ANTHELMINTIC EFFECTIVENESS OF SELECTED MEDICINAL PLANTS USED IN TREATING SHEEP HELMINTHIASES IN KOIBATEK AND MOGOTIO SUB COUNTIES, BARINGO COUNTY, KENYA

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DECLARATION

To the best of my knowledge, the material contained in this thesis is my original work and has not been presented for a degree or for other awards in any other University.

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DEDICATION

This work is dedicated to the resident farmers of Koibatek and Mogotio sub-counties, Baringo County who immensely contributed their knowledge on the medicinal plants used in treating sheep helminthiases and allowing me to document them.

I also dedicate this work to my loving wife, Naomi, my children, Joy, Ryan and Lynne for their patience, moral support, financial assistance and encouragement through the entire period of the study.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AR	Anthelmintic Resistance
ASAL	Arid and Semi Arid Land
BZ	Benzimidazole
CE	Cold method of aqueous extracts of plant material
CT	Condensed Tannins
EAH	East African Herbarium
g	Grams
HE	Hot method of aqueous extracts of plant material
L3	Larval instar 3 (infective larval stage)
KALRO	Kenya Agricultural and Livestock Research Organization
Kg	Kilogram
Ml	Milliliters
Nacl	Sodium chloride (table salt)
NACOSTI	National Council for Science, Technology and Innovation

Rpm	Revolution per minute
Mg/µl	Milligram per microlitre
SD	Standard Deviation
WAAVP	World Association for the Advancement of Veterinary Parasitology

ABSTRACT

Helminthiasis is one of the most important diseases worldwide that cause heavy production losses in livestock. The disease is prevalent all over the world especially in developing countries and associated with poor management practices, lack of access to conventional anthelmintic drugs as a control or curative strategy and also greatly hampered by drug resistance exhibited by parasites. Farmers therefore resort to traditional medicinal plants for helminthiases treatment which lack information on their effectiveness, toxicity levels, dosages and safety. The current study aimed to determine anthelmintic effectiveness of six selected medicinal plants used in the traditional management and treatment of sheep helminthiases in Koibatek and Mogotio sub counties, Baringo County, Kenya. Field work was conducted in nine administrative units of Koibatek and Mogotio sub-counties. Demographic information on age and sex of informants was collected to check the existing knowledge and attitude on the use of medicinal plants. Further, field work was conducted to assess the prevalence of helminths in the two sub counties. Anthelmintic activities of six selected medicinal plants were tested at KALRO - Muguga North Laboratories *in-vitro* system using eggs and larvae of *Haemonchus contortus.* Five concentrations (6.25 mg/ul, 12.5 mg/ul, 25 mg/ul, 50 mg/ul and 100 mg/ul) of methanolic extract were tested, which involved determination of egg hatching and larval development. Levamisole (10mg/ml) was included as positive control and distilled water as negative control. The results indicated that out of 130 respondents interviewed, 49 out of 83 men and 23 out of 47 female had knowledge on the use of medicinal plants but there was no significant association in the knowledge of medicinal plant with the gender (X^2=63.33, d.f=48; P=0.068). Methanolic and water extracts from the six medicinal plants under investigation, showed biological activities in egg hatching and larval development in varying concentrations as compared to positive and negative controls. The findings indicated a significant difference in mean of eggs hatched (F = 65.31; P = 0.0001) in varying methanolic concentrations with the lowest concentration being significantly different from negative controls. *Olea capensis* displayed the least mean of eggs hatched (mean 1.00±1.00 larvae); followed by *Leucas calostachys* (mean 5.67±2.31 larvae). *Jasminum floribundum* had the highest mean of eggs hatched (mean 25.33±3.51) followed by *Vepris simplicifolia* (mean 24.33±2.52) and *Olinia rochetiana* (mean 22.00±1.73) at concentration of 50 mg/ul. In larval development, there was no significant difference (F=2.613; P=0.080) in the mean number of larvae killed by the various methanolic plant extracts at 100 mg/ul. Plant extract from *O. capensis* had the highest number of dead larvae (mean of 9.33±0.577 larvae) followed by extracts from *V. simplicifolia* (mean of 9.0±1.0 larvae) and *O. rochetiana* (9.0±1.0). *A. aethiopicum* had the least mean larvae killed (7.0±1.0). Prevalence of helminthes was higher in the year 2006 (mean 28.13±1.73 animals) than all the years under consideration (2006-2012). Lowest prevalence rate was recorded in 2012 with a mean of 19.70±1.50 animals infested with worms. Sheep had the highest percentage of infestation with helminths (mean 27.31±1.34) followed by goats (24.01±1.59) and least was cattle with mean of 18.21±1.54 animals. There was significant difference (F=9.55; P=0.001) in helminth infestation among livestock. The findings of this study provide evidence on the potential use of medicinal plants for anthelmintic drug development from the plants in the study area. It is recommended that livestock farmers use the six medicinal plants to manage and treat sheep helminthiases and drug development. Bioactive substances from these plants should be identified.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Parasitic nematodes are among the most common and economically important infectious disease organisms of grazing livestock, especially in ruminants around the world causing helminthiases (Alawa *et al.*, 2001; Perry *et al.*, 2002). In developing countries, the disease may be attributed to lack of resources to regularly deworm affected livestock, in addition to development of parasite resistance to conventional drugs resulting to poor use of drugs. Moreover, parasites infections are likely to increase in the face of climate change (Weaver *et al.*, 2010; Tinsley *et al.*, 2011). It is well documented that parasites undergo evolution to adapt to opportunities presented by climate change or anthelmintic use or undoubtedly as a manifestation of "Survival of the fittest" (Sargison *et al.*, 2007; Davey *et al.*, 2009).

Different control strategies including the use of anthelmintics, grazing management in livestock and improvements in sanitation are available for gastrointestinal nematode infections but these control methods are associated with development of resistance to available chemotherapeutic anthelmintic drugs (Kaplan, 2004; Wolstenholme *et al.*, 2004; Gasbarre *et al.*, 2009). Consequently, rural communities resort to using medicinal plants to treat symptomatic clinical signs of which they have continued to claim effectiveness. In most developing African countries like Kenya, livestock production remains crucial and represents a major asset among resource-poor small scale farmers by providing milk, meat, skin, manure and traction. However, the economic benefits of livestock populations remain marginal due to prevailing livestock diseases which are among the bottle neck of livestock

performance and cause of high economic losses to the resource - poor farmers (Mesfine and Lemma, 2001).

Ethno-veterinary medicine is a system that is based on folk beliefs, traditional knowledge skills, methods and practices used for curing diseases and maintaining health of animals (Mathias-Mundy and McCorkle, 1989; Tabuti *et al.*, 2003). Traditional medicine knowledge like all other traditional knowledge systems is handed down orally from generation to generation and it may disappear because of rapid social-economic, environmental, technological changes and as a result of the loss of cultural heritage under the guise of civilization (Mathias –Mundy and McCorkle, 1989; Nfi *et al.*, 2001). The only solution is that it must be documented and conserved through systematic studies before it is lost forever. In third world countries, there is poor extensive network to provide modern veterinary facilities. Dwindling financial resources and poorly developed necessary infrastructure like roads, laboratories and cold chains to keep heat-sensitive vaccines refrigerated at all times/makes government-run veterinary services un-able to provide good quality animal health services (McCorkle and Green., 1998; Matekaire and Bwakura, 2004; Fajimi and Taiwo, 2005).

The use of ethno-veterinary practices to treat and control livestock diseases is an old practice in large part of the world, particularly developing countries where animal health services are still poor and found scarcely located at urban areas (Kokwaro, 1976; McCorkle, 1995; Sinha *et al.*, 2002). Still, those in close proximity to

conventional drugs also use traditional medicinal drugs to treat their animals (Gamechu *et al.*, 1997) due to cultural acceptability, efficacy against certain diseases and economic affordability (Teklehaymanot and Giday, 2007). A great variety of traditional medicinal plants are used to treat and prevent livestock health problems. Medicinal plants (Bekele and Musa, 2009) which have been used both for prevention and cure of various diseases of human and animals from time immemorial occupy the largest portion (Giday and Ameni, 2003). Similar to other forms of traditional knowledge, medicinal plants knowledge is not compiled (Fullas, 2010). Thus unraveling the information and documentation of ethno-veterinary medicinal plants that treat animal helminthiases is urgent so that the medicinal plants knowledge can be available and conserved from deterioration and loss for the sustainable control of helminthiases.

Control of helminthiases has been the centre of focus in biomedical research for a long time. Both the medical and veterinary professionals have tried to control helminthiases by administration of conventional drugs (Ssebuguzi, 2000). However, these drugs are becoming increasingly expensive while some have serious side effects (Siddiqui, 1992). The demand for herbal medicine has steadily increased over the past decade worldwide but, majority of them have not been assessed for their quality, safety or licensed as medicines (Alte, 1993). Little is known or documented about usefulness, effectiveness or potential of such medicines. With onset of modernization of agriculture and other western influence, such knowledge is greatly threatened and could totally be lost with the passing generations. It is prudent therefore to research more on this field to generate vital data that could be

necessary to revitalize and preserve such knowledge. The objective of the present study was to determine anthelminthic effectiveness of selected medicinal plants used in the traditional management and treatment of sheep helminthiases in Koibatek and Mogotio sub-counties, Baringo County, Kenya.

1.2 Problem statement

Most livestock farmers in Kenya, especially in arid and semi arid lands (ASAL) are resource - poor and cannot afford conventional drugs to treat livestock helminthiases. Koibatek and Mogotio sub-counties are such areas where helminthiases are one of the major menaces for livestock which cause obstacles in the development of profitable livestock industry. There are inadequate veterinary services and unaffordable conventional anthelmintics which have been resisted by parasites, a combination of which leads farmers to use indigenous cure (medicinal plants) which lacks scientific information on their efficacy, dosage and toxicity levels of these herbal remedies. It is therefore, important that disease treatment and control strategies are aimed at making veterinary services affordable and readily available to farmers. This cannot be achieved by adopting disease control strategies that are totally dependent on conventional imported drugs which are expensive and unavailable to farmers. Effective helminthic control should be those based on cheaper, safer and sustainable methods of helminthic treatment and control, such as the use of locally and naturally derived drugs from plants. The indigenous knowledge underlying this kind of science has not been exploited to the benefit of farmers to cause significant improvement in their well being. A scientific intervention is required to enhance and develop the existing knowledge into more

reliable and widespread strategy for the control and management of helminthic infections. Validation studies of the locally used medicinal plants in the Koibatek and Mogotio sub-counties will help to identify potential sources of herbal based drugs that could confidently be used to treat and manage sheep helminthiases.

Much as the local use of medicinal plants for treating helminths infections exist in the sub-counties, their conservation status is not known. Motivation and value to study the conservation status of such plants can be realized if their local use is scientifically proven. Otherwise, potentially useful medicinal plant species may become prone to threats of endangerment before scientific discoveries are made. Ethnobotanical information like any other form of traditional knowledge is orally transmitted from generation to generation and is in danger of extinction as older people die and younger generation fails to learn the traditional way of life because no documentation of such knowledge has taken place. Therefore, validation studies of selected plants for treating helminthic infections will lead to research initiative to study conservation issues.

1.3 Justification of the study

In Koibatek and Mogotio sub counties, helminthic infections are rampant and cause heavy production losses in livestock. These in turn affect farmers directly or indirectly. Indirectly, financial losses are occasioned by livestock death and decreased production and direct losses are due to adverse effects to human health after consumption of animal products with infective stages of helminths. The use of

traditional medicine would reduce such losses. There is little information documented in the study area on the use, safety and efficacy of traditional medicine prepared from medicinal plants to combat these worms. This study will provide more information on the existing herbal plants used to treat livestock helminthiases and the type of helminths causing helminthiases in Koibatek and Mogotio sub-counties. Knowledge about the use of plants for purposes of treating helminthiases is dwindling rapidly due to changes towards a more western lifestyle and also due to loss of personnel with the knowledge because of old age. Ethnopharmacology and natural products discovery is significant hope in the improvement of poor livelihoods of rural communities of Koibatek and Mogotio sub-counties. The two sub counties were chosen for the study because there are diverse vegetation structures, remote with inadequate veterinary services and helminthic infections are rampant causing helminthiases. Sheep were considered for the study because they are small ruminants; grazers hence have got high probability of consuming infective stages of helminths larvae leading to helminthiases. Also sheep is mostly reared livestock due to its unique ability to perform well on forage and terrains which cattle cannot.

1.4 Research questions

(i) What are the types of helminths reported in Koibatek and Mogotio sub-counties that cause helminthiases in sheep?

(ii) What is the level of knowledge amongst livestock owners on medicinal plants used in treating livestock helminthiases in Koibatek and Mogotio sub-counties?

(iii) Which are the medicinal plants used in the traditional treatment of helminthic infection in Koibatek and Mogotio sub-counties?

(iv) What are the pharmacological activities of herbal extracts in managing livestock helminthiases in Koibatek and Mogotio sub-counties?

1.5 Hypotheses

(i) There are no helminths reported in Koibatek and Mogotio sub-counties that causes sheep helminthiases.

(ii) There is no knowledge amongst livestock owners on medicinal plants used in treating livestock helminthiases in Koibatek and Mogotio sub-counties.

(iii) There are no medicinal plants of value used in traditional treatment of helminthic infections in Koibatek and Mogotio sub-counties.

(iv) There are no herbal plant extracts that have pharmacological activity in Koibatek and Mogotio sub-counties used in treating helminthic infections.

1.6 Objectives

1.6.1 General objectives

To determine anthelminthic effectiveness of six selected medicinal plants used in the traditional management and treatment of sheep helminthiases in Koibatek and Mogotio sub-counties, Baringo County, Kenya.

1.6.2. Specific objectives

(i) To determine the types of helminths existing in Koibatek and Mogotio sub-counties that causes livestock helminthiases in sheep.

(ii) To assess the existing local knowledge on the use of medicinal plants for treating sheep helminthiases in Koibatek and Mogotio sub-counties.

(iii) To document medicinal plants used in traditional treatment of helminthic infections in Koibatek and Mogotio sub-counties.

(iv) To determine anthelmintic activity of selected medicinal plants used in treatment of helminthes in sheep using *in vitro* experiments.

