*IN VITRO S*TUDY OF THE EFFICACY OF SIX ETHNOVETERINARY PLANTS SPECIES AGAINST PARASITIC NEMATODES AND *Caenorhabditis elegans* IN NIGERIA

by

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LIST OF ABBREVIATIONS

Conc.	Concentration
CT	Condensed tannin
DMSO	Dimethyl sulfoxide
EPG	Egg per gram
FEC	Faecal egg count
GAE	Gallic acid equivalent
GCMS	Gas chromatography mass spectrometry
GFP	Green fluorescent protein
GIN	Gastrointestinal nematode
GIT	Gastrointestinal tract
GluClα	Glutamate gated chloride channel
HT	Hydrolysable tannin
hrs	hours
IC	Inhibitory concentration
L1	First stage larva/larvae
L3	Third stage larva/larvae
L4	Fourth stage larva/larvae
nACHRs	Nicotinic acetylcholine receptors
Neg.	Negative
NGM	Nematode growth medium
rmp	Revolution per minute
RNA	Ribonucleic acid
RNAi	Ribonucleic acid interference
SPSS	Statistical package for social science

TAE	Tannic acid equivalent
TPC	Total phenolic content
TSBP	Thiol Sepharose binding fraction
TTC	Total tannins content
v/v	Volume/volume
w/v	Weight/volume
WAAVP	World Association for the Advancement of Veterinary Parasitology
WHO	World Health Organisation

KAJIAN *IN VITRO* KEBERKESANAN ENAM SPESIES TUMBUHAN ETNOVETERINAR TERHADAP NEMATOD PARASIT DAN *Caenorhabditis elegans* DI NIGERIA

ABSTRAK

Kawalan nematod trikostrongil menggunakan dadah sintetik telah mengakibatkan beberapa komplikasi seperti peningkatan kerintangan nematod kepada dadah sintetik, perubahan kualiti produk dari haiwan yang dirawat akibat residu dadah dalam tisu haiwan. Ini menyebabkan penyelidikan untuk meneroka alternatif kepada dadah konvensional. Tujuan kerja ini adalah untuk menjalankan kajian *in vitro* daripada ekstrak akueus dan ekstrak metanol *Detarium microcarpum, Guiera senegalensis, Cassia siamea, Parkia biglobosa, Gmelina arborea* dan *Boswellia dalzielii* terhadap nematod trikostrongil ruminan kecil dan strain *Caenorhabditis elegans* DA1316 rintang ivermektin. Serbuk batang kulit kayu setiap tumbuhan telah diekstrak secara maserasi dalam air dan metanol. Pemeriksaan fitokimia ekstrak menunjukkan kehadiran alkaloid, flavonoid, tanin, sebatian fenolik, steroid dan terpenoid tetapi taburannya tidak sama rata di antara jenis tumbuhan dan antara ekstrak akueus dan metanol. Ekstrak tumbuhan telah diuji secara *in vitro* terhadap kemotilan larva L3 nematod trikostrongil pada kepekatan 0.2, 0.4, 0.6, 0.8, 1.0 dan 2.0 mg / ml. *D. microcarpum* adalah tumbuhan yang paling poten mengikut piawaian “World Association for the Advancement of Veterinary Parasitology (WAAVP)”. kerana ekstrak akueus dan ekstrak metanol merencat kemotilan larva sehingga 90%. *G. senegalensis* adalah tumbuhan kedua paling potent merencat 80% dan 90% dalam ekstrak akueus dan metanol masing-masing. Ekstrak *C. siamea* yang sederhana berkesan pada 2.0 mg / ml adalah tumbuhan ketiga yang paling berkesan. Ekstrak *D.*

microcarpum, G. senegalensis, C. siamea tertakluk kepada ujian selanjutnya terhadap penetasan telur, perkembangan larva, penghijrahan larva dan kemotilan larva *C. elegans* DA1316 (strain rintang ivermektin) dan *C. elegans* Bristol N2. Ekstrak akueus dan ekstrak metanol *D. microcarpum* telah disahkan yang paling poten berbanding ekstrak tumbuhan lain terhadap kedua-dua strain *C. elegans* pada 2.0 mg / ml. *G. senegalensis* adalah tumbuhan poten kedua terhadap *C. elegans* walaupun ekstrak akueus tidak berkesan terhadap penetasan telur pada 2.0 mg / ml. Aktiviti larvisid dan ovisid ekstrak tumbuhan terhadap *C. elegans* Bristol N2 adala sama jika dibanding aktiviti lavisid dan ovisid ekstrak terhadap dengan *C. elegans* DA1316 ($P > 0.05$). Kombinasi kepekatan terendah (0.2 mg / ml) ekstrak metanol *D. microcarpum* dan 0.02 μg / ml ivermektin menghasilkan ke potenan tertinggi terhadap kemotilan kedua-dua strain *C. elegans*. Ekstrak *D. microcarpum* dan *G. senegalensis* telah diuji terhadap penghijrahan larva L3 *Haemonchus contortus*, *Trichostrongylus colubriformis, Ostertagia circumcincta* dan *Oesophagostomum* spp. *Haemonchus contortus* dibuktikan yang paling sensitif terhadap semua ekstrak tumbuhan jika dibandingkan spesies yang lain. *Oesophagostomum* spp adalah yang kurang sensitive jikadibandingkan dengan spesies yang lain. Semua spesies lebih sensitif kepada *D. microcarpum* daripada *G. senegalensis* ($P < 0.05$). Ekstrak *D. microcarpum* mengandungi jumlah fenol dan tanin yang tertinggi diikuti oleh ekstrak *G. senegalensis*. Ekstrak metanol mengandungi kuantiti fenol dan tanin yang lebih tinggi daripada ekstrak akueus. Kajian ini mengesahkan *D. microcarpum* dan *G. senegalensis* sebagai tumbuhan paling poten yang boleh berfungsi sebagai sumber sebatian utama untuk antelmintik semulajadi terhadap nematod trikostrongil. Ekstrak itu juga disahkan berfungsi dengan baik dalam kombinasi dengan ubat sintetik.

