

***IN VITRO AND IN VIVO EVALUATION OF
ANTHELMINTIC ACTIVITIES OF CRUDE
EXTRACTS OF SELECTED MEDICINAL PLANTS
AGAINST HAEMONCHUS CONTORTUS***

By

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fulfillment of the requirement of the Degree of Master of Science in
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Declaration

I, the undersigned, declare that this thesis is my own work and has not been presented in other universities, colleges or institutions, seeking for similar degree or other purpose.

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Acronyms

AL-IPB- Alkali Lemma Institute of Pathobiology
ANOVA- Analysis of variance
COWP – Copper oxide wire particles
DMSO- Dimethyl sulfoxide
ED₅₀ – Effective dose 50
EHA- Egg Hatch Assay
EHNRI – Ethiopian Health and Nutrition Research Institute
EPG- Egg per gram of faeces
EVM – Ethnoveterinary medicine
FECR- Faecal egg count reduction
GABA- Gama amino buteric acid
GI – Gastrointestinal
Ip - Interaperitoneal
L3 – 3rd stage larva
L4 – 4th stage larva
LD₅₀ . Lethal dose 50
LDT- Larval development test
LMIT – Larval migration inhibition test
m asl - meters above sea level
nAChR- Nicotinic acetyl choline receptor
PBS - Phosphate Buffered Saline
PCV- Packed Cell Volume
RPM- revolution per minute
SEM- Standard error of the mean
SPSS- Statistical package for social sciences
TWCR – Total worm count reduction
WAAVP- World Association for Advancement of Veterinary Parasitology

Abstract

In the current study, *in vitro* experiments were conducted to determine the possible anthelmintic effects of crude aqueous and hydro-alcoholic extracts of the seeds of *Croton macrostachyus*, *Ekebergia capensis*, *Coriandrum sativum*, *Acacia nilotica*, *Terminalia schimperiana*, *Jatropha curcas*, leaves of *Lawsonia inermis*, *Chenopodium ambrosioides*, ripe berries of *Hedera helix*, and bark of *Albizia gummifera* on eggs and adult *Haemonchus contortus*. Aqueous extracts of *C. sativum* and *H. helix* were also investigated for toxicity (LD₅₀ determination) in Albino mice and for *in vivo* anthelmintic activity in sheep infected with *H. contortus*. Both extract types of *C. macrostachyus*, *E. capensis*, *C. sativum*, *J. curcas*, *A. gummifera* and aqueous extract of *A. nilotica* inhibited hatching of eggs at concentration less than or equal to 2 mg/ml. Based on their ED₅₀, the six most potent extracts were aqueous extract of *E. capensis* (0.06mg/ml), Hydro-alcoholic extract of *C. ambrosioides* (0.09mg/ml), aqueous extract of *C. macrostachyus* and *J. curcas* (0.1mg/ml) aqueous extract of *C. sativum* and *H. helix* (0.12mg/ml), in decreasing order of potency. Hydro-alcoholic extract of *A. nilotica*, both extracts of *T. schimperiana* and *L. inermis* did not inhibit hatching of eggs of *H. contortus* significantly and in dose dependent manner at all concentrations tested. Hydro-alcoholic extracts of most of the plants have shown better *in vitro* activity against adult parasites compared to the aqueous extract. Hydro-alcoholic extracts of *C. macrostachyus*, *A. gummifera*, *C. sativum* and *H. helix* produced mortality of adult *H. contortus* significantly to the level of 90, 86.67, 85 and 66.67% at concentration of 8 mg/ml while aqueous extracts produced only 36.67, 33.33, 45, and 29.17% respectively at the same concentration. Like their activity on eggs, extracts of *A. nilotica*, *T. schimperiana*, *J. curcas* and *L. inermis* have shown no statistically significant effect on survival of the adult parasites at the concentrations tested, and a few mortality cases recorded were not dose dependent ($p < 0.05$). Oral administration of aqueous extract of *C. sativum* didn't produce mortality and no clinical sign of toxicity was detected in mice despite the high dose (15000 mg/kg) given, while intraperitoneal (IP) administration caused mortality at lower doses. IP LD₅₀ for *C. sativum* was 2177.5 mg/kg. Oral LD₅₀ for *H. helix* was 3846.09 mg/kg. *In vitro* anthelmintic evaluation was conducted in total of 36 male sheep artificially infected with *H. contortus*. The sheep were randomly divided into six groups of six animals each. The first four groups were treated with crude aqueous extract of *C. sativum* (0.45g/kg), *C. sativum* (0.90g/kg),

H. helix (1.13g/kg), *H. helix* (2.25g/kg) respectively. The fifth group was treated with albendazole at 3.8mg/kg and the last group was left untreated. Efficacy was tested by faecal egg count reduction (FECR) and total worm count reduction (TWC). On day 2 post treatment, significant FECR was detected in group treated with higher dose of *C. sativum*, both doses of *H. helix* ($p<0.05$) and albendazole ($p<0.001$) compared to untreated control group. The maximum efficacy of the extracts observed on day 2 post treatment was 46.71% for higher dose of *H. helix* (2.25g/kg) and 24.79% for higher dose of *C. sativum* (0.9g/kg). On day 7 post treatment, significant reduction was detected only for higher dose of *H. helix* ($p<0.05$) and albendazole ($p<0.001$). The percentage reduction of FEC of sheep treated with both plant extracts decreased gradually on day 7 and day 14 post treatment, while that of albendazole increased from 97.8 on day 2 to 100% on day 14 post treatment. The percentage of larvae recovered from culturing faeces obtained from group of sheep treated with plant extracts was reduced in dose dependant manner compared to faeces obtained from untreated control group. Significant reduction ($p<0.05$) in TWC was detected for higher dose of *C. sativum* and both dose levels of *H. helix* compared to the untreated group. Reduction in male worm count was significant ($p<0.05$) in all treatment groups except for lower doses of *C. sativum*, while significant reduction of female worm count was detected only in the case of higher doses of *H. helix*. No worm was detected in the group treated with albendazole, indicating significant susceptibility of the strain of parasites employed in the current study. Treatment with both doses of *H. helix* helped the animals maintain their PCV while PCV of animals treated with *C. sativum* decreased significantly. Treatment with albendazole showed significant increase in PCV ($p<0.05$). The overall findings of the current study indicated that most of the plants have potential anthelmintic effect and further *in vitro* and *in vivo* evaluation is warranted to make use of these plants in the future.

1. INTRODUCTION

Animal diseases remain a major constraint to livestock productivity across all agro-ecological zones and production systems in Africa. Among these, helminth infections play a crucial role mainly in small ruminants leading to enormous economic losses particularly in areas where extensive grazing is practiced (Tembely *et al.*, 1994, Waller, 1997). The major classes of endoparasitic helminthes include gastrointestinal nematodes, trematodes and cestodes. Gastrointestinal nematodes of the order Strongylida are the most common causes of clinical helminthosis. These parasites infest the wall or the lumen of abomasum, small intestine and large intestine. The most common gastrointestinal nematodes of small ruminants belong to the following genera: *Haemonchus*, *Trichostrongylus*, *Ostertagia*, *Oesophagostomum*, *Cooperia*, *Nematodirus*, *Marshallagia*, *Strongyloides* and *Trichuris*. Mixed infection of several genera is common in most of the natural infections (Soulsby, 1986).

The transmission, incidence, and intensity of infections are determined by several factors such as climate, methods of husbandry, systems of production, host age, nutrition, acquired immunity and concurrent infections (Soulsby, 1986). Worms predominantly affect young animals. In small ruminants the most commonly affected age groups are weaned lambs and yearlings. Helminth infection is of most importance when the plane of nutrition is low but massive infections can also affect well-fed animals (Radostits *et al.*, 1994).

Helminth infection causes loss of production directly and indirectly. The direct loss is manifested through mortality, loss of blood and plasma protein by blood sucking behavior of the parasites and leakage into the gastrointestinal tract, depression of mineral level and diarrhea, all contributing to weight loss, milk and wool production (Soulsby, 1986). The indirect economic impact is manifested by increased cost of control strategies (anthelmintics, labor, drenching equipments) and other parasite related penalties such as delay in achieving target weights, increased feed requirements to achieve target weight, and reduced quality of carcass and predisposition to other diseases (Kassai, 1999; Soulsby, 1986).

Economic assessments show enormous financial loss due to helminth infections. Herlich (1978) reported a loss of more than 30 million sheep and goats throughout the world because of helminth infection. In the United States alone parasites cause an estimated loss of 2 billion

dollars per year through decreased productivity and increased operational costs (Sonstergard and Gasbarre, 2001). In Kenya, haemonchosis alone was estimated to cause an annual loss of 26 million dollars in sheep and goat production (Allonby and Urquhart, 1975). Although adequate and up-to-date estimates of economic impact of helminth infections are lacking in Ethiopia, it is supposed to be very high due to the occurrence of the parasites in all agroecological zones of the country. Helminthosis is responsible for 28% of mortality and 3-8% of weight loss in Ethiopian highland sheep (Bekele *et al.*, 1992). The annual loss due to helminthosis in Ethiopia was also estimated to be about 700 million Birr (Habte-Silasie *et al.*, 1991).

1.1. *Haemonchus*

The genus is among the largest in the family of Trichostrongylidae ranging from 10-30mm in length. In fresh specimen the worms can be easily detected due to their bright red color and considerable large size. In both sexes there is a pair of wedge-shaped cervical papillae in the esophageal region and a tiny lancet inside the buccal capsule, which is used for piercing small blood vessels. The species of *Haemonchus* include *H. contortus*, *H. placei*, *H. longistipes* and *H. similis* which usually infect sheep/goats, cattle, camel and deer/cattle respectively. These parasites may also be found in related hosts when they graze together (Soulsby, 1986).

Compared to other nematodes, *Haemonchus contortus* is highly pathogenic parasite of small ruminants, and is capable of causing acute disease and high mortality in all classes of stock (Allonby and Urquhart, 1975). It is one of the top 10 constraints of sheep and goat production in East Africa (Perry *et al.*, 2002).

1.1.1. *Life Cycle*

An understanding of the life cycle of *Haemonchus* is important for effective control programs. Adult *Haemonchus* worms live in the abomasum and lay eggs that are passed in the faeces (Fig. 1). Each adult female parasite has a tremendous egg laying potential (5000 -10000 eggs per day). The eggs that are excreted together with faeces hatch and pass through three larval stages, the third stage (L3) being infective to host. The period required for hatching of the egg and development of the larvae ranges from 5 days to several months depending on the weather conditions. Ingestion of the L3 together with grass while grazing leads to infection of the host.

L3 then penetrates the mucus membrane of the abomasum and molt to L4 within the next few days. L4 remain in the mucus membrane for 10-14 days after which they emerge and molt into adult stage (L5) and females start egg production within 14-21 days post infection (Soulsby, 1986, Whittier *et al.*, 2003).

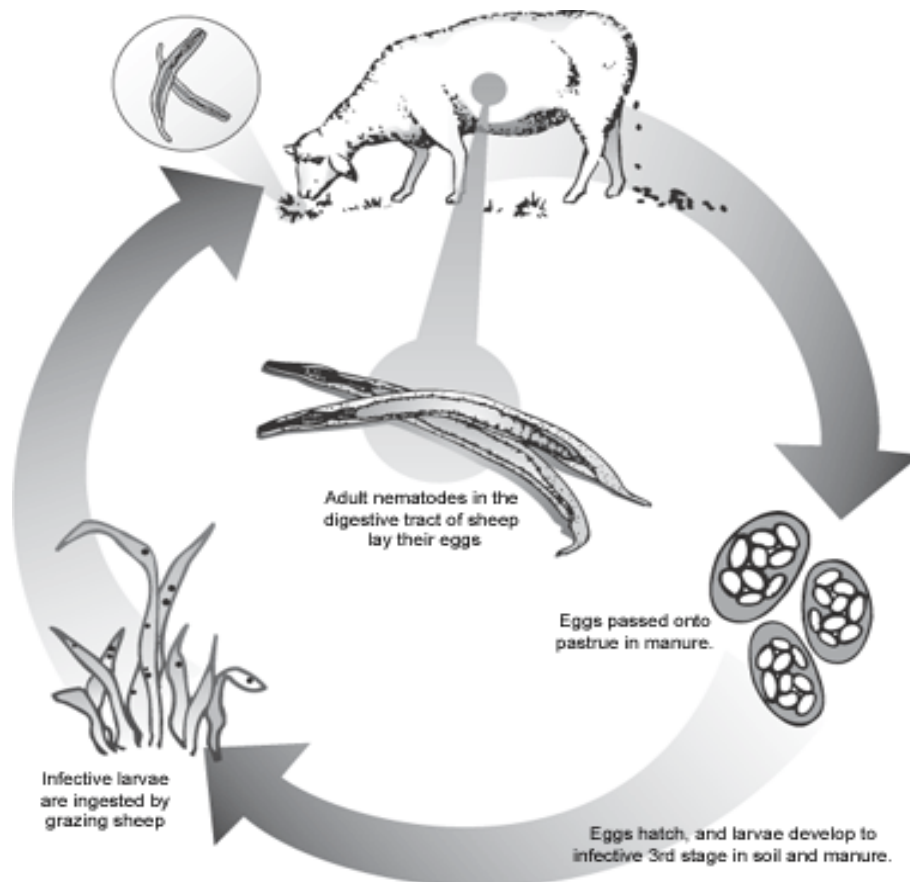


Figure 1 Life cycle of nematode parasite *Haemonchus contortus* (Whittier *et al.*, 2003).

1.1.2. Pathogenesis

Haemonchosis is characterized by hemorrhagic anemia attributable to blood loss via the blood-sucking activities of worms in the abomasum. The mechanism of blood sucking involves the worm attaching to the mucosa and extruding its oral lancet to slit capillaries in the abomasal mucosa. Fourth stage larvae (L4) as well as adults ingest blood flowing from these slit capillaries (Johnstone *et al.*, 1998). They also secrete anticoagulant into the bleeding lesion ensuring the continual bleeding after the worm has moved away, thus causing hemorrhagic anemia. Each worm removes about 0.05 ml of blood per day through ingestion and seepage from lesions so that a sheep with 5000 parasites may lose about 250 ml of blood per day

(Urquhart *et al.*, 1996). The pathogenic effect of *H. contortus* results from the inability of the host to compensate for blood loss. The spectacular depression of hemoglobin level accompanied by weakness and death are the classical features of haemonchosis (Allonby and Urquhart, 1975).

The clinical manifestation of the disease usually involves hyperacute, acute and chronic forms. In hyperacute haemonchosis, sudden deaths in a flock of previously healthy sheep are seen. This syndrome results from ingestion of large numbers of infective larvae by sheep grazing on a heavily infested pasture. It often occurs following a period of warm and wet weather during which massive numbers of *Haemonchus* eggs develop rapidly to infective stages. At necropsy, large number of worms ranging from 20,000 - 50,000 could be found in the abomasum (Johnstone *et al.*, 1998, Soulsby, 1986).

In acute haemonchosis, grazing sheep develop a sudden onset of anemia. In the absence of treatment, the situation of the animals will progressively worsen. At first, the packed cell volume (PCV) drops gradually, followed by a rapid drop signaling exhaustion of the erythropoietic system. Death is the usual outcome if not treated. At necropsy, mucus membranes of these animals become pale and edematous due to loss of blood and plasma protein respectively. 2,000 -20,000 worms may be found in the abomasum. Abomasal content will be brownish due to the presence of blood. Hemorrhagic lesions are also detected on the abomasal mucosa (Johnstone *et al.*, 1998).

Chronic haemonchosis usually results from a combination of infection with small number of worms and poor nutrition that persist for a prolonged period of time. Infected sheep suffer daily loss of small amount of blood, which is exacerbated by a diet poor in protein. As a result, these animals are unable to replace all their lost serum proteins from their deficient diet. Consequently, they lose weight as they mobilize muscle proteins to provide the amino acids necessary to synthesize vitally important proteins such as plasma proteins and hemoglobin (Whittier *et al.*, 2003).

1.1.3. Major control strategies of haemonchosis

Commercial anthelmintics have been used for some decades throughout the world to minimize the losses caused by helminth infections (Baker *et al.*, 1992, Waller, 1997). However, the threats of anthelmintic resistance, risk of residue, availability and high cost especially to farmers of low income in developing countries have led to the notion that sustainable helminth control cannot be achieved with commercial anthelmintics alone. Therefore, today the strategy of helminth control has shifted to integrated control scheme involving grazing management, utilization of natural immunity together with anthelmintics for sustainable control of helminth parasites (Barger, 1996). Other options like, biological control, vaccine and traditional medicinal plants are being examined in different parts of the world (Githiori, 2004).

The common control methods for Haemonchosis are discussed below. Most management and therapeutic methods apply equally to most gastrointestinal nematodes and the principles discussed for *Haemonchus* also apply for other gastrointestinal nematodes (Whittier *et al.*, 2003).

1.1.3.1. Anthelmintics

As of early 1960's there have been three major classes of broad-spectrum synthetic anthelmintics commercially released for the control of nematode parasites of ruminant livestock namely:

- A) benzimidazoles and probenzimidazoles
- B) tetrahydropyrimidines and imidazothiazoles
- C) macrocyclic lactones

In addition to these classes, there are other drugs, with specific anthelmintic activities. They are generally classified as narrow spectrum anthelmintics. These drugs include organophosphates, substituted phenols and salicylanilides (Kassai, 1999).

A) Benzimidazoles and probenzimidazoles

Benzimidazoles are widely used anthelmintic drugs. Some of the drugs under this class include thiabendazole, oxibendazole, fenbendazole, flubendazole, mebendazole and albendazole. Two common probenzimidazoles are netobimin and febantel. The mode of action

of benzimidazoles/ probenzimidazoles is by interference with polymerization of microtubules of nematodes. They act by binding to tubulin dimers, preventing their polymerization to microtubules and thus causing the disassembly of cytoplasmic microtubule structures of the parasite. This action reduces the absorption of nutrients and excretion of metabolites leading to death of the parasite (Kohler, 2001).

B) Tetrahydropyrimidines and imidazothiazoles

The two most important tetrahydropyrimidine drugs (pyrantel and morantel) and the two imidazothiazoles (levamisole and tetramisole) affect the neuromuscular transmission of the nematode. The target site of these drugs is a pharmacologically distinct ion channel that forms a nicotinic acetylcholine receptor (nAChR) on the body muscle of nematodes. These drugs act as acetylcholine agonists. The nAChRs that are found on surface of somatic muscle cells of nematodes can be opened by nicotinic anthelmintics. By binding to these receptors, they affect acetylcholine neurotransmission producing depolarization and spastic paralysis of the nematode muscle (Harder, 2001). Once paralysed, the nematodes are expelled out of the host along with the ingesta. These drugs are active against the major intestinal parasites of small ruminants and cattle (Kohler, 2001).

