exposed population. There are guidelines for conducting such tests in laboratory animals.

Subacute toxicity and chronic studies are tests that are carried out for up to three months and beyond respectively. In addition to the observation of clinical symptoms, organ function tests are done to assess possible damage and finally postmortem lesions are observed in case of death or euthanasia. A routine procedure in live animals is the clinico-pathological assessment and testing. Hematology, liver and kidney function tests are the most frequently performed, and are usually closely correlated with both the clinical and histo-pathological findings (Kaneko *et al.*, 1997; Cornelius, 1987).

CHAPTER THREE

ETHNOPHARMACOLOGICAL STUDY OF ANTHELMINTIC AND OTHER MEDICINAL PLANTS TRADITIONALLY USED IN LOITOKTOK DISTRICT

3.1 Introduction

Ethnobotanical studies are often significant in revealing locally important plant species especially for the discovery of crude drugs. Right from its beginning, the documentation of traditional knowledge, especially on the medicinal uses of plants, has provided many important modern drugs (Cox 2000; Flaster 1996). The modern pharmacopoeia still contains in the order of 25% of the drugs derived from plants and many others are synthetic analogues built on prototype compounds isolated from plants. Traditional medicine still remains the main resource for a large majority (80%) of the people in developing countries for their primary health care needs (Danøe and Bøgh, 1999; WHO, 2002). There has been a resurgence of interest in traditional health practices throughout the world, which mainly encompasses ethnobotany and the use of herbal remedies. The forces responsible for this momentum include the perception that "natural is nice", concerns of synthetic drug residues in the environment and the food chain, and particularly the spectre of rapid emergence of multiple resistant pest organisms through misuse and overuse of these modern drugs. A case in point is the effectiveness of artemisinin from the Chinese herb, *Artemisia annua*, against multi-drug resistant malaria (WHO, 2002).

More than 50,000 flowering plants are used for medicinal purposes across the world (Govaerts, 2001; Schippmann *et al.*, 2002). In Kenya, more than 1200 plants are described as medicinal from a flora of approximately 10,000 members (Kokwaro, 1993). The wide spread use of

traditional medicine among both urban and rural population in Kenya could be attributed to cultural acceptability, efficacy against certain types of diseases, physical accessibility and affordability as compared to modern medicine. Kenyan traditional medical system is characterized by variation and is shaped by the ecological diversities of the country, socio-cultural background of the different ethnic groups as well as historical developments, which are related to migration, introduction of foreign culture and religion. In Kenya, the knowledge from herbalists is often passed secretly from one generation to the next verbally.

The study of African medicinal plants has not been realized as fully as that of India, China or other traditional communities elsewhere (Iwu, 1993). In Kenya, though there has been some organized ethnomedicinal studies, there has been limited development of therapeutic products and the indigenous knowledge on usage of medicinal plants as folk remedies are getting lost owing to migration from rural to urban areas, industrialization, rapid loss of natural habitats and changes in life style (Njoroge and Bussman, 2006). In addition, there is a lack of ethnobotanical survey carried out in most parts of the country. In view of these, documentation of the traditional uses of medicinal plants is an urgent and important matter in order to preserve the knowledge (Fratkin, 1996). Thus, the purpose of this study was to investigate and document the traditional uses of medicinal plants by the people of Loitoktok District and to provide baseline data in an ongoing study whose aim is to formulate a plant based anthelmintic using both indigenous technical knowledge and scientific pharmacognosy technique.

3.2 Materials and methods

3.2.1 Choice of Study area

A reconnaissance survey was undertaken to Kajiado district headquarters, in May 2009, to identify key informants in the study. The district cultural officer, under whose jurisdiction the registration of herbalists fall and the local administrators were chosen as the key sources of information about the herbal practitioners. It is from discussions with these key informants that Loitoktok District district, formerly part of Kajiado district, was chosen as the most suitable area of the study due to its widespread use of traditional medicine and relatively less modernity.

3.2.2 Study area description

Loitoktok District comprises an area of 6,300 km² and is home to the Ilkisonko subgroup of the Maasai people. However, several non-Maasai groups, of which the Kikuyu and Kamba are the most numerous, now live in Loitoktok District. Figure 3.1 shows Loitoktok District in relation to the map of Kenya. It is located in the southwestern part of the Rift valley province of Kenya and borders Kajiado central district to the north, Namanga district to the northwest, Tanzania to the southwest, Taita - Taveta and Makueni districts to the southeast and northeast respectively. Its highest points are the slopes of, the snow-capped, Mount Kilimanjaro and the Chyulu hills, while its lowest point is the Amboseli basin.

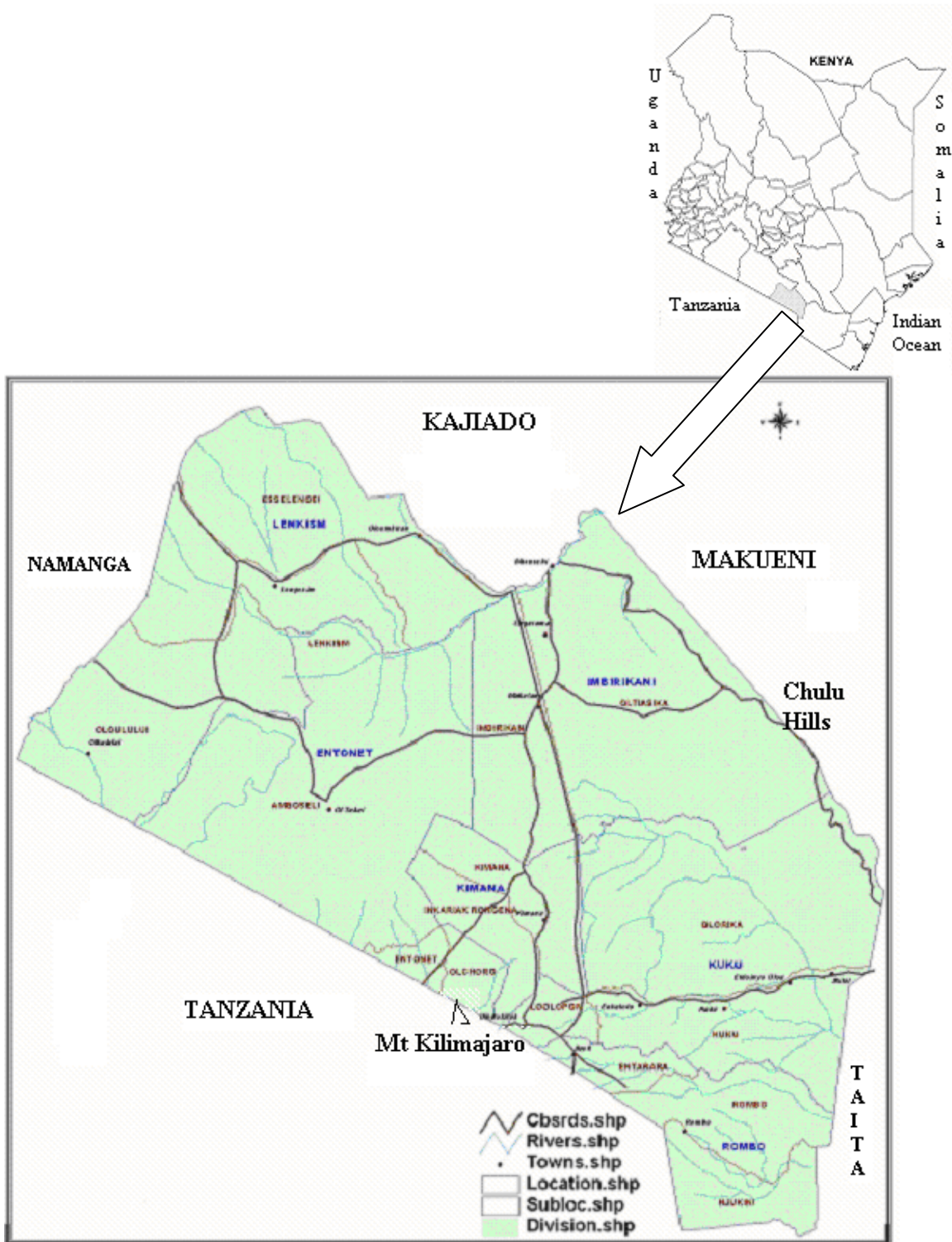


Figure 3.1: Map of Kenya showing the location of Loitokitok District

Loitoktok District has a bimodal rainfall pattern with the long rains falling between March and May and the short rains between October and December. High rainfall occurs around the slopes of Mt. Kilimanjaro and the Chyulu hills. Other areas, especially the rangelands are characterized by lower rainfall. The October-December rainfall accounts for 45% and the March-May for 30% of the total rainfall. The temperatures in Loitoktok District, like rainfall, also vary with altitude and season. The hottest temperatures of 30°C have been recorded around Lake Amboseli and the lowest mean minimums of 10°C are experienced on the eastern slopes of Mt. Kilimanjaro. The coolest period is June-August and the hottest is September-February. The vegetation of the Amboseli plains is dominated by bushland and open grasslands (*Acacia* – commiphora mosaic). Swamps are found at the base of Mt. Kilimanjaro. The vegetation composition has changed significantly over the last decade (Ntiati, 2002). Most of the woodland has been converted into marginal crop farming areas, swamps into irrigated land and grassland to bush land due to overgrazing and overstocking.

3.2.3 Data Collection

Data on medicinal plants traditionally used to treat worm infestation and other ailments was collected through interviews, stakeholder meetings; transect walks, focus group discussion (Figure 3.2) and administration of semi-structured questionnaires to herbalists. The approach was for the herbalists to mention the plants used in anthelmintic herbal remedies and then those used in remedies for other diseases and conditions. The information sought included the herbalists' biodata, diseases treated with herbal remedies, harvesting of medicinal plants and parts used in herbal remedies, methods of their preparation and administration.



Figure 3.2. Focus group discussion during the ethnopharmacological study in Loitoktok District.

Thirty herbalists from across the locations in Loitoktok District were recruited and 80% of them cooperated and fully participated in the study.

3.2.4 Collection of plant samples and identification

Plants reportedly used in herbal remedies were collected for identification and voucher specimens deposited at the University of Nairobi Herbarium. The information gathered included the vernacular name of the plant, species, habitat, parts used, ailments they cure and methods of preparation; dosage and routes of administration.

3.2.5 Data analysis and reporting

The data collected was analyzed and reported using proportions and percentages. The relative importance of individual plant species, for medicinal use by the community, was assessed by calculating their use values (UV_s) by a slight modification of the method described by Philips and Gentry (1993):

Use value of a species (UV_s) = $\sum_i UV_{is} / n_s$, where UV_{is} is the use value of one plant species to one informant and n_s is the number of informants interviewed for the species (in our case the number of informants citing use of the species). Our assumption was that every informant had equal chances of mentioning any of the species used in medicinal purposes in the area because of the way we framed our questions.

$UV_{is} = \sum U_{is} / n_{is}$, where U_{is} is the number of uses mentioned by an informant for a particular plant species and n_{is} is the number of interviews by the informant (in our case only one interview for each informant).

The value of a botanical family (FUV) = UVs / n_f, where n_f is the number of species reported in the family. Calculation of the consensus factor (F_{IC}) for the use of herbal remedies in the treatment of helminthosis was done by the method provided by Trotter and Logan (1986), where $F_{IC} = N_{ur} - N_t / (N_{ur} - 1)$ and N_{ur} is the number of use-reports by informants for a particular illness usage, where a use-report is a single record for use of a plant mentioned by an individual, and N_t refers to the number of species used for a particular illness category for all informants.

3.3 Results and Discussion

The traditional healers had registered an association with the Ministry of State on National Heritage and Culture for regulatory and advocacy purposes. Out of the 23 participating herbalists, 21 were men (87%) and three (13%) were women including one traditional midwife. Majority of the herbalists (62.5%) treated only human ailments while 37.5% attended both to human and livestock. The ages of the herbalists ranged from 29 to 81 years with a median of 53 years. This is probably an indication of how long it takes for the knowledge to be acquired or that the practice is not readily being passed on to the younger generations and therefore the urgency to document it before it disappears. This finding is quite similar to that reported by Minja and Allport (2001) among the Maasai of Simanjiro district in Tanzania. Fifty four percent (54%) of the herbalists had no formal education whereas 29% and 12.5% had primary and secondary education respectively. This is low compared with the average national adult literacy rate of 71% in Kenya (Ndemo, 2005). However, the most educated herbal practitioner in the group had a university degree in Botany. Ninety two percent (92%) of the herbalists were of the Christian faith while 8% were traditionalists. Fifty eight percent (58%) of the herbalists inherited the practice from relatives while 42% acquired the knowledge by observation and apprenticeship

with older herbalists. This pattern of knowledge transfer and the tendency of secrecy are also reported in similar studies elsewhere (Mesfin *et al.*, 2009; Nanyingi *et al.*, 2008).

The conditions treated included stomach disorders and helminthosis, malaria, sexually transmitted diseases, infertilities, injuries, aches, coughs and colds (Table 3.1). Some of the remedies were used for recreational purposes under various categories with regard to the effect they exert. Some of these are excitants, digestives, aphrodisiacs, emetics, bio-stimulators and fat emulsifiers. The most common methods of preparation included boiling or soaking in water, drying and grinding while the preferred route of administration was oral. These methods of remedy preparation and dosing are quite similar to those reported by others (Nanyingi *et al.*, 2008; Teklehaymanot and Giday 2007). The informants' responses indicated that there were variations in the dosages of remedies, units of measurement, duration and time that were prescribed for the same kind of health problems. The major factors that determine the amount to be given are age, physical fitness, stage of illness, pregnancy and presence or absence of any disease other than the disease to be treated. The lack of precision and standardization as a major drawback on the traditional health care system has been widely discussed (Abebe 1986; Getahun, 1976; Sofowora, 1993). The majority of the herbalists (67%) interviewed were aware that overdosing could result in undesirable effects and some of the antidotes they frequently used were milk, finely ground charcoal, sorghum/millet porridge, beef soup and water.

