PHARMACOLOGICAL AND CLINICAL EVALUATION OF THE ANTHELMINTIC ACTIVITY OF *ALBIZIA ANTHELMINTICA* BROGN, *MAERUA EDULIS* DE WOLF AND *MAERUA SUBCORDATA* DE WOLF PLANT EXTRACTS IN SHEEP AND MICE

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DECLARATION

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

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DEDICATION

TO MY LOVING PARENTS, THE LATE JAMES GAKUYA AND MUM MARY WANJIKU

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ABSTRACT

The use of medicinal plants for the control of helminthoses has been in practice for centuries and there are many plants claimed to have anthelmintic activity. However, it is only a few of these plants that have their anthelmintic activity scientifically evaluated. These studies were done to evaluate the anthelmintic activity of *Albizia anthelmintica* Brogn, *Maerua edulis* De Wolf and *Maerua subcordata* De Wolf in sheep and mice and to determine the bioactivity of these plants using brine shrimp lethality test. An attempt was also made to separate the pharmacological active ingredient in *Albizia anthelmintica* water extract.

A questionnaire was used to obtain medicinal plants used by farmers and herbalists in Kibwezi Division of Makueni district and Tunyo division of Marakwet district as anthelmintic. Out of 51 useful plants identified in Kibwezi, *Albizia anthelmintica* (Kyoa in Kikamba) and *Maerua edulis* (Munatha in Kikamba) were singled out by herbalists as very potent anthelmintics. In Tunyo division *Albizia anthelmintica* (Kitwongwo in Marakwet) and *Maerua subcordata* (Liswa in Marakwet) were identified as potent anthelmintics. For this reason, they were collected and botanically identified for further testing and analysis.

The anthelmintic activity of the three plants was evaluated in the present study. Crude extraction of the plant samples was done as described by the herbalists. An aqueous extract from both unground and ground material of each plant material was prepared using boiling water. Twenty one clinically healthy sheep of mixed breeds and sexes were randomly allocated to four treatment groups of four animals each.

The control group had three sheep. Faecal egg counts were done for all the sheep on day 0. A single oral dose of 1.2 ml/kg body weight (*Albizia anthelmintica*) and 0.8 ml/kg body weight (*Maerua edulis*) was administered to the sheep in the 4 treatment groups. The control was left untreated . *Albizia anthelmintica* extract was the only one which reduced faecal egg count. At double the dose, the percentage faecal egg count reduction was 55, 49, 38, 16 and 14 for powdered *Albizia anthelmintica*, powdered *Maerua edulis*, fresh *Maerua subcordata,* fresh *Maerua edulis* and fresh *Albizia anthelmintica* respectively. Therefore, the crude product could control helminthoses to a reasonable extent and maintain the animal at clinically healthy state.

Brine shrimp assay was used to detect bioactivity (LC_{50}) in the various extracts of *Albizia anthelminitica, Maerua subcordata* and *Maerua edulis*. The various extracts were made using water, methanol and chloroform and immediately freeze dried. Brine shrimp eggs obtained from pet shops were hatched using marine salt solution as media and yielded a large number of larvae. Serial dilution of the plant extracts were put into tubes with 10 brine shrimps each. The number of live larvae was determined after 24 hours. Probit method of the Finney computer programme was used to determine the lethal concentration fifty (LC_{50}) and 95% confidence intervals . It was evident that the chloroform extract of the three plant extracts was the most toxic to the brine shrimps compared to water and methanol extracts. *Albizia anthelmintica* extracts of all the solvents was the most potent compared with the two *Maerua* species.

The anthelmintic efficacy of the three plants was studied in mice experimentally infected with *Heligmosomoides polygyrus*. The results indicated a percentage faecal

Heligmosomoides polygyrus egg count reduction of 72%, 69%, 50%, 42% using water extracts of *Albizia anthelmintica* at 10gm/kg bodyweight, *Maerua edulis* at 20gm/kg bodyweight, *Albizia anthelmintica* at 20gm/kg bodyweight and *Albizia anthelmintica* at 5gm/kg bodyweight respectively. Seven days after treatment there was a reduction in worm counts at postmortem of 68%, 36%, 20%, 19%,16% and 14% for water extracts of *Albizia anthelmintica* at 5gm/kg bodyweight, *Maerua edulis* at 10gm/kg bodyweight, *Albizia anthelmintica* at 10gm/kg bodyweight, *Albizia anthelmintica* at 20gm/kg bodyweight, *Maerua edulis* at 20gm/kg bodyweight and *Maerua edulis* at 5gm/kg bodyweight respectively. Mice treated with *Albizia anthelmintica* at 5gm/kg bodyweight had a significantly lower mean worm counts than the rest of the treatment groups and the control ($p<0.05$).There was insignificant reduction in worm counts for other treatment groups compared with the control ($p<0.05$).

The column and thin layer chromatography done on the aqueous extract of *Albizia anthelmintica* yielded only one fraction which was active and had a relative fraction (Rf) of 0.75. The results therefore indicate that the plant have some anthelmintic activity though low with *Albizia anthelmintica* being most efficacious. The bioactive fraction in *Albizia anthelmintica* obtained through chromatographic techniques indicate that there is a rationale in the use of this plant as an anthelmintic by the pastoralists. There is therefore a need to identify the active ingredient in *Albizia anthelmintica* for future commercial use.

CHAPTER ONE

INTRODUCTION AND OBJECTIVES OF THE STUDY ON PLANT ANTHELMINTICS

1.0.Introduction

The livestock production sector in Kenya plays a major role in human nutrition as it provides meat and milk, farmers income and employment. It is the only economic activity for about 25% of the population living in arid areas (Grandin *et al*,1991). According to FAO records of 1994, combined Kenyan exports of both meat and dairy products were more than the imports though there were more imports of dairy products compared to exports (FAO, 1995b). However, Kenya has the option of becoming more self-reliant and exporter of dairy products, with potential foreign exchange saving (Gakuya, 1996). The current status of livestock production and productivity is due to a number of constraints.

The population increase has led to migration of people from high potential agriculture areas which are densely populated, to low potential areas. This has resulted in cultivation in areas previously used by pastoral communities for grazing of animals. Shortage of grazing areas has led to overstocking, malnutrition and increased disease incidence e.g. helminthoses, due to filth build up as opposed to traditional nomadic system (Chiejina, 1986).

Among the important diseases of livestock in sub-saharan Africa, helminthoses is a disease of economic importance which reduces production in livestock. Despite this , the disease is usually not given much attention by farmers because of intercurrent diseases

and with obvious signs which are well identified and also because of it's chronic and enzootic nature.

In the tropics and Kenya in particular among the modern methods of helminth control, use of synthetic anthelmintics is currently being viewed as the most effective strategy (Hammond *et al*,1997). Their use has contributed a lot to livestock development as more people are realising that nematodes are important causes of morbidity and mortality (Kinoti *et al*, 1994).

The use of synthetic anthelmintic is faced with a number of constraints, which include lack of foreign exchange to import the drugs; farmers lacking capital to purchase them; the associated anthelmintic resistance; unavailability in the rural areas; improper use of the anthelmintics by extension staff and farmers; environmental pollution and unreliable manufacturers who often produce anthelmintics with little or no efficacy at all (Kinoti *et al*, 1994).

This then calls the search for alternative methods of helminth control. Among the alternative methods used, i.e. grazing management, improved nutrition, use of helminth resistant animals and helminth vaccines, the use of plant anthelmintics is a realistic and rational alternative. This is because, the use of plants is sustainable, environmentally friendly and the plants are accessible to farmers (Gakuya, 1996). Among the many plants claimed to act against helminths, it is only a few which have been scientifically tested.

Some veterinarians often view the traditional healers and herbalists with contempt (Mesfin and Obsa, 1994). This is supported by the observation that some of the plants which are toxic to helminths, can also be toxic to livestock at high doses (Mbaria *et al*, 1994). In other studies by Ibrahim *et al* (1984), it was reported that some of the plants used as anthelmintics had no efficacy at all. In studies by Kliks (1995), it was reported that there was no significant anthelmintic effect of *Chenopodium ambrosioides* L. used in Mexico. However, some authors have scientifically tested some plant extracts and found them to have anthelmintic efficacy, some up to 100% (Ibrahim *et al*, 1984; Satrija *et al*, 1994).

A number of methods have been used to identify these plants with anthelmintic activity and pharmacological active ingredient as well as testing them for efficacy, toxicity and optimum dosage rate (Dhar *et al*, 1968; Farah,1991; Mbaria *et al,* 1994; Mehrotra, 1984). These range from the use of questionnaires, determination of LD_{50}, solvent extraction and determination of faecal egg count and worm reduction in animals. Toxicity to the brine shrimp has also been used to screen medicinal plants for biological activity (Meyer *et al*, 1982). On the other hand , column chromatography and thin layer chromatography (TLC)have been used for the separation of the active compound in plant materials (Bobbit *et al*, 1968; Harbone, 1973; Kiptoon, 1981; Maitai, 1973).

In order to integrate the plant anthelmintics in the overall helminthoses control, there is need to assess the indigenous knowledge, to carry out experiments to validate pharmacological value so as to assist farmers in discarding plant products which are

ineffective and put emphasis on those plants that have appreciable efficacy and are non toxic.

1.1. Objectives

The objectives of this study were to :-

1. Identify plants used as anthelmintics.
2. Evaluate the efficacy of crude water extracts of *Albizia anthelmintica* Brogn and *Maerua* spps against gastrointestinal nematodes in sheep.
3. Determine the bioactivity of *Albizia anthelmintica*, *Maerua edulis* and *Maerua subcordata* extracts using the brine shrimp lethality test.
4. Evaluate the anthelmintic efficacy of *Albizia anthelmintica* and *Maerua edulis* extracts against patent *Heligmosomoides polygyrus* infections in mice.
5. Separate the pharmacological active ingredient in *Albizia anthelmintica* water extract using brine shrimp lethality guided fractionation

CHAPTER TWO

LITERATURE REVIEW

2.0 Helminthoses in Kenya

2.0.1. Economic importance of helminthoses

Allonby and Preston (1979) estimated the economic loss due to helminthoses in sheep alone in Kenya to be US $26 million per year. In fact, worm infections is the single greatest constraint to sheep and goat production in the tropics (Allonby and Urquhart, 1975). Other losses are due to the costs of anthelmintics, molluscicides, labour, fences and unutilised lands (e.g. swamps infested by snails, the intermediate host of *Fasciola* and *Schistosoma*).

Studies done by Cheruiyot (1980) and Cheruiyot and Onyango-Abuje (1984) on carcass condemned at meat inspection in slaughter houses in Kenya showed that *Taenia saginata* cysticercosis, *Fasciola gigantica*, *Echinococcus* and *Stilesia hepatica* were the major helminth of economic importance. Grindle (1978) estimated an annual loss of US$1.38 million due to *Taenia saginata* cysticercosis alone.

Cheruiyot (1980) estimated an annual loss due to *Stilesia hepatica* between 1975 and 1978 to be US$ 16,700-38,460. It was estimated that the annual economic loss due to fasciolosis was US$4.2. million and this included losses from mortality , reduction in body weight, lowered fertility, liver condemnation and reduction in milk yields (Agriculture Research Foundation , 1986).

2.0.2. Helminth control methods

There are a number of methods that have been used to control helminth infections in livestock and these include;

2.0.2.1 Anthelmintics

The use of anthelmintics is the most effective method of helminth control. Most of the anthelmintics used are broad spectrum and are aimed at eliminating the parasitic stages of the helminth in the host as well as preventing the discharge and build up of eggs and larvae into the environment at the same time allowing the animal to develop premunition (Shavulimo, 1986). The major anthelmintics used are classified and their efficacy to the various classes of livestock helminth are shown in appendices 2.1, 2.2, and 2.3.

2.0.2.2. Nutrition

Well nourished animals are less susceptible to helminth infections (Preston and Allonby,1978). Helminths cause more pronounced detrimental effects in malnourished animals. Generally, poorly fed animals are more susceptible to the effects of internal parasites and are more inclined to carry heavy worm burden due to failure of throwing off infestations. Specific nutritional deficiences such as colbalt, copper, phosphorus or protein are known to lead to decline in animals resistance to worm infestation (Blood *et al*, 1985).

2.0.2.3 Management practices

Husbandry practices that prevail in a farm have a lot of impact on helminth diseases (Torto, 1989). In extensive systems (traditional), the incidence of helminthosis is different from that of intensively managed herds with high stocking rates and limited

housing areas (Chiejina, 1986). Larvae build up is higher in intensive than in extensive systems due to increased dung accumulation compared to the scattered distribution in the extensive system.

2.0.2.4. Grazing management

This control method is aimed at preventing the build up of dangerous numbers of larvae on pastures, minimising acquisation of infection by predicting the periods when there is large build up of larvae and removing susceptible animals from heavily contaminated pastures before these periods (Brunsdon,1980).

There are five major methods of grazing management for helminth control. These are: reduction of stocking density; rotational grazing; zero grazing; dilution and mixed grazing (Arundell and Hamilton, 1975, Sewell, 1976, Shavulimo,1986). The reduction of the stocking density prevents build up of parasites especially those with high fecundity (Shavulimo,1986). Rotational grazing involves limiting intake of infective larvae by allowing animals to graze on a particular area of pasture no longer than three days and not allowing the animals to the same pasture until all the infective larvae have died .

In zero-grazing, cattle do not graze on pasture but are confined in a shed or yard thus serving as a helminth control method (Sewell, 1986). The dilution of pasture involves grazing helminthologically inert stock with susceptible one, e.g. a single suckled calf running with it's cow in a dual purpose or beef ranches. In mixed grazing, two or more different animal species are involved, with one species reducing the number of parasites of the other species, e.g. when cattle are mixed with sheep (Arundel and Hamilton,1975).

2.0.2.5 Helminth resistant breeds

It has been reported that certain breeds of livestock posses a high level of innate resistance to parasites. One breed of sheep in Kenya that has been shown to exert helminth resistance is the Red Maasai sheep (Preston and Allonby, 1978).

2.0.2.6. Destruction of intermediate host.

This is mainly for the control of trematodes i.e. *Fasciola* and *Schistosoma* which require snails as an intermediate host. The control of snails is done by using chemical and plant molluscicides (Broberg, 1982; Cheruiyot *et al,* 1981; Cheruiyot and Wamae, 1988; Hammond *et al*,1994; Hammond and Sewell, 1995). Other management practices are drainage of water, fencing infected areas and providing alternative source of clean water (Anon, 1986).

2.0.2.7. Helminth vaccines

Dineen (1978) and Munn (1993) reported a success in the control of *Haemonchus contortus* using molecular vaccine based on proteins of the parasite's gut membrane, including polymerase proteins, contortin and intregral membrane protein H11 against *Haemonchus contortus*. This immunisation is important for young stocks against *Haemonchus contortus* before they develop immunity (Illes, 1993).

2.0.3. Constraints to helminth control

2.0.3.1. Finance

The shortage of foreign exchange to import synthetic anthelmintics is the major limitation of availability of anthelmintics. Mwamachi *et al* (1995) reported cases where

farmers opted to slaughter their sheep or sell at reduced prices because they were unable to treat them due to expensive veterinary services.

The situation in Kenya is such that the private practice is yet to develop fully and as such most of the veterinary services are left with the Department of Veterinary Services. According to Grandin *et al* (1991), this Department is constrained by lack of transport facilities, inconsistent supply of drugs , low staffing levels at 1:1000 Tropical Livestock Unit (TLU) in high potential areas to 1:13,000 TLU in pastoral areas.

2.0.3.2. Anthelmintic resistance

Kinoti *et al* (1994), observed that there is increased usage of anthelmintics as farmers are becoming aware of the importance of helminth control. This potentially pose a risk of developing anthelmintic resistant especially in major livestock producing areas which rely entirely on synthetic anthelmintics.

There are two possibilities for development of anthelmintic resistance. A number of species of gastrointestinal nematodes of sheep and goats can be resistant to one anthelmintic. One example is the benzimidadole resistance in *Haemonchus contortus, Ostertagia spp., Nematodirus spp., Trichostrogylus spp., Strongyloides spp.* and *Oesophagostomum spp* as reported by Mwamachi *et al* (1995) and McKenna (1989). The other type of resistance is where one species may develop resistance to many anthelmintics as with the case with *Haemonchus contortus* to benzimidazoles, levamisole and Ivormectin (Maingi, 1991; Maingi , 1993 and Mwamachi *et al*, 1995 ;).

According to Taylor and Hunt (1989), resistance to available anthelmintics has been reported throughout the world. In Kenya, anthelmintic resistance has also been reported in sheep and goats for the last 14 years (Maingi, 1991; Maingi, 1993; Maingi *et al*, 1998; Mwamachi *et al* , 1995; Ndarathi, 1992; Njanja *et al*, 1987; Wanyangu *et al*, 1996; Waruiru *et al*, 1991; Waruiru, 1994; Waruiru *et al*, 1994; Waruiru *et al*, 1996; Waruiru *et al* , 1997; Waruiru *et al,* 1998). According to Mwamachi *et al* (1995) , there is resistance of sheep and goat nematodes to a number of anthelmintics in the Kenyan market thus potentially risking farmers abandoning sheep and goat enterprises for lack of effective anthelmintics.

2.0.3.2.1. Causes of anthelmintic resistance

The high frequency of anthelmintic use and possibly incorrect dosage cause anthelmintic resistance. The spread of resistance amongst farms is attributed to introduction of animals with nematode resistance that are purchased from government farms into commercial and small scale farms (Kinoti *et al*, 1994; Mwamachi *et al,* 1995). The keeping of sheep and goats together especially by small scale farmers and pastoralists may also lead to increased chances of development of anthelmintic resistance . Resistant nematodes can be transmitted from goats and sheep if grazed together (Coles and Rousch, 1992). Some farmers use the same dosage for sheep and goats despite the fact that some anthelmintics (albendazole, oxfendazole, levamisole and morantel) are less effective on gastrointestinal nematodes of goats than in sheep (Elliot, 1987; McKenna and Watson, 1987). The regular use of one anthelmintic over a long period of time contributes also to development of anthelmintic resistance in nematodes (Kinoti *et al* ,1994).

All these are further complicated by the paucity of knowledge amongst many farmers in Kenya. It is only the veterinarians and animal health attendants who know the proper dosages of different anthelmintics, factors favouring anthelmintic resistance and frequency of use of the anthelmintics. The illiteracy level amongst farmers is another complications, as they may not be able to read instructions written on the drug packages and clearly understand advices given by the professionals. These constraints indicate that relying entirely on synthetic anthelmintics presents serious difficulties and there is a need to search for alternative methods of helminth control.

2.1. Background on the usage of medicinal plants

2.1.1. Develoment of Ethnoveterinary medicine

The study of folk beliefs, knowledge, skills, practices relating to the care of animals is called ethnoveterinary medicine (McCorkle, 1986). According to the World Health Organization, 80% of the world's population depend on plants for the primary health care (Farnsworth *et al*, 1985). Similarly, according to WHO (1993), it was estimated that about 80% of the population of most developing countries still relies on traditional forms of medicine for daily health care.

Ethnoveterinary medicine is important in Kenya particularly in the pastoral communities such as Rendille, Turkana, Maasai, Samburu, Marakwet and Pokot who are rich in traditional knowledge of disease control (Ohta, 1984). For instance, studies done by Illes (1990) among the Samburu found that out of 104 goats and sheep surveyed 38 % were managed through ethnoveterinary practices. According to Ohta (1984), the Turkana people are not interested in the etiology of the disease but the Samburu pastoralists are very specific and describe the disease according to the clinical signs (Bizimana,1994).

Leakey (1977), reported a lot of ethnoveterinary practices among the Kikuyu who are mainly agro-pastoralists.

In Kenya, there are many plants listed as used for both human and animal disease control. Kokwaro (1993) listed thousands of plants used in human and livestock disease control in Kenya and in other parts of East Africa. Similarly Intermediate Technology Development Group (ITDG) and International Institute of Rural Reconstruction (IIRR) (1996) list medicinal plants used in many parts of Kenya for treatment of livestock diseases.

The Hausa of Nigeria use certain plants as both food and medicine (Bodeker, 1999). Similarly, the Maasai of East Africa cook the bark of *Acacia goetzi* (*Leguminosae*) and *Albizia anthelmintica* with their traditional diet of boiled meat, milk and blood. The bark is claimed to lower cholesterol. This may be the reason why cholesterol levels among the Maasai are about one third of that of the average Americans.

In other countries such as India, as much as 15,000- 20,000 plants posses proven medicinal value (Krisna Kumar,1996). Of these, it is only 7,000 to 7,500 which have medicinal value and are used by rural communities (Chaundri, 1996). Today India exports ten of thousands of medicinal plants a year and is the main supplier of European market.

The use of plant anthelmintics in Britain faded just recently. According to British Veterinary codex (1953, 1965), oil of Chenopodium is listed for use against *Ascaris* in horses and pigs and *Strongylus* in horses. Other plants used as listed in the same British veterinary Codex are the male fern *Dryopteris filix-mas* against *Monezia* , *Ascaridia* and

other gastrointestinal nematodes, i.e. *Cooperia, Haemonchus , Nematodirus, Ostertagia* and *Trichostrongylus. Artemisia cina* and other *Artemisia spp.* are used against *Ascaris suum*, *Toxocara* and tapeworms in poultry.

2.1.2. Plant anthelmintics

2.1.2.1. Plant that have been used to treat livestock helminthoses

There are many plants used for the control of helminthoses in Kenya and other tropical countries. Of the many plants with anthelmintics properties used in many parts of Africa, it is only a few of them which have undergone clinical trials. There are those used specifically against nematodes, cestodes and trematodes (Appendix 2.4 and 2.5).

According to Bizimana (1994), there are several general anthelmintics used in Africa for the control of gastrointestinal nematodes. In Cameroon, the bark of *Terminalia mollis*, roots of *Vermonia guiniensis*, whole plants of *Eygerium canadensis*, root of *Dryopteris athamanticum,* leaves and roots of *Pseudospondias microcarpa*, fruit of *Solanum torvum* and the bark of *Prosopsis africana* are used as anthelmintic against gastrointestinal nematodes. In Tanzania, the leaves of "Ifurufuru" or the root of "Ikingili" are boiled and used against roundworms (Bizimana,1994). In Kenya, pyemarc a by-product of pyrethrum and a commercial ruminant feed is considered to act against gastrointestinal nematodes of ruminants (Mbaria *et al*,1994; Rottcher, 1994).

Bizimana (1994) reported various plant used against liver flukes in Africa. For instance liver fluke infestation in cattle has been treated using the leaves of *Aspilia ciliata* and *Crassocephalum vitellinum* as a drench in Burundi; the bark of *Khaya senegalensis* in

Niger; the bark of *Boswelli delzii*, bark of *Erythrina senegalensis*, leaves of *Lawsonia inermis* and *Allum sativum* in Nigeria (Bizimana,1994).

Elsewhere, according to Minja (1989), *Cissampelos mucromata, Senecio lyrratipartitus* and *Croton microstachys* in Tanzania are very potent, while among the Chaggas, the sap of young shoots of *Musa sapientum* is said to be very effective anthelmintic (Minja, 1991). Bizimana (1994) also reported that in Mauritania, the bark from trunks or rind from roots of *Anogeissus leiocarpus* mixed with leaves and stems of *Securinega virosa* ; the bark from stem of *Khaya senegalensis* and roots of *Nauclea latifolia* produce very effective anthelmintic preparations.