C) Macrocyclic lactones

Compounds of this class are extremely potent antinematodal drugs, insecticides, and acaricides. These drugs interact with invertebrate specific, glutamate-gated chloride channels and chloride channels of helminth gamma aminobutyric acid (GABA) receptor complexes. They induce flaccid paralysis of somatic worm musculature and block pharyngeal pumping affecting motility, fecundity and feeding of the parasite resulting in elimination of parasites from the host (Harder, 2001). Disruption of ingestive activity and worm starvation is the real nematocidal action of these drugs. Five analogues of macrocyclic lactones are commercially available for the treatment of nematodes in animals, namely: ivermectin, avermectin B1, doramectin, moxidectin and milbemycin A4-5-oxime (Kohler, 2001).

Despite the extremely high level of efficacy of modern broad-spectrum anthelmintics, a total reliance on anthelmintics to control nematode parasites of livestock is no longer tenable mainly due to an increasing frequency of anthelmintic resistance worldwide. Studies have shown that

anthelmintic resistance has developed to most of these drugs in different parts of the world, especially in major sheep producing countries, like Australia, Newzealand, South Africa, and Kenya. For instance worms in 80% of sheep farms in Australia have developed resistance to benzimidazole, imidazothiazole and tetrahydropyrimidine (Waller, 1997). In South Africa 90% of the sheep farms have parasite strains resistant to at least one anthelmintic group and 40% have parasite strains resistant to all the three major groups of anthelmintics (Maingi *et al.*, 1998; van Wyk *et al.*, 1999).

The major anthelmintic drugs commonly used in Ethiopia for the control of helminth parasites in small ruminants are benzimidazolees (albendazole, triclabendazole, fenbendazole) and levamisole (tetramisole) and Oxytoclozanide. In spite of long use of anthelmintics, there are a few reports of suspect for development of anthelmintic resistance against these drugs. Hussein *et al* (1999) and Bayu (2003) reported resistant nematodes to tetramisole in goats at Adami Tulu and around Addis Ababa, respectively whereas Ademe, (2003) reported the occurrence of albendazole resistant population of *Haemonchus contortus* in goats at Awassa. Biffa *et al* (2004) also reported low activity of albendazole in sheep with mixed nematode infection, speculating the development of resistance. Since country wide surveys for anthelmintic resistance have not yet been carried out, the current prevalence of anthelmintic resistance in Ethiopia might be underestimated.

1.1.3.2. Grazing management

Grazing management practices involve rotational grazing, tethering, and zero grazing, which separate the host from infective larvae on pasture. However, rotational grazing may not be feasible due to limitations in farm/plot size in smallholder farms in Ethiopia. Furthermore, communal grazing and common watering points, which are shared in most pastoralists and smallholder flocks, could serve as a source of parasitic infection (Githiori, 2004).

1.1.3.3. Biological control

Various workers have reported *Duddingtonia flagrans*, a nematophagous fungus, is capable of inhibiting the development of larvae in the faecal matter. It is able to survive gut passage and have the ability to grow rapidly in fresh faeces. This microfungus has been shown to occur in the same environment that favors larval development (Larsen, 2000). Field evaluations have

shown promising results (Chandrawathani *et al.*, 2003). However, according to Githigia *et al* (1997) this method is most unlikely to be appropriate for use by smallholder farmers because of lack of suitable application system. Moreover, the nematode trapping fungi are only effective against larvae in faecal pats but not on those that have migrated neither to vegetation nor on worm burdens in the animals.

1.1.3.4. Copper oxide wire particles (COWP)

Studies conducted in sheep (Knox, 2002) and goats (Chartier *et al.*, 2000), revealed that COWP administered as capsules has shown to be effective in reducing the establishment and fecundity of *H. contortus*. The anthelmintic mechanism of action is assumed to be based on the lethal effects of copper ions liberated from the COWP induced by the acid secreted from mucosa of the abomasum. However, the concentration necessary for an anthelmintic effect and the potential for toxicity in copper sufficient animals, or those exposed to copper accumulating plants, are not yet established. In some areas, animals suffer from copper deficiency while in other areas copper toxicity is a problem. Therefore, the use of COWP would have to be evaluated in different areas. In addition, high costs of COWP make them unaffordable and unavailable to financial resource poor farmers (Githiori, 2004).

1.1.3.5. Exploitation of host immunity

Host resistance to nematode parasites is the ability of an animal to prevent infection or reject established parasites in the animals, by utilizing both innate and acquired immune responses (McClure, 2000). The protective effect is expressed by rejection of incoming larvae, depression of worm fecundity and expulsion of adult parasites. Most adult ruminants exhibit acquired protective immunity to gastrointestinal nematodes following natural infection. However, young sheep infected by gastrointestinal nematodes are unable to respond as early or as intensely as required (McClure, 2000). Immunity is also subject to physiological and external factors such as age, pregnancy, lactation, health, sex, genotype, and nutrition. Host immunity has significant influence on use of vaccines, breeding for resistance and nutritional supplementation for control of nematode infection (McClure, 2000).

Breeding of animals that have natural resistance to parasite infection is one of the control strategies. Considerable research has been undertaken in many sheep rearing countries to

identify breeds that have natural resistance to GI nematode parasite infections (Woolaston and Baker, 1996). In Kenya, it has been demonstrated that the local Red Massai sheep are more resistant than the Dorper sheep (Mugambi *et al.*, 1997). However, the immune responses of such resistant breeds are inadequate under low plane of nutrition, and these animals can then succumb to effects of GI nematode infections (van Houtert and Sykes, 1996).

The use of vaccine is another control strategy that utilizes host immunity. Irradiated larval vaccine against the cattle lungworm *Dictyocaulus viviparus*, and *H. contortus* of sheep showed promising results. Irradiated *H. contortus* L3 was however found to offer good protection only in sheep older than six months and those with no worm exposure prior to vaccination (Bain, 1999). Current research on helminth vaccines has mainly concentrated on the production of synthetic or recombinant vaccines (Schallig *et al.*, 1997, Smith *et al.*, 2003). Irrespective of the progress that has been made, and the promise for the future, it will take a long time before these vaccines have a place in the control of *H. contortus* and other parasites (Githiori, 2004).

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1.1.3.6. Ethnoveterinary medicine

Ethnoveterinary medicine (EVM) refers to people's beliefs, knowledge, skills and practices relating to the care of their animals. Only 15-20% of the livestock population in developing nations has enjoyed regular and affordable access to modern veterinary medicine while the remaining 80-85% relies on traditional medicine (McCorkle, 1986). Traditional veterinary practice is based on indigenous knowledge passed on from generation to generation. The use of EVM suffers from, the ineffectiveness of some treatments, the existing harmful practices and inadequate ethno-diagnosis. There is lack of pathophysiological understanding of the disease, which results in poor diagnosis (Schillhorn van Veen, 1997). Disease classification by traditional healers is based on observed signs and abnormalities on the animal, and treatment is usually offered to alleviate these symptoms (Alawa *et al.*, 2002). Although EVM is widely used in many parts of East Africa, there is lack of wider acceptance by scientists and veterinarians, because it is believed to be associated with superstition and without a place in reality (Mesfin and Obsa, 1994). This is mainly because EVM does not follow the paradigms of scientific evidence-based demonstration of efficacy (Githiori, 2004).

Plant/ herbal remedies were extensively used as anthelmintics in the developed world before the

era of broad-spectrum anthelmintics (British Veterinary Codex, 1965, cited in Githiori, 2004). For instance compounds considered as herbal anthelmintics like essential oils (Ascaridole) and Aritimisin are derived from *Chenopodium ambrosioides* and plants of the genus *Aritimisia* respectively. Moreover, many currently available therapeutic compounds are plant derived and/or synthetic analogues derived from plants (Githiori, 2004).

Several references are available on ethnoveterinary use of medicinal plants as anthelmintics. In most of these sources, however, there is only a brief description of the plants used, and the purported conditions that they treat. Most of these reports did not provide information on the part of the plants used and method of preparation. Often no validation of the effect against the disease conditions is provided. There is considerable and apparently expanding interest worldwide in traditional herbal dewormers in both industrialized and developing countries of the world (Waller and Thamsborg, 2004). Recently several workers have tried to screen and validate the anthelmintic activity of herbal preparations used by traditional healers in different parts of the world (Ketzi *et al.*, 2002, Ademola *et al.*, 2004, Githiori *et al.*, 2002, Hounzangbe-Adote *et al.*, 2005).

1.1.3.6.1. Screening of medicinal plants for anthelmintic activity

Screening of claimed products for anthelmintic activity involves both *in vitro* and *in vivo* methods. *In vitro* evaluation is mainly performed on egg, larvae and/or adult parasites. Most of these methods have been used for detection of anthelmintic resistance to the conventional anthelmintics and they are modified for evaluation of natural products (Coles *et al.*, 1992, Hubert and Kerboeuf, 1992). The common *in vitro* methods include: Egg Hatch Assay (EHA), Larval Development Test (LDT), Larval Migration Inhibition Test (LMIT) and *In vitro* effect on adult parasites (Ademola *et al.*, 2004; Hounzangbe-Adote *et al.*, 2005).

Egg Hatch Assay (EHA) is the method by which different concentrations of the test substance is evaluated for inhibition of egg hatching. Larval Development Test (LDT) is the test, which is used to evaluate the effect of the drug on the development of first stage larvae to the 3rd stage infective larvae. Larval Migration Inhibition Test (LMIT) is performed to measure the inhibition of the motility of the infective larvae by test substance. *In vitro* effect on adult parasite involves the application of different concentration of drugs to parasite for its effect on

motility and survival in petridishes. Adult parasites of *Haemonchus contortus*, Ascarides of human and free living roundworm *Caenorhabditis elegans* have been used for evaluation of anthelmintics in drug development by this method (Hounzangbe-Adote *et al.*, 2005, Iqbal, *et al.*, 2004). *H. contortus* has been proved to be a good test worm for *in vitro* screening because of its longer survival in phosphate buffered saline after being removed from the host. Several workers have used this parasite for screening of traditional medicine claimed for anthelmintic activity (Iqbal, *et al.*, 2004, Hounzangbe-Adote *et al.*, 2005).

In vivo screening is conducted by treating naturally or artificially infected animals by the required medicinal preparations. Some workers use mice infected by cestode parasite like *Hymenolepis nana* and nematode parasites like *Heligmosomoides polygyrus* as model for parasites in higher animals and humans (Githiori *et al.*, 2003, Endale *et al.*, 1998). Recently there are several reports on both *in vitro* (Ademola *et al.*, 2004, Pessoa *et al.*, 2002, Hounzangbe-Adote *et al.*, 2005) and *in vivo* (Ketzi *et al.*, 2002, Ademola, *et al.*, 2004, Githiori *et al.*, 2002) evaluation of traditional medicinal plants claimed for anthelmintic properties against *H. contortus* in different parts of the world. Several other workers have evaluated different herbal preparations against different mixed natural infections of nematode parasites (Biffa *et al.*, 2004, Satirijia *et al.*, 2001, Gathuma *et al.*, 2004, Jost *et al.*, 1996).

Some of the screening studies conducted have shown promising results. For example, dried fruit of *Morinda citrifolia* resulted in 73.6 - 88.8% reduction of *H. contortus* egg production in sheep (Satirijia *et al.*, 2001). Papaya latex (*Carica papaya*) resulted in significant reduction (80-100%) in the egg produced by the adult worms of *Ascaris suum* in pigs (Satirijia *et al.*, 1994). *Myrsine Africana*, *Albizia anthelminthica* and *Hilderbrandtia sepalosa*, resulted in 77, 89.8 and 90% reduction, respectively in faecal egg count in sheep infected with mixed natural helminthosis in Kenya (Gathuma *et al.*, 2004).

On the other hand, reports indicated that some herbal remedies that had been traditionally used for deworming internal parasites have little value of reducing faecal egg production or parasite burden in the host. For example, evaluation of six medicinal plants that have been commonly used by pastoralists and smallholder farmers in Kenya as deworming agents for their livestock showed that none of the plant preparations had any biologically significant anthelmintic effect

in mice infected by *Heligmosomoides polygyrus* (Githiori *et al.*, 2003). Githiori *et al* (2002) reported no significant anthelmintic effect of *Albizia anthelmintica* in sheep infected with *H. contortus* and mice infected with *H. polygyrus* even if the plant had long been traditionally claimed for anthelmintic activity.

1.1.3.6.2. Ethnoveterinary/ Ethnomedicine studies in Ethiopia

Ethnoveterinary medicine studies in Ethiopia focused mainly on survey and documentation of traditional practices used for various animal health problems. Tollosa (1997) has identified and documented 37 herbal plant species from central highlands of Ethiopia used as remedy for various animal diseases. Another study conducted in central highlands of Ethiopia recorded lists of 39 plant species for treatment of different diseases of which nine of them were claimed to be used for treatment of internal parasites. These Plant species include *Clematis simensis*, *Ekebergia capensis*, *Ferula communis*, *Impatiens rothii*, *Nicotina tobacum*, *Pentanisa auranogyne* and *Plecthranthus tonginosus* (Wirtu *et al.*, 1999).

Abebe *et al* (2003) reported the presence of 19 species of plants claimed globally for their anthelmintic activity in human and animals in Ethiopia. Some of these plants include *Albizia anthelmintica*, *Tamarandus indica*, *Hagenia abisynica*, *Ccubiata pepo*, *Thymus serulatus* etc. Deressa *et al* (2003) also reported the use of 24 Ethnoveterinary medicinal plants in central Ethiopia of which seven of them used against helminth parasites. Gidey and Amini (2003) also documented several medicinal plants used in Tigray region of North Ethiopia.

In spite of these valuable documentations of medicinal plants in the country, very few efforts have been made to scientifically evaluate these plants for their claimed medicinal properties. Most of a few works in this regard are concentrated on medicinal plants against cestodes and little is done on nematodes. Desta (1995) have investigated 33 traditional herbal drugs used as tanicides in human. On the basis of lower toxicity, higher potency and shorter period of tapeworm expulsion, *Embelia scheperie*, *Thymus serulatus* and *Hagenia abysynica* were the first three best preferred traditional tanicides in human. Endale *et al* (1998) also reported the effectiveness of seeds of *Glinus lottoides* in mice infected with *Hymenolepis nana*.

Mesfin *et al* (1995) have evaluated 14 medicinal plants for their anthelmintic activity in sheep infected with mixed helminth parasites in central Ethiopia. According to this study, none of

these plants demonstrated reliable and consistent anthelmintic effect except the seed of *Cucurbita pepo*, which showed complete effectiveness against *Moniezia* in sheep. However this plant was toxic to sheep at therapeutic doses resulting in death of the experimental animals within two weeks of treatment. This study has some drawbacks like low number of animals per treatment group (as low as 2 animals) which is very much lower than the minimum number per group recommended by WAAVP, use of animals shading very few eggs (as low as 100 eggs) and unknown species of parasites infecting the sheep to ascertain the percentage of the affected species by the plant preparation.

Biffa *et al* (2004) evaluated anthelmintic activities of three herbal preparations namely fresh leaves of *Dodonea viscosa*, *Albizia gummifera* and *Vernonia amygdalina* against mixed natural infections in sheep. They reported that *Dodonea viscosa* and *Vernonia amygdalina* have no significant anthelmintic effect while sheep treated with *A. gummifera* exhibited significant faecal egg reduction and increase in weight gain. This experiment also used only four animals per group.

Screening and proper evaluation of the claimed medicinal plants could offer the possible alternatives that may both be sustainable and environmentally acceptable. Moreover, plants claimed for anthelmintic activity but with no such effect could also be identified and their use be discouraged (Githiori, 2004).

1.1.3.6.3. Description of plants used in the current study

Croton macrostachyus, Del (1848) (Euphorbaceae) is locally known as “Besana” and “Bekenisa” in Amharic and Oromifa respectively. It is a shrub or tree ranging from 2-25m in height. It is a dioecious plant widely distributed in most part of Ethiopia (Vollesen, 1995a). The fruit and decoction of the roots are used for the treatment of venereal diseases and seed for induction of abortion. The leaves are used for treatment of constipation in Ethiopia. The pulverized bark mixed with *Hagenia abyssinica* is used as purgative and vermifuge in different parts of Africa (Getahun, 1976). The leaves are reported to be used traditionally as remedy for constipation and for treatment of internal parasites of cattle in Tanzania (Nsekuye, 1994). In Ethiopia, the bark of *C. macrostachyus* is used for the treatment of tapeworm infection,

syphilis, and asthma in humans (Dessisa, 2001). The seed is used by local population of the Bonga area of South Ethiopia for treatment of tapeworm infection in humans (FARM Africa and SOS, 2004).



Plate 1. *Croton macrostachyus*, near Asela

Ekebergia capensis, Sparrm (1779) (Meliaceae) locally called “Sombo” is a dioecious tree up to 30m of height. It is widely distributed in tropical Africa from Senegal to Ethiopia and South Africa. In Ethiopia, it is widely distributed throughout the country (Styles and White, 1989). The bark mixed with other plants is used for the treatment of anaplasmosis in Tanzania (Nsekuye, 1994). Extracts from *E. capensis* are used to facilitate labor in pregnant women and have been shown to have significant uterotonic activity in *in vitro* study. Decoctions made from chopped bark are also used as an emetic agent, for treatment of coughs and other respiratory complaints while leaves are used in an infusion as purgative parasiticide in South Africa (Sewarm *et al.*, 2000). The concoction of this plant is used traditionally as anthelmintic for treatment of animals in central Ethiopia (Wirtu *et al.*, 1999). *Ekebergia capensis* together with *Olea capensis* are used to treat abdominal cramps by local population of the Bonga area of South Ethiopia (FARM Africa and SOS, 2004). Chemicals isolated from different parts of this plant include Limonoids, Squalene, triterpenoids and cumarins (Moholland *et al.*, 2000).



Plate 2. *Ekebergia capensis*, near Asela.

Coriandrum sativum, Linnaeus (1753) (Apiaceae) locally called “Dinbilal” in Ethiopia is erect annual herb 20-70cm tall with strong smell. It is widespread throughout the world as a result of cultivation for its aromatic seeds. It is cultivated in fields and home gardens at altitude of 1700-2500masl in Eastern, Western, Southern and Central Ethiopia where there is sufficient rainfall. The seed has a wide range of daily use in foodstuff eg for preparation of “berbere”, as flavoring agent in “wot”, “injera”, cakes and bread. It is also used against stomach ache (Hedberg and Hedberg, 2003). It has been reported to have several pharmacological effects such as anti-fertility, anti-diabetic anti-hyperlipidemic, antioxidant, and hypotensive activities (Al-Said *et al.*, 1987, Chithra and Leelamma, 1997, Melo, *et al.*, 2003, Gray and Flatt, 1999). According to Dessisa (2001), seeds of *C. sativum* are used by some traditional healers for treatment of hepatitis and ascaris infection in Ethiopia. Phytochemical screening of *C. sativum* has shown the presence of chemicals such as quercetin 3-glucoronide linalool, camphor, geranyl acetate, geraniol and coumarins. The amount of total lipid was 28.4% of seed weight. The major fatty acid was petroselinic acid (65.7% of the total fatty acid methyl esters) followed by linoleic acid (Ramadan and Morsel, 2002).