*IN VITRO S*TUDY OF THE EFFICACY OF SIX ETHNOVETERINARY PLANTS SPECIES AGAINST PARASITIC NEMATODES AND *Caenorhabditis elegans* IN NIGERIA

ABSTRACT

Control of trichostrongylid nematodes using synthetic drugs have resulted to several complications such as increasing resistance of the nematodes to synthetic drugs, change in the quality of the products from the treated animals due to drugs residues in the tissues of the animals. These called for research to explore alternative to conventional drugs. The purpose of this work was to carry out *in vitro* study of the aqueous and methanol extracts *Detarium microcarpum, Guiera senegalensis, Cassia siamea, Parkia biglobosa, Gmelina arborea* and *Boswellia dalzielii* against trichostrongylid nematodes of small ruminants and the ivermectin resistant strain of *Caenorhabditis elegans* DA1316. The powdered stem bark of each plant was extracted by maceration in water and methanol. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, steroids and terpenoids but were not equally distributed among the types of plants and between aqueous and methanol extracts. The plant extracts were tested *in vitro* against the motility of L3 larvae of trichostrongylid nematodes at the concentrations of (0.2, 0.4, 0.6, 0.8 1.0 and 2.0 mg/ml). *Detarium microcarpum* was the most potent plant according to the standard of World Association for the Advancement of Veterinary Parasitology (WAAVP), as the aqueous and methanol extracts inhibited up to 90% motility of the larvae. *Guiera senegalensis* was the second most potent plant for inhibiting 80% and 90% by aqueous and methanol extracts respectively. The extracts

of *C. siamea* which were moderately effective at 2.0 mg/ml was the third most effective plant. The extracts of *D. microcarpum*, *G. senegalensis, C. siamea* were subjected to further test against the egg hatch, larval development, larval migration and larval motility of *C. elegans* DA1316 (ivermectin resistant strain) and *C. elegans* Bristol N2. The aqueous and methanol extracts of *D. microcarpum* were confirmed the most potent of all the extracts against both strains of *C. elegans* at 2.0 mg/ml. *G. senegalensis* was the second potent plant against the *C. elegans* though the aqueous extract was ineffective against egg hatch at 2.0 mg/ml. The larvicidal and ovicidal activities of the plants extracts against *C. elegans* Bristol N2 where the same compared to that of *C. elegans* DA1316 ($P > 0.05$). Combination of the lowest concentration (0.2 mg/ml) of the methanol extract of *D. microcarpum* and 0.02 µg/ml of ivermectin yielded the highest potency against the motility of both strains of *C. elegans*. Extracts of *D. microcarpum* and *G. senegalensis* were tested against the migration of *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Ostertagia circumcincta* and *Oesophagostomum spp* L3 larvae. *Haemonchus contortus* proved the most sensitive of all the species to the extracts from both plants. *Oesophagostomum spp* was the less sensitive of all the species. All the species were more sensitive to *D. microcarpum* than *G. senegalensis* ($P < 0.05$). Extracts of *D. microcarpum* contained the highest quantities of phenols and tannins followed by extracts of *G. senegalensis*. Methanol extracts contained a higher quantity of phenols and tannins than aqueous extracts. This study confirmed *D. microcarpum and G. senegalensis* as the most potent plants that can serve as a source of lead compounds for natural anthelmintic against trichostrongylid nematodes. The extract was also confirmed to work best in combination with the synthetic drug.

CHAPTER 1

INTRODUCTION

1.1 Background

One of the major global veterinary health problem today is gastrointestinal nematodes (GIN). They cause infections in livestock and wildlife leading to morbidity and mortality, reducing the population of the host animals, thereby threatening food security through the reduction of productivity of livestock especially of the ruminant animals (Rose *et al.,* 2015). Loss of productivity of livestock as result of GIN infection could be due to inducement of changes in certain mechanisms leading to low food intake, gastrointestinal dysfunction poor metabolism of protein, energy, and minerals. The degree of these effects is dependent on the size, number and species of the worms (Fox, 1997; Coop *et al.,* 2001). Reduction in livestock and it productivity could result from the pathogenic effects of the worms such as severe anaemia, severe damage to gastric mucosa and villous, reduction in functional gastric gland mass on the animals and death may occur in the severe case of any of the above mention ailment (Amulya *et al.*, 2015).

Economic loss arising from the infection of livestock with gastrointestinal nematodes often results from spending on research and treatment, especially when handling resistant cases of GIN. Also low milk production, poor quality of meat and wool could all contribute to economic loss (Miller *et al.*, 2012; Amulya *et al.*, 2015). For instance in Australia, the estimated amount of 1 billion dollars has been spent on the control of GIN of ruminants and 10 of billions of dollars have been proposed annually for the control of GIN of ruminant animals (Sackett *et al.*, 2006).

Sanitation and the used of synthetic anthelmintic drugs have been adopted as the major approach by the developed nations towards the eradication of helminths parasite's infections (Sutherland *et al.*, 2002; Stepek *et al.*, 2006). Efforts to eradicate nematodes parasites within animals involved the use of some of these classes of narrow and broad spectrum commercial antihelmintics which include: imidazoles, imidazothiazoles, tetrahydropyrimidines and macrocyclic among others (Sutherland *et al.,* 2002; Beech *et al.,* 2011; Sutherland *et al.,* 2011). Amino-acetonitrile derivatives (AAD) has been in the recent time employed as a new class of commercial anthelmintic to control GIN but field resistance has been observed (Scott *et al.,* 2013). Also partial or full resistance to classes of anthelmintics such as levamisole, pyrantel, mebendazole, macrocyclic lactose, benzimidazole has been reported in recent times (Sutherland *et al.,* 2011 Scott *et al.,* 2013; Werner *et al.,* 2013; Magzoub, 2015).