1.7 Significance of the study

The study is aimed at generating useful data on medicinal plants that could confidently be used for treatment of helminthic infections. The results of this study will provide some baseline data, which could lead to further research and

development of natural products for control and management of livestock helminthic infections. This in long run could help poor farmers improve and maintain good health care and improve production of their livestock through enhanced use of cheaper and locally available medicinal plants.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of medicinal plants

Preparations derived from plants were the original therapeutic interventions used by man to control diseases in both human and livestock. Introduction of western medicine in the developing countries by missionaries and colonizing powers displaced local systems of animal and human health care (Mathias *et al.*, 1999). However, the expensive costs of conventional drugs and their short supplies make their affordability and availability to farmers difficult. As a result, the majority of the world's poor population find themselves seeking medication from local health care systems. World Health Organization (WHO) estimates that 80% of the world's people, mostly in poor and less developed countries depend on traditional medicine for their primary health care requirements (WHO, 2000).

In Samburu District, a research study was carried out to investigate the use of plant extracts against livestock intestinal worms (*ntubui*). The main plant species used were seeds of *Myrsine Africana* (*seketet*)-45%, *Albizia anthelmintica* (*lmungutan*)-30% and *Warburgia ugandensis* (*sokorioi)*-18%. *Carissa edulis* and *Rapanea melanophloeios* were used in smaller percentages (Nanyingi *et al.*, 2008). Potential medicinal plants were identified but a gap existed on their efficacy, quality, safety, toxicology and approximate dosage per animal. In East Africa, parts of *Aerva persiaca* and *Clerodendrum myricoides* are used in the treatment of East coast fever (ECF), while *Erythrococca bongensis* and *Monsania anguistifolia* are used for black quarter disease (Kokwaro, 1976). In Tanzania, traditional medicine used by the Maasai pastoralist was reported for treatment of livestock diseases such as

brucellosis, rinderpest and foot and mouth diseases (Marecik, 1998). The bark extracts of *A. anthelmintica* locally used by pastoral farmers in Kenya to treat worms, was found to decrease feacal eggs counts of *Haemonchus contortus* in sheep by 34% (Githiori *et al.*, 2003). In Kenya, the use of traditional plants among the Samburu and Turkana has shown that several cattle diseases such as diarrhea, cough and mange are treated using traditional plants (ITDG and IIRR, 1996; Wanyama, 1997).

In Kenya, a number of plants used against all major groups of helminths (cestodes, trematodes and nematodes) (Anonymous, 1996) were identified and methods of preparation noted. Similarly, Kokwaro (1993) listed twenty one plants used against hookworms (*Ancyclostoma ssp),* six against roundworms, twenty two against tapeworms, one plant used against threadworms (*Strongyloides ssp*) and a list of 79 plants that are used as general anthelmintics in human in East Africa. Five plants (*Drysopteris inaequalis, A. anthelmintica, Albizia gummifera, Olea africana and M. africana)* were reportedly used by the Marakwet people of Kenya as anthelmintics (Lindsay, 1978). The Kikuyu of central Kenya purportedly used *Cissampelos pareira* roots, *Vernonia lasiopus* roots and leaves, *Myrsine africana* fruits, *Rapanea melanophloeios* fruits, *Ficus thonningi ssp*, *Albizia anthelmitica* roots and *Ficus sycomorus ssp* sap for treatment of gastrointestinal parasites of small ruminants (Gachathi, 1993). They also used the bark of *Acacia mellifera* against the gastrointestinal parasites of cattle. Based on the plant species mentioned, little information is known about their safety and efficacy.

The bulk of material used for medicinal purposes are collected from the natural vegetation stock that continues to shrink, with majority of claimed ethno-veterinary medicinal plants collected from natural habitat (wild) without cultivation. The fact that the remedies are only found in the wild possesses a big threat to their existence as long as the mass destruction of their habitat continues (Giday *et al.*, 2003) and the mode of transfer of indigenous knowledge is verbal from generation to generation. There is also an increase in threat to these medicinal plants due to frequent drought, agricultural expansion and cultivation of marginal land. The stock of vegetation of medicinal plants is shrinking rapidly and hence poses a big threat to their extinction due to combined effects of these factors (Bekele, 2006). Medicinal plants are cost-saving replacement of commercial drugs (Mathias, 2004). Not only the resource poor farmers, but also the intensive production units use the medicinal plants. Market and public demand of medicinal plants have been increased and there is great risk that many medicinal plants today face either extinction or loss of genetic diversity (Kudi, 2003). In European Union and other countries where the use of antibiotics and other drugs is increasingly restricted in food animals, plant medicines are gaining importance (Mathias, 2004). In developing countries, an interest in botanicals is reviving and different developmental organizations are supporting commercial or backyard cultivation. This is an established fact not only in developing countries, but even in the industrialized countries where medicinal plants will remain an integral part of veterinary therapeutics (Waller, 2006).

Different reports have shown that some plants used in helminthiases control are toxic to animals when used in high doses (Toma *et al.*, 2009). Efficacy studies

carried out in different parts of Africa on different medicinal plants species and worm species have revealed varying efficacies (Gakuya, 2001; Githiori *et al.*, 2005; Sujon *et al.*, 2008) while others have revealed no activity at all. Plant secondary metabolites like alkaloids, tannins, flavonoids, glycosides, phenolic compounds, steroids and volatile oils are responsible for the physiological effects in the body (Makut *et al.*, 2008). Studies conducted showed that herbal remedies possess significant pharmacological activity and these have potential adverse effects, which may vary from relatively safe to potentially lethal (Pribitkin, 2005).

Previous studies indicated several plants in Uganda are used in treatment of livestock helminthiases although some were considered more potent (Nalule *et al.*, 2011). However, efficacies of the claimed potent plants have not been investigated to validate their traditional use as anthelmintics. One such is *Euphorbia heterophylla Linn* (Euphorbiaceae), commonly called milk-weed (Parsons and Cuthbertson, 1992). The plant is native to Central and South America, but widely distributed throughout the tropics and sub- tropics and is a common crop weed across the world (Parsons and Cuthbertson, 1992; Mosango, 2008). Ugandan agro-pastoralists also use the plant to treat livestock and human constipation while the pigs feed on it (Tabuti, 2003). In West Africa and India, *E. heterophylla* and a related species, *Euphorbia hirta* are traditionally used to treat constipation, bacterial and inflammatory disease conditions such as arthritis and rheumatism (Ogueke *et al.*, 2007; Falodum *et al.*, 2008; Anilkumar, 2010; Karimi *et al.*, 2010).

Ethnopharmacology and natural product drug discovery remains a significant hope in improving the poor livelihoods of rural communities. Many modern pharmaceuticals have their origin in ethnomedicine and ethnoveterinary medicine, which rely upon a local pharmacopoeia (Tamboura *et al.*, 2005). The ethnopharmacology knowledge is a holistic system approach that can serve as an innovative and powerful discovery engines for newer, safer and affordable medicines (Patwardhan, 2005). Cultural acceptability of traditional practices, along with perceptions of affordability, safety and efficiency play a role in stimulating scientific research and validation of traditional medicines (WHO, 2002). Therefore, validation studies of selected herbal therapies for the treatment of sheep helminthiases can lead to the discovery of new alternative chemical ingredient for use.

2.2 Anthelmintic plant products

The most extensive research on plants as alternatives to commercial anthelmintics has focused on those plants that are rich in condensed tannins. Condensed tannins (CTs) are secondary metabolites that have been reported to have direct and indirect effects on gastrointestinal helminths. An indirect effect may be due to the capacity of CTs to complex with proteins in the rumen so that they pass the abomasums and small intestine for digestion (Nguyen *et al.*, 2005). This increase in digestible protein supports improved growth and resistance against gastrointestinal nematodes (Iqbal *et al.*, 2007). However, too high concentration of CTs in the diet has adverse effects on intake, ruminant function and digestion due to un-palatability of high CTs in plants, destruction of microbes and binding of digestive enzymes by CTs

respectively (Nguyen *et al.*, 2005). Reports of direct anthelmintic effects of CTs have included claims such as inhibited egg hatching and larval development decreased faecal egg count, hindrance of exsheathment and reduced worm count burdens. The mechanisms of anthelmintic activity are likely to vary between different CTs from different species (Nguyen *et al.*, 2005) and the difference in antiparasitic effects could be related to the various chemical structures of CTs (Brunet and Hoste, 2007).

Pessoa *et al.*, (2002) noted that eugnol, the main component of *Ocimum gratissimum*, was efficient in inhibiting egg hatching as the essential oil of the plant, indicating that eugnol may be active constituent. *Spigelia anthelmia* was reported to contain spiganthe, a compound capable of causing paralysis in worms (Assis *et al.*, 2003) and spigelline, a toxic alkaloid (Ademola *et al.*, 2007). *Nicotiana tabacum*, a species of tobacco plant, contains nicotine, an alkaloid that binds nicotinic receptors on nematode muscles causing paralysis (Iqbal *et al.*, 2006b).

Terpenes are the largest group of plant secondary metabolites and they are the primary constituents of many essential oils. Numerous references list terpenes as compounds identified in the plants of interest (Hordegen *et al.*, 2003; Gathuma *et al.*, 2004; Iqbal *et al.*, 2005; Equale *et al.*, 2007; Jabbar *et al.*, 2007. While the main components of a plant are not necessarily responsible for an observed biological activity, terpenes have exhibited a wide range of medicinal activities that include the inhibition of parasites. Souza *et al.* (2008) showed that acetogenins from *Annona*

squamosa had activity against *H. contortus* eggs *in- vitro*. Ethyl acetate, methanol water and aqueous extracts of the seed of the plant and an isolated acetogenin were incubated at various concentrations with *H. contortus* eggs for 48 hours. Both the isolated acetogenin and the ethyl acetate extracts completely inhibited egg hatching; the methanol-water extract caused 81% inhibition and the aqueous extract caused only 52% inhibition. These results showed that acetogenins do have anthelmintic activity against at least one stage of *H. contortus*.

2.3 Helminthic infection in livestock

One of the most important set back in livestock production is parasitic diseases. In the tropics and sub tropics, parasitic gastrointestinal nematodes are prevalent and remain a major constrains to ruminant productivity. Parasitic nematodes rank as number one in causing production losses arising from stock mortality, severe weight loss and poor production (Perry and Randolph, 1999; Chiejina, 2001; Perry *et al.*, 2002. The parasitic infections in grazing animals mainly belong to the phyla platyhelminthes and Nematohelminthes. Flatworms have two classes of parasites, the cestoda (tapeworms) and trematoda (flukes). These parasites are flattened dorso-ventrally and are hermaphroditic. The most common and abundant cestodes parasite in grazing animals is *Monienza* ssp (Soulsby, 1982). The most important trematodes in livestock in Kenya are *Fasciola gigantica*, which is endemic in marshy areas with poor drainage and high rainfall (Wamae *et al.*, 1990). Nematohelminthes consist of class nematoda. Some of the super families of veterinary importance in the phylum include Ancylostomatoidea, Ascaridoidea, Oxyuroidea, Rhabditoidea, Strongyloidea and Trichostrongyloidea (Anderson, 1992).

Ovine gastrointestinal parasites are important pathogens affecting the health of animals and the income of their farmers (Fitzgerald, 1980; Chartier and Parand, 2012). Clinical signs of diseases such as diarrhea, and even mortalities affect mainly young animals (Hansen and Perry, 1994; Chartier and Parand, 2012). Sub clinical effects such as long term weight loss and reduced growth, are probably more important considerations to modern livestock production aiming for improvement of production through healthier animals (Fitzgerald, 1980; Foreyt, 1990; Taylor, 2009). To achieve such a lasting effect the farmers and veterinarians require knowledge of parasites affecting the sheep, risks affecting the presence of parasites and methods to detect and treat the infections in a suitable way (Sargison, 2011; Chartier and Parand, 2012).

In various surveys, *Haemonchus contortus* and *Trichostrongylus colubriformis* have been listed among the top ten most common nematodes hampering production of goats and sheep in tropical countries (Anon, 1992; Arosemena *et al.*, 1999; Horak, 2004). Haemonchosis caused by *H. contortus*, is characterized by anaemia, hemorrhagic gastroenteritis, hypoproteinemia, sudden death or chronic emaciation, whereas infection with *T. colubriformis* causes protracted diarrhea with dark stool, weakness, loss of production and death (Soulsby, 1982; Urquhart *et al.*, 1996). The high fecundity, combined with the high rainfall and temperatures of the tropics, favours permanent larval development in the environment leading to the development of the free living stages of infective larval forms (L3) throughout the year. Control of gastrointestinal nematodes infections in small ruminants is exclusively by use of conventional drugs which are expensive and sometimes

unavailable or unaffordable by rural farmers in developing countries and they end up using poor quality products (Monteiro *et al.*, 1998).

Alternatively, the widespread intensive use of anthelmintic drugs by commercial farmers has created multiple drugs resistance that has led to failure to control helminthes in ruminants (Prichard, 1998; Wolstenholme *et al.*, 2004; Jabbar *et al.*, 2006). Therefore, strategies employed include use of plants as alternative anthelmintic compounds. Use of effective indigenous plant preparations as livestock dewormers would appear to be a sustainable and affordable method readily adaptable to rural farming communities (Hammond *et al.*, 1997). Several books have been written on ethnoveterinary medicine, for example, Mathias-Mundy and McCorkle (1989), Anonymous (1994), Bizimana (1994), Anonymous (1996), McCorkle, Mathias and Veen (1996), Mathias-Mundy (2001), Martin *et al.*, (2001) and a few data bases and websites on the subject exist. In most of these sources, there is only a brief description of the plant used and the purported conditions that they treat and often no validation on the effects against these conditions is provided. Based on this, a gap exists in ethnoveterinary medicines that need to be looked into to validate some of the plants.

2.4 Anthelmintic resistance

Drug resistance is the heritable ability of the parasite to tolerate a normally effective dose of the anthelmintic. The parasite is considered resistant if it survives exposure to the standard recommended dose of anthelmintic and the ability to survive and pass the genes to its offspring. Resistance can be viewed as drug tolerance, since "resistant" individuals can often be removed by exposure to higher dose rates of anthelmintic up to the maximum tolerant dose (Geert *et al.*, 2000). Genetics modifications that confer resistance may reflect different biochemical modifications such as cellular changes that affect the capacity of the drug to accumulate into the cell, alteration of enzymatic systems and/or alteration of cellular receptors. In veterinary practice, frequent treatment of closed population has led to serious problem of anthelmintic drug resistance which is now largely irreversible (Geert *et al.*, 2000).