In the current study, the medicinal plants were found in a wide range of habitats including woodlands, rocky surfaces, forests, grazing and farmlands, home gardens, road and riversides,

farm borders and hedges. However the majority of these plants were found growing in the wild and this is in conformity with findings from a similar study done in Ethiopia (Mesfin *et al.*, 2009). A total of 81 plants were cited as being useful in various ethno-medical/veterinary remedies. These plants belonged to 46 different families and 71 genera as shown in Table 3.1. The Plant families Fabaceae, Euphorbiaceae and Rutaceae were cited at 10%, 6% and 5% respectively, while others varied between 1 and 4% (Figure 3.4). However, the six most important families by their medicinal use values in decreasing order were Rhamnaceae, Myrsinaceae, Oleaceae, Liliaceae, Usneceae and Rutaceae. The habits of the medicinal plants in the area were 48%, 38%, 7%, 6% and 1% trees, shrubs, herbs, lianas and lichens respectively. Some of the medicinal plants recorded in Loitoktok are also used in remedies in other parts of Kenya and elsewhere in Africa (Anonymous, 1996; Beentje, 1994; Gathuma *et al.*, 2004; Kokwaro, 1993; Mesfin, 2009). This work is also published in the journal of ethnopharmacology (Muthee *et al.*, 2011)

Table 3.1: Plants used in herbal remedies in Loitoktok District.

Plant family	Species	Vn	Local name	Habit	Medicinal uses	pu	Nh	UVs
Amaranthaceae	<i>Sericocomposis hildebrandtii</i> Schinz.	JK26	Olaisai	shrub	Malarial complications	R	1	0.04
Anacardiaceae	<i>Rhus natalensis</i> Bernh	JK58	Olmusigiyo	shrub	Endometritis, foot and mouth disease	Sb,R,L	3	0.13
	<i>Ozoroa insignis</i> Del.	JK64	Olokunonoi	tree	Tooth ache, snake bite	R	1	0.09
Apocynaceae	<i>Carissa edulis</i> (Forsk.) Vahl	JK27	Olamuriaki	shrub	Gonorrhoea, syphilis	R	1	0.04
	<i>Acokanthera schimperi</i> Schewnf.	JK54	Olmorijoi	tree	Blood pressure, ectoparasites, AIDS	R,Sb	1	0.13
Araliaceae	<i>Cussonia holstii</i> Harms ex Engl.	JK73	Oltimaroi	tree	Abdominal pains	B	1	0.04
Asclepiadaceae	<i>Mondia whytei</i> (H.f.) Skeels	JK53	Olmokongora	liana	Aphrodisiac, respiratory	R	1	0.09
Balanitaceae	<i>Balanites glabra</i> Meldbr & Schlectr	JK60	Olngosua	tree	Cowpox, stomach upsets	R,B,T	3	0.17
Boraginaceae	<i>Cordia africana</i> Lam.	JK22	Muringa	tree	Vitamins	L	1	0.04
	<i>Kigelia africana</i> (Lam.) Benth.	JK71	Oltarpoi	tree	Measles	F	1	0.04
	<i>Cordia monoica</i> Roxb.	JK77	Oseki	tree	Backaches	R	1	0.04
Burseraceae	<i>Commiphora swynnertonii</i> A. B. Burtt	JK72	Oltenuai	shrub	Gonorrhoea	Sb	1	0.04
	<i>Commiphora africana</i> (A. Rich) Engl.	JK78	Osilalei	tree	skin disorders	Sb	1	0.04
Cannellaceae	<i>Waburgia ugandensis</i> Sprague	JK80	Osokonoi	tree	Respiratory	Sb	4	0.17
Capparidaceae	<i>Caparis tomentosa</i> Lam.	JK28	Olarunduudiai	shrub	Respiratory	L, R	1	0.04
Celastraceae	<i>Maytenus senegalensis</i> (Lam.) Exell	JK25	Olaimurunyai	shrub	Gynecological conditions	R	1	0.04
	<i>Elaeodendron buchananii</i> Loes.	JK67	Olparsento	tree	Cuts and wounds	R	1	0.04
Combretaceae	<i>Terminalia brownii</i> Fres.	JK31	Olbukoi	tree	Skin disorders	Sb	1	0.04
	<i>Combretum molle</i> R.Br. exG.Don	JK50	Olmaroroi	tree	Respiratory, kidney, backache	R,Sb	3	0.13
Commeliaceae	<i>Comelina africana</i> L.	JK06	Enkaiteteyia	herb	Stillbirths, fever	T	1	0.04

Table 3.1 (continued)

Plant family	Species	Vn	Local name	Habit	Medicinal uses	pu	Nh	UVs
Compositae	<i>Psiadia punctulata</i> (DC.) Vatke	JK24	Olabaai	shrub	Abdominal pains	L	1	0.04
	<i>Artemesia afra</i> Willd.	JK34	Olchanipus	herb	East coast fever		1	0.04
Cucurbitaceae	<i>Zehneria scabra</i> L.f. Sond.	JK19	Lesitani	liana	Malaria	F	1	0.04
Ebenaceae	<i>Euclea divinorum</i> Hiern.	JK41	Olkinyei	tree	Constipation	R	1	0.04
Euphorbiaceae	<i>Ricinus communis</i> L.	JK35	Oldule	shrub	Ruminal impaction / Constipation	F	1	0.04
	<i>Euphorbia cuneata</i> Vahl.	JK48	Olmame	shrub	Gonorrhea	R	1	0.04
	<i>Croton megalocarpus</i> (Hutch.)	JK52	Olmerguet	tree	Respiratory conditions	L	1	0.04
	<i>Croton dichogamus</i> pax.	JK61	Oloibor benek	shrub	Gonorrhea, arthritis	R	2	0.09
	<i>Euphorbia candelabrum</i> Kotschy	JK68	Olpopongi	tree	Joints, venereal, infertility	R	1	0.13
Fabaceae	<i>Indigofera arrecta</i> A. Rich.	JK01	Eiyemiyem	shrub	Stomach disorders	R	1	0.04
	<i>Acacia drepanolobium</i> Harms.	JK02	Eluaai	shrub	Uterine cleaning	Sb	3	0.13
	<i>Cajanus cajan</i> (L.) Mill sp.	JK03	Enchuko	shrub	Allergies	L	1	0.04
	<i>Ormocarpum kirkii</i> S. moore	JK08	Enkike empari	shrub	Cuts and wounds	L, R	1	0.04
	<i>Acacia xanthophloea</i> Benth.	JK36	Olerai	tree	Skin disorders, fatigue	Sb,R	2	0.09
	<i>Acacia nilotica</i> (L.) Del.	JK40	Olkilorit	tree	Stimulant / excitant	Sb	1	0.04
	<i>Albizia anthelmintica</i> Brongn	JK57	Olmugutan	shrub	Helminthiasis, malaria, stomachache, emetic	Sb, R	16	1.09
	<i>Erythrina abyssinica</i> DC	JK65	Oloponi	tree	Infertility, urinary tract infections	Sb,R	2	0.09
Flacourtiaceae	<i>Dovyalis abyssinica</i> (A. Rich.) Warb.	JK55	Olmorogi	shrub	Indigestion	L	1	0.04
Labiatae	<i>Leucas pododiskos</i> Bullock	JK15	Entialongo	shrub	Dysentery, stomachache	L	3	0.13
	<i>Leucas calustachys</i> Oliv.	JK30	Olbibiai-oibor	shrub	Respiratory	L	1	0.04
	<i>Iboza multiflora</i> (Benth) E.A, Brunce	JK47	Olmalaka	tree	Malaria, appetizer	L	1	0.09

Table 3.1 (continued)

Plant family	Species	Vn	Local name	Habit	Medicinal uses	pu	Nh	UVs
Liliaceae	<i>Aloe secundiflora</i> Engl.	JK81	Osukuroi	herb	Malaria, wounds	R, L	3	0.35
Loganiaceae	<i>Strychnos henningsii</i> Gilg.	JK75	Oltipilikwa	tree	Arthritis, stomachache	R	2	0.09
Meliaceae	<i>Azadirachta indica</i> A. Juss	JK21	Muarubaini	tree	Malaria, stomach problems	L,Sb,F	2	0.13
	<i>Turraea mombassana</i> Hiern ex Dc	JK32	Olchani narok	shrub	Emetic, malaria	R	2	0.09
Moraceae	<i>Ficus sycomorus</i> L.	JK37	Olgaboli	tree	uterine bleeding	B	1	0.04
	<i>Ficus thonningi</i> Blume.	JK70	Olreteti	tree	Respiratory	Sb, R	1	0.04
Musaceae	<i>Musa acuminata</i> Colla	JK49	Olmarikoi	herb	Blood pressure	Sb	1	0.04
Myrsinaceae	<i>Rapanea melanophloes</i> (L.) Mez.	JK12	Enkodwai	shrub	Worms, gonorrhea	F	4	0.22
	<i>Myrsine africana</i> L.	JK20	Loodwa/Seketet	shrub	Worms, gonorrhea, constipation	F	17	1.22
	<i>Embelia schimperi</i> L.	JK33	Olchani onyokie	tree	Worms, gonorrhea, heartburn	F	4	0.22
Olacaceae	<i>Ximenia americana</i> L.	JK07	Enkamai	tree	Uterine bleeding, stomachache	R,F	2	0.13
Oleaceae	<i>Olea africana</i> L.	JK62	Oloirien	tree	Worms, retained afterbirth, respiratory, anaplasmosis	Sb,L	6	0.48
Oliniceae	<i>Olinia usambarensis</i> Gilg.	JK42	Olkirenyi	tree	Respiratory	F,Sb	1	0.04
Polygonaceae	<i>Rumex usambarensis</i> Dammmer	JK05	Enkaisijoi	herb	Worms, constipation	T	1	0.13
Proteaceae	<i>Faurea saligna</i> Harv.	JK59	Olngeriantus	tree	Stomach problems	R	2	0.09
Rhamnaceae	<i>Rhamnus staddo</i> A.Rich.	JK43	Olkokola	shrub	Gonorrhea, diabetes, endometritis	R,Sb,F,L	15	0.74
	<i>Rhamnus perinoides</i> L' Herit	JK44	Olkonyil	shrub	Gonorrhea, prostate, Malaria, brucellosis	R,Sb,F,L	15	1.22
Rosaceae	<i>Prunus africana</i> (Hk.f.) Kalkman	JK45	Olkujuk	tree	Prostate, urinary tract infections	Sb, R, F	4	0.22

Table 3.1 (continued)

Plant family	Species	Vn	Local name	Habit	Medicinal uses	pu	Nh	UVs
Rubiaceae	<i>Galium aprimnoides</i> Forsk.	JK04	Engeriantus	liana	Stomach ulcers, urinary infections	R	2	0.13
	<i>Vangueria tomentosa</i> Hochst.	JK39	Olgumi	tree	Snake bites	R	1	0.04
	<i>Hymenodictyon parvifolium</i> Oliv.	JK66	Olosholo	tree	Constipation	R	1	0.04
Rutaceae	<i>Calodendrum capense</i> (L.f.) Thunb.	JK10	Enkirashai	tree	Stomach upsets, emetic	Sb	2	0.09
	<i>Teclea simplicifolia</i> (Engl) Verdoon.	JK38	Ogilai	tree	Pneumonia, allergy, gonorrhoea	R, L	4	0.22
	<i>Clausena anisata</i> (Wild) Hook.f.	JK51	Oلماتاسيا	shrub	Worms, stomach problems, prostate	Sb	3	0.26
	<i>Zanthoxylum usambarense</i> (Engl.)Kokwaro	JK63	Oloisuki	tree	Malaria, respiratory, diarrhea	R,F	11	0.57
Salvadoraceae	<i>Salvadora persica</i> L.	JK69	Olremit	tree	Worms, malaria, stomachache	R	4	0.17
Samydaceae	<i>Trimelia bakeri</i> Gilg.	JK17	Lasipeta	shrub	Brucellosis, malaria	R,L,F,Sb	3	0.22
Santalaceae	<i>Osyris lanceolata</i> Hochst. & Steud	JK29	Olasesiai	tree	Ringworm, impotence, fatigue	R,F,Sb,L	3	0.17
Sapindaceae	<i>Pappea capensis</i> Eckl. & Zeyh.	JK74	Oltimigomi	tree	Aphrodisiac	B	1	0.04
Simaroubaceae	<i>Harisonia abyssinica</i> Oliv.	JK11	Enkisar ngatuny	shrub	Venereal diseases	R	1	0.04
Solanaceae	<i>Solanum taitanse</i> Vatke	JK14	Entemelua	shrub	Respiratory	R, F, L	1	0.04
	<i>Withania somnifera</i> (L) Dunal	JK18	Lesayiet	shrub	Gonorrhoea	R	3	0.13
	<i>Solanum incanum</i> L.	JK76	Ondulele	shrub	Throat infections	R	1	0.04
Sterculiaceae	<i>Dombeya rotundifolia</i> (Hochst.) Planch	JK56	Olmotoo	tree	Dysentery	R	1	0.04
	Urticaceae	<i>Urtica dioica</i> L.	JK13	Entamejoi	herb	Low butterfat in milk, diabetes, ulcers	L, R	2

Table 3.1 (continued)

Plant family	Species	Vn	Local name	Habit	Medicinal uses	pu	Nh	UVs
Usneceae	<i>Usnea</i> spp.	JK23	Ntana oosoito	lichen	Malaria, heart burn, fever	W	4	0.3
Verbenaceae	<i>Clerodendrum myricoides</i> (Hoc) Vatke	JK46	Olmakutkut	shrub	Gonorrhoea, syphilis	R	3	0.17
	<i>Lippia kituiensis</i> Vatke	JK79	Osinoni	shrub	Induce vomiting	L	1	0.04
Vitaceae	<i>Rhoicissus tridentata</i> (L.f.) Wild & Drum.	JK09	Enkilenyai	liana	Cuts and wounds	S, R	2	0.09
	<i>Cissus quadrangularis</i> L.	JK16	Esukurtuti	liana	Cuts and wounds	S	2	0.09

Key:

F: fruits; L: leaves; R: roots; Sb: stem bark; W: whole plant

Vn: voucher specimen number

Pu: part of the plant used in the preparation of remedies

Nh: number of herbalists citing the use of the plant in remedies

UVs: species use values

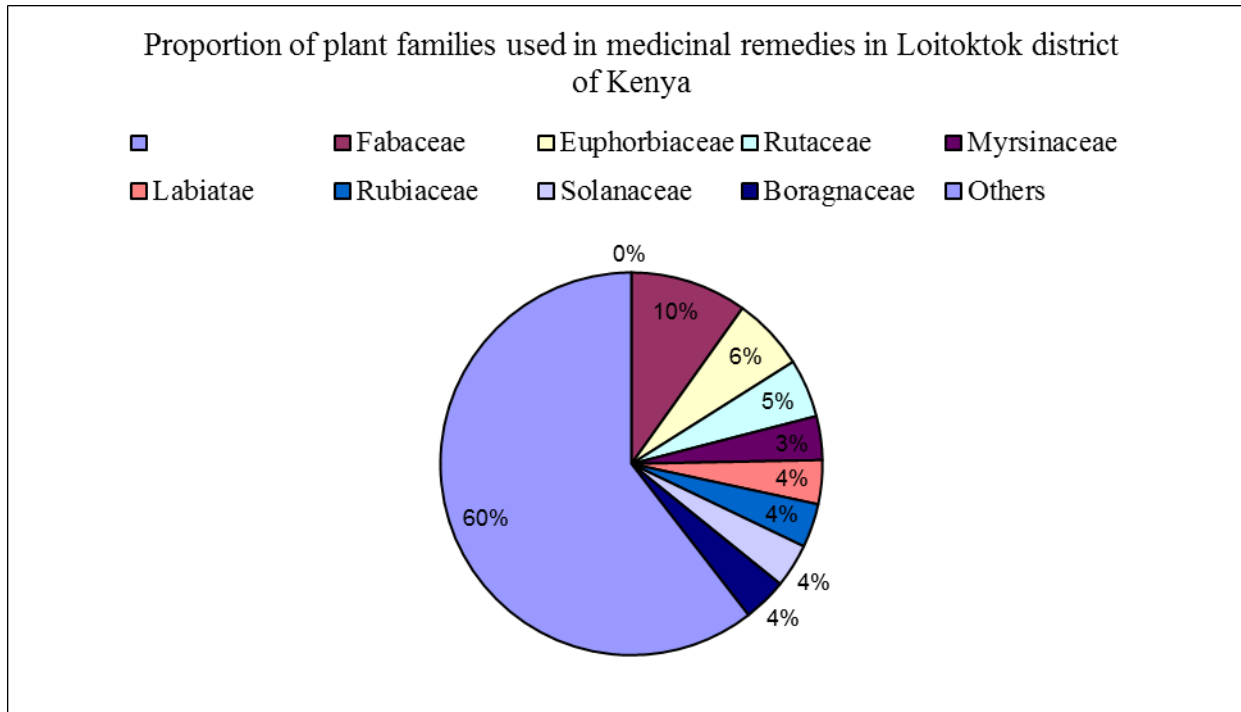


Figure 3.3: Medicinal plant families used in Loitoktok District

Twenty one herbalists (91%) used one or more plants for the treatment of helminthosis, which is probably an indication of the importance of the disease in the area. Seven plants belonging to 7 genera and 6 families were cited for their anthelmintic use (Table 3.2) in addition to other uses (Table 3.1). There was a high informant consensus factor (0.85) on the use of medicinal plants for the treatment and control of helminthosis. The most frequently used anthelmintic plants were *Albizia anthelmintica* (Fabaceae), *Myrsine africana* (Myrsinaceae), *Rapanea melanophleas* (Myrsinaceae), *Embelia schimperi* (Myrsinaceae), *Clausena anisata* (Rutaceae), *Olea africana* (Oleaceae), *Rumex usambarensis* (Polygonaceae) and *Salvadora persica* (Salvadoraceae) by 70, 70, 17, 17, 13, 9, 4 and 4 percent of the respondents respectively. These plants have been reviewed in Table 3.2, and all of them have been cited in one or more other studies for their anthelmintic and other uses.

The most widely sought plant parts in the preparation of remedies were the root, bark, leaves, stems and seeds in that order. The popularity of these parts has serious consequences from both ecological point of view and the survival of the medicinal plant species (Mesfin *et al.*, 2009). The main threat for medicinal plants in the natural vegetation was increasing population pressure and agricultural expansion due to the continuing subdivision of the group ranches in the area (Ntiati, 2002). These factors combined with the natural vulnerability of such arid and semi-arid lands may lead to further reduction in natural habitats of the medicinal plants. Pressure from agricultural expansion, wide spread cutting for fuel wood combined with seasonal drought is also reported in other studies (Balemie *et al.*, 2004; Lulekal *et al.*, 2008; Nanyingi *et al.*, 2008 and Yineger *et al.* 2008) as main factors in environmental degradation.

Table 3.2: Plants used in anthelmintic remedies by herbalists in Loitokitok district

Plant species/family	Parts used	Active principles	References
<i>Albizia anthelmintica</i> (Fabaceae)	Stem and root barks	Sesquiterpenes, kosotoxin	Beentje (1994), Gakuya (2001), Gathuma <i>et al.</i> (2004), Grade & Langok (2000), Koko <i>et al.</i> (2000), McCorkle <i>et al.</i> (1996), Minja & Allport (2001), Minja (2002), Nanyingi <i>et al.</i> (2008), Ole-Miaron (2003).
<i>Myrsine africana</i> (Myrsinaceae)	Fruits	Benzoquinones	Anonymous (1996), Beentje (1994), Desta (1995), Gachathi (2007), Gathuma <i>et al.</i> (2004), Kokwaro (1993), McCorkle <i>et al.</i> (1996), Nanyingi (2008).
<i>Embelia schimperi</i> (Myrsinaceae)	Fruits	Embelin (benzoquinone)	Bøgh <i>et al.</i> (1996), Githiori (2004).
<i>Rapanea melanophloeos</i> (Myrsinaceae)	Fruits, stem and root barks	Benzoquinones	Anonymous (1996), Beentje (1994), Gachathi (2007), Kokwaro (1993), Midiwo <i>et al.</i> (2002).
<i>Olea africana</i> (Oleaceae)	Stem bark		Fratkin (1996), Kokwaro (1993).
<i>Clausena anisata</i> (Rutaceae)	Leaves	Essential oils, Clausenol (carbazole alkaloid)	Ole-Miaron & Mapenay (2004), Senthilkumar & Venkatesalu (2009).
<i>Rumex usambarensis</i> (Polygonaceae)	Tuber	Anthraquinones	Midiwo <i>et al.</i> (2002)
<i>Salvadora persica</i> (Salvadoraceae)	Root		Paliwal <i>et al.</i> (2007).

3.4 Conclusion

It was established that herbal remedy is crucial for primary health care in Loitoktok District. Traditional medicinal plants were harvested mostly from natural vegetation area but also home gardens; roadsides, farmlands and live fences. The medicinal plants in the area are becoming scarce and traditional healers had resulted to planting some in their home gardens and sourcing the plants from distant places including across the border in Tanzania. However, traditional healers still depend largely on naturally growing species in their locality because of their belief that those species in the natural vegetation are more effective in the prevention and treatment of diseases and health problems. Furthermore, the documented medicinal plants can be used as a basis for further studies on the regional medicinal plant knowledge and for future phytochemical and pharmacological studies.

CHAPTER FOUR

BRINE SHRIMP LETHALITY TEST AND PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANTS USED AS ANTHELMINTICS IN LOITOKTOK DISTRICT

4.1 Introduction

Medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediaries and chemical entities for synthetic drugs (Ncube *et al.*, 2008). Phytochemicals are non-nutritive plant secondary metabolites that have protective or disease preventive properties. Plants produce these chemicals to protect themselves but recent research demonstrates that many of these phytochemicals can protect humans and animals against diseases (Kumar *et al.*, 2009).

Extraction methods used pharmaceutically involves the separation of bioactive portions of plant tissues from the inert components by using selective solvents. During extraction, solvents solubilize compounds with similar polarity resulting in relatively complex mixtures of metabolites such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Handa *et al.*, 2008). These are in the forms of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such extracts have been popularly called galenicals, named after the famous Roman physician, Claudius Galenus of Peragon -129-200 A.D (Paulsen, 2010).

The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components

follows a logical pathway. Plants are selected either randomly or by following leads from local healers in geographical areas where the plants are found (Prakesh *et al.*, 2006). Fresh or dried plant materials can be used for the extraction of phytochemicals. However, due to differences in the water contents at harvesting, plants are usually air dried to a constant weight before extraction. Plant materials can also be dried in the oven at about 40 °C for 72 hours. Reduction of the particle size to increase the surface area of adsorption is also standard practice.

Successful determination of phytochemicals from plant materials is largely dependent on the type of solvent used in the extraction process. The factors affecting the choice of solvent are the quantity of phytochemicals to be extracted, rate of extraction, diversity of compounds to be extracted, ease of handling, toxicity of solvent in the bioassay system, and the potential health hazard of the extractants (Eloff, 1998).

Bioassays offer special advantage in the standardization and quality of heterogeneous herbal products. Physical analytical methods, such as chromatography, are by themselves not very useful for this purpose as they are usually insensitive to the chemical complexities found in crude botanical extracts (McLaughlin *et al.*, 1998). Most often a desired biological response is due to not one but a mixture of bioactive plant components and the relative proportion of single components can vary from batch to batch while the bioactivity still remains within tolerable levels. Thus, physical or chemical analysis of a single component in such a mixture may not be adequate and for practical application in health care, today's work in medicinal plant chemistry should include bioassays (McLaughlin *et al.*, 1998).

The brine shrimp lethality test (BST) is a rapid general bioassay described by Meyer *et al.*, (1982) and refined by McLaughlin *et al.* (1991). This *in vivo* lethality test in a simple zoologic organism can be used as a convenient tool for screening and fractionation in the discovery and monitoring of natural products (McLaughlin *et al.*, 1998). The eggs of brine shrimp, *Artemia salina* (Leach), are readily available in pet shops at low cost and remain viable for years in a dry state. Upon being placed in sea water, the eggs hatch within 48 hours to provide large numbers of larvae (nauplii) for experimental use.

The first objective of this study was to qualitatively screen for the presence of phytochemicals in the crude aqueous and organic solvent extracts of the plants used in anthelmintic remedies by traditional medicinal practitioners in Loitoktok District. The second objective of the study was to determine the bioactivity of the crude aqueous and organic solvent extracts of said anthelmintic plants using the Brine shrimp lethality test (BST).

4.2 Materials and methods

4.2.1 Collection and preparation of the Plant materials

The plant samples used in the study were collected from Loitoktok District with the aid of the local traditional health practitioners and identified by taxonomists at the University of Nairobi herbarium, where voucher specimens were deposited. The plant species (*Albizia anthelmintica*, *Myrsine africana*, *Embelia schimperi* and *Rapanea melanophloeos*) were chosen based on their ethnopharmacological uses, as anthelmintics, by the traditional health practitioners. The information gathered included parts of the plant used, method of preparation of anthelmintic

remedies and route of administration. The chopped stem bark of *Albizia anthelmintica*, and the fruits of the other species were air dried under shade and separately ground into fine powder using a laboratory mill.

4.2.2 Preparation of crude plant extracts

The aqueous extract of each of the plants was prepared by soaking 100 grams of powdered plant material in 500 ml of distilled water, with regular stirring and shaking at least 3 times daily for 3 days. The material was then filtered through muslin gauze and the filtrate frozen for 24 hrs before lyophilization. The lyophilized dry powder was then weighed and stored in airtight bottles at -20 °C until used. The organic solvent (hexane, ethyl acetate and ethanol) extraction was done on fresh sample for each by soxhlet apparatus for 6 hours. The extract was then concentrated in vacuum by use of a rotavapor and further dried in an oven at 40 °C. The dry solid extracts were then weighed and stored at -20 °C in airtight bottle containers until utilized. The (methanol: chloroform 1:1) extraction was done by cold maceration for 72 hours, filtered and then concentrated by rotavapour followed by oven drying at 40 °C.

4.2.3 Preparation of the test extracts for Brine Shrimp Lethality Test

Stock solutions of aqueous extracts (10, 000 µg/ml) were made in distilled deionized water. The organic extracts were dissolved in a little dimethyl sulphoxide (DMSO) and further diluted with distilled water to make up stock solutions of 10, 000 µg/ml. The DMSO concentration in the final test solution was less than 1%, to avoid solvent toxicity. Test extracts at appropriate amounts (5, 50, and 500 µl for 10 µg/ml, 100 µg/ml and 1000 µg/ml, respectively) were

transferred into 10 ml vials and the volume topped up to 5ml using brine with 5 replicates at each dose level.

4.2.4 Culture and harvesting of *Artemia salina*

Artemia salina cysts, batch number DE RP 33801, were purchased from JBL GmbH & Co.KG (Neuhofen, Germany) and the product was labeled as JBL Artemio Pur Brand. The cysts had been harvested from Great Salt Lake, Utah, USA and were zoogeographically identified as *Artemia salina*. *Artemia salina* eggs were incubated to hatch in a rectangular dish (14 cm x 9 cm x 5 cm) filled with 225 ml of a 3.3% w/v solution of artificial sea water. A plastic divider with several 2 mm holes was clamped in the dish to make two unequal compartments. The eggs (1.11 grams) and yeast (0.08827 grams) were sprinkled into the larger compartment which was darkened. The smaller compartment was illuminated by a tungsten filament light. After 48 hours, hatched *A. salina* larvae were ready for the tests. The phototropic nauplii were collected by pipette from the lighted side, having migrated through the pores on the divider leaving the shells on the darker compartment.

4.2.5 Bioassay of *Artemia salina*

For bioactivity/toxicity tests, ten *A. salina* nauplii were transferred into each sample vial using 230 mm disposable glass Pasteur pipettes and filtered brine solution was added to top up to 5 ml. The nauplii were counted macroscopically in the stem of the pipette against a lighted background. A drop of dry yeast suspension (3 mg in 5 ml artificial sea water) was added as food to each vial. All the vials were maintained under illumination. The surviving nauplii were counted with the aid of a 3x magnifying glass, after 24 hours, and the percentage of deaths at the

three dose levels and the control determined. In cases where control deaths occurred, the data was corrected using the formula by Abbott (1925) as follows:

Percent (%) deaths = [(Test – control)/control] x 100. The surviving nauplii were then killed by the addition of 100 µl of 5% (v/v) phenol to each vial.

4.2.6 Determination of LC₅₀

The lethal concentration fifty (LC₅₀), at 95% confidence interval and slope were determined from the 24 hour counts using the probit analysis method as described by Finney (1971). The bioactivity was classified weak when the LC₅₀ is between 500 and 1000 µg/ml, moderate when it was between 100 and 500 µg/ml and strong when it was between 0 and 100 µg/ml (Meyer *et al.*, 1982).