In Zaire, Kasonia *et al* (1991) reported 11 plants used as anthelmintics, while Chavunduka (1976) in Zimbabwe listed two plants used as anthelmintics. In Nigeria, 18 plants were reported by Nwude and Ibrahim (1980) to be used as anthelmintics and the herbal treatment against helminths in calves less than a year old is a usual programme for the Fulani herdsmen in Nigeria (Ibrahim *et al*, 1984).

In Asia, 23 plants were reported being used as anthelmintics against internal helminths (Anon, 1994). In Kenya, about 60% of the plants listed as anthelmintics above are available though not all are used as anthelmintics (Lindsay, 1978; Kokwaro, 1993, ITDG and IIRR,1996). Among medicinal plants of the Marakwet district in Kenya, five of them are used as general anthelmintics (Lindsay,1978). These are *Dryopteris inaequalis, Albizia anthelmintica, Albizia gummifera, Olea africana* Mill and *Myrsine africana* .

ITDG and IIRR (1996) list eighteen plants used as anthelmintic. They are ; *Lantana trifolia, Albizia anthelmintica, Albizia coriavera, Diospyros scabra, Trichilia emetica* for lung worms.; *Rhus vulgaris, Cassia spectabilis, Tamarindus indica, Rapanea melanophloeos, Carissa edulis, Cucurbita maxima, Launaea cornuta, Ricinus communis, Ocimum basilicum, Myrsine africana, Tedea nobilis, Allium sativum, Hagenia abyssinica* for roundworms. Kokwaro (1993) list 21 plants used against hookworm; 6 plants for roundworms; 22 plants for tapeworm and one plant for threadworm in humans. He also lists 79 plants used as general anthelmintics.

2.2. Methods used to identify plants of medicinal value and pharmacological parameter

2.2.1. Survey questionnaire

A survey questionnaire has been proposed by Farah (1991) which contains questions for the traditional herbalists or farmers in order to identify medicinal plants and their use. Minja (1994) used this questionnaire format proposed by Farah (1991) in three regions of Tanzania and collected medicinal plants of value. Gakuya (1996) also adapted Farah (1991) questionnaire format intended for study of medicinal plants with anthelmintic activity . The format is very useful in identifying plants used as anthelmintic, how often they are used; which helminth infections they are used for; the parts used; extraction procedures and dosage rates.

Mehrotra (1984) proposed guidelines on the collection of medicinal plants (Appendix, 2.6). Identification of the plant should be done by a botanist (Harbone, 1973). He suggested that a fresh plant tissue is ideal for phytochemical analysis and should be plunged into boiling alcohol within minutes of collection (Harbone, 1973). However,

plants can be dried before extraction and if so, drying should be done under controlled conditions to avoid too many chemical changes. It is important that the plant tissue collected should be free from disease, i.e. not affected by fungal, viral or bacterial infection.

2.2.2. Methods for extraction of active ingredient

The mode of extraction depends on the texture and water content of the plant material being extracted and on the type of substance being isolated (Harbone, 1973). To obtain organic constituents from a dried plant material, a continuos extraction of the powdered material using soxhlet apparatus with a wide range of solvents, starting with ether, petroleum, and chloroform and then using alcohol and ethyl acetate should be used (Harbone, 1973). Dhar *et al* (1968) proposed the use of 50% ethanol solution for extraction of active ingredients. Thaiyah (1991) also used methanol for extraction on a ground leaf material of *Cassia didymobotrya* Fres held at 37°C for 48 hours. Whereas Ibrahim *et al* (1984) used the same reagent using powdered dry plant material.

Maitai (1973) isolating alkaloids from ground *Catha edulis* material used acidified water on it and boiled it for one hour and simmered it at 80°C for another three hours. The residue was again extracted using acidified water and the process repeated three times. All the aqueous extract from the four extracts was combined and filtered using Whitman paper. The tannins and pigments were precipitated using lead acetate solution and by centrifuging. To extract alkaloids, excess lead ions were removed using 0.1 N sulphuric acid and ether was used on the clear detannated extract. The ether extract, which contained acids and neutrals was set aside leaving the acidified aqueous solution, which had alkaloids. The acidified aqueous solution was alkalinised using sodium bicarbonate or sodium hydroxide and ether again used to extract alkaloids.

Kiptoon (1981) used methanol, water, chloroform and ethanol to extract powdered *Gnidia latifolia* (Meisn) material. The procedure involved adding the respective solvent to the powdered material and after stirring to mix, was left overnight in a water bath at 45°C. The solvent was recovered after 24 hours and soluble extract separated.

2.2.3. Detecting bioactivity in plant material using brine shrimp lethality test

Among the many plants claimed to have medicinal value, it is only a few that have been screened to demonstrate their medicinal value. There is therefore very little quantitative data available on the activity of the plant extracts. The major limitation of obtaining such data is the cost of screening such medicinal plants. It is only large advanced pharmaceutical companies and large laboratories which have the necessary equipment and funds to do complicated bioassays to screen plant extracts. The procedures are expensive, time consuming and the equipment are not available especially in sub-Saharan Africa. At the same time, analysis done on plants by phytochemists are mainly to analyse the constituents and not to test their biological activity. Therefore, the possibility of knowing their medicinal value is rather dim (Meyer *et al*, 1982). There is therefore a need for a reliable, less complicated bioassay for testing medicinal plants for activity.

A simple bioassay method for screening plant extracts using brine shrimp (*Artemia salina* LEACH) is ideal for screening plant material for activity. The eggs of brine shrimp available at low cost in pet shop and used as food for fish hatch large numbers of larvae within 24 hours after being placed on brine solution. The toxicity of plant extracts against the larvae is determined in the brine shrimp assay. Briefly, serial concentrations of the plant extract are prepared by dissolving a given amount of the material in certain

amount of marine salt solution. Then for every concentration, five tubes are prepared and ten brine shrimp larvae are transferred to the extract solution in each tube and the number of surviving larvae determined after 24 hours.

This assay has been used by Meyer *et al* (1982) to evaluate the toxicity of ethanol extracts of seeds (known to contain physiologically active principle) of 41 species of *Euphorbiaciae* of which 18 were toxic in the brine shrimp assay at $LC_{50} < 1000$ug/ml. Similarly, Mwangi *et al* (1999) screened 78 plant samples from twenty one families using brine shrimp lethality test out of which 36 showed toxicity to the brine shrimp at $LC_{50} < 1000$ ug/ml.

2.2.4. Toxicity tests and determination of LD_{50}

It is important to carry out toxicity tests if the plant extract is to be used with safety as opposed to doses that are used in the traditional way. This is because in high dosages, medicinal plants may have systemic toxicity as was reported in many studies (Kiptoon (1981); Kellerman *et al* (1988); Mugera (1970); Mbaria *et al* (1994); Shone and Drummond (1965) and Thaiyah (1991).

The LD_{50} is the median lethal dose of a drug, which kills half of the population, and a comparison of LD_{50} is a measure of acute toxicity. It is an important parameter in acute toxicity studies of any drug and it's determination and that of median effective dose (ED_{50}) gives the therapeutic index which is a measure of safety margin and hence the usefulness of a drug (Maitai, 1973).

Mbaria *et al* (1994) studied the toxicity of pyrethrins (an extract from pyrethrum) using sheep and rabbits. The clinical signs of acute pyrethrins toxicity after oral administration in sheep and rabbit in a 24 hours were hyper-excitation, tremors, convulsions, paralysis and death. They calculated the LD_{50} using sheep (8 animals of each) divided into four groups of two animals corresponding to four dosage levels of pyrethrins and found it to be 595mg/kg with the median effective dose (ED_{50}) required to induce toxic signs in sheep being 420mg/kg. Blood samples collected from treatment groups and controls for evaluation of haematological and biochemical parameters using routine laboratory procedures showed no significant changes after administration of pyrethrins to the sheep. However, post- mortem done on all dead animals after 24 hours revealed extensive pulmonary congestion and oedema and ecchymotic haemorrhages in respiratory and cardiovascular systems. The authors suggested that the cause of death was due to respiratory failure.

Thaiyah (1991) studied the toxicity of *Cassia didymobotrya* Fres in rats. The rats were divided into two sets of five groups each. The first set group 1,2,3, and 4 were fed on powdered leaves mixed with chick mash at 80%, 40%, 20% and 10% respectively and group 5 was fed on chick mash alone and left as control. In the second set, they were treated as in set one and fed on powdered stems. The rats showed clinical signs, which varied in acuteness depending on the concentration of *C.didymobotrya* Fres but eventually all succumbed and died. In determining the LD_{50}, the rats were injected intra-peritoneally with the methanol extract of *C. didymobotrya* Fres reconstituted with physiological saline at varying dosages and observed for 24 hours. The LD_{50} was calculated from the results according to Reed and Muench (1938) and was 5.80gm/kg bodyweight.

Maitai (1973) determined the LD_{50} of d- nor pseudoephedrine, the active principle in *Catha edulis* FORSK (miraa) in mice. Using 24 male albino mice divided randomly in four groups of six, they were randomly injected intra- peritoneally with a graded dose of d- norpseudoephedrine and observed for a period of 10 hours before being left overnight. The LD_{50} was determined using Litchfield and Wilcox (1949) method and was 178mg/kg bodyweight.

Kiptoon (1981) studied the toxicity of *Gnidia latifolia* (Meisn) using calves and rats. The LD_{50} was determined using 42 albino wistar rats by intra- peritoneal injection with ethanol extract at varied dosage rates. The percentage death was plotted on a graph against dosage rate and the LD_{50} estimated from the graph was 278mg/kg bodyweight.

Muchiri (1987) studied the pharmacological and toxicological properties of *Paddiae volkensii* and *Scutia myrtina* (BURM.F.) KURZ using male mice. After intra-peritoneal injection of the extract on the mice, the number that died within 24 hours after injection were recorded and the LD_{50} calculated using the method of Reed and Muench (1938). The LD_{50} calculated at 24 hours was 6.3 g/kg bodyweight for *Paddie volkensii* and 6.1g/kg bodyweight for *Scutia myrtina* (BURM.F(KURZ).

2.2.5. Determination of the percentage faecal egg counts reduction

The percentage faecal egg count reduction is the efficacy and is defined as the difference between the group mean eggs/gram pre- and post treatment and expressed as a percentage of the pre-treatment value (Waruiru *et al*, 1991). FECR% = (1-T2/ T1x C1/C2) where T and C are geometric means for the treated and control groups and subscripts 1 and 2 designate counts before and after treatment respectively. The

percentage efficacy can also be calculated using the following formula of (Njanja *et al* ,1987);

$$\% \text{ Efficacy} = \frac{\text{mean EPG control} - \text{mean EPG treated}}{\text{Mean EPG controls}} \times 100$$

According to the World Association for the Advancement of Veterinary Parasitology (Coles *et al*, 1992), the percentage reduction (PR) = 100 (1-XT/XC), where XT and XC are the average arithmetic mean faecal egg counts for the sample on day 10-14 for the treated and control groups respectively.

Several authors have determined the percentage faecal egg count reduction using various plant anthelmintics in domestic animals. In India, Sharma (1993) reported that Jantana, a commercial anthelmintic preparation tested on 26 crossbred cattle with mild to moderate infections of *Haemonchus spp., Strongyloides spp., Trichostrogylus spp.* and *Nematodirus spp.* had a 100% anthelmintic efficacy on day 7 post- treatment. The Jantana capsules contain extracts *of Artemisia maritima, Brassica nigra, Cassia lanceolata, Vermonia anthelmintica, Cuprium sulphas* and *Embelia ribes*.

Akhtar and Riffat (1984) studied the anthelmintic activity of *Melia azedarach* against natural gastrointestinal nematodosis comprising of *Haemonchus, Trichostrongylus, Trichuris* and *Charbertia spps* in goats. After treatment of the goats with 30 mg/kg of the powdered fruit, the percentage faecal egg count reductions of these nematodes were 70%, 96%, 99% on days 3, 10 and 15 respectively.

The efficacy of Santonin manufactured from *Artemisia maritima* flower heads and commercially available was evaluated and compared with that of piperazine against *Toxocara vitulorum* in bufallo calves after natural infection (Akhtar,1984). It was observed that the efficacy of the two preparation was comparable.

The seeds of *Caesalpina crista* in Pakistan were tested against *Toxocara vitulorum* in buffalo calves by Akhtar *et al* (1985). In naturally infected calves, oral administration of the powdered seeds at 4 gm/kg body weight or equivalent amount of methanol extract showed slower but similar effect in reducing faecal egg counts when compared to 0.01 gm/kg of morantel tartarate. A reduction of 62%, 91% and 100% was observed on days 3,10 and 15 after treatment respectively and the mild and transient side effects compared similarly to those of morantel tartarate.

The effect of *Melia azedarach* and its ethanol extracts against *Ascaridia galli* in experimentally infected chickens was studied by Akhtar and Riffat (1985). At a dosage of 20 mg/kg of powdered fruit, the reduction in faecal egg count after 15 days post-treatment was 58% whereas the ethanol extract produced a 68% reduction. However, some poultry showed signs of toxicity of the central nervous system when 20mg/kg was used.

Ibrahim *et al* (1984) screened 18 plants traditionally used as human and animal anthelmintics using *Nippostrongylus* infections in rats as a model. Plants that showed significant anthelmintic activity in the treated group compared to the untreated controls were *Aloe barteri, Terminallia avicemoides, Annona senegalensis, Cassia occidentalis, Annoggeissus leiocarpus* and *Diospyros mespiliformis* .

The efficacy of nicotine from tobacco leaves against *Haemonchus contortus* was studied in 27 goats and at a dosage of 310.5 mg/goat, the faecal egg count declined by 78% but at 465.8 mg/goat, toxic signs were recorded (Karo-karo,1990).

The anthelmintic properties of aqueous extract of *Colliandra portiricensis* (JACQ) Benth against *Toxocara canis* in dogs was tested and showed significant egg count reduction from 0.34 million to 0 after treatment (Adewunmi and Akubue, 1981). However, this preparation showed no effect on *Ancylostoma caninum* and *Hymenolepsis diminuta* in rats.

The therapeutic efficacy of *Punica granarum* and *Cucurbita maxima* against clinical nematodosis in calves less than 6 months old was studied by Pradhan *et al* (1993). They reported that after oral treatment of the calves with the extracts, the average efficacies of *Punica granarum* at a dose of 8-10 gm per calf and *Cucurbita maxima* at 10-15 mg per calf were 78.2% and 40.6% respectively.

Carica papaya is among the plants claimed in tropical and subtropical countries to have anthelmintic activity against *Ascaris lumbricoides* in humans but it's efficacy has never been assesed in humans (Satrija *et al*, 1994). There are however experiments that have been done to test its efficacy against *Ascaris spps. in vivo* in chickens and pigs and *in vitro* using *Heligmosomoides polygyrus* from mice.

The efficacy of papaya sap against *Ascaridia galli* in laying hens was tested by Mursof (1990). The use of sap from young papaya fruits in different dosages of 20% watery solution resulted in a significant ($p< 0.05$) increase in body weight and egg production

with best results at 1120 mg per chicken of watery solution. In other studies, papaya latex has been shown to be effective against *Ascaris suum* in pigs (Satrija *et al*,1994) and *Heligmosomoides polygyrus* in experimentally infected mice (Satrija *et al*, 1995).

The mechanism of anthelmintic action of Benzyl isothiocyanate (BITC) (an anthelmintic principle in *Carica papaya*) comparing it with that of mebendazole using *Ascaridia galli* as *in vitro* model was studied by Kumar *et al* (1991). They studied the effects of glucose uptake, glycogen content depletion and toad rectus muscle contraction. Benzyl isiothiocyanate and mebendazole inhibited glucose uptake and glycogen content in the presence of glucose. The motility of *Ascaridia galli* was reduced after 20 hours of incubation by BITC. The mechanism of anthelmintic actions of Benzyl isithiocyanate is in both inhibiting energy metabolism and in inhibiting motor activity of *Ascaridia galli*.

It has been reported that some pasture plants have anthelmintic activity against gastrointestinal nematodes in ruminant. For instance, Niezen *et al* (1995) reported that *Heydesarium coronarium* (Surra) which contains condensed tannins decreased faecal egg count ($p< 0.05$) and lowered worm burdens ($p< 0.05$) in parasitised lambs (with *Trichostrongylus species*) compared to those grazed on *Medicago sativa* (Lucerne) which has no condensed tannins. They thus suggested that the condensed tannins or some other plant components might have direct effect on the *Trichostrongylus spp.* establishment or persistence and perhaps also other nematodes.

In studies carried out by Pustovoi (1968), it was showed that *Ferula foelidissima* which is abundant in pasture during spring in Tadzhik SSR decreased gastrointestinal infections in sheep. When fed *F. foelidissima ad libitum*, two out of five sheep were

cleared of *Haemonchus*; one out of four of *Bunostomum* and *Charbertia* and two out of five of *Nematodirus* infections. However, the extract was found to be ineffective. In other studies (Eminov,1982) it was reported that several plant species growing naturally on pasture in Azerbaidzhan had anthelmintic activity against *Bunostomum, Trichostrongylus, Ostertagia* and *Marshallagia* infections and *Sibbaldia spp.* was found to be most active against *Oesophagostomum circumcincta in vitro.* Gadzhiev and Eminov (1986) also in Azerbaidzhan reported that powdered *Heracleum sosnowskyi* which is a common pasture plant cured 60% of sheep with natural nematode infections and reduced infection levels in others by 87.6% when fed over a 10 day period.

The anthelmintic properties of *Tribulus terrestris* Linn against *Ascaridia galli in vitro* and *in vivo* was studied by Chakraborty *et al* (1979). They reported that the alcoholic extract and the mixture of alkaloid effectively inhibited contractions of *Ascaridia galli in vitro* tests and caused expulsion of worms and reduced faecal egg counts in chickens.

Traditionally, the Maasai of Kenya and Tanzania use *Embelia schimperi* Vatke as a cestocide against *Taenia saginata* in humans. *In vivo* and *in vitro* studies using diammonium salt of Embelin isolated from the dried fruit against the tapeworms (*Hymenolepsis diminuta* and *Hymenolepsis microstoma*), trematode (*Echnostoma caproni*)and the nematode *Heligmosomoides polygyrus* was done by Bogh *et al* (1996). They reported that *in vivo*, only the tapeworm *Hymenolepsis diminuta* was killed and no significant effect was noted on the rest. They concluded that the crushed seeds of *Embelia schimperi* used by the Maasai as anthelmintic has an anthelmintic effect against the human intestinal tapeworm.

Msolla *et al* (1987) studied nicotine the principle alkaloid found in high concentrations in tobacco plant (*Nicotiana tobaccum* and *Nicotiana rustica*) for the control of bovine parasitic otitis, a disease reported in Kenya, Tanzania, Uganda and Zimbabwe. They reported that 2 ppm of nicotine in 0.28% toxaphene used as an acaricide twice weekly for 12 weeks was 95.3% effective in treatment and control of bovine parasitic otitis in cattle.

The effect of *Mallotus philippinensis* (Kamala), a fruit which is normally dried for use as an anthelmintic for humans and animals in Indo-Pakistan against natural gastrointestinal cestode infections in 42 beetal goats was studied by Akhtar and Ahmad (1992). They reported that the powdered *Mallotus philippinensis* fruit, it's aqueous extract and it's glycoside had significant activity against cestode and was safe for treatment of gastrointestinal tapeworm infections in beetal goats. The oral dose of 375 mg/kg of Kamala powder or 100mg/kg bodyweight of it's glycoside had similar effects as Nilzan$^{(R)}$(Levamisole Hcl,1.5% and Oxyclozanide,3% w/v of Cooper Kenya) given at a dosage of 5ml/15kg body weight for mixed gastrointestinal cestodes on day 15 after treatment. The anthelmintic effect of the resin of *Mallotus philippinensis* was also evaluated in albino rats infected with tapeworm. At 60 and 120 mg/kg doses, it had a lethal effect on 35.69% and 78.21% population of tapeworms respectively (Gupta *et al* ,1984).

Pyrethrum (*Chrysanthemum species*) which contains pyrethrin as major pharmacological active ingredient has been claimed to have anthelmintic properties (Hammond *et al*, 1997) and low toxicity (Landberg and Accousti,1940; McLellan and Mbaria *et al*,1994). In his review of pyrethrum as an anthelmintic, McLellan(1964) reported that pyrethrum

has in vivo activity against *Ascaris lumbricoides*, *Taenia spps*, *Cyathostomes*, *Parascaris equorum* and *Oxyuris equi* when used at a rate of 1 gm of pyrethrin per animal.

Pyrethrin was reported to reduce mortalities in sheep infected with *Monezia*, *Trichuris*, *Charbertia* and *Trichostrongyles* from 60-70% to 1.5 when used three times a year at a dose rate of 300 mg/ animal. Pyrethrin was also shown to be 71.4% effective in poultry experimentally infected with *Ascaridia galli* when mixed in food at a rate of 2 % for seven days (Zarnowski and Darski, 1957). In other studies, 200 mg of powdered pyrethrin in gelatin capsules containing 0.8 % pyrethrin was 95% effective in treating *Ascaridia galli* infected chickens (Rebrassier,1934).

In Kenya, experiment done on water extract of seeds, leaves, flowers, stems and roots of *Glinus lotoides* in the family of *Aizoaceae* showed that it killed the miracidium of *Fasciola gigantica* (Anon, 1981). There are also five species of *Solanum* in *Solanaceae* family that have been reported to have effect against miracidia of *Fasciola.*

The stem bark decoction of *Zanthoxylum liebmannianum* (Engelm.) P. Wilson (*Rutaceae*) has been observed to decrease intestinal nematodes egg count in naturally infected sheep (Navarret and Hong, 1996).

Studies on the anthelmintic effect of an alcoholic extract from *Diospyrus mollis* (Ma – Klua in Thailand) on adult and larvae of *Hymenolepsis nana* in mice was done in comparison with flubendazole (Maki *et al*, 1983). A single oral dose of 10-1000 mg of Ma-Klua alcoholic extract or flubendazole/ kg body weight was given on day 1,2,3,4, or

12 post infection and post mortem done on day 14. It was observed that Ma-Klua extract was effective in the elimination of adult but not larvae and decreased egg output whereas flubendazole had minimal effect on adult and larval stages.