Plate 3. Seeds of *Coriandrum sativum*

Acacia nilotica, Widex Del (1813) (Mimosoideae) locally called “Bekko” is a tree 2.5-14m high with bright yellow flowers. The plant is distributed in Afar region, Central Highlands and South East part of Ethiopia. The bark extract is used as soothing agent and for treatment of diabetes. The seed is used as dye (Asfaw and Thulin, 1989). The powdered seed of *A. nilotica* macerated in fresh water is claimed to be useful against diarrhea in Mauritania. It is also used as anthelmintics in Nigeria in sheep and goats. Powdered seeds after removal of the outer layer are applied locally as antiseptic to treat wounds. All plants under this genus have tannin in their bark and often are source of hydrocyanic acid (Nwude and Ibrahim, 1980).



Plate 4. *Acacia nilotica*, Nazareth area.

Terminalia schimperiana, Hochest (1894) (Combretaceae) locally called “Aballo” is a tree about 10m high. It is widely distributed from West Africa to Ethiopia. It grows at an altitude range of 1000-2200masl (Vollesen, 1995b). *T. schimperiana* is used to treat cattle gastrointestinal helminthes together with other plants in Cameroon (Toyang *et al.*, 2005). *T. avicenniodes*, another species in the same genus has also been reported to be effective against natural worm infections in sheep. The seed oil of the plant has been used as an ingredient in the treatment of rheumatic conditions, parasitic skin diseases, in the treatment of fever, jaundice, gonorrhoea, as a diuretic agent, as a mouthwash and laxative in certain African countries. The leaves have been used as a haemostatic agent and the bark as fish poison. (Nsekuye, 1994).



Plate 5. *Terminalia schimperiana*, around Wolkite.

Lawsonia inermis, Linnaeus (1753) (Lythraceae) locally called “Henna” is evergreen shrub or tree 2-7m tall. It grows in alluvial soils along rivers or near water holes, from sea level to 1100masl in Ethiopia and throughout the old world tropics (Vollesen, 1995a). The leaves boiled together with *Alium sativum* leaves are drenched to sheep and goats for treatment of worm infection (Nwude and Ibrahim, 1980). Similar preparation is also used for treatment of febrile conditions caused by infectious agents in Nigeria. The powdered leaves mixed with tea, produce a red dye used for coloring hair, beards and nails (Vollesen, 1995a). The filtrate of

handful of leaves soaked in a liter of water for 12-24 hours is used for treatment of trypanosomiasis in camel. The paste formed from the pulverized leaves is also used for treatment of wounds (Nsekuye, 1994). In India its bark is reported to be useful in treatment of jaundice, enlargement of spleen and reported of having anti-inflammatory, antipyretic and analgesic effects (Ali *et al.*, 1995). It has also been proved to have hepatoprotective effect. The bark extract was reported to have a broad-spectrum antifungal and antimicrobial activity (Singh and Pandey, 1989; Malekzadeh, 1968). The phytochemical investigations have shown the presence of the β -sitosterolglucosides, flavonoids, quinonoids, naphthalene derivatives, luteolin, betulin, lupeol, garlic acid, coumarins, xanthenes and phenolic glycosides (Dasgupta *et al.*, 2003)



Plate 6 *Lawsonia inermis* source: <http://www.hennaforhair.com/>

Jatropha curcas, Linnaeus (1753) (Euphorbiaceae) locally called “Sudan gullo” and “Ayderke” is a shrub or small tree 4.5 to 8m high. It has smooth bark and milky latex. It is cultivated as ornamental plant and live fencing at an altitude of 450-1300masl. It is commonly found in southern part of Ethiopia. It was known to be native to tropical America later introduced to old world tropics where it is now widely cultivated and naturalized (Vollesen, 1995a). The roots, stems, leaves seeds and fruits of the plant have been widely used in traditional folk medicine in many parts of West Africa. The seeds have been used as purgative,

anthelmintic, abortifacient, for treating ascites, gout and skin diseases (Vollesen, 1995a). Seeds have also been reported to be effective against *Strongyloides papillosus* infection in goats (Nsekuye, 1994). The seeds are good sources of oil, which can be used as a diesel substitute. They are used also in manufacturing of soap and cosmetics in various tropical countries (Makkar *et al.*, 1998). In Ethiopia, study by Dessisa, (2001) revealed that the fruit is commonly used as purgative and anthelmintic in human patients by traditional healers.

The seed of some provenances of *J. curcas* is toxic to rats, mice and ruminants. Several cases of *J. curcas* poisoning in humans after accidental consumption of the seeds were reported. Phorbol esters have been identified as the main toxic agent of *J. curcas* (Makkar *et al.*, 1998). Extraction using 92% alcohol was reported to remove the toxic and heat-stable factors and the residue was found to be non-toxic to rats (Makkar and Becker, 1999). The defatted product was found to contain 50 to 62% of protein. High proportion of trypsin inhibitor, lectin activities, antimetabolic metal-chelating agent, heat-stable factor and phytic acid, was isolated from the seed of *J. curcas* (Makkar, *et al.*, 1998).



Plate 7. *Jatropha curcas*, around Gojeb area.

Chenopodium ambrosioides, Linnaeus (1753) (Chenopodiaceae) locally called “Amedmado” is strongly aromatic herb, about 1.2 m high, sometimes with woody base. It is a common weed

of cultivated areas, often in seasonally wet sites at altitude of 950-2500masl. The origin is supposed to be from America but now it has spread throughout the tropics and subtropics (Friis and Gilbert, 2000). Oil of *C. ambrosioides* was used for many years to treat parasite infections in human and animals in different parts of the world (Githiori, 2004). Its use was discontinued when more effective and less toxic anthelmintics became available. Recently, Ketzis *et al* (2002) reported ineffectiveness of this plant extract against *H. contortus* infection in goats.

Ascaridole, a monoterpene essential oil, was reported to be the principal active component found in the extract of *C. ambrosioides* and responsible for anthelmintic activity. The other components include isoascaridole, P-cymene, limonene and x-terpinene. The level of the different compounds varies depending on the part of the plant, age of the plant and whether it is dried or fresh plant material (<http://www.ansci.cornell.edu/plants/medicinal/epade.html>).



Plate 8 *Chenopodium ambrosioides*, premises of AL-IPB, Addis Ababa.

***Hedera helix*:** (Araliaceae) is an evergreen, often climbing or creeping shrub, whose wooden aerial stems often attach by numerous, small root like structures to trees, rocks, walls and buildings. Through these roots, the branching stem is able to climb up to 30 meter in height. The leaves are dark green with waxy and leathery appearance arranged alternately along the stem. Mature fruits are black with a fleshy outer covering (Prenner, 2004). In Ethiopia, *H.*

helix is not endemic and found mainly in cities like Addis Ababa as natural fencing. It is an ornamental plant imported from abroad.

Traditionally leaves and fruits of this plant have been used in Europe against diseases of the gastro-intestinal tract and gall-stones since the antique. It was also indicated for treatment of inflammations and burns, cough as well as neuralgia and rheumatism. It is also claimed for anti-fungal, anthelmintic, molluscicidal, and anti-mutagenic properties (Prenner, 2004). Topical preparations of *H. helix* based products are indicated for use in weight loss and in skin care due to their itch-relieving and anti-cellulite properties (Dermaxime, 2005).

Different fractions of fruits of *H. helix* phenolic compounds (flavonoids, caffeoylquinic acids) and saponins (hederacoside C, alpha-hederin, and hederagenin) showed significant antispasmodic activity (Trute *et al.*, 1997). Extract of *H. helix* also showed secretolytical, spasmolytical, anti-elastase, anti-hyaluronase, hepatoprotective and antimicrobial activities (Prenner, 2004). It also showed significant anthelmintic activity against liver flukes (Julien *et al.*, 1985). All parts of the plant contain several triterpenoid saponins as a major chemical constituent (Bedir *et al.*, 2000). Although the plant has been claimed for its anthelmintic activity, its effect on nematode parasite has not been evaluated scientifically.



Plate 9. Leaves and ripe fruits of *Hedera helix*, garden of Yekatit 12 Hospital, Addis Ababa.

Albizia gummifera Gamel (1930) (Mimosaceae), locally called 'Ambabesa', is an umbrella-

like medium or large size tree to about 50 m high commonly growing in upland and reverine forest at altitude range of 1700-2400masl. It is commonly found in eastern tropical Africa. In Ethiopia, it is widely distributed in the southwest highland of the country (Thulin, 1989). The plant under this genus, *Albizia anthelmintica*, has been traditionally used as an anthelmintic in different parts of the world in both human and animals. Extract from bark of this plant was reported to produce 89.8% faecal egg reduction in sheep infected with mixed gastrointestinal parasites, while it had got 100% efficacy against monezia spp. (Gathuma *et al.*, 2004) although another worker reported that this plant has no anthelmintic effect (Githiori *et al.*, 2003). The same plant was reported to be 95.5% efficient against trematode parasite *Fasciola gigantica* in goats (Koko *et al.*, 2000). Biffa *et al* (2004) reported significant anthelmintic activity of the decoction of *A. gummifera* against mixed nematode infection in sheep. Extract from *A. gummifera* was also reported to have significant *in vitro* antimalarial effect (Ofulla *et al.*, 1995). Stem bark extract was reported to contain a wide range of triterpene saponins (Debella *et al.*, 2000).



Plate 10. *Albizia gummifera*, around Bedele

2. OBJECTIVES

General

To screen and evaluate the anthelmintic properties of the crude aqueous and hydro-alcoholic extracts of some medicinal plants of Ethiopia.

Specific

1. To conduct qualitative phytochemical screening of the medicinal plants.
2. To investigate the *in vitro* anthelmintic activity of plant extracts on the eggs of *Haemonchus contortus*.
3. To determine *in vitro* inhibitory effects of the plant extracts on survival of adult *Haemonchus contortus*.
4. To determine LD₅₀ of the two promising plants in mice.
5. Based on the *in vitro* results, to evaluate *in vivo* anthelmintic activity of two plant extracts on *Haemonchus contortus* infection in sheep.

3. MATERIALS AND METHODS

3.1. Plant collection

Selection of plants was based on the literature survey on the traditional uses of the plants in Ethiopia and other parts of the world. Those plants with claimed anthelmintic activity but not scientifically evaluated for the purported activity were selected. For *in vivo* evaluation it was predetermined to select two plants based on performance in *in vitro* evaluation, absence of information on their *in vivo* activity and easy availability of the plant material in the required amount. Most of the plant materials were collected from their natural habitat while seeds of *Coriandrum sativum* and leaves of *Lawsonia inermis* were purchased from the markets at Debre Birhan and Awassa, respectively, from November 2004 – April 2005. Parts of all the plant species were collected and transported to Alkalilu Lemma Institute of Pathobiology, (AL-IPB) AAU. All the plants collected were identified and voucher specimens of each species were deposited at the Herbarium of the Addis Ababa University, Biology Department. The garbled plants were air dried at room temperature, grinded and kept in amber colored bottle until processed. List of the plant species used in this study, parts used and areas of collection are shown in Table 1.

Table 1. Species, herbariums voucher No, areas of collection and parts of medicinal plants used in the current study (* plants used for both *in vitro* and *in vivo* studies)

Species(Family)	Herbarium voucher No	Area of collection	Parts used
<i>Croton macrostachyus</i> (Euphorbiaceae)	MG-02/05	Kuyera	seeds
<i>Ekebergia capensis</i> (Meliaceae)	MG-03/05	Asela	seeds
<i>Coriandrum sativum</i> (Apiaceae)*	MG-04/05	Debre Birhan	seeds
<i>Acacia nilotica</i> (Mimosoideae)	MG-05/05	Nazreth	seeds
<i>Terminalia schimperiana</i> (Combretaceae)	MG010/05	Wolkite	seeds
<i>Lawsonia inermis</i> (Lythraceae)	MG-012/05	Awassa	leaves
<i>Jatropha curcas</i> (Euphorbiaceae)	MG-013/05	Gojeb	seeds
<i>Chenopodium ambrosioides</i> (Chenopodiaceae)	MG-031/05	Addis Ababa	leaves
<i>Hedera helix</i> (Araliaceae)*	HH-2038/04	Addis Ababa	Ripe berries
<i>Albizia gummifera</i> (mimosaceae)	Ag-2006/04	Bedele	bark

3.2 Extraction methods and phytochemical screening

A. Aqueous extraction

Extraction was conducted at the Drug Research Department of Ethiopian Health and Nutrition Research Institute (EHNRI). A weighed amount of the dry powder (50 - 100g) was soaked in distilled water and shaken for three hours by electric shaker. The suspension was filtered through mucellin gauze and the filtrate kept in deep freezer for 24 hours, which was then lyophilized using lyophilizer. The lyophilized dry powder was then collected in a stoppered sample vial, weighed and kept in a desiccator to avoid absorption of water until used for the assay.

B. Hydro-alcoholic extraction

200-300g of the dried and powdered plant material was percolated using 80% methanol for 5 days, which was then filtered through whatman filter paper No.1. The solvent was then evaporated using a Rota vapor to give a sticky gummy residue. The extracts were then kept in a stoppered sample vial at 4 °C until used for the assay.

C. Phytochemical screening

Preliminary qualitative screenings for major secondary metabolites of the medicinal plants were conducted according to Debella (2002) from aqueous and hydro-alcoholic extracts. Plant extracts were screened for the presence of polyphenols, cyanogenic glycosides, saponins, phytosteroides and withanoids, phenolic glycosides, flavonoids, glycosides, tannins, alkaloids and antraquinone glycosides. The methods used were chemical tests involving color changes through reaction with different standard reagents. Reagents used and color changes observed are presented in Annex 1.

Thin Layer Chromatography (TLC) was also employed to determine the number of chemical components found in some of the plant extracts. Both silica and Alumina were initially used as adsorbants. Silica was found to be convenient for better separation of the components. Different proportions of chloroform, methanol, diethylamine and water were used as solvent system. TLC spraying agents used were Dragendroff reagent, 1% FeCl₃+K₃Fe (CN)₆ + 1% Fast Blue B and 5% vanillin for alkaloids, polyphenols and saponins, respectively.

3.3. Parasites

Adult female parasites of *Haemonchus contortus* were collected from abomasum of infected sheep obtained from Addis Ababa Abattoir. The worms were washed and crushed to liberate eggs. The eggs were then cultured in a glass jar filled with autoclaved sheep faeces for eight days at room temperature. At the end of 8th day, infective larvae were harvested by rinsing the side of the culture jar with a drop of water. About 3000 larvae were inoculated to two worm free sheep that were kept indoor in separate house in the animal facilities of the AL- IPB throughout the study period. These sheep served as *Haemonchus contortus* egg donors for subsequent *in vitro* and *in vivo* trials.

3. 4. *In vitro* experiments

3.4.1. Egg Hatch Assay

The Egg Hatch Assay was conducted according to the World Association for The Advancement of Veterinary Parasitology (WAAVP) guidelines, (Coles *et al.*, 1992). Briefly, faecal pellets were collected from the rectum of donor sheep and placed in small bucket. Warm water was slowly added to the faeces and the pellets were stirred until a relatively liquid suspension was obtained. The suspension was mashed through sieve with 3mm aperture. The suspension that passed through the sieve was collected and washed through 100-mesh (150µm pore size) sieve. The suspension was then poured into 15ml test tubes and centrifuged for 2 minutes at 1000 RPM and the supernatant decanted. The tube was agitated by vortex mixer to loosen the sediment. Saturated sodium chloride was then added to the test tube until the meniscus forms above the tube on which the cover slip was placed. After 3-5 minutes the cover slip was carefully taken off the tube and eggs washed into glass centrifuge tubes, filled with water and centrifuged for 2minutes at 1000 RPM. Most of the water was then decanted and the number of eggs per ml was determined and diluted to the required concentration.

Aqueous and hydro-alcoholic extracts of the plant materials were used as the active treatment. Albendazole (99.8% pure standard reference) obtained from Drug Administration and Control Authority (DACA) was used as positive control while untreated eggs in water were used as negative control. The test was conducted in 5ml test tubes. In the assay, approximately 150 - 250 eggs in 1.5ml of water were placed in each test tube. Various serial concentration of each plant extract in total volume of 0.5ml in distilled water was added to make concentrations of 2,

1, 0.5, 0.25, 0.125 0.0625 and 0.03125mg/ml together with water containing the eggs. Albendazole originally dissolved in Dimethyl sulfoxide (DMSO) and diluted in distilled water at the concentrations of 5, 2.5, 0.125, 0.0625, 0.03125 and 0.0156 μ g/ml was used. The test tubes were then covered and kept in incubator at 27 °C for 48 hrs. The experiment was conducted in duplicates for each concentration and replicated three times. Hatched larvae (dead or alive) and unhatched eggs were then counted under dissecting microscope at 40X magnification.

3.4.2. Effect of plant extracts on adult worms

Adult *Haemonchus contortus* were collected from the abomasum of the sheep slaughtered at the Addis Ababa Abattoir. Immediately after death, the abomasums were collected and transported to the laboratory. The collected parasites were then washed and kept in phosphate buffered saline (PBS). The test was performed in 5cm diameter plastic petridish. Eight to ten worms were then placed in petridishes filled with 8, 4, 2, 1, 0.5, and 0.25mg/ml of the aqueous and hydro-alcoholic extracts of plant materials in PBS and PBS alone for the control group in total volume of 4 ml. Albendazole originally dissolved in DMSO and diluted in PBS at the concentrations of 0.5, 0.25, 0.125, 0.0625, 0.03125mg/ml was also used as a positive control. Three replications per each treatment concentration were employed. After 24 hrs the extract is washed away and the parasite resuspended in PBS for 30 minutes for possible recovery of the parasite motility. Finally the number of motile (alive) and immotile (dead) worms were counted under dissecting microscope and recorded for each concentration. Death of worms was ascertained by absence of motility for observation period of 5-6 seconds. A mortality index was calculated as the total number of dead worms divided by the total number of worms per petridish.

3. 5. In vivo study

3. 5.1. LD₅₀ determination

Toxicity of the aqueous extract of two plants (*H. helix* and *C. sativum*) that were selected for *in vivo* anthelmintic evaluation were used for LD₅₀ determination on 6 to 8 week old albino mice purchased from the Ethiopian Health and Nutrition Research Institute (EHNRI). The mice weighed between 26 and 37 gm. The animals were allocated into five treatment groups for each extract. Each group contains five male and five female mice. Before being treated with

different dose levels of the extract, they were devoid of food and water for 18 hrs. After preliminary trial, aqueous extract of *Hedera helix* (2500, 3000, 3500, 4000 and 4500mg/kg) and *C. sativum* (5000, 7500, 10000, 12500 and 15000mg/kg) dissolved in distilled water was drenched orally. For *C. sativum* doses of 1500, 2000, 2500, 3000 and 3500mg/kg were also given for groups of mice intraperitoneally (IP). They were observed for any clinical manifestations for 24hrs and animals that died were opened and the visceral organs were examined for any gross pathological changes. Finally, the number of dead and alive mice at each dose level was recorded at the end of 24 hrs.