The introduction of modern anthelmintics was a double-edge sword, particularly for the small ruminant's production industry. On the other hand, anthelmintics provided broad spectrum, safe, highly effective elimination of helminths that resulted in more productive and more profitable animals. Great dependability of these drugs to kill the parasites within the host caused a shift in production practices such that producers relied on application of anthelmintics alone and began to abandon other control methods to minimize parasite population in the environment. Several years following the advent of modern dewormers, resistance of strains of *H. contortus* were reported (Craig, 2006). The first broad spectrum anthelmintic to become

associated with resistance parasite strains in North America was thiabendazole, and benzimidazole, followed in the 1980s by Levamisole and ivermectin.

As early as 1997, reports of resistance to all three major classes of drugs were confirmed in multiple countries in Africa and 80% of sheep farms in high rainfall areas of Australia reported resistance to benzimidazole and Levamisole (Waller, 1997). In south states in US, such as Louisiana and Florida, no major drug class could effectively control *H. contortus*. In South America, even combination products were failing and hence were reputed to have the worst resistance problem in the world. Since then, due to the fact that few new drugs have emerged to provide relief, resistance is on the rise (Kaplan, 2004). Modern commercial anthelmintics were initially so effective that the efficacy standard for these drugs was a parasite reduction of 90% or more. However, in light of the current resistance problem, many producers are forced to settle for medicinal plant products.

Genetic variation is responsible for resistance; spontaneous mutations or pre-existing alleles render a worm invulnerable to the mechanism of action of a drug (Silvestre and Humbert, 2002). When all susceptible worms are killed by treatment, resistant worm survive and thus pass resistant worm alleles to further generations. When a worm is resistant to one member of a drug class, it is also resistant to the other members of that drug class because of the similar mode of action of the drugs, a phenomenon known as side resistance (Sangster, 1999). Mutation can also result in cross resistance where drugs from different classes with different mechanisms are

ineffective. Finally, drugs with long residuals eventually reach a sub-therapeutic level in the body that kills only incoming worms without resistant alleles and favors heterozygote's, allowing only resistant worms to establish in the animal (Craig, 2006).

Anthelmintic resistance (AR) has been a global issue in the small ruminant industry during the past few decades. Most probably, AR is of great concern in goats than in sheep (Demake *et al.*, 2012). Sheep and goats differ in many aspects; as goats have a higher metabolic rate and require higher dose rates for drugs (Bankunzi, 2003; Cabaret *et al.*, 2008). The immune system of goats is also different. The modern broad-spectrum anthelmintic is currently used in prophylaxis and treatment of helminth infections in farm animals (Easwaran *et al.*, 2009). Anthelmintic resistance is due to traditional treatment, low diet and inadequate dose levels of antiparasitic agents (Harikrishnan, 2012).

An unsatisfactory response of parasitized animals to treatment is usually the first apparent signs of presence of anthelmintic resistance nematodes on a given farm. AR threatens the sustainability of sheep production if it is allowed to reach high levels (Sargison, 2011b). On most sheep farms, the first indications of AR are failure of lambs to reach recommended finished weights by late autumn, scouring and even deaths due to parasitic gastroenteritis, despite preventive anthelmintic treatment (Sargison, 2011a). AR can result in clinically in apparent, sub-optimal growth rates for sometimes before these overt signs of disease are seen.

2.5 Importance and distribution of sheep

Sheep are a worldwide commodity. They provide a number of products: meat, milk, skin, and wool (Zygoyiannas, 2006). While in most cases they cannot compete with cattle in meat and milk production, sheep have characteristics that give them value over cattle. One main advantage is wool production. A second value is their small size, which is beneficial on small farms that do not possess the land or resources required for cattle. Furthermore, sheep have a unique ability to perform well on forage and terrain on which cattle would not. In 2006, Zygoyiannas estimated the world population of sheep to be 1024 million, with major sheep producing regions located in Europe, the Near East of South America, Australia and New Zealand. For countries where sheep are economically critical, such as Syria, indigenous sheep meat is the second ranked commodity (FAO, 2005), and decline in production could seriously impact the livelihood of millions.

Where there are sheep, there are parasites; so based on its pathogenicity, it is not surprising that *Haemonchus contortus* is the most important parasite of sheep in numerous regions throughout the world. In the past 10 years, estimates for cost of treatment to *H. contortus* alone have approached $30 million, $50 million and $105 million in Kenya, South Africa and India respectively (Waller and Chandrawathani, 2005). *Haemonchus Contortus* is a problem in tropical and sub tropical areas with high temperatures and abundant rainfall. *Haemonchus contortus* is distributed throughout the US. It is predominant Ovine helminths in the Eastern US whereas in the more arid Western US, it is more likely to be a problem in irrigated areas. Eggs will not hatch below 9^0C, and high humidity is important especially at high

temperatures where desiccation becomes a serious threat to both eggs and larvae (O`Connor *et al.*, 2006).

2.6 Biological screening of medicinal plants (*in-vitro* experiments)

In-vitro screening of medicinal plants is done using egg hatch assay and larval development test. The egg hatch test was originally developed for the detection of benzimidazole (BZ) resistance in livestock helminths. It is based on the ovicidal activity of BZ. However, the test has also been used for screening of plants and or other compounds for their anthelminthic activity (Molan *et al.*, 1999; Molan *et al.*, 2000; Waghorn and Molan, 2001; Molan *et al.*, 2002; Min *et al.*, 2004). The test was originally described by Le Jambre (1976). A standard protocol was adapted by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles *et al.*, 1992). The reliable data can be obtained by freshly collected faecal samples (within 3 hours of being shed). This is because of a false positive result due to development of eggs beyond the ventral indentation stage leading to embryonation (Le Jambre, 1976; Weston *et al.*, 1984; Riou *et al.*, 2005). If fresh collection of feaces is not possible, samples must be stored anaerobically. Larval development test is an *in vitro* assay for the detection of resistance to benzimidazole, Levamisole and macrocyclic lactones in nematodes parasite of sheep (Taylor, 1990; Varady *et al.*, 1996), horses (Kerboeuf, 1994), pigs (Varady *et al.*, 1996) and cattle. The assay can also be used to detect effectiveness or resistance of anthelmintic herbal plant extracts. Nematodes are isolated from faecal samples, placed into wells of micro-titre plate and allowed to develop through to infective L3 larvae in the presence of a range of concentration of anthelmintics.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was carried out in Koibatek and Mogotio sub-counties which rely heavily on livestock production as a source of income. The area, being remote with inadequate modern veterinary health care facilities and diverse vegetation structures forms the criteria for selection of the study site. The study area is located approximately 250 km from Nairobi the Capital city of Kenya. The two sub-counties have a total area of 2,306.4 Km^2 and a population of 138,168 people, with a population of 445,338 livestock (Cattle - 130,935; Sheep - 106, 221 and Goats - 208,182) according to 2009 census. The vegetation consists of thickets, bush lands, and an area of about 512.4 km^2 is covered by forest. The dominant land use is for livestock rearing and subsistence crop Agriculture. Livestock reared include sheep, cows, goats, donkeys, cats and dogs. It borders Baringo North and Baringo Central to the North and North West, Nakuru County to the East and South, Kericho and Uasin Gishu to the west. It is located between longitudes 35^o 30' and 35^o 15' East and between latitudes 0^o 10' South and 0^o 25' North. The equator cuts across at the Southern tip of Mogotio sub-counties. The study area is further divided into Mumberes, Torongo, Emining, Kisanana, Sirwa, Kimngorom, Esageri, Mogotio and Eldama Ravine administrative units/divisions (Appendix III).

3.2 Study design

The study was divided into three components: Questionnaire based cross-sectional study aimed at establishing knowledge levels on the use of medicinal plants for management and treatment of sheep helminthiases and documenting the plants used

for treatment of sheep helminthiases. The nine administrative units in the two sub counties, namely Mumberes, Torongo, Emining, Kisanana, Sirwa, Kimngorom, Esageri, Mogotio and Eldama Ravine were purposively selected for the study and from the nine administrative units, 10 villages were selected of which five were from highland (Mumberes, Tugumoi, Sirwa, Kiplombe and Benonin) and five were from lowland (Radad, Molos, Kipsogon, Nyalilpuch and Saos). In each village 13 semi structured questionnaires were issued randomly to individuals, local village elders and herbalist giving a total of 130 informants to be able to establish levels of knowledge and the medicinal plants used traditionally in treating sheep helminthiases. Plants were collected at various localities mentioned by the informant with their guidance. The second part of the study documented meat inspection records aimed at identifying helminths associated with livestock infections and the prevalence. This was carried out by obtaining data kept in the Veterinary Offices and abattoirs in the two sub-counties on the number of slaughtered livestock (sheep, goats and cattle) infected with helminths and the types of helminths from the year 2006 to 2012. The third part of the study was based on testing the efficacy on the six of the identified plants as anthelmintic agents in an *in vitro* system using *Haemonchus contortus* eggs and larvae (Appendix XVII). This was carried out at KALRO - Muguga North Veterinary Laboratories as an experimental laboratory based design.

3.3 Identification of medicinal plants

Medicinal plants were identified in the Month of March to April 2013 to obtain an impression on vegetation characteristic (plants being leafy and flowery) of the study

area using semi-structured questionnaires and interviews (Appendix I). A focused group discussion was held with the community members in each of the study villages namely Mumberes, Tugumoi, Radad, Molos, Sirwa, kiplombe, Kipsogon, Nyalilpuch, Saos and Benonin. This was supplemented with interviews and questionnaire surveys (guided open - and close - ended questionnaires). Discussions were conducted using structured questionnaires with individuals, local village elders and traditional medicine practitioners (Appendix II). The questionnaires included questions on demography like sex, age, tribe, religion, education, knowledge and attitudes about helminthic infections, plant used, practices for treating and preparations, place of plant harvest, any reported side effect, application and dosage of anthelmintic herbal medicine. The questionnaires were translated into Kalenjin; the principal language spoken in the study area. All plant materials mentioned by respondent in the study were identified in the field. After obtaining several plant species, the plants with the highest frequency of their being mentioned (at least two) by farmers and herbalist were considered to be more effective and usage were established based on traditional methods of extraction and administration to livestock.

3.4 Plant collection

Collection of plants was undertaken with the help of farmers and traditional healers/herbalist. The parts of the sub-county where plants were collected and the parts of the plant used as well as the dosage used in sheep were noted. All plant material mentioned by respondents in the study area were identified; voucher specimen of each plant species was collected and preserved by pressing and drying

under the shade /indoor. Local names of the plants were noted and plants collected were transported to East African Herbarium at National Museums of Kenya (NMK) for identification.

3.5 Selection of the medicinal plants

The medicinal plants were selected mainly on the basis of frequency of their mention by the respondents (farmers, herders and herbalist), where six plants with the highest percentage frequencies were picked. A total of one hundred and thirty (n-130) respondents were interviewed and fifteen (15) plants were mentioned of which six (6) plants were selected for extraction namely: - *Leucas calostachys, Olinia rochetiana, Vepris simplicifolia, Olea capensis, Jasminum floribundum* and *Asplenium aethiopicum* (Appendix XVI).

3.6 Field collection of prioritized medicinal plants

Fresh plant parts used by the farmers in the treatment of helminthiases were collected from the field, dried in the shade and kept in polythene bags after being chopped into small pieces. The polythene bags were labeled and transported to Kenyatta University- Department of Pharmacy for processing and extraction.

3.7 Processing and extraction of selected medicinal plant material

Each dry plant material (parts used) was grounded using an electric grinder into powder and stored in air tight bottles. Exposure to sunlight was avoided to prevent the loss of active components.

3.7.1 Crude extraction (water extracts)

Plants material grounded into fine powder was weighed and extracted in distilled water using the methods described by Anonymous (1996). Ten grams of the powder from each selected plant was immersed in 100 ml of water in labeled beakers and soaked overnight. The extracts were then filtered using a muslin cloth and filter papers to provide the dose and stored in labeled bottles at 4^0C until used. For *Leucas calostachys,* ten grams of grounded plant material was mixed with 200 ml of water because it absorbed a lot of water. Treatment was similar to the other plant material. Water extraction yielded the volumes of filtrates as shown below (Table 3.1).

Table 3.1: Volumes of filtrates (water extract) from each plant extracts

Plant sample (10 grams)	Volume of filtrate in ml
Jasminum floribundum	60
Vepris simplicifolia	70
Asplenium aethiopicum	60

Leucas calostachys	120
Olea capensis	80
Olinia rochetiana	80

3.7.2. Solvent extraction (methanol extracts)

For each of the dry sample of the plant material (powder), 50 grams from each plant material was soaked in 200 ml of methanol extraction fluid. The mixtures were kept for 2 days (48 hours) in tight sealed conical flasks (labeled) at room temperature protected from sunlight and mixed daily with a sterile glass rod. The mixtures were then filtered into conical flasks using whatman filter paper No. 10. The filtrate was then concentrated on a rotary evaporator at 50^0C to yield semi solid masses as shown below (Table 3.2). The extracts were then stored in labeled bottles in a refrigerator at 4^0C until used.

Table 3.2: Weights of methanol extracts from each medicinal plant

Medicinal plant samples	**Weight of extracts (g)**
Jasminum floribundum	5.54
Vepris simplicifolia	4.34
Asplenium aethiopicum	2.28

Leucas calostachys	3.13
Olea capensis	6.72
Olinia rochetiana	3.67

3.8 Determination of helminths presence

Information on the prevalence of helminths and the type was collected from veterinary offices and slaughter houses in various divisions of the two sub-counties (Table 3.3). Meat inspectorate quarterly reports compiled at the veterinary offices from the various divisions of the study area were used to determine the presence of helminths.

Table 3.3: Slaughter houses and points visited to check the number of carcasses with helminths infestation and the type of helminths

Division	**Sub county**	**Slaughter house/point**
Esageri	Koibatek	Esageri/Muserechi
Torongo	Koibatek	Torongo slaughter house
Mumberes	Koibatek	Ilotea, Timboroa and Equator
Eldama Ravine	Koibatek	Saos, Solian, Maji Mazuri and Makutano

Emining	Mogotio	Emining
Mogotio	Mogotio	Chemogoch and Noiwet
Kisanana	Mogotio	Kisanana and Ngendalel
Sirwa	Mogotio	Sirwa

3.9 Evaluation of anthelmintic activity of the selected medicinal plants

In vitro experiments were carried out at Kenya Agricultural and Livestock Research Organization (KALRO) - Muguga laboratories to test the biological activities of water and methanolic extracts of the six priority plants used by farmers to treat sheep helminthiases.

3.9.1 Collection of fresh eggs of *Haemonchus contortus*

At KALRO-Muguga North Veterinary Laboratories, at least 10 sheep were randomly selected from a flock of 36 and 100 grams of faecal samples were collected from rectum of each animal using gloved finger (Appendix XV) three times at an interval of three days. The faecal matter was then placed in plastic polythene bags and transported to the laboratory for extraction.