Evaluation of the fasciolocidal efficacy of oral doses of *Albizia anthelmintica* Brogn *Mimosaceae* stem bark water extract at 9g/kg body weight and of *Balanites aegyptiaca* (L) Del.(*Balanitaceae*) fruit mesocap water extract at the same dosage was carried out by comparing them with albendazole at 20 mg/kg body weight against *Fasciola gigantica* adult worm (Koko *et al*, 2000). On determining the percentage reduction in liver fluke counts at postmortem two weeks post treatment, the efficacies of *Albizia anthelmintica*, *Balanites aegyptiaca* and albendazole was 95.5, 93.2 and 97.7% respectively. In other studies, *Albizia anthelmintica* bark aqueous extract administered orally to rats experimentally infected with *Hymenolepis diminuta* at a concentration of 50-150g/kg bodyweight (Galal *et al*,1991) was found to be safe and eliminated the infection successfully. At a dosage of 25-150g/kg bodyweight, the butanolic extract of *A. anthelmintica* bark administered to the same rats using a stomach tube was reported to be highly toxic and inactive against *H. diminuta.*

The root of the fern *Matteuccia orientalis* was reported to have fasciolocidal activity in bovine (Shiramizu *et al* ,1993). A crude preparation of the root given orally to 18 cattle with fasciolosis, *Fasciola* egg count was reduced to zero in 1-2 weeks after treatment in 14 cattle and remained so for 8 weeks. In the other four, faecal egg counts were also reduced but not to zero. Seven controls had variable faecal egg count during the trial.

Shilaskar and Parashar (1989) evaluated the *in vitro* anthelmintic activity of 15 extracts of the following medicinal plants; *Andrographis paniculata, Azadirachta indica, Butea frondisa, Caesalpinia crista, Piper betle, Psoralia corylifolia, Swertia chirata, Vernenia anthelmintica* against *Ascaridia galli* from birds after postmortem. Motility loss of the worms after incubation at 41°C with the test material was examined at 1,2,3 and 24 hours and was used as an indicator of mortality and percentage mortality determined. A high significant anthelmintic activity was reported in ether and alcohol extracts of seeds of *B. frondisia* and *V. anthelmintica*, ether extract of seeds of *C. crista,* alcohol extract of seeds of *P. corylifolia* and essential oils from leaves of *Piper betle.* A significant activity was reported in alcoholic extract of whole plant of *Swertia chirata.*

The fasciolocidal activity of Pinocembrine (5,7,dihydroxyflavanone) the only active compound from *Teloxys graveolens* was studied by Rayo-Camacho *et al* (1991). The compound was reported to have fasciolocidal, ovicidal and larvicidal activities on newly encysted *Fasciola hepatica*, infective eggs of *Ascaridia galli* and on stage three larvae of *Stomoxys calcitrans* respectively.

Akhtar and Javed (1991) used *Monezia* infected sheep to study the efficacy of powdered *Nigella sativa* Linn seeds. The experimental sheep were either given *N. sativa* powder suspended in 2% gum tragacanth at a dosage of 1.5, 2.0 or 2.5 g/kg body weight, ethanol or water and the control group was treated with niclosamide at 0.01g/kg. Faecal egg counting was done on days 3,10, and 15-post treatment. The result revealed that on day 15, the powdered seeds at 2.5g/kg of 2% gum tragacanth, equivalent of ethanol extract and niclosamide were equally effective in treating sheep.

Akhtar and Ahmad (1991) studied the antinematodal efficacy of the alkaloid tetra-hydroharmine isolated from *Peganum harmala* (Rutaceae) using 30 beetal goats with mean faecal egg count of 3400-8650 eggs per gram. Oral drenching was given to a group of 6 animals at 5,10 or 20 mg/kg bodyweight and 6 goats were given morantel at 10 mg /kg bodyweight. It was found out that the reduction in nematode egg count 15 days post treatment for tetra-hydroharmine at 20mg/kg bodyweight was similar to that of morantel (99%). It was further evident that tetrahydroharmine was also effective on oocyst burden with oocyst reduction count being 99%.

Minced fresh garlic given at 200mg/litre of water for two days and an equivalent concentration of hexane extract were shown to be 100 and 75 % effective respectively against natural *Capillaria* species infections in 250 Carp (Pena *et al,* 1988). The effectiveness was judged by egg counts and presence of worms at necropsy. On the other hand the aqueous extract of the garlic material was ineffective. It was further shown that minced garlic was more effective than ammonium- potassium tartarate (Pena *et al*,1988).

The anthelmintic studies on *Combretum quadrangulare* KURZ were done by Euswas *et al* (1988). A single dose of 54, 27 and 18 mg of ground ripe seeds/ kg bodyweight of *Combretum quadrangulare* Kurz was shown to result into a decrease in the number of *Neoscaris vitulorum* eggs in the faeces of buffalo calves to zero within 1,2 and 3 weeks respectively.

In vivo and *in vitro* anthelmintic activity of three extracts from *Hedera helix* (saponin complex 60% - CS60, purified saponin complex 90% - CSP 90 and alpha-hederin) was done by Julien *et al* (1985). *In vitro* test of the three extracts was done against *Fasciola*

hepatica and *Dicrocoelium* species and against *Dicrocoelium spps* in naturally infected sheep. After 24 hours *in vitro,* both *Fasciola* and *Dicrocoelium* spps were killed by alpha hederin at concentrations of 0.005 and 0.001 mg/ml respectively. It was further observed that CS60 and CS90 were capable of eliminating *Dicrocoelium* spps parasites from naturally infected sheep that were treated orally three times at a dose rate of initially 500 and then 800 mg/kg twice.

Powdered shoots of *Artemisia herba- alba* were reported to have anthelmintic activity against haemonchosis in Nubian goats (Idris-Um-Ei *et al* ,1982). Four of the six treated goats with 2, 10 or 30 g of *Artemisia* shoots showed no clinical signs of haemonchosis. in the abomasum.

Another plant product that has been studied include the herbal product Taenil (male fern, kamala and senna). At 600mg/kg given orally once per day for three consecutive days to 8-10 week old pups infected with *Cysticercus tenuicollis,* scolices were reduced to 0 on autopsy (Siya and Tripathy, 1981). Compared to niclosamide at 100mg/kg-body weight and mebendazole at 100mg/kg-body weight, they reduced scolices to 7 and 0 respectively. Five untreated controls had 23 scolices at autopsy.

Sharma (1979) reported that in 4 of 8 birds inoculated with 15 *Railletina spp* cysticercoids and treated with aqueous extract of dried *Punica granarum* root given at 5 ml of decoction for three days, ceased to pass segments within 24 hours post treatment. Four untreated birds continued to pass the segments. On increasing the dose to 10 ml for two days, although death occurred in birds, no worms were recovered in any of the birds treated at postmortem whereas 1 to 4 worms were recovered in control birds. Qureshi

and Sabir (1979) also reported a complete effective treatment of poultry infected with cestodes using *Embelia* seeds.

The essential oils of *Nigella sativa* tested against *Taenia solium, Bunostomum trigonocephalum* and *Oesophagostomum columbianum* was reported by Agarwal *et al* (1979) to have similar results as piperazine phosphate against *Taenia solium* and hexylresorcinol against *Bunostomum trigonocephalum* and *Oesophagostomum columbianum*. At 0.01% emulsion of *N. sativa*, death of *T. solium*, *B. trigonocephalum* and *O. columbianum* occurred at 76, 43, and 36 minutes and at 0.6% it occurred in 19, 13 and 20 minutes respectively.

Cyathocline lyrata Cass essential oils was reported by Shrivastava (1979) to have better anthelmintic activity than that of piperazine and hexylresorcinol against tapeworms and hookworms respectively.

2.2.6. Separation and identification of the pharmacological active ingredient in the plant anthelmintic

The separation and purification of plant constituent is done using one or a combination of four chromatographic techniques namely; paper, thin layer, gas liquid and column chromatography. For instance Maitai (1973) used thin layer chromatography (TLC) technique on basic fractions followed by use of gas liquid chromatography to determine the amount of d-norpseudoephedrine recoverable from *Catha edulis.*

In order to identify a plant constituent after isolation and purification, the class of compound must be determined. This can be done by running a single spot in several thin

layer chromatography (TLC) systems, testing it's response to colour tests, testing it's solubility and relative fractions (Rf) properties and it's ultraviolet(UV) spectral characteristics (Harbone, 1973). For complete identification within the class, other properties are measured and compared with those in the literature, e.g. melting (for solids), boiling (for liquids) points, optical rotation and Rf. Also informative of a plant substance are its spectral characteristics, which include; ultraviolet (UV), infrared (IR), nuclear magnetic response (NMR) and mass spectral (MS) measurements. The final confirmation is done by direct comparison with authentic material.

2.2.7. Limitations of plant anthelmintics

2.2.7.1. Ethnodiagnosis

In order to use plant anthelmintics effectively, an accurate diagnosis of helminthoses is essential. Herbalists and herders lack the basic diagnostic techniques, which only the western veterinary medicine can provide (McCorkle and Mathias-mundy,1992). Laboratory techniques are useful tools to differentiate helminthoses and other conditions, e.g., malnutrition, mineral deficiencies, toxicoses and other infections (Hammond *et al*, 1997).

There is a possibility of misdiagnosis because some helminth are not obviously visible in the faeces and their eggs are microscopic. Another complication is that some herbalists and farmers may assume that their plant anthelmintics are effective when worms are shed spontaneously due to self-cure phenomenon of *Haemonchus contortus* in sheep or in calves infected with *Toxocara vitulorum* where a lot of these worms are shed spontaneously at 6 months of age (Hammond *et al*,1997). In the case of tapeworm

segments, *Ascaris spp.* and *Toxocara*, the segments or the tapeworm may be shed after using plants that act as purgatives and can be confused as having anthelmintic activity.

2.2.7.2. Toxicity

Some of the plants used against helminths may be toxic to animals depending on the dosage and the part of the plant used. Pastoralists in Africa have been reported to have a lot of knowledge and skills on plant toxicoses (McCorkle and Mathias-mundy,1992), however Mugera (1970) reported that plants e.g. *Maesa lanceolata* whose leaves are used as anthelmintic and as a purgative could kill calves when given as a daily drench for three weeks. Kellerman *et al* (1988) and Shone and Drummond (1965) reported that *Solanum incanum* used as anthelmintic and *Phytolacca dodecandra* used as molluscicide are poisonous to livestock .

Pyrethrum which is considered safe and has been used for a long time as an anthelmintic was reported by Mbaria *et al* (1994) to be slightly toxic to sheep and rabbits in levels beyond 420 mg/kg. They recommended that its use should not exceed 420 mg/kg-body weight.

2.2.7.3. Availability

Herbalists are increasingly getting concerned about the scarcity of medicinal plants. In India, they harvest 90% of it's medicinal plants from natural sources while China harvest 80 % (Lange, 1996). This implies that within a short period there will be scarcity of these plants unless cultivation and conservation methods are undertaken. This overexploitation of economically valuable medicinal plants along with other deforestation activities leads to soil erosion besides loss of natural habitat.

Most of the plants which were formerly ever found in the backyards are scarce and now grow singly in the wild and are difficult to locate (Minja, 1989). The population pressure and the need for more food has led to bush clearing for crop production and has affected the availability of plants with medicinal value. The change of soil texture and preference of cash and food crops to indigenous plants are other factors that affect biodiversity of plants and hence scarcity of medicinal plants. Similarly, the introduction of exotic plants to replace the indigenous ones has also affected the availability of medicinal plants especially in the agro-pastoral communities.

2.2.7.4. Sustainability

The importance of sustainability in developing countries is that it promotes self-reliance, increased use of renewable resources and promotes systems that require minimum external inputs (Hammond *et al*, 1994). The methods of harvesting and processing some medicinal plants may lead to their use being unsustainable as in the case of *Neurautanenia pseudopachrhiza*, a molluscicide which require hard labour to dig out the roots, transport them and apply to target organisms (Teesdale,1954).

2.2.7.5. Traditional beliefs

Although some plants have been traditionally held to have medicinal value, the traditional knowledge may have been passed on from one generation to the next without any scientific validation. Kliks (1995) studied the ethnopharmacology of the use of powdered *Chenopodium ambrosioides* (American wormseed, goosefoot, epazote, and paico) and related species which are used as anthelmintics against *Ascaris* by native people of Chiapa in Mexico. He observed no significant anthelmintic effect even at twenty times the traditionally used dose. According to Kliks (1995), the belief held in

the efficacy of *C. ambrosioides* as used may be due to spontaneous or peristalsis-induced passage of worms after a drench or the amount of Ascaridol content within the plant may have decreased with time. Therefore, the efficacy of indigenous plants should be validated before they are recommended for use as anthelmintics.

The knowledge of medicinal plants is passed orally from one generation to the next. This knowledge is kept secret and confidential and most of the times is only passed at the time of the death of the herbalist (Kokwaro, 1993). Therefore the recipient may not get all or clear information, may forget and mistake various plant species leading to therapies which are not effective and hence open criticism to the use of medicinal plants.

The aspect of getting wealthy especially in modern days may lead to some herbalist claiming to know the cure of all diseases. They therefore apply one drug after another until the sick animal dies and again they may lead the people to have low opinion of medicinal plants.

CHAPTER THREE

IDENTIFICATION OF MEDICINAL PLANTS USING PARTICIPATORY RURAL APPRAISAL TOOLS

3.0. Introduction

Several authors have listed many plants with medicinal value after collection and botanical identification. Kokwaro (1993) listed over 2000 medicinal plants found in East Africa which he collected and were botanically identified. Of these plants collected, 21 plants are used against hookworm ; 6 plants for roundworms; 22 plants for tapeworm and one plant for threadworm in humans. He also listed 79 plants used as general anthelmintics (Appendix 2.5).

Lindsay (1978) collected medicinal plants of the Marakwet district of Kenya (located in the Kerio valley) used in human herbal therapies. Among the 269 plants collected and botanically identified, 5 were used as anthelmintic. These were *Albizia anthelmintica* Brogn, *Albizia gummifera*, *Dryopteris inaequalis*, *Myrsine africana* and *Olea africana.* Similarly ITDG & IIRR (1996) listed many plants used for livestock disease control. Eighteen of these plants are used for the control of livestock helminthoses in Kenya. They are ; *Lantana trifolia, Albizia anthelmintica, Albizia coriavera, Diospyros scabra, Trichilia emetica* for lung worms.; *Rhus vulgaris, Cassia spectabilis, Tamarindus indica, Rapanea melanophloeos, Carissa edulis, Cucurbita maxima, Launaea cornuta, Ricinus communis, Ocimum basilicum, Myrsine africana, Tedea nobilis, Allium sativum, Hagenia abyssinica* for roundworms. In her studies, Bizimana (1994) listed many

plants used for livestock disease control in Africa. Among these, 74 plants are used specifically for the control of livestock helminthoses (Appendix 2.4).

Farah (1991) proposed a questionnaire to be used in surveys meant to identify medicinal plants followed by collection and subsequent botanical identification. The questionnaire identifies the type of plant, locality, their use, part used extraction procedures and their therapeutic purposes. This questionnaire proposed by Farah (1991) was used by Minja (1994) in three regions of Tanzania where he collected medicinal plants of value. Factors such as dosage rate and side effects such as toxicity need to be identified. The questionnaire also allows for assessment of the availability of the plant.

Mehrotra (1984) and Harbone (1973) proposed guidelines on the collection of medicinal plants (Appendix 2.2.). There is a need to carry out a survey when studying medicinal plants because most of the plants used may be common in many parts of the selected study area and therefore details will need to be verified easily.

There is a lot of knowledge of medicinal plants especially among the older generation among the Marakwet and prior study had been done on medicinal plants used by humans (Lindsay,1978). In Kibwezi there is limited knowledge on medicinal plants especially among the young generation. The purpose of this study was to assess the knowledge and identify plants of medicinal value in Kibwezi division in Makueni district and Tunyo division of Marakwet district.

3.1. Materials and methods

A prior study on medicinal plants had been conducted by Dryland Husbandry Project in Machakos and Makueni districts and three renowned herbalists had been identified. In this study, the three herbalists were interviewed using a modified version of the questionnaire described by Gakuya (1996) and adapted from that of Farah (1991).

A survey was carried out involving a herbalist and a community based animal health attendant in Kibwezi area in order to gather information about medicinal plants used for other diseases other than helminthoses.

The questions tried to identify the various uses of the plants and parts of the plants used. They were also meant to identify methods of extraction, dosage rates and species of animal they are used for. The species of helminth they are used for, the frequently of use and duration from one dosing to the next was also questioned. The questionnaire also tried to identify the toxicity, storage methods, expiring date after extraction and practical experience on its use. The sample questionnaire used in the studies is given in (Table 3.1). The medicinal plants used as anthelmintics were identified and photographed in their natural environment and collected for further identification . The second study used the same format but tried to identify other medicinal plants apart from those used as anthelmintic. In Tunyo division , a previous interview had been done by a priest who had identified one prominent herbalist who uses medicinal plants for both human and animal diseases. The herbalist was interviewed in a similar manner as in Kibwezi using standard PRA tools. Several people in the area were also interviewed to assess their knowledge of medicinal plants and to confirm the report from the herbalist.

Table 3.1. A sample questionnaire

Date:	Plant vernacular name:	Collection SMP no*
Name of informer:		
1.Locality:	District:	Village:
Distance and direction from major town:		
Agro-ecological zone:		
2. Plant description		
Tree	Herb	Parasite
Shrub	Liana	Aqueous plant
3. For trees: Height and bulk:		Bark description
4. Flower colour:		5. Fruit description
6. Latex present		
7. Provisional description:		
8. Extraction procedure:		
Plant part:	Fresh:	Dried:
Amount collected:		
Crushed:	Powdered:	Mixed with water (amount):
Other preparation:		Storage Expiry date
9. Dosage rate:		
10: Species of animal the plant is used to treat		
11: Type of helminth infection the plant is used to treat(in order of importance)		
12: How often the plant is used		
Very often	Occasionally	Rarely
13: Interval between dewormings		
14:Other comments		
15: Recommendations:		

SMP* Serial medicinal plant number.

Adapted from Farah (1991)

3.1. Results

In the two surveys done at Kibwezi, fifty one plants were claimed to have medicinal properties (Table 3.2). Two medicinal plants which were identified as supposedly used to control helminths and described as very potent were *Maerua* species or Munatha (Kinatha) and *Albizia* species or Mwoa (Kyoa) in Kikamba, the local language (Fig 3.1.)

The herbalists reported that the main use of Munatha is as an anthelmintic though it was also noted that people near Tana river put the tuber in river water to control schistosomosis and amoebiosis (Musimba, 1999). The three herbalists interviewed reported that the active principle is in the root (Fig. 3.2.). The traditional extraction involves first peeling the outer cover of a fresh tuber with a knife and then slicing it into pieces (chips) before boiling for extraction. A kilogram of the tuber is mixed with a litre of water and boiled for 20-30 minutes where approximately a litre of the yellowish extract is made by filtering with a cloth or fine sieve. One table spoonful of the table salt is added as an appetiser.

The species of animals drenched with the extract were reported to be goats, sheep and cattle. The amounts given to goats and sheep were 5,10,15 and 20 mls for suckling , weaners , breeders and adult respectively, while that of cattle was 150- 200ml depending on the size. The animals were given a single drench after every six months. According to the herbalists, the tuber was claimed to be a broad spectrum anthelmintic with a high efficacy on *Haemonchus contortus*. One of the herbalist claimed to have cured 500 out of 600 goats which had diarrhoea due to haemonchosis and a 100 of them had died prior to the treatment.

Table 3.2. A check list of some useful plants of Kibwezi Dryland Field Station

A. Control of Helminthoses in human and livestock

Plant species	Family	Life form category	Kikamba name	Use
Albizia anthelmintica, Brogn	*Mimosaceae*	Tree	Kyoa, Mwoa	Dewormer
Acacia brevispica, Harms.	*Mimosaceae*	""	Mukuswi	Intestinal worms
Maerua edulis De Wolf	*Capparidaceae*	Shrub	Kinatha	Anthelmintic
Ocimum basilicum, L.	*Labiatae*	Herb	Mutaa	Stomach and intestinal worms
Balanites aegyptiaca, L.	*Balanitaceae*	Tree	Mulului	Anthelmintic, purgative

B.Treatment of other livestock diseases

Grewia villosa, Willd.	""	""	Mulawa	Foot and mouth, retained after birth
Pappea capensis, Spreng.	*Sapindaceae*	Tree	Muva	Indigestion in goats
Cissus rotundifolia, Forsk	*Vitaceae*	Shrub	Ituuru	Foot and Mouth , poultice, purgative, diarrhoea
Barleria acanthoides Vahl.	*Acanthaceae*	Herb		Medicine for goats
Lepidigathis scariosa, Nees	""	"		Browse for livestock
Justicia striata, Kl	""	""		Browse for livestock
Maerua crassifolia, Forsk	*Capparidaceae*	""		Browse for livestock
Anthericum subpetiolatum Bak.	*Liliaceae*	Herb		Browse for livestock
Loranthus rufescens, DC	*Lorantheceae*	Herb		Browse for livestock
Dioscorea schimperiane, Kunth	*Dioscoreaceae*	""		Browse for livestock
Lannea alata, Engl.	""	Shrub	Kitungu	Browse for livestock
Acacia nilotica L.	*Mimosaceae*	Tree	Kisemei	Abortion and Orf
Duosperma kilimandscherica L.	*Acanthaceae*	Shrub	Ithande	Browse for livestock
Commiphora schimperi, Berg.	*Burseraceae*	Tree	Iulu, yeulu	Browse for livestock and medicinal
Ochna inermis, Forsk	*Ochneceae*	Shrub	Mutandi	Calving problems in cattle
Premna hilderbrandtii, Gurke.	*Verbenaceae*	""		Browse for livestock
Acacia mellifera, Vahl.	*Mimosaceae*	Tree	Muthia	Eye problems and diarrhoea
Hermania uliligii, Engl.	*Malvaceaea*	""		Browse for livestock
Aloe secundiflora, Engl.	*Liliaceae*	Herb	Kiluma	Lumpy skin disease, Salmonellosis
Benthium spr.	"	"	Muiwa-ivia	Browse for livestock and general medicinal

C. Treatment of other human diseases

Boscia coriacea Pax.	""	Herb		Stomach-ache and gonorrhoea
Capparis tomentosa, Lam.	*Capparidaceae*	Tree	Kitandambo	Asthma, chest pains, cough and abdominal pains
Grewia bicolor, Juss	*Tiliaceae*	""	Ilawa	Treatment of chest pains,colds
Grewia villosa, Willd.	""	""	Mulawa	Stomachache and eyeache, spleen troubles
Maerua kirkii Oliv.	*Capparidaceae*	Shrub	Ivovotwe	Stomach disorders in human
Grewi a similis, K. selium	*Tiliaceae*	Shrub	Mutubu	Cure for sores
Cassia kasneri, Bak. F.	*Caesalpinaceae*	""	Mwala ndathe	Malaria treatment
Entada abyssinica, Steud.	*Mimosaceae*	Shrub	Mwaitha	Medicinal- Rheumatic pains in humans
Lannea stulhumanii, Engl.	*Anacardiaceae*	Tree	Mwethi	Headache, stomach pains, poultice for childbirth
Hoslundia opposita, Vahl.	*Labiatae*	Shrub	Musovi	Coughs,cold,fever , stomach-ache, aphrodisiac
Ormocarpum Kirkii, S. Moore	*Papilionaceae*	Shrub	Muema nzou	Malaria treatment
Ochna inermis, Forsk	*Ochneceae*	Shrub		Stomach troubles in human
Acacia nilotica L.	*Mimosaceae*	Tree	Kisemei	Sore throats and coughs, pneumonia
Plectranthus barbatus, Andr.	*Labiatae*	Shrub	Maiya	Stomach-ache and purgative
Acacia mellifera, Vahl.	*Mimosaceae*	Tree	Muthia	Stomach troubles in human, pneumonia, malaria
Dichrostachyus cinerea, L.	*Mimosaceae*	Shrub	Mundua	Conjuctivitis, stomach troubles, snake bites,
Tephrosia villosa, L.	*Papilionaceae*	Herb		Liver and spleen pain
Commelina africana L.	*Commelinaceae*	""	Kikowe	Fever, relieve eye irritant latex drops, ear-ache
Combretum exalatum, Engl.	*Combretaceae*	Shrub	Mukokola	Rheumatism
Boscia anqustifolia, A. Rich.	*Capparidaceae*	Shrub	Isivu	Malaria treatment
Boscia coriacea Pax.	""	Herb	Kisivu	Stomach-ache and gonorrhoea
Achyranthes aspera, L.	*Acanthaceae*	Herb	Uthekethe	Headache, stop bleeding, constipation
Justicia flava, Vahl.	*Acanthaceae*	Herb	Mutaa	Emetic, eye lotion, stomach-ache, diarrhoea and cough
Capparis tomentosa, Lam.	*Capparidaceae*	Tree	Kitandambo	Asthma,chest pains, cough and abdominal pains
Cyphostema orondo, Desc.	*Vitaceae*	Herb	Kyuungu	Treatment of abscess and boils
Aloe secundiflora, Engl.	*Liliaceae*	Herb	Kiluma	Lack of appetite, anti-emetic, malaria, headache
Aspilia mossambicensis, Oliv.	*Compositae*	""	Muliti	Cystitis and gonorrhoea
Adenium obesum, Forsk	*Apocynaceae*	Shrub	Mwatha	Lice infestation and fish poison
Sonchus schwenfurthii, Oliv.	*Compositae*	Herb	Uthungu	Emetic and chest troubles
Tridax procumbens, L.	*Compositae*	""	Mumela	Malaria and stomach-ache
Rhoicisus tridentata, Lif.	*Vitaceae*	Herb	Muvelengwa	Indigestion, abdominal pains during menstruation

Kokwaro(1993), ITDG & IIRR (1996), Gakuya and Awala1999)

Fig. 3.1. *Maerua edulis* (munatha) photographed in Kibwezi field station

Maerua edulis (Munatha) shrub

Fig.3.2. The tuber of *Maerua edulis* (munatha) partly dug from the ground

Maerua edulis tuber

Albizia spps which was reported to be less efficacious than *Maerua* spps have the active principle in the bark, though the roots are also claimed to have some efficacy. It is used as an anthelmintic for both human beings and animals. The inner part of the bark which looks stringy is removed and crushed. The extraction method involves boiling of the crushed material with water at a rate of 1 kg of the material in a half litre of water for 20-30 minutes. After filtration of the suspended material, the supernatant is used as a drench. The herbalist reported that the species of animals in which it is used are goats and sheep at a dosage of 30 ml for adult and 10-20 ml on the rest of the animals depending on the size. It was reported to be used mainly against tapeworm and other gastrointestinal nematode infections.

