STUDIES ON ANTHELMINTIC ACTIVITY OF *TITHONIA DIVERSIFOLIA* IN MBINGA DISTRICT, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

Gastrointestinal nematode parasitism is a global problem in both sub-tropical and tropical countries. Due to frequent administration of chemical anthelmintics the gastrointestinal nematodes have developed resistance hence giving rise to the search of alternative anthelmintics. This study was carried out to evaluate anthelmintic effects of *Tithonia diversifolia* in Mbinga district, Ruvuma region, Tanzania. The study specifically dealt with evaluation of the efficacy of *T. diversifolia* extracts against adult *Haemonchus contortus* worms in a controlled critical test. Further, the toxicity of the plant was evaluated using the brine shrimp lethality test. A total of fifteen goats which were free from helminthosis were purchased and quarantined for 60 days. They were then administered 1250 larvae of *Haemonchus contortus*. On day 29 after infection the egg per gram of faeces (epg) count was done. The goats were randomly divided into three groups of five goats each .The groups were negative control, treated and positive control. The treatment group was administered 50 mg/kg of *T. diversifolia* orally and the positive control group was administered 8mg/kg of albendazole orally. The epg count was then carried out on day 4, 7, 10 and 14, after which animals were sacrificed for total worm count. The results show that *Tithonia diversifolia* is not effective against adult *Haemonchus contortus* worms based on epg count and post-mortem worm counts reduction tests.From the study, it is recommended that more studies should be carried out so as to validate the anthelmintic effects of *T. diversifolia* by investigating its activity on other specific species of the nematodes which parasitize animals.

DECLARATION

I, **Lutfrid Samwel Komba**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

…… **……**

Lutfrid Samwel Komba Date

(MSc. Pharmacology candidate)

The above declaration is confirmed

…… **……..……..**

Prof. A. J. Ngomuo Date

(Supervisor)

…… **……**

Prof. A.A. Kassuku Date

(Co-Supervisor)

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DEDICATION

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LIST OF ABBREVIATIONS

AADs	Amino-acetonitrile derivatives
AIDS	Acquired immunodeficiency syndrome
AR	Anthelmintic resistance
ARU	Animal Research Unit
ARVs	Antiretroviral drugs
BSL	Brine shrimp lethality
CP	Cysteine proteinase
DASP	Department of Animal Science and Production
DMSO	Dimethyl sulphoxide
FECR	Faecal egg count reduction
FECRT	Faecal egg count reduction test
GABA	Gamma amino butyric acid
HIV	Human immunodeficiency virus
MUHAS	Muhimbili University of Health and Allied Sciences
NAChR	Nicotinic acetylcholine receptor
NaCl	Sodium Chloride
PCR	Polymerase-chain reaction
Rpm	Revolutions per minute
USA	United States of America
WAAVP	World Association for Advancement of Veterinary Parasitology
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

For centuries, medicinal plants have been used in different parts of the world as source of both preventive and curative traditional medicine preparations for both human and livestock .The traditional medicine also creates income to the indigenous people by exporting the dried form of the medicinal preparations (Yirga, 2010). The plants were used by people without the knowledge of their active ingredients. The plant materials used include seeds, berries, roots, leaves, barks or flowers (Oreagba *et al*., 2011). Those people took the crude extract orally, the practice which was extremely hazardous since the extracts may contain some toxic constituents (Egwaikhinde *et al.,* 2009). They acquired knowledge of medicinal plants by methods of trial and error (Okigbo *et al*., 2008; Qureshi *et al*., 2010). The indigenous people used the medicinal plants for medicine (de Boer *et al*., 2005).

Currently it is estimated that more than 80% of the people living in developing countries rely on traditional medicine (WHO, 2002b; Mbwambo *et al*., 2007; Sharafzadeh and Alizadeh, 2012; Pfoze *et al*., 2012). Most of the people in developing countries continue to rely on traditional medicines due to its accessibility and affordability. For example the research which was conducted in Uganda showed that the ratio of traditional practitioner to population was between 1:200 and 1:400 compared to ratio of the allopathic practitioner to population which was 1:20,000 or less. From this difference most of the traditional practitioners are easily available

compared to the allopathic ones (WHO, 2002b). On the other hand the cost of the modern drugs are extremely high compared to the traditional ones as it was revealed in a research carried out in Ghana, Kenya and Mali whereby it was observed that the course of sulfadoxine/pyrimethamine was estimated to be about several dollars although in the actual fact the per-capita-out-pocket health expenditure was US$ 6 per year while the traditional medicines used in malaria treatment were cheaper and sometimes it was just charged according to the wealth of the patients (WHO, 2002b). Some people in developing countries do not go straight to the public health centres as it has been shown that in Dar es Salaam (Tanzania) more than 21% of the patients who attended public health services had consulted a traditional healer before going to the hospitals (de Boer *et al*., 2005).

The traditional medicines practices are also evident in developed countries, where the reports show that different countries have been documented with percentage in brackets; China (40%), Australia (48%), Canada (70%), USA (42%), Belgium (38%) and France (75%) (Mbwambo *et al*., 2007).

In some African countries people have been using herbal medicine as primary treatment for Human immunodeficiency virus (HIV) related problems. There are also evidences of some people taking anti-retroviral drugs (ARVs) and traditional medicinal plants simultaneously (Namuddu *et al*., 2011). Research has shown that some traditional medicines have powerful immune-stimulant effects which have raised hope to the HIV victims (WHO, 2002b).

Some people believe that there are some diseases which cannot be cured in the hospitals so they use to go and consult the traditional healers (Muella *et al*., 2000).