3.9.2 Extraction of eggs from feaces

Forty milliliters of tap water was added to 50 grams of faecal material in a polythene bag and kneaded thoroughly by compression using a stomacher. The faecal suspension was passed through a series of sieves-strainer, 150 μm and 38 μm as minimum and retentate containing fine faecal debris was poured into polyallomer centrifuge tubes and centrifuged at 1000 revolutions per minute (rpm) for 5 minutes. The supernatant was removed/discarded using a vacuum line, leaving approximately 1 ml of faecal debris and eggs. The sediment was re-suspended using 10-12 ml saturated sodium chloride solution and mixed thoroughly but gently (violent mixing causes more faecal debris to float); it was again centrifuged at 500 rpm for 5 minutes. Top third of supernatant (2 ml) was removed and retained by pipetting into another centrifuge tube. Water was added and centrifuged at 1000 rpm for five minutes, the supernatant was discarded. It was then resuspended in water and centrifuged again at 1000 rpm for 5 minutes and a repeat of resuspending in water was done to obtain final volume of approximately 2 ml. Twenty five microlitre (μl) was withdrawn and number of eggs counted. The extracted eggs were pooled to make up a total of 10 ml in a volumetric flask and the number of eggs counted in 100 μl (contains approximately 100 eggs) (Hunt *et al.*, 1989).

3.9.3 Preparation of stock and working solutions

One gram (1g) each of methanolic extracts stored at 4^0C were removed, transferred into a 100 ml volumetric flask and dissolved in 10 ml of DiMethylsulphoxide (DMSO) on the day of the experiments. It was mixed thoroughly and distilled water added to make 100 ml of stock solutions which was used to prepare different

suitable range of dilutions for the purposes of evaluating anthelmintic activity. The stock solutions made were used to make the following concentration of working solutions for each methanolic plant extract in 96 micro-titre plates. The concentrations of 6.25 mg/µl, 12.5 mg/µl, 25 mg/µl, 50 mg/µl and 100 mg/µl were prepared and using clean pipettes 100 µl distilled water was added to each well. With the water extract 10 ml each was removed and placed in beakers, then 200 µl was obtained from each beaker for egg hatch assay and larval development assay respectively (Coles *et al.*, 1992).

3.9.4 Egg hatch test

Suspensions (0.2ml) containing approximately 100 eggs were distributed in 96 micro-titre plates in three replicates and mixed with same volume of different concentration (6.25 mg/µl, 12.5 mg/µl, 25 mg/µl, 50 mg/µl and 100 mg/µl) of plant extracts with control plates having 200 µl of distilled water and Levamisole10 mg/ml. Eggs were incubated in these mixtures for 48 hours at 25^0C. One drop of lugols iodine solution was added to stop the eggs from hatching. Eggs (dead or embryonated) and first stage larvae (L1) in each plant extracts were counted as described before (Coles *et al.*, 1992).

3.9.5 Larval development test

The isolated eggs that had been extracted were incubated in a micro-titre plate for seven days at 25^0C. To prevent dehydration, a wet sponge was introduced under the plate and the system covered with a pouch. The water in the wells was checked

every day during incubation. After hatching, wells were supplemented with 20 µl of growth nutritive medium and 10 µl of distilled water was added to the control well. Plant extracts were prepared by diluting them with 1% DiMethylsulphoxide (DMSO) to make different dilutions of 6.25 mg/µl, 12.5 mg/µl, 25 mg/µl, 50 mg/µl and 100 mg/µl and 3 replicates were made for each set up. Ten living *H. contortus* larvae were introduced to each well and the plates were then returned into the incubator for 6 days. On the seventh day, all the larvae were counted as either living third stage larvae (L3) or dead larvae a modification as described by Hubert and Kerboeuf (1992).

3.10 Data analysis

Chi square test was used to determine the difference in knowledge of medicinal plants species known and used by female and male practitioners in the two sub-counties. One way analysis of variance was used to determine whether there was significant difference in the prevalence of helminthes infestation in the 2006 to 2012, determine significant difference amongst animals (sheep, goats and cattle) infested with helminths and to determine if there was significant difference in mean number of animals tested for organ infestations. Comparison of the knowledge levels among the gender of various ages was done using Mann-Whitney test and one way analysis of variance (ANOVA) was used to determine whether there was a significance difference in plants extracts of varying/different concentration in egg hatchability and larval development test.

CHAPTER FOUR: RESULTS

4.1 Intestinal helminths infestations in Koibatek and Mogotio sub-counties

Results on intestinal helminths indicated that a total of 19,856 out of 81,438 (24.38%) of livestock slaughtered were infested with helminths. Sheep had the highest percentage of infestations with helminths having a mean of 27.31±1.34 followed closely by goats with a mean of 24.01±1.59 and least number of infested livestock was cattle with mean of 18.21±1.54. Using ANOVA, there was significance difference (F=9.55; P=0.001) in helminth infestation among the livestock (sheep, goats and cattle). When helminthic infestations amongst livestock were compared (sheep versus goats; sheep versus cattle and goats versus cattle) using paired t-test (Appendix IV), the result indicated that there was no significant difference in sheep versus goats infestations (t=2.05; P=0.086), but there was significance difference between sheep versus cattle (t=5.33; P=0.002) and cattle versus goats (t=2.85, P=0.030) (Appendix V).

The prevalence of helminthes was higher in the year 2006 (mean 28.13±1.73) than in all the years under consideration (2006-2012). The lowest prevalence recorded was in the year 2012 (mean 19.70±1.50) and the year 2011 (mean 20.63±2.95). However, analysis of variance shows mean prevalence for the years under study were not significantly different (F=0.78; P= 0.602) (Table 4.1).

Table 4.1: Meat inspectorate reports on the number of livestock infested with helminths for the year 2006-2012 in Koibatek and Mogotio sub-county

Year	Livestock infested with helminths			Mean% infestations per year ± SD
	Bovine (cattle)	Ovine (sheep)	Caprine (goats)	
2006	479/1812 (26.4%)	1517/4795 (31.6%)	1538/5817 (26.4%)	**28.13 ± 1.73**
2007	233/1612 (14.5%)	1046/3781 (27.9%)	1538/5428 (28.3%)	**23.57 ± 4.53**
2008	288/1631 (17.6%)	1752/5118 (34.2%)	1211/5104 (23.7%)	**25.17 ± 4.85**
2009	299/1873 (15.9%)	1081/4442 (24.3%)	1498/5520 (27.1%)	**22.45 ± 3.35**
2010	391/1972 (19.8%)	982/3812 (25.8%)	1065/4794 (22.2%)	**22.60 ± 1.74**
2011	407/2718 (14.9%)	1006/4069 (24.7%)	1167/5222 (22.3%)	**20.63 ± 2.95**
2012	374/2039 (18.3%)	964/4250 (22.7%)	1020/5628 (18.1%)	**19.70 ± 1.50**

Totals	**2471/13,657**	**8348/30,268**	**9037/37,513**	
Mean ±SD	**18.21a±1.54**	**24.01ab±1.59**	**27. 31±1.34**	

Mean values denoted by similar letters are not significantly different

Spleen, kidney, lungs, and liver were harboring the worms. Liver was the most infested organ with a total of 18,409 animals with worms in this organ, followed by lungs (619 animals) then kidney (35 animals) and spleen had the least number of helminths (14 animals). Basing on the individual worms, *S. hepatica* (72.67%) and *F. hepatica* (19.42%) were rampant in liver while Echinocyst were more in the lungs (n=619) than in the liver (n=518). Hydatidosis (n=17) and calcified cyst (n=304) were only localized in the liver. Chi-square test revealed that among the organs affected by the helminths, liver and lungs had significantly high infestation by the helminths (χ^2=3.722; d.f=4, P<0.005 and χ^2=6.13; d.f=1, P<0.005 respectively across all the years under study, with *S. hepatica* being dominant worm in the liver (72.7%) and Echinocyst being the dominant in the lungs (3.2%). Helminthic infestations were higher in the year 2006 which recorded 19.66% infestation of the animals and followed by the year 2008 which had an infestation of 16.60%. Least infestations were realized in the year 2010 with 11.50% of animals infested with helminths (Table 4.2).

Table 4.2: Meat inspectorate reports on the number of livestock infected with specific type of helminths for the year 2006-2012 in Koibatek and Mogotio sub-counties

Year	Organs									Percentage of animals infested
	Liver					Lungs		Kidney	Spleen	
	Fasciola hepatica	*Stilesia hepatica*	Calcified cyst	Echinocyst	Hydatidosis	Echinocyst	Hydatidosis	Echinocyst	Echinocyst	
2006	399	2726	43	158	17	221	0	3	1	**19.7**
2007	135	2012	21	101	0	85	0	2	1	**13.2**
2008	266	2753	50	35	0	110	0	0	0	**16.6**
2009	948	1509	35	48	0	44	0	4	4	**12.9**
2010	558	1652	31	24	0	37	0	2	6	**11.5**
2011	232	2090	68	107	0	56	0	24	2	**13.2**
2012	1171	1136	56	45	0	66	0	0	0	**12.9**
Total	3709	13878	304	518	17	619	0	35	14	
%	**19.42%**	**72.67%**	**1.59%**	**2.71%**	**0.089%**	**3.24%**	**0%**	**0.183%**	**0.073%**	

4.2 Farmers knowledge on medicinal plants used in treating sheep helminthiases according to gender and age

Knowledge on medicinal plants used for treating sheep helminthiases amongst the farmers in Koibatek and Mogotio sub-counties was transmitted orally to individuals secretly. A great majority of respondents interviewed consisted of men totaling 83 (63.8%) as compared to women 47 (36.2%). Out of the 83 men interviewed, only 49 (59%) were aware of plants of medicinal value and 23 (48.9%) women interviewed knew the plants used in treating sheep helminthiases (Table 4.3). Thus, more men than women were conversant with the plants used. Also from the survey, young generation did not know or were not aware of traditional plants used for treating sheep helminthiases. When the knowledge of the males and the females were established using a chi square test (Appendix VI), the results showed that there was no significant association in the knowledge of the medicinal plants to the gender (χ^2 = 63.33, d.f=48; P = 0.068). A further comparison of the knowledge levels among the gender of various ages was done using Mann-Whitney test (Appendix VII) with an assumption that knowledge among the male to that among the female was equal. The results showed that the hypothesis could not be rejected at P<0.05, W=69, the test was significant at 0.9577 which implied that knowledge by the male to that by the female was the same. Male respondents knowledge levels were however slightly higher (mean 9.8%) than the females (mean 8.2%).

Table 4.3: Knowledge of medicinal plants used to treat sheep helminthiases according to gender and age bracket

Age	No of male respondents		No of female respondent	
	With knowledge n=49/83 (59%)	**Without knowledge n=34/83 (41%)**	**With knowledge n=23/47 (49%)**	**Without knowledge n=24/47 (42%)**
80 – above	2 (4.1%)	0 (0%)	4 (17.4%)	0 (0%)
70-79	6 (12.2%)	0 (0%)	2 (8.7%)	0 (0%)
60-69	17 (34.7%)	6 (17.6%)	2 (8.7%)	0 (0%)
50-59	6 (12.2%)	6 (17.6%)	10 (43.5%)	0 (0%)
40-49	11 (22.4%)	3 (8.9%)	4 (17.4%)	4 (16.7%)
30-39	7 (14.2%)	8 (23.5%)	1 (4.34%)	8 (33.3%)
20-29	0 (0%)	7 (20.5%)	0 (0%)	11 (45.8%)
10-19	0 (0%)	4 (11.7%)	0 (0%)	1 (4.16%)
Total	**49/130 (37.7%)**	**34/130 (26.2%)**	**23/130 (17.6%)**	**24/130 (18.5%)**

4.3 Medicinal plants used by farmers in Koibatek and Mogotio sub-counties

A total of 15 plants from eleven families were recorded as useful in managing sheep helminthiases by the farmers in the two sub-counties. Out of eleven families encountered, Oleaceae, Labiatae and Leguminosae had the highest frequency of species being used (Table 4.4).

Table 4.4: Medicinal plants used for management of sheep helminthiases in Koibatek and Mogotio Sub-Counties

Family name	Local name	Scientific name	Life form	Parts of the plant	Mode of preparation	Dosage
Oleaceae	Masaita	*Olea capensis*	Tree	Bark	Bark soaked in water to make cold water extract	Bottle of soda (300ml) orally given in the morning
Oliniaceae	Nerkwe	*Olinia rochetiana*	Tree	Bark	Boiling the bark in water	Bottle of soda (300ml) given orally
Labiatae	Ngechebchat	*Leucas calostachys*	Shrub	Leaves	Leaves crushed and water added	Bottle of soda (300ml) given orally
Oleaceae	Emtit	*Olea europaea*	Tree	Bark	Boiling the bark in water	Bottle of soda (300ml) given orally
Oleaceae	Kiptere	*Jasminum floribundum*	Shrub	Leaves	Leaves bounded and mixed with water	Bottle of soda (300ml) given orally
Labiatae	Ngwanderet	*Ajuga integrifolia*	Herb	Leaves	Leaves bounded and water added	Bottle of soda (300ml) given orally
Compositae	Mororwet	*Vernonia lasiopus*	Shrub	Leaves	Bounding the leaves in water	Bottle of soda (300ml) given orally
Aspleniaceae	Sugumerie	*Asplenium aethiopicum*	Herb	Rhizome	Rhizome crushed then mixed with water	Bottle of soda (300ml) given orally
Myrsinaceae	Seketet	*Myrsine africana*	Shrub	Seeds	Seeds grounded and soaked in water	Bottle of soda (300ml) given orally

Family name	Local name	Scientific name	Life form	Parts of the plant used	Mode of preparation	Dosage
Canellaceae	Soket	*Warburgia ugandensis*	Tree	Leaves	Leaves bounded in water	Bottle of soda (300ml) given orally
Leguminosae	Senetwe	*Senna didymobotrya*	Shrub	Leaves	Leaves bounded and soaked in water	Bottle of soda (300ml) given orally
Meliaceae	Mwarubaini	*Melia azedarach*	Tree	Leaves	Leaves bounded and boiled in water	A third bottle of soda (100ml) given orally
Ebenaceae	Uswet	*Euclea divinorum*	Tree	Bark	Bark boiled in water and cooled	Quarter bottle of soda (70ml) given orally
Rutaceae	Kurionte	*Vepris simplicifolia*	Tree	Leaves	Leaves bounded and mixed with water	Bottle of soda (300ml) given orally at night
Leguminosaea	Barmukute	*Albizia anthelmintica*	Tree	Bark	Bark boiled in water	Half bottle of soda (150 ml) given orally

4.4 Medicinal plants used by farmers from different habitats

Four medicinal plants species were commonly used by the farmers, namely *Albizia anthelmintica* (14.66%), *Leucas calostachys* (12.93%), *Olea capensis* (12.07%) and *Olea europaea* (10.34%) and the least medicinal plants used were *Myrsine africana* (1.72%), *Senna didymobotrya* (1.72%), *Vernonia lasiopus* (1.72%) and *Melia azedarach* (0.86%). Out of the 15 plants recorded to be of value in treating sheep helminthiases, ten plants were from highland and five were from lowland (Table 4.5).