Kitwongwo or Kitangw'a tentatively identified as *Albizia spps* using the method of Lindsay (1978) is claimed to be a broad spectrum anthelmintic. The part of *Albizia spps* used was the bark and the extraction procedure involved crushing of the bark, grinding and drying. It can then be administered as a drench by mixing the powder with water or fed as powder in cattle , sheep and goats. The dosage rate of cattle is four table spoonful or more depending on the size of the animal whereas for the sheep and goats, one table spoonful is enough. Half of the dose is recommended for the young ones. The administration is repeated after three months.

The questionnaire also revealed that "Kiteria" which is a climber has the active principle in the leaves and are crushed, mixed with water and administered at the same dosage rate as *Albizia spps*. It is a broad spectrum anthelmintic and is claimed to treat hydatidosis in livestock normally. All plant materials were collected except for the climber kiteria which was not available as it was during the dry season. The *Albizia spps*

were collected and their bark stripped, while the tubers of *Maerua* species were dug out from the soil. The leaves of both plants were pressed for botanical identification, which was done in the Department of Range management in the University of Nairobi. The *Maerua spps* and *Albizia spps* from Kibwezi were both botanically identified *as Courbonica glauca* or *Courbonica edulis* or the new name *Maerua edulis (*Gilg) De Wolf and *Albizia anthelmintica* Brogn respectively. Those from Tunyo division were identified as *Maerua subcordata* (Gilg and Bened) De Wolf and *Albizia anthelmintica* Brogn respectively.

3.2. Discussion

The survey conducted in Tunyo division of Marakwet district was not extensive and relied on one herbalist and non herbalists. However, *Albizia anthelmintica* which was identified as the most efficacious anthelmintic was also identified by other studies in the area by Lindsay (1978). Similarly *Maerua subcordata* had been identified by the same author. In this area, reports from reliable source indicates that 75 % of the livestock owners opt for medicinal plants as first priority when their animals fall sick whereas 50% of the people seek herbal therapy as their first priority after sickness. From the studies done by Lindsay(1978), this area has a wide range of plant life and has the potential for studies on medicinal plants for human and livestock.

In Kibwezi area , the survey conducted was the first one which was specific for the area. The number of people with the knowledge on medicinal plants is limited especially for the young people but the area has potential plants for human and livestock disease control. The number of livestock keepers who use medicinal plants as first priority when their animals are sick are about 40% and similarly, sick people using herbal

remedies as first priority are the same percentage (Ikutwa, 2001). Previous studies done by DHP in Kibwezi and missionaries in Marakwet had observed that in many circumstances the human and livestock herbalists are the same. There is therefore a need to have a joint approach when dealing with the medicinal plants because most plants are commonly used for treating both human and animal diseases. There is a wide knowledge among livestock keepers of their medicinal plants and there is a chance of studying many plants for livestock disease control apart from those used as anthelmintics.

CHAPTER FOUR

DETERMINATION OF THE EFFICACY OF CRUDE WATER EXTRACTS OF *ALBIZIA ANTHELMINTICA* BROGN AND *MAERUA* SPECIES ON GASTROINTESTINAL NEMATODES IN SHEEP

4.0.Introduction

There are several plants used for the control of human and livestock helminthoses in Kenya. Some have been reported by herbalist to be very potent. However the efficacy of most of them has not been investigated to validate their use. The efficacy of these anthelmintic plants can be evaluated by determining their percentage helminth faecal egg count reduction after administration . This is done by taking a faecal egg count before and after treatment and comparing it with infected and untreated control. The efficacy of plant anthelmintics may also be determined by counting the total worm burden after treatment. This is done by euthanising animals and emptying the gut content and using standard procedures to count the worms in infected and untreated control group and determining percentage worm count reduction.

The percentage faecal worm egg count reduction indicates the efficacy of medicinal plants which is the difference between the group mean eggs per gram pre- and post treatment and is expressed as a percentage of the pre-treatment value (Waruiru *et al,* 1991). According to the World Association for the Advancement of Veterinary Parasitology (WAAVP) , the percentage reduction (PR) is calculated using the formula PR= 100(1-XT/XC) with XT and XC being the average arithmetic mean faecal egg counts on days 10 -14 respectively.

The efficacy of various plant anthelmintics against gastrointestinal nematodes in livestock has been reported by several authors. The anthelmintic activity of *Melia azedarach* against gastrointestinal nematodosis in goats was studied by Akhtar *and* Riffat (1984). They reported a percentage faecal egg count reduction of nematodes of 70%, 96% and 99% after treating the goats with 30 mg/kg body weight of the powdered fruit on days 3,10 and 15 respectively.

Heracleum sosnowskyi, a common pasture plant was reported by Gadzhiev and Eminov (1986) in Azerbaidzhan to have cured 60% of sheep with natural nematode infections and reduced infection levels by 87.6 % when fed over a 10- day period. The plant also reduced the prevalence of *Trichostrongylus, Ostertagia*, *Bunostomum* and *Charbertia spps* in sheep within a duration of 10 days from 40, 38, 10, 10% to 22, 20, 2 and 6% respectively.

Heydesarium coronarium (Surra) which contains condensed tannins was shown to decrease faecal egg count at slaughter ($p< 0.05$) and lower worm burden($p<0.05$) in parasitised lambs (with *Trichostrongylus* species) in constrast to lambs grazed on *Medicago sativum* (Lucerne) which has no condensed tannins (Niezen *et al*,1995). They suggested that the condensed tannins or some other plant component might have direct effect on the establishment or persistence of *Trichostrongylus* species and perhaps other nematodes. Similar pasture studies by Pustovoi (1968) showed that *Ferula foelidissima*, an abundant pasture in Tadzhikstan (Russia) decreased gastrointestinal infections in sheep.

A number of plants are known to be used as medicinal herbs for livestock and human diseases in Kenya especially in rural areas. In livestock, these are mainly used in the treatment and control of helminth infections. *Albizia anthelmintica*, *Maerua edulis* and *Maerua subcordata* are some of the medicinal plants that are normally used by a number of herbalists in the study areas (Kibwezi and Tunyo divisions) to treat animals against helminth infections. Their efficacy against gastrointestinal nematodes has never been evaluated before. Therefore, this study was aimed at evaluating the efficacy of the extracts of *Albizia anthelmintica* , *Maerua edulis* and *Maerua subcordata* in the treatment of gastrointestinal nematodes in sheep.

4.1.Materials and methods

4.1.0.Plant material

The extraction procedure of the *Maerua* species was done by peeling the outer cover of the tubers and then slicing the peeled tuber into small chips. A fresh water extract was obtained by boiling one kg of the chips in two litres of water for 40 minutes. The filtered extract was cooled and stored in glass bottles at 4°C. Additional chipped materials were sun dried and stored in polythene sheets and later ground in a posho mill into a powder that was stored in glass jars. Extraction was done on the powdered material by boiling one kg of the material in three litres of water for 30 minutes and the extract stored as with the fresh material.

The *Albizia anthelmintica* bark was similarly cut into fine pieces and a fresh extract made by boiling one kg of chips in two litres of water for 30 minutes. The filtered extract was cooled and stored in glass bottles at 4°C. Part of the chopped material was sun dried and stored in plastic bags. A portion of the dried material was ground in a

posho mill and stored in glass jars. Extraction of the powdered material was done by boiling the material in water at a rate of one kg of material in three litres of water for 30 minutes. The filtered extract was stored in a similar manner as the previous samples.

4.1.1.Animals

The study was carried out at the large animal clinic in the Department of Clinical Studies of the University of Nairobi. Twenty one clinically healthy sheep of mixed breed and sexes were used. They weighed between 14.5 kg and 37.5 kg (Appendix 4.1).

4.1.2. Experimental design

A preliminary egg count was done for all sheep (Appendix 4.2.). For this and all other counts in this study, the modified McMaster counting technique (MAFF,1977) was used. The counts ranged from 0 up to 4000 eggs per gram. The sheep were given one week to pick more larvae in the pastures, aiming that the least worm egg count would be 400 epg. A second faecal egg count was done for all the sheep an day 9 which was taken as day 0 of the experiment (Appendix 4.3). The least count was 300, with the highest being 4800 eggs per gram. The sheep were randomly assigned without restriction into five(5) groups i.e. 4 treatment groups (each having 4 sheep) and one control group with 3 sheep and weighed (Appendix 4.4). The dose rates in all the treatment groups were derived from those used by the herbalists. Group1 was drenched with a water extract of fresh *Maerua edulis,* while group 2 was drenched with water extract of powdered *Maerua edulis* at a dosage rate of 0.8ml per kg body weight. Groups 3 and 4 were drenched with a water extract of fresh and powdered *Albizia anthelmintica* respectively at a dosage rate of 1.2 ml per kg body weight. In all the four treatment groups, the weight of the

heaviest animal was used to calculate the dose rates. Sheep in group 5 were not treated and served as controls.

Further faecal egg count was done for all the groups on days 7 and day 14 post treatments (Appendix 4.5). The mean faecal egg counts of days 7 and 14 post treatment were determined and compared with that of day 0 in order to establish the percentage faecal egg count reduction for each of the treatment groups. From the results obtained, the experiment was repeated using the same sheep after allowing them a week in between and the dosage was doubled. *Maerua subcordata* was also included in this experiment. The faecal worm egg count on days 0 and 16 were recorded (Appendix 4.6). The mean faecal worm egg counts and percentage faecal egg count reduction for the treated groups and the control on days 0 and 16 were determined.

4.1.3.Statistical analysis

The student's t-test , Duncans Multiple Range Test for variable and Tukeys Studentized Range were used to test the difference between the means of the control and the treatment groups and also between the treatment groups themselves. A significant difference was considered at $p<0.05$.

4.2. Results and discussion

The results of faecal worm egg count reduction by fresh and powdered crude water extracts of *Maerua edulis* and *Albizia anthelmintica* are given in Table 4.1. Both fresh and powdered water extracts of *Maerua edulis* at 0.8ml/kg bodyweight showed no effect on the mean faecal worm egg counts on days 7 and 14 post treatment($p<0.05$). Similar results were observed with fresh and powdered *Albizia anthelmintica.*

At double the dosage (*M. edulis* at 1.6ml/kg, *A. anthelmintica* at 2.4ml/kg and *M. subcordata* at 1.6ml/ kg bodyweight) (Table 4.2.) the percentage reduction of faecal egg count was 55%, 49%, 38%, 16% and 14% for powdered *Albizia anthelmintica*, powdered *Maerua edulis,* fresh *Maerua subcordata,* fresh *Maerua edulis* and fresh *Albizia anthelmintica* respectively.

In all these plants tested, both fresh and powdered extracts indicated some efficacy though it varied from one plant to another. At a dosage rate of 0.8ml/kg bodyweight of *Maerua* species and 1.2 ml/kg bodyweight of *Albizia anthelmintica*, there was no faecal egg count reduction. After doubling the initial dose, there was significant reduction in all the treatment groups (Table 4.2). This implies that in the case of these plants the effect is dose dependent.

The faecal egg counts of the control animals reflected some bias as the animals had a very low epg count on day 0 and still a low count on day 16 compared with the treated groups despite having done the grouping at random. This led to a low percentage faecal egg count reduction because the percentage egg count reduction (PR) was determined as PR= 100(1-XT/XC), where XT is the arithmetic mean faecal count for the treatment group and XC is for the control (Coles *et al*, 1992).

Table 4.1. Mean faecal worm egg counts in sheep before and after treatment with single dose of *Maerua edulis* (0.8ml/kg bodyweight) and *Albizia anthelmintica* (1.2.ml/kg bodyweight)

Treatment group	No. of sheep	FEC Day 0 (epg)	Average day 7 and 14	PR (%)
1. Fresh *Maerua edulis*	3	1100	2700	0
2. Powdered *Maerua edulis*	4	875	1138	0
3. Fresh *A. anthelmintica*	3	1167	1467	0
4. Powdered *A. anthelmintica*	4	1125	825	0
5. Control	3	533	700	0

Table 4.2. Mean and percentage reduction of faecal egg counts in sheep before and after treatment with a double dose of *Maerua edulis* (1.6ml/kg),*Maerua subcordata* (1.6ml/kg) and *Albizia anthemintica* (2.4ml/kg bodyweight.

Treatment group	No. of sheep	FEC Day 0 (epg)	Day 16 (epg)	PR (%)
1. Fresh *Maerua edulis*	3	3033	1033	16
2. Powdered *Maerua edulis*	4	1100	625	49
3. Fresh *A. anthelmintica*	3	1466	1067	14
4. Powdered *A. anthelmintica*	4	925	550	55
5. Fresh *Maerua subcordata*	2	3600	900	38
6. Control	3	367	1233	

Key

FEC means faecal egg count
PR means percentage reduction

Feacal egg count reduction is an estimate of anthelmintic efficacy by comparing worm egg counts before and after treatment. In this experiment the sampling days and frequencies were done twice on day 7 and 14 in the first part of the experiment and once on day 16 in second part of the experiment. Coles *et al* (1992) recommended that post treatment should be collected 10-14 days after treatment in sheep and goats when evaluating efficacy of synthetic drugs. However there is a lot of variation on the number of sampling and frequency when testing both synthetic and herbal anthelmintics as reported by several authors e.g. Akhtar and Riffat (1984), Maingi(1991), Mwamachi *et al*(1995) , Sharma (1993), Waruiru (1991). There is also a variation in the use of epg evaluation only or in combination with worm counts reduction when testing both synthetic and herbal anthelmintics. In this experiment only epg evaluation was used due to financial constraint. However, the combination of both methods is more reliable as the reduction in egg counts denotes a reduction in worm counts.

The result of these experiments shows that although the percentage egg count reduction was not high (with the highest being 55%), but the crude extracts could control the helminths. The initial mean faecal egg count for all the sheep before the study compared to the mean after the experiments was more , indicating the potency of the crude extracts. If the sheep had not been treated with the plant extracts, the counts would have been very high with clinical signs as was the case of one sheep that had to be withdrawn from the experiment due to high epg counts, diarrhoea and emaciation. At this form of crude extract, the plant anthelmintics are only for controlling the helminths in such a way that the helminths do not affect the body condition of the sheep but there is no 100% cure. After these pilot studies, further studies are warranted to test the efficacy of these plants using a more purified extract.

CHAPTER FIVE

DETERMINATION OF THE BIOACTIVITY OF PLANT EXTRACTS USING THE BRINE SHRIMP LETHALITY TEST

5.0. Introduction

There are many plants used in Kenya that are claimed to be capable of controlling human and livestock helminthoses. However, only a few of them have been evaluated for their pharmacological activity. Screening of medicinal plants for their biological activity is expensive and laborious. It is only large advanced pharmaceutical companies and laboratories that have the required equipment and funds for complicated bioassays needed to screen plant extracts. The cost of such equipment, special reagents and aseptic techniques required is not within the research funds available in Kenya. This may be the reason why there is limited data on the bioactivity of many potential herbal medicaments. There is therefore a need for a reliable, less complicated bioassay for testing medicinal plants.

Meyer *et al* (1982) proposed and used the brine shrimp lethality test as a convenient method of solving such limitations. As most active plant constituents are toxic in high doses, screening their toxicity to zoological systems is an indicator of their bioactivity. The brine shrimp bioassay test is based on this principle. It utilises the larvae of brine shrimp, *Artemia salina* Leach. The eggs that are readily available at low cost in pet shops and are used worldwide for feeding tropical fish remain viable for years upon being placed in brine solution and when incubated, they hatch within 48 hours, providing large numbers of larvae.

The test which is inexpensive, rapid and simple has been used to detect and isolate active plant constituents through guided screening and pytochemical fractionation. Only basic training and simple equipments are required. Mwangi *et al* (1999) successfully used brine lethality test to screen 78 plant samples obtained from 34 plants used for medicinal purposes in Kenya. They reported that out of the 78 samples, 36 showed toxicity to the brine shrimp (LC_{50}< 1000ug/ml).

This study was therefore conducted to determine the bioactivity of the water, methanol and chloroform extracts of *Albizia anthelmintica* Brogn, *Maerua edulis* De Wolf and *Maerua subcordata* De Wolf using brine shrimp lethality test.

5.1. Materials and methods

5.1.1. Acquisition and preparation of plant material

The plant materials of *Albizia anthelmintica* Brogn and *Maerua edulis* De Wolf were obtained from Kibwezi in Makueni district whereas *Maerua subcordata* De Wolf was obtained from Tunyo division of the Marakwet district. The tubers of *Maerua* species were peeled, chopped, and dried using an ELE oven at 60° C for 6 days. The dried material was ground into a powder using an electric mill (Christy Hunt 8-inch Lab mill, Essex England) and stored in airtight plastic bags . The *Albizia anthelmintica* bark was sun- dried for three days and later ground as above and stored in airtight plastic bags.

5.1.2.Extraction of active ingredient from plant material

The active ingredient(s) from the prepared plant material were extracted in water, methanol and chloroform.

Water extraction

One kilogram of the powdered plant material was put in a five-litre conical flask. Distilled water was added up to the 5-litre mark and the mixture stirred well and boiled for two hours. An additional 3 litres of water were then added to compensate for the evaporation. The mixture was then boiled further for one hour and centrifuged. The mixture was filtered using Whatman no.1 filter paper. The volume of the filtrate was further reduced using rotary evaporator bath, then freeze- dried for three days using Edwards high vacuum freeze drier (Manor Royal Crawley Sussex, UK).

Methanol extract

The powdered material of each of the three-plant material was weighed to 1 kilogram and the material put into a 5- litre conical flask. Two litres of methanol (Kobian Limited) was added. The flask was placed into a water bath at 50°C for 12 hours. The methanol extract was filtered through absorbent cotton wool and later filtered through filter paper Whatman no.1. The methanol was then evaporated and recovered using Soxhlet apparatus. The filtered methanol extract was freeze- dried and then stored at 4°C in plastic airtight containers.

Chloroform extraction

One kilogram of each powdered plant material was put into a 5- litre conical flask. Two litres of re-distilled chloroform was then added and the mixture was stirred and left

standing at room temperature for 12 hours. It was then filtered through cotton wool. Approximately 1.2 litres of chloroform extract were obtained. The filtrate was filtered further through Whatman no.1 filter paper and the chloroform was recovered by distillation using a Soxhlet apparatus and re-used in other extraction. The remaining 20-50 ml of the filtrate were transferred into a paper boat made of aluminium foil and evaporated for twelve hours in a Griffin Incubator at 40°C. The filtrate was then freeze-dried for 36 hours.

5.1.3.Hatching of the brine shrimp

The materials and reagents used included *Artemia salina* Leach eggs bought from Yaya centre pet shop (Nairobi); marine salt; a rectangular plastic container with two unequal chambers divided by a wall with 2 mm holes; a 40 watt electric bulb and yeast granules .

Marine salt solution was prepared by dissolving thirty three grams of marine salt in 1 litre of distilled water. The plastic container was filled with the marine salt solution and rectangular hole (24cm squared) was made on the part of the lid covering the smaller chamber leaving the other side intact . Dry yeast granules were added at a rate of 6mg of dry yeast per litre of marine salt solution on the covered chamber of the container. Fifty milligrams of brine shrimp eggs were sprinkled on the same compartment. A fourty watt bulb was hung on the other chamber of the container with an opening . The bulb was kept lit to provide light to attract the larvae from the dark side to the illuminated side leaving their eggshells behind. The larvae were allowed 48 hours to hatch.

5.1.4. Bioassay

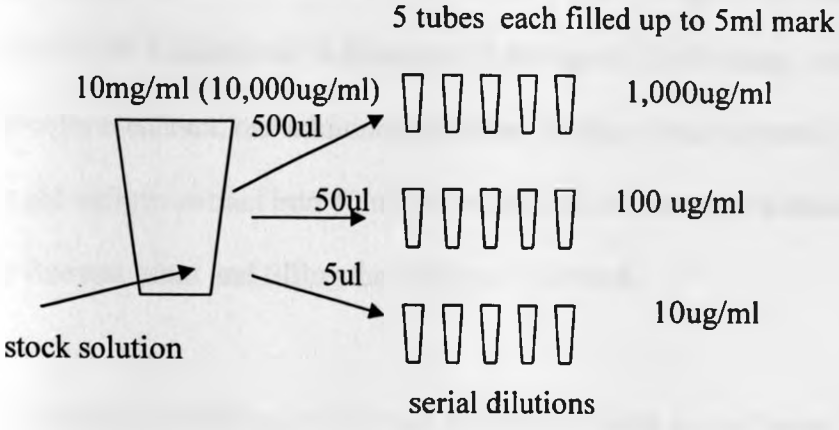
Serial dilutions of water, methanol and chloroform extracts were prepared using the marine salt solution. The dilutions were prepared by first dissolving 0.1 grams of the extract into 10 ml of marine salt solution. Three dilutions were prepared by transferring 500ul, 50ul and 5 ul to the set of five graduated vials for each dilution respectively to make dilutions of 1000 ug/ml, 100ug/ml and 10ug/ml by adding marine salt solution to 5ml mark (Figure 5.1). Five graduated vials were set for every dilution and a further five for the control.