4.2.7 Preliminary phytochemical screening

One gram of the dried extract was dissolved in 100 ml of their mother solvents to obtain stocks of concentration 1% w/v. The reconstituted extracts thus obtained were subjected to phytochemical screening following the methods of Harbone (1998) and Kokate (2001)

4.3 Results and Discussion

4.3.1 Brine Shrimp Lethality and LC₅₀

The LC₅₀ values for the aqueous extracts ranged from 149 to 616 µg/ml while those for the organic solvents ranged from 11 to 581 µg/ml (Tables 4.1 to 4.3). All the plant extracts demonstrated a dose-dependent bioactivity. In bioassay studies, the LC₅₀ values less than 1000 µg/ml (ppm) are considered significant (Meyer *et al.*, 1982; Rieser *et al.*, 1996)). In this study,

crude aqueous and organic plant extracts were evaluated for their brine shrimp lethality (bioactivity). Four plants, belonging to two families, frequently used in anthelmintic remedies in Loitoktok District were evaluated. The aqueous extracts of three of them (*Albizia anthelmintica*, *Embelia schimperi* and *Myrsine africana*) had significant activity, suggesting the presence of bioactive compounds and further supporting the results of the earlier phytochemical screening. The bioactivity of all the organic extracts was significant with most of them categorized as strongly bioactive. The exceptions were ethanolic extracts of *Myrsine africana* and *Rapanea melanophloeos* which were moderate. These results indicate that majority of the bioactive components in these plants are non-polar and merit further investigation. The current observation is in agreement with the findings of Cantrell *et al.* (2003) and Nguta (2011) who found organic extracts to be more toxic than aqueous extracts of the same plant species, in a brine shrimp bioassay.

The bioactivity of the extracts of the plants in this study is an indication of the presence of potent compounds and may explain some of their traditional uses. These could be of particular interest in relation to their unexplored efficacy and can be potential sources of chemically interesting and biologically important drug candidates.

Brine shrimp lethality test is a simple and cheap benchtop bioassay which detects a broad range of biological activities and a diversity of chemical structures. It is very useful in screening extracts, for bioactivity, in the drug discovery process (McLaughlin *et al.*, 1991). Currently there is growing pressure from human rights' advocates to limit the use of higher animals for

toxicological studies and since the brine shrimp are crustacean, and sensitive to a variety of substances, the BST is rapidly gaining value as a quick and simple test for predicting the bioactivity/toxicity of plant extracts and guiding phytochemical fractionation in the search for novel compounds useful in health care (McLaughlin, 1991; Cáceres, 1996; Parra *et al.*, 2001).

Table 4.1: Brine shrimp lethality of crude aqueous plant extracts

Plant species	Plant part	Percent deaths at 24 hrs					
		10 µg/ml	100 µg/ml	1000 µg/ml	LC ₅₀ µg/ml	Limits 95% CI (µg/ml)	Slope
<i>Albizia anthelmintica</i> Brongn	Stem bark	2	10	94	259	107-658	0.3729
<i>Embelia schimperi</i> L.	Fruits	0	30	100	149	60-498	0.5686
<i>Myrsine africana</i> L.	Fruits	10	20	60	616	ND	0.7391
<i>Rapanea melanophloeos</i> L.	Fruits	0	8	20	NA	ND	2.2706

Table 4.2: Brine shrimp lethality of crude organic (Ethanol) plant extracts

Plant species	Plant part	Percent deaths at 24 hrs					
		10 µg/ml	100 µg/ml	1000 µg/ml	LC ₅₀ µg/ml	Limits 95% CI (µg/ml)	Slope
<i>Albizia anthelmintica</i> Brongn	Stem bark	3	82	96	23	2-74	0.4909
<i>Embelia schimperi</i> L.	Fruits	50	68	100	14	0-54	0.6294
<i>Myrsine africana</i> L.	Fruits	6	46	76	178	51-947	0.4071
<i>Rapanea melanophloeos</i> L.	Fruits	0	20	60	581	ND	0.5362

Key: ND = Not detectable; NA = Not active; CI = confidence interval; µg/ml = micrograms per mililitre

Table 4.3: Brine shrimp lethality of organic (CHCL3: MeOH, 1:1) crude plant extracts

Plant species	Plant part	Percent deaths at 24 hrs					
		10 µg/ml	100 µg/ml	1000 µg/ml	LC ₅₀ µg/ml	Limits 95% CI (µg/ml)	Slope
<i>Albizia anthelmintica</i> Brongn	Stem bark	50	80	100	11	0-39	0.6307
<i>Embelia schimperi</i> L.	Fruits	30	60	100	36	6-112	0.4036
<i>Myrsine africana</i> L.	Fruits	22	64	100	42	11-118	0.3469
<i>Rapanea melanophloeos</i> L.	Fruits	40	52	88	36	0-142	0.7919

Key: ND = Not detectable; NA = Not active; CI = confidence interval; µg/ml = micrograms per mililitre

4.3.2 Preliminary phytochemical screening

The yields and phytochemicals detected from the aqueous and organic extracts of three plants, commonly used in anthelmintic remedies in Loitoktok District are presented in Table 4.4. The yields of the aqueous extracts ranged from 8.1 to 17% w/w, while those of the organic solvents varied from 0.5 to 32.6% w/w. The phytochemicals found to be present were alkaloids, anthraquinones, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids.

The preliminary screening results in this study confirm the presence of constituents which are known to exhibit medicinal and physiological activity (Sofowora, 1993) For example, phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects (Liu, 2003; Akindele and Adeyemi, 2007; Ilkay Orhan *et al.*, 2007). Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities (Oliver, 1980; Cherian and Augusti, 1995). Rupasinghe *et al.* (2003) have reported that saponins possess hypocholesterolemic and anti-diabetic properties. The steroids and triterpenoids have been shown to have analgesic properties (Sayyah *et al.*, 2004; Malairajan *et al.*, 2006). Steroids and Saponins are known to have central nervous system activities (Argal and Pathak, 2006). Terpenes and tannins, especially condensed tannins, have been shown to have potent anthelmintic activity (Khan and Diaz-Hernandez, 1999; Hostettmann, *et al.*, 2000).

Table 4.4: Phytochemicals in plants used in anthelmintic remedies in Loitoktok District

Phytochemicals screened	<i>Albizia anthelmintica</i> (bark)				<i>Embelia schimperi</i> (fruits)				<i>Myrsine africana</i> (fruits)			
	Aqua	Eth	Ethy	Hex	Aqua	Eth	Ethy	Hex	Aqua	Eth	Ethy	Hex
Alkaloids	+	+	-	-	-	-	-	-	+	+	+	-
Anthraquinones	-	-	-	-	-	-	-	-	+	-	+	+
Flavone aglycone	-	-	-		+	-	-	-	+	-	+	+
Flavonoids	+	+	+	-	-	-	-	+	+	-	-	+
Glycosides	+	+	+	+	+	-	+	+	+	-	+	+
Reducing sugars	-	-	-	-	+	+	+	+	-	+	+	-
Saponins	+	+	+	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	-	+	+	+	+	+	+
Tannins	-	-	-	-	-	+	-	-	+	+	+	-
Triterpenoids	+	+	+	+	+	+	+	+	+	+	+	+
Percentage yield	8.1	9.5	3.4	0.5	17	32.6	20.6	7.8	10.4	31.8	18.6	14.3

Key: Aqua = Water extract; Eth = Ethanol extract; Ethy = Ethyl acetate extract; Hex = Hexane extract; + = Presence; - = Absence.

Tannins are polyphenolic compounds that have been reported to have anthelmintic properties (Butter *et al* 2000; Athanasiadou *et al.*, 2000, 2001; Max *et al.*, 2005a, 2005b, 2007). The mechanisms by which tannins produce anthelmintic effect remain unclear. Actions similar to those of synthetic phenolic anthelmintics such as niclosamide, oxiclozanide and nitroxynil, which interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation, have been reported (Martin, 1997; Mali *et al.*, 2005; Praveen *et al.*, 2010; Jitendra *et al.*, 2011). Other reports have suggested tannins' ability to bind free proteins in the gastrointestinal tract of host animals (Niezen *et al.*, 1993; Wang *et al.*, 1996; Athanasiadou, 2001) or to glycoproteins on the cuticle of parasites leading to their death (Thompson and Geary, 1995).

A form of terpene known as palasonin (terpene anhydride) was reported to produce anthelmintic activity by inhibiting glucose uptake and hence depleting the glycogen content of parasites (Kumar *et al.*, 1995). Similarly, gentistein (a form of flavone aglycone) was reported to produce anthelmintic effect which was linked to its activity on nitric oxide synthetase (Kar *et al.*, 2002). The anthelmintic effects of flavonoids have also been reported (Lahlou, 2002; Trease and Evans, 2002). Other studies have indicated that alkaloids and flavonoids also have anthelmintic properties (Praveen *et al.*, 2010; Jitendra *et al.*, 2011; Rubini *et al.*, 2012).

All the five myrsinaceae (*Myrsine africana*, *Maesa lanceolata*, *Rapanea melanophloeos*, *Embelia schimperi* and *Embelia keniensis*) in Kenya are widely used across ethnic groups as anthelmintics and have been found to be rich in benzoquinones (Midiwo *et al.*, 2002). The

bioactivity of isolated individual phytochemicals may not be in doubt; however, the better therapeutic effect of crude extracts may result from a combination of active principles in each plant (Villasenor *et al.*, 1998; Cho *et al* 2003). Lactones such as santonin have been shown to have a strong anthelmintic activity against *Ascaris* and other nematode species of livestock and humans (Waller *et al.*, 2001). Alkaloids have also exhibited strong nematocidal activity against *Stroglyoides ratti* and *Strongyloides venezuelensis*, two rat nematodes used as models for human nematodes (Satou, *et al.*, 2002). The nematocidal activity of tannins has been reported as early as the 1960s (Taylor and Murant, 1966), and more recently evidence on the anthelmintic properties of condensed tannins has been supported by a series of *in vitro* (Dawson *et al.*, 1999; Athanasiadou *et al* 2001; Molan *et al.*, 2003b; Ademola and Idowu, 2006) and *in vivo* studies (Athanasiadou *et al.*, 2000; Butter *et al.*, 2001; Paolini *et al.*, 2003a and 2003b).

4.3.3 Conclusion

- 1) Medicinal plants used in anthelmintic remedies in Loitoktok District have bioactivity against brine shrimp larvae and that organic extracts are generally more potent than the aqueous extracts
- 2) Medicinal plants used in anthelmintic remedies in Loitoktok District are rich in phytochemicals that could be responsible for their bioactivity and merit further analysis to isolate the actual active components and determine their mode of action with a view of discovering novel products for use in health care.

CHAPTER FIVE

EVALUATION OF ANTHELMINTIC EFFICACY OF SELECTED MEDICINAL PLANTS USED AS ANTHELMINTICS IN LOITOKTOK DISTRICT

5.1 Introduction

Medicinal plants have been used to combat parasitism and other human and veterinary ailments for centuries and in many parts of the world (including Kenya), they are still used for this purpose. The World Health Organization estimates that 80% of the populations of developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs (WHO, 2008). There has been a resurgence of interest in traditional health practices throughout the world, which mainly encompasses ethnobotany and the use of herbal remedies. The forces responsible for this momentum include the perception that "natural is nice", concerns of synthetic drug residues in the environment and the food chain, and particularly the spectre of rapid emergence of multiple resistant pest organisms through misuse and overuse of conventional drugs.

Renewed interest in traditional pharmacopoeias has meant that researchers are more concerned, not only with determining the scientific rationale for the plant's usage but also, with the discovery of novel compounds of pharmaceutical value. Instead of relying on trial and error as in random screening procedures, traditional knowledge helps scientist to target plants that may be of medicinal value (Fennel *et al.*, 2004; Fabricant and Farnsworth, 2001). Reports from around the world include exhaustive list of medicinal plants that have been reported to have anthelmintic properties (Akhtar *et al.*, 2000; Waller *et al.*, 2001; Gathuma *et al.*, 2004; Fajmi and Taiwo,

2005; Githiori *et al.*, 2006; Athanasiadou *et al.*, 2007; Hussain, 2008; Iqbal and Jabbar, 2010; Gakuya *et al.*, 2011). Although the majority of the evidence on the antiparasitic activity of these plants is based on anecdotal observations, there is growing number of controlled studies that aim to scientifically verify, validate and even quantify such bioactivity. Two main approaches have traditionally been used in efficacy studies against helminths. The first one is through feeding plants or their parts to naturally or experimentally infected animals (Iqbal *et al.*, 2004; Chandrawathani *et al.*, 2006). The second one is by testing extracts and concoctions from medicinal plants via *in vivo* and *in vitro* systems (Githiori, 2004; Gathuma *et al.*, 2004).

Kenya is endowed with a variety of indigenous medicinal plants which are used by the local herbalists for the treatment of various diseases among them helminthosis (ITDG and IIRR, 1996; Kokwaro, 2009; Kigen *et al* 2013). However, most of these herbal remedies have not yet been scientifically validated or developed into viable products for the market. The purpose of the current study was to determine the anthelmintic efficacy of four medicinal plants most frequently used to treat and control helminthosis in Loitoktok District of Kajiado County in Kenya. The four plants studied were *Albizia anthelmintica* Brongn, *Embelia schimperi* L., *Myrsine africana* L. and *Rapanea melanophloeos* (L.) Mez. The first plant belongs to the Fabaceae family while the last three belong to Myrsinaceae. These plants were selected from an earlier ethnopharmacological study (chapter 3) conducted in the area involving renowned traditional healers identified through key informants.

5.2 Materials and methods

5.2.1 The experimental design

The study was done in two phases namely, the field and the controlled efficacy trials. The field experiment was done by treating 50 parasitized sheep, of mixed breeds under natural grazing conditions in Loitoktok District, with herbal anthelmintic remedies as prepared and administered by the traditional healers. The second experiment was conducted by treating 42 Dorper lambs, artificially infected with mixed gastrointestinal parasites, with enhanced dosages of herbal anthelmintic remedies obtained from traditional healers in Loitoktok District. Both experiments had negative (untreated) and positive (treated with albendazole) control groups. The efficacy in both experiments was determined using the faecal egg count reduction test (FECRT), but in addition, the controlled experiment analyzed hematological parameters and worm counts.

5.2.2 Experimental sites

The general area of the field experiment is as described in chapter 3 of this work, but the 2 flocks used were near Kimana market, about 15 Km North of Loitoktok town. The experiment was done from mid-November, 2010 and had been timed to coincide with the short rains in the area which usually occurs between October and December. The controlled clinical efficacy trial was conducted at the University of Nairobi, Faculty of Veterinary Medicine with Dorper lambs purchased from a ranch, in Kiambu County, about 40 Km North East of the study site.