Table 4.5: Frequencies and percentages of medicinal plants mentioned for the treatment of helminthiases in the study area

Scientific name	Habitat	Number of times a plant was mentioned (frequency)	Percentage frequency (%)	Ranking of plants based on frequency of use
Leucas calostachys	Highland	15	12.93	2
Euclea divinorum	Highland	5	4.31	9
Olea europaea	Highland	12	10.34	4
Myrsine africana	Highland	2	1.72	12
Senna didymobotrya	Lowland	2	1.72	12
Vepris simplicifolia	Highland	7	6.03	7
Ajuga integrifolia	Highland	7	6.03	7
Olinia rochetiana	Highland	11	9.48	5
Warburgia ugandensis	Lowland	3	2.59	11
Asplenium aethiopicum	Highland	5	4.31	9

Jasminum floribundum	Lowland	11	9.48	5
Olea capensis	Highland	14	12.07	3
Vernonia lasiopus	Highland	2	1.72	12
Melia azedarach	Lowland	1	0.86	15
Albizia anthelmintica	Lowland	17	14.66	1

4.5 Medicinal plants selected for *in vitro* experiments

From the analysis of medicinal plants mentioned, some plants were being mentioned more often than others and basing on this criterion, percentages of plants being mentioned were calculated and plants with the highest frequencies were selected for *in-vitro* experiments (Table 4.6). These included: *Leucas calostachys, Olinia rochetiana, Vepris simplicifolia, Olea capensis*, *Jasminum floribundum* and *Asplenium aethiopicum* (Appendix XVI).

Table 4.6: Selected plants for *in vitro* experiments based on the frequency used for treating sheep helminthiases

Scientific name	Percentage frequency of use
Albizia anthelmintica	14.66
Leucas calostachys	12.93
Olea capensis	12.07
Olea europaea	10.34
Olinia rochetiana	9.48
Jasminum floribundum	9.48

Vepris simplicifolia	6.03
Ajuga integrifolia	6.03
Asplenium aethiopicum	4.31

4.6 Growth life forms of the medicinal plants used

The majority of medicinal plants recorded were trees (53.3%) followed by shrubs (33.3%) and herbs (13.3%). The vegetation of the area was predominated by shrubs and trees as well as herbs. Local community knowledge of medicinal plants seemed to be generally influenced by nature of vegetation/flora in the surrounding environment because more medicinal plants were derived from highlands (66.67%) and few were from lowland (33.33%).

4.7 Medicinal plant parts used

Most of the plants parts used as source of medicine were leaves (53.33%). This was followed by the bark (33.33%). Seeds and rhizomes were least frequently used with only one plant for each that is *M. africana* and *A. aethiopicum* respectively. From the survey use of roots was 0%.

4.8 Traditional methods of processing herbal plants in the two sub counties

Main methods of processing herbal medicine by herbalist in Koibatek and Mogotio sub- counties were decoction and maceration. Decoction was used when working with tough and more fibrous plants parts such as the bark of the stem and rhizomes.

Materials were boiled in the water for a long period of time to soften the material to release the active ingredients. Maceration was preferred for very soft/tender fresh plant material for example, leaves that tend to lose their active compounds upon heating. Seeds like *Myrsine africana* (seketet) were grounded to form powder and soaked in water to form concoction for oral formulation.

4.9 Medicinal plants extinction

Many plants in the study area are threatened by anthropogenic activities and natural factors. The majority of medicinal plants decline in number due to deforestation for timber used in construction, making of furniture, firewood, fodder, agricultural expansion, drought, overgrazing and bushfire. Majority of the local tradition healers preferred to collect medicinal plants solely to preserve their secrecy though sometimes accompanied by their chosen family members. Ethnobotanical knowledge is transferred to that trustworthy family member by a word of mouth rather than through a well organized written script. Some traditional herbalists were reluctant to pass on their plant use knowledge even to their families and thus leading to the loss of indigenous knowledge and eventually the medicinal plants upon their death.

4.10 *In vitro* anthelminthic activity of the medicinal plant extracts

4.10.1 Effect of concentrations of plant extracts in egg hatchability

The result showed that, *Olea capensis* had the least mean of eggs hatched (1.0 ± 1.00) at concentration of 50 mg/µl which was lower than the positive control (Levamisole) with a mean of 3.0 ± 5.02, while plant extract from *Jasminum floribundum* (mean 25.33 ± 3.51) had the highest mean of hatched eggs than the other plant extracts. *Olinia rochetiana* had the highest mean of eggs hatched (64.0 ± 2.65) at a concentration of 6.25 mg/µl and the least mean of eggs hatched at concentration of 100 mg/µl, indicating that effectiveness of plant extract increases with an increase in concentration which was showing the same trend for the six plants tested. In the experiment, two controls were used in three replicates which included distilled water (negative control) and Levamisole (positive control). In distilled water, a mean of 94.0 ± 5.0 eggs hatched while a mean of 3.0 ± 5.02 eggs hatched in Levamisole (Table 4.7)

Table 4.7: The mean of eggs hatched after treatment with various methanolic concentrations of the plant extracts, water extract and controls

Olinia rochetiana	*Olea capensis*	*Leucas calostachys*	*Asplenium aethiopicum*	*Vepris simplicifolia*	*Jasminum floribundum*	**Plant extract**	
64.0± 2.65c	18.67±2.52a	44.33±4.73b	17.67± 3.51a	51.63±4.16b	52.0± 4.58b	**6.25 mg/µl**	**Concentrations of methanolic extracts**
52.67±4.16c	13.33±2.08a	25.00±6.56b	16.00±2.00a	35.33±3.79b	30.67± 1.53b	**12.5 mg/µl**	
47.67±2.89c	10.00±2.00a	14.33± 1.53a	13.67±2.31a	31.67± 4.16b	28.67± 2.52b	**25 mg/µl**	
22.00± 1.73c	1.00 ± 1.00a	5.67± 2.31a	14.00± 1.00b	24.33±2.52c	25.33 ± 3.51c	**50 mg/µl**	
4.67 ± 2.08a	6.00 ±1.00a	15.33±1.16b	18.67± 7.51b	29.33±5.86c	19.00± 2.65b	**100 mg/µl**	
43.00± 6.08d	6.33± 1.53a	22.00±2.65bc	16.67±5.13b	28.67± 6.81c	29.33±3.06c	**Water extract**	
94.0±5.0	94.0±5.0	94.0± 5.0	94.0± 5.0	94.0±5.0	94.0± 5.0	**Distilled water**	
3.0±5.02	3.0± 5.02	3.0± 5.02	3.0± 5.02	3.0± 5.02	3.0±5.02	**Levamisole**	

Mean values in same column denoted by similar letters are not significantly different at $P \leq 0.05$.

Using One-way ANOVA to establish the variations in the effect of the plant extracts on the mean of eggs hatched at concentration of 50 mg/µl, the results indicated that there was a significant difference (F = 65.31; P = 0.0001) in the mean eggs hatched following treatment in 50 mg/µl concentration of plant extracts (Appendix VIII). A Scheffe post hoc test revealed that *J. floribundum* had statistically higher mean of eggs hatched (25.3 ± 3.51) compared to *A. aethiopicum* (mean 14.0 ± 1.0); P=0.02, *L. calostachys* (mean 5.67 ± 2.31); P=0.000 and *O. capensis* (mean 1.0 ± 1.0); P=0.000. *Vepris simplicifolia* (24.3 ± 2.51) had a statistically higher mean of eggs hatched as compared to *A. aethiopicum* (P=0.004), *L. calostachys* (P=0.000) and *O. capensis* (P=0.000). *Asplenium aethiopicum* had a statistically lower mean of eggs hatched as compared to *J. floribundum, V. simplicifolia and O. rochetiana* (22.0 ± 1.73). *Asplenium aethiopicum* also had statistically a higher mean of eggs hatched as compared to *L. calostachys* (P=0.018) and *O. capensis* (P=0.000). *Asplenium aethiopicum* had a statistically different mean of eggs hatched as compared to all the other plants tested (Appendix XIX).

There was no statistical difference between the mean of eggs hatched by *J. floribundum* at the concentration of 50 mg/µl and the mean eggs hatched by *V. simplicifolia* or *O. rochetiana* (P = 0.641). Further, there was also no significant difference between the mean of egg hatched by *L. calostachys* and *O. capensis* (P = 0.308). A scheffe post hoc test created three groups: (C) the greatest number of egg hatching occurred in plant *J. floribundum, V. simplicifolia* and *O. rochetiana*; (B) the second moderate number of egg hatching in plants occurred in *A. Aethiopicum;*

(A) the lowest number of egg hatching occurred in *L. calostachys* & *O. capensis* (Table 4.8).

Based on mean for groups in homogeneous subsets, the most potent plant was *O. capensis*, followed closely by *L. calostachys* and then *A. aethiopicum*. Least potent was *J. floribundum* though not statistically different from *O. rochetiana* and *V. Simplicifolia*

Table 4.8: Mean for groups of methanolic plant extracts in homogeneous subsets at the concentration of 50 mg/µl

Plant		Subset for alpha = 0.05		
		A	B	C
Scheffe[a]	***O. capensis***	1.00		
	L. calostachys	5.67		
	A. aethiopicum		14.00	
	O. rochetiana			22.00
	V. simplicifolia			24.33
	J. floribundum			25.33
	Sig.	.308	1.000	.641

4.10.2 Number of larvae recovered after treatment with various concentrations of plant extracts

Olea capensis had statistically the lowest mean of larvae recovered (1.0 ± 1.0) compared to *V. simplicifolia* (17.0 ± 1.0), *O. rochetiana* (16.0 ± 1.0) and *A.*

aethiopicum (11.0 ± 4.58). *Vepris simplicifolia* had statistically the highest mean of larvae formed at concentration 50 mg/µl (17.0 ± 1.0) as compared to *A. aethiopicum* (11.0 ± 4.58) and *O. rochetiana* (16.0 ± 1.0). *Jasminum floribundum* (9.0 ± 2.0) had the highest mean of larvae recovered as compared to *L. calostachys* (4.0 ± 1.0) (Table 4.9).

Table 4.9: Mean number of larvae recovered in methanolic plant extracts at concentration of 50 mg/µl

Plant	Mean ± Standard Deviation (SD)
J. floribundum	9.0 ± 2.0
V. simplicifolia	17.0 ± 1.0
A. aethiopicum	11.0 ± 4.582576
L. calostachys	4.0 ± 1.0
O. capensis	1.0 ± 1.0
O. rochetiana	16.0 ± 1.0

Number of larvae formed in the treatments with different methanolic plant extracts at concentration of 50 mg/µl were significantly different ($F = 25.24$; $P = 0.0001$) according to ANOVA test. A Scheffe post hoc test showed that *J. floribundum* had a significantly lower mean of larvae formed as compared to *V. simplicifolia* ($P =$

0.023) and significantly higher compared to *O. capensis* (P = 0.023). *Vepris simplicifolia* had a significantly higher mean of larvae formed at a concentration of 50 mg/µl as compared to *J. floribundum* (P = 0.023), *L. calostachys* (P = 0.000) and *O. capensis* (P = 0.000). *Olea capensis* had statistically the lowest mean of larvae recovered as compared to *L. calostachys* but the two were not significantly different (P = 0.73) (Appendix XX). There was no significant mean difference in larvae recovered between *O. capensis* and *L. calostachys* (P = 0.730). There was no significant mean difference between *L. calostachys, J. Floribundum* and *A. aethiopicum* (P = 0.053). There was no significant mean difference between *J. Floribundum, A. aethiopicum* and *O. rochetiana* (P = 0.053). Finally, there was no statistical difference between *A. aethiopicum, O. rochetiana* and *V. simplicifolia* (P = 0.118).

A scheffe post hoc test created four groups: (D) - the greatest number of larvae recovered occurred in plant *V. simplicifolia, O. rochetiana* and *A. Aethiopicum*; (C) - the second greatest number of larvae recovered in plants *O. rochetiana, A. Aethiopicum* and *J. floribundum*; (B) - the second lowest number of larvae recovered in plants *J. floribundum, A. Aethiopicum* and *L. calostachys*; (A) - the lowest number of recovered larvae in plants *L. calostachys* & *O. capensis* (Table 4.10). The most potent plant among the six plants was *O. capensis* followed by *L. calostachys* though not significantly different, *J. floribundum, A. aethiopicum, O. rochetiana* respectively, and least potent was *V. simplicifolia*

Table 4.10: Mean for groups of methanolic plant extracts in homogeneous subsets for larvae recovered at the concentration of 50 mg/µl

Plant		Subset for alpha = 0.05			
		A	B	C	D
Scheffe[a]	*O. capensis*	1.00			
	L. calostachys	4.00	4.00		
	J. Floribundum		9.00	9.00	
	A. aethiopicum		11.00	11.00	11.00
	O. rochetiana			16.00	16.00
	V. simplicifolia				17.00
	Sig.	0.730	0.053	0.053	0.118

4.10.3: Comparing various concentrations of methanol extract with the water extracts

Using independent t-test for the comparison of the various means of methanol extract in egg hatching at 50 mg/µl concentration from the six plants tested and the water extracts (Appendix XVIII) showed that, there was a significant difference in methanolic extract versus water extract in egg hatchability in plant extract *Leucas calostachys* (t = 7.00; P = 0.020), *Olea capensis* (t = 16; P = 0.004) and *Olinia rochetiana* (t = 6.53; P = 0.023), while there was no significance difference in methanolic versus water extract in *Jasminum floribundum* (t = 1.33; P = 0.314),

Vepris simplicifolia (t = 1.00; *P* = 0.423) and *Asplenium aethiopicum* (t=0.79; *P*=0.513) (Table 4.11).