3.5.2. *In vivo* anthelmintic efficacy test

Study animals and infection

Thirty-six 6-8 month old male Menz sheep were purchased from Debre Birhan, which is located 138 km north of Addis Ababa. They were brought to animal house of AL-IPB and ear tagged. They were kept indoors on concrete floor throughout the study period, fed with hay and concentrate provided with water *adlib*. The animals were provided adaptation period of 3 weeks before initiation of the experiment. During this period the animals were examined for parasite infestation and some of them were found positive for strongile eggs and all of them were dosed with albendazole (Expitol[®], ERFARs.a., Greece) at 10mg/kg and dipped with acaricide, Fenvalerate(VAPCOCIDIN 20 EC, Jordan) according to manufacturers instruction. The animals were examined for nematode faecal egg production on the 3rd and 6th days post anthelmintic treatment and all were negative for parasite eggs on the 6th day post treatment. Each animal was inoculated orally with 1750 *Haemonchus contortus* infective larvae (L3) on the 7th day. The infective larvae were obtained by culturing *H. contortus* eggs collected from previously mentioned two mono-species infected donor sheep.

Experimental design and treatment

Four weeks after infection, the sheep were divided into 6 groups of 6 animals each by blocking based on live weight and faecal egg count taken one day ahead. The groups were randomly allocated to two dose level of each plant, one positive control treated with albendazole and the last group served as negative untreated control. The findings on LD₅₀ study and percentage yield of the aqueous extraction were used to determine the dosage of the plant materials. The

ground powder at the dose level of (6 g/kg and 12 g/kg) for *Coriandrum sativum* and (3.5g/kg and 7g/kg) for *Hedera helix* was soaked in water overnight, which was stirred occasionally. The mixture was then filtered through tea strainer and the filtrate drenched to each animals via stomach tube according to their body weight. The dose level for aqueous extract based on the extraction efficiency (extrapolated from Table 3) for each group is indicated in Table 2.

Table 2. Experimental layout for *in vivo* anthelmintic efficacy test

Group(dose)	Treatment	Dose (dry powder)	Dose (aqueous extract)
1(I)	<i>Coriandrum sativum</i>	6g/kg	0.45g/kg
2(II)	<i>Coriandrum sativum</i>	12g/kg	0.9g/kg
3(I)	<i>Hedera helix</i>	3.5g/kg	1.13g/kg
4(II)	<i>Hedera helix</i>	7g/kg	2.25g/kg
5	Albendazole	3.8mg/kg	
6	<i>Untreated control</i>	_____	

a) Determination of Eggs per gram of faeces (EPG): Faecal sample was collected on day zero pretreatment and on day 2, 7, and 14 post treatment directly from the rectum. Faecal egg count was determined by modified McMaster Technique according to Coles *et al* (1992). Briefly, 3 gram of faeces was soaked in 42 ml of water for few minutes until the faeces softened. Homogenization by mortar and pistil was performed and then poured through 150µm pore size mesh. The filtrate was thoroughly mixed and 15 ml was poured in to 17ml centrifuge tube that was then centrifuged for 2 minutes at 1500 RPM on a bench top centrifuge. The supernatant was gently sucked off and saturated sodium chloride was added to give original volume (15ml). It was then mixed gently and immediately a sample was withdrawn with Pasteur pipette to fill the two chambers of the McMaster slide. Then the eggs in the two chambers were counted under 40X magnification. The number of eggs per gram of faeces (EPG) was determined by multiplying the total number of eggs in the two chambers by 50.

b) Live weight measurements: animals were weighed on day 0 before treatment as well as on day 7 and day 14 post treatment.

c) **PCV determination:** The animals were bled from the marginal ear vein to microhaematocrite capillary tubes on Day 0 before treatment, on day 7 and 14 after treatment. Packed red cell volume (PCV) was determined by microhaematocrit method.

d) **Worm recovery and Total Worm Count (TWC):** On day 15-post treatment all the animals were humanely killed and the abomasum was ligated at the junctions of the abomasum to the omasum and the abomasum to the small intestine. The abomasum was removed, and opened up with a blunt-tipped pair of scissors and the contents emptied into a bucket. The abomasal mucosa was washed gently with running tap water and the parasites washed off into the bucket. The contents of the bucket were adjusted to make two liters and thoroughly mixed. An aliquot of 200 ml was then taken and the numbers of male and female *H. contortus* in the aliquot were counted. The abomasal mucus membrane was scraped and parasites in the scraping counted under dissecting microscope. Only tail parts were counted in the cases when intact parasites were not recovered. The total number of parasites was calculated by multiplying the number of parasites found in the aliquot by 10 and adding the number of parasites found from mucosal scrapings.

e) **Faecal culture assay:** Faecal culture assay was performed on the faecal sample collected from groups of sheep employed by the *in vivo* experiment in the current study. Fresh faeces from the rectum of all sheep from group 1 (*Coriandrum sativum* dose I), group 2 (*C. sativum* dose II), group 3 (*Hedera helix* dose I), Group 4 (*H. helix* dose II) and group 6 (untreated control) were collected on the second day post treatment. Pooled faecal sample of each sheep in a group was thoroughly mixed and egg per gram of faeces (EPG) was quantified for each group. Then 5 g of each pooled faecal samples were put in small plastic container loosely packed into the bottom of the containers covered and allowed to stand at room temperature. Each treatment was replicated 3 times. Larvae was then harvested on the 8th day by rinsing the sides of each container with 10 ml of water and counted under dissecting microscope at 40X magnification.

3.6. Data management and statistical analysis

Data from EHA were transformed by probit transformation against the logarithm of extract concentration. The extract concentration required to inhibit 50% (ED₅₀) and 90% (ED₉₀) egg

hatching was calculated after correction was made for natural mortality by probit analysis. Comparison of mean percentages of egg hatch inhibition, mortality of adult parasites and larval recovery in faecal culture assay at different concentrations with the control, was performed by one-way ANOVA. 50% lethal concentration (LD₅₀) of mice in acute toxicity study was determined from the regression curve by probit analysis. Mean of the EPG, PCV and body weight of groups of sheep at different days, and Mean worm burden at necropsy were compared with the control group by one-way ANOVA. Variation in mean EPG, PCV and body weight for each group over time was analyzed using General Linear Model by repeated measures analysis of variance.

Faecal egg count reduction test (FECRT) was determined at days 2, 7 and 14 post treatment according to the method described by Coles *et al* (1992) using the formula:

$$\text{FECR}\% = 100 \times (\text{C}-\text{T}/\text{C})$$

Where, T = arithmetic means of FEC in the treated group

C = arithmetic means of FEC in the control group

A similar formula was used for percentage Worm Count Reduction (WCR) except the use of geometric mean in this case. All statistical analysis was performed by SPSS version 13.0. The Post hoc statistical significance test employed was List Square Difference (LSD), the difference between the means were considered significant at $p < 0.05$.

4. RESULTS

4.1. Extraction and screening of plant materials

Variation in yield among different plant species in both aqueous and hydro-alcoholic extracts was observed (Table 3). The lowest yield was observed for aqueous extract of the bark of *Albizia gummifera* (2.43%) and the highest yield was observed for hydro-alcoholic extract of seed of *Acacia nilotica* (61.92%). Some plants showed higher yield in aqueous extract while others in hydro-alcoholic extract.

Table 3 Percentage yield of the different plants using aqueous and hydro-alcoholic extraction methods.

Plant species	Parts used	Extract type	% Yield (w/w)
<i>Croton macrostachyus</i>	seeds	Aqueous	10.32
		Hydro-alcoholic	16.81
<i>Ekebergia capensis</i>	seeds	Aqueous	17.48
		Hydro-alcoholic	5.86
<i>Coriandrum sativum</i>	seeds	Aqueous	7.75
		Hydro-alcoholic	5.54
<i>Acacia nilotica</i>	seeds	Aqueous	18.15
		Hydro-alcoholic	61.92
<i>Terminalia schimperiana</i>	seeds	Aqueous	10.51
		Hydro-alcoholic	17.1
<i>Lawsonia inermis</i>	leaves	Aqueous	12.45
		Hydro-alcoholic	30.53
<i>Jatropha curcas</i>	seeds	Aqueous	14.77
		Hydro-alcoholic	4.48
<i>Chenopodium ambrosioides</i>	leaves	Aqueous	12.67
		Hydro-alcoholic	4.38
<i>Hedera helix</i>	ripe fruits	Aqueous	32.2
		Hydro-alcoholic	22.5
<i>Albizia gummifera</i>	bark	Aqueous	2.43
		Hydro-alcoholic	10.75

The major secondary metabolites identified from the medicinal plants are indicated in Table 4. Most of the plants have major secondary metabolites like alkaloides, flavonoids, phytosteroides and withanoids. Polyphenol was found in all plants tested. Saponin was detected in *Chenopodium ambrosioides*, *Hedera helix* and *Albizia gummifera* whereas tannin, cyanogenic glycosides and antraquinone glycosides were found only in *Acacia nilotica*. Screening of some

of the plants by Thin Layer Chromatography (TLC) showed that the plant extracts have several components that gave different colors and retention factors (R_f) when sprayed with spraying agents (Table 5).

Table 4. Major classes of secondary metabolites found in the aqueous and hydro-alcoholic extracts of medicinal plants used in the current study.

Plant species/ Parts used	Extract type	<i>C. macrostachyus</i> /seeds	<i>E. capensis</i> /seeds	<i>C. sativum</i> /seeds	<i>A. nilotica</i> /seeds	<i>T. schimperi</i> /seeds	<i>L. inermis</i> /leaves	<i>J. curcas</i> /seeds	<i>C. ambrisooides</i> /leaves	<i>H. helix</i> /ripe fruits	<i>A. gummi fera</i> /stem bark
Polyphenols	aq	+	+	+	+	+	+	+	+	+	+
Cyanogenic glycosides	aq	-	-	-	+	-	-	-	-	-	-
Saponins	aq	-	-	-	-	-	-	-	+	+	+
	ha	-	-	-	-	-	-	-	-	+	+
Phytosteroides and Wthanoides	ha	+	+	+	-	+	+	-	-	+	+
Phenolic glycosides	ha	-	-	-	+	+	-	-	-	-	-
Flavonoids	aq	+	+	+	+	+	+	-	-	+	+
	ha	+	+	+	+	+	+	-	-	+	+
Glycosides (Oligosaccharids)	aq	-	-	-	+	+	+	-	+	-	+
	ha	-	-	-	+	-	+	-	-	-	+
Tannins	ha	-	-	-	+	-	-	-	-	-	-
Alkaloides	aq	+	+	+	-	+	-	-	-	+	+
	ha	+	+	+	-	+	-	-	-	+	+
Antraquinone glycosides	aq	-	-	-	+	-	-	-	-	-	-

Note + = present aq- aqueous extract
- = absent ha- hydro-alcoholic extract

Table 5. TLC pattern on silica gel-60 F₂₅₄ on aluminum foil plates of the crude hydro-alcoholic and aqueous extracts of some of the plants

Plant	Extract type	Solvent system	Color formed using TLC spraying agents	Number of Spots	R _f values of the components separated
<i>C. macrostachyus</i>	ha	CHCl ₃ -MeOH-diethyl amine (10:4:0.05)	Orange and violet	5	0.06, 0.25, 0.86, 0.91, 0.95
	ha	CHCl ₃ -MeOH- Water (12:3:0.25)	Violet	7	0.06, 0.12, 0.18, 0.58, 0.81, 0.88, 0.97
<i>E. capensis</i>	ha	CHCl ₃ -MeOH-Diethyl amine (10:4:0.05)	Orange, brown and violet	5	0.06, 0.25, 0.81, 0.9, 0.95
	ha	CHCl ₃ -MeOH- Water (12:3:0.25)	Brownish	7	0.06, 0.13, 0.36, 0.75, 0.84, 0.9, 0.97
<i>C. sativum</i>	ha	CHCl ₃ -MeOH-Diethyl amine(10:4:0.05)	Orange, yellow	7	0.06, 0.25, 0.41, 0.63, 0.8, 0.86, 0.91
	ha	CHCl ₃ -MeOH- Water (12:3:0.25)	Yellowish brown	7	0.06, 0.16, 0.36, 0.52, 0.69, 0.81, 0.91
<i>C. sativum</i>	aq.	CHCl ₃ -MeOH-Diethyl amine(10:4:0.05)	Violet	2	0.06, 0.86
	aq.	CHCl ₃ -MeOH- Water (12:3:0.25)	Brownish	5	0.13, 0.33, 0.39, 0.84, 0.97
<i>H. helix</i>	ha	CHCl ₃ -MeOH-Diethyl amine(10:4:0.05)	Yellow, violet	5	0.06, 0.82, 0.86, 0.95, 0.96
	ha	CHCl ₃ -MeOH- Water (12:3:0.25)	Brownish	7	0.08, 0.16, 0.35, 0.46, 0.52, 0.62, 0.83
<i>H. helix</i>	aq.	CHCl ₃ -MeOH-Diethyl amine(10:4:0.05)	Violet	1	0.97
	aq.	CHCl ₃ -MeOH- Water (12:3:0.25)	Violet	5	0.13, 0.31, 0.36, 0.86, 0.96
<i>C. ambrosioides</i>	ha	CHCl ₃ -MeOH-Diethyl amine(10:4:0.05)	Yellow	4	0.06, 0.19, 0.89, 0.95
	ha	CHCl ₃ -MeOH- Water (12:3:0.25)	Yellow	6	0.16, 0.33, 0.4, 0.71, 0.81, 0.91

R_f- Retention factor aq- aqueous extract, ha – hydro-alcoholic extract (80% methanol extract)

4.2 In vitro study

4.2.1 Egg Hatch Assay

Both aqueous and hydro-alcoholic extracts of most of the plants exhibited good activities against eggs of *Haemonchus contortus* although there is variation in doses required for each type of extract. Both extract types of some plant like *Terminalia schimperiana* and *Lawsonia inermis* have shown low activity and the inhibition is not dose dependent (Table 6).

The maximum concentration required to induce total (100%) egg hatch inhibition for most of the plant extracts fall below 2mg/ml. Aqueous extracts of *Ekebergia capensis* and *Coriandrum sativum* required maximum of 0.25mg/ml whereas aqueous extract of *Croton macrostachyus*, hydro-alcoholic extracts of *C. sativum* and *Chenopodium ambrosioides* required maximum concentration of 0.5mg/ml to induce 100% egg hatch inhibition. Albendazole induced 100% egg hatch inhibition at a concentration of 0.125µg/ml (Table 6).

Table 6 Mean percentage inhibition of egg hatching after 48 hours exposure of eggs of *H. contortus* to different concentrations of plant extracts (mg/ml) and albendazole ($\mu\text{g/ml}$)

Plant type	Ext	Mean \pm SE at different concentrations								
		0.0	0.01565	0.03125	0.0625	0.125	0.25	0.5	1	2
Albendazole		3.87 \pm 1.18	21.16 \pm .74	25.61 \pm 3.47	86.4 \pm 1.9	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
<i>C. macrostachyus</i>	Aq	0 \pm 00		6.77 \pm 1.25	15.89 \pm 7.9	56.08 \pm 16.3	93.34 \pm 3.82	100 \pm 0	100 \pm 0	100 \pm 0
	Ha	0.61 \pm .61		0 \pm 0	0.99 \pm .5	3.5 \pm 2.21	66.99 \pm 18.45	96.82 \pm 2.32	97.26 \pm 2.11	98.32 \pm 1.68
<i>E. capensis</i>	Aq	0.51 \pm .51		20.94 \pm 12.9	11.64 \pm 1.29	91.38 \pm 6.48	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
	Ha	0.41 \pm 0.41		1.5 \pm .25	2.74 \pm 2.08	6.49 \pm 3.83	10.46 \pm 3.52	9.97 \pm 3.85	15.23 \pm 4.84	97.22 \pm 2.7
<i>C. sativum</i>	Aq	0.56 \pm .56		1.24 \pm .64	19.42 \pm 4.86	18.88 \pm 4.57	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
	Ha	1.42 \pm .73		1.78 \pm 1.79	3.37 \pm 2.33	15.36 \pm 8.72	74.68 \pm 15.91	100 \pm 0	100 \pm 0	100 \pm 0
<i>A. nilotica</i>	Aq	0.81 \pm .81		0 \pm 0	0 \pm 0	0.33 \pm .33	2.5 \pm .25	12.4 \pm 1.56	40.63 \pm 3.21	97.13 \pm 2.87
	Ha	0.48 \pm .48		5.5 \pm .0	30.23 \pm 17.23	5.89 \pm 3.11	40 \pm 21.10	47.99 \pm 18.61	64.48 \pm 17.9	26.34 \pm 13.4
<i>T. schimperiana</i>	Aq	0.52 \pm .52		0 \pm 0	0 \pm 0	1.62 \pm 1.62	0 \pm 0	0 \pm 0	1.67 \pm 1.67	0.99 \pm .49
	Ha	1.03 \pm 1.03		0.52 \pm .52	6.67 \pm 1.51	10.19 \pm 1.96	55.21 \pm 19.67	66.86 \pm 13.3	8.74 \pm 1.85	21.07 \pm 1.5
<i>L. inermis</i>	Aq	0.68 \pm .68		0.55 \pm .55	2.17 \pm .36	5.72 \pm 2.8	6 \pm 3	10.84 \pm 2.95	10.47 \pm 2.62	29.47 \pm 1.19
	Ha	0.77 \pm .77		0.66 \pm .66	0.56 \pm .56	2.38 \pm .35	3.75 \pm 2.08	6.23 \pm 1.73	10.86 \pm 6.78	22.87 \pm 3.12
<i>J. curcas</i>	Aq	1.12 \pm .6		0.67 \pm .67	68.75 \pm 10.85	52.28 \pm 5.15	64.98 \pm 5.27	97.09 \pm .51	99.66 \pm .34	100 \pm 0
	Ha	0.66 \pm .33		6.51 \pm 1.19	27.81 \pm 8.5	12.4 \pm 4	19.31 \pm 5.7	98.71 \pm 1.3	77.46 \pm 22.4	100 \pm 0
<i>C. ambrosioides</i>	Aq	1.02 \pm .59		2.57 \pm 2.37	8.76 \pm 3.01	52.73 \pm 10.77	96.0 \pm 4	99.75 \pm .25	100 \pm 0	100 \pm 0
	Ha	0.34 \pm .34		12.02 \pm 4.03	50.21 \pm 25.24	91.43 \pm 7.67	94.69 \pm 4.59	100 \pm 0	100 \pm 0	100 \pm 0
<i>H. helix</i>	Aq	0.67 \pm .67		2.17 \pm 1.09	5.81 \pm 1.71	46.24 \pm 18.63	94.8 \pm 4.76	98.4 \pm 1.6	99.2 \pm .8	100 \pm 0
	Ha	0.47 \pm .29		2.87 \pm 1.47	8.61 \pm 3.52	16.47 \pm 8.2	75.47 \pm 21.49	91.0 \pm 9	95.0 \pm 5	100 \pm 0
<i>A. gumifera</i>	Aq	0.56 \pm .2		0.6 \pm .59	0.70 \pm .7	0.98 \pm .59	4.08 \pm .67	23.32 \pm 6.51	84.03 \pm 12.32	99.3 \pm .27
	Ha	0.73 \pm .42		0.78 \pm .39	0.974 \pm .56	6.67 \pm 2.26	11.9 \pm 2.64	50.33 \pm 13.15	62.01 \pm 2.73	99.3 \pm .7

Ext. =extract type, Ha= hydro-alcoholic, Aq= aqueous

In addition to inhibition of egg hatching at higher concentrations, some of the plant extracts have shown effect on the survival of the hatched larvae at concentrations below complete egg hatch inhibition. This includes aqueous extract of *Acacia nilotica*, hydro-alcoholic extract of *Terminalia schimperiana*, aqueous extract of *Jatropha curcas*, aqueous and hydro-alcoholic extracts of *Albizia gummifera*.