5.2.3 Plant collection and preparation

The plant materials were collected with the help of the traditional healers (THs) from different parts of Loitoktok district and transported to the University of Nairobi. Pieces of the stem bark of

Albizia anthelmintica were hived off the tree trunks using a matchette in the area between Loitoktok town and Kimana market (figure 5.1). These were later chopped into smaller pieces and left to dry under the shade for several days. The seeds of *Embelia schimperi*, *Myrsine africana* and *Rapanea melanophloeos* were obtained from Kuku area, foot of Chyulu hills and the foot of Mt. Kilimanjaro respectively. The seeds were also dried under shade in a well aerated enclosure for several days. Representative samples for each plant were collected and placed into a field press for transportation and identification at the University of Nairobi herbarium where voucher specimens were deposited.

5.2.4 Preparation of the anthelmintic remedies for the field clinical efficacy trial

The dry plant materials were crushed into powder using the traditional wooden pestle and mortar and anthelmintic remedies prepared and dosed according to the traditional methods as follows:

- 1) *Albizia anthelmintica* remedy was prepared by boiling 130 grams of powder in 5 litres of water for about 30 minutes and letting it cool and then sieving it with tea strainer. The dose for adult sheep was 300 ml of the filtrate
- 2) *Embelia schimperi* remedy was prepared by boiling 130 grams of powder in 5 litres of water for about 30 minutes and letting it cool and then sieving it with tea strainer. The dose for adult sheep was 300 ml of the filtrate
- 3) *Myrsine africana* remedy was prepared by boiling 800 grams of powder in 5 litres of water for about 30 minutes and letting it cool and then sieving it with tea strainer. The dose for adult sheep was 300 ml of the filtrate



Figure 5.1. A traditional herbal practitioner harvesting the stem bark of *Albizia anthelmintica* near Kimana market in Loitoktok district.

- 4) One cup (approximately 300 ml) of cow milk was added to each of the five litres of the remedies before administering to the animals. The reason for this, according to the healers, was to forestall abortion in case some the animals involved in the trial were pregnant.

5.2.5 Preparation of the anthelmintic remedies for the controlled clinical efficacy trial

The dry plant materials were milled into powder using a laboratory mill (Christy and Norris Ltd, England) and anthelmintic remedies prepared as follows:

- 1) *Albizia anthelmintica* remedy was prepared by soaking 800 grams of powder in 3.2 litres of water (25% w/v) and shaken vigorously at least 3 times every 24 hours for 72 hours. The mixture was then filtered through a piece of gauze repeatedly until it was clear. The dose to be administered was fixed at 150 ml (equivalent to 3 g of freeze dried extract) from a controlled pre-trial where a dose of 4.4 g of freeze dried powder per lamb caused death from severe respiratory embarrassment but doses below 3 g were tolerated.
- 2) *Embelia schimperi* remedy was prepared by soaking 600 grams of powder in 2.4 litres of water (25% w/v) and shaken vigorously at least 3 times every 24 hours for 72 hours. The mixture was then filtered through a piece of gauze repeatedly until it was clear. The dose to be given was fixed at 200 ml (equivalent to 8.5 g of freeze dried extract) based on pre-trials and the result of the field trial.
- 3) *Myrsine africana* remedy was prepared by soaking 600 grams of powder in 2.4 litres of water (25%) and shaken vigorously at least 3 times every 24 hours for 72 hours. The mixture was then filtered through a piece of gauze repeatedly until it was clear. The dose

to be given was fixed at 200 ml (equivalent to 5.2 g of freeze dried extract) based on the results of the field trial and a controlled pre-trial.

- 4) *Rapanea melanophloeos* remedy was prepared by soaking 800 grams of powder in 3.2 litres of water (25% w/v) and shaken vigorously at least 3 times every 24 hours for 72 hours. The mixture was then filtered through a piece of gauze repeatedly until it was clear. The dose to be given was fixed at 200 ml (equivalent to 5 g of freeze dried extract) based on the results of a controlled pre-trial and doses cited by the traditional practitioners since this plant was not tested in the field due to unavailability of the plant material at the time of the trial.

5.2.6 Animals used in the field clinical efficacy trial

The participating sheep flocks, used for the field trial, were identified through the local Veterinary office in Loitoktok District. The main breeds of the sheep flocks kept in the area are the Red Maasai and the Persian blackhead. Most of the sheep flocks are mixed and grazed together with goats. The selected flocks had not been dewormed for at least 90 days prior to the study. They were screened for the presence of gastrointestinal nematodes by examining faecal samples obtained directly from the rectum. A pooled faecal sample was cultured (Hansen and Perry, 1994) in the laboratory and 100 larvae identified to estimate the prevalence of the nematodes that were present. The experimental animals were selected, based on the faecal egg counts of samples obtained on the day of the treatment, and marked on easily visible areas of the body using oil based paints of different colours. The selected animals (male and female of different breeds and ages) were randomly divided into five groups of 10 animals each. The test

groups were for three anthelmintic herbal remedies (*Albizia anthelmintica*, *Embelia schimperi* and *Myrsine africana*), positive and negative controls. The *Rapanea melanophloeos* remedy was not tested due to unavailability in the area (fruits were out of season) at the time. The Faecal egg count (FEC) of the sheep ranged from 100 to 1500 epg while the group means varied from 238 to 438 before treatment (Table 5.1).

The positive control group was given a synthetic commercial anthelmintic product valbazen[®] (2.5% albendazole formulation by Pfizer/Ultravetis) at the recommended dosage rate of 10 mg/kg body weight orally. The negative control group was left untreated. The treatment for each group was administered by the traditional healers (THs) together with the researcher. The test animals remained with the rest of the flock, under normal grazing conditions, as before for 10 days. On day 11 the faecal samples were obtained directly from the rectum for FEC determination. Pooled group faecal samples were cultured to determine the species of the nematodes still shedding the eggs post treatment.

Table 5.1: Groups of sheep used in the anthelmintic efficacy against natural infection of mixed gastrointestinal nematodes

Group	Treatment	Parts used (where specified)	Dose/adult sheep (or as specified)	Mean eggs/gram ±SD
A	<i>Albizia anthelmintica</i>	Stem bark	300 ml	238±220
E	<i>Embelia schimperi</i>	Fruits	300 ml	238±104
M	<i>Myrsine africana</i>	Fruits	300 ml	438±196
V	Valbazen [®] (Albendazole)		10 mg/Kg body weight	357±1289
C	Untreated control		-	400±509

SD: Standard deviation

5.2.7 Animals used in the controlled clinical efficacy trial

Forty two (42) male Dorper lambs, with the age between 6 and 8 months, were purchased from a ranch in Ruiru, Kiambu County approximately 40 Km North East of the study site. The lambs were identified by use of numbered plastic eartags, weighed and faecal samples collected from the rectum for screening of endoparasites. About half of them were shedding gastrointestinal (GI) nematode eggs. They were all treated using Closamectin[®] (combination of closantel and ivermectin by Norbrook, Kenya) at the recommended dosage rate of 200 µg and 5 mg per kilogram body weight for ivermectin and closantel respectively by subcutaneous injection. The lambs were housed in clean dry pens with concrete floors that were regularly cleaned. They were put in groups of four by body weight to prevent the bigger ones from out-competing the others for feed and especially the concentrate feed. Hay, water and mineral lick were provided *ad libitum*. Ewe and lamb mash (Pembe Feeds Ltd) were provided at the rate of 200 g per head per day. The health of the lambs was monitored on a daily basis. After two weeks of arrival the lambs were screened again for gastrointestinal nematodes and all were found to be negative.

5.2.8 Helminth cultures, infection and treatment

Mixed Infective larvae (L3) were obtained by culturing faeces from, prescreened naturally parasitized, sheep reared outdoors in an institutional farm in Tigoni, Kiambu County, about 25 Km North West of the experimental site. The faeces were collected directly from the rectum and then cultured at about 26 °C for 10 days. The larvae were recovered by a Baermann technique (MAFF, 1986) and stored in water in tissue culture bottles. One hundred larvae were identified to determine the proportion of the nematode genera in the culture (Haemonchus: 77%;

Trichostrongylus: 20%; Oesophagostomum: 3%). The larvae used for infection were usually less than one week old.

The larvae were estimated by the method used by Mugambi *et al* (2005) with slight modifications. Briefly, 50 µl aliquots of larval suspension were spread in drops onto a glass slide and larvae counted in each drop under a microscope. From counts in 10 aliquots an estimate of number larvae in 1 ml of larval suspension was arrived at. The volumes were adjusted so that the larval dose required per animal would be contained in 2 ml. The larvae were given per os using a 5 ml syringe. The first infection was at the rate of 3000 L3 per animal 3 weeks after the arrival of the lambs. From the third week post infection the lambs were screened for GI nematode eggs on a weekly basis. Animals found to be negative for GI nematodes 5 weeks post infection or with less than 200 epg were given another dose of 2000 L3 per animal and this was repeated again at the end of the 7th week. All animals were shedding GI nematode eggs by the tenth week since the first infection and hence ready for the treatment.

The lambs were divided into two blocks by FEC from where they were randomly allocated into 7 groups of 6 animals each, the minimum recommended, per group, by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) for such efficacy trials (Wood *et al.*, 1995). The FEC on the day of treatment ranged from 100 to 37100 epg and the group means from 1417 to 8725 epg (Table 5.2).

Table 5.2: Groups of sheep used in the anthelmintic efficacy against artificial infection of mixed gastrointestinal nematodes

Group	Treatment	Parts used (where specified)	Dose/lamb (or as specified)	Mean eggs/gram \pm SD
A	<i>Albizia anthelmintica</i>	Stem bark	150 ml	2067 \pm 2775
AR	<i>Albizia anthelmintica</i> + <i>Rapanea</i> <i>melanophloeos</i>	Stem bark Fruits	100 ml + 100 ml	2400 \pm 3165
E	<i>Embelia schimperii</i>	Fruits	200 ml	2100 \pm 3005
M	<i>Myrsine africana</i>	Fruits	200 ml	2283 \pm 5007
R	<i>Rapanea</i> <i>melanophloeos</i>	Fruits	200 ml	1417 \pm 1522
V	Valbazen [®] (albendazole)		10 mg/Kg body weight	8725 \pm 15360
C	Untreated control		200 ml tap water	2100 \pm 3813

SD: Standard deviation

5.2.9 Determination of weights, biochemical and haematological parameters

The lambs were weighed on days 0, 21 and 35; faecal samples were taken directly from the rectum on days 0, 10, 18 and 35 while blood was obtained via jugular venipuncture for haematology and biochemistry on days 0, 8, 21 and 35. Faecal egg counts were determined using modified McMaster method (Henriksen and Aagaard, 1976). Hematological analysis was done using a fully automatic haematology cell-counter (Melet Schloesing Laboratories-BP 508-95528 cergy-Pontoise Cedex – France). The differential cell counts were done manually. Total protein, albumin and aspartate amino transferase (AST) were analyzed from plasma with a spectrophotometer (Biomerieux Sa 69280 Marcy iEtoile/France).

5.2.10 Worm counts and identification

From day 35 post treatment the lambs were humanely slaughtered and the abomasum ligated at both ends and removed. The abomasum was opened along the greater curvature using a blunt tipped pair of scissors and the contents emptied into the bucket. The abomasal mucosa was washed gently by running water into the bucket and the contents adjusted to make 2 litres. After thorough mixing a 10% aliquot (200 ml) was taken and all worms inside counted under a stereo microscope. They were preserved in 70 % ethanol for identification later using procedure by MAFF (1986). The small and the large intestine were removed separately and each of them opened and mucosa washed into a separate bucket and the above procedure repeated.

5.2.11 Statistical analysis

The parameters analyzed were Live weight (LWT), faecal egg count (FEC), Total worm count (TWC), packed cell volume (PCV) and other haematological components. Others were total

protein (TP), albumin and the enzyme aspartate aminotransferase (AST). The data were subjected to one way analysis of variance using SPSS 17 testing whether there is significance ($P < 0.05$) between treatments. Because of a skewed distribution, FEC and TWC were analysed on logarithm transformed data (Snedecor and Cochran, 1989). For example,

$$\text{LFEC} = \log_{10} (\text{FEC} + 25); \text{LTWC} = \log_{10} (\text{TWC} + 1).$$

5.2.12 Estimation of anthelmintic efficacy

The anthelmintic efficacies were estimated through percentage faecal egg count reduction (FECR%) and post-mortem worm count reduction percentages. The FECR% was calculated using the following equation:

$$\text{FECR}\% = (1 - (T_2/T_1 \times C_1/C_2)) \times 100.$$

Where, T and C are the arithmetic means of the eggs per gram of faeces for the treated and control groups and subscripts 1 and 2 designate the counts before and after treatment, respectively (Campbell *et al.*, 1978; Presidente 1985). The confidence interval for the albendazole reduction was calculated according to the formula by Coles *et al* (1992) to find out whether there was resistance as follows:

$$95\% \text{ CI limits; upper limit} = 100[1 - \bar{Y}_t/\bar{Y}_c \exp (-2.048\sqrt{Y^2})] \text{ and lower limit} = 100[1 - \bar{Y}_t/\bar{Y}_c \exp (+2.048\sqrt{Y^2})]$$

Where, \bar{Y}_t and \bar{Y}_c are the arithmetic means of the treated and control groups respectively, and Y^2 is the variance of the reduction (log scale).

The percentage total worm count reduction (TWCR%) was calculated using the formula:

$TWCR\% = (1 - TWC_t / TWC_c) \times 100$ (Wood *et al.*, 1995), where the subscripts t and c designate the treatment and the untreated control groups respectively. The same formula was also used for the percentage differential worm count reductions

5.3 Results and Discussion

5.3.1 Field anthelmintic clinical efficacy trial

The results of the faecal egg count reduction test (FECRT) indicated that only the *Myrsine africana* remedy and albendazole had anthelmintic efficacy at 58.9% and 87.2% respectively as shown on Table 5.3. However, the FECR by albendazole was less than 95% and a 95% confidence level of more than 90%, making it suspect for resistance (Coles *et al.*, 1992).

The results for the *Myrsine africana* anthelmintic remedy looked promising and together with others, became the subject of a further study under controlled conditions that followed this field clinical trial. The genera of the gastrointestinal nematodes, cultured from pooled faecal samples, remained the same in all the groups before and after the treatments. They were *Haemonchus* (80%), *Trichostrongylus* (17%) and *Oesophagostomum* (3%).