Table 4.11: Comparison of mean eggs hatched in the different concentration of methanol extract with water extract

	Mean of Methanolic plant extracts at 50mg/µl ± SD	Water extract	t- value	P values
Jasminum floribundum	25.33 ± 3.51	29.33 ± 3.06	1.33	0.314
Vepris simplicifolia	24.33 ± 2.52	28.67 ± 6.81	1.00	0.423
Asplenium aethiopicum	14.00 ± 1.00	16.67 ± 5.13	0.79	0.513
Leucas calostachys	5.67 ± 2.31	22.00 ± 2.65	7.00	0.020*
Olea capensis	1.00 ± 1.00	6.33 ± 1.53	16.00	0.004*
Olinia rochetiana	22.00 ± 1.73	43.00 ± 6.08	6.53	0.023*

*Indicate a significant independent t value at 95% CI between the rows (the plant extracts and water extracts)

4.10.4 Effects of plants extract concentrations on larval development

The results showed that *Olea capensis* extracts killed the highest number of living *H. contortus* larvae at 50 mg/µl and 100 mg/µl concentrations, while *Leucas calostachys* extracts killed all living larvae, when water extract was used. Rate of

larvae killed increased as the plant extract concentration was increased. For the controls, distilled water killed none of the larvae while Levamisole killed all the living larvae (Figure 4.1).

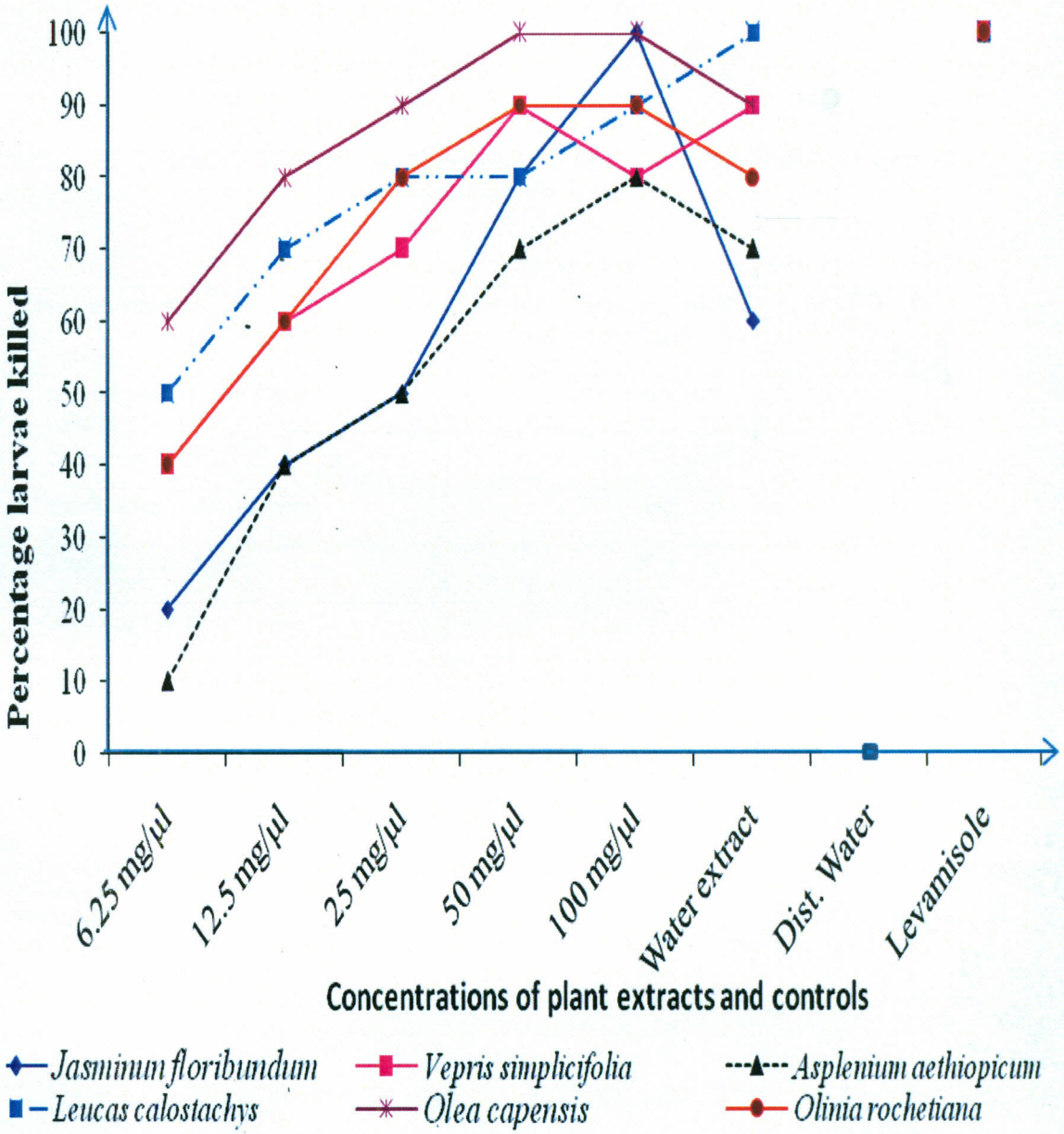

Figure 4.1: Percentage larvae killed by different concentrations of methanol plant extracts, water extracts and controls (distilled water and Levamisole)

When the number of larvae killed was determined, there was no significant difference (F = 2.613; $P = 0.080$) in mean of larvae killed by the various methanolic plant extracts tested at 100 mg/µl concentration. *Olea capensis* plant extract killed the highest number of larvae with a mean of 9.33 ± 0.577 followed closely by *V. simplicifolia* and *O. rochetiana* with mean of 9.0 ± 1.0 (Table 4.12).

Table 4.12: Mean of *H. contortus* larvae killed by methanolic plant extracts at 100 mg/µl

Plant extract	**Mean larvae killed ± SD in methanolic extract**
Jasminum floribundum	8.0 ± 1.0
Vepris simplicifolia	9.0 ± 1.0
Asplenium aethiopicum	7.0 ± 1.0
Leucas calostachys	8.0 ± 1.03
Olea capensis	9.33 ± 0.577
Olinia rochetiana	9.0 ± 1.0

CHAPTER 5: DISCUSSION

5.1 Prevalence of helminths

In the present study between 2006 and 2012, a total of 19,856 (23.438%) livestock were infested with various types of helminths in Koibatek and Mogotio sub counties. The study indicated a high prevalence and widespread distribution of helminthic infestations in the two sub counties, which caused a reduction in animal production, financial loses as well as high rate of livestock infections by parasites. This concurs with an earlier study which indicated high prevalence of flukes and gastrointestinal nematodes in livestock in tropical countries (Kaufmann and Pfister, 1990; Moyo, 1990; Anene *et al.*, 1994).

Helminthic infestations of liver (being a major organ that plays many vital functions in the body) led to poor production. Also, the organ is highly demanded by the people due to its high nutritive value and when condemned in slaughter points, it causes very high financial loses. Therefore, there is an urgent need to combat helminthiases by introducing ethnoveterinary medicinal plants to supplement the conventional drugs which have been resisted by nematodes (Kaplan, 2004). A study on the prevalence of *Cytic echinococcosis* in slaughtered animals was conducted in three divisions of northern- Turkana- Kenya. Livestock were examined and *Echinococcus granulosus metacestodes* were found in large numbers (Njoroge *et al.*, 2002) which agrees with the current study findings. In respect to the current study on the prevalence of helminths, the year 2006 had the highest infestation than all the years under consideration because of heavy rainfall that was experienced through

year. It is clear that helminthic infections are rampant and there is need to counter them in order to increase production amongst livestock farmers.

The current findings conform to earlier study carried out in semi- arid coastal region in Taita Taveta Division in Kenya to assess the prevalence and economic significance of *Fasciola gigantica* and *Stilesia hepatica* in slaughtered animals (Mungube *et al.*, 2006). *Fasciola gigantica* and *S. hepatica* constrained ruminant productivity. Ruminant productivity systems of Taita Taveta division were estimated in retrospective appraisal of the slaughter records on the total number of animals slaughtered and liver condemned due to helminthic infections over a period of 1989 to 2004. Liver condemnation rates differed significantly between bovine, caprines and ovines for *F. gigantica* and for *S. hepatica* in the present study.

5.2 Knowledge of ethno veterinary plants

Knowledge on the use of indigenous medicinal plants was more bestowed on men than women, with young generation being unaware on the use of ethno veterinary medicinal plants. This is because knowledge was fundamentally rooted among men whose social responsibility traditionally was livestock keeping and management hence acquired more information on use of traditional medicinal plants for helminthiases treatment than women. Only a few women had medicinal plants knowledge because it is believed that some might have acquired the knowledge through observation as medicinal plants were being prepared by men to treat animal helminthiases. Due to western kind of culture currently, young generation is losing

the knowledge rapidly This supports an earlier study by Kemonga (2010) that nearly two times more males than females were knowledgeable about medicinal plants used for treating livestock helminthiases. Despite the available veterinarians, farmers rely on their knowledge for prevention and treatment of helminthiases as reported elsewhere (Walzer *et al.*, 1991). They acquire the knowledge of medicinal plants against helminths from parents and grandparents (ancestors), contemporary practitioners or from experience. This was analogous with studies carried out in Ethiopia (Giday *et al.,* 2009), Pakistan (Farooq *et al.*, 2008) and Brazil (Barboza *et al.*, 2007; Monteiro *et al.*, 2011). The traditional knowledge was passed orally from generation to the next and sometimes within the family to constitute the basis for traditional bio prospect (Cox, 2000). Traditional bio prospecting forms the foundations for ethnomedicine (Sindiga *et al.*, 1993) and ethnoveterinary practices (Ole-Miaron, 1997). Further, according to Nanyingi (2008) the average numbers of medicinal plants known and used by female and male practitioners were similar which disagree with the current study. The present study concurs with Nanyingi (2008) observation that informants between 58 and 77 years old mentioned more species than younger informants and ethnobotanical knowledge was passed on by word of mouth.

5.3 Medicinal plants used

All the six plants selected and evaluated, namely: *Jasminum floribundum*, *Vepris simplicifolia, Asplenium aethiopicum, Leucas calostachys*, *Olea capensis* and *Olinia rochetiana* exhibited anthelmintic activity. The anthelmintic activity of the plants had different degree of action and this could be attributed to chemistry of the plants,

place of collection and target parasite to exert anthelmintic effects (Pribitkin, 2005). The vegetation of the area was predominated by trees and shrubs as well as herbs. The majority of medicinal plants recorded were trees followed by shrubs and herbs. The present study disagrees with earlier findings in parts of Ethiopia (Tessema *et al.*, 2001; Sori *et al.*, 2004; Teklehaymanot *et al.*, 2007) that herbaceous medicinal plants were widely used for treatment of helminthiases, followed by trees and shrubs. Leaves were predominantly used plant part for herbal preparation followed by bark. The findings from the current study were in conformity with previous study of Buuri District, Meru County, Kenya, where mostly trees constituted the largest category, followed by herbs and shrubs (Gakuubi and Wanzala, 2012). According to Nanyingi (2008), analysis of growth forms partially differs with the current study in that, the largest form of medicinal plants used for treating animal helminthiasis constitute shrubs, followed by trees and herbs. Leaves were the most frequently used plant part constituting 40%, followed by roots (30%), stem (10%), seeds/fruits (18%) and whole plant (4%).

Extinction of medicinal plants is brought about by anthropogenic activities and natural factors according to the present findings. Also, transmission of knowledge from one generation to another by elderly to young contributes to loss of information when older generation dies before conveying the knowledge. This agrees with a similar study carried out (Giday *et al.*, 2005), that medicinal plants remedies were found only in the wild possession and there is threat to their existence as long as there is mass destruction of their habitat continues and that the mode of transfer is from generation to generation.

5.4 Pharmacological activities of plant extracts

The present study shows that all the six plants extracts used in the tests (*in-vitro*) as ethno-veterinary medicine could be of value in treatment of livestock helminthiases irrespective of solvent used to extract the active ingredients because it showed levels of effectiveness in reducing egg hatching and larval development of *Haemonchus contortus* eggs and larvae respectively. The present study indicated that medicinal plants; *Jasminum floribundum*, *Vepris simplicifolia, Asplenium aethiopicum, Leucas calostachys*, *Olea capensis* and *Olinia rochetiana* could be of value in the treatment of helminthiases because they yielded appreciably positive results. Most potent among the six plants were *Olea capensis* and *Leucas calostachys* which showed high level of activity in both tests indicating presence of active components. These findings agree with previous reports that indigenous plants are useful in treatment of helminthiases (Akhtar and Riffat, 1984). Two of the studied plants (*Cannabis sativa* and *Moringa oleifera*) however, did not give satisfactory results in the initial screening. The present findings also agrees with previous studies in Samburu District of Kenya on efficacy of *Myrsine africana*, *Albizia anthelmintica* and *Hilderbrantia sepalosa* herbal remedies against mixed natural sheep helminthiases (Gathuma *et al*., 2004). According to Gathuma (2004), all the herbal remedies had some efficacy against both nematodes and monezia species of helminths. The effects of plant extracts concentrations against nematodes were significantly different as compared to untreated control group, the efficacy against nematodes was 77%, 89.8% and 90% for *M. africana, A. anthelmintica* and *H. sepalosa*, respectively while albendazole had 100% efficacy. The current findings also concur with the previous study to assess herbal anthelmintics used by farmers in Kirinyaga County, Kenya, for treatment of helminthiases in cattle where *Entada leptostachya* exhibited

highest *in-vitro* activity while *Erythrina abyssinica* had lowest activity. Active ingredients that caused anthelminthic activity were present (Njonge *et al.*, 2013).

Extraction efficiency of methanol and water was relatively high for both solvents. This demonstrated that both solvents could equally be good for bioactive ingredients for extraction. Although the yields were different, their biological activity did not display the same trend, probably indicating the bulk of the extracts may not be biologically active. It is likely that the active molecules are more soluble in methanol than water solvent compared to compounds responsible for bioactivity. This could be due to high solubility of reducing sugars that may not participate in pharmacological activities of chemical compounds. According to Gakuya (2001), water, methanol and chloroform solvents were used to extract *Albizia anthelmintica* for lethality test on shrimps. The present study findings were similar with these findings, in that difference in method of bioactive ingredients extraction may have affected ingredients extracted thus affecting their bioactivity. Further in conformity to the current study, *Albizia anthelmintica* from different parts of Kenya (Githiori, 2003) and *Hedera helix* from Ethiopia (Equale *et al.*, 2007) showed difference in efficacy between solvents preparations. But active ingredients responsible for parasite destruction may not be affected by different solvents since different compounds have different solubility and perform different roles. In the present study, both extracts achieved relatively the same maximum effects despite the difference in potency.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

(i) Results obtained indicate that helminthic infections in Koibatek and Mogotio Sub- counties are rampant. The helminths/stages of development causing havoc in the two sub-counties include *Fasciola hepatica*, *Stilesia hepatica*, Echinocyst, Calcified cyst and Hydatid cyst, which are causing helminthiases.