The effective doses required to induce 50% and 90% (ED₅₀ and ED₉₀) inhibition of egg hatching, calculated by probit analysis are shown in Table 7. ED₅₀ for egg hatch inhibition of albendazole was 0.04µg/ml. Aqueous extracts of *Croton macrostachyus*, *Ekebergia capensis*, *Coriandrum sativum*, *Jatropha curcas* and *Hedera helix* induced 50% and 90% inhibition at lower concentration compared to hydro-alcoholic extract of the same plants, while hydro-alcoholic extracts of *Acacia nilotica*, *Terminalia schimperiana*, *Chenopodium ambrosioides* and *Albizia gummifera* induced 50% and 90% inhibition at lower concentration compared to their aqueous counterparts. Based on their Ed₅₀, the six most potent extracts were aqueous extract of *E. capensis* (0.06mg/ml), Hydro-alcoholic extract of *C. ambrosioides* (0.09mg/ml), aqueous extracts of *C. macrostachyus* and *J. curcas* (0.1mg/ml), aqueous extracts of *C. sativum* and *H. helix* (0.12mg/ml) (Table 7).

Table 7. *In vitro* anthelmintic activity of plant extracts and albendazole expressed in ED₅₀ and ED₉₀ on the eggs of *H. contortus* exposed for 48 hrs.

Plant type	Extract	ED ₅₀ (LCL-UCL)	ED ₉₀ (LCL-UCL)
Albendazole (µg/ml)		0.04 (0.026-.051)	0.074 (0.06-0.103)
<i>C. macrostachyus</i> (mg/ml)	Aqueous	0.10 (0.08-0.12)	0.22 (0.19-0.28)
	Hydro-alcoholic	0.32 (0.15-0.34)	0.51 (0.32-1.46)
<i>E. capensis</i> (mg/ml)	Aqueous	0.06 (0.05-0.07)	0.13 (0.11-0.17)
	Hydro-alcoholic	1.03 (0.52-1.33)	3.9 (1.63-173.06)
<i>C. sativum</i> (mg/ml)	Aqueous	0.12(0.09-0.19)	0.26 (0.18-0.74)
	Hydro-alcoholic	0.18 (0.14-0.26)	0.41 (0.28-0.81)
<i>A. nilotica</i> (mg/ml)	Aqueous	0.87 (0.81-1.15)	2.23 (1.51-2.62)
	Hydro-alcoholic	0.74 (0.22-785.04)	22.23 (2.89- 1034.01)
<i>T. schimperiana</i> (mg/ml)	Aqueous	233.19(201-379 .06)	107594.8(179.3-126473)
	Hydro-alcoholic	39.71(28.54-76.34)	287159.68(1343-3234)
<i>L. inermis</i> (mg/ml)	Aqueous	11.74 (3.41-51.65)	333.58 (27.19-6564.31)
	Hydro-alcoholic	13.8(3.79-75.53)	237 (18.30-6678.8)
<i>J. curcas</i> (mg/ml)	Aqueous	0.1 (0.02-0.26)	0.41 (0.18-87.79)
	Hydro-alcoholic	0.23 (0.08-0.65)	0.75 (0.35-53.45)
<i>C. ambrosioides</i> (mg/ml)	Aqueous	0.15(0.09-0.22)	2.77(1.93-3.32)
	Hydro-alcoholic	0.09 (0.08-0.1)	0.21 (0.18-0.23)
<i>H. helix</i> (mg/ml)	Aqueous	0.12 (0.09-0.16)	0.25 (0.19-0.43)
	Hydro-alcoholic	0.17 (0.13-0.23)	0.43 (0.29-0.79)
<i>A. gummifera</i> (mg/ml)	Aqueous	0.67 (0.36-1.54)	1.53 (0.84-23.11)
	Hydro-alcoholic	0.48 (0.39-0.65)	1.59 (1.08-2.81)

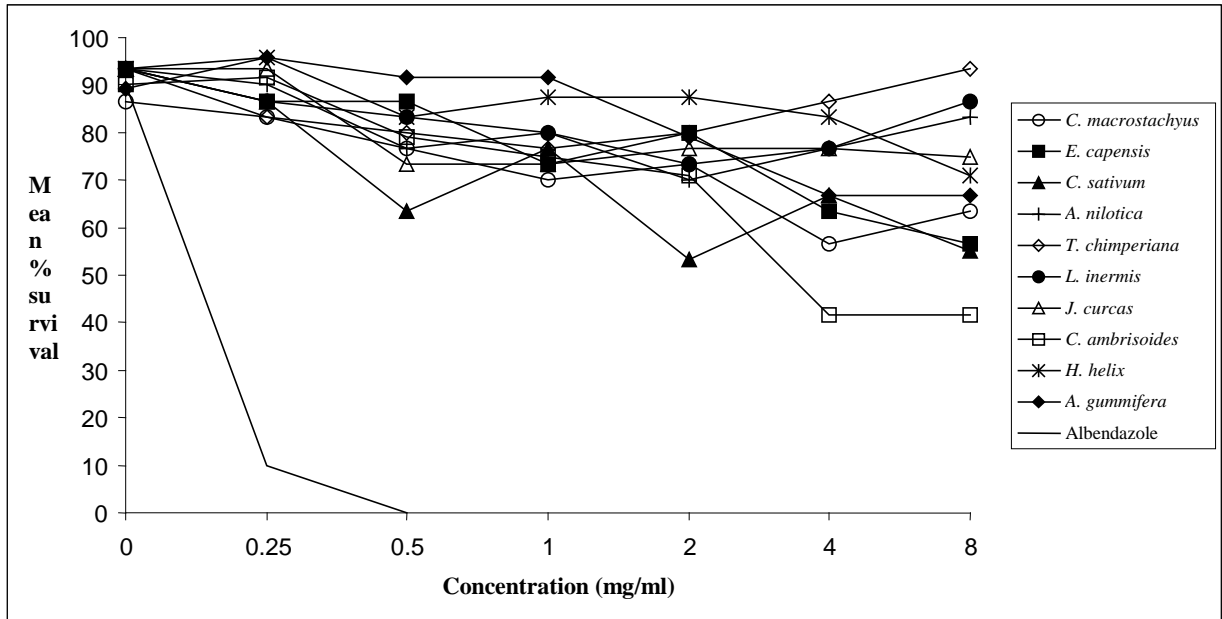
LCL- lower confidence limit, UCL-upper confidence limit

4.2.2. *In vitro* effects on adult parasites

Both aqueous and hydro-alcoholic extracts of most of the plants showed inhibitory effect on the survival of *Haemonchus contortus* in a dose dependent manner. However, most hydro-alcoholic extracts were more potent than the aqueous extracts. Hydro-alcoholic extracts of *Croton macrostachyus*, *Albizia gummifera*, *Coriandrum sativum* and *Hedera helix* produced mortality of adult *H. contortus* significantly to the level of 90, 86.67, 85 and 66.67 % at concentration of 8mg/ml while aqueous extract produced only 36.67, 33.33, 45 and 29.17 %, respectively at the same concentration (Fig. 2). The first 3 extracts killed the worms at statistically significant level ($P < 0.05$) at all concentrations tested compared to the mortality recorded in the control group. Aqueous extract of *Acacia nilotica*, *Terminalia schimperiana* and *Lawsonia inermis*

produced few mortality cases which was not statistically significant. Hydro-alcoholic extracts of these plants caused parasite mortality, although the effects were not dose dependent. Other plants killed the parasites in somewhat dose dependent manner. Albendazole, on the other hand killed the parasites in a dose dependent manner and all the worms were dead at a concentration of 0.5mg/ml with in 24 hours.

a) Aqueous extract



b) Hydro-alcoholic extract

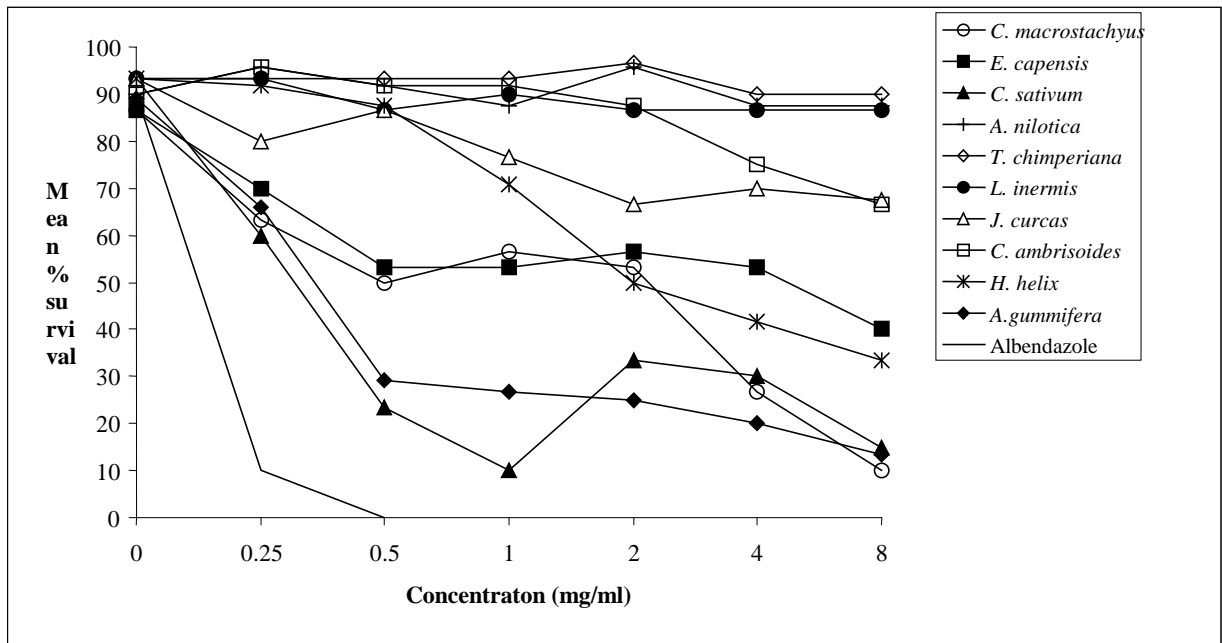


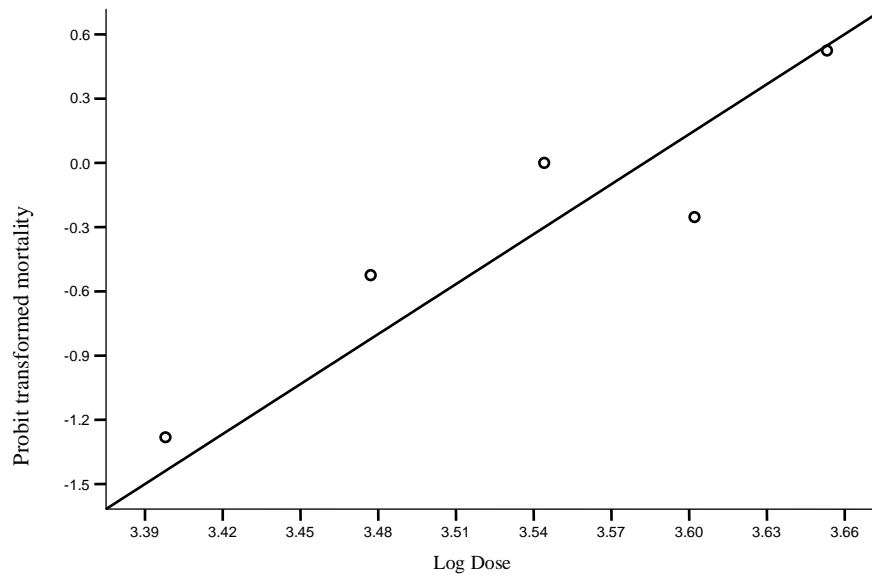
Figure 2. Effect of different plant extracts and albendazole on survival of adult *Haemonchus contortus* after 24 hours of exposure: a) aqueous and b) hydro-alcoholic extracts

4.3. In vivo studies

4.3.1. LD₅₀ Determination

In all doses tested, neither death of mice nor physical clinical symptom of toxicity was detected in groups administered with extracts of *Coriandrum sativum* orally. However, administration of the same extract intraperitoneally (IP) exhibited mortality at lower doses and the IP LD₅₀ calculated by probit analysis was 2177.5 mg/kg with 95% CL of (1374.09-2776.27). Group of mice administered with extracts of *Hedera helix* exhibited mortality in a dose dependent manner. The oral LD₅₀ calculated by probit analysis was 3846.09 mg/kg with 95%CL of (3504.15-5563.63). Probit transformed mortality rates of both plant extracts are shown in Fig. 3. Mice given higher doses of *H. helix* were restless, which was followed by depression and standing hair coat. Some of them stretch their legs and necks followed by loss of motility and death. Dead mice were dissected and examined for gross pathological changes of the visceral organs by comparing with untreated control mice. The subcutaneous tissues of these animals were hyperemic; the lungs congested while the livers were dark red in color compared to the control group.

a) *Coriandrum sativum*



b) *Hedera helix*

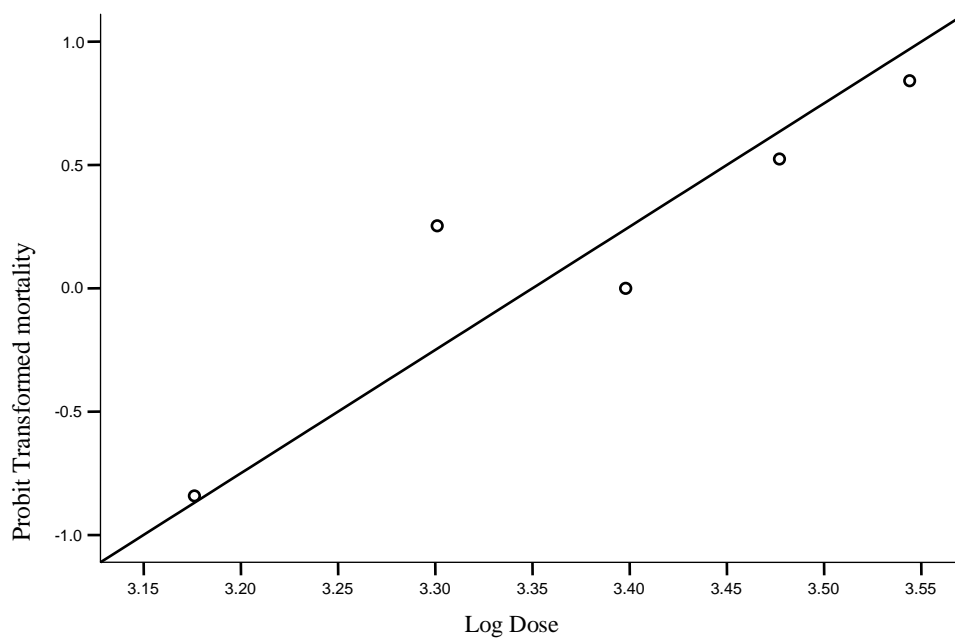


Figure 3. Probit transformed mortality of mice administered with aqueous extracts of a) *C. sativum* (Ip) b) *H. helix* (oral)

4.3.2. Faecal egg count reduction test

There was no physical clinical sign of toxicity in all groups of sheep treated with plant extracts except a transient bloat observed in a sheep treated with *Hedera helix* dose I which recovered within short period without any interference. Mean \pm SEM of faecal EPG counts before and after treatment with crude aqueous extract of *H. helix*, *Coriandrum sativum* and albendazole are shown in Table 8. On day 2 post treatment both plant extracts reduced egg count in a dose dependent manner although the reduction caused by *C. sativum* dose I was very low. On the same day, significant decrease in faecal egg count was detected in group of sheep treated with *C. sativum* dose II, *H. helix* dose I, *H. helix* dose II ($p < 0.05$) and albendazole ($p < 0.001$) compared to untreated control group. However, significant difference was detected on day 7 post treatment for *H. helix* dose II ($p < 0.05$), albendazole ($p < 0.001$) and on day 14 only for albendazole ($p < 0.001$) compared to EPG counts of the untreated control group. The maximum efficacy observed was 100% for albendazole on day 14, 46.71% for *H. helix* dose II, and 24.79% for *C. sativum* dose II at day 2 post treatment. The percentage reduction of faecal egg count of sheep treated with both plant extracts decreased gradually on day 7 and day 14 post treatment, while that of albendazole increased from 97.8 on day 2 to 100% on day 14 (Table 9).

Table 8. Mean faecal EPG counts before and after treatment with aqueous extracts of *H. helix*, *C. sativum* and albendazole

Group	Dose	Mean [†] EPG counts			
		Pretreatment	post treatment		
		Day 0	Day 2	Day7	Day 14
<i>C. sativum</i> (dose I)	0.45g/kg	10491.67±1046.06	11483.33±772.298	9258.33±1538.366	9000±334
<i>C. sativum</i> (dose II)	0.90g/kg	10625.00±1017.25	9766.67±772.30*	9450.00±1252.20	8825.00±1242.23
<i>H. helix</i> (dose I)	1.13g/kg	10466.67±782.48	7758.33±1351.75**	8000±1084.44	7675±1018.72
<i>H. helix</i> (dose II)	2.25g/kg	9816.67±1030.35	6891.67±719.42**	6258.33±1538.37**	7183.33±763.40
Albendazole	3.8mg/Kg	10900.00±1464.87	266.67±189.15**	16.67±16.67**	0****
Untreated control	—	10758.33±1528.31	12933.33±1137.37	10258.33±443.74	8983.33±347.67

* P<0.05, ** P<0.01, *** P<0.001 † mean±SEM

Table 9. Percentage efficacy based on FECRT

Group	Dose	Post treatment % reduction		
		Day 2	Day7	Day 14
<i>C. sativum</i> (dose I)	0.45g/kg	11.21	9.75	-0.19
<i>C. sativum</i> (dose I I)	0.90g/kg	24.49	7.88	1.76
<i>H. helix</i> (dose I)	1.13g/kg	40.00	22.01	14.56
<i>H. helix</i> (dose II)	2.25g/kg	46.71	38.99	20.04
Albendazole	3.8mg/kg	97.79	99.84	100

4.3.3. Worm count

The geometric mean counts of *Haemonchus contortus* recovered from abomasums of sheep of all treatment groups and untreated negative control group is indicated in Table 10. Significant variation in total worm count was detected for *Coriandrum sativum* only at dose II while both dose levels of *H. helix* resulted in significant reduction (p<0.05) compared to the untreated

group. Reduction in male worm count was significant in all treatment groups except for *C. sativum* dose I, while significant variation in female worm count was detected only in the case of *H. helix* dose II compared to untreated control. In the cases of sheep group treated with albendazole, no worm was detected ($p < 0.001$).