The rate of increase and spread of GIN coupled with the increase in their resistance to drugs is a serious problem and a matter of greater concern especially in the third world countries (Eguale *et al.*, 2007; Kundu *et al.*, 2014). A research on co-infection of *Haemonchus contortus* and *Trichostrongylus spp*. in five farms located in three states in Malaysia revealed that 118 goats out of 416 (28.4%) were infected with trichostrongylid nematodes. PCR analysis indicated 93 out 416 (22.4%) were infected with *H. contortus,* 99 of 416 (23.8%) infected with *Trichostrongylus spp* and 2 of 416 (0.48%) are co-infection with *H. contortus* and *Trichostrongylus spp* (Tan *et al.,* 2014).

The result of the investigation of prevalence of parasitic infection of small ruminant farms in Kampar, Larut Matang and Selama, Kuala Kangsar and Manjung districts all in Perak, Malaysia revealed that *Eimeria spp* was found in 162 (92.57%) of the animals, *Theileria spp* recorded 25 (14.30%) of the animals *and Haemonchus* *contortus* was found in 152 (86.86%) of the animals (Zainalabidin *et al.*, 2015). *Haemonchus contortus* was shown to be the second most important cause of helminthiasis in small ruminant within Malaysia proven by recent studies (Zainalabidin *et al*., 2015).

Helminths infection is a serious veterinary health problem in Africa today, owing to poor sanitary conditions, abject poverty, ignorant of the diseases transmission mechanisms among other several factors. An investigation was carried out on detection of anthelmintic resistance of gastrointestinal helminths in sheep in Khartoum State, Sudan, Africa, using the drugs; levamisole powder and abamectin injection. Parasites recovered within the animals were; *Strongyloids papillosus, Oesophagostomum columbianum, H. contortus and Trichostrongylus* spp. The results of anthelmintic tests on these nematodes revealed the resistance of the parasites to levamisole but there was no resistance observed with abamectin (Magzoub, 2015).

Evaluation of the effectiveness of albendazole, levamisole, ivermectin and *Vernonia amygdalina* was performed on West African dwarf goats in Ibadan, Oyo state, Nigeria. The helminths parasites recorded during the investigation included *Oesophagostomum spp.* (56%), *H. contortus* (70%), *Trichostrongylus spp* (61%) and mixed infection were found in all the animals. The results of treating the infected animals with commercial dewormers and the extract of *Vernonia amygdalina* indicated that extract from *V. amygdalina* was more effective against the helminths (100%), compared to the synthetic drugs; albendazole (99%) and ivermectin (96%). Lower resistance was detected in levamisole and ivermectin (Adediran *et al.*, 2015).

Because of the increasing resistance of gastrointestinal parasites to synthetic drugs, cost of drugs, the side effects displayed in the treated animals and environmental hazards associated with the use of synthetic drugs and the possible toxic effects of the residues on food, the need arose for the search of alternative natural, safe, and cheap alternative therapy from plant against helminths' infections and multi drugs resistant GIN using *Caenorhabditis elegans* as an experimental model. Therefore, the search for natural plants therapy against drugs resistant parasitic nematodes by testing plants extracts *in vitro* on ivermectin resistant strain of *C. elegans* (DA1316) becomes the ultimate goal of this research. In addition, ethnoveterinary application of some Nigerian plants as dewormers on ruminant animals has been practiced by the traditional animal breeders without scientific validation. Therefore, this research is also targeted at validating the use of some of these plants as dewormers.This becomes necessary with a view to adding to some existing information that will form the basis for the development of new lead chemicals for pharmaceuticals and for producing new anthelminthic drugs to tackle the problem of resistance of parasitic nematodes to commercial anthelmintics. Plants provide a natural source of lead compounds that can be used in the development of new anthelmintics.

Screening for the anthelmintic activity of plant extracts for a veterinary application requires testing the extracts on the ruminant host naturally infected with the parasites. However effective screening would be expensive as it would require livestock facilities, huge amounts of plant materials and consideration of animal welfare (Katiki *et al.*, 2011; Kumarasingha *et al.*, 2014). Several *in vitro* screening of active compounds against parasitic helminths using egg hatch assay, larval development, larval motility and larval migration assay among others are being used as techniques in search of effective anthelmintic drugs (Katiki *et al.*, 2011; Kumarasingha *et al.*, 2014). These assays are not possible on adult parasitic nematodes and there are also complications on screening the adult parasitic nematodes outside the host because the adult worms cannot survive outside the definitive host (Marie-Madeleine *et al.*,2009). These difficulties have been overcome by using the free-living nematode *C. elegans* as a model for screening anthelmintic drugs. Screening therapeutic compounds against nematodes create ways for the advantage of cheap *in vitro* assay and provide the possibility for a demonstration of the potentials of compounds against adult stage of the related nematodes species.

In Malaysia, only little has been done concerning the application of *C. elegans* as a model in veterinary research. Also, there are scarcely documented evidence of research on the veterinary applications of *C. elegans* in Nigeria as well. Therefore, it is hoped that the outcome of this research will throw more light on the veterinary applications of *C. elegans* in solving the problems of anthelmintic drugs resistance by parasitic nematodes of ruminant animals and to encourage more research in this area. Also because of little or absence of documented evidence of research on the veterinary application of *C. elegans* in Nigeria. The outcome of this work is hope, to provide baseline information that will stimulate the proliferation of more research in the field of veterinary health requiring the application of *C. elegans* as an experimental model.

1.2 Objectives

This study was based on achieving the following objectives: -

1- To carry out preliminary phytochemical studies on the extracts of the six pants; *Detarium microcarpum, Guiera senegalensis, Cassia siamea, Parkia biglobosa, Gmelina arborea,* and *Boswellia dalzielii* use as animal dewormers in Nigeria ethnoveterinary practice.