Table 5.3: Anthelmintic efficacy against natural infection of mixed gastrointestinal nematodes of sheep in Loitoktok District

Treatment Group	Arithmetic mean eggs per gram \pm SD (Range)				
	Before treatment (day 0)	After treatment (day 11)	FECR%	95% CI (Albendazole)	Remarks
A	238 \pm 220 (100-500)	1029 \pm 559 (200-1700)	-11.3	-	No efficacy
E	238 \pm 104 (100-400)	1213 \pm 978 (100-2800)	-31.1	-	No efficacy
M	438 \pm 196 (100-1100)	700 \pm 673 (0-2000)	58.9	-	Some efficacy
V	357 \pm 1289 (100-1400)	200 \pm 115 (0-300)	87.2	67.9 – 94.9	Suspects resistance
C	400 \pm 509 (100-1500)	1543 \pm 1611 (100-4800)	0	-	Untreated control

Key: A = *Albizia anthelmintica*; AR = mixture of *Albizia anthelmintica* and *Rapanea melanophloeos*; E = *Embelia schimperi*; M = *Myrsine africana*; R = *Rapanea melanophloeos*; V = Valbazen (albendazole); C = Untreated control; SD = Standard deviation; FECR % = Percentage faecal egg count reduction and CI = Confidence interval for the reduction

5.3.2 Controlled anthelmintic clinical efficacy trial

5.3.2.1 Effects of anthelmintic treatments on general health of the animals

Some irritation and coughing followed by transient bloating occurred in animals dosed with *A. anthelmintica* preparation but this was over in about 12 hours. However, these animals slightly reduced feed intake during the following week but went back to normal thereafter. A few animals developed bottle jaw in groups A, AR, C and V. Two animals died of conditions unrelated to helminthosis (one in group AR of pneumonia and one in group V of GIT blockage by numerous phytobenzoars that were encountered on post mortem). Otherwise the parameters of general health of all the other animals remained within the normal range. Transient bloating of sheep treated with aqueous extracts of *A. anthelmintica* in a similar study were reported by Githiori (2004). In the same study it also caused deaths in mice, infected with *Heligmosomoides polygyrus*, when given at the dose of 33 g/Kg body weight. Gakuya (2001) also reported deaths in all groups of mice infected with *H. polygyrus* and treated with 5, 10 and 20 g/Kg body weight of methanolic extracts of *A. anthelmintica*. *Albizia anthelmintica* in this study was found to have high amounts of saponins. Saponins, which are triterpenoids, have been considered responsible for reducing food intake, causing nutritional deficiencies, hemolysis and extreme cases death of herbivores (Applebaum and Birk, 1979; Milgate and Roberts, 1995).

5.3.2.2 Effects of anthelmintic treatments on faecal egg counts

The faecal egg counts (FEC), before and 10 days post treatment, and FECR% in the artificially infected Dorper lambs are displayed on Table 5.4, while the changes up to day 35 are shown on figure 5.2. The FEC varied from 0 to 10,700 in the treated groups while in the untreated control group it varied from 200 to 6,100 on day 10. The FECR% was -55, 25.8, 7.6, 34.2, 69.3 and 83.3% for *Albizia anthelmintica*, mixture of *A. anthelmintica* and *R. melanophloeos*, *Embelia schimperi*, albendazole and *Myrsine africana* respectively. The *Myrsine africana* remedy had the best FECR of 83% even surpassing that of albendazole and the FECR of 59% obtained during the field trial in Loitoktok District. It was also the only group that had significantly ($P < 0.05$) fewer epg than the untreated control group. This FECR is slightly higher but comparable to the FECR of 77% reported by Gathuma *et al.* (2004) in sheep naturally infected with mixed GIN in Samburu County; though, the sheep in the Samburu study had much lower mean pretreatment epg (300) than in the current study (2283). The Samburu study also reported 100% efficacy against *Monezia* tapeworms (Gathuma *et al.*, 2004). Further, extracts from the fruits of this plant have been reported to have good efficacy against the cestode *Taenia solium* and the nematodes *Bunostomum trigonocephalum* and *Oesophagostomum columbianum* (Kakrani and Kalyani, 1983). *Rapanea melanophloeos* and *E. schimperi* (both Myrsinaceae) had insignificant FECR and this result for all the three plants is consistent with another study done in Kenya by Githiori (2004) using Dorper lambs artificially infected with a monoculture of *H. contortus*.

Table 5.4: Anthelmintic efficacy against artificial infection of mixed gastrointestinal nematodes in sheep

Group	Arithmetic mean epg \pm SD (Range)		FECR %	95% CI (Albendazole)	Remarks
	Pre- treatment (day 0)	Post- treatment (day 10)			
A	2067 \pm 2775 (100-7400)	2817 \pm 4129 (300-10700)	-55		No efficacy
AR	2400 \pm 3165 (100-7600)	1560 \pm 3213 (0-7300)	25.8		Little efficacy
E	2100 \pm 3005 (100-7900)	1700 \pm 2032 (0-5400)	7.6		Negligible efficacy
M	2283 \pm 5007 100-12500)	333 \pm 137 (200-500)	83.3		Good efficacy
R	1417 \pm 1522 (100-3400)	817 \pm 588 (0-1500)	34.2		Some efficacy
V	8725 \pm 15360 (200-31700)	2350 \pm 3813 (0-8000)	69.3	-560.8 – 75.3	Resistance
C	2100 \pm 3813 (100-8900)	1840 \pm 2520 (200-6100)	0		Untreated control

Key: A = *Albizia anthelmintica*; AR = mixture of *Albizia anthelmintica* and *Rapanea melanophloeos*; E = *Embelia schimperi*; M = *Myrsine africana*; R = *Rapanea melanophloeos*; V = Valbazen (albendazole); C = Untreated control; FECR % = Percentage faecal egg count reduction and CI = Confidence interval for the reduction).

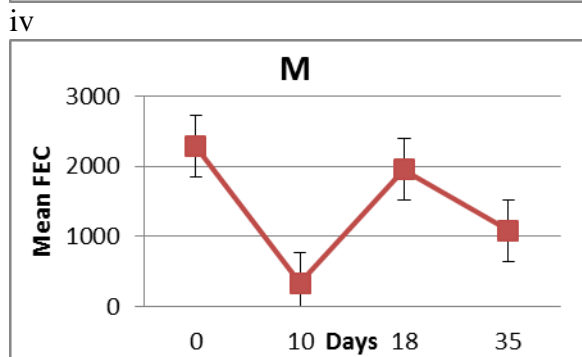
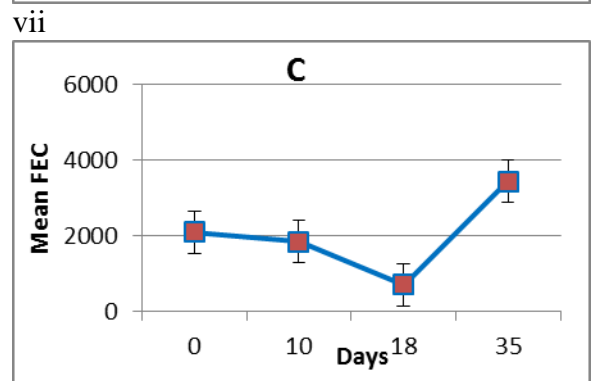
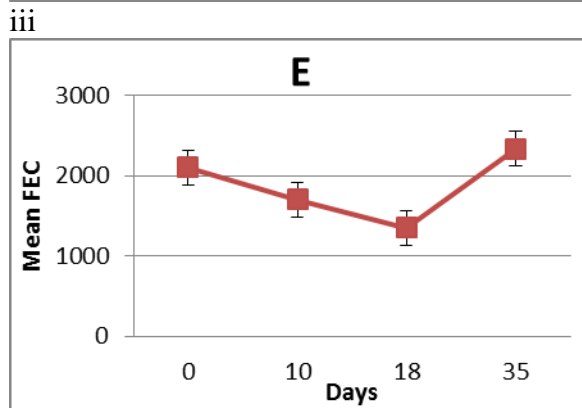
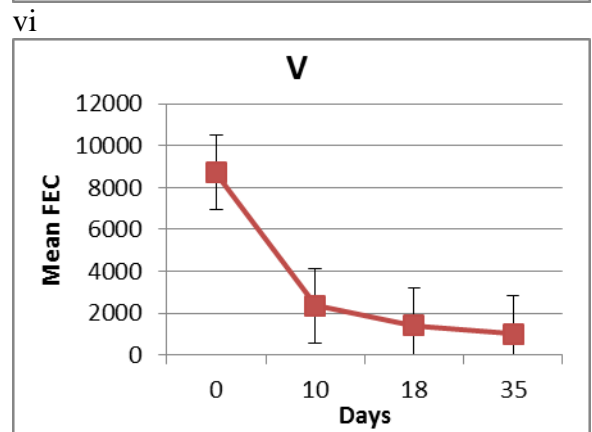
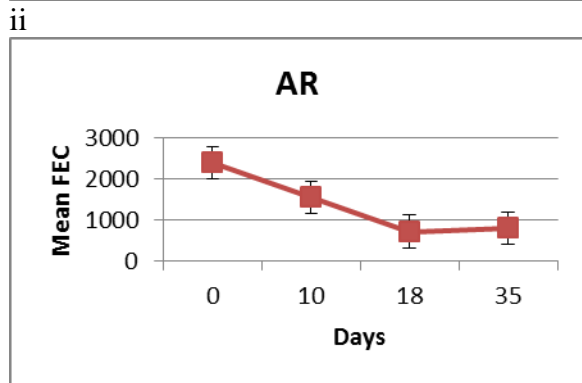
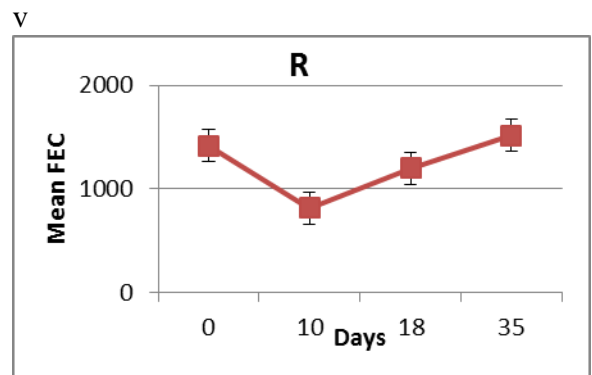
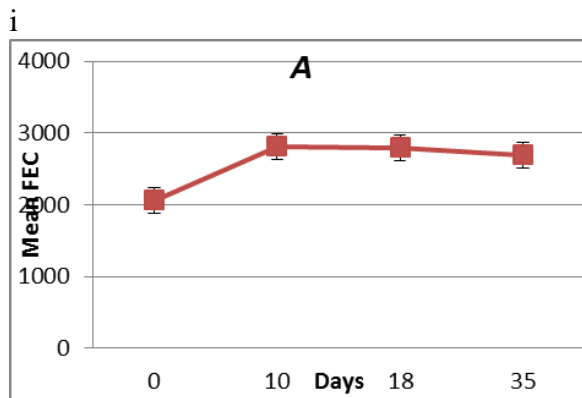


Figure 5.2 (i-vii) Effect of anthelmintic treatment on the faecal egg counts (FEC) in sheep artificially infected with mixed gastrointestinal nematodes

Key: A: *Albizia anthelmintica*; AR: *Albizia anthelmintica*+*Rapanea melanophloeos*; E: *Embelia schimperi*; R: *Rapanea melanophloeos*; M: *Myrsine africana*; V: Valbazen (albendazole); C: Untreated control

The *A. anthelmintica* group was the only one that had higher mean eggs per gram of faeces than its pre-treatment levels and the untreated control. This increase in FEC could possibly have been a result of higher concentration in faeces due to the observed transient bloating and reduction in feed intake in the days post treatment of the animals. However, the Samburu study by Gathuma *et al.* (2004) reported high FECR of 89.8% using about 26.5g of the root bark of *A. anthelmintica* per adult sheep naturally infected with mixed GI nematodes while the current study used about 37.5g of stem bark from Loitoktok per animal. The differences in the reported efficacies could be as a result of the variation in the dosages given or the phytochemical composition of the plants obtained from different areas and ecosystems and also the methodologies, and possibly the composition and species of GI nematodes in the study animals (Athanasiadou *et al.*, 2005; Tzamaloukas *et al.*, 2005). Variable conditions of collection and storage of the plants have also been shown to affect the physical and chemical properties of the plant secondary metabolites (PSM) and probably their bioactivity as well. In addition, seasonal and environmental variability will have an impact on the synthetic pathways of the PSM, which can potentially affect their physical and chemical properties (Mueller-Harvey and McAllan, 1992).

The FECR for albendazole of 69% with a 95% CI of -560.8 to 75.3 is an indication of resistance by the GIN used to artificially infect the animals in this study. In the earlier field trial, resistance was only suspected because, though the FECR was less than 95%, the upper 95% CI was more than 90%. However, in this case resistance is confirmed because both conditions, FECR <95% and CI < 90% have been met (Coles *et al.*, 1992).

5.3.2.3 Effect of anthelmintic treatments on live weight, haematological and biochemical parameters

The changes in the animal live weights and PCV in relation the different treatment groups are displayed in figures 5.3 and 5.4 respectively. All the groups lost live body weights by a mean of 0.4 and 2.4 Kg by day 35. However, only the loss by group A (*A. anthelmintica*) was statistically significant ($P < 0.05$) compared to the control group. This could possibly have been due to the reduced feed intake that was accompanied by bloating immediately after treatment. This could also explain the increase in the mean FEC as compared to the rest of the groups as discussed. There were no statistically significant ($P > 0.05$) changes on all the haematological and biochemical parameters analyzed. However, the PCV levels dropped in all other groups of animals by day 8 except the *M. africana* group that increased marginally by 0.9%. By day 21 of treatment the PCV levels improved marginally except in groups A and R and again the *M. africana* treatment had the highest improvement of 2.2% of the pretreatment levels. This could mean that apart from reducing GI nematodes burdens in the parasitized animals, it could also be having stimulatory effects on the haemopoietic tissue or even other body systems leading to increased resistance and even resilience. There is recent evidence suggesting that the consumption of medicinal plants or plant extracts has improved the immune response (immunomodulatory effects) of parasitized hosts, by increasing the number of specific effector cells (Huffman *et al.*, 1997; Niezen *et al.*, 2002; Tzamaloukas *et al.*, 2006).