Table 10. Geometric mean worm count of groups of sheep treated with aqueous extracts of *C. sativum*, *H. helix*, Albendazole and untreated control 15 days post treatment.

Group	Treatment type	Dose	Geometric Mean [†] worm count		
			male	female	Total
1	<i>C. sativum</i> dose I	0.45g/kg	426.56±64.40	611.69±96.43	1039.72±158.1
2	<i>C. sativum</i> dose II	0.90g/kg	288.95±32.36*	551.39±33.74	850.18±32.58*
3	<i>H. helix</i> dose I	1.13g/kg	400.29± 20.93*	490.89±44.30	892.96±61.38*
4	<i>H. helix</i> dose II	2.25g/kg	203.02± 15.96*	431.63± 31.99*	637.24±40.07*
5	Albendazole	3.8mg/kg	0.0***	0.0***	0.0***
6	Untreated control	—	507.74±12.37	632.90±32.70	1141.99±41.23

* $p < 0.05$, *** $p < 0.001$ †mean±SEM

As shown in Table 11, higher doses of both treatment groups, *C. sativum* and *H. helix* induced significant percentage reduction in total worm count (25.56 and 44.2%), respectively. Male parasites were more susceptible to both plant extracts compared to female parasites except in the cases of the group treated with *H. helix* dose I.

Table 11. Efficacy of the treatment based on percentage reduction of worm burden.

Group	Treatment type	Dose	% reduction		
			Male	Female	Total
1	<i>C. sativum</i> dose I	0.45g/kg	15.99	3.35	8.96
2	<i>C. sativum</i> dose II	0.90g/kg	43.09	12.88	25.56
3	<i>H. helix</i> dose I	1.13g/kg	21.16	22.44	21.81
4	<i>H. helix</i> dose II	2.25g/kg	60.02	31.8	44.2
5	Albendazole	3.8mg/kg	100	100	100

4.3.4. Effect of treatment on PCV

Mean PCV of all treatment groups except the group treated with albendazole has shown reduction from day 0 to day 14 post treatment. However, significant reduction was not detected in groups of sheep treated with both dose levels of *Hedera helix* ($p>0.05$), while significant reduction in PCV was detected in group treated with *Coriandrum sativum* dose I as of day 7 post infection and *C. sativum* dose II on day 14. In contrast to other treatment groups, PCV of the group of sheep treated with albendazole, showed statistically significant increase ($p<0.05$) on day 14 post treatment. No significant difference was found between the groups on days 0 and 7. On day 14, group of sheep treated with albendazole had significantly highest PCV when compared with other groups. Groups treated with *C. sativum* doses I and II as well as the untreated group had significantly low ($p<0.05$) PCV (Table 12).

Table 12. Mean PCV of sheep treated with aqueous extracts of *C. sativum*, *H. helix*, and albendazole.

Group	Dose	Mean [†] PCV before and after treatment		
		Day 0 <i>Bt</i>	Day 7 <i>Pt</i>	Day 14 <i>Pt</i>
<i>C. sativum</i> (dose I)	0.45g/kg	24.75±0.36*	21.67±1.36	19.83±1.72 ^b
<i>C. sativum</i> (dose II)	0.90g/kg	22.67±0.76	23.08±1.39	19.33±1.02 ^{b*}
<i>H. helix</i> (dose I)	1.13g/kg	24.58±1.94	22.92±2.04	22.67±0.91
<i>H. helix</i> (dose II)	2.25g/kg	23.92±1.74	22.58±1.41	22.67±0.91
Albendazole	3.8mg/kg	21.83±1.82	24.67±0.72	26.00±1.32 ^{a*}
Untreated control	—	25.33±1.05*	21.33±1.59	21.67±0.88 ^b

Across rows, * indicate means with significant difference at p<0.05

Within columns, means with different letters have significant difference at P<0.05

[†] mean±SEM, *Bt*- before treatment, *pt*- post treatment

4.3.5. Effect on body weight

Although there is minor reduction in mean live weight of the sheep in all treatment groups, treatment has induced no statistically significant difference (p>0.05) when compared with untreated control (Table 13).

Table 13. Mean live weight of sheep treated with aqueous extracts of *C. sativum*, *H. helix*, and albendazole compared to untreated control group.

Group	Dose	Mean [†] Body wt. before and after treatment(kg)		
		Day 0 <i>Bt</i>	Day 7 <i>Pt</i>	Day 14 <i>Pt</i>
<i>C. sativum</i> (dose I)	0.45g/kg	14.58±0.5	14.63±0.51	14.02±0.62
<i>C. sativum</i> (dose I I)	0.90g/kg	14.68±0.71	14.53±0.79	14.01±0.78
<i>H. helix</i> (dose I)	1.13g/kg	14.95±0.71	14.88±0.64	14.58±0.68
<i>H. helix</i> (dose II)	2.25g/kg	14.75±0.87	14.78±1.01	14.45±0.93
Albendazole	3.8mg/kg	14.55±1.02	14.83±1.14	14.15±1.12
Untreated control	—	14.51667±0.79	14.28±0.78	14.25±0.97

[†] mean±SEM, *Bt*- before treatment, *pt*- post treatment

4.3.6. Faecal culture assay

Mean percentage of larvae recovered from faeces collected 2 days post infection from sheep treated with 2 dose levels of plant extracts and untreated control group are indicated in Fig. 4. With increasing dose of both plant extracts, there was significant decline in the proportion of larvae recovered from the original eggs determined by Mc Master Technique. More than 60 % of total eggs in the control group were developed to 3rd stage larvae and recovered after 8 days of culture while 52.16, 40.72, 47.52 and 36.07% of eggs developed to third stage larvae from faeces collected from groups 1, 2, 3 and 4 respectively. Significantly lower proportion of larvae was recovered in the Groups 2, 3 and 4, compared to untreated control group ($p < 0.05$).

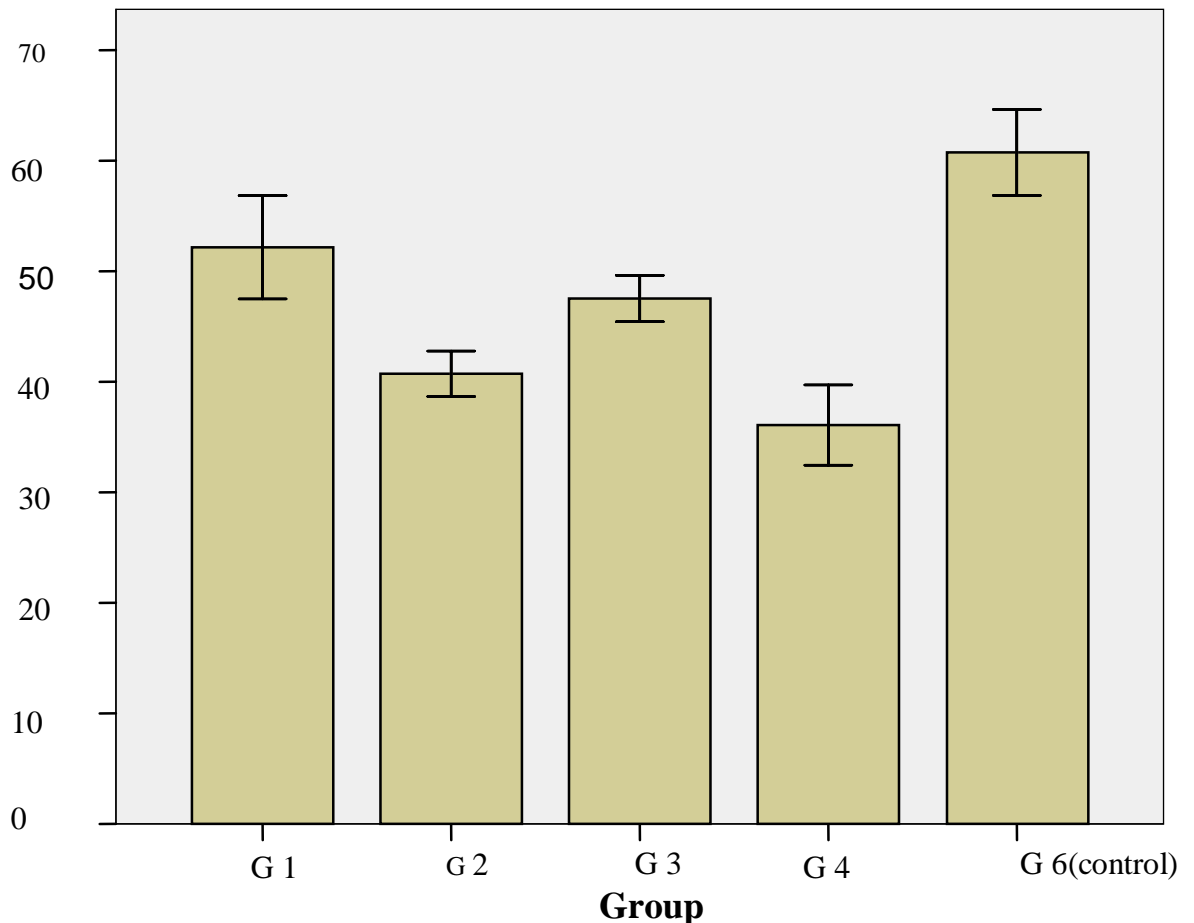


Figure 4. Mean \pm SEM % of larvae recovered from *H. contortus* eggs in faeces of sheep treated with crude aqueous extracts of *C. sativum*, *H. helix* and untreated control.

5. DISCUSSION

In the current study, significant variation in the yield of plant extracts was observed. The reason for variation in the yield of the aqueous and hydro-alcoholic extract of the medicinal plants could be due to a difference in the chemical composition of each plant so that some plants may contain active components that are more soluble in organic solvents while others may contain chemicals that are more soluble in water. The highest extraction efficiency was that of hydro-alcoholic extract of *Acacia nilotica*, while the aqueous extract was three times lower. The reason for this variation could be due to high concentration of less polar organic compounds in the seeds of *A. nilotica*, which are capable of dissolving in relatively less polar solvent methanol than water (Siddhuraju *et al.*, 1996).

The lowest extraction efficiency was observed for the aqueous extract of *Albizia gummifera*. This might be due to the thicker walls of the cells from the bark reducing the extraction efficiency. It was extracted from the bark of the plant unlike other plants, which were extracted from the seeds and leaves. Extraction efficiency of this type of plant could be improved by boiling in water (decoction) for a relatively longer time (Debella, 2002). However, this method may result in loss of heat labile components of the plants. Unlike the aqueous extract, the hydro-alcoholic extract of the same plant had higher extraction efficiency, which could be due to presence of some additional organic chemicals, capable of dissolving in methanol. Parts of the plants used, age of the plant, season and area of harvest are responsible for variation in the biochemical profiles of plants, which in turn, lead to difference in percentage yield of extracts from different or the same plant species (Raskin *et al.*, 2002).

Qualitative Phytochemical screening of the medicinal plants revealed that most of the plants with good anthelmintic activity have secondary metabolites like alkaloids, flavonoids, saponin, and all of them were positive for polyphenol. These classes of plant secondary metabolites are considered the sources of chemical components responsible for wide therapeutic activities of several medicinal plants (Debella, 2002). The active principles that induced the observed anthelmintic activity might be found in one or more of these classes of chemicals. The presence of flavonoids and saponin in *Hedera helix*, saponin in *Albizia gummifera*, tannin in *Acacia nilotica*, and flavonoids in *Lawsonia inermis* is in agreement with earlier works (Trute *et al.*, 1997, Debella *et al.*, 2000, Nwude and Ibrahim, 1980, Dasgupta *et al.*, 2003). Dasgupta *et al* (2003) reported phenolic glycosides in *L.*

inermis, however, it was not found in the current study. It might be decomposed before extraction due to prolonged time after collection or improper storage. The dry leaves with unknown date of collection were purchased from the market.

The variation in activity of the extract type of the plants might be due to difference in the proportion of the active components responsible for the tested anthelmintic activity resulting from the difference in solubility either in water or methanol. The activity of botanical compounds found from plant materials largely depends on the type of extractant and the method of extraction (Eloff, 1998). Most of the plant extracts in the current study inhibited egg hatching at low concentration compared to other plants studied previously. For example, 7.1mg/ml of aqueous extract of *Annona senegalensis* inhibited only 11.5% eggs (Alawa *et al.*, 2003) and 2.5mg/ml of essential oil of *Ocimum gratissimum* induced 96.94% of egg hatching (Pessoa *et al.*, 2002). In the current study, the aqueous extract of *Ekebergia capensis* and *Coriandrum sativum* required a maximum of 0.25mg/ml; the aqueous extract of *Croton macrostachyus*, hydro-alcoholic extract of *C. sativum* and *Chenopodium ambrosioides* required maximum concentration of 0.5mg/ml to induce 100% egg hatch inhibition. Increasing the concentration of the plant extracts resulted in increased inhibition of egg hatching indicating dose dependent activity.

Plant materials evaluated in the current study had been identified from various sources to serve as anthelmintic agents by traditional healers or farmers in different parts of the world. Except for *Chenopodium ambrosioides* and *Albizia gummifera* literature survey indicated no adequate previous scientific evaluation conducted for other medicinal plants tested in the current study. There are only putative reports of the traditional use of the rest of plants for deworming of humans and animals.

The oil of *C. ambrosioides* had been used for many years to treat parasite infections in human and animals in different parts of the world before the emergence of more effective and less toxic modern anthelmintics (Githiori, 2004). A monoterpene (ascaridole) is believed to be the active principle in this plant (Ketzis *et al.*, 2002). Kato (1997) reported that the dry powder resulted in 33-36% reduction in mixed parasite infection in sheep. Ketzis *et al* (2002) reported that short-term administration of oil or freshly ground plant material of *C. ambrosioides* was ineffective in reducing adult *Haemonchus contortus* populations in goats although oil of *C. ambrosioides* at concentration of 3.3 μ l/ml induced 100% egg hatch inhibition. This finding is in agreement with the

current finding in which both aqueous and hydro-alcoholic extracts of *C. ambrosioides* produced complete egg hatch inhibition at concentrations lower than 1mg/ml. Long term treatment of animals in a given farm may reduce hatchability of eggs excreted with faeces resulting in both reduced re-infection and lighter worm loads by decreasing pasture contamination levels.

The current *in vitro* activity of *Albizia gummifera* on the eggs of *Haemonchus contortus* is in line with the *in vivo* anthelmintic activity demonstrated against mixed nematode infection in sheep (Biffa *et al.*, 2004). Although extracts from *A. gummifera* could not produce complete egg hatch inhibition at lower concentrations, the hatched larvae were found dead in all concentrations of aqueous extract and at concentrations higher than 0.125 mg/ml for hydro-alcoholic extract. This observation indicated that the plant extract has larvicidal effect on the first stage larvae (L1) of *H. contortus* which shows additional anthelmintic benefit of this plant. Complete larvicidal effect observed at the lowest concentration tested (0.01565mg/ml) for aqueous extract compared to the hydro-alcoholic extract might be due to the higher concentration of the active principle responsible for this activity in the aqueous extract than in hydro-alcoholic extract.

Higher egg hatching inhibition was recorded for the aqueous extract of *A. nilotica*, compared to the hydro-alcoholic extract. Moreover most of the first stage larvae hatched from eggs treated with aqueous extract were found dead. Both extracts of *T. schimperiana* did not inhibit egg hatching significantly and the minor inhibition observed was not in dose dependent manner; however the hatched larvae treated with higher doses of aqueous extract of *T. schimperiana* were found dead indicating the larvicidal property of the extract. *T. schimperiana* together with *Vernonia amygdalina* are considered principal remedies against gastrointestinal helminthes of cattle and sheep in Cameroon (Toyang *et al.*, 2005). Extracts from *Lawsonia inermis* are claimed for various activities including anthelmintic properties (Nwude and Ibrahim, 1980, Singh and Pandey, 1989). In the current study, however, *L. inermis* has shown no efficacy against eggs of *H. contortus* at the concentrations tested. This might be because of the difference in the composition of the plant in the current study from that had been used traditionally due to strain variation or lack of activity of the extract on the egg of *H. contortus* while having effect on other parasites (Raskin *et al.*, 2002).

Based on the concentration required to produce 50% egg hatch inhibition (ED₅₀), the six most potent extracts in a decreasing order were the aqueous extracts of *Ekebergia capensis*, Hydro-

alcoholic extract of *Chenopodium ambrosioides*, aqueous extracts of *Croton macrostachyus*, *Jatropha curcas*, *Coriandrum sativum* and *Hedera helix*. Although there were differences in the ED₅₀ for the extract types of the same plant, the differences were not statistically significant ($p>0.05$) for *C. sativum*, *J. curcas*, *H. helix* and *Albizia gummifera*. This could be due to the presence of similar or related chemicals having ovicidal property in both extract types in nearly equivalent proportion. The minimum concentration of albendazole required (0.125 µg/ml) to induce 100% egg hatch inhibition as well as the ED₅₀ value of 0.04 µg/ml is a good indication of susceptibility of strain of *Haemonchus contortus* employed in the current study to benzimidazole anthelmintics. According to WAAVP, (Coles *et al.*, 1992), eggs with an ED₅₀ value less than 0.1µg/ml are indicative of benzimidazole susceptibility.

Anthelmintic drugs can reach target site in nematode parasites either by oral ingestion or by uptake/diffusion through the external surface, termed cuticle. However studies have shown that transcuticular diffusion is a common means of entry for non-nutrient and non-electrolyte substances in nematodes (Geary *et al.*, 1999). It has also been shown that this route is predominant for the uptake of major broad-spectrum anthelmintics; benzimidazole, levamisole and ivermectin by different nematodes, cestode and trematode parasites as opposed to oral ingestion. Lipophilic anthelmintics such as albendazole have a greater capability to cross the external surface of the helminthes than the hydrophilic compounds. Transcuticular passive diffusion across the lipid component of the parasite cuticle is considered as the rate-limiting step in the process of drug entry into helminthes (Geary *et al.*, 1999).

The effect of hydro-alcoholic extract of *Albizia gummifera*, *Croton macrostachyus*, *Coriandrum sativum*, *Hedera helix* and *Ekebergia capensis* on the survival of the adult parasite is significantly higher compared to their aqueous counterparts and other plants. The possible explanation for the better activity of the hydro-alcoholic extracts compared to the aqueous extracts on adult parasites could be due to easy transcuticular absorption of the hydro-alcoholic extracts into the body of the parasite more than the aqueous extracts. Although distinct chemical profiles of the plant extracts are not known, in general, hydro-alcoholic extracts of plants may contain some non-polar organic chemicals with wide range of polarity than the aqueous extracts (Debella, 2002), rendering them higher lipid solubility than the aqueous counterparts. On the other hand, nematodes maintain a strongly buffered environment in the aqueous spaces of the cuticle structure, with a pH value of this

compartment being around 5.0 due to accumulation of organic acids, the end products of carbohydrate metabolism, including lactate, acetate, propionate, butyrate, etc. (Sims *et al.*, 1994). The interaction between these products and plant extracts may influence the polarity and the extent of absorption of the active components and hence *in vitro* anthelmintic activity.