Many drugs used in conventional medicine are derived from plants (de Boer *et al*., 2005, Chin *et al.,* 2006; Oreagba *et al*., 2011). The medicinal plants contribute about 90% of the newly discovered pharmaceuticals (Moshi, 2005). For example, many years ago a plant chemical was discovered in a tropical plant, *Cephaelis ipecacuanha,* and the chemical was named emetine. A drug was developed from this plant chemical called *Ipecac* which was used for many years to induce vomiting mostly if someone accidently swallowed a poisonous or harmful substance (Taylor, 2000).

Cynarin is a plant chemical found in the common artichoke (*Cynara scolymus*). In Germany, a cynarin drug is sold for liver problems and hypertension. Other modern commercial medicines derived from plants are such as atropine (*Atropa belladonna*), codeine (*Paparva somniferum*), digoxin (*Digitalis purpurea*), ephedrine (*Ephedra sinica*), nicotine (*Nicotiana tabacum*), quinine (*Cinchona ledgeriana*), artemisinin (*Artemisia annua*), vincristine (*Catharanthusrosea*),arecoline (*Areca catechu*), caffeine (*Camellia sinensis*), camphor (*Cinnamomum camphora*), cocaine (*Erythroxylum coca*), oubain (*Strophanthus gratus*), pilocarpine (*Pilocarpus jaborandi*), strychnine (*Strychnos nux-vomica*), yohimbine (*Pausinystalia yohimbe*), tubocurarine (*Chondodendron tomentosum*) and glaucine (*Glaucium flavum*).

So there is a need to make strong collaboration between traditional medicine and conventional medicine so as to fight diseases of priorities such as tuberculosis, malaria, hypertension, diabetes mellitus and HIV/AIDS (Mbwambo *et al*., 2007).

The use of traditional medicine in developing countries such as Tanzania is empowered due to its accessibility and affordability in the majority of resource-poor communities. Sometimes ethnoveterinary medicine can be obtained free of charge among the traditional healers found in African villages (Prabuseenivasan *et al*., 2006, Moreki *et al*., 2012).

Due to non-regulated use of several herbal medicines the health of the users may be at risk due to toxicity. This is due to the fact that there is limited scientific evidence in evaluating the safety and effectiveness of traditional medicine products and practices (Oreagba *et al*., 2011).

The uses of plants as medicine differ from one place to another. Therefore there is the need to study the plants so as its traditional knowledge can be validated by taking small locality and a number of plants, in each study. Based on above facts researches have been carried out for many plants and their preparations, to investigate the pharmacological activities. It is therefore important to validate traditional medicine knowledge so as to conserve the plants as well as documenting them for future generations. The present study focused on *Tithonia diversifolia* as an anthelmintic used by people living in Mbinga district, and the aim of the study is to provide scientific validation on the alleged activity of the plant.

1.2 Objectives

1.2.1 Main objective

The main objective of the study is to examine anthelmintic activity of the leaf extracts of *Tithonia diversifolia.*

1.2.2 Specific objectives

i. To conduct informal interviews in 3 wards of Mbinga district to collect information on the different uses of the plant.
ii. To study the efficacy of *Tithonia diversifolia* using the Faecal Egg Count reduction test.
iii. To study the efficacy of *Tithonia diversifolia* using a controlled critical test (worm count reduction test).
iv. To study the toxicity of the plant using Brine Shrimp Lethality test.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Medicine

Traditional medicine is defined as health practice, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercise so as to treat, diagnose and prevent illnesses and thus maintaining well being (Nxumalo *et al*., 2011; Oreagba *et al*., 2011).

Traditional medicine and the knowledge of their use provide an essential contribution to human and livestock health care. The herbalists, midwives and spiritual healers are the ones constituting the main source of assistance with health problems for about 80% of rural population in developing countries (Yirga, 2010). Similarly, in Latin America and Asia, there is also an extensive use of traditional medicine, for example in China 40% of all health care is delivered by traditional health practitioners (Nxumalo *et al*., 2011).

The western drugs used worldwide in the treatment of different diseases are either too expensive (Qureshi *et al*., 2010), toxic or not available in most of rural areas (Bakari *et al*., 2012). On the other hand, the number of service providers is very low compared to the number of patients (Qureshi *et al*., 2010). Due to this fact the people living in these areas have turned to the use of plants as alternative medicines in human and livestock diseases (Maphosa *et al*., 2010). A study by Dilshad *et al*. (2010) showed that clinical mastitis in dairy buffaloes and cows in Pakistan

developed resistance against synthetic antibiotics; when medicinal plants such as *Allium sativum* were given to the animals the results were better. The use of medicinal plants as alternative to western drugs is only possible if the safety and efficacy is well studied and known (Soetan *et al*., 2011). From ancient times people from different parts of the world have been using plants in the treatment of different problems (Iqbal *et al.,* 2006; Behnke *et al*., 2008). The discovery of these medicaments was done depending on the experience of the common people which was based on long and dangerous self experiment (Chhetri *et al*., 2008). The discovery of these plants was done by trial and error (Okigbo *et al*., 2008). For example, during ancient times people noticed that if they had aches and pains, the conditions disappeared as soon as they drank tea made up from the barks of willow tree (*Salix* sp.), later on the scientists discovered that the tree contained salicylic acid which is the active ingredient also referred to as aspirin (Okigbo *et al*., 2008; Oreagba *et al*., 2011).

During the 19th century European doctors used papain and papaya latex for the treatment of worms without knowing exactly how the two brought the effects. Until 1930s it was established that the two had the ability of digesting the nematodes. Later on the active principle was isolated from different parts of the plants and it is known as Cysteine proteinase (CP) (Behnke *et al*., 2008).

Ethnoveterinary medicine has been used for a long treatment by livestock keepers in treatment of different animal diseases. The ethnoveterinary medicine has been used either as prophylaxis or treatment (Dilshad *et al*., 2009; Adedeji *et al*., 2013).