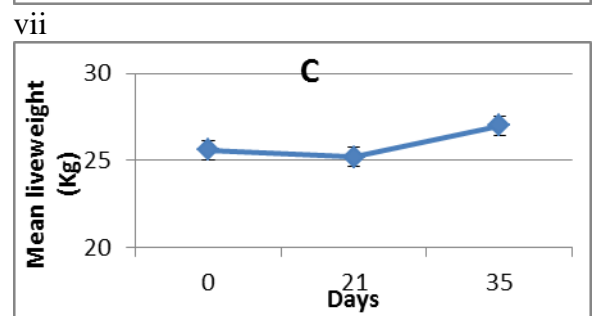
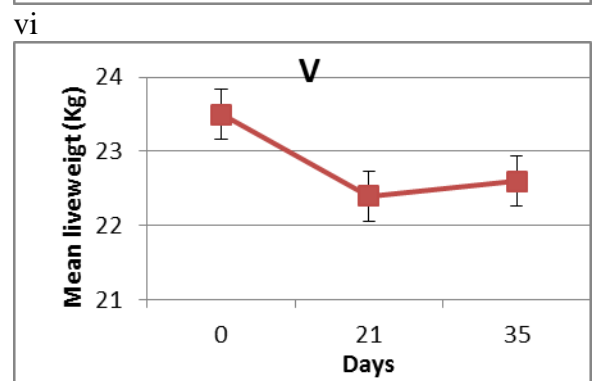
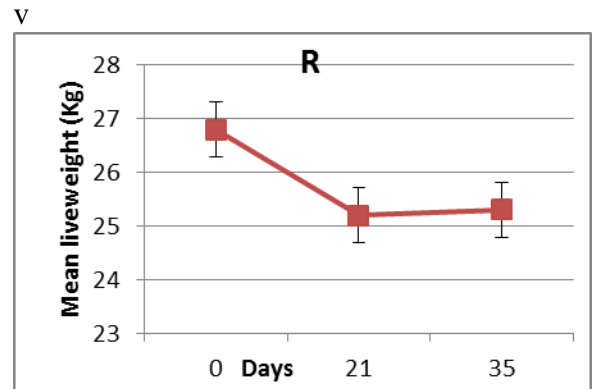
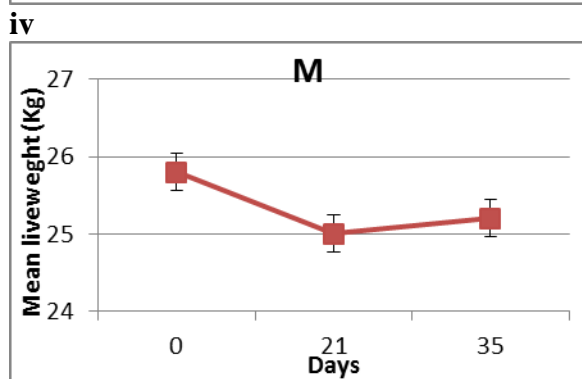
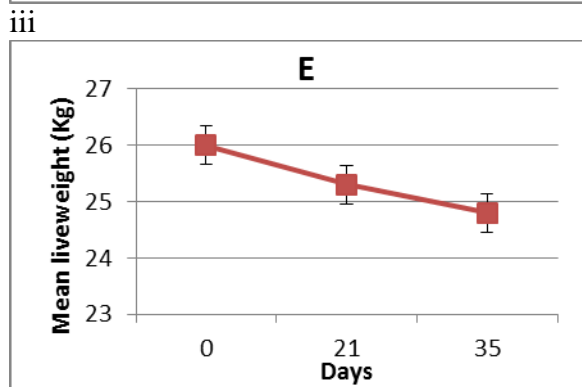
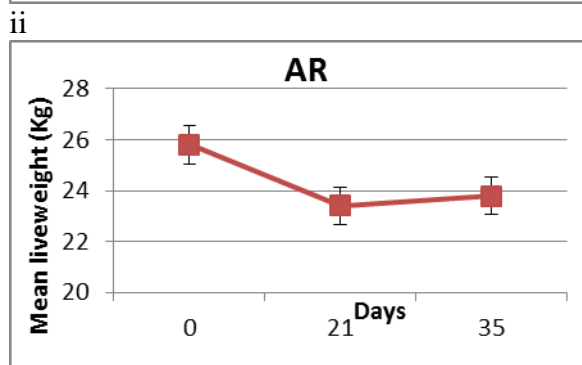
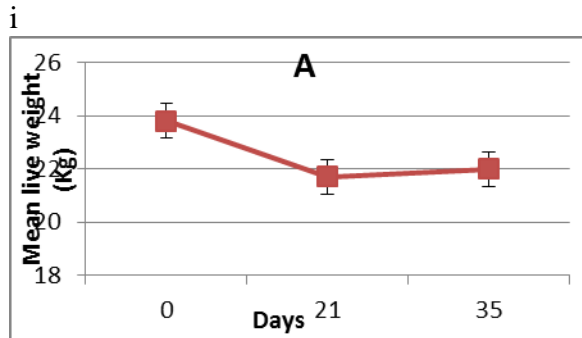


Figure 5.3. (i-vii) Effect of anthelmintic treatment on the liveweight of sheep artificially infected with mixed gastrointestinal nematodes

Key: A: *Albizia anthelmintica*; AR: *Albizia anthelmintica*+*Rapanea melanophloeos*; E: *Embelia schimperi*; R: *Rapanea melanophloeos*; M: *Myrsine africana*; V: Valbazen (albendazole); C: Untreated control.

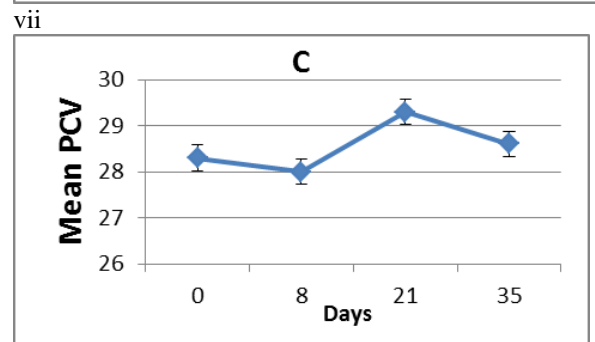
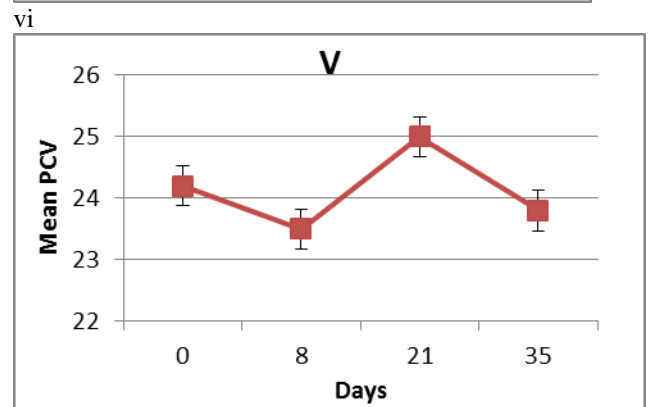
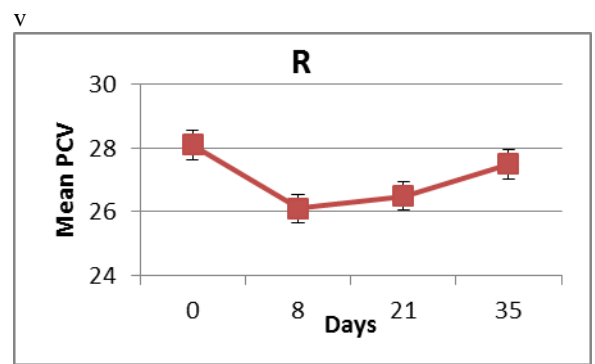
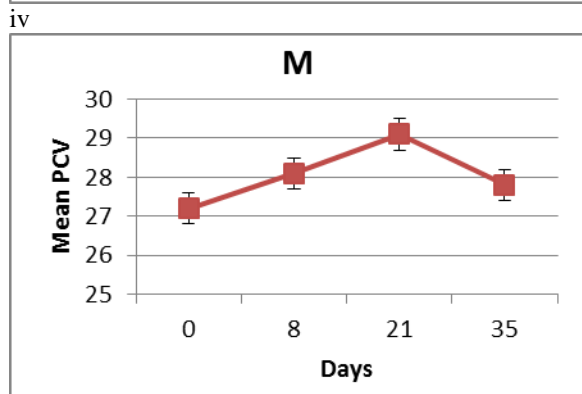
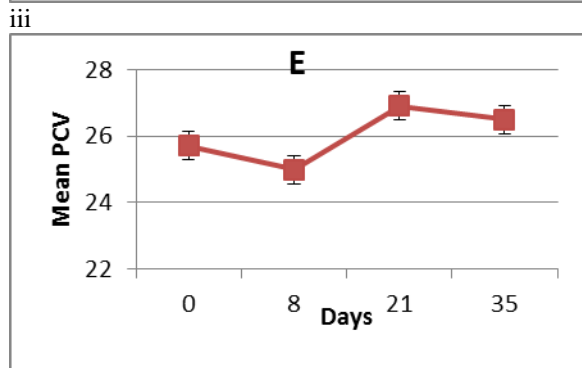
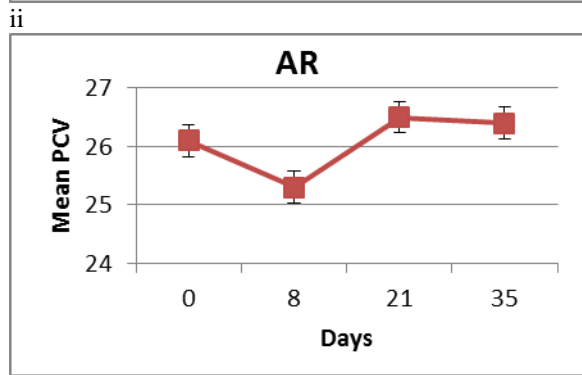
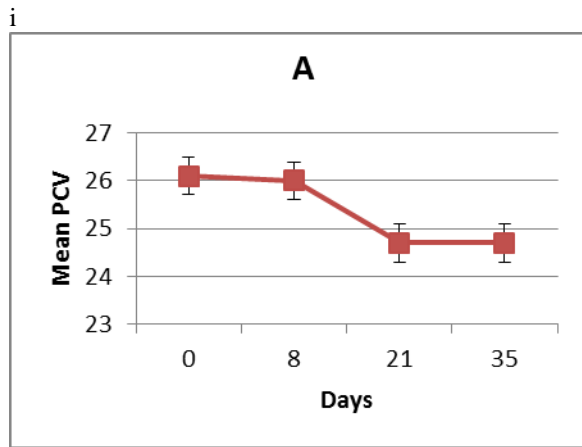


Figure 5.4. (i-vii) Effect of anthelmintic treatment on the packed cell volume (PCV) of sheep artificially infected with mixed gastrointestinal nematodes

Key: A: *Albizia anthelmintica*; AR: *Albizia anthelmintica*+*Rapanea melanophloeos*; E: *Embelia schimperi*; R: *Rapanea melanophloeos*; M: *Myrsine africana*; V: Valbazen (albendazole); C: Untreated control

5.3.2.4 Effect of anthelmintic treatments on total and differential worm count

The total and differential worm counts and percentage reductions are shown in Table 5.5. The TWCR% of 60.7, 55, 44.6, 66, 69.7 and 35.6 percent were recorded for groups A, AR, E, M, R and V respectively. All the treatments caused a reduction of TWC but only those of *R. melanophloeos*, *M. africana* and *A. anthelmintica* were significant ($P < 0.05$). In addition these three treatments had significant activity against *H. contortus* with reductions ranging from 73 to 78%. *A. anthelmintica* (Fabaceae) showed substantial activity against the abomasal *T. axei* but not the intestinal *T. columbriformis* or any other intestinal nematode. However, *M. africana* and *R. melanophloeos* (both Myrsinaceae) had little or no activity at all on the intestinal nematodes except for 100% reduction of *T. ovis* in the case of *R. melanophloeos* and *E. schimperi*. Moreover, the *T. ovis* numbers were so few across the groups for any meaningful deductions to be made in this study.

The high TWCR (70%) for *R. melanophloeos* compared to its low faecal egg count reduction of 34% can only be speculated. One possible reason would be that the active phytochemicals in this plant only exert a quick effect on the worms like paralyzing them but no longterm effect on the surviving ones which then continue laying eggs normally or probably have a selective effect on the male worms. The former explanation is further supported by the the fact that FEC for this treatment group increased faster than all the others and by day 35 it had a mean FEC of 4100 against the runners up (group AR) with 2680 epg.

Table 5.5: Effect of anthelmintic treatment on the total and differential worm counts in sheep artificially infected with mixed gastrointestinal nematodes

Nematode species	Treatment groups						
	C	A	AR	E	M	R	V
<i>Haemonchus contortus</i>	809	222 (73)	337 (58)	422 (48)	198 (76)	176 (78)	630 (22)
<i>Trichostrongylus axei</i>	8	3 (63)	0 (100)	0 (100)	10 (-25)	5 (38)	1 (84)
<i>Trichostrongylus columbriformis</i>	21	49 (-127)	20 (7)	31 (-45)	42 (-95)	15 (32)	0 (100)
<i>Oesophagostomum columbianum</i>	147	112 (24)	86 (41)	93 (57)	85 (43)	103 (30)	3 (98)
<i>Trichuris ovis</i>	0.4	3 (-525)	0.2 (50)	0 (100)	0.5 (-25)	0 (100)	0 (100)
Mean nematode worm count	986	389 (61)	444 (55)	546 (45)	336 (66)	299 (70)	634 (36)
± SD	±1385	±315	±752	±616	±407	±209	±816

Key: A = *Albizia anthelmintica*; AR = mixture of *Albizia anthelmintica* and *Rapanea melanophloeos*; E = *Embelia schimperi*; M = *Myrsine africana*; R = *Rapanea melanophloeos*; V = Valbazen (albendazole); C = Untreated control; SD = Standard deviation

Numbers in brackets represent the percentage total worm count reduction

Embelia schimperi (Myrsinaceae) also showed some activity on *H. contortus*, *T. axei* and *O. columbianum*. It is only *R. melanophloeos* and the AR combination treatment of *A. anthelmintica* and *R. melanophloeos* that had activity across all the nematode species. The significant TWCR by *A. anthelmintica* further supports the earlier observation that the negative FECR value could have been a result of concentration in faeces due to the reduced feed intake other than increased fecundity by the GI nematodes.