Ten shrimps were transferred into each of the vial using a Pasteur pipette. Then the marine salt was added to 5 ml mark. Ten shrimps were also transferred to each of the five vials set as the control and marine salt solution added to 5 ml mark. The tubes were left at room temperature and the number of live larvae counted after 24 hours. The percentage mortality was determined. From the trends of the percentage mortality additional dilutions were prepared for each extract.

Additional dilutions

Five serial dilutions of the water extract of *Albizia anthelmintica* were prepared by first dissolving 0.1 gram of the extract in 10 ml of marine salt solution. Five graduated vials were set for each dilution and further 5 tubes for the control. Using a micropipette, 200ul, 150ul, 100ul, 10ul, and 1 ul were transferred into separate five test tubes which were to make dilutions of 400ug/ml, 300ug/ml, 200ug/ml, 20ug/ml and 2ug/ml after adding the marine salt solution to a 5-ml mark. A similar procedure was used to prepare five additional dilutions of 200ug/ml, 150ug/ml, 50ug/ml, 20ug/ml and 2ug/ml for methanol extract and four (20ug/ml, 7.5ug/ml, 5ug/ml and 2.5ug/ml) for chloroform extract.

Figure 5.1. Preparation of serial dilutions



Serial dilutions of water and methanol extracts of *Maerua edulis* were prepared by dissolving 1 gram of the extract into 10 ml of distilled water. Using one 1 ml syringe and micropipette, volumes of 1ml, 825ul, 750ul, 625ul, 500ul, 250ul, 100ul and 10ul of the water extract- marine salt mixture was transferred into vials to make dilutions of 20,0000ug/ml, 17,500ug/ml, 15,000ug/ml, 12,500ug/ml, 10,000ug/ml, 5,000ug/ml, 2,000ug/ml and 200ug/ml after adding marine salt solution to 5 ml mark. A set of five vials were prepared for each dilution. The same was done for methanol to obtain five dilutions of 5,000ug/ml, 4,000ug/ml, 3,000ug/ml, 2,000ug/ml and 200ug/ml. For the chloroform extract, one additional dilution of 2ug/ml was prepared by dissolving 0.1g of the chloroform extract into 10 ml of marine salt solution and transferring 1 ml to each of the five test tubes and filling the vial to a 5ml mark.

The additional dilutions of *Maerua subcordata* water extract were prepared in the same way as those of water extract of *Maerua edulis* but only six dilutions were prepared and they were 15,000ug/ml, 12,500ug/ml, 10,000ug/ml, 5,000ug/ml, 2,000ug/ml and 200ug/ml. For the methanol extracts one gram of the methanol extract was dissolved in 10 ml of marine salt solution and dilutions were made by transferring volumes of 500ul, 400ul, 300ul, 200ul and 100ul into vials (five for each dilution) to make dilutions of 10,000ug/ml, 8,0000ug/ml, 6,000ug/ml, 4,000ug/ml, and 2,000ug/ml respectively on adding marine salt solution to 5ml mark. Further dilutions were made by dissolving 0.1g of methanol extract into 10ml of marine salt solution and volumes of 100ul, 10ul and 1ul were transferred to vials (each dilution had five vials each) to make dilutions of 200ug/ml, 20g/ml and 2ug/ml respectively.

Bioassays were done for all dilutions. The number of live larvae and percentage mortality for each dilution and controls were calculated. Where control deaths occurred within 24 hours, the data was corrected using the equation:

% deaths = ((test- control)/ control)x 100

The results were interpreted using probit method of Finney computer program acquired from the Department of Pharmacology and Pharmacognosy in the Faculty of Pharmacy, University of Nairobi. The program uses the number of dose level , number of brine shrimps used for every concentration, percentage mortality for every concentration and the dose level. The lethal concentration fifty (LC_{50}) and 95% confidence intervals were determined using the computer program.

5.2. Results and discussion

The results of the brine shrimp bioassay are presented in Figures 5.2., 5.3 and 5.4. These shows the percentage mortality of the brine shrimp caused by serial dilutions of water, methanol and chloroform extracts. They are also shown in Tables 5.1, 5.2 and 5.3. The LC_{50} and the 95% confidence interval determined using probit method of Finney computer programme are shown in Table 5.4.

According to the trends of the percentage mortality rates of brine shrimps, *Albizia anthelmintica* serial dilutions were found to be most toxic with 50% mortality in concentrations less than 300ug/ml. It was further observed that serial dilutions of the chloroform extract were more toxic to brine shrimps than water and methanol extracts for the three plant materials. The least toxic to brine shrimps was found to be water extracts for the three plants.

The water, methanol and chloroform extracts of *Albizia anthelmintica* bark were active (LC_{50} <1000ug/ml) with LC_{50} of 101ug/ml, 18ug/ml and 5ug/ml (Table5.4). The 95% confidence intervals of these LC_{50} were 19-768ug/ml, 16-35ug/ml and 2-6 ug/ml respectively. The water and methanol extracts of *Maerua edulis* and *Maerua subcordata* were not active at LC_{50}<1000ug/ml except for the chloroform extract of *Maerua edulis* which was active at LC_{50} of 275ug/ml and 95% confidence interval of 45-1722 ug/ml. The LC_{50} of the chloroform extract of *Maerua subcordata* was not determined due to shortage of material.

These results indicate that the chloroform extracts are more toxic than the extracts of methanol and water. This may be based on the basic features of the solvent with chloroform being less polar than methanol and water and with the later being most polar. However , Mwangi *et al* (1999) reported that polarity is not a determinant of activity of plant extract. In their studies, out of the 36 active plants extracts (LC_{50} <1000) reported, 44% were from polar fractions using methanol while 56% were from non-polar fractions using petroleum ether.

Although brine shrimp lethality test indicates the biological activity in plant extracts, lack of lethality does not mean that the extract has no biological activity as cited by Mwangi *et al* (1999). For instance, *Prunus africana*, atropine and phentolamine drugs, have pharmacological activity but they are not toxic to the brine shrimps. Overall, albeit the differential toxicity to brine shrimps by the three extracts of chloroform, methanol and water used in this study, the results indicate that the three plants have bioactivity which need to be assessed thoroughly using more effective bioassay.

Table 5.1. Brine shrimp mortality rate caused by serial dilutions of water extracts

Serial dilutions of water extracts (ug/ml)	Percentage mortality		
	Albizia anthelmintica	*Maerua edulis*	*Maerua subcordata*
2	18		
20	26		
200	40	0	8
300	72		
400	74		
2000		4	24
5000		28	40
10000		46	48
12500		52	52
15000		56	74
17500		66	
20000		86	

Table 5.2. Brine shrimp mortality rate caused by serial dilutions of methanol extracts

Serial dilutions of methanol extracts (ug/ml)	Percentage mortality		
	Albizia anthelmintica	*Maerua edulis*	*Maerua subcordata*
2	16		
20	44		
50	58		8
100	90		
150	92		
200	96	0	0
1000		12	
2000		20	40
3000		50	
4000		66	50
5000		94	
6000			60
8000			96
10000			100

Table 5.3. Brine shrimp mortality rate caused by serial dilutions of Chloroform extracts

Serial dilutions of chloroform extracts (ug/ml)	Percentage mortality	
	Albizia anthelmintica	*Maerua edulis*
2.5	28	
5.0	48	
7.5	54	
10	64	22
20	78	
100	98	26
1000	100	52
2000		90

Fig 5.2. Brine shrimp mortality caused by serial dilutions of water extracts

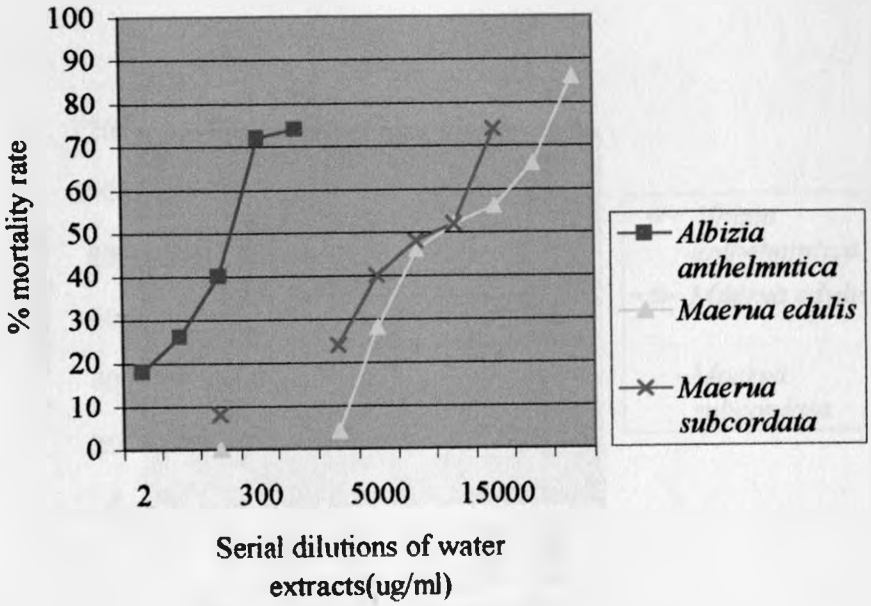


Fig.5.3. Brine shrimp mortality caused by serial dilutions of methanol extracts

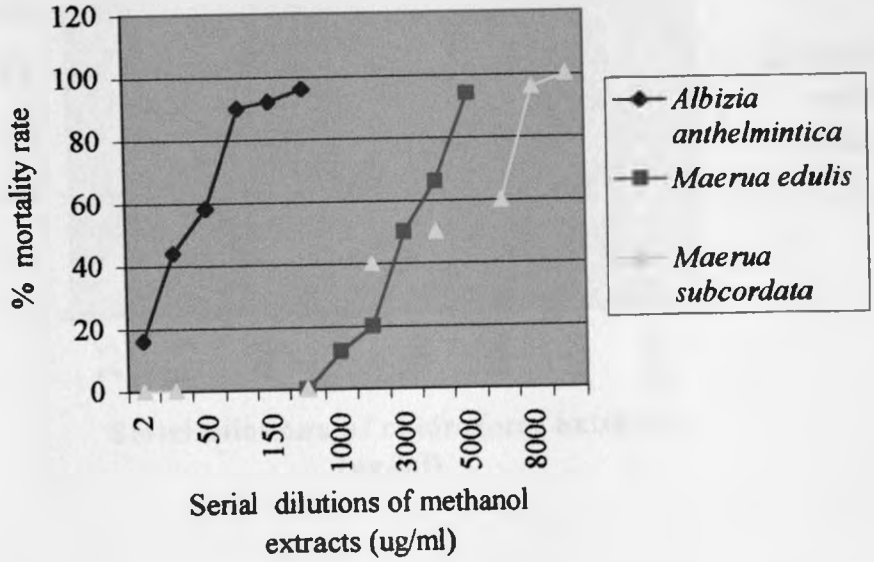


Fig. 5.4.Brine shrimps mortality rate caused by serial dilutions of chloroform extracts

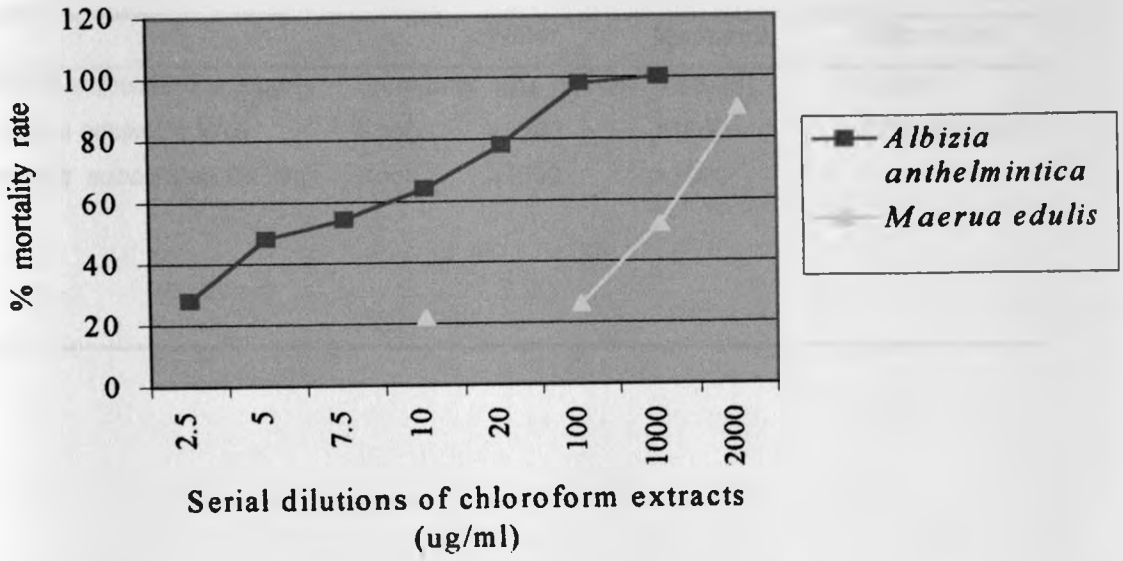


Table 5.4. Brine shrimp bioassay results of water, methanol and chloroform extracts (LC_{50} and 95 % confidence interval)

Botanical name	Part used	LC_{50} (ug/ml)		
		Water	Methanol	Chloroform
Albizia anthelmintica Brogn	Stem bark	101(19-768)	18(6-35)	5(2-6)
Maerua edulis De Wolf	Root	>1000	>1000	275(45-1722)
Maerua subcordata De Wolf	Root	>1000	>1000	

CHAPTER SIX

EVALUATION OF THE EFFICACY OF WATER, METHANOL AND CHLOROFORM PLANT EXTRACTS AGAINST NEMATODE *HELIGMOSOMOIDES POLYGYRUS* INFECTIONS IN MICE.

6.0 Introduction

The bark of *Albizia anthelmintica* has been reported by many authors to have anthelmintic activity. According to Watt and Breyer-Brandwijk (1962), *Albizia anthelmintica* has been reported in West Africa to have efficacy against tapeworms at a dosage of 60gm or more in humans and this has also been reported in Ethiopia and Somalia. According to the same authors, the bark has been used for treatment of hookworm infestation in humans in Usambara, Tanzania whereas the Maasai people mix the bark with milk, blood or soup and use the preparation as an anthelmintic. It has also been reported that sheep nibble the bark when they suffer from worm infestation

The Samburu and Turkana use the bark of this plant for treatment of many kinds of helminth infestations and some external parasitosis (ITDG and IRRR, 1996).

Several authors have studied the anthelmintic activity of the bark of *Albizia anthelmintica*. Koko *et al* (2000), studied the fasciolocidal efficacy of *Albizia anthelmintica* stem bark water extract at 9g/kg body weight in 6- months old goats. They reported that *Albizia anthelmintica* and *Balanites aegyptiaca* each at 9 g/kg body weight and albendazole at 20mg/kg body weight had a percentage reduction in liver fluke counts of 95.5%, 93.2.% and 97.7%. respectively.

Galal *et al* (1991) evaluated the efficacy of *Albizia anthelmintica* bark aqueous extract using *Hymenolepsis diminuta* in rats at a dosage of 50-150g/kg body weight. They reported that the extract was safe and eliminated the infestation successfully. The butanolic extract of *Albizia anthelmintica* bark administered to the same rats using a stomach tube was reported to be highly toxic to the rats and ineffective against *Hymenolepsis diminuta.*

The tuber of *Maerua edulis* has been reported to have anthelmintic activity against nematodes in livestock. Studies done by Gakuya *et al* (2000) have reported a 49 % reduction of worm egg count using an aqueous extract of the powdered tuber material of *Maerua edulis* in sheep.

Heligmosomoides polygyrus is a stronglye nematode living in the lumen of the small intesine of mice. *H. polygyrus*- experimentally infected mice have been used to evaluate the anthelmintic activity of papaya latex (Satrija *et al,* 1995). In this experiment *Heligmosomoides polygyrus* experimentally infected in mice were also used to evaluate the anthelmintic efficacy of *Albizia anthelmintica* and *Maerua edulis* plants as a model for the control of gastrointestinal nematodes in livestock.

6.1. Materials and methods

6.1.0. Experimental design

Fifty four mice of Swiss breed aged 10 weeks and of both sexes were brought to the Department of Clinical Studies, Kabete campus from International Livestock Research Institute(ILRI)-Nairobi-Kenya. The mice were housed in standard cages placed in well

ventillated room at 20°C and allowed five days to acclimatise. They were fed on rabbit pellets obtained from Unga Ltd and provided with drinking water *ad libitum*.

On day one of the experiment, a pooled faecal sample was collected from the mice to establish the helminth burden of the mice. The mice were randomly allocated without restriction into nine groups of six mice each. The groups were further randomly assigned into eight treatment groups (A,B,C,D,E,F,G,H) and one control(I).

Each mouse was infected with 100 larvae of *Heligmosomoides polygyrus* obtained from helminthology section of ILRI and allowed 11 days for infection to establish. The larvae were administered to the mice per os using a 18 gauge needle which had a bulb at the end (Fig.6.1). A faecal egg count for the mice were done on the 11^{th} and 17^{th} day post infection (Appendix 6.2,6.3). Two mice died in group C and one in groups D and F before the treatment day.

6.1.1. Treatment regime

Acquisation of plant material

A freeze dried, water, methanol and chloroform extracts of *Albizia anthelmintica* bark and *Maerua edulis* root prepared and stored as in Chapter 5 were used. A suspension of the plant material was prepared by dissolving the required dosage per one mouse with distilled water . A volume of 0.4 ml of distilled water was used per every dosage given to each mouse. The chloroform extracts of *Albizia anthelmintica* extracts had a problem with dissolving and therefore one ml of distilled water was used to dissolve every dose of extract required by the mouse. A Voltex machine (Assistent, Reamix 2789) was used for mixing the extract in distilled water, alternating with warming in a water bath at 37°C.

Fig 6.1. Infecting a mouse orally with the larvae of *Heligmosomoides polygyrus*

Treatment

On 18th day after infection , all the mice were weighed and the weight of the heaviest mouse in the group recorded (Appendix 6.1). The dose rates were based on a previous studies done to evaluate the efficacy of *Albizia anthelmintica* against helminths in rats by reducing the dose rates ten times. Group A, B and C were given a dose of chloroform extracts of *Albizia anthelmintica* at dosage rate of 10gm/kg, 20gm/kg and 30gm/kg bodyweight per mouse respectively. The extracts which were suspended in distilled water and thoroughly mixed with peanut butter and rabbit pellets were fed to the mice for a period of two days.

Group D mice were given an oral dose of water extract of *Albizia anthelmintica* at a dosage 10gm/kg body weight divided into two doses of 0.2 ml each using an 18 gauge needle four hours apart. Similarly group E was given methanol extract of *Albizia anthelmintica* at a dosage of 10gm/kg bodyweight divided into two doses per os. Groups F and G were given water and methanol extracts of *Maerua edulis* at 10gm/kg bodyweight per os respectively in two doses. Group H was given chloroform extract of *Maerua edulis* at 10gm/kg bodyweight per os as two doses (Fig 6.1). Group I was given 0.4 ml of distilled water in two doses.

6.1.2. Parasitological techniques

6.1.2.1. Faecal egg concentrations (EPG) determination.

On 4th and 6th day post infection, the faecal worm egg counts were done for all the mice and percentage faecal egg counts reduction determined. Fresh faeces were collected by transferring an individual mouse from their group cages into a clean plastic container having a lid.

They were allowed 2-3-hours and their faecal pellets collected. One gram of faecal pellets was homogenised in 30 ml of saturated salt solution and the other half used to rinse the container. The homogenised and rinsed material were mixed and seived. McMaster slide was used to count the eggs immediately . From these faecal worm egg counts, the group means and percentage egg reduction were determined .In cases where the faecal samples were less than one gram , the volume of the saturated salt solution was reduced proportionately with the weight of the sample.

6.1.2. 2. Accurate determination of total adult worm burden

On the 7th day post infection, the mice were euthanised by cervical dislocation to allow for determination of total worm counts and the percentage worm count reduction in the treatment groups. Briefly, after euthanising the mice, the gut cavities were opened and entire loops of the small intestines exposed. The loops of the small intestine were recovered by separating it from the mesenteries and cutting the proximal part of the loop as far as the end of the stomach and distally to the entrance of the caecum. The loops of each individual mouse were put in a separate petri dish to recover the nematode, *Heligmosomoides polygyrus*.

The caecum and the distal loop to the anal opening was also recovered to determine the infections of pinworms, *Aspiculuris tetraptera* and *Syphacia muris* and put into a separate petri dish for each mouse.

The recovered small intestinal loops placed on nettings that had been spread on petri dishes were opened longitudinally with a set of scissors and all the contents and tissues wrapped with the net. The contents were then suspended in a 50ml beaker containing

about 25 ml of physiological saline. The beakers were incubated at 37°C for 4 hours to allow the worms to migrate from the lumen of the small intestines. After 4 hours, the gauzes were removed and the tissues discarded.

The gauzes were placed on a petri dish, scanned under low power dissecting microscope and the worms that were trapped counted. The temperature was then increased up to 42 °C and thereafter increased by 2 degrees every five minutes up to 50°C. This caused the worms to dissociate from their interlocking knots and made counting easier . Part of the supernatant was poured out after allowing the beaker to settle and the contents poured on a petri dish. Worms (Fig 6.2.) in the supernatant were also counted using a dissecting microscope. The total number of worms was added to the number of worms earlier obtained from the gauze strips.

The caecum and distal loops placed on petri dishes were opened longitudinally to expose the worm and about 5ml of physiological saline was poured on the petri dish and the tissues and content agitated. The pinworms (Fig 6.3.) recovered were counted using a dissecting microscope.