2- To determine the most effective plant against *Haemonchus contortus, Trichostrongylus colubriformis, Ostertagia circumcincta* and *Oesophagostomum* spp of the family trichostrongylidae nematodes *in vitro.*

3- To evaluate the efficacy of *Detarium microcarpum, Guiera senegalensis* and *Cassia siamea* against *C. elegans* DA1316 (ivermectin resistant strain) and *C. elegans* Bristol N2 (susceptible to ivermectin).

4- To investigate the anthelmintic efficacy of *Detarium microcarpum* and *Guiera senegalensis* extracts against the larval migratory activities of selected species (*Haemonchus contortus, Trichostrongylus colubriformis, Ostertagia circumcincta* and *Oesophagostomum* spp) of trichostrongylid nematodes.

CHAPTER 2: LITERATURE REVIEW

2.1 General characteristics of trichostrongylid nematodes

Nematodes worms are equally called the roundworms. They constitute one of the largest diversity of animal organisms which are cosmopolitans (distributed over a wide range of habitats). They inhabit almost every ecosystem which includes mountain, depth of the sea, soil, fresh and marine water, trees, mud walls, desert as well as the lowest and highest elevation, gold mines and several other parts of the earth (Borgonie *et al.,* 2011). Some members of this phylum exist as free-living organisms. Some exist as plants and animal's parasites (Hickman *et al.,* 2001; Zhang, 2011). About 250,000 nematodes species have so far been identified (Hickman *et al.,* 2006). Nematodes play very important role in the stabilization of ecosystem due to their numerical dominance on the earth, diversity of life cycles and adequate representation at every trophic level (Lorenzen, 1994).

Parasitic nematode infection in small ruminants is normally caused by trichostrongylid nematodes of the family Trichostrongylidae. The most important economically influential species responsible for the infection include *Haemonchus* spp, *Trichostrongylus* spp, *Ostertagia* spp, *Cooperia spp, Oesophagostomum spp* among others (Wyrobisz *et al.,* 2015; Zarrin *et al.,* 2015). These parasites cause one or several pathogenic effects that may sometimes lead to the death of the infected host animal, hence very important from the viewpoint of veterinary practice (Pestechian *et al.,* 2014).

On the outline, trichostrongylid nematode worms are cylindrical, threadlike with both ends pointed and approximately less than 2.5 mm and at least 0.1 mm long with the thickness of 100 µm (Brady *et al.,* 2000   Trichostronyglid nematodes are microscopic but some attained the size of more than 1 m in length (Ruppert *et al.,* 2004). Other distinctive structures include the bristle, rings, and ridges covering the body (Weischer *et al.,* 2000). The nematodes are characterized by the exhibition of distinct head which is radially symmetrical and the body is bilaterally symmetrical. The head exhibits many sensory bristles and in many cases contained 3-6 mouth lips with series of teeth on their inner edges. The oral cavity leads into the pharynx which is lined with cuticle. This region bears the digestive glands which produce digestive enzymes that digest the food (Barnes, 1987). The tip of the tail bore an adhesive caudal gland. Collageneous cuticle covers a single layer of cells which form the epidermis. A layer of longitudinal muscles cells lies beneath the epidermis (Barnes, 1987). Nematodes lack stomach, however, the pharynx is directly connected to the intestine thereby forming the gut. Digestion further takes place in the intestine as it produces enzymes that digest the food. Absorption of digested nutrients also takes place through the single cell lining of the intestine. The intestine terminates into a cuticular rectum which formed the major outlets for waste products (Barnes, 1987; Hickman *et al.*, 2006).

2.2 General life cycle of trichostrongylid (parasitic) nematode of ruminants

The generalized life cycle of the trichostrongylid nematodes could be demonstrated using the life cycle of the economically most influential ruminant parasitic nematode of the family Trichostrongylidae. Most genera under this family are of veterinary importance. The genera under this family include *Haemonchus, Cooperia, Ostertagia, Trichostrongylus* and *Nematodirus* (Wharton, 2012; Foreyt, 2013). The adult organisms are found to inhabit the gastrointestinal tract of the ruminant host. They duel in the intestine or abomasum of the hosts. Trichostrongylid are dioecious, meaning sexes are separately given rise to separate male and female. They exhibit direct life cycle because the life cycle does not require an intermediate host (Sissay, 2007).

The life cycle begging with eggs lying by the matured worm in the host organism. eggs Most female organisms can produced up to 200 eggs per day, within the alimentary canal of the host. The eggs spend about 3-4 days within the gastrointestinal tract of the host. The host voided out the eggs together with faeces in the environment. Under favourable conditions of temperature and relative humidity, the eggs hatch into free-living first stage rhabditiform (L1) larvae. With optimum conditions for development, the L1 larvae moult to L2 larvae after about 4-6 days. Both the L1 and L2 larvae feed on the bacteria found on the faecal remains of the host and require damp condition with an appropriate temperature of about 27-28^{o}C for further development. The L2 larvae moult into infective L3 larvae and migrate from the faeces onto the grass blade. The L3 larvae remain with the cuticle of the L2 larvae which offer them protection against harsh environmental conditions and prevent them from feeding. Meanwhile, the L3 larvae depend on the food reserved for their survival as they wait for hosts to be infected. The L3 larvae may be ingested by a grazing animal (sheep or goat) alongside grasses as they swallow them. The ingested larvae move through the first three chambers to abomasum. After about 2- 3 days within the host, the larvae shed their cuticles burrow into the wall of the abomasum and moult to L4 larvae.

The L4 larvae moult in young adults L5 larvae after another 10-14 days. The L5 (young adult) grow to maturity and mating occurs between male and female in the abomasum after which the gravid female begging another round of eggs lying. The period between the ingestion of the L3 larvae to when the eggs are laid is termed as a prepatent period

(Sissay, 2007). The general life cycle of a typical trichostrongylid (parasitic) nematode can is illustrated in Figure 2.1.