(ii) Farmers knowledge on the use of medicinal plants for helminthiases treatment in the two sub-counties was limited to few people especially traditional herbalist and more so to the elderly generations who rear livestock. This knowledge was only transferred secretly by word of mouth to confidants and close family members after a well thought consideration by herbalist.

(iii) In Koibatek and Mogotio sub-counties, there are medicinal plants of value for treating sheep helminthiases. The plants that were mentioned, selected and finally tested include: *Olea capensis*, *Olinia rochetiana*, *Leucas calostachys*, *Jasminum floribundum*, *Asplenium aethiopicum* and *Vepris simplicifolia.*

(iv) The preliminary results on the six selected medicinal plant species (*Jasminum floribundum*, *Vepris simplicifolia, Asplenium aethiopicum, Leucas calostachys*, *Olea capensis* and *Olinia rochetiana*) used in traditional treatment of sheep helminthiases in *in-vitro* system displayed degrees of anthelmintic activity in reducing egg hatching and killing of larval stages (L3) of *Haemonchus contortus* worms, when both methanolic and water extract were used. This is a scientific proof of the presence of active ingredients in the plants studied.

6.2 Recommendations

(i) The study encourages the use of *J. floribundum, V. simplicifolia, A. aethiopicum, L. calostachys, O. capensis* and *O. rochetiana* plants species in treating sheep helminthiases because they have an anthelminthic activity.

(ii) There is need for research and development of herbal drugs from the six studied medicinal plants species for integration into existing, yet inadequate and cost ineffective modern veterinary drugs that are hardly affordable to many livestock owners. Investigations are needed to be undertaken to identity active compounds (ingredients) present and standardization of dosage by conducting *in vivo* trials (using sheep) for bioactivity of the medicinal plants.

(iii) Toxicity studies on the six species of plants need to be conducted to develop confidently used herbal remedies. Further mechanisms of action, compatibility with other drugs, side effects and other important parameters should be carried out.

(iv) Conservation efforts should be undertaken on the plants mentioned in order to preserve them due to the pressure of increased population leading to overgrazing, land clearing for farming expansion and other human activities. This would ensure availability of the plants for future research work and generation of novel drugs.

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APPENDICES

APPENDIX I: Questionnaire for Koibatek and Mogotio sub-counties residence for data acquisition

Determination of anthelmintic activities of selected medicinal plants used in treating sheep helminthiases in Koibatek and Mogotio sub-counties, Baringo County, Kenya

PART 1

a) Respondent's particulars

i) Name of respondent .....

Sex (M/F) .....

Age ..... Tribe .....

Religion .....

ii) Education level (illiterate, primary, secondary or post secondary education)

.....

iii) Location ..... Sub location .....

District ..... County..... Sub county .....

iv) Occupation .....

b) Particulars about medicinal plants

i) Do you know of any medicinal plants for treating livestock helminthiases (yes / No) .....

ii) If yes, list the plant as below

Table of medicinal plants used in the Koibatek and Mogotio sub- counties

S/NO	Plant name (local name)	Parts of plant used	Mode of preparation of use	Approximate quantity used per animal	Any known side effects	Place of harvest
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

ii) What are the people's feelings towards using medicinal plants in livestock deworming.

.....

.....

iii) Response of interviewee: - Good ☐ Fair ☐ Poor ☐

(iv) Which parts of the plant named is used for treating animals?

.....

(v) What are the routes of application? .....

(vi) What is the approximate dosage for each animal?

.....

PART II

Respondent Consent agreement

I..... Hereby agrees to participate in this study with my full consent and conscience and declare that to the best of my knowledge. The information that I have provided is true, accurate and complete.

Signature / thumb print ..... date:

.....

PART III

Researcher's declaration

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual proprietary of the Kalenjin community.
2. I will at no time initiate or conduct practices that are deemed to obtain information from the respondents by intimidation, coercion or false pretence.
3. The respondent will be informed of the intended project elaborately prior to questionnaire administration and in confidence to eliminate any degrees of conspiracy.
4. I will be under no obligation to edit / temper the information from the respondents.
5. Translation and transcription will be necessary for clarification due to the language barriers.
6. The information collected will be used for the described research purpose and not any undisclosed intentions.

Appendix II: Check list questions for focused group discussions

(i) Do farmers in Koibatek and Mogotio Sub-Counties have access to veterinary services?

.....

(ii) Which type of services do they offer?

.....

(iii) Apart from lack of pastures, water and mineral supplements, what else can lower the production of livestock?

.....

(iv) Are you aware of worms or helminths prevalent in livestock in Koibatek and Mogotio Sub-Counties?

.....

(v) What do farmers use to combat helminthiases at the locality?

a) Conventional drugs (yes/no).....

List them.....

b) Herbal medicinal plants (yes/no).....

List them.....

.....

(vi) Between conventional and herbal drugs, which one is reliable and economical to farmers?

.....

(vii) Does ethnoveterinary medicine used for helminthes eradication effective?

.....

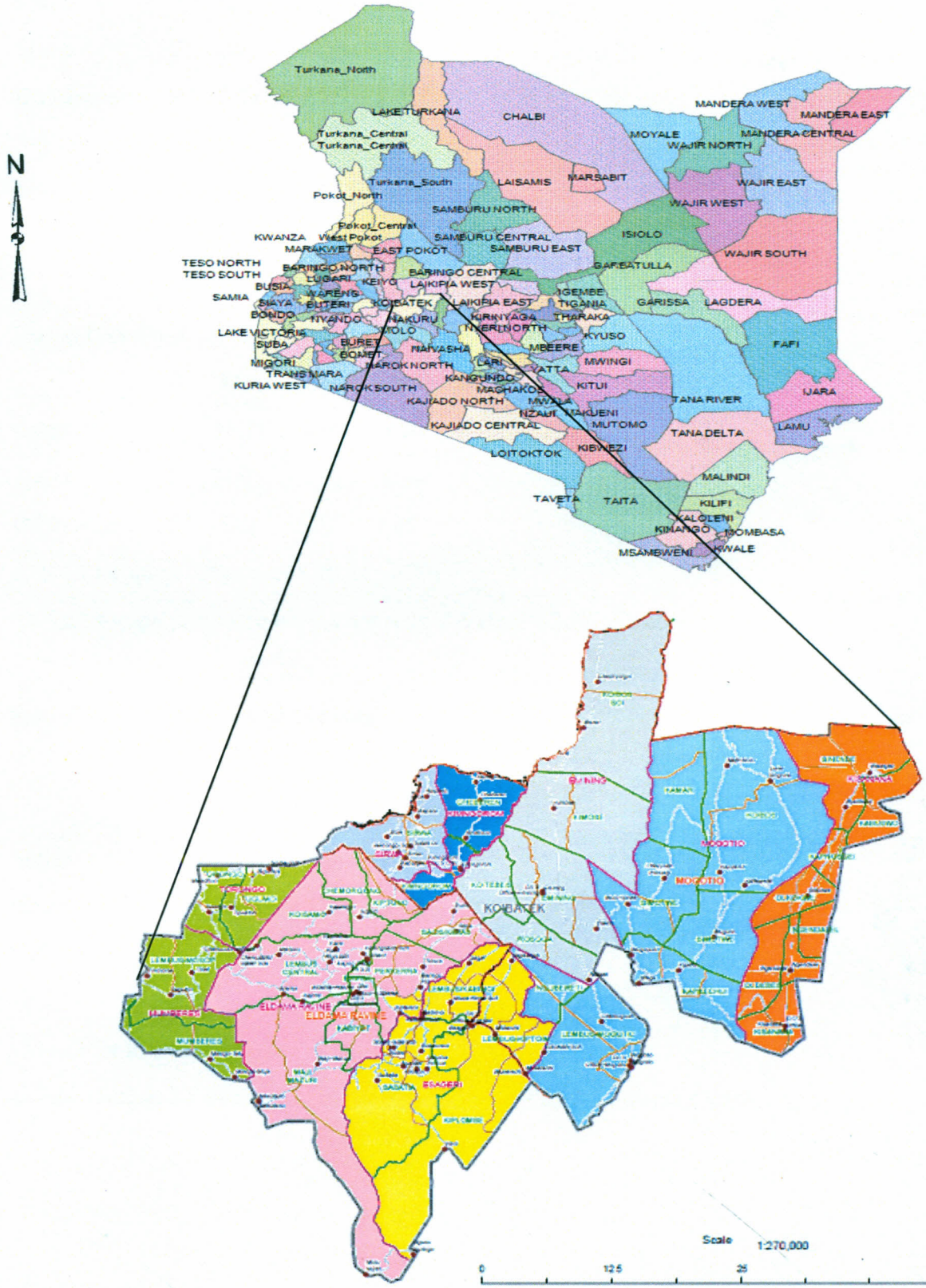
.....

(viii) What should be done to conserve such knowledge for generations to come?

.....

.....

Appendix III: Map of study area. Top: Map of Kenya illustrating the geographic position of Koibatek and Mogotio Sub-Counties. Bottom: Koibatek and Mogotio Sub-Counties indicating the divisional boundaries. Source (Kenya Population & Housing Census, 2009).



Appendix IV: Paired t- test for the livestock

Paired T-Test and CI: cattle versus sheep

	N	Mean	St Dev	SE Mean
Cattle	7	18.21	4.08	1.54
Sheep	7	27.31	4.20	1.59
Difference	7	-9.11	4.52	1.71

95% CI for mean difference: (-13.29, -4.92)

T-Test of mean difference = 0 (vs not = 0): T-Value = -5.33, *P*-Value = 0.002

Paired T-Test and CI: cattle versus goats

	N	Mean	St Dev	SE Mean
Cattle	7	18.21	4.08	1.54
Goats	7	24.01	3.53	1.34
Difference	7	-5.81	5.42	2.05

95% CI for mean difference: (-10.82, -0.79)

T-Test of mean difference = 0 (vs not = 0): T-Value = -2.83, *P*-Value = 0.030

Paired T-Test and CI: sheep and goats

	N	Mean	St Dev	SE Mean
Sheep	7	27.31	4.20	1.59
Goat	7	24.01	3.53	1.34
Difference	7	3.30	4.26	1.61

95% CI for mean difference: (-0.64, 7.24)

T-Test of mean difference = 0 (vs not = 0): T-Value = 2.05 *P*, -Value = 0.086

Appendix V: Comparisons of helminthic infestations among sheep versus goats; cattle versus goats and cattle versus sheep between the year 2006 and 2012

	Cattle	**Sheep**	**Goat**
Cattle		t = 5.33 , *P* = 0.002*	t = 2.85, *P* = 0.030*
Sheep	t = 5.33 , *P* = 0.002*		t = 2.05, P = 0.086
Goat	t = 2.85, *P* = 0.030*	t = 2.05, *P* = 0.086	

*indicate significant different in the mean values of the two animals at 95% CI

Appendix VI: Chi-Square Tests on farmers knowledge on medicinal plants

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	63.333(a	48	.068
Likelihood Ratio	43.995	48	.638
Linear-by-Linear Association	1.423	1	.233
N of Valid Cases	16		

63 cells (100.0%) have expected count less than 5. The minimum expected count is .06.

Appendix VII: Mann-Whitney Test and CI: male, female

Male N = 8 Median = 12.20

Female N = 8 Median = 8.70

Point estimate for ETA1-ETA2 is -0.00

95.9 Percent CI for ETA1-ETA2 is (-13.29, 13.70)

W = 69.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.9581

The test is significant at 0.9577 (adjusted for ties)

Cannot reject at alpha = 0.05

Appendix VIII: One way analysis of variance (ANOVA) table for eggs hatched

One-way ANOVA: 50 mg/versus Plants

Analysis of Variance for 50

Source	DF	SS	MS	F	P
Plants	5	1578.28	315.66	65.31	0.000
Error	12	58.00	4.83		
Total	17	1636.28			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	--+---------+---------+---------+----
J. floribundum	3	25.333	3.512	(-*--)
V. Simplicifolia	3	24.333	2.517	(-*--)
A. aethiopicum	3	14.000	1.000	(--*--)
L. calostachys	3	5.667	2.309	(--*-)
O. capensis	3	1.000	1.000	(--*--)
O. rochetiana	3	22.000	1.732	(--*--)

--+---------+---------+---------+----

Pooled St Dev = 2.198 0 10 20 30

Appendix IX: One way analysis of variance (ANOVA) - Table for larvae that hatched from eggs.