From the worm counts of the control and the treated groups, percentage efficacy of the plant extract was estimated according to Satrija *et al* (1995) as:

$$\frac{(\text{mean no. of worms in control group}) - (\text{mean no. of worms in treated group})}{\text{mean number of worms in control group}} \times 100$$

Fig 6.2. Adult worms of *Heligmosomoides polygyrus* from the loops of small intestine of a mouse treated with *Albizia anthelmintica* water extract

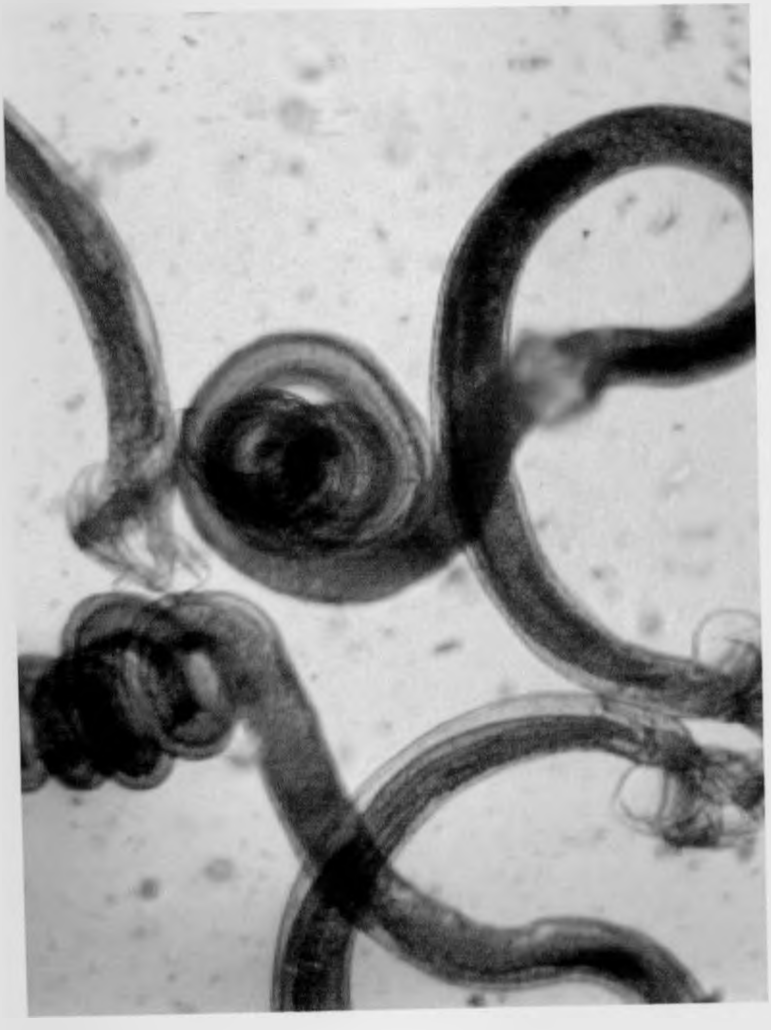
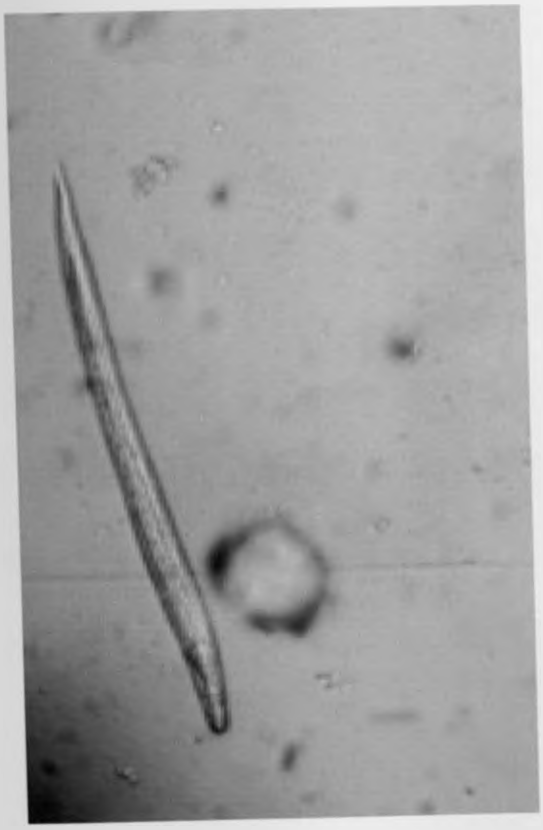


Fig 6.3. Pinworm from the caecum of a mouse treated with *Albizia anthelmintica* chloroform extract



6.1.3. Statistical analysis

The SAS computer program was used. The significant differences between different means of faecal worm egg counts and worm counts in the groups were determined using Student - t test, Duncan's Multiple Range Test for variable and Tukeys Studentized Range . A contrast test was also done between the control and other treatment groups.

6.2.Results

A preliminary egg count of a pooled sample of mice faeces on day one was 4,800 egg per gram and all were pinworms. Eleven days after infection with *Heligmosomoides polygyrus* the type of eggs and faecal egg count showed that all were infected with the *H. polygyrus* except one mouse in group F (Appendix 6.2.). For the pinworms the number of mice infected at day eleven were; one in group A , 4 in group B, 4 in group C, 4 in group D, 5 in group E, 4 in group F, 3 in group G, 4 in group H and 3 in group I.

The results of faecal worm egg counts for *H. polygyrus* and pinworms on day 17 and days 4 and 6 post treatment are shown in Appendix 6.3. and 6.4. On day seventeen the faecal worm egg count in individual mice showed that all mice were infected with *Heligmosomoides polygyrus* with burdens ranging from 100 to 16,500 epg. The epgs of pinworms ranged from 0 to 1,100.

After administration of the initial dose of the *Maerua edulis* chloroform extracts, all the mice in group H died within a period of one and a half hours. They showed signs of incordination and dysponea before death. In group G, one mouse died three hours after

administration of the initial dose of *Maerua edulis* methanol extract and three died four hours after the second dose.

In group F one mouse died five and a half hours after administration of the *Maerua edulis* water extract at 10gm/kg bodyweight. In group E, two mice died two and a half hours after administration of the initial dose of methanol extract of *Albizia anthelmintica* and the other four died two hours after the second dose. In group D one mouse died after administration of the initial dose of *Albizia anthelmintica* water extract and two more died after the second dose. In group C one mouse died after administration of *Albizia anthelmintica* chloroform extract at 30gm/kg bodyweight. In group A and B all the mice survived. The results of the worm counts on 7^{th} day post treatment for *Heligmosomoides polygyrus* and pinworms are shown in Appendix 6.5 and 6.6.

The results of mean faecal egg counts of *Heligmosomoides polygyrus* and pinworms after treatment with *Albizia anthelmintica* and *Maerua edulis* chloroform, methanol and water extracts at different dose are shown in Tables 6.1 and 6.2. The mean worm counts and anthelmintic efficacy of the various extracts of *Albizia anthelmintica* and *Maerua edulis* at different doses are shown in Tables 6.3 and 6.4.

Table 6.1. Mean faecal *H. polygyrus* egg counts in mice before and after treatment with various extracts of *A. anthelmintica* and *M. edulis* at different dosages

		FEC		
Treatment group	No. of mice-day6	Day 0* (epg)	Day 4	Day 6
A	6	11700	6100	9033
B	6	9617	5020	7150
C	4	5280	5975	5900
D	2	6633	5900	7150
E	All died	8850		
F	4	1750	6325	5033
G	2	5763	5250	4200
H	All died	2200		
I	6	3767	6367	4500

Key

* Means of day 0 are taken as the baseline data before treatment

Groups

A. *Albizia anthelmintica* chloroform extract at 10gm/kg bodyweight
B. *Albizia anthelmintica* chloroform extract at 20gm/kg bodyweight
C. *Albizia anthelmintica* chloroform extract at 30gm/kg bodyweight
D. *Albizia anthelmintica* water extract at 10gm/kg bodyweight
E. *Albizia anthelmintica* methanol extract at 10gm/kg bodyweight
F. *Maerua edulis* water extract at 10gm/kg bodyweight
G. *Maerua edulis* methanol extract at 10 gm/kg bodyweight
H. *Maerua edulis* chloroform extract at 10gm/kg bodyweight
I. Control

Table 6.2. Mean faecal pinworm egg counts in mice before and after treatment with various extracts of *A. anthelmintica* and *M. edulis* at different dosages

Treatment group	No. of mice-day6	FEC Day 0* (epg)	Day 4	Day 6
A	6	0	0	67
B	6	34	33	33
C	4	240	375	50
D	2	317	0	0
E	All died	17		
F	4	283	425	150
G	2	9	0	0
H	All died	211		
I	6	100	300	180

Key

* Means of day 0 are taken as the baseline data before treatment

Groups

A. *Albizia anthelmintica* chloroform extract at 10gm/kg bodyweight
B. *Albizia anthelmintica* chloroform extract at 20gm/kg bodyweight
C. *Albizia anthelmintica* chloroform extract at 30gm/kg bodyweight
D. *Albizia anthelmintica* water extract at 10gm/kg bodyweight
E. *Albizia anthelmintica* methanol extract at 10gm/kg bodyweight
F. *Maerua edulis* water extract at 10gm/kg bodyweight
G. *Maerua edulis* methanol extract at 10 gm/kg bodyweight
H. *Maerua edulis* chloroform extract at 10gm/kg bodyweight
I. Control

Before treatment the mean faecal egg count for *Heligmosomoides polygyrus* for the control was significantly less than the means of group A, B and E and similar to the rest using Student t-test and Duncan's Multiple Range Test for variable at $p<0.05$. Four days post treatment there was no significant difference between the mean faecal egg counts of *H. polygyrus* of the treatment groups and the control and in between the treatment groups using Duncan's Multiple Range Test for variable at $p<0.05$. On 6^{th} day post treatment , there was no significant difference between the mean faecal worm egg counts of *H. polygyrus* in the treated and the control groups ($p< 0.05$) .

A day before treatment there was no significant difference between the mean group faecal pinworm worm egg counts for the treated groups and the control. On 3^{rd} day post treatment group F (*Maerua edulis* water extract) had significantly high mean faecal egg count than group A(*Albizia anthelmintica* chloroform extract), D (*Albizia anthelmintica* water extract) and G (*Maerua edulis* methanol extract) ($p<0.05$). On 6^{th} day post treatment the mean faecal pinworm egg count of the control group was significantly greater than the treatment groups ($p<0.05$).

On 7^{th} day group A (*Albizia anthelmintica* chloroform at 10gm/kg), B(*Albizia anthelmintica* chloroform at 20gm/kg) and G (*Maerua edulis* methanol extract at 10gm/kg) worm counts were significantly higher than group F (*Maerua edulis* water extract at 10gm/kg). There was an anthelmintic efficacy of 59, 29 and 24 % for group F (*Maerua edulis* water extract at 10gm/kg), D (*Albizia anthelmintica* water extract at10gm/kg bodyweight) and C (*Albizia anthelmintica* chloroform extract at 30gm/kg body weight) respectively.

For the pinworms counts at 7th day post treatment, the treatment groups had significantly less worm counts than the control ($p<0.05$). There was an anthelmintic efficacy of 100, 95, 85, 25 and 16% in group G (*Maerua edulis* methanol extract at 10gm/kg bodyweight, A (*Albizia anthelmintica* chloroform extract at 10gm/kg),B (*Albizia anthelmintica* at 20gm/kg), C (*Albizia anthelmintica* chloroform extract at 30gm/kg), and D (*Albizia anthelmintica* water extract at 10gm/kg bodyweight) respectively.

Table 6.3. Mean worm counts and percentage reduction of H. polygyrus after treatment of of mice with *A. anthelmintica* and *M. edulis* extracts at different dosages.

Treatment group	No. of Mice(day7)	Total worm count 7 days post treatment	Anthelmintic efficacy (%)
A	6	60	
B	6	62	
C	4	37	24
D	2	35	29
E	All died		
F	4	20	59
G	2	69	
H	All died		
I	6	49	

Key

Groups

A. *Albizia anthelmintica* chloroform extract at 10gm/kg bodyweight
B. *Albizia anthelmintica* chloroform extract at 20gm/kg bodyweight
C. *Albizia anthelmintica* chloroform extract at 30gm/kg bodyweight
D. *Albizia anthelmintica* water extract at 10gm/kg bodyweight
E. *Albizia anthelmintica* methanol extract at 10gm/kg bodyweight
F. *Maerua edulis* water extract at 10gm/kg bodyweight
G. *Maerua edulis* methanol extract at 10 gm/kg bodyweight
H. *Maerua edulis* chloroform extract at 10gm/kg bodyweight
I. Control

Table 6.4. Mean worm counts and percentage reduction of *H. polygyrus* after treatment Of mice with *A. anthelmintica* and *M. edulis* extracts at different dosages.

Treatment group	No. of mice(day6)	Total worm count 6 days post treatment	Anthelmintic efficacy (%)
A	6	11	95
B	6	35	85
C	4	174	25
D	2	195	16
E	All died		
F	4	269	
G	2	2	100
H	All died		
I	6	231	

Key

Groups

A. *Albizia anthelmintica* chloroform extract at 10gm/kg bodyweight
B. *Albizia anthelmintica* chloroform extract at 20gm/kg bodyweight
C. *Albizia anthelmintica* chloroform extract at 30gm/kg bodyweight
D. *Albizia anthelmintica* water extract at 10gm/kg bodyweight
E. *Albizia anthelmintica* methanol extract at 10gm/kg bodyweight
F. *Maerua edulis* water extract at 10gm/kg bodyweight
G. *Maerua edulis* methanol extract at 10 gm/kg bodyweight
H. *Maerua edulis* chloroform extract at 10gm/kg bodyweight
I. Control

6.3.Discussion

The results of this experiment showed that *Maerua edulis* water extract had the highest percentage reduction of *Heligmosomoides polygyrus* egg counts whereas the methanol extract of *Maerua edulis* had the highest anthelmintic efficacy against pinworms. The chloroform extracts of *Albizia anthelmintica* had an anthelmintic efficacy of 95% and 85% against pinworms at a dosage of 10gm/kg and 20gm/kg respectively.

These results were affected by some constraints during the experiment. The *Albizia anthelmintica* chloroform extracts had a problem in dissolving in distilled water. The suspension that resulted was too thick to pass through gauge 18 needle which was used for oral drenching in mice. Therefore, peanut butter and rabbit pellets were used to make a paste with the *Albizia anthelmintica* chloroform extract. This paste was fed to the mice in a period of two days. The disadvantage of feeding the mice in a group is that there is no control of the amount of the extract each mouse takes. This may have affected the results of this trial. The other constraint was the total volume the mouse had to be drenched with as one dose. The maximum amount being 0.2ml per mouse which, implied that extracts which required higher volumes to dissolve in water must be given either as feed or have to be given in many doses.

The mortality rate of mice in group H (*Maerua edulis* chloroform extract and E (*Albizia anthelmintica* methanol extract) was 100%. Whereas that of Group G (*Maerua edulis* methanol extract) and D (*Albizia anthelmintica* water extract) was each 66.7%, indicating the preparations were toxic. Acute toxicity has also been reported in plants used for medicinal purposes by others (Mugera,1970; Kellerman *et al*,1988; Shone and Drummond, 1965; Mbaria *et al*, 1994; Thaiyah, 1991 and Muchiri, 1987).

It is possible that the chloroform and methanol solvents must have extracted some substances from *Maerua edulis* and *Albizia anthelmintica* which were very toxic to the mice but not to the *Heligmosomoides polygyrus* as 90% of the worms were found still surviving on postmortem.

Although the chloroform extracts of *Albizia anthelmintica* showed high efficacy against pinworms (group A-95% and B-85%), they had low efficacy to *Heligmosomoides polygyrus*. These extract were well tolerated by the mice but the method of administration was a problem due to low solubility in water. In the other hand mice treated with *Maerua edulis* water extract had a mortality of 33 % but the extract had the highest efficacy towards *Heligmosomoides polygyrus*. Therefore, further studies of the water extract of *Maerua edulis* at varied dosages are required in order to elucidate it's potential.

The water extract of *Albizia anthelmintica* was found to be toxic to the mice with a mortality rate of 50% and with low anthelmintic efficacy of 29% and 16% against *Heligmosomoides polygyrus* and pinworms respectively. Since it has been reported that aqueous extracts of *Albizia anthelmintica* have been used in humans, livestock and in rats and that they are safe (Watt and Breyer-Brandwijk,1962, Koko *et al*,2000, Galal *et al*, 1991), it is important to further evaluate it's efficacy and safety for use in animals.

Some plants extracts also showed anthelmintic activity against pinworms indicating their activity is broad and hence their potential for use against a variety of helminths. However this calls for further studies.

According to Coles *et al* (1992) synthetic anthelmintics are considered therapeutically effective if the percentage egg per gram count reduction is 95% and above. Several studies done on medicinal plants to evaluate their anthelmintic efficacy have shown a wide variation in efficacy as compared to synthetic drug. This may depend on the different methods of extraction and how refined is the extract. In this experiment there was a variation from zero to 100%. There is therefore a need for those studying plant anthelmintics to standardize the minimum efficacy before a herbal medicament could be accepted as therapeutically effective.

Some plants extracts caused toxicity in animals evidenced by the mortality but seemed to have no effect on helminth as they were found alive upon postmortem. This phenomenon may be indication of problems due to extraction methods as well as dosages that were used. In an attempt to elucidate the possible explanation another experiment was carried out using water extracts which are cheap and claimed to be safe.

CHAPTER SEVEN

EFFICACY OF AQUEOUS EXTRACTS OF *ALBIZIA ANTHELMINTICA* AND *MAERUA EDULIS* AGAINST EXPERIMENTAL *HELIGMOSOMOIDES POLYGYRUS* INFECTIONS IN MICE

7.0 Introduction

Among the many plants used for medicinal purposes, some have been reported to have acute toxicity to the host depending on the dose level and the part of the plant used. African pastoralist have been reported to have a lot of knowledge and skills on plant toxicoses (McCorkle and Mathias-mundy, 1992). However Mugera (1970) reported that plants, e.g. *Maesa lanceolata* whose leaves are used as anthelmintic and a purgative could kill calves when given as a daily drench for three weeks. Kellerman *et al* (1988) and Shone and Drummond (1965) also reported that *Solanum incanum* used as anthelmintic and *Phytolacca dodecandra* used as molluscicide are poisonous to livestock in South Africa.

Pyrethrum which is considered safe and has been used for long as an anthelmintic was reported by Mbaria *et al* (1994) to be slightly toxic to sheep and rabbits in levels beyond 420 mg/kg. The clinical signs of acute pyrethrin toxicity after oral administration in sheep and rabbit in a 24 hours duration was hyper-excitation, tremors, convulsions, paralysis and death. The post- mortem done on all dead animals after 24 hours revealed extensive pulmonary congestion and oedema, ecchymotic haemorrhages in respiratory and cardiovascular systems. The authors suggested that the cause of death was due to respiratory failure.

Galal *et al* (1991) reported that at a dosage of 25-150g/kg bodyweight, the butanolic extract of *Albizia anthelmintica* bark administered to rats using a stomach tube was highly toxic and inactive against *Hymenolepsis diminuta.* In other studies, Thaiyah (1991) studied the toxicity of *Cassia didymobotrya* Fres in rats which is used as a strong purgative and antimalarial by the Maasai. He observed clinical signs which varied in acuteness depending on the concentration of *C. didymobotrya* Fres but eventually all succumbed and died. Muchiri (1987) studied the pharmacological and toxicological properties of *Paddiae volkensii* and *Scutia Myrtina* (BURM.F.) KURZ which are medically used as laxatives. He reported that rats administered up with the plants developed rough hair coat, diarrhoea and weight loss and on postmortem they showed pulmonary haemorrhage with severe alveolar thickening.

In the previous study (Chapter 6) the chloroform and methanol extracts of *Albizia anthelmintica* and *Maerua edulis* were toxic to mice. The water extracts of *Albizia* anthelmintica has been reported to be safe in humans and in rats (Galal *et al* ,1991; Koko *et al*, 2000; Watt and Breyer-Brandwijk,1962). *Maerua edulis* aqueous extract has been reported to be safe and having anthelmintic efficacy of 49% against gastrointestinal nematodes in sheep (Gakuya *et al*,2000). The objective of this experiment was therefore to evaluate the efficacy of water extracts of *Albizia anthelmintica* and *Maerua edulis* in mice following the previous experiment where they were found to be less toxic at low dose level than chloroform and methanol extracts. The aqueous extracts were tested at three dose levels to determine their efficacy and the safe dose and toxic levels.

7.1. Materials and methods

7.1.0. Experimental design

Fifty five (55) mice aged 10 weeks of SWR and CBA crosses of both sexes (sixth generation) obtained from International Livestock Research Institute(ILRI) Nairobi Kenya, were brought to the Department of Clinical studies, Kabete. They were housed in cages and placed in a well ventilated room at 20°C . They were allowed a week to acclimatise and fed on rabbit pellets from Unga Ltd and provided with water *ad libitum*.

On day one of the experiment, the mice were randomly allocated without restriction to seven groups, comprising of six treatment groups (1,2,3,4,5,6,) with eight mice each and one control with seven mice. They were then infected with 150 larvae of *Heligmosomoides polygyrus* obtained from helminthology section of ILRI and allowed 11 days for infection to establish. On the 11^{th} day, a pooled faecal sample for each group was taken and worm egg counts done to determine the level of infection (Appendix 7.1). A second faecal sample was taken on day 17^{th} which was considered optimal infection status.

7.1.1. Treatment regime.

On day eighteenth of the experiment the mice were weighed and the mean weight determined (Appendix 7.2.). The mean weight was used to calculate the number of grams of the extract to be given to the mice at three dose levels of 5gm/kg, 10gm/kg and 20gm/kg bodyweight which was based on the previous experiment. The calculated weight of the freeze dried water extracts of *Albizia anthelmintica* and *Maerua edulis* was then dissolved in distilled water allowing 0.4 ml per mouse to be administered as two

doses four hours apart. A voltex machine (Assistant, Reamix 2789) was used to facilitate the mixing.

Groups 1, 2 and 3 were given an oral dose of water extracts of *Albizia anthelmintica* at 5 gm/kg, 10 gm/kg and 20 gm/kg bodyweight respectively using a syringe and gauge 18 needle with a bulb at the tip. Groups 4, 5 and 6 were given water extract of *Maerua edulis* at dose rates of 5 gm/kg, 10 gm/kg and 20 gm/kg bodyweight respectively. Group 7 was the control and was given twice a dose of 0.2ml of distilled water four hours apart.

7.1.2. Parasitological techniques

7.1.2.1. Faecal worm egg count determination

Faecal *Heligmosomoides polygyrus* egg counts were done on 5th day post treatment. A pooled faecal sample for each treatment group was taken by transferring each group in a clean cage. Food was withdrawn during that period. They were allowed two hours before faeces were collected. One gram of the faecal pellets was mixed thoroughly with 30 ml of saturated salt solution and the container rinsed with another 30 ml. After homogenising, the mixture was seived and the filterate used to fill a McMaster slide. Egg counts were done for all the groups. Faecal egg counts for day 0 and 5 pre and post-treatment are shown in Appendix7.3.

7.1.2.2. Total adult worm counts

Seven days after treatment (day 24) the mice were euthanised by cervical dislocation. The gut cavities were opened and the entire loops of small intestine exposed. The loops were recovered by separating them from the mesenteries and severing them proximally

to the end of the stomach and distally to the entrance of the caecum. The loops were put into petri dishes covered with nets and opened longitudinally. All the contents and tissues were wrapped in a net and then suspended in a beaker containing about 25 ml of physiological saline. The beakers were incubated for 4 hours at 37°C to allow the worms to migrate from the lumen of the small intestines.