People from Mbeere and Embu districts in Eastern province of Kenya have been using different plants in the treatment of various conditions (Kareru *et al.,* 2008). Most of plants which are found in most parts of Africa have been used by livestock keepers in the treatment of East coast fever (ECF), pneumonia and helminthosis (Gakuubi and Wanzala, 2011). Some of plants found in Africa are used to treat both livestock and humans. For example, the roots of *Peltophorum africanum* is used in Botswana for fertility promotion in cattle, its ash is also used to control mites and lice in domestic birds while its barks are used to relieve stomach disorders in humans (Moreki *et al*., 2012).

The use of ethnoveterinary in Africa is gaining popularity due to the toxic effects of synthetic drugs on humans, development of resistance to synthetic drugs by target parasites, high cost of synthetic drugs and these low cost and environmentally friendly (Adedeji *et al*., 2013).Since there is great overlap between indigenous and western science then there is the need of creating cooperation between ethno medicine and biomedicine which in turn would create a great benefit to the local population and their environment (Calvet-Mir *et al*., 2008).

The traditional medicine has different names in Indo-Pakistan subcontinent where it is referred as Ayuverda, Unani, Eastern or indigenous medicine (Iqbal *et al.,* 2006). The knowledge of traditional medicine is handed down through generations that are socially shared by the members of the same generation (Calvet-Mir, *et al*., 2008).
Unlike the Western medicine where the patient has to first give the complaint to the doctor before the doctor diagnoses the illness, in traditional medicine the treatment is

done by traditional healer based on symptoms (Calvet-Mir, *et al*., 2008, Taye, 2009,). Although many modern pharmaceuticals used today for our various ailments originate from plants and plant-based medicaments very few active ingredients have been isolated so far (Behnke *et al*., 2008).

Plants have a number of bioactive compounds which are used either as medicine or in preparation of new drugs. Nowadays about 30% of worldwide drugs are based on natural products isolated from medicinal plants. Many efforts have been made to extract new antimicrobial bioactive compounds from various kinds of sources (Khan *et al*., 2011a).

2.2 Helminthosis

Small ruminant industry is found in almost all countries in the world simply because it needs small amount of money to invest in it. It is the most important part in the mixed farming in both the tropical and sub-tropical countries. The industry is suitable for resource-poor farmers simply because resource-poor farmers can afford to buy goats and sheep as compared to cattle. It requires low input requirement such as small initial capital and maintainance costs also it is free of social, religious and cultural taboos (Terefe *et al*., 2012).

However the industry is faced with a great challenge of different diseases. Among the diseases helminthosis is the commonest condition found in all countries with ruminants. This condition is caused by endoparasitic worms which reside within

different parts of the hosts including gut, lungs, blood, gallbladder, tissues, internal cavities and the cells (Bashir, 2009).

The prevalence of helminthosis worldwide is very high (Lone, 2012) and it depends on humidity, temperature, rainfall, vegetation and management practices (Degefu *etal.*, 2011). The condition is severe in the countries with little or no access to modern animal health care facilities (Farooq *et al*., 2012). Helminthosis in livestock sector causes high economic losses through lowered fertility, reduction of food intake, reduced weight gain, lower milk production, treatment costs and mortality in severely parasitized animals (Degefu *et al*., 2011; Zeryehun, 2012). The condition affect all age groups but its severity is very high in sheep and goats of 0-1 year. On the other hand the mal-nourished animals are also highly affected (Lone *et al.*, 2012). The helminths which affect ruminants are grouped into cestodes, trematodes and nematodes (Soulsby, 1982).

2.2.1 Cestodes

The main genera of veterinary importance in this group infecting ruminants are *Moniezia* spp. and *Taenia* spp. (Joshi, 2000). In ruminants cestodes are acquired by feeding on contaminated food or water. In cattle for example *Moniezia* is acquired through ingestion of herbage contaminated with mitescarrying the infective stage of the parasite. If the lambs get infected with *Moniezia,* diarrhoea is the main clinical manifestation (Bashir, 2009).

Taenia saginata which is commonly known as beef or buffalo tapeworm has two hosts namely humans and cattle. In this aspect the definitive host is man and the intermediate host is the cattle. The worms (segments) are passed out through the human faeces and later on the eggs are ingested by the cattle. In the alimentary canal of the cattle the eggs hatch out as larvae (oncosphere) thus penetrating the gut wall and enter the mesenteric lymphatics and finally reach the circulation. Later on these larvae invade the muscular tissues so as to undergo further development to obtain the infective stage which is *Cysticercus bovis* cyst. Human beings are infected when they eat undercooked beef containing live *Cysticercus bovis* cyst (Bashir, 2009).

2.2.2 Trematodes

These are commonly known as flukes. They are found in the bile duct or small intestine although sometimes they may affect the lungs. The trematodes of veterinary importance include *Fasciola* spp., *Schistosoma* spp. and *Paramphistomum* spp. (Bashir, 2009).

Fasciolosis causes great loss in the agricultural sector worldwide. It is estimated that the loss caused by the condition world wide is about US$ 200 million per year and more than 600 million animals are affected per year (Bashir, 2009). The vulnerable group for this condition is mainly the young animals and the infection may manifest itself as clinical or subclinical. The severity of the condition varies according to the number of parasites present and nutritional status of the animal (Soulsby, 1982).

Transmission of this condition involves snails. The cattle acquire infection by ingesting moist and raw aquatic plants and grasses contaminated with infective metacercariae. The metacercaria excyst into immature Fasciola in the abomasum and enters peritoneum and into liver where it matures to become an adult worm and it lays eggs which are then passed through the faeces. When the eggs come into contact with water they mature and invade the molluscan snail host. The mature cercaria emerges out of the snail and gets encysted on aquatic grasses and they develop into metacercariae which is the infective stage of the parasite (Bashir, 2009).