In contrast, the aqueous extracts of the above mentioned plants have shown better activity in Egg Hatch Assay than the hydro-alcoholic. The possible explanation could be variation in the composition of active components responsible for Egg Hatch Assay and adult parasite effect in the two types of extracts. The other reason could be due to difference in structure of the eggshell and cuticle of *H. contortus* through which absorption of chemicals take place. From these findings it is evident that extract types of plants effective against one developmental stage of the parasite may not be efficient against other developmental stage.

From the aqueous extracts of all plants, only *Chenopodium ambrosioides* has induced greater than 50% mortality of adult parasites at the largest concentrations tested (8mg/ml). Both types of extracts of *Acacia nilotica*, *Terminalia schimperiana* and *Lawsonia inermis* have shown minor effect on the survival of the parasite at the concentrations tested, and the few deaths recorded were not in a dose dependent manner. This finding is in agreement with the *in vitro* Egg Hatch Assay result of the current study discussed earlier except the larvicidal effect observed for *A. nilotica* and *T. schimperiana*. Despite these findings, these plants have been claimed for their anthelmintic activity by traditional healers (Nwude and Ibrahim, 1980, Toyang *et al.*, 2005). The reason for the lack of efficacy in the current study might be due to difference in localities and age of the plant, method of preparation of the extract, species of parasite tested and/or the total absence of the real efficacy against nematodes. Worms treated by traditional healers are mainly those that are macroscopically visible after application of plant remedies. Most of the time what are considered expelled worms by traditional healers and owners of livestock are likely destrobilated segments of tapeworms, although this could be shade irrespective of treatment (Gthiori, 2004).

The faecal culture trial was carried out as a follow up of the activity of both plants in the Egg Hatch Assay. In Egg Hatch Assay, aqueous extract of both plants inhibited egg hatching completely at the concentration less than 2mg/ml. In faecal culture assay, although there was dose dependent reduction in the percentage of larval recovery, the maximum reduction observed was 63.93% in group of sheep treated with *Hedera helix* dose II. This finding indicates that in addition to their

effect on the adult parasites in the host, feeding of these plant materials could contribute to inhibition of development of excreted eggs to infective larvae on pasture. The probable reason for low efficacy in this regard compared to the eggs employed in the Egg Hatch Assay could be due to digestion, degradation or absorption of some of the active components responsible for inhibition of egg hatching or development within the digestive tract of the host. Some of the active components responsible for inhibition of egg hatching may have already been biotransformed to other chemicals or it may interact with different chemicals in the gut content (Raskin *et al.*, 2002). Moreover, close contact of each egg with the active component is not as easy as in the case of Egg Hatch Assay, which is conducted in the aqueous solution while faecal culture assay is conducted in the presence of faecal matter. Even in the control group, 40% of eggs originally determined in the faeces were not recovered on the 8th day, developed to 3rd stage larvae. This is because the larval recovery technique used in the current study is not exhaustive and there might be more larvae left in the faeces.

Toxicity tests showed no mortality and sign of toxicity in a group of mice administered orally with aqueous extract of *Coriandrum sativum*. This revealed the safety of this extract at doses as high as 15000mg/kg. This is in line with the daily use of this plant as spice in the preparation of various foodstuffs and for treatment of different animal and human ailments (Hedberg and Hedberg 2003). However administration of the same extract intraperitoneally exhibited mortality at lower dose. This variation could mainly be because of the higher extent of absorption from intraperitoneal space than the gastrointestinal tract. Moreover, orally administered drugs are exposed to enzymatic degradation and interaction with feed before being absorbed to systemic circulation. Orally the aqueous extract of *Hedera helix* on the other hand started killing the mice at a concentration as low as 2500mg/kg with some gross pathological observations as the dose increased. *H. helix* is considered a potential toxic plant in Europe and USA. Ingestion of this plant was reported to cause vomiting, diarrhea, nervous conditions and dermatitis in sensitive individuals (Swearingen and Dedrch, 2005).

The *in vitro* methods provide a means to screen rapidly for potential anthelmintic activities of different plant extracts. However due to considerable variation in conditions encountered *in vivo* like; metabolic biotransformation, interaction with feed materials and absorption, results obtained by *in vitro* method could not be extrapolated for *in vivo* activity. The results should be ascertained

by *in vivo* evaluation. In the current study, the aqueous extract of two of the plants that have demonstrated good *in vitro* activity were evaluated for *in vivo* activity in a ruminant- host parasite model using sheep artificially infected with *Haemonchus contortus*. *In vivo* efficiency of aqueous extracts of both plants on *H. contortus* infection in sheep based on FECRT on day 2 post treatment, did not persist for the following days, especially for *Coriandrum sativum*. This might be due to decrease in fecundity of female parasites because of treatment at early days when concentration of the extract is high in the animals, which gradually increased after excretion of the plant materials. The other reason might be faecal egg count reduction of the plant extracts could have been exaggerated at early days post treatment, due to dilution of faeces of the experimental animals by the large volume of plant materials administered (Githiori, 2004).

Unlike FECRT, efficacy test based on total worm count confirmed that except for *Coriandrum sativum* dose I, significant level of reduction was recorded in all treatment groups compared to untreated control group ($p < 0.05$). Groups of animals treated with plant extracts except for *C. sativum* dose I, have shown significantly higher reduction ($p < 0.05$) in number of male parasites compared to untreated control. On the other hand, significant reduction in proportion of female parasites was detected only for group of sheep treated with *Hedera helix* dose II. Increasing the dose of both plant extracts improved the efficacy against male parasites than female parasites. The possible explanation for higher susceptibility of male parasites than female parasites could be due to smaller size of the male parasites than the female parasites increasing their surface area to volume ratio. The fact that there was significant reduction in EPG on day 2 post treatment irrespective of absence in significant reduction in female worm count in groups of sheep treated with *C. sativum* dose II and *H. helix* dose I, justifies the effect of the plant extracts on fecundity of the parasites (Athanasiadou *et al.*, 2001).

Although significant efficacy was recorded based on total worm count reduction, the achieved reduction in groups of sheep treated with plant extracts is not to the level of therapeutically required amount. The lower efficacy could be due to low amount of active chemicals in the crude solution of the plant extracts. This could probably be improved by increasing the dose. However increasing the dose may cause toxicity to the host animals and care should be taken especially for *H. helix*, which was lethal to mice at dose of 2500mg/kg and above. The other possible means to

improve the efficacy could be repeated dosing for a few days (Prichard *et al.*, 1978). This could increase the extract and parasite contact time and presumably improve reduction in fecundity of the parasites observed in the current study during early days of treatment and may increase nematocidal efficacy. The 100% efficacy by FECRT as well as the complete absence of adult parasite in all sheep treated with albendazole suggested the susceptibility of the strain of *H. contortus* used in the current study to this drug and confirm the result found by Egg Hatch Assay.

Both plant extracts were not able to improve the PCV of *Haemonchus contortus* infected animals. However, Groups of sheep treated with both doses of *Hedera helix* were able to maintain their PCV, although they could not improve it, probably because of low reduction in worm burden (20-40%). The improvement in PCV of group of sheep treated with albendazole is due to complete removal of the parasite *H. contortus*. Each worm is responsible for daily loss of about 0.05ml of blood through ingestion and seepage from lesions (Urquhart *et al.*, 1996)

Treatment with plant extracts as well as albendazole has not shown statistically significant effect on live weight of the animals, rather all groups have shown minor reduction in weight. The reason behind this could be due to confinement of animals to dry feed together with leakage of blood and plasma protein to gastrointestinal tract by the parasites preventing weight gain (Johnstone *et al.*, 1998).

6. CONCLUSION AND RECOMMENDATIONS

Traditional medicine or ethno-veterinary medicine is widely practiced for treatment of various diseases of human and animals in different parts of the world and it is the predominant method in developing countries like Ethiopia. Anthelmintic medication has its origin in the use of plant preparations, although they were rapidly disappeared due to toxicity and low efficacy from human and veterinary use with the discovery of synthetic anthelmintic compounds.

In the current study, extracts from plants like *Croton macrostachyus*, *Ekebergia capensis*, *Coriandrum sativum*, *Hedera helix*, *Albizia gummifera* and *Chenopodium ambrosioides* have shown promising *in vitro* anthelmintic activity against eggs and adult *H. contortus*. Extracts from *Jatropha curcas*, *Acacia nilotica*, *Terminalia schimperiana*, and *Lawsonia inermis* have shown poor activity on survival of adult parasites at concentrations tested; however both extracts of *J. curcas* and *A. nilotica* have shown good activity on egg hatch inhibition.

Aqueous extracts of both *C. sativum* and *H. helix* demonstrated dose dependent reduction in the worm burden of *H. contortus* in sheep although not to the therapeutically required level. *H. helix* demonstrated better activity than *C. sativum*. The activity might be improved by increasing the dose or by repeated treatment.

Based on the above concluding remarks, the following recommendations are made:

- Since *in vitro* anthelmintic evaluation methods are simple and cost effective than *in vivo* method, preliminary *in vitro* screening of several other claimed medicinal plants so as to identify the ones with promising anthelmintic properties should be conducted before directly going to *in vivo* evaluation
- *In vitro* evaluation of different plant parts and extracts that has demonstrated promising activities in the current study should be done so as to reach at the most active part.
- *In vivo* evaluation of the rest of plant extracts which has shown promising *in vitro* anthelmintic activity should be conducted in different animal models and different helminth parasites especially in rodent parasites such as *Heligmosomoides polygyrus* before evaluation in higher animals
- Activity guided fractionation of the total extract of the plants that demonstrated good

activity should be carried out in order to facilitate the isolation and characterization of the most active components.

- Documentation of Ethnoveterinary/medicine information on the traditional use of the plants, like parts used, method of preparation and the health problem for which they use should be obtained before beginning evaluation of a given traditional medicine.
- Toxicological evaluation (acute, subacute and chronic) of the most active plant extracts should be carried out in order to promote their ultimate utilization by the community or facilitate dosage formulation by herbal drug industries.

7. REFERENCES

- Abebe D, Debella A, Urga K. (2003) Illustrated checklist of medicinal plants and other useful plants of Ethiopia. National Health and Nutrition Research Center, Addis Ababa, Ethiopia. pp 39 - 53.
- Ademe M. (2003) Observation on Albendazole resistant Haemonchosis in Awassa Agricultural college farm. *Ethiop. Vet. J.*, **7**: 78-79
- Ademola I O, Fagbemi B O, Idowu S O. (2004) Evaluation of Anthelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *In vitro* and *in vivo* studies. *Vet. Parasitol.* **122**:151-164.
- Alawa C B, Adamu A M, Gefu J O, Ajansui O J, Abdu PA, Chiezey N P, Alawa J N, Bowman D D. (2003) *In vitro* screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. *Vet. Parasitol.* **113**: 73-81.
- Alawa J P, Jokthan G E, Akut K. (2002) Ethnoveterinary medical practice for ruminants in the subhumid zone of northern Nigeria. *Prev.Vet. Med.* **54**: 79-90.
- Ali B H, Bashir A K, Tanira M O. (1995) Anti-inflammatory, antipyretic, and analgesic effects of *Lawsonia inermis* L. (henna) in rats. *Pharmacol.* **51**: 356–363.
- Allonby E W, and Urquhart M. (1975) The epidemiology and the pathogenic significance of Haemonchosis in Merino Folk in Eastern Africa. *Vet. Parasitol.* **1**: 1001-1007.
- Al-Said M S, Al-Khamis K I, Islam M W, Parmar N S, Tariq M, Ageel A M. (1987) Post-coital antifertility activity of the seeds of *Coriandrum sativum* in rats. *J. Ethnopharmacol.* **21**: 165-73.
- Asfaw H. and Thulin M. (1989) Fabaceae: In Flora of Ethiopia; Pisstosporaceae to Araliaceae, Hedberg I. and Edwards S. (eds), Vol 3, Addis Ababa, Ethiopia, pp.86.
- Athanasiadou S, Kyrazakis I, Jakson F, Coop RL. (2001) Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol.* **99**: 205- 219.
- Bain R K. (1999) Irradiated vaccines for helminth control in livestock. *Int. J. Parasitol.* **29**: 185-191.
- Baker R L, Reynolds L, Lahlou K A, Rege J E, Bekele T, Mukassa M, Rey B. (1992) Prospects of breeding for resistance to endoparasites in small ruminants in Africa. Proceedings of the 2nd conference of Small Ruminant Research Network, Arusha, Tanzania.
- Barger I A. (1996) Prospects for integration of Novel parasite control options into grazing system. *Int. J. Parasitol.* **26**: 1001-1007.

- Bayu K. (2003). Efficacy of Duexamine against field infections of GI parasites in sheep and goats. Proceedings of the Ethiopian Pharmaceutical Association, Addis Ababa, Ethiopia.
- Bedir E, Kirmizipekmez H, Sticher O, Calis I. (2000) Triterpene saponins from the fruits of *Hedera helix*. *Phytochemistry*. **53**: 905-909.
- Bekele T, Weldeab T, Lahlou-kassi A, sherngton J.(1992) Factors affecting morbidity and mortality on –farm and on –station in the Ethiopian highland sheep. *Acta Tropica*, **52**: 99-109.
- Biffa D, Nurfeta A. Yilma Jobre Y. (2004) Evaluation of anthelmintic activities of crude leaf extracts of three indigenous herbal plants against ovine gastrointestinal nematodes. *Ethiop. Vet. J.* **8**: 57-58.
- Chandrawathani P, Jamnah O, Waller P J, Larsen M, Gillespie A T, Zahari W M. (2003) Biological control of nematode parasites of small ruminants in Malaysia using the nematophagous fungus *Duddingtonia flagrans*. *Vet. Parasitol.* **117**: 173-183.
- Chartier C, Etter E, Hoste H, Pors I, Koch C, Dellac B. (2000) Efficacy of copper oxide needles for the control of nematode parasites in dairy goats. *Vet. Res. Commun.* **24**: 389-399.
- Chithra V, and Leelamma S. (1997) Hypolipidemic effect of coriander seeds (*Coriandrum sativum*): mechanism of action. *Plant Foods for Human Nutrit.* **51**: 167-172.
- Coles G C, Bauer C, Borgsteede F H, Geerts S, Klei T R, Taylor M A, Waller P J. (1992) World Association for Advancement of Veterinary Parasitology (WAAVP) Methods for detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* **44**: 35-43.
- Dasgupta T, Rao A R, Yadava P K. (2003) Modulatory effect of Henna leaf (*Lawsonia inermis*) on drug metabolising phase I and phase II enzymes, antioxidant enzymes, lipid peroxidation and chemically induced skin and fore stomach papillomagenesis in mice. *Mol. Cell. Biochemist.* **245**: 11–22.
- Debella A. (2002) Manual for phytochemical screening of medicinal plants, Ethiopian health and Nutrition Research Institute, Addis Ababa, Ethiopia
- Debella A, Haslinger E, Schmid M G, Franz B F, Michl G, Abebe D, and Kuner O. (2000) Triterpenoid saponins and saponin lactones from *Albizia gummifera*. *Phytochemistry* **53**: 885-892
- Deressa A, Mekonnen S, Tollosa A. (2003) Ethnoveterinary use of plants in North Shoa zone, Ethiopia. *Ethiop. Vet. J.* **7**: 11-17.
- Dermaxime Ltd. (2005) Dermaxime International Cosmetics and skin care products. URL:

<http://www.dermaxime.co.za>

- Dessisa D. (2001) A preliminary economic evaluation of medicinal plants in Ethiopia: trade, volume and price In: proceedings of the National Workshop on Biodiversity Conservation and Sustainable use of Medicinal Plants in Ethiopia, Addis Ababa, Ethiopia, Medhin Z, and Abebe D. (eds), 28th April-May 1st, 1998, pp 176-188.
- Desta B. (1995) Ethiopian traditional herbal drugs. Part I: Studies on the toxicity and therapeutic activity of local taenicidal medications. *J. Ethnopharmacol.* **45**: 27-33.
- Eloff J N. (1998) Which extractant should be used for screening and isolation of antimicrobial components from plants? *J. Ethnopharmacol.* **60**: 1-8.
- Endale A, Kassa M, Gebre-Mariam T. (1998) *In vivo* anthelmintic activity of the extract of the seeds of *Glinus lotoidus* in Albino mice infested with *Hymenolepis nana* worms. *Ethiop. Pharma. J.* **16**: 34-41.
- FARM Africa and SOS Sahel International/ UK (2004) Participatory Forest Management Programme (PFMP), Commercialization of Medicinal Trees in Bonga (Project Profile), March 2004, Addis Ababa. URL: <http://www.pfmp-farmsos.org>.
- Friis I and Gilbert M G. (2000) Chenopodiaceae. In: Flora of Ethiopia and Eritrea; Magnoliaceae to Flacourtiaceae, Edwards S, Mesfin T, Sebsebe D, Hedberg I. (eds), Vol. 2, Published by National Herbarium of Addis Ababa University and Uppsala University, Uppsala, pp.277.
- Gathuma J M, Mbaria J M, Wanyama J, Kaburia H F, Mpoke L, Mwangi J N. (2004). Efficacy of *Myrsine africana*, *Albizia anthelmintica* and *Hilderbrandtia sepalosa* herbal remedies in Samburu district, Kenya. *J. Ethnopharmacol.* **91**:7-12.
- Geary T G, Sangster N C, Thompson D P. (1999) Frontiers in anthelmintic pharmacology. *Vet. Parasitol.* **84**: 275-295.
- Getahun A. (1976) Some common medicinal and poisonous plants used in Ethiopia folk medicine, AAU, URL: [www//http://ip.aaas.org/tekindex.nsf/](http://ip.aaas.org/tekindex.nsf/)
- Gidey M, and Amini G. (2003) An ethinobothanical survey on plants of veterinary importance in two woredas of southern Tigray, northern Ethiopia. *SINET Eth .J. science.* **26**:123-136.
- Gilbert M G. (2000) Lythraceae In: Flora of Ethiopia and Eritrea; Magnoliaceae to Flacourtiaceae, Edwards S, Mesfin T, Sebsebe D, Hedberg I. (eds) Vol. 2, Published by National Herbarium of Addis Ababa University and Uppsala University, Uppsala, pp.394.