Gauzes were removed from the beaker after 4 hours and tissues discarded. The gauzes were placed on a petri dish and scanned under low power dissecting microscope to count worms. The temperature was then increased up to 42°C and thereafter increased by two degrees every five minutes up to 50°C. Part of the supernatant was poured out after allowing the beaker to settle and the contents poured on a petri dish. A dissecting microscope was used to count the worms.

The total number of worms counted was a sum of those counted in the gauze and counts of the petri dish (Appendix 7.4.). The anthelmintic efficacy was determined from the worm counts of the control and that of the treated groups according to formula used by Satrija *et al* (1995): i.e. anthelmintic efficacy:

$$\frac{\text{(Mean no. of worms in control group)- (mean no. of worms in treated group)}}{\text{Mean number of worms in control group}} \times 100$$

7.1.3. Statistical analysis

The SAS computer programme was used. The significant differences between different means of faecal worm count in the groups were determined using Student t-test,

Duncan's Multiple Range Test for variable and Tukey's Studentized Range test . A contrast test was also done between the control and other treatment groups.

7.2. Results

A pooled faecal *Heligmosomoides polygyrus* egg count on day 11 showed that all animals were infected with *Heligmosomoides polygyrus*. On day 0, the faecal egg counts ranged from 4,500 to 12,000. After treatment with *Albizia anthelmintica* water extract one mouse out of eight died in group 1(5gm/kg extract), four mice died out of eight in group 2 (10gm/kg extract)), three mice died out of 7 in group 3 (20gm/ kg extract) . In group 6, five mice out of eight died after administration with 20gm of *Maerua edulis* water extract . There were no mortalities in other groups.

The results of mean faecal *Heligmosomoides polygyrus* egg counts and percentage faecal egg reduction after treatment with *Albizia anthelmintica* and *Maerua edulis* water extracts at different doses are shown in Table 7.1.whereas the mean worm counts and anthelmintic efficacy after treatment with *Albizia anthelmintica* and *Maerua edulis* at different doses are shown in Table 7.2.

Table 7.1. Faecal *H. polygyrus* egg counts and percentage egg count reduction in mice before and after treatment with water extracts of *Albizia anthelmintica* and *Maerua edulis* at different dosages.

Treatment group	No. of Mice	FEC Day 0* (epg)	FEC Day 5 post-treatment (epg)	PR %
1	7	10,300	6,100	42
2	4	9,400	2,900	72
3	4	4,500	5,300	50
4	8	6,100	11,300	0
5	8	12,000	18,000	0
6	3	8,600	3,300	69
7	7	6,600	10,500	

Key

* Baseline egg count before treatment

Group

1. *Albizia anthelmintica* water extract at 5gm/kg bodyweight
2. *Albizia anthelmintica* water extract at10 gm/kg bodyweight
3. *Albizia anthelmintica* water extract at 20 gm/kg bodyweight
4. *Maerua edulis* water extract at 5gm/kg bodyweight
5. *Maerua edulis* water extract at 10gm/kg bodyweight
6. *Maerua edulis* water extract at 20gm/kg bodyweight
7. Control

epg means egg per gram
FEC means Feacal egg count

Table 7.2. Mean worm counts and percentage reduction of *H. polygyrus* after treatment of mice with *A.anthelmintica* and *M. edulis water* extracts at different dosages.

Treatment group	No. of Mice	Total worm count 7th day post treatment	Anthelmintic efficacy (%)
1	7	25	68
2	4	60	20
3	4	61	19
4	8	65	14
5	8	48	36
6	3	63	16
7	7	75	

Key

Group
1.*Albizia anthelmintica* water extract at 5gm/kg bodyweight
2. *Albizia anthelmintica* water extract at10 gm/kg bodyweight
3. *Albizia anthelmintica* water extract at 20 gm/kg bodyweight
4. *Maerua edulis* water extract at 5gm/kg bodyweight
5. *Maerua edulis* water extract at 10gm/kg bodyweight
6. *Maerua edulis* water extract at 20gm/kg bodyweight
7. Control

There was a percentage reduction in faecal *H. Polygyrus* egg counts of 72%, 69%, 50%, 42% when mice were treated with water extracts of *Albizia anthelmintica* at 10gm/kg body weight, *Maerua edulis* at 20gm/kg body weight, *Albizia anthelmintica* at 20gm/kg body weight and *Albizia anthelmintica* at 5gm/kg bodyweight respectively.

After determining the mean worm counts and anthelmintic efficacy, the water extract of *Albizia anthelmintica* at 5gm/kg bodyweight was the most efficacious with 68% worm count reduction. Using Duncan's Multiple Range test this same dosage had significantly ($p<0.05$) lower mean worm counts than all the rest of the treatment groups and the control. The mean worm counts in all other groups except group 5 were statistically similar at $p<0.05$.

7.3. Discussion

The results shows a variation in both egg and worm counts before and after treatment when the effect of different dosage levels are analysed. This variation may have been caused by the different strains of mice used. The CBA and SWR crosses have been reported by (Behnke *et al*, 2000) to show a wide variation in responses because the parental strains are very susceptible (CBA) and very resistant (SWR) to *Heligmosomoides polygyrus* infection. It may be possible that the extracts act like levamisole by paralysing the worm and allowing peristalsis to push the worms. Therefore if the extract affects gut mucosa , there will be a higher worm count.

From the above results the *Albizia anthelmintica* water extracts showed efficacy against the worm *Heligmosomoides polygyrus* however there was a mortality of mice in the three dose rates. From the previous experiment (Chapter 6), the water extract of

Albizia anthelmintica at 10gm/kg body weight had an anthelmintic efficacy of 29% and a mortality of 50% of the mice which is similar to the current experiment. Although in the current experiment the efficacy is not dose related, it shows that doses exceeding 5gm/kg bodyweight are toxic to the mice and not necessary more toxic to the worms.

In the case of *Maerua edulis*, the water extracts at 10gm/kg body weight had an anthelmintic efficacy of 36% and none of the mice died whereas at 20gm/kg bodyweight, five mice died. These results are also similar to those of previous experiment and it implies that doses of *Maerua edulis* water extracts exceeding 10gm/kg bodyweight are toxic to mice. From this experiment the water extracts of *Maerua edulis* at 20gm/kg bodyweight killed 5 mice out of 8. In the previous experiment (Chapter 6) chloroform extract of *Maerua edulis* at 5gm/kg killed 6 out of 6 mice and methanol extract of *Maerua edulis* at 10gm/kg killed 4 out of 6 mice. It implies that *Maerua edulis* is not an effective anthelmintic and is toxic to mice.

This current experiment shows that water extracts of *Albizia anthelmintica* at dosages of 5gm/kg are more efficacious with anthelmintic efficacy of 68% against *Heligmosomoides polygyrus* in mice. Similarly in Chapter 4, the powdered *Albizia anthelmintica* was more efficacious at 55 % against gastrointestinal nematodes in sheep. There is therefore a need to investigate the active compound in the water extract of *Albizia anthelmintica* and methods of increasing the efficacy.

CHAPTER EIGHT

BRINE SHRIMP LETHALITY GUIDED FRACTIONATION OF *ALBIZIA ANTHELMINTICA* WATER EXTRACT.

8.0.Introduction

In order to obtain fractions of plant extracts which are bioactive, there is need to first detect the active fractions and then isolate them from the rest of the fractions. The separation and purification of plant constituent is done using one or a combination of four chromatographic techniques namely; paper, thin layer, gas liquid and column chromatography. For instance, Maitai (1973) used thin layer chromatography (TLC) technique on the basic fractions obtained from solvent – solvent extraction of *Catha edulis* using five solvents systems to result into only one compound with the same Rf value as d-norpseudoephedrine. Using the gas liquid chromatography technique on the basic fraction from the *Catha edulis* material and other chemical compounds for comparison, Maitai (1973) determined the amount of d-norpseudoephedrine recovered from *Catha* material from the ratio of the peak height or area under curve of the standard to that of *Catha* material sample. In other studies Kiptoon (1981) investigated the chemical fractions of crude ethanol extract of *Gnidia latifolia* (Meisn) using thin layer chromatography.

The lethality of some fractions of plant extract to some zoologic organisms and parasites has also been used to detect and isolate bioactive compounds through bioactivity- guided screening and fractionation. For instance, Meyer *et al* (1982)

proposed the use of brine shrimp larvae for bioactivity -guided fractionation of bio-active plant extracts.

According to Watt and Breyer-Brandwijk (1962) several authors have tried to isolate the active principle in *Albizia anthelmintica* bark and even tried to find out the anthelmintic principle in it. The bark has been reported to contain saponins, phyloglucinol and musennin which has echinocystic acid in the structure and thought to have the anthelmintic principle. Lindsay (1978) reported kosotoxin-mussenin to be the active principle in *Albizia anthelmintica* bark.

In this experiment brine shrimp larvae was used to guide the fractionation of *Albizia anthelmintica* bark water extract by running it on a column. The active fraction thus obtained was run on the thin layer chromatographic plates and Rf values determined.

8.1. Materials and methods.

8.1.1. Column chromatography

8.1.1.1.Cold extraction of active ingredient from powdered *Albizia anthelmintica*

A hundred (100) grams of powdered *Albizia anthelmintica* bark was placed in a conical flask containing 500 ml of distilled water, agitated to dissolve and left to stand for one hour. The mixture was left standing for one hour. The mixture was then filtered through cotton wool and then through filter papers. The volume was reduced by boiling until a white precipitate appeared followed by filtration through filter papers. The volume was reduced further until the sample was dry. This was the sample run on the column.

8.1.1.2. Preparation of the column and sample introduction

The UltraRac 7000 fraction collector of LKB Sweden was used to run gel filtration. The Sephadex gel G-200 was suspended in distilled water to swell for 2 hours. Afterwards, the gel was poured into the column and distilled water was allowed to pass through. The column was packed within two hours. Distilled water at the top of the column was allowed to pass through the gel until all disappeared .

To run the column, 100mg of the sample obtained as above was dissolved in 2ml of distilled water. Using a pipette about 0.5ml of the sample was carefully introduced at the top of the column. As it passed through more distilled water was added to prevent the gel from drying. Another volume of distilled water was added when the sample was inside the column to put some pressure for the sample to pass through. The fractions started emerging form the column after two hours.

8.1.1.3. Sample collection

Ten test tubes were placed in the each of the ten racks of UltraRac 7000 fraction collector. Approximately 10 live brine shrimps larvae were put in each tube by taking about 0.5 ml of marine salt mixture containing the brine shrimp larvae. The collector was programmed to collect 30 drops per tube of the fractions as they drop from the column. The tubes were left for 24 hours at room temperature to determine in which tubes the fractions showed lethality to brine shrimp larvae.

8.1.2. Thin layer chromatography (TLC)

8.1.2.1. Acquisation of the plant extract

Cold extraction of powdered *Albizia anthelmintica* was done by mixing 100 gm of the powdered bark of *Albizia anthelmintica* in 0.5 litres of water in a conical flask and left standing for one hour. The extract was filtered through cotton wool and then through Whatman no.1. filter paper. The volume was reduced by boiling until a white precipitate formed. It was then filtered using a Whatman no.1 filter paper and then the volume was then reduced until the sample was dry. The resultant sample was dissolved in 0.5 litres of methanol forming a white precipitate. This was filtered through whatman no.1 filter paper and the volume of methanol reduced up to 5 ml.

8.1.2.2. Preparation of TLC plates

Glass plates of 20X 20cm were thoroughly cleaned and dried. Silica gel for thin layer chromatography was weighed , allowing 15 gm per plate and put into a beaker. Distilled water was then added at the rate of 20 ml per 15 gm of silica gel. The mixture was shaken thoroughly until all the lumps dissolved. The slurry was poured into the glass plates placed on Desaga spreading apparatus and spread to give a thickness of 25 mm. The plates were then left overnight in an oven at 100°C.

8.1.2.3.Running a two dimensional thin layer chromatography

The sample was spotted with a capillary tube (75mm long, 1mm bore) at one corner of the plate, 2 cm from the edges of each side. The chromatography tank of Desaga chromatography systems . was used to develop the chromatogram. The development solvent was made of methanol and chloroform at a rate of 90:10. In the development tank, 200 ml of the development solvent was put and thoroughly shaken. The plate was

placed in the tank and then covered with it's lid. The chromatogram was allowed one hour to develop. The plate was removed from the tank and viewed under long U.V. light of 365nm using Chromato-vue machine of Ultraviolet products, INC California, USA. The distances the solvent and the spots had travelled were measured and Rf values determined.

The plate was placed into a second tank containing methanol:distilled water (90:10) as developing solvent. The spots developed as in the first chromatograph were placed at the bottom of the tank. They were allowed one hour to develop and the plate removed and examined under U.V. light as in the first run. Measurement of the solvent front and the spots from the extract were taken and relative fraction (Rf) values determined (Rf is the ratio between the distant the spot has travelled to that of the solvent front). Several two-dimensional TLC were run and their Rf values determined (Table 8.1.)

8.2. Results and discussion

After running the column, the extract passed through the column as one fraction and was lethal to the brine shrimp. The mortality was 100% after 24 hours. On running the two dimensional thin layer chromatography, there was only one blue spot viewed under ultraviolet light confirming that the sample had only one fraction. The relative fraction (Rf) values of all the runs are shown in Table 8.1. The mean Rf value was calculated as 0.75.

By use of the column chromatography and TLC in these studies, the *Albizia anthelmintica* bark water extract was found to have one fraction. The fraction which had a mean Rf value of 0.75 was 100% lethal to brine shrimp. These results are in

agreement with those of chapter five where the water extract of *Albizia anthelmintica* bark was shown to be toxic to brine shrimp with LC_{50} of 101ug/ml. In this fraction lies the bioactive compound of the plant material. The results therefore indicate that there is a rationale in the use of this plant for medicinal value as is practiced by pastoralists. It is suggested that the active ingredient could be identified for commercial use. This would warrant studies on several TLC systems, testing the response of the water extract to colour test, testing it`s solubility and UV spectral characteristics. This would then be followed by determining other properties such as boiling point , optical rotation, infra-red , nuclear magnetic response and mass spectral measurements of the plant extract.

Table 8.1. Rf values of *Albizia anthelmintica* water extract run on a two dimensional TLC and viewed under long U.V. light.

	Distance moved(mm)		
No. of runs	solvent front	*A. anthelmintica* spot	Rf values
1.	85	67	0.79
2.	78	56	0.72
3.	75	53	0.71
4.	75	55	0.73
5.	60	45	0.75
6.	60	48	0.80

Mean Rf 0.75.

CHAPTER NINE

GENERAL CONCLUSIONS

The use of Partipatory Rural Appraisal(PRA) tools is very useful when studying medicinal plants. The survey conducted in Kibwezi division revealed 51 useful plants and the herbalists and farmers were able to identify *Albizia anthelmintica* and *Maerua edulis* as potent anthelmintics. There is a wide knowledge on the use of medicinal plants among farmers and herbalist and more studies are indicated in other diseases apart from helminthoses.

The evaluation of the anthelmintic efficacy of *Albizia anthelmintica* and *Maerua* species against gastrointestinal nematodes in sheep showed that the crude extracts have some anthelmintic efficacy. The water extract of *Albizia anthelmintica* was more potent than that of *Maerua edulis* and *Maerua subcordata.* Although the percentage faecal egg count reduction was only 55%, more studies are indicated to purify the extract and test further it's efficacy.

The determination of the bioactivity of water, methanol and chloroform extracts of *Albizia anthelmintica*, *Maerua edulis* and *Maerua subcordata*, using brine shrimp lethality test showed that the three plants had appreciable bioactivity feautures. This could be the basis of their use as anthelmintics. The *Albizia anthelmintica* extracts had LC_{50} less than 1000ug/ml and were more toxic to the brine shrimp than *Maerua* species. However this calls for more bioassays for *Albizia anthelmintica* extracts.

The evaluation of the anthelmintic efficacy of chloroform , methanol and water extracts of *Albizia anthelmintica* and *Maerua edulis* against *Heligmosomoides polygyrus* in mice showed that chloroform and methanol extracts of these plants were very toxic to the mice than water extracts. The plant extracts had more anthelmintic efficacy to pinworms than to *Heligmosomoides polygyrus*. More studies are therefore indicated to determine the efficacy of the plants extracts against pinworms and other helminths.

The evaluation of the efficacy of the aqueous extracts of *Albizia anthelmintica* and *Maerua edulis* against nematode *Heligmosomoides polygyrus* infections in mice showed that *Albizia anthelmintica* extracts were more potent than *Maerua edulis*.

The column and thin layer chromatography of *Albizia anthelmintica* water extracts showed one fraction which was 100% lethal to the brine shrimp with an Rf value of 0.75. There is thus a need to identify the active ingredient if the extract is be used commercially.

Further studies are generally required to obtain the scope of perception, attitude and practice of herbalist and users and to evaluate the therapeutic efficacies of the claimed herbal medicinal preparations.

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APPENDIX 2.1

CLASSIFICATION OF ANTHELMINTICS USED AGAINST NEMATODES, CESTODES AND TREMATODES

A. Benzimidazole/ Probenzimidazoles:
Thiabendazole,Parbendazole,Cambendazole,Mebendazole, Fenbendazole,Oxibendazole, Oxyfendazole, Albendazole, Triclabendazole

B. Imidazothiazoles
Levamisole

C. Tetrahydropyramidines
Pyrantel, Morantel

D. Macrocyclic lactones
Ivermectin, Moxidectin, Nemadectin, Milbemycin, Doramectin

E. Salicylanilides and substituted phenols
Oxyclozanide, Rafoxanide, Nitroxynil, Bithionol and Brotianide

F. Diphenoxyalkyl ethers
Diamphenethide

G. Organophospahtes
Dichlorvos, Haloxon, Metriphonate

H. Piperazine
Diethylcarbamazine

I. Others
Nitroscanate, Praziquantel, Bunamidine, Arecholine.

After Hammond and Sewell (1995)

APPENDIX 2.2

Efficacy of the anthelmintics against the most important adult gastrointestinal nematodes in cattle

Classi-Fication		Anthelmintics	Dosage in mg/kg Body weight (oral)	Abomasum		
				Ostertagia	Haemonchus	Trichostro-ngylus
I		Nitroxynil	10	-	+++	-
ii.	1.	Tiabendazole	100	+++	+++	+++
	2.	Parbendazole	30	+++	+++	+++
	3.	Fenbendazole	7.5	+++	+++	+++
	4.	Oxibendazole	10	+++	+++	+++
	5.	Oxfendazole	2.5	+++	+++	+++
	6.	Albendazole	7.5	+++	+++	+++
iii		Rafoxanide	7.5	-	+++	-
iv	I	Pyrantel	25	+++	+++	-
	2	Morantel	10	++	+++	+++
v		Levamisole	or. 7.5,s.c., i.m. 5	+++	+++	+++
vi	1	Thiophanate	50	+++	+++	+++
	2	Febantel	7.5	+++	+++	+++
vii		Ivermectin	0.2	+++	+++	+++

Classification of efficacy : +++, 95%- 100%; ++, 80%-100%;+,0%-100%;-,not effective or insufficient data.
Adapted from Boersama (1985)

Small intestine					Large intestines
Trichostrongylus	Cooperia	Nematodirus	Bunostomum	Strongyloides	Oesophagostomum
-	-	-	+++	-	+++
+++	+++	+++	+++	+++	+++
+++	+++	+++	-	+++	+++
+++	+++	+++	+++	++	+++
+++	+++	++	+++	+++	+++
+++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++
-	-	-	+++	-	+++
-	+++	+++	-	-	-
+++	+++	+++	+++	-	+++
+++	+++	+++	+++	-	+++
+++	+++	++	-	-	-
-	+++	-	-	-	-
+++	+++	++	-	-	-

APPENDIX 2.3

Efficacy of the anthelmintics against the most important adult gastrointestinal nematodes in sheep and goats

Classi-Fication		Anthelmintics	Dosage in mg/kg Body weight (oral)	Abomasum			Small Intestine						Large intestines	
				Haemonchus	Ostertagia	Trichostro-gylus	Trichostro-gylus	Cooperia	Nematodirus	Bunosto-mum	Gaigeria	Strongy-loides	Oesopha-gostomum	Charbetia
I	1.	Disophenol	10	+++	-	-	-	-	-	-	-	-	-	-
	2.	Nitroxynil	10	+++	-	-	-	-	-	-	-	-	+++	-
ii	1.	Tiabendazole	75	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2.	Perbendazole	30	+++	+++	+++	+++	+++	++	+++	_	++	+++	+++
	3.	mebendazole	15	+++	+++	+++	+++	+++	++	+++	+++	+	++	+++
	4..	Fenbendazole	5	+++	+++	+++	+++	+++	+++	++	+++	++	+++	+++
	5..	Oxybendazole	10	+++	+++	+++	+++	+++	+++	+++	_	+++	+++	+++
	6.	Oxfendazole	5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	7.	Albendazole	5	+++	+++	+++	+++	+++	+++	-	+++	-	+++	+++
iii	1	Rafoxanide	7.5	+++	-	-	-	-	-	-	-	-	-	-
	2	Closantel	5	+++	-	-	-	-	-	-	-	-	-	-
iv	1	Pyrantel	25	+++	+++	+++	++	-	+++	+++	+++	-	-	+++
	2	Morantel	10	+++	+++	-	+++	-	+++	-	+++	-	+++	+++
v		Levamisole	or 7.5 i.m. and sc. 5	+++	+++	+++	+++	+++	+++	+++	-	++	+++	+++
vi	1	Thiophanate	50	+++	+++	+++	+++	+++	++	-	-	-	-	+++
	2	Febantel	5	+++	+++	+++	+++	+++	++	+++	-	++	+++	-
vii		Ivermectin	0.2	+++	+++	+++	+++	+++	-	-	-	-	+++	-

Classification of efficacy : +++, 95%- 100%; ++, 80%-100%;+,0%-100%;-,not effective or insufficient data.
Adapted from Boersama (1985)

APPENDIX 2.4

PLANT ANTHELMINTICS USED IN DIFFERENT PARTS OF AFRICA

Acacia brevisca
Albizia anthelmintica
Ani toro
Annona senegalensis
Aspilia ciliata
Bali kaylay
Bersama abyssinica subsp. abyssinica
Buren
Carissa edulis
Cassia diymobotrya
Cassia occidentalis
Cassine aethiopica
Cissampelos mucromata
Cissus petiolana
Clausena anisata
Clerodendrum myricoides
Clerodendrum schweinfurthii var.lonitubum
Crassocephalum vitellinum
Croton macrostachys
Cynodon nlemfuensis
Dem bani fida
Dicoma anomala
Diosprus mespiliformis
Disan
Dryopteris athamanticum
Dryopteris pentheri
Embelia schimperi
Embelia kilimandscarica
Eygerum canadiensis
Fern
Ficus wakefieldii
Gombocarpus physocarpus
Harungana madagascarensis
Iboza riparia
Jasminum dichotomum
Khaya senegalensis
Lenkeh
Leonotis nepetifolia
Lime
Munsunetu
Musa sapientum
Myrica alicifolia
Nicotiana tobaccum
Nonokendeh
Opilia celtidifolia
Othonna natalensis
Opilia celtidifolia
Parinari benna
Pelargonium sidaefolium
Pelargonium sidoides
Pennisetum purpureum
Pennisetum trachyphyllum
Periploca linearifolia
Phytolacca dodecandra
Plectranthus barbatus
Porpor
Pramnia maxima
Prosopsis africana
Pseudospondiasis microcarpa
Pteleopsis myrtifolia
Rhamnus princides
Rhoicissus capensis
Rumex usambarensis
Senecio lyratripartus
Simba bali
Solanum anguivi
Solanum incanum
Solanum torvum
Sunsun
Synadenium grantii
Tephrosa nana
Terminalia mollis
Tragia brevipes
Walisa