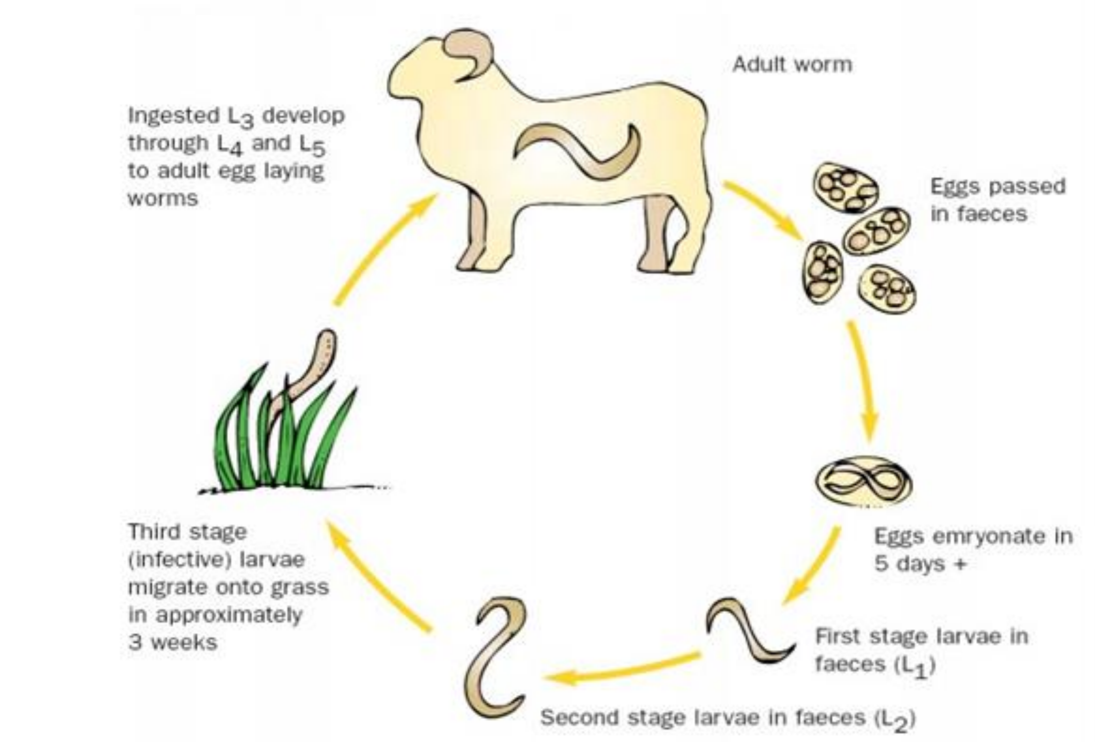

Figure 2.1: Lifecycle of a typical trichostrongylid nematode (adapted from Sissay, 2007).

2.3 Pathogenecity of Trichostrongylidae

Worm's infection causes serious problem to animal health, poor meat quality and quantity, which may in turn affects the farms and the generality of veterinary based industries that can results to overall economic loss by the farmers and the affected community or nation at large (Kumsa *et al.*, 2011; Mavrot *et al.*, 2015).

Pathogenic effects of the family trichostrongylidae depend on the species of trichostrongylid nematode, the burden of the worms and it is more pronounced among the young ruminants. On a general note, clinical manifestation of trichostrongylid infections includes: diarrhoea, loss of weight, anaemia, loss of appetite, severe dehydration, reduced milk production, rough hair coat, uncoordinated movement, stunted growth, lethargy, untreated and serious cases may result in death. The pathogenic cases arising from the infection of small ruminants by *H. contortus, O. circumcincta* and *T. colubriformis* demonstrate typical pathogenicity of trichostrongylid infections (Roeber *et al.,* 2013).

2.3.1 *Haemonchus contortus*

This is recognized worldwide as the most pathogenic and economically important small ruminant nematode parasite. Haemonchosis is a term used to described infections caused by *Haemonchus contortus*. Infections with haemonchosis cut across animals of all ages among cattle, sheep, goats and other small ruminants. The adult organisms that inhabit the abomasum usually on the gastric wall or sub-mucosa are blood feeders (Vatta *et al.*, 2001; Tehrani *et al.*, 2012; Roeber *et al.*, 2013). Infection by *H. contortus* leads to severe anaemia due to loss of blood, oedema, loss of appetite, body weakness, emaciation, pale mucous membrane and drastic fall in the level of haemoglobin of the infected animal (Vatta *et al.,* 2001; Gebresilassie *et al.,* 2015). The infection by this worm may results in the average loss of 0.05 ml of blood per day by a single worm in the affected animal due to ingestion and seepage by the worm. Therefore, approximately 250 ml of blood loss can result from the effect of infection by 5000 adults *Haemonchus contortus.* Oedema is usually developed within the jaw tissue, a phenomenon described as bottle jaw. Oedema may also be in the thoracic cavity, abdomen, gut mucosa among several clinical symptoms (Githigia *et al.,* 2001; Vatta *et al.,* 2001; Qamar *et al.,* 2009).

2.3.2 *Ostertagia circumcincta*

This parasite is identified as one of the economically important nematodes of small ruminant within the tropical region and as the most abundant of all the species of trichostrongylid in the temperate region of the world (Bishop *et al.*, 1996; Akhter *et al.*, 2011; Nabavi *et al.*, 2011). The morphological length of male *O. circumcincta* is about 7.0-8.5 mm while the female is about 9.8 - 12.2 mm. The adult organisms inhabit the abomasum of ruminant animals such as cattle, sheep, camels and goats. The symptoms displayed due to *O. circumcincta* infection in the ruminants are lethal. Some of these symptoms include watery diarrhoea, raised in abomasal pH; loss of appetite, plasma pepsinogen levels become elevated and several changes in the functions of gastrointestinal tracts, mucosal hyperplasia among others (Fox, 1997; Scott *et al.*, 2000; Simpson *et al.*, 2009).