The differences in activity of various plant remedies on different nematode species can be explained by the possible variations in the active phytochemicals in the different plants as shown on Table 4.1 (chapter 4). Furthermore, other issues like bioavailability of the active compounds at different parts of the gastrointestinal tract (GIT), the host-plant interactions and the parasite specificity could explain why some plants are more active against specific parasite species and not others (Athanasiadou *et al.*, 2005; Tzamaloukas *et al.*, 2005). This might be related to the parasite niche or the bioavailability of the compound in the different compartments of the GIT of the parasitized host (Athanasiadou *et al.*, 2007). Condensed tannins, for example, have been shown to form complexes with macromolecules, such as proteins (Mueller-Harvey, 2006). Due to physiological conditions, tannins are expected to be in complexes in the abomasum of parasitized hosts and hence unavailable to exert their anthelmintic activity there but may do so in the intestine, though poorly, when animals are on high protein diets (Athanasiadou *et al.*, 2001).

The TWCR value of only 36% for albendazole further confirms its resistance by the GI nematodes used in this study and especially by *H. contortus* whose reduction by the albendazole

used was only 22%. According to the criterion set by the WAAVP, resistance is declared when TWCR is less than 90% (Wood *et al.*, 1995). Resistance in human and animal pathogenic helminths has been spreading in prevalence and severity to a point where multidrug resistance against the 3 major classes of anthelmintics (benzimidazoles, imidazothiazoles and macrocyclic lactones) has become a global phenomenon in GI nematodes of farm animals (Kaplan, 2004; Jabbar *et al.*, 2006; Kaminsky *et al.*, 2008). In Kenya, resistance has mainly been reported in institutional farms which also happen to be the source of breeding stock for other smaller farms with potential danger of spreading this problem (Wanyangu *et al.*, 1996; Waruiru *et al.*, 1998; Gakuya *et al.*, 2007). The source of the GI nematode parasites used to infect sheep in this study is an institutional farm just like the ones mentioned by the previous authors.

5.4 Conclusion

The results of this study have clearly shown some of the plant anthelmintic remedies used in Loitoktok District of Kajiado County like *M. africana* have good efficacy at safe levels and could continue to be used with satisfactory outcomes. However, some of them like *A. anthelmintica* may be toxic at the same levels that they could be effective against GI nematodes. The GI nematodes used in this study were resistant to albendazole and that the farm of origin and the herders of Loitoktok should be advised accordingly. Further studies are necessary to properly evaluate any possible adverse effects and the most optimum dosages, especially against such resistant strains of GI nematodes, for the very efficacious *M. africana*. The actual phytochemicals responsible for this anthelmintic activity and their possible modes of action also need to be further investigated with a view of formulating a novel anthelmintic product.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

The first objective of this study was to document plants, which are commonly used in the treatment and control of helminthosis in Loitoktok District of Kajiado County in Kenya. The other objectives were to determine the phytochemistry, bioactivity and anthelmintic efficacy of herbal remedies made using selected plants from the study area. The ethnopharmacological study documented 81 medicinal plants belonging to 46 families and 71 genera for the treatment and control of various diseases and conditions in both human and animals. However, the six most important families by their medicinal use values in decreasing order were Rhamnaceae, Myrsinaceae, Oleaceae, Liliaceae, Usenaceae and Rutaceae. The results of the current study are in concurrence with the observation that ethnopharmacological / ethnobotanical surveys are more successful and cost effective in identifying plants with biological activity than random collections, taxonomic or chemical relationships between plants (Farnsworth *et al.*, 1985). This becomes even better when targeted selection of informants is done to obtain specialist information (Martin, 1995).

The results of the phytochemical screening indicated that the plants contain alkaloids, anthraquinones, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids. All these constituents are known to exhibit medicinal and physiological activity (Sofowora, 1993). The anthelmintic activity exhibited by the plant remedies is possibly due to individual phytochemicals or a combination of them through similar or different pathways but this can only

be elucidated by further research. Lactones such as santonin have been shown to have a strong anthelmintic activity against *Ascaris* and other nematode species of livestock and humans (Waller *et al.*, 2001). Alkaloids have also exhibited strong nematocidal activity against *Strongyloides ratti* and *Strongyloides venezuelensis*, two rat nematodes used as models for human nematodes (Satou, *et al.*, 2002). The nematocidal activity of tannins has been reported as early as the 1960s (Taylor and Murant, 1966), and more recently evidence on the anthelmintic properties of condensed tannins has been supported by a series of *in vitro* (Dawson *et al.*, 1999; Athanasiadou *et al.* 2001; Molan *et al.*, 2003b; Ademola and Idowu, 2006) and *in vivo* studies (Athanasiadou *et al.*, 2000; Butter *et al.*, 2001; Paolini *et al.*, 2003a and 2003b). However, it should be noted that these same phytochemicals, either in plant parts or extracts, can have detrimental effects on animals and humans if consumed in high doses. For example, excessive consumption of tannins, which are polyphenolic compounds, has been associated with reduction of food intake and digestibility, impairment of rumen metabolism and mucosal toxicity (Hageman and Butter, 1991; Rittner and Reed, 1992; Reed, 1995; Dawson *et al.*, 1999). Saponins, which are triterpenoids, have been considered responsible for reducing food intake, causing nutritional deficiencies, hemolysis and extreme cases death of herbivores (Applebaum and Birk, 1979; Milgate and Roberts, 1995). Excessive consumption of cyanogenic glycosides, terpenes or alkaloids can result in neurological and other defects (Conn, 1979). The plant *Albizia athelmintica* in this study contained alkaloids, glycosides, flavonoids, steroids, triterpenoids and particularly high amounts of saponins in both aqueous and organic extracts. It was also the only treatment that produced noticeable adverse effects during the controlled study. Indeed during the pre-trial stage of the study one sheep died suddenly of severe respiratory embarrassment when

given 4.4 grams of freeze dried aqueous extracts of stem bark. The traditional healers in Loitokitok District had indeed cautioned us of its toxicity and further observed that certain subspecies growing in certain areas were known to be more toxic than others. The traditional signal to alert would be users of medicinal plants known to have resulted in deaths during treatment was to fence around those particular plants.

Other effects of medicinal plants on animals, besides being antiparasitic and antinutritional, should also be taken into account when considering their use in parasite control. In this study the remedy *Myrsine africana*, besides having the best FECR and second best TWCR, also had the least weight loss and improved the PCV by 2.2% by day 21 after treatment an indication of other stimulatory effects on the animals. An emerging aspect of the potential benefits of medicinal plants is their contribution towards the development of host resistance to parasites. Evidence suggests that the consumption of medicinal plants or their extracts can improve the immune response of infected animals by increasing the number of effector cells (Niezen *et al.*, 2002; Tzamaloukas *et al.*, 2006). There is also the possibility that the improvement in the immunity is generic, which would offer an added benefit to the well being of the host and this is an area that needs to be further investigated. There is no published report of the development of resistance to any type of medicinal plants (Athanasiadou *et al.*, 2007). It is possible that due to the lower anthelmintic efficiency of plants compared with the synthetic anthelmintic drugs; selection pressure on the resistant parasite population is not strong enough. Alternatively, anthelmintic drugs seem to exert anthelmintic activity through a single mechanism in the parasites (Geary, 2005), whereas plant compounds may demonstrate a variety of effects, due to their multiplicity

of bioactive phytochemicals. Consequently, it would be expected that resistance would develop at lower rates, compared to anthelmintic drugs, if it developed at all. Determination of the mechanisms of action of specific compounds will greatly contribute towards making safe assumptions on the development of resistance to medicinal plants (Athanasiadou *et al.*, 2007).

The interpretation of the observed inconsistencies in the efficacy of medicinal plants between different controlled studies and also claims by traditional practitioners is not straightforward. In some cases it may be due to misinterpretation of facts by local communities, often due to lack of scientific knowledge. For example, traditional healers are not always familiar with the parasite species that are most pathogenic. In a participatory study in Northern Kenya, traditional healers identified plants as being very effective anthelmintics if they expelled tapeworm segments (Githiori *et al.*, 2004). The latter are easy to identify, as they are visible to the naked eye, but not as pathogenic as helminth nematodes, whose both parasitic and non-parasitic stages require specialized knowledge and equipment to be identified. In other cases observed inconsistencies might be due to methodological variations, for example *in vitro* versus *in vivo*, different animal models, different sources of the plant materials, collection, storage and preparations. Furthermore, compound bioavailability and thus efficacy of medicinal plants may also be related to the host species. For example, in study by Paolini *et al* (2003a) condensed tannins did not have any effects on the abomasal worm burden in sheep but did in goats, probably because a number of physiological adaptations have taken place in the gastrointestinal tract of goats to counteract the presence of plant secondary metabolites in the browse material (Hoste *et al.*, 2006).

In the field trial in Loitoktok District, albendazole was suspected for resistance by the gastrointestinal nematodes in sheep. From the results of the controlled trial the GI nematodes used were completely resistant to albendazole and especially *H. contortus* having met all three indicators by WAAVP, for the confirmation of resistance namely, FECR of less than 95% and confidence interval of less than 90%, and TWCR of less than 90% (Coles *et al.*, 1992; Wood *et al.*, 1995). Resistance in human and animal pathogenic helminths has been spreading in prevalence and severity to a point where multidrug resistance against the 3 major classes of anthelmintics (benzimidazoles, imidazothiazoles and macrocyclic lactones) has become a global phenomenon in GI nematodes of farm animals (Kaplan, 2004; Jabbar *et al.*, 2006; Kaminsky *et al.*, 2008). In Kenya, resistance has mainly been reported in institutional farms which also happen to be the source of breeding stock for other smaller farms with potential danger of spreading this problem (Wanyangu *et al.*, 1996; Waruiru *et al.*, 1998; Gakuya *et al.*, 2007). The source of the GI nematode parasites used to infect sheep in this study is an institutional farm just like the ones mentioned by the authors. From the findings of this study there is an opportunity to further evaluate the efficacious herbal remedies against these albendazole resistant gastrointestinal nematodes.

6.2 Conclusions

The current study concludes the following:

1. Loitoktok District is endowed with a wide variety of medicinal plants that are crucial for the primary health care of both human and animals
2. Traditional healers in Loitoktok largely depend on naturally growing medicinal plants in the wild which are rapidly becoming scarce due to continued subdivision of communal lands and expanding agricultural and other anthropogenic activities
3. Anthelmintic medicinal plants used in Loitoktok District are rich in phytochemicals which could be responsible for their cytotoxicity/bioactivity.
4. *Myrsine africana* used in anthelmintic remedies in Loitoktok District had significant ($P<0.05$) FECR and TWCR of 83.3 and 66% respectively in sheep artificially infected with mixed gastrointestinal nematodes.
5. *Albizia anthelmintica* and *Rapanea melanophloeos* had significant ($P<0.05$) TWCR of 61 and 70% respectively but had insignificant effects on FECR in sheep artificially infected with mixed gastrointestinal nematodes
6. *Albizia anthelmintica* from Loitoktok District caused reduced feed intake and weight gain in sheep fed with aqueous extract at a dose equivalent to 3 grams of the freeze dried extract of the stem bark
7. The gastrointestinal nematodes used to artificially infect sheep in this study were resistant to albendazole and especially *H. contortus*.
8. Brine shrimp bioassay may be used in isolation of bioactive compounds from the studied plants

6.3 Recommendations

The current study recommends the following:

1. There should be concerted efforts by the relevant sectors to educate and help the people of Loitoktok District to rehabilitate and conserve the rapidly disappearing medicinal plants and traditional knowledge
2. The bioactive extracts should be further characterized with bioassay guided fractionation with the view of isolating novel anthelmintic compounds and determining their mode of action
3. *Myrsine africana* remedy should be further evaluated to determine the most optimum dosage and especially against these resistant strains of gastrointestinal nematodes

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APPENDIX

Questionnaire administered to herbalists in Loitoktok district

ETHNOPHARMACOLOGICAL SURVEY FOR ANTHELMINTIC AND OTHER MEDICINAL HERBAL REMEDIES IN LOITOKTOK DISTRICT OF KENYA

Questionnaire serial no..... Area.....

Name of the interviewer..... Date.....

PART ONE: CONSENT

A. RESEARCHERS' DECLARATION

1. The following research will be undertaken with recognition and all due respect for the indigenous knowledge and the rights of the traditional health practitioners
2. The information shall at no time be obtained from the respondents by intimidation, coercion or false pretence
3. The researchers shall be under no obligation to edit or tamper with the information provided by the respondents
4. The information collected will be used for the intended research and not for any other undisclosed purposes

Name and Signature of Researcher(s):

1.
2.
3.

B. RESPONDENT'S CONSENT AGREEMENT

The undersigned agrees to participate in this study out of own free will and further declare that the information provided shall only be true, accurate and complete to the best of their knowledge

Signature/Thumb print..... Date.....

PART TWO: HERBAL PRACTICE AND PRACTITIONERS

A. BIODATA

Name..... Age (yrs)..... Gender.....

Type of practice (Veterinary, human or both).....

Location of practice..... in..... Division

Number of years in practice.....
Cultural background.....
Religion.....
How was this knowledge and skills acquired.....
Formal education (None; Primary; Secondary; Other.....)
Contact (Telephone).....

B. COLLECTION AND PREPARATION OF HERBAL REMEDIES

i) Signs and symptoms observed to arrive at a diagnosis of helminthiosis.....
.....
.....
.....

ii) Which plants are used to treat helminthosis

Vernacular name	Botanical name	Plant availability	Part(s) used and state of harvest	Preparation of the remedy	Dosage given	Other diseases treated with plant

iii) Which of the plants/remedies above are most commonly used in the treatment of helminthosis.....

.....

iv) Are the plants growing in the wild, cultivated or both.....

- v) If wild, are they readily available.....
.....
- vi) If cultivated, how easy are they to establish and how long do they take to mature.....
.....
.....
- vii) How is the medicine/remedy prepared.....
.....
.....
.....
.....
.....
.....
.....
.....
.....
- viii) What is the shelf life of the preparation.....
- ix) How is the preparation administered to the animal.....
.....
.....
- x) What antidote is given in case of overdose.....
.....
- xi) What are the common challenges encountered in the practice of herbalism.....

.....
.....
xii) Does the traditional health practitioner belong to any association of healers?
(Yes or no).

xiii) Do traditional healers in the area share knowledge and experiences with their
colleagues? (yes or no)

xiv) Other plants commonly used for the treatment of various diseases in the area

Vernacular name of plant	Botanical name	Plant part(s) used	Disease(s) treated