- Githigia S M, Thamsborg SM, Larsen M, Kyvsgaard NC, Nansen P. (1997) The preventive effect of the fungus *Duddingtonia flagrans* on trichostrongyle infections of lambs on pasture. *Int. J. Parasitol.* **27**: 931-939.
- Githiori J B, Hoglound J, Waller P J, Baker R L. (2002) Anthelmintic activity of Preparations from *Myrsine africana* and *Rapanea melanophloeos* against the nematode parasite, *Haemonchus contortus*, of sheep. *J. Ethnopharmacol.* **80**: 187-191.
- Githiori J B, Hoglound J, Waller P J, Baker R L. (2003) Evaluation of anthelmintic properties of some plants used as livestock dewormers by pastoralists and smallholder farmers in Kenya against *H. polygyrus* infection in mice. *Vet. Parasitol.* **30**: 1-12.
- Githiori J B. (2004) Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastoralists and smallholder farmers in Kenya. Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Gray A M and Flatt P R. (1999) Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant *Coriandrum sativum* (coriander). *Br. J. Nutrit.* **81**: 203-209.
- Habte-Silasie M, Mesfin T, WoldeMichael G, Getaneh K, Tilahun G, Mesfin T, Kinfe G, Yehualashet T. (1991) The significance of helminth parasites in livestock production. In: Proceedings of the 3rd National Livestock Improvement Conference, held in Addis Ababa, May 24-26, 1989..
- Harder A. (2001) Mechanism of action and resistance of antiprotozoal and anthelmintic drugs. In: encyclopedic reference of parasitology, diseases, treatment, therapy, Mehlhorn H. (ed) ,2nd ed, Springer, Berlin, Heidelberg, Newyork , 213-216.
- Hedberg I. and Hedberg O. (2003) Apiaceae In: Flora of Ethiopia and Eritrea; Apiaceae to Dipsacaceae, Hedberg I, Edwards S and Sileshi N. (eds) Vol 4, Published by National Herbarium of Addis Ababa University and Uppsala University, Uppsala, pp. 1-45.
- Herlich H. (1978) The importance of Helminth infection in Ruminants. *World Anim. Rev.* **26**: 22-26.
- Hounzangbe-Adote S, Fouraste I, Moutairou K, Hoste H. (2005) *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode *Haemonchus contortus*. *Res. Vet. Sci.* **78**: 155-160.
- Hubert J. and Kerboeuf D. (1992) A microlarval development assay for the detection of anthelmintic resistance in sheep nematode. *Vet. Rec.* **130**: 442-446.
- Hussein N, Hassen A, Asaye Z. (1999) Efficacy of Panacour, Rintal and Duaxamine against field infections of GI parasites in goats. *Ethiop. Vet. J.* **3**: 9-16.

- Iqbal Z, Lateef M, Ashraf M, Jabbar A. (2004) Anthelmintic activity of *Artemisia brevifolia* in sheep. *J. Ethnopharmacol.* **93**: 265-268.
- Johnstone C, Guerrero J, Chou S, Melissa D, Howe-Smith R, Eisenberg A. (1998) The Nematodes, *Haemonchus*– pathogenesis In: Parasites and Parasitic Diseases of Domestic Animals an online book of text and images URL: [http://www. cal.vet.upenn.edu/merial/Trichos/trich_5b.htm](http://www.cal.vet.upenn.edu/merial/Trichos/trich_5b.htm)
- Jost C C, Sherman D M, Thomson E F, Hesselton R M. (1996) Kamala (*Mallotus philippinensis*) fruit is ineffective as an anthelmintic against gastrointestinal nematodes in goats indigenous to Balochistan, Pakistan. *Small Rum. Res.* **20**:147-153.
- Julien J, Gasquet M, Maillard C., Balansard G, and Timon D P. (1985) Extracts of the Ivy plant, *Hedera helix*, and their anthelmintic activity on liver flukes. *Planta Medica.* **51**: 205-208.
- Kassai T. (1999) Veterinary helminthology. Read Educational and Professional Publishing LTD, London, 260 pp.
- Kato S. (1997) Effects of the oil of *Chenopodium ambrosioides* on sheep: efficacy as an anthelmintic for treatment of gastrointestinal nematodes in sheep, DVM thesis, Cornell University, NY, USA.
- Ketzis J K, Taylor A, Bowman D D, Brown D L, Warnick L D, and Erb H N. (2002) *Chenopodium ambrosioides* and its essential oil as treatments for *Haemonchus contortus* and mixed adult-nematode infections in goats. *Small Rum. Res.* **44**: 193-200
- Knox M R. (2002) Effectiveness of copper oxide wire particles for *Haemonchus contortus* control in sheep. *Australian Vet. J.* **80**: 224-227.
- Kohler P. (2001) The biochemical basis of anthelmintic action and resistance. *Int. J. Parasitol.* **31**:336-345.
- Koko W S, Galal M, Khalid H S. (2000) Fasciolicidal efficacy of *Albizia anthelmintica* and *Balanites aegyptiaca* compared with albendazole. *J. Ethnopharmacol.* **71**:247-252
- Larsen, M. (2000) Prospects for controlling animal parasitic nematodes by predacious micro fungi. *Parasitology.* **120**: 121-131
- Maingi N, Bjorn H, Gichohi V M, Munyua W K, Gathuma J M. (1998) Resistance to bendimisasoles and levamisole in nematode parasites of sheep in Nyandarua District of Kenya. *Acta Tropica.* **69**:31-40.
- Makkar H P S, Aderibigbe A O, Becker K. (1998) Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and

- toxic factors. *Food Chemist.* **62**: 207–215.
- Makkar H P S. and Becker K. (1999) Nutritional studies on rats and fish (carp *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of a non-toxic provenance. *Plant Foods for Human Nutrit.* **53**:183–192.
- Malekzadeh F. (1968) Antimicrobial activity of *Lawsonia inermis* L. *Appl Microbiol*, **16**: 663–664.
- McClure SJ. (2000) Sheep immunity to gastrointestinal nematode parasites- review. URL: <http://www.csiro.au/scips>.
- McCorkle C M. (1986) An introduction to ethnoveterinary research and development. *J. Ethnobiol.* **6**: 129-149.
- Melo E A, Bion F M, Filho J M, Guerra N B. (2003) *In vivo* antioxidant effect of aqueous and etheric coriander (*Coriandrum sativum* L.) extracts. *Europ. J. of Lipid Sci. and Technol.* **105**:483-487.
- Mesfin T, and Obsa T. (1994) Ethiopian traditional veterinary practices and their possible contribution to animal production and management. *Rev. Sci. and Technol.* **13**: 417-424.
- Mesfin T, Sisay L, Gebru B, Gebrenigus T, Zeleke A.(1995)Evaluation of some traditoal herbs in the highlands of Ethiopia for their anthlmintic value against helminth parasites in sheep. Phase I Research report, Pastoral and environmental network in Africa, Addis Ababa, Ethiopia.
- Moholland D A, Parel B, Coombes H. (2000) The chemistry of Meliaceae and Pateroxylaceae of southern and eastern Africa and Madagascar, *Current Organic Chemist.* **4**:1011-1054.
- Mugambi J M, Bain R K, Wanyangu S W, Ihiga, M A, Duncan J L, Murray M, and Stear M J.(1997). Resistance of four sheep breeds to natural and subsequent artificial *Haemonchus contortus* infection. *Vet. Parasitol.* **69**: 265-273.
- Nsekuye B. (1994). Traditional Veterinary Practice in Africa, Technical cooperation –Federal republic of of Germany, Eschaborn.
- Nwude N, and Ibrahim M A. (1980) Plants used in traditional veterinary medicinal practice in Nigeria. *J. vet. pharm. Therapeut.* **3**: 261-273.
- Ofulla A V, Chege G M, Rukunga G M, Kiarie F K, Githure J I, Kofi-Tsekpo M W.(1995).In vitro antimalarial activity of extracts of *Albizia gummifera*, *Aspilia mossambicensis*, *Melia azedarach* and *Azadirachta indica* against *Plasmodium falciparum*. *Afr J Hlth. Sci.* **2**: 309-311.
- Perry B, Randolph T, McDermott J, Sones K, and Tornton P K.(2002) investing in animal health research to alleviate poverty ILRI (International Livestock Research Institute, Nairobi, Kenya, pp. 148 .

- Pessoa L M ,Morias S M, Bevilaqua C M L, Luciano J H. (2002). Anthelmintic activity of essential oil *Ocimum gratissimum* Linn.and euginol against *Haemonchus contortus*. *Vet. Parasitol.* **109**: 59-63
- Prenner L. (2004) *Hedera helix* L URL:www// <http://institut.physiochem.uni-bonn.de/>
- Prichard P K, Hennessy D R, Steel J W. (1978). Prolonged administration: a new concept for increasing the spectrum of and effeciveness of anthelmintics. *Vet. Parasitol.* **4**: 309-315.
- Radostits O M, Blood D C, and Gay C C. (1994). Vetrinary Medicine. A text book of the diseases of cattle, sheep, pig, Goats and Horses. 8th ed., Billiere Tindall, London PP.1228
- Ramadan M. and Morsel J. (2002) Oil composition of coriander (*Coriandrum sativum* L.) fruit-seeds. *Europ. Food Res. Technol.* **215**: 204-209
- Raskin I, Ribnicky M, Komamytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A. Moreno DA, Ripoll C, Yakoy N, O'Neal JM, Cornwell T, Pastor I, Fridlender B. (2002) Plants and human health in the twenty-first century, *Trends Biotechnol.* **20**: 522-531.
- Satirijia F, Nansen P, Bjorn H, Murtini S. (1994) Effect of Papaya latex (*Carica papaya*) against ascaris suum in naturally infected pigs. *J. Helminthol.* **68**: 343-346.
- Satirijia F, Retnani E, Ridwan Y, Tiuria, R. (2001) Potential use of Herbal anthelmintics as alternative antiparasitic drugs for smallholder farmers in developing countries, Livestock community and environment, proceedings of the 10th conference of the association of institutions for Tropical Vet. Medicine, Copenhagen, Denmark.
- Schallig H D, van Leeuwen M A, Cornelissen A W. (1997) Protective immunity induced by vaccination with two *Haemonchus contortus* excretory secretory proteins in sheep. *Parasite Immunol.* **19** : 447-453.
- Schillhorn van Veen, T W. (1997) Sense or nonsense? Traditional methods of animal parasitic disease control. *Vet. Parasitol.* **71**: 177-194.
- Sewarm V, Raynor W, Mulholland A, Raidoo M. (2000) The uterotonic activity of compounds isolated from supercritical fluid extract of *Ekebergia capensis*. *J. Pharmaceut. Biomed. Analy.* **24**: 133-145.
- Siddhuraju, P, Vijayakumari K, Janardhanan K. (1996) Chemical composition and nutritional evaluation of an underexploited legume, *Acacia nilotica* (L.) Del., *Food Chemist.* **57**: 385-391
- Sims S M, Ho N F, Magas L T, Geary T G, Barshum C L, Thompson D P. (1994) Biophysical model of the transcuticular excretion of organic acids, cuticle pH and buffer capacity in

gastrointestinal nematodes. *J. Drug Target.* **2**:1-8

- Singh V K, Pandey D K. (1989) Fungitoxic studies on bark extract of *Lawsonia inermis* against ringworm fungi. *Hindustan Antibiot Bull.* **31**: 32–35.
- Smith W D, Newlands F J, Smith S K, Pettit D, and Skuce P J. (2003) Metalloendopeptidases from the intestinal brush border of *Haemonchus contortus* as protective antigens for sheep. *Parasite Immunol.* **25**: 313-323.
- Sonstergard, T S and Gasbarre L C. (2001) Genomic tools to improve parasite resistance. *Vet. Parasitol.* **101**:387-403.
- Soulsby E J. (1986) Helminths, arthropods and protozoa of domesticated animals Balliere, Tindall, London, 809pp.
- Styles T. and White F. (1989) Meliaceae. In: Flora of Ethiopia; Pisstosporaceae to Araliaceae, Hedberg I. and Edwards S. (eds) Vol 3, Addis Ababa, Ethiopia pp.489.
- Swearingen J M, and Dedrch S. (2005) English ivy *Hedera helix* L. Ginseng family (Araliaceae). Plant Conservation Alliance, Alien Plant Working Group. URL:<http://www.nps.gov/plants>
- Tembely S, Bengaly K, Berchmoes W. (1994) Effect of strategic anthelmintic treatment on growth performance and survival rate of native lambs in the sub humid environment in Mali, Small Ruminant Research And Development in Africa, proceedings of the second Biennial Conference of African Small ruminant Research Network, AICC Arusha, Tanzania
- Thulin M.(1989) Fabaceae. In: Flora of Ethiopia; Pisstosporaceae to Araliaceae, Hedberg I. and Edwards S. (eds) Vol 3, Addis Ababa, Ethiopia pp.96.
- Tollosa A. (1997) Ethnoveterinary knowledge in central highlands of Ethiopia, Sheno, North Showa. Proceedings, Ethiopian Vet. Association, 11th annual conference, Addis Ababa, Ethiopia.
- Toyang N J, Nuwanyakpa M, Ndi C, Django S, Kiyuy W C. (2005) Ethnoveterinary medicine practices in the Northwest Province of Cameroon. URL: <http://www.nuffic.nl/ciran/ikdm/3-3/articles/toyang.html>
- Treating livestock with medicinal plants, Beneficial or toxic? *Chenopodium ambrosioides*. URL: <http://www.ansci.cornell.edu/plants/medicinal/epade.html>
- Trute A, Gross J, Mustscler E. (1997) *In vitro* antispasmodic compounds of the dry extract obtained from *Hedera helix*. *Planta Medica.* **63**:125-129
- Urquhart G M, Armour J L, Duncan J L, Dunn A M, Jennings F W. (1996) Veterinary Parasitology. Longman scientific and technical paper, 264pp.

- van Houtert M F. and Sykes A R. (1996) Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *Int. J. Parasitol.* **26**: 1151-1167.
- van Wyk J A, Stenson M O, Van Dar J S, Vorster R J, Vilson P G. (1999) Anthelmintic resistance in South Africa, surveys indicated serious situation in sheep and goat farms *Trop. Anim. Hlth. Prod.* **46**: 265-270.
- Vollesen K. (1995a) Euphorbaceae. In: Flora of Ethiopia and Eritrea; Canellaceae to Euphorbaceae, Edwards S, Mesfin T, Hedberg I.(eds), Vol. 2 Addis Ababa, pp.324
- Vollesen K. (1995b) Combretaceae. In: Flora of Ethiopia and Eritrea; Canellaceae to Euphorbaceae, Edwards S, Mesfin T, Hedberg I.(eds), Vol. 2 Addis Ababa, pp.128
- Waller P. (1997) Sustainable helminth control of ruminants in developing countries, *Vet. Parasitol.* **71**:195-207.
- Waller P J, and Thamsborg S M. (2004) Nematode control in green ruminant Production systems, *Trends Parasitol.* **20**: 493-497.
- Whittier D, Zajac A, Umberger H. (2003) Control of Internal Parasites in Sheep, Virginia Cooperative Extension, Publication No 410-027, October 2003
- Wirtu G, Adunga G, Samuel T, Kelbessa E, Geleto A. (1999) Aspects of knowledge attitude and practices of animal health problems in central Ethiopia, Ethnoveterinary medicine: Alternatives for livestock development. Proceedings of an International Conference, 4-6 November 1997, Pune, India.
- Woolaston R R and Baker R L. (1996) Prospects of breeding small ruminants for resistance to internal parasites. *Int. J. Parasitol.* **26**: 845-855.

Annex 1. Phytochemical screening methods (Debella, 2002)

	Secondary metabolite	Chemicals /method	Indicator
1	Polyphenols	1% FeCl ₃ + 1ml of K ₃ Fe (CN) ₆	green blue color
2	Cyanogenic glycosides	1% picric acid + 10% Na ₂ CO ₃	brick red color
3	Saponins	heat	formation of persistent Honey comb froth
4	Phytosteroides and withanoids	CHCl ₃ + H ₂ SO ₄ (conc.)	red, reddish brown or violet color
5	Phenolic glycosides	ferric sulphate	dark violet color precipitate
6	Flavonoids	2% lead acetate	yellow or orange color
7	Tannins	2% Sodium Chloride, gelatin	white precipitate
8	Alkaloids	Dragendorffs, Mayers reagent and 0.5% Tannin solution	yellow orange, white and yellowish-white precipitate
9	Glycosides (oligosaccharids)	2N hydrochloric acid, chloroform, benedict's solution	red color
10	Antraquinone glycosides	2N HCL, benzene, 10% ammonia	red color

Annex 2. *In vitro* assays of different plant preparations evaluated against developmental stages of nematode *H. contortus*.

Plant spp.	Parts used	Extract type	Type of test	Result	Reference
<i>Ocimum gratissimum</i>	Aerial part	Essential oil	EHA	100% inhib. At 0.5% conc.	Pessoa <i>et al.</i> , 2002
<i>Vernonia amygdalina</i>	Leaves	aqueous	EHA	No sig. effect	Alawa <i>et al.</i> , 2003
<i>Annona senegalensis</i>	Stem barks	aqueous	EHA	11.5% inhibition	Alawa <i>et al.</i> , 2003
<i>Artemisia brevifolia</i>	The whole plant	Aqueous	Effect on adult parasite	Significant inhibition at conc. 25mg/ml	Iqbal <i>et al.</i> , 2004
		Hydro-alcoholic	'' ''	No sig. effec.	
<i>Zanthoxylum zanthoxyloides</i> <i>Newbouldia laevis</i> <i>Morinda lucida</i> <i>Carica papaya</i>	Leaves Leaves Leaves seeds	Hydro-alcoholic '' '' ''	EHA LMI and Effect on adult parasite	Each has shown significant anthelmintic effect	Hounzangbe-Adote <i>et al.</i> , 2005

Annex 3. *In vivo* activity of some plant preparations evaluated against *H. contortus*

Plant spp.	Parts used	Extract type	host	efficacy	Reference
<i>Albizia anthelmintica</i>	Bark	aqueous	sheep	34% (EPG)	Githiori <i>et al.</i> , 2003
<i>Myrsine Africana</i> <i>Rapanea melanophlores</i>	Leaves and fruits Fruits	decoction	sheep	Not effective	Githiori <i>et al.</i> , 2002
<i>Atemisia brevifolia</i>	Whole plant	Crude plant material Aqueous	Sheep sheep	62.1% 67.2%	Iqbal <i>et al.</i> , 2004
<i>Chenopodium ambrosioides</i>	Whole plant and essential oil	oil	goats	Not effective	Ketzis <i>et al.</i> , 2002

Annex 4. *In vivo* evaluation of some plant preparations against different nematode infections in small ruminants.

Plant spp.	Parts used	Extract type	host	Efficacy (FECR)	Reference
<i>Albizia gummifera</i>	Barks	aqueous	sheep	34%	Biffa <i>et al.</i> , 2004
<i>Morinda citrifolia</i>	fruits	decoction	sheep	73.6-88.8%	Satirijia <i>et al.</i> , 2001
<i>Myrsine Africana, Albizia anthelminthica</i> <i>Hilderbrantia sepalosa</i>	Leaves Leaves Leaves	Crude product	Sheep	77% 89.9% 90%	Gathuma <i>et al.</i> , 2004
<i>Khaya senegalensis</i>	barks	Ethanol	sheep	88.82%	Ademola <i>et al.</i> , 2004
<i>Mollotus philippinesis</i>	fruits	Crude powder	goats	No sign. effect	Jost <i>et al.</i> , 1996