2.3.3 *Trichostrongylus colubriformis*

This parasite is well known to inhabit the small intestine and the abomasa of cattle sheep, camels and goat. However, the parasite is also found in dogs, rabbits, pigs and man. Morphologically the male is 4-5.5 mm and the female could be up to 5-7 mm long (Anderson, 2000). The adult worms mostly infect the upper portion of the small intestine and occasionally the abomasa. Several lethal symptoms are displayed in the infected human and animals such as loss of weight, loss of appetite, frequent dark coloured diarrhoea, hypoalbuminemia, hypophosphataemia, oedema, atrophy of skeletal and myocardial muscles, swollen of the duodenum, entrities, haemorrhage, weakness of the limbs, lethargy and death becomes the ultimate end in a severe case with less medication (Trapani *et al.*, 2013; Watthanakulpanich *et al.*, 2013).

2.3.4. *Oesophagostomum* spp

Oesophagostomum species occurs in Africa, Brazil, China, Indonesia and Philippines. The genus infects both human and animals. However, species such as *O. bifurcum* infects only animals and none human primates. The parasites usually inhabit the large intestine of host. The adult worms exhibit cephalic groove as well as visible secretory pore at esophagus. The organism has well developed multinucleate digestive tract as well as digestive system. The larvae can invade colon wall thereby causing nodular pathology. The multi nodular is characterized by the formation of many nodular lesions containing worms and pus along the colon wall. The nodules can give rise to further complications such as bowel obstructions peritonitis and intestinal volvulus. In rare cases, emaciation, fluid in pericardium, cardiomegaly, hepatosplenomegaly, perisplenitis and enlargement of the appendix may occur (Verweij *et al.,* 2007).

2.4 Treatment and control of parasitic nematode

The current control of parasitic nematodes of ruminant animals relies on two main approaches which include chemical and none chemical approach. However, vaccine is one of the approaches to the control of parasitic nematodes although, it has not been fully developed and made readily available.

2.4.1 Chemical control

This is treatment and control of nematode parasites of ruminant using conventional anthelmintic. Those chemical substances that eliminate or kill parasitic worms within the body of host animals are simply referred to as anthelmintic. Anthelmintic drugs are introduced into the animals through oral administration in the form of a suspension or as pellets, powders, granules for mixing with animals' food or injection (Magzoub, 2015). Anthelmintics are also called dewormers or drenches which are still preferred and widely used in the veterinary healthcare today (McArthur *et al.*, 2011). Therefore, the reduction of high incident of a large population of nematodes infections is achieved through anthelmintic therapy (Köhler, 2001). The various groups of conventional anthelmintics exert more effects against the immature stages of the parasitic nematodes in ruminant's animals. The effects of the drugs lead to biochemical and physiological changes such as changes in structural proteins, ion channels, enzymes activities and transportation of molecules in the nematodes (Köhler, 2001).

2.4.1a Benzimidazoles

This is the second generation anthelmintics groups after piperazine and Phenothiazine which reign as the first-generation group. These are made up of heterocyclic aromatic compounds which have undergone several modifications to improve their spectrum of activity and safety which resulted into drugs of high potency against nematodes within the several years of used from 1960's to date. The group benzimidazole is a broad spectrum with a large family which includes several effective members with a wide margin of safety such as fenbendazole, albendazole, flubendazole, oxfendazole, thiabendazole and triclabendazole (Kahn *et al.*, 2005; Magzoub, 2015). Some members of benzimidazoles have long half-life and are not quickly metabolized, which make their effective concentration to remain in the gut and plasma for a long time that conferred on their total evaluation as the most effective members of the group. These include oxfendazole, albendazole, and fenbendazole which have their activity more pronounced against the immature and the adult stage of the nematodes. The activity of benzimidazole is less in cattle compared to small ruminants like sheep and goat because they are metabolised into inactive compounds and excreted faster in cattle unlike in sheep and goat and this account for higher dose rate in cattle than in sheep and goat (Kahn *et al.*, 2005). The elimination of most of the larval and adult stages of gastrointestinal parasites of ruminants such as those of the genus *Cooperia, Nematodirus, Trichostrongylus, Haemonchus, Ostertagia* and *Bunostomum* are effectively accomplished through administration of any of benzimidazoles (Brander *et al.*,1982).

The most widely used member of benzimidazole is albendazole, with high efficacy against parasitic nematodes, tapeworms and flukes. Albendazole in interact against the activity of worms and flukes as it rapidly metabolized to sulphone and sulphoxide which prevent the activity of the ruminant parasites. It takes several days after administration before the residue of the drug cleared from the treated animal system.

Therefore at least 10 days should elapse after the treatment before the animal is slaughtered (Moreno *et al.,* 2008).

Mechanism of action of benzimidazole

The benzimidazoles bind to β-tubulin, with a 25–400-fold greater inhibition constant for nematode tubulin compared with that of mammals. Tubulin is a protein subunit of the microtubules that have a fundamental and ubiquitous role in the mitotic spindle. Benzimidazole-specific binding sites on β-tubulin lead to local unfolding of the protein with the resulting abnormal conformation inhibiting further polymerization of α- and β-tubulin subunits to form microtubules and in rapidly dividing cells, this results in a lethal effect. However, in nondividing cells, a variety of effects on homeostatic mechanisms is elicited, often leading to nonlethal expulsion of nematodes from their sites of predilection. At higher concentrations, benzimidazoles have a variety of nonspecific effects on nematodes, e.g. the inhibition of fumarate reductase. Depending on specific molecular characteristics, the spectrum of antiparasitic activity of the benzimidazoles can include nematodes, cestodes, trematodes and certain protozoa (Stephen W. 2008).