Source	DF	SS	MS	F	Prob.
Extracts	5	2464.6	492.91	3.3	0.016
Residual	30	4429.7	147.66		
Total	35	6894.2			

Overall Mean = 18.78 s (Residual) = 12.15

Coefficient of Variation = 64.7 %

The following observations have large residuals

Observation	Value	Residual	se	Ratio	%RSS
6	59	25.33	11.1	2.28	14.5
12	51	26.67	11.1	2.4	16.1

MAIN EFFECTS

Extracts

Level	Mean	Count	S.E.
J. floribundum	33.67	6	4.961
V. simplicifolia	24.33	6	4.961
A. aethiopicum	15.67	6	4.961
L. calostachys	9.833	6	4.961
O. capensis	10.5	6	4.961
O. rochetiana	18.67	6	4.961

Appendix X: One way analysis of variance (ANOVA) - Table for larval development

Source	DF	SS	MS	F	Prob.
Plants	5	3031.9	606.38	4.7	0.003
Residual	30	3862.3	128.74		
Total	35	6894.2			

Overall Mean = 18.78 s (Residual) = 11.35

Coefficient of Variation = 60.4 %

The following observations have large residuals

Observation	Value	Residual	se	Ratio	%RSS
6	59	27.17	10.4	2.62	19.1
30	4	-27.83	10.4	-2.69	20.1

MAIN EFFECTS

Plants

Level	Mean	Count	S.E.
J. floribundum	20.83	6	4.632
V. simplicifolia	28	6	4.632
A. aethiopicum	11.67	6	4.632
L. calostachys	15	6	4.632
O. capensis	5.333	6	4.632
O. rochetiana	31.83	6	4.632

Appendix XI: One way analysis of variance (ANOVA) - Larvae versus plant extract

Analysis of Variance for Larvae

Source	DF	SS	MS	F	P
Plant ex	5	3032	606	4.71	0.003
Error	30	3862	129		
Total	35	6894			

Individual 95% CIs for Mean

Based on Pooled St Dev

Level	N	Mean	St Dev	---+---------+---------+----
J. floribundum	6	20.83	10.21	(-----*-----)
V. simplicifolia	6	28.00	10.64	(------*-----)
A. aethiopicum	6	11.67	1.63	(------*-----)
L. calostachys	6	15.00	9.38	(-----*-----)
O. capensis	6	5.33	5.92	(------*-----)
O. rochetiana	6	31.83	20.72	(-----*------)
				---+---------+---------+----
Pooled St Dev = 11.35		0	15	30 45

Appendix XII: Analysis of Variance for Living larvae

Source	DF	SS	MS	F	P
Plant	5	57.89	11.58	2.55	0.049
Error	30	136.00	4.53		
Total	35	193.89			

Individual 95% CIs for Mean

Based on Pooled St Dev

Level	N	Mean	St Dev	---+---------+---------+---------+--
-				
J. floribundum	6	4.167	2.858	(--------*--------)
V. simplicifolia	6	2.833	1.941	(--------*--------)
A. aethiopicum	6	5.167	2.483	(--------*--------)
L. calostachys	6	2.167	1.722	(--------*--------)
O. capensis	6	1.333	1.506	(--------*--------)
O. rochetiana	6	2.667	1.966	(--------*--------)
				---+---------+---------+---------+---
Pooled St Dev = 2.129		0.0	2.0	4.0 6.0

Appendix XIII: Analysis of Variance for Living larvae

Source	DF	SS	MS	F	P
Conc	5	114.89	22.98	8.73	0.000
Error	30	79.00	2.63		
Total	35	193.89			

Individual 95% CIs for Mean based on Pooled St Dev

Level	N	Mean	St Dev	--+---------+---------+---------+----
6.25 mg/ul	6	3.000	1.673	(----*----)
12.5 mg/ul	6	1.500	1.049	(----*----)
25 mg/ul	6	6.333	1.862	(----*-----)
50 mg/ul	6	1.000	0.894	(----*----)
100 mg/ul	6	4.167	1.602	(-----*----)
Water extracts	6	2.333	2.251	(----*-----)
				--+---------+---------+---------+----
Pooled St Dev =	1.623			0.0 2.5 5.0 7.5

Appendix XIV: Analysis of Variance for % Killed larvae

Source	DF	SS	MS	F	P
Plants	5	5789	1158	2.55	0.049
Error	30	13600	453		
Total	35	19389			

Individual 95% CIs for Mean

Based on Pooled St Dev

Level	N	Mean	St Dev	-----+---------+---------+---------
J. floribundum	6	58.33	28.58	(--------*--------)
V. simplicifolia	6	71.67	19.41	(--------*--------)
A. aethiopicum	6	48.33	24.83	(--------*--------)
L. calostachys	6	78.33	17.22	(--------*--------)
O. capensis	6	86.67	15.06	(--------*--------)
O. rochetiana	6	73.33	19.66	(--------*--------)

```
                                    -----+---------+---------+---------+-
Pooled St Dev =   21.29              40        60        80       100
```

Appendix XV: One way analysis of variance (ANOVA) -Table for larvae

Source	DF	SS	MS	F	Prob.
Extracts	5	2235.4	447.08	0.4	0.875
Residual	42	52712	1255.1		
Total	47	54948			

Overall Mean = 67.29 s (Residual) = 35.43

Coefficient of Variation = 52.6 %

The following observations have large residuals

Observation	Value	Residual	se	Ratio	%RSS
39	0	-77.5	33.1	-2.34	11.4

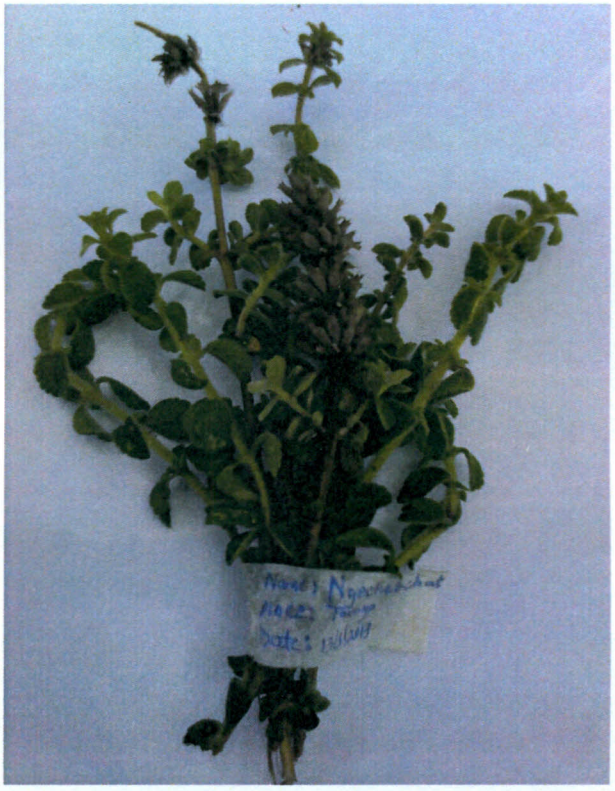
MAIN EFFECTS

Extracts

Level	Mean	Count	S.E.
J. floribundum	61.25	8	12.53
V. simplicifolia	62.5	8	12.53
A. aethiopicum	58.75	8	12.53
L. calostachys	72.5	8	12.53
O. capensis	77.5	8	12.53
O. rochetiana	71.25	8	12.53

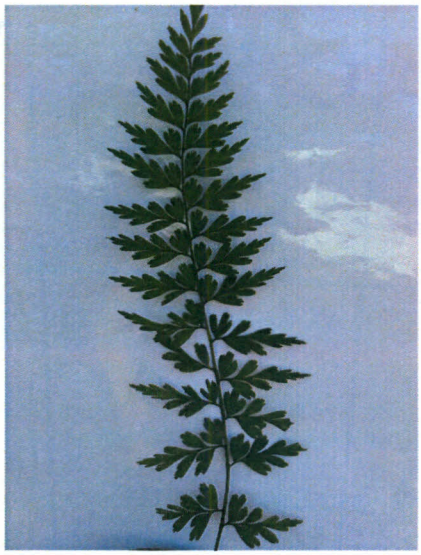
Appendix XV: Extraction of faecal samples at KALRO

Appendix XVI: Photographs of medicinal plants selected for extraction



a) Ngechebchat (*Leucas calostachys)*

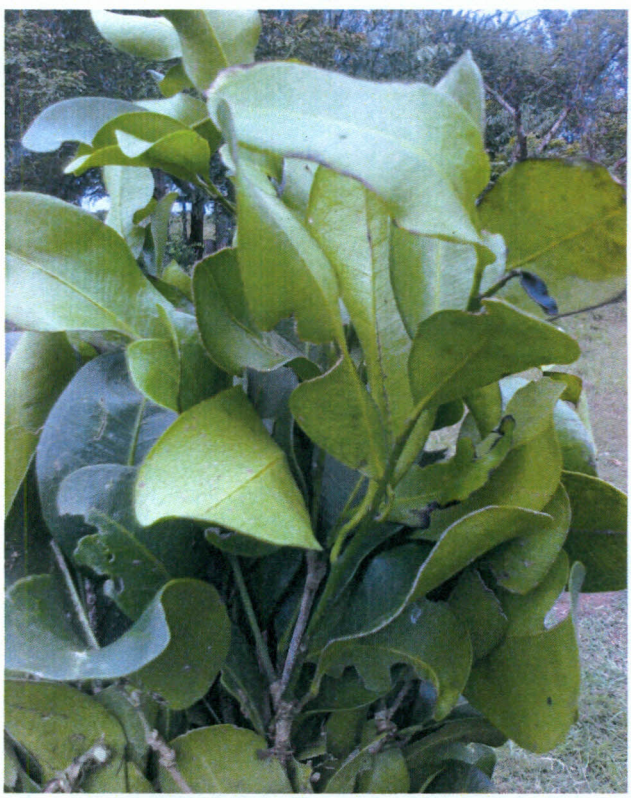
b) Kiptere (*Jasminum floribundum*)



Leaves

Rhizomes

c) Sugumerie (*Asplenium aethiopicum)*

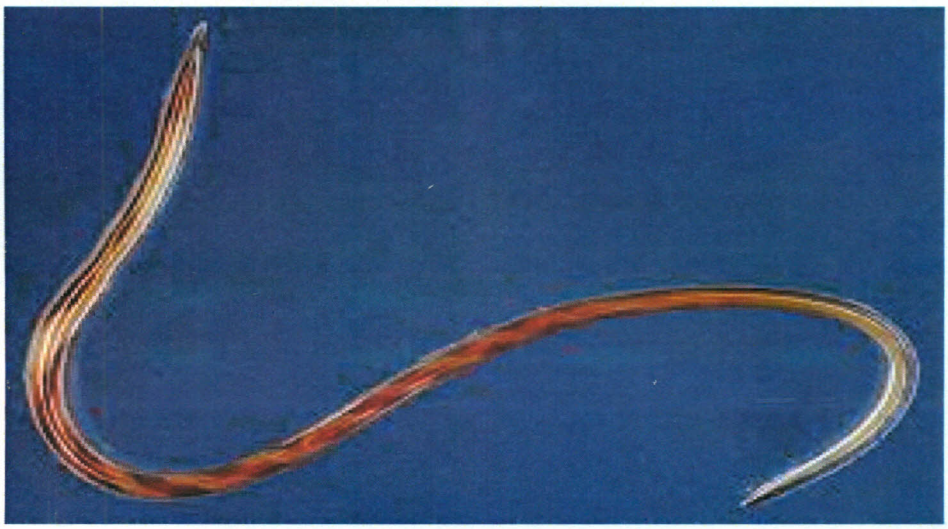


d) Kurionte (*Vepris simplicifolia*)

e) Masaita (*Olea capensis)*

f) Nerkwe (*Olinia rochetiana*)

Appendix XVII: *Haemonchus contortus* larvae



Appendix XVIII: Comparison of mean eggs hatched in the different concentration of methanol extract with water extract

Independent t -Test and CI: *J. floribundum* methanol ext Vs water extract

Independent t- test for Plant A ext - water

	N	Mean	StDev	SE Mean
Plant A ext	3	25.33	3.51	2.03
Water ext	3	29.33	3.06	1.76
Difference	3	-4.00	5.20	3.00

95% CI for mean difference: (-16.91, 8.91)
T-Test of mean difference = 0 (vs not = 0): T-Value = -1.33; P-Value = 0.314

Independent t -Test and CI: *V. simplicifolia* methanol ext Vs water extract

Independent t- test for Plant B ext - water_1

	N	Mean	St Dev	SE Mean
Plant B ext	3	24.33	2.52	1.45
water_1	3	28.67	6.81	3.93
Difference	3	-4.33	7.51	4.33

95% CI for mean difference: (-22.98, 14.31)

T-Test of mean difference = 0 (vs not = 0): T-Value = -1.00; *P*-Value = 0.423

Independent t -Test and CI: *A. aethiopicum* methanol ext Vs water extract

Independent t - test for Plant C ext - water_2

	N	Mean	St Dev	SE Mean
Plant C ext	3	14.00	1.00	0.58
water_2	3	16.67	5.13	2.96
Difference	3	-2.67	5.86	3.38

95% CI for mean difference: (-17.22, 11.89)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.79; *P*-Value = 0.513

Independent t -Test and CI: *L. calostachys* methanol ext Vs water extract

Independent t - test for Plant D ext - water_3

	N	Mean	St Dev	SE Mean
Plant D ext	3	5.67	2.31	1.33
water_3	3	22.00	2.65	1.53
Difference	3	-16.33	4.04	2.33

95% CI for mean difference: (-26.37, -6.29)
T-Test of mean difference = 0 (vs not = 0): T-Value = -7.00; *P*-Value = 0.020

Independent t -Test and CI: *O. capensis* methanol ext Vs water extract

Independent t – test for Plant E ext - water_4

	N	Mean	St Dev	SE Mean
Plant E ext	3	1.000	1.000	0.577
water_4	3	6.333	1.528	0.882
Difference	3	-5.333	0.577	0.333

95% CI for mean difference: (-6.768, -3.899)
T-Test of mean difference = 0 (vs not = 0): T-Value = -16.00; *P*-Value = 0.004

Independent t -Test and CI: *O. rochetiana* methanol ext Vs water extract

Independent t – test for Plant F ext - water_5

	N	Mean	St Dev	SE Mean
Plant F ext	3	22.00	1.73	1.00
water_5	3	43.00	6.08	3.51

Difference 3 -21.00 5.57 3.21

95% CI for mean difference: (-34.83, -7.17)
T-Test of mean difference = 0 (vs not = 0): T-Value = -6.53; *P*-Value = 0.023

Appendix XIX: Multiple comparison of egg hatched in various methanolic plant extract at concentration of 50 mg/µl

Row mean – column mean		*J. floribundum*	*V. simplicifolia*	*A. aethiopicum*	*L. calostachys*	*O. capensis*
Vepris simplicifolia	i-j	-1				
	P	0.997				
Asplenium aethiopicum	i-j	-11.33	-10.3333			
	P	0.002	0.004			
Leucas calostachys	i-j	-19.6667	-18.6667	-8.33333		
	P	0.000	0.000	0.018		
Olea capensis	i-j	-24.3333	-23.3333	-13	-4.66667	
	P	0.000	0.000	0.000	0.308	
Olinia rochetiana	i-j	-3.33333	-2.33333	8	16.3333	21
	P	0.641	0.88	0.023	0.000	0.000

Appendix XX: Multiple comparison of larvae recovered in various methanolic plant extract at concentration of 50 mg/µl

Row mean - column mean		*J. floribundum*	*V. simplicifolia*	*A. aethiopicum*	*L. calostachys*	*O. capensis*
V. simplicifolia	i-j	8				
	P	0.023				
A. aethiopicum	i-j	2	-6			
	P	0.933	0.118			
L. calostachys	i-j	-5	-13	-7		
	P	0.246	0.000	0.053		
O. capensis	i-j	-8	-16	-10	-3	
	P	0.023	0.000	0.005	0.73	
O. rochetiana	i-j	7	-1	5	12	15
	P	0.053	0.997	0.246	0.001	0.000