Schistosoma spp. is the only trematode which is found in the blood stream of the warm-blooded hosts. The reason which makes them to live there is that the blood is rich of glucose and amino acids. The amino acids and glucose are highly required by *Schistosoma* spp. for the egg laying process. The worms cause a condition known as schistosomosis or commonly known as bilharziasis. The infection is often manifested by acute intestinal signs; the mucosa of the intestine is severely damaged and so the animal develops profuse bloody diarrhoea, dehydration and loss of appetite. The condition is also zoonotic because it also affects humans (Joshi *et al*., 2001). The most prevalent *Schistosoma* spp. includes *S. japonicum*, *S. bovis* and *S. spindale* (Joshi *et al*., 2001).

2.2.3 Nematodes

Nematodes are the most pathogenic helminths in both tropical and sub-tropical countries. This group contains many helminths, but those of veterinary importance in

Tanzania include *Haemonchus* spp., *Trichostrongylus* spp., *Nematodirus* spp. and *Cooperia* spp. (Degefu *et al*., 2011).

Trichostrongylus spp. affects the ruminants thus causing loss to the small ruminant industry. The worm lays microscopic eggs which are passed through the faeces and then within a few days the eggs hatch into larvae. The larvae develop via second and third larval stages. The larvae then infest pastures so the goats acquire the infection upon grazing on the contaminated pastures. In the intestine of ruminants the larvae mature into adults. The male and female adult worms mate hence fertile female starting laying the eggs (Soulsby, 1982). Adult roundworms cause anaemia, diarrhoea, poor growth and even death (Bashir, 2009).

In the subtropical and tropical countries the most pathogenic among the nematodes is *Haemonchus contortus*. The worm is a small and slender abomasal nematode, commonly known as ‘barber worm’. It belongs to the family Trichostrongylidae (Soulsby, 1982). This is the most pathogenic among all gastro-intestinal worms (Soetan *et al*., 2011). It causes severe disease in small ruminants and substantial economic loss to producers (Max *et al*., 2007; Sargison, 2011; Mwale *et al*., 2012). The losses are due to mortality and high costs of treating the animals (Miller and Horohov, 2006; Max *et al*., 2007). The animals are affected due to depression of feed intake, lower growth rates, lower weight gain (Jittapalapong *et al*., 2011) and anaemia (Qamar *etal*., 2009). According to Qamar and others (2009) it is estimated that one worm can suck 0.05 ml of blood per day. The deaths of small ruminants are

very common in tropical and subtropical regions and this is attributed to marginal level of nutrition (Ademola and Eloff, 2010).

However the severity of infection depends on physiological status, breed and weather (Keyyu *et al*., 2005; Al-Shaiban *et al*., 2008). Swai and others (2006) reported that the prevalence of nematode in Ngorongoro district was 14.2% in young stock (1-3 years), 35% immature (>3-6 years) and 21.7% in adult cattle.

The study will concentrate on *H. contortus* since it is the most pathogenic gastro-intestinal worm in Tanzania.

2.3 Life Cycle of *Haemonchus contortus*

Haemonchus contortus has a direct life cycle (Fig. 1). Adult male and female worms are found in the abomasum where they reproduce sexually (Miller and Horohov, 2006). The female *Haemonchus contortus* is very prolific; it may produce up to 10,000 eggs per worm per day (Siddiqui, 2009). The eggs are passed with faeces out of the host. If environmental conditions are conducive (warm and humid), a first stage larva (L_1) will hatch from an egg within a day. L_1 is small and slender and feeds only on faecal bacteria (Squires, 2009). The larvae moult to a larger second stage larva (L_2) which continues to feed on bacteria before moulting to the third larval stage (L_3). The duration from hatching to the third stage larva (L_3) under ideal conditions is roughly 7 days (Soulsby, 1982). The cuticle from L_2 is retained thus covering its own cuticle. This serves as a protective sheath. The sheath increases the resistance of the larva to adverse weather conditions. During this stage the L_3 is prevented by its sheath, thus it is unable to feed so it survives on its energy reserves.

It is the L_3 which is infective to hosts. Once it is either raining or there is any mechanical disruption the larvae are dispersed from faecal pellets and they are able to move onto vegetation via surface films of moisture.

Once ingested by a grazing host, the larva emerges from its sheath in the rumen and then moves into the gastric glands of the abomasal mucosa to feed. While in the mucosa it causes damage to the gastrointestinal tract thus resulting into inflammation and painful condition (Maphosa *et al*., 2010). Another series of moults occur as the parasite moves through a fourth and fifth larval stage to become an adult in the abomasal lumen and this happens 14 - 21 days after ingestion of L_3(Miller and Horohov, 2006).

The cuticle of *H. contortus* forms a small lancet in its oral opening and this is used for piercing the mucosa thus causing capillary bleeding on which the worm feeds. Blood feeding begins at the fourth larval stage. The *H. contortus* is also capable of undergoing a period of developmental arrest which is known as hypobiosis (O'Connor *et al*., 2006). During this period the larvae in the host do not develop directly into adults instead they remain as L_4 in the gastric glands of the abomasum for weeks or months.

The period is prolonged if the condition outside the host is unfavourable for the development of the larvae. This phenomenon prevents the shedding of eggs into the environment which would be unlikely to develop and survive (O'Connor *et al*., 2006). The underlying mechanism of hypobiosis is not yet fully understood,

although responses to environmental conditions, host immunity, and genetic programming (Capitini *et al.*, 1990) have all been implicated.

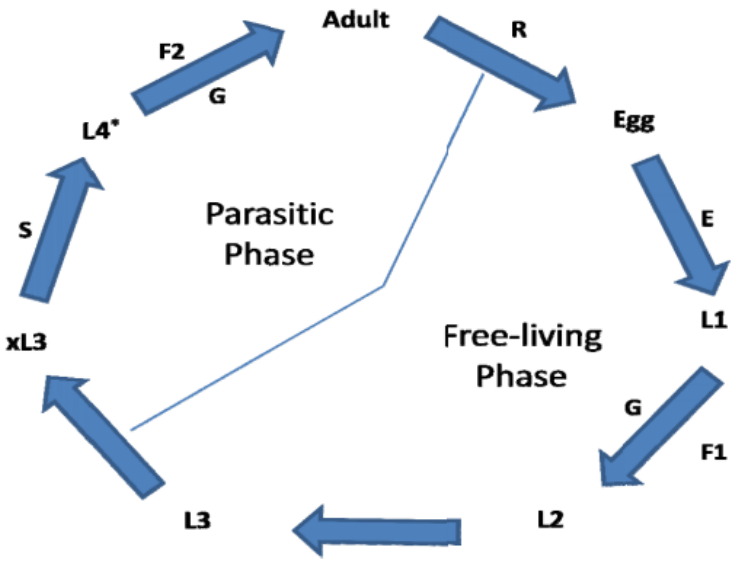

Figure 1: Life cycle of *Haemonchus contortus.*

Specific features of development: E, embryogenesis; S, sexual differentiation; R, reproduction; F_1, bacterial-feeding phase; F_2, blood-feeding phase; G, rapid growth phases; *, indicates potential to undergo hypobiosis. L_1, L_2, L_3, exsheathed L_3 and L_4 larval stages.(Adapted from Siddiqui, 2009).

2.4 Control of *Haemonchus contortus*

For many years the main way of controlling the nematodes has relied upon mass administration of anthelmintics to sick animals (Nayebzadeh *et al*., 2008; Jackson *et al*., 2009; Ihler, 2010). Anthelmintics which are recommended for the control of nematodes are the benzimidazoles, macrocyclic lactones, imidazolthiazoles and tetrahydropyrimidines (Kotze *et al.*, 2009).

2.4.1 Benzimidazoles

Benzimidazoles were originally developed as fungicides in plants but later on they were developed as veterinary anthelmintics (Horton, 2000). The first benzimidazole discovered and used as anthelmintic in sheep in USA was thiabendazole. It was discovered during 1961 (Lacey *et al*., 1994). More benzimidazoles with improved efficacy have been developed since then. These include mebendazole, oxibendazole, albendazole and fenbendazole (Martin *et al*., 1997; McKeller and Scott, 2008; Reddyjarugu, 2008; Sharma *etal*., 2012).).

The mode of action of these drugs is by binding to the cycloskeletal heterodimeric protein tubulin within the parasite thus disrupting tubulin polymerization leading to cell starvation (Taylor, 2000; WHO, 2002a; Pourgholami *et al*., 2006; Reddyjarugu, 2008). They are used due to their high therapeutic index and absence of toxic residues in food animals (Townsend *et al*., 1990; Reddyjarugu, 2008). They are available as oral pastes and as suspension due to poor aqueous solubility. The slow transit through the rumen makes it ideal for use in ruminants (Harder *et al*., 2003).

Spectrum of activity includes most nematodes such as*H. contortus, O. ostertagi, Nematodirus* spp. and *Dictyocaulus viviparous.* In addition to that, albendazole is also effective against *Fasciola hepatica*. Albendazole and oxfendazole are not recommended to be used in pregnant animals due their teratogenic effects.

2.4.2 Macrocyclic lactones

The members of this class are avermectin and milbemycins. Both classes were isolated in the 1970s from the soil living *Streptomyces* species (Yates *et al*., 2003; Siddiqui, 2009). These are the fermentation products of *Streptomyces avermilitis* and *Streptomyces cyanogriseus* microorganism. They are regarded as the most effective antinematodal and safest parasiticides yet developed (Tarpoff, 2010).

The active compounds within this group include ivermectin, eprinomectin and doramectin. The avermectins differ from each other by side chain substitution on the lactone ring while milbemycins (moxidectin) differ from avermectin by missing sugar on the lactone skeleton. These groups act by opening a glutamate-dependent chloride channel in neuromuscular membranes of the parasite resulting in paralysis of the parasite (Wolstenholme and Rogers, 2005; Boekh, 2009). The group is effective against most nematodes of domestic ruminants, horses and dogs; nematodes include *Cooperia spp*., *Haemonchus* spp., *Nematodirus* spp. and *Dictyocaulus viviparous* (Tarpoff, 2010). The drugs are effective against ectoparasites and onchocerciasis in human but they are not effective against tapeworms and flukes (Prichard, 2007).

2.4.3 Imidazothiazoles and tetrahydropyrimidines

The members of this class include levamisole and morantel (Martin *et al*., 1997). These compounds are nicotinic agonists acting as cholinergic agonists thus causing depolarization of the nematode muscle bag membranes with outflow of Sodium.

Levamisole spectrum includes *H. placei*, *O. ostertagi*, *Cooperia oncophora* and *Dictyocaulus viviparous*.

Morantel only comes in feed premix and has spectrum against *Haemonchus,Ostertagia*, *Trichostronglus* and *Cooperia*. Levamisole causes toxic effects such as salivation, restlessness and muscle fasculation.

Lastly there is a new class of anthelmintics that has been recently discovered. This class is known as amino-acetonitrile derivatives (AADs). AADs are thought to act on a nematode specific group of acetylcholine receptor subunits and exhibit broad spectrum of activity on sheep and goats. The prototype of AADs is monepantel (Kaminsky *et al*., 2008). It is effective against larval and adult stages of nematodes.

Despite the availability of all these anthelmintics, control of the worms has become difficult due to development of resistance against all classes of the anthelmintics (Edward and Hoffman, 2008).

2.5 Anthelmintic Resistance

This is genetically transmitted loss of sensitivity of a drug in worm population which is caused by change in gene frequency of the population. This results into drug selection; alleles for resistance are inherited by the next generation (Prichard *et al*., 1980). In this phenomenon certain β-tubulin sequence polymorphism results in amino acid sequence changes and the loss of high affinity receptor binding sites, thus resulting into resistance. In this situation a single nucleotide polymorphism (SNP) at codon 167 or 200 of the β –tubulin isotype-1 results in the change to amino acids

tyrosine (TAC) instead of phenylalanine (TTC) in resistant isolates as compared to the susceptible worm (Prichard, 2001). The mutated populations are the ones which increase the anthelmintic resistance provided there is a survival advantage for the parasite carrying these alleles (Miller and Horohov, 2006; Ihler, 2010). Anthelmintic resistance is the global problem especially in sheep (Nabavi *et al*., 2011), goats and cattle (Edward and Hoffmann, 2008). This has been a global problem in small ruminant industry throughout the world.

Due to repeated use of the anthelmintics the worms have developed resistance against most of the anthelmintic classes (Kotze *et al*., 2009; Ihler, 2010; Domke *et al.*, 2012). This has led to the emergence of resistant *H. contortus* (Ihler, 2010).

H. contortus has become resistant to all known anthelmintics with the exception of the newly discovered anthelmintics AADs. The first case of resistance against avermectins was reported in 1988 in South Africa and until today the anthelmintic resistance is common in Australasia, Africa and South America (Siddiqui, 2009).

Currently there are tests which have been developed so as to detect anthelmintic resistance. These include faecal egg count reduction test whereby the faecal egg count in pre-and post-drug treatment are compared to indicate the percentage reduction in egg count due to treatment. The second method is phenotypic assay whereby the effects of drugs on free-living life cycle stages are examined *in vitro* bioassays. The third method employed is the molecular test in which the genotypic

changes associated with drug resistance are monitored using polymerase-chain reaction (PCR)-based method (Sharma *et al*., 2012).

The emergence of anthelmintic resistance has led scientists to develop alternative methods in controlling helminthosis. This can be done either by the use of medicinal plants such as garlic (Athanasiadou *et al*., 2007) or combination of drugs. The resistance has caused great losses to the poor farmers in both tropical and sub-tropical countries due to the cost of treatment and mortality of their livestock (Siddiqui, 2009).

Therefore, due to the development of anthelmintic resistance and awareness of the consumers on drug residues investigation of alternative anthelmintics is required in order to control the nematodes (Siddiqui, 2009; Shamar *et al*., 2012). One of the alternative anthelmintics is from the medicinal plants. A large number of medicinal plants are claimed to have anthelmintic property in traditional systems of medicine and they are used throughout the world by the ethnic groups.

2.6 Review of Anthelmintic Plants

Modern synthetic medicines are very effective in curing diseases but on the other hand they cause a number of side effects. Various medicinal plants have been scrutinized either *in vivo* or *invitro* for their anthelmintic effects. Some of the plants with anthelmintic activities are described briefly below:

***Coldenia procumbens* Linn**.

The plant belongs to the family Boraginaceae. It is an annual herb and is a common weed found in India. In traditional system of medicine the plant was used as anti-inflammatory, antimicrobial, analgesic, antidiabetic and as central nervous system depressant. It is commonly used as anthelmintic and the principle active constituent is known as wedelolactone (Aleemuddin *et al.,* 2012).

***Ocimum sanctum*Linn.**

The plant belongs to the family Lamiaceae. Commonly known as sacred Basil. It contains volatile oil of which the chief constituents are Eugenol (about 51%), β-caryophyllene (37%) and a number of sesquiterpenes and monoterpes. The essential oil and eugenol have potent *in vitro* anthelmintic effects against nematodes (Mali and Mehta, 2008).

***Azadirachta indica* A.Juss. (Meliaceae)**

This plant is commonly known as neem, neem-tree, Indian lilac, or white cedar. It is a hardy tree growing to a height of 15 to 20 m, with a dense leafy, oval-shaped canopy. The bark is rough, pale grey-brown in colour. The plant has shiny compound leaves, with 5 to 8 pairs of leaflets, crowded towards the end of branches. The flowers are scented, small creamy white and hang down in long sprays, while the fruits are oval and yellow when ripe, yielding aromatic oil. It is found in arid and semi-arid areas of Eastern Africa (Dharani, 2002). The plant is used in various ailments including helminth parasites (Githiori, 2004).

***Annona squamosa* L. (Annonaceae)**

This is commonly known as sugar apple. It is a small tree of about 3 to 5 m high. The leaves are hairy when young, oblong, and 8 to 15 cm in length with petioles 1 to 1.5 cm long. The flowers occur singly in the axils of the leaves and are about 2.5 cm long. They are pendulous, hairy, three-angled, and greenish-white or yellowish. The fruit is heart-shaped, and 6 to 9 cm in length. The ripen fruit is light yellowish green. The leaves of the tree were reportedly used in Asia and South America (Vieira *et al*., 1999) as an anthelmintic for livestock.

Piliostigma thonningii

The plant belongs to the family Caesalpiniaceae. Stem bark of this plant is traditionally used in the treatment of dysentery, snake bite, toothache as well as anthelmintic.

***Moghania vestita*Kuntze**

The plant belongs to the family Fabaceae. It is a leguminous tuberous root crop commonly found in the north-eastern regions of India where it is consumed by the people together with its peels for curing intestinal helminths. It is very effective against *Paramphistomum*(Mali and Mehta, 2008).

Calotropis procera

The plant belongs to the family Asclepiadaceae. It is commonly used for the treatment of syphilis, eczema, and leprosy. It is effective against *H. contortus* where it acts by decreasing the number of larvae and eggs produced. The plant also is

effective against larvae of *Ostertagia*, *Fasciola*, *Nematodirus*, *Taenia* and *Dictyocaulus*(Mali and Mehta, 2008).

***Carica papaya* Linn.**

The tree is distributed worldwide. The active ingredient is known as benzyl isothiocyanate. The benzyl isothiocyanate exerts its action by inhibiting energy metabolism and hence affecting motor activity of the parasite (Mali and Mehta, 2008).

***Salvadora persica* L. (salvadoraceae)**

This is widely distributed in Africa and Asia. In Tanzania it is found in every district and has different uses (Mbuya *et al*., 1994). Various plant parts are edible. It is believed to possess antimicrobial and other medicinal properties and it is important in dental hygiene thus the plant has been used as toothbrush for centuries (Ruffo *et al*., 2002).

***Veronica amygdalina* (Compositae)**

This is a shrub or small tree of 2 to 5 m. The leaves are green with a characteristic odour and a bitter taste. The roots and the leaves are used in traditional medicine for treatment of fever, kidney problems and stomach discomfort (Ojiako and Nwanjo, 2006; Erasto *et al*., 2007).

***Albizia anthelmintica* Brongn (Mimosaceae)**

This plant is a deciduous shrub or small tree, ranging in height from 3 to 11 m. It has a characteristic grey bark, which can be smooth or deeply reticulated in texture. The plant bears fruits which are glossy pale brown (Githiori, 2004). The infusion of the bark decoction of this plant has been used against a range of parasites in both human and livestock in East Africa (Galal *et al*., 1991; Koko *et al*., 2000).

Areca catechu

This is commonly known as betel nut. It is grown mainly in India, Malaysia, Taiwan and other Asian countries (Jaiswal *et al*., 2011). It grows up to 30 m tall; its leaves are 1.5 to 2 m long. Its seeds contain alkaloids such as arecaidine and arecoline. These alkaloids are the ones which are responsible for its medicinal activity. It is used as taeniafuge for tapeworms, in treatment of urinary and diarrhoea disorders, as an antioxidant, as anti-inflammatory and as analgesic (Khan *et al*., 2011b).

2.7*Tithonia diversifolia*

The plant is commonly known as Mexican sunflower (Obafemi *et al*., 2006), tree marigold, shrub sunflower (Oyewole *et al*., 2006). The plant belongs to the family Asteraceae (Jama *et al*., 2000; Obafemi *et al*., 2006). It is a stout, shrubby herb (Liasu and Ogunkunle, 2007). The plant is an annual and it may grow to about 2-3m high (Olabode *et al*., 2007) and bears leaves which are sub-ovate and about 5cm in length. The leaves alternate, blade contains 3 to 5 lobes. The flowers are yellow orange in colour. The central part of the flower is made up of tight tubes each one is responsible for giving one seed after fertilization. The active ingredients of the plant

are concentrated in storage organs such as leaves, stems, barks and roots (Ogundare, 2007). The plant is native to tropical America (Mexico). However, it has been introduced to other parts of the world as an ornamental plant. It is grown in areas with temperature of about 15-31ºC and with mean rainfall of about 550-1950 mm. The plant tolerates drought and heat (Orwa *e*t *al*. 2009). It can be propagated either by cuttings or seed (Ipou *et al*., 2011).

2.7.1 Uses of the plant

In Egypt the plant has been used for lowering of blood sugar in animals (Liasu and Ogunkunle, 2007), in protection of crops from termites, as pig feed, soil erosion control, building materials, shelter for poultry (Fasuyi and Ibitayo, 2011; Olabode *et al*., 2007), antimalarial (Bouberte *et al*., 2006; Taiwo *et al*., 2007), treatment of pimples and stomach-aches (Taiwo *et al*., 2007), treatment of the wound and haematoma (Oyewole *et al*., 2006), as antiviral, in gastrointestinal disorders, as anti-inflammatory when dried leaves are applied externally on wounds (Obafemi *et al*., 2006; Bouberte *et al*., 2006); flowers have been used in the treatment of eye diseases while it has also been reported to have anti-rheumatic effects (Ogundare, 2007). The plant also has been used as green manure crop in Central and South America, Asia and Africa (Ogundare, 2007). Orwa *et al*., (2009) reported that the plant has been used as medicine for constipation and liver pain.

2.8 Brine Shrimp, *Artemia salina*Leach

Artemia is a member of fairly primitive crustaceans belonging to the genus *Artemia*, sub-class Branchipoda and order Anostraca. They are found throughout the world in

saline lakes and ponds. They have well developed mechanisms of surviving under harsh and variable environmental conditions. The mechanisms include the formation of very resistant ‘eggs’ (cysts). The cysts are embryos arrested at the stage of gastrula and they are encased in chitinous shells. The cysts only hatch if they have dried out to extremely low water content. During the dehydrated state metabolism is completely arrested and the embryos are in a state known as cryptobiosis. If they are exposed to water the eggs become active. In a few hours or days larval nauplii emerge and swim actively by rhythmic movements of the head appendages. For few days the nauplius feeds on yolk then begin to feed and develop through a series of moults and successive instars to the reproductive stage.

The exceptional physiological capacity of the *Artemia salina* is its ability to tolerate extremes of environmental salinity. *Artemia* can survive and even thrive in media ranging from1/2 the tonicity of sea water (about 1.5% NaCl) to concentrated brines in which salts actually crystallize out; this would be approximately at 15% NaCl concentration.

2.9 Brine Shrimp Lethality Assay

Brine shrimp has been a useful tool to researchers in genetics, histology, toxicology, biochemistry, molecular biology and ecology due its characteristics and short life span. It has also been used in screening pharmacological activities of chemical compounds and plant extracts (Carballo et al., 2002). The organism has been used in laboratory bioassay of toxicity and other biological actions through estimation of median lethal concentration (LC50 values). The test for the toxicity of chemicals

against the *Artemia salina* was developed by Michael *et al*. (1956) and was adapted and improved by others (Meyer *et al*., 1982; McLaughlin *et al*., 1991; Solis *et al*., 1993). Due to its sensitivity to a variety of chemical substances brine shrimp lethality test is a convenient preliminary toxicity test. The brine shrimp lethality (BSL) bioassay has been shown to be a useful and quick *in vitro* test for predicting toxicity of plant extracts and hence guiding their fractionation (Meyer *et al*., 1982).

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1 Study Area

The field work which included gathering of the information on plant parts used and preparations of the plant was done at Mbinga district, Ruvuma Region. The laboratory work was done at the Faculty of Veterinary Medicine, and in the Department of Animal Science and Production (DASP), Sokoine University of Agriculture (SUA).

3.2 Collection of Plant Samples

Informal interviews were conducted in three wards namely Litembo, Myangayanga and Mbaha at Mbinga District, Ruvuma Region. The interviews were carried out among farmers based on the uses of the parts of the plant, and how the plant parts were prepared for administration to animals (Appendix 1**).** The specimens were photographed, collected and carried to the Department of Crop Science and Production, SUA for further identification and confirmation by a botanist.

3.3 Preparation and Extraction of the Plant Materials

The leaves of *Tithonia diversifolia* were cleaned with water, chopped into small pieces and air-dried for 14 days under the shade. The air-dried leaves were ground into fine powder using laboratory mill (Christy Hunt Engineering Ltd, England) at the DASP, SUA.

3.3.1 Preparation of aqueous extracts

The aqueous extracts of the leaves were prepared by mixing 100g of the ground powder of the leaves with 1000ml of distilled water. The mixture was left to soak over-night at room temperature. The mixture was then filtered using a filter funnel fitted with Whatmann® filter paper No. 1 (Whatman International Ltd, England).

3.3.2 Preparation of ethanolic extracts

This was done by dissolving 138 g of *T. diversifolia* leaves in 250 ml of 80% ethanol overnight at room temperature. After 24 hours the mixture was filtered using a filter funnel fitted with Whatmann®filter paper No. 1 (Whatmann International Ltd, England). The filtrates were concentrated on water bath at 50°C using Rotavapor (BUCHI Labortechnik AG, Switzerland).

3.4 Experimental Animals and their Management

Fifteen young goats were purchased from Kingolwira and Mkundi villages in Morogoro municipality, Morogoro region, and transferred to the Animal Research Unit (ARU) at the Faculty of Veterinary Medicine, SUA. The animals were divided into three groups of five goats each and kept in separate pens with slatted floor (Appendix 2). The animals were treated with albendazole (Albandazole®, Hebei Yuanzheng Pharmaceutical Company Ltd, China) at a dose of 8 mg/kg body weight; sulfadimidine (S-Dime®, Cosmos company, Kenya) at a dose of 99 mg/kg body weight, ivermectin (Kelamectin®, Kela company, Belgium) at a dose of 0.2mg/kg body weight. The drugs were given for the control of endoparasites and ectoparasites. The animals were left to acclimatize for 2 months while being

provided with worm free hay and grasses (fetched from areas known to be free of nematode larvae infestation). The animals were also supplemented with maize bran and minerals (Superlick®, Farmers centres, Tanzania). The meal was prepared by mixing 2 kg of Superlick® with 38 kg of maize bran and 10 kg seed cake. Each animal was given 0.25 kg per day. The ingredients of the Superlick are given below:

Vitamin A	12 000 000 IU
Vitamin D_3	4 000 000 IU
Vitamin E	10 000 mg
Iron	250 000 mg
Manganese	200 000 mg
Copper	40 000 mg
Zinc	80 000 mg
Cobalt	6 000 mg
Iodine	10 000 mg
Selenium	200 mg
Calcium	22.5%
Chloride	13.2%
Sodium	8.8%
Phosphorus	9.0%

3.5 Analysis of Faeces for Eggs Per gram (epg) Count

The egg count per gram (epg) of faecal sample from each goat was done by using the McMaster method with slight modification. Four grams (4 g) of faeces were weighed and placed in container 1 and 56 ml of flotation fluid was added. The contents were

mixed thoroughly with a stirring device. The faecal suspension was filtered through a tea strainer or a double-layer of cheese cloth into container 2. While stirring the filtrate in container 2, sub-samples were taken using a Pasteur pipette. Both sides of the McMaster counting chamber were filled with sub-sample. The counting chamber was allowed to stand for 5 minutes. The sub-sample of the filtrate was examined under a microscope at 10x10 magnification. Eggs and coccidian oocysts within the engraved area of both chambers were counted.

The epg was calculated as follows:

Add the egg count of the two chambers together. Multiply the total by 50.

3.6 Solvents

Distilled water and ethanol were used for aqueous and ethanolic extracts preparation respectively. Ethanol (99.9%, Harris reagent, Philip Harris limited, Sheristone, England) was purchased from a local dealer in Morogoro. Distilled water was obtained from the Department of Veterinary Microbiology and Parasitology, SUA.

3.7 The Test Organisms

Artemia salina Leach (*Artemia salina* sanders^{TM} Great Salt Lake, Brine Shrimp Company L.C., USA) was used for brine shrimp lethality bioassay. The eggs were donated by Dr. Joseph Jangu Magadula, Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences (MUHAS).

3.8 Larvae

Adult *Haemonchus contortus* worms were isolated from the abomasa of goats purchased from Melela slaughter slabs in Mvomero District in Morogoro region from December 2011 to February 2012. The worms were isolated by incising the greater curvature of the abomasa. The female worms were then ground using mortar and pestle to liberate the eggs. The suspension was centrifuged (Kubota 5100, Japan) for 5 minutes at 1500 rpm and the supernatant was discarded (Hussien *et al*., 2011). The sediment which contained eggs was then incubated in a conical flask for 7 days (Appendix 3) and 3rd stage larvae were then harvested, and stored at 4ºC. The larvae were used to infect goats in Groups 1-3 as described in section 3.10.

3.9 Brine Shrimp lethality Assay for *T. diversifolia*

3.9.1 Hatching of the brine shrimp