2.4.1b Imidazothiazole

This group consists of water-soluble anthelmintics which offer them more advantage above other conventional therapeutic agents besides been excellent anthelmintics administered through injection (McKellar *et al.,* 1990; Magzoub, 2015). Drugs in this group have been used against nematodes of ruminants, pigs and poultry since 1968, but it is ineffective on flukes and tapeworms. A major member of this class "levamisole" is a broad spectrum with excellent efficacy against the adults and larval stages of the nematodes and relatively safe but it has no ovicidal activity (Martin, 1997; Kahn *et al.,* 2005). The cholinergic property of levamisole qualified it for rapid paralysis of worms such that it can expel the worms within 24 hours after dosing the animal (Brander *et al.,*1991). Levamisole has a high rate of absorption in the body irrespective of mode of administration (orally or through injection) and as such it has a plasma half-life of not more than 4 hours after dosing. About 46% of the drugs are rapidly eliminated in the body through urination and 32% eliminated through faeces within 24 hours; hence it has 3 days withdrawal period for meat and a day for milk (Brander *et al.,* 1991).

Mechanism of action of imidazothiazole

This group is characterized by the composition of nicotinic receptors agonist which acts on nicotinic acetylcholine receptors (nACHRs) located within the cell membranes of somatic muscles cells. The ion channel which regulates the influx of potassium, chloride and sodium ion is linked to nicotinic acetylcholine nACHRs. The nACHRs is attacked and stimulated by the nicotinic receptor agonist from the drugs thereby leading to long-term activation of the ion channel to remain open which will automatically increase the influx of ions that in turn leads to spastic paralysis of the neuromuscular junction of the worm (Wolstenholme *et al.,* 2004; Abongwa *et al.,* 2017). Further more, Levamisole as a member of imidazothiazole act as nicotinic agonist that selectively gate acetylcholine ion-channels on nematode muscle and on nematode neurons to produce spastic paralysis and inhibition of egg-laying (Martin *et al.,* 2010).

2.4.1c Macrocyclic lactones

Members of this group are derived from the bye product of fermentation of the genera actinomycetes of soil microbes by *Streptomyces avermilitis* (Burg *et al.,* 1979). Drugs in this group comprise of mainly milbemycin and avermectin which exist in circulation as anthelmintic in the 70's. The most important derivative of avermectin is ivermectin which was introduced into use as anthelmintic in 1981 with a high level of global acceptance (James *et al.,* 2009). It displays high potency against all stages of parasitic nematodes of ruminant animals and a potent ectoparasiticides of animals but completely has no activity against tapeworms and flukes. The absorption rate of ivermectin is very high when dosed through parenteral injection.

The absorption rate reached peak plasma concentration (with 100% bioavailability) at about 4.4 hrs after abomasum direct dosing. Despite the speed in the metabolic action of the drugs, it has the longest half-life in the body among other anthelmintics with their residues concentrated within the fatty tissues and the liver and far less in the muscle. Ivermectin has the withdrawal period of about 28 days in cattle and approximately 21 days in sheep and goats before slaughter. Because of its persistence level in the tissue, it should not be used for farm animals that provide milk for human consumption. Only 2% of the drug is excreted in the urine, the remaining 98% excreted alongside faeces (Brander *et al.,* 1982).

Mechanism of action of macrocyclic lactones

Macrocyclic lactones are selective agonists of glutamate-gated chloride channels (GluCls) which are present in neurons and pharyngeal muscles of nematodes and arthropods, but absent in humans. Macrocyclic lactone causes the GluCl to open allowing the influx of chloride ions. This leads to the paralytic effect on the neuromuscular system of the nematodes and subsequent paralysis of pharynx, the body wall, and the uterine muscles of nematodes. Paralysis (flaccid) of body wall muscle may be critical for rapid expulsion, even though paralysis of pharyngeal muscle is more sensitive. As the macrocyclic lactone concentration decreases, motility may be regained, but paralysis of the pharynx and resultant inhibition of feeding may last longer than body muscle paralysis and contribute to worm deaths. In addition to GluCl effects, the avermectins also act as antagonists of 4-aminobutyric acid (GABA) and nicotinic receptors expressed on somatic muscle cells of parasitic nematodes (Puttachary *et al.,* 2013; Magzoub, 2015).

2.4.1d Acylanilides

These groups are amide derived from salicylic and aniline acids. The group consists of narrow spectrum nematicide. Members of this group are only effective against *Haemonchus contortus.* The most important member of this group is Closantel which has a lasting effect of 2-3 months in the treated animal. Closantel has been confirmed to be very effective against immature and matured stages of *Haemonchus contortus* (Kahn *et al.,* 2005; Köhler, 2001). Combination of Closantel with other broad-spectrum therapeutic agents such as albendazole and levamisole has been confirmed to broaden its spectrum of activity (Maingi *et al.*, 1997; Maingi *et al.*, 2002).

Mechanism of action of acylanilides

The molecular mode of action of **acylanilides** is not completely elucidated. They all are **uncouplers of the oxidative phosphorylation** in the cell mitochondria, which disturbs the production of ATP, the cellular "fuel". This seems to occur through suppression of the activity of succinate dehydrogenase and fumarate reductase, two enzymes involved in this process. This impairs the parasites motility and probably other processes as well. Closantel disturbs the liquid and ion transport mechanisms in the parasites membranes Novobilský *et al.,* 2015).

2.4.2 Non-chemical control

2.4.2a Grazing management

The practice of good grazing management strategies has gone a long way in reducing the rate of infection of ruminant by nematode parasites and to some extent reduced the parasites population and the number of infected individual host (Waller, 2006). Grazing management as a strategy aimed at reducing the rate of infection by GIN among ruminant animals was classified by (Michel, 1985) as follows:

-Preventive: In these principles, the uninfected animals are reared on nematode free pasture, the animals are given anthelmintic treatment to suppress worms egg output at the onset of the grazing season continuously up to the time that the population of the infective larvae of the worms on the pasture decline to safer levels (Michel, 1985).

-Evasive: The principle here does not consider elimination of the infective stages of the parasites from the grazing pasture instead the strategies of moving the animals to a fresh grazing land before the population of the infective larvae of the parasite becomes geometrically high on the infected original pasture land (Waller, 2006; Hoste *et al.*, 2011).

-Diluting: This involved concurrent grazing of a large number of livestock that are naturally resistance to parasitic nematode infection with susceptible animals of the same species or different species aim at decreasing the infestation of the pasture from the combined output of nematodes eggs voided out from their faeces (Michel, 1985).

-Defensive sanitary measures: This method employed the principle of space or time scale in managing or reducing the rate of spread of GIN infections. This is an efficient form of dilution where the stocking rate in time per space is reduced thereby reducing the parasitic risk. This practice has been proven effective in the tropical and temperate region despite the non-linear relationship that exists between the worm burden and the stocking rate of the livestock (Saul, 1996; Torres-Acosta *et al.,* 2008).

-Offensive: This strategy is centered on the elimination of the parasitic nematodes on the grazing pastures using chemical, physical and biological means. However, the use of chemical substances such as urea, sodium hypochlorite and calcic Cyanamid under laboratory condition yielded effective results but less active when applied to the grazing field. This could be due to interactions with organic matter. (Alvarez-Calderón *et al.,*2007; Gonzalez Garduno *et al.,*2010). Physical measures such as the disinfection of pasture using UV light as a means of eliminating the infective L3 larvae was found to be very effective (Napoleone *al.,* 2012).

2.4.2b Biological control

This is the principle of introducing the natural enemies of the parasitic nematodes or other animal's activities with the aim of decreasing the parasites population in the grazing unit lower than what the population would have been if the biological control organism(s) was not there (Gross *et al.*, 1999; Waller, 2006). The separation of the animals away from their faeces or away from the infective larvae of the parasites before they reach the peak of larval pick-up periods is still interpreted as an indirect biological control in a broader sense (Waller, 2006). Man's activity involving the collection and utilization of dung as fuel, building materials among others in the developing nations leading to break in the life cycle of the nematode parasites is portrayed as biological control.

Certain birds that scatter large deposits of animal faeces in search of coprophilous arthropods as food speed up the desiccation of the animal's dung which will, in turn, killed the eggs and free stages of the parasites thereby breaking off the life cycle of the parasites (Barger *et al.,* 1984; McCracken, 1993). The feeding activities of dung beetles and earthworms which involve the removal and scattering of animal dung help to expose the free-living larvae of the nematodes to desication thereby breaking their life cycle (Holter, 1979; Waller, 1993). Various microorganisms which include viruses, predacious nematodes, mites, fungi and bacteria have found free-living stages of GIN as favourable food thereby helping to depopulate them by breaking their life cycle (Waller, 1993; Grønvold *et al.,* 1996; Waller, 2006).

2.4.2c Nutrition

The nutritive quality of feeds determined the resilience and resistance of the ruminant host organism(s) to gastro intestinal nematodes. The effect of nutrient quality is manifested more on host resilience than resistance (Coop *et al.,* 1999; Hoste *et al.,* 2011). Sheep feed supplemented with protein busted the immunity of the host ruminants accompanying a drastic decrease in parasites larvae burden (Coop *et al.,* 1999; Marume *et al.,* 2012). Faecal egg count (FEC) and the nematodes expulsion reduction rate was confirmed to be directly proportional to the quantity of undegradable protein in the animal's diets. Confirmation of the protein influence in host resilience but low effect on host resistance was determined in the urea experiment which the results revealed a greater pack cell volume, higher albumin concentrations and weight gain with less change in resistance as demonstrated by the test and control sheep as both showed comparable worm burden and faecal egg count (Wallace *et al.,* 1998). Protein-rich feeds generate long-term antibody responses, weight gain, low FEC and higher wool output (Datta *et al.,* 1999; Stear *et al.,*2000).

Haemonchus contortus burden has been proved to be reduced by supplement of copper oxide wire particles in an established case of infections in a sheep host in Sweden 6 weeks post infections (Knox, 2002). However, copper oxide supplement displayed minimal effects on the newly infected case in the host organisms (Waller *et al.,* 2004). Only 2 g dose of copper oxide supplement demonstrated a remarkable reduction in the FEC in pregnant ewes than any dose higher which may have a negative potential impact on multiple-born offspring (Waller *et al.,* 2004; Burke *et al.,* 2005). Contrarily, several researchers found supplement of copper oxide wire with little or no effect on other major nematodes such as *Teladorsagia* or *Trichostrongylus* in small ruminant species (Bang *et al.,*1990; Chartier *et al.,* 2000).

2.4.2d Herbal anthelmintics

This is purely dependent on concoctions with low anthelmintic potency in ruminant animals. The preparation of the concoctions is usually made from the wild and domestic botanical floras, some of which are commonly found and use for common ailments globally. Patronage of herbal anthelmintic becomes low in recent time with the advent of synthetic therapeutic dewormers (Waller *et al.*, 2001). However, there is currently a global resurgence driving force towards the use of herbal remedy due to threat pose by the development of multi-resistant worms and the diminishing public perceptions of the conventional drugs as a remedy to veterinary health and disease control.

The major problem of the ethnobotanical veterinary drugs is lack of scientific validation on its biochemical composition, dosages and other regulatory guides in its applications (Waller *et al.*, 2001). With the renew global interest in the development of natural therapeutic agents from plants for use in the veterinary health care, studies are now underway to evaluates some of the best candidates' floras used by pastoralists in many parts of the global communities as livestock's de-wormers (Githiori *et al.*, 2003; Githiori *et al.*, Baker, 2004).

2.4.2e Nutraceuticals

Nutraceuticals are crops rich in secondary metabolites otherwise referred to as nutricines which are purposely grown for their beneficial effects in satisfying veterinary health interest of curing infections in animals than their consideration for nutrients. Research has confirmed that sheep infected with nematodes grazed on tannins rich leguminous crops demonstrated reduced level in the GIN (Niezen *et al.*, 1998). Sheep and goats feed on dietary condensed tannin (CT) demonstrated a reduction in FEC in the order of 50- 60% based on the combination of worm fecundity and expulsions of adult worms (Kamiya *et al.*, 2003; Paolini *et al.*, 2003). Such tanneferous crops may inhibit the establishment of new larvae and such effects on worm burden could vary depending on species of the nematodes. For instance, extracts