After Bizimana 1994

APPENDIX 2.5

PLANT ANTHELMINTICS USED FOR THE CONTROL OF INTESTINAL WORMS IN HUMANS

Hookworms

Anisopappus africanus
Antidesma venosum
Aspilia mossambicensis
Calotropis procera
Combretum padoides
Cyathula cylindica
Dissotis rotundifolia
Dombeya praetermissa
Euclea natalensis
Hibiscus cannabinus
Lonchocarpus capassa
Maytenus putterlickiodes
Myrica kilimandscharia
Phyllanthus reticulatus
Plumbago zeylanica
Rhus natalensis
Solanum daspyllum
Vangueria rotundata
Ximenia caffra

Roundworms

Achyrospermum radicans
Bersama abyssinica
Myrsine africana
Phyllanthus nummulariifollius
Rauvolfia caffra
Tacca leontopetaloides

Tapeworms

Albizia anthelmintica
Amaranthus caudatas
Bridelia micrantha
Clerodendrum rotundifolium
Croton macrostachyus
Dryopteris inaequalis
Embelia schimperi
Enicostema hyssopifolia
Erythrophloleum guineens
Fuerstia africana
Hagenia abyssinica
Lippia javanica
Myrsine africana
Olea africana
Olinia usambarensis
Phytolacca dodecandra
Pentas longiflora
Punica granarum
Rapanea rhododendroides
Rauvolfia caffra
Vernonia karaguensis

Threadworms

Gynandropsis gynandra

General anthelmintics

Acacia brevisca
Adenia cissampeloides
Aframomum sanguineum
Albizia anthelmintica
Albizia versicolor
Alstonia boonei
Ammannia prieureana
Ampelocissus africana
Aphloia theiformis
Artemisia afra
Asparagus falcatus
Aspilia mossambicensis
Asystasia gangetica
Blanites aegyptica
Begonia oxyloba
Bersama abyssinica
Bidens pilosa
Canthium crassum
Canthium rubrocostamum
Canthium venosum
Cassia petersiana
Cissus producta
Clerodendrum ugandense
Combretum molle
Combretum paniculatum
Combretum xanthothyrsum
Crassocephalum mannii
Crotalaria sp.
Croton megalocarpus
Cyathea stuhlmannii
Cylicomorpha parviflora
Dalbergia melanoxylon
Deinbollia kilimandschariea
Embelia schimperi
Erythrina abyssinica
Erythrococca fisheri
Erythrophleum suaveolens
Euclea natalensis
Evolvulus alsinoides
Gomphocarpus semilunatus
Grewia holstii
Hagenia abyssinica
Helichrysum odoratissimum
Hugonia castaneifolia
Jasminum floribundum
Jateorhizza palmata
Juniperus procera
Justicia exigua
Kalanchoe spp.
Khaya senegalensis
Leonotis mollissima
Maesa lanceolata
Maytenus heterophylla
Momordica foetida
Mysine africana
Newtonia hildebrandtii
Ozoroa mucronata
Pavetta crassipes
Piper capense
Plectranthus elegans
Rapanea rhododendroides
Rumex bequaertii
Scorodophloeus fischeri
Scutia myrtina
Strophanthus eminii
Syzygium guineense
Tacca leontopetaloides
Teclea nobilis
Trichilia subcordata
Vangueria acutiloba
Vangueria apiculata
Vangueria tomentosa
Vernonia amygdalina
Ximenia americana
Zimmermannia capillipes

Kokwaro, 1993

APPENDIX 2.6.

COLLECTION OF MEDICINAL PLANTS

General guidelines

1.To make prior arrangements with local authorities to guide in the collection, by providing accommodation and transportation.

2.To liase with local herbaria which will aid in the botanical survey for ease of location of particular species.

3.To keep in mind the geographical location and prevailing climatic conditions at the time of visit. However, collection trips are not advisable during rainy months because unmanageable factors namely: drying of sample is not satisfactory, there is every chance of rotting of the sample during drying, transportation and inaccessibility of most areas.

4. An ideal plant collecting team should consist of a systematic botanist

Specific guidelines

1.Ensure exhaustive documentation of field data on the plants being collected.

2. Chopping of fresh plant should be undertaken to speed drying and to effect reduction in bulk so that they can occupy less than 60% of the space required for unprocessed samples. While chopping, care should be taken to protect the eyes from injurious liquids from plants viz: latex etc. by wearing safety goggles.

3. Drying: Under ideal conditions when there is no threat of rains and the wind is dry, the samples can be spread under shade, properly covered with thick polythene sheet to avoid absorption of moisture by the samples. Once the material is collected and chopped it must be exposed to air until fully dry.

4. Garbling: This is the removal of extraneous matter such as other parts of the same plant or any other foreign matter. In rhizomes, tubers and roots, aerial portions or stem

bases must be removed. All the materials must be dried completely before packing for transportation.

5. Packing and transportation: The packing should provide protection as well as economy of space. The material should preferably be packed in water proof round canvas kit bags. The use of Jute kit bags with half dried samples is advantageous in that they get some air to circulate, thus avoiding any rotting.

6. Storage: Proper preservation is necessary for maintaining high quality plant sample. Protect samples from rodents. Storage room should be cool and dark with no possibility of any moisture absorbed by the material. Moisture will cause not only increase in the weight of the material but reduce the percentage of the active principle and also favour enzymic activity and facilitate microbial destruction of the samples. Destruction of samples by insects during storage can be prevented by use of a few drops of tetrachloride or chloroform.

7. Voucher samples: Before passing the samples for grinding, the crude chopped sample about (100-200g) is kept in glass jars of uniform size for future references. This is called voucher sample.

8. Grinding: This should be done by experienced personnel. After grinding, the material is passed to a chemist for extraction.

9. Recollection of biological active plants: Plant samples which show some biological activity are required to be recollected in larger quantities about 10-20kg dry weight for obtaining pure compounds.

Source: Mehrotra (1994)

APPENDIX 2.7.

APPARATUS FOR HELMINTHOLOGICAL FAECAL EGG COUNT

1.A compound microscope with x 100 eyepiece(s) and low power objectives, giving x 40-50 and x 100 total magnifications. A mechanical stage is useful, but not essential.

2.A small centrifuge.

3.A balance and weights to weigh 3g plus or minus 0.1g or a teaspoon with which to estimate this amount of faeces.

4.A stainless steel fork.

5. A coffee strainer- preferably nylon, with a large meshed area.

6.Small bowls to accept the strainer- preferably lipped.

7.Small plastic bottles, marked at 45 ml.

8.Conical 15ml centrifuge tubes.

9.Spatula or twiddle stick.

10.Scissors.

11.Grease pencil or 'magic marker'

12.Pasteur pipettes and teats.

13.McMaster slides.

14.Saturated Salt solution- S.G. 1.18.

15.Hydrometer.

Source: Urquhart and Sewell (1995)

APPENDIX 2.8

Preparation of a standard solution (Centrifuge available)

1.Break up specimen with a fork.

2.Estimate 3g of faeces and place in a coffee- strainer in a bowl.

3.Pour about 30 ml of water into the bowl and macerate the faeces in the seive in this water.

4.Wash the suspension into a bottle marked 45 ml and add water to the mark.

5.Thoroughly mix the suspension and fill one or two round -bottom plastic centrifuge tubes.

6.Centrifuge briefly and discard the supernatant(s). DO NOT over centrifuge.

7.Refill the centrifuge tube(s) with saturated salt solution(but use water for Stoll count). Suspend the sediment with a twiddle stick and pour the fluid (s) into another bottle.

Preparation of a standard solution (Centrifuge not available)

1.Break up the specimen with a fork

2.Estimate 3g of faeces and place in a coffee- strainer in a bowl.

3.Pour about 30 ml of water into a bowl and macerate the faeces in the seive in this solution.

4.Wash the suspension into a bottle marked at 45 ml and make up to the mark with saturated salt solution.

5.Use this suspension directly to perform a McMaster count.

Source: Urquhart and Sewell (1995)

APPENDIX 2.9.

Modified McMaster counting technique

1.While keeping the standard suspension well stirred, but without excess shaking, withdraw fluid with a Pasteur pipette and fill one chamber of a McMaster slide.

2.Allow the slide to stand for 3 minutes and then count all the eggs under the ruled square. Do not count any eggs outside the square.

3. The total number of eggs counted x 100 indicated the number of eggs per gram of faeces.

Modified Stoll counting technique

1.Estimate the volume of the drops from a vertically held Pasteur pipette. This can be done by weighing or by using a graduated conical centrifuge tube. Once this has been done, the pipette can be used for many counts (usually about 0.03 ml per drop).

2. Prepare a standard suspension in water instead of a flotation fluid. Include a centrifugal wash if possible.

3.Thoroughly mix the washed suspension, quickly fill the pipette and place 2 to 5 drops on a microscope slide in pools of 2-3-drops each.

4. Cover each pool with a cover slip, and examine under the low power of the microscope, counting all eggs , larvae or oocysts seen.

Source: Urquhart and Sewell (1995)

APPENDIX 4.1.

WEIGHT OF THE SHEEP BEFORE EXPERIMENT

Sheep no.	Sex	Weight (kg)
3101	F	14.5
3103	M	25.0
3104	F	14.5
3105	M	23.0
3106	M	31.5
3107	M	32.0
3108	M	20.0
3109	F	27.5
3110	F	30.0
3111	M	20.5
3112	F	17.0
3113	M	23.0
3114	M	16.0
3115	M	21.5
3116	F	24.0
3358	M	21.0
4881	M	37.5
4883	F	27.0
4884	M	30.0
4885	M	27.0
4886	F	22.0

APPENDIX 4.2.

FIRST FAECAL WORM EGG COUNT

Sheep no.	EPG counts
3101	100
3103	1300
3104	900
3105	1100
3106	3800
3107	200
3108	300
3109	200
3110	0
3111	1000
3112	300
3113	1100
3114	4000
3115	3300
3116	500
3358	1300
4881	800
4883	100
4884	0
4885	100
4886	2400

Key:
EPG means egg per gram

APPENDIX 4.3

SECOND FAECAL WORM EGG COUNT
(day 0 of experiment)

Sheep no.	EPG counts
3101	500
3103	2100
3104	800
3105	2300
3106	6000
3107	300
3108	600
3109	2200
3110	1700
3111	1100
3112	400
3113	900
3114	4000
3115	4400
3116	700
3358	500
4881	1200
4883	400
4884	300
4885	400
4886	700

Key
EPG means egg per gram

APPENDIX 4.4.

WEIGHT OF THE SHEEP ON DAY 0 OF EXPERIMENT

Sheep no.	Sex	Weight (kg)
3101	F	15.0
3103	M	24.5
3104	F	16.5
3105	M	23.0
3106	M	21.0
3107	M	31.0
3108	M	20.0
3109	F	29.0
3110	F	29.0
3111	M	21.5
3112	F	17.0
3113	M	22.5
3114	M	16.5
3115	M	21.5
3116	F	23.0
3358	M	21.5
4881	M	38.0
4883	F	27.0
4884	M	29.5
4885	M	27.0
4886	F	22.0

APPENDIX 4.5.

FAECAL WORM EGG COUNTS ON DAY 0,7 AND 14

Group	Sheep no.	Epg counts Day 0	Day 7	Day14
	3112	400	900	2700
1	3110	1700	5000	3400
	4881	1200	1200	3000
	3107	300	300	1000
2	3108	600	300	1000
	3103	2100	1300	1900
	3358	500	1400	1200
	3105	2300	800	1400
3	4884	300	400	1000
	3113	900	1400	2000
	3109	2200	1000	2300
4	3111	1100	1300	1000
	3104	800	200	400
	4883	400	400	0
	3116	700	1000	400
5	4885	400	500	400
	3101	500	1600	300

Key:
EPG means egg per gram

APPENDIX 4.6.

FAECAL WORM EGG COUNTS ON DAY 0 AND 16

Group	Sheep no.	Epg counts Day 0	Day 16
	3112	2700	200
1	3110	3400	1600
	4881	3000	1300
	3107	1000	100
2	3108	300	900
	3103	1900	400
	3358	1200	1100
	3105	1400	500
3	4884	1000	500
	3113	2000	1300
	3109	2300	1300
4	3111	1000	500
	3104	400	300
	4883	0	100
	4886	3000	1600
5	3114	4200	200
	3116	400	1000
6	4885	400	1400
	3101	300	1300

Key:
EPG means egg per gram

APPENDIX 6.1
WEIGHT OF THE MICE

GROUPS	MOUSE NO.	WEIGHT(gm)	WEIGHT OF HEAVIEST MICE
A	1	29.22	
	2	29.34	
	3	30.66	30.66
	4	29.05	
	5	28.52	
	6	27.65	
B	1	27.71	
	2	27.97	
	3	29.24	29.27
	4	26.61	
	5	31.31	
	6	29.27	
C	1	21.54	
	2	28.07	
	3	28.78	30.36
	4	30.36	
	5	20.24	
D	1	32.32	
	3	28.40	
	4	26.46	33.54
	5	32.55	
	6	29.20	
E	1	24.78	
	2	29.65	
	3	33.95	33.95
	4	30.36	
	5	33.13	
	6	23.71	
F	1	23.72	
	2	33.62	
	3	32.10	35.7
	4	26.28	
	5	33.35	
	6	35.70	

Appendix 6.1. continued

G	1	28.54	
	2	26.17	
	3	27.95	30.91
	4	30.32	
	5	30.91	
	6	29.03	
H	1	30.50	
	2	35.57	
	3	28.94	35.57
	4	29.96	
	5	27.59	
	6	30.90	
I	1	34.46	
	2	29.97	
	3	35.30	36.67
	4	28.38	
	5	36.67	
	6	32.27	

APPENDIX 6.2
FAECAL EGG COUNT AT DAY11

GROUP	MOUSE NO.	*H.POLYGYRUS* EGG COUNT	PINWORMS EGG COUNT
A	1	1,600	0
	2	1,400	0
	3	4,900	0
	4	1,100	0
	5	3,000	0
	6	3,900	100
B	1	5,300	0
	2	4,500	0
	3	4,100	100
	4	3,000	100
	5	3,800	1,200
	6	1,300	100
C	1	1,600	0
	2	1,700	600
	3	700	200
	4	1,300	1,500
	5	3,600	100
D	1	4,200	200
	2	1,600	800
	3	2,600	100
	4	700	300
	5	4,200	0
	6	900	0
E	1	3,200	200
	2	7,900	0
	3	2,500	200
	4	1,200	100
	5	3,900	600
	6	2,000	200
F	1	900	100
	2	2,400	200
	3	5,000	100
	4	0	0
	5	700	0
	6	1,400	300

Appendix 6.2 continued

G	1	400	0
	2	2,300	200
	3	1,400	0
	4	500	500
	5	200	0
	6	1,900	300
H	1	2,900	100
	2	1,200	1,800
	3	2,600	100
	4	1,900	600
	5	1,600	0
	6	1,000	0
I	1	1,300	0
	2	400	100
	3	1,600	400
	4	1,800	0
	5	1,800	100
	6	3,900	0

APPENDIX 6.3

HELIGMOSOMOIDES POLYGYRUS EGG COUNTS ON DAY 0,4and 6

GROUP	MOUSE NO		EPG COUNT	
A		DAY 0	DAY 4	DAY 6
	1	10,700	6,900	9,400
	2	6,700	3,200	5,800
	3	16,500	5,200	10,600
	4	14,200	8,300	15,800
	5	12,400	5,200	4,800
	6	9,700	7,800	7,800
B	1	13,200	5,020	6,400
	2	13,200	1,900	2,800
	3	8,300	8,100	5,300
	4	8,200	5,100	4,500
	5	7,900	4,600	9,500
	6	6,900	5,400	4,400
C	1	3,800	3,400	6,100
	2	4,700	9,600	7,400
	3	3,300	7,800	4,300
	4	10,100	died	
	5	4,500	3,100	5,800
D	1	10,600	died	
	2	8100	2100	6400
	3	5800	died	
	4	1100	died	
	5	8800	9700	7900
	6	5400	died	
E	1	12200	died	
	2	15500	died	
	3	9500	died	
	4	1400	died	
	5	4900	died	
	6	9600	died	
F	1	1300	died	
	2	900	4900	2800
	3	5500	10800	7500
	4	100	400	0
	5	1400	9200	4800
	6	13000	died	

Appendix 6.3. continued

G	1	5700	2600	1700
	2	6700	died	
	3	4700	7900	6700
	4	3200	died	
	5	8500	died	
	6	5775	died	
H	1	2200	died	
	2	1800	died	
	3	2500	died	
	4	1800	died	
	5	2700	died	
	6	2200	died	
I	1	4700	7800	3800
	2	1300	1500	1200
	3	4300	4000	1900
	4	5000	5800	5600
	5	2400	8600	4100
	6	4900	10500	10400

Key
EPG means egg per gram

APPENDIX 6.4

PINWORM EGG COUNTS ON DAY 0, 4 and 6 POST TREATMENT

GROUP	MOUSE NO	EGG COUNT		
		DAY 0	DAY 4	DAY 6
A	1	0	0	100
	2	0	0	0
	3	0	0	0
	4	0	0	300
	5	0	0	0
	6	0	0	0
	1	100	0	0
	2	0	0	100
B	3	0	0	0
	4	100	0	0
	5	0	100	0
	6	0	100	100
	1	0	0	100
	2	700	700	100
C	3	0	600	0
	4	0	d	0
	5	500	200	d
	1	100	d	d
	2	1100	0	0
D	3	600	d	d
	4	100	d	d
	5	0	0	0
	6	0	d	d
	1	0	d	d
	2	0	d	d
E	3	100	d	d
	4	0	d	d
	5	0	d	d
	6	0	d	d
	1	0	d	d
	2	600	700	0
F	3	1000	800	100
	4	100	200	100
	5	0	0	400
	6	0	d	d

Appendix 6.4. continued

	1	0	0	0
	2	200	d	d
G	3	0	d	0
	4	500	d	d
	5	0	d	d
	6	0	d	d
	1	0	d	d
	2	600	d	d
H	3	200	d	d
	4	100	d	d
	5	400	d	d
	6	0	d	d
	1	0	800	100
	2	100	400	100
I	3	0	200	0
	4	0	400	500
	5	200	0	200
	6	300	0	0

Key

d means dead

APPENDIX 6.5.

HELIGMOSOMOIDES POLYGYRUS COUNT ON DAY 7 POST TREATMENT

GROUP	MOUSE NO	WORM COUNT
A		DAY /
	1	48
	2	31
	3	65
	4	65
	5	73
	6	75
B	1	89
	2	67
	3	84
	4	55
	5	73
	6	5
C	1	55
	2	66
	3	8
	4	d
	5	20
D	1	d
	2	41
	3	d
	4	d
	5	29
	6	d
E	1	d
	2	d
	3	d
	4	d
	5	d
	6	d
F	1	d
	2	44
	3	11
	4	2
	5	21
	6	d

Appendix 6.5. continued

G	1	74
	2	d
	3	64
	4	d
	5	d
	6	d
H	1	d
	2	d
	3	d
	4	d
	5	d
	6	d
I	1	77
	2	30
	3	66
	4	15
	5	40
	6	68

Key
d means dead

APPENDIX 6.6.

PINWORM COUNTS DAY 7 POST TRE ATMENT

GROUP	MOUSE NO.	WORM COUNT
		DAY 7
A	1	1
	2	2
	3	22
	4	4
	5	1
	6	35
B	1	14
	2	16
	3	40
	4	4
	5	100
	6	34
C	1	78
	2	227
	3	152
	4	238
	5	d
D	1	d
	2	316
	3	d
	4	d
	5	74
	6	d
E	1	d
	2	d
	3	d
	4	d
	5	d
	6	d
F	1	d
	2	386
	3	273
	4	296
	5	119
	6	d

Appendix6.6 continued

G	1	1
	2	d
	3	3
	4	d
	5	d
	6	d
H	1	d
	2	d
	3	d
	4	d
	5	d
	6	d
I	1	141
	2	285
	3	300
	4	78
	5	382
	6	198

Key
d means dead

APPENDIX 7.1.

FAECAL EGG COUNT AT DAY 11

GROUP		*H.POLYGYRUS* EGG COUNT
A		12,200
B		9,400
C		10,900
D		14,400
E		11,400
F		6,500
G		10,600

APPENDIX 7.2.

WEIGHTOF THE MICE

GROUPS	MOUSE NO.	WEIGHT(gm)	AVERAGE
A	1	19.22	
	2	20.00	
	3	18.86	
	4	16.89	
	5	18.71	18.90
	6	16.40	
	7	18.20	
	8	23.20	
B	1	13.42	
	2	15.83	
	3	16.50	
	4	20.10	
	5	17.80	17.30
	6	19.85	
	7	18.05	
	8	16.77	
C	1	21.66	
	2	19.78	
	3	20.35	
	4	19.37	20.40
	5	21.63	
	6	21.04	
	7	19.01	
D	1	19.00	
	2	23.81	
	3	18.32	
	4	19.82	20.20
	5	20.44	
	6	21.25	
	7	18.42	
E	1	15.34	
	2	24.25	
	3	16.25	
	4	22.87	
	5	22.66	17.80
	6	21.60	
	7	16.80	
	8	18.55	

Appendix 7.2 continued

F	1	20.57	
	2	17.84	
	3	18.39	
	4	20.12	
	5	23.09	20.00
	6	16.65	
	7	24.82	
	8	18.86	
G	1	19.63	
	2	14.81	
	3	22.81	
	4	24.23	
	5	21.71	20.70
	6	25.94	
	7	18.86	
	8	17.41	

APPENDIX 7.3.

FAECAL EGG COUNT AT DAY 0 and 5 PO ST TREATMENT

		H.POLYGYRUS	EGG COUNT
GROUP		DAY17	DAY 5
A		10,300	6,100
B		9,400	2,900
C		4,500	5,300
D		8,600	3,300
E		6,100	11,300
F		12,000	18,000
G		6,600	10,500

APPENDIX 7.4

***HELIGMOSOMOIDES POLYGYRUS* COUNTS ON DAY 7 POST TREATMENT**

GROUP	MOUSE NO	WORM COUNT
		DAY 7
A	1	7
	2	2
	3	34
	4	7
	5	36
	6	50
	7	39
B	1	68
	2	60
	3	40
	4	72
C	1	59
	2	68
	3	45
	4	73
D	1	48
	2	64
	3	60
	4	80
	5	63
	6	65
	7	82
	8	61
E	1	56
	2	70
	3	41
	4	27
	5	57
	6	51
	7	9
	8	78

Appendix 7.4. continued

F	1	65
	2	76
	3	48
G	1	75
	2	67
	3	74
	4	64
	5	101
	6	86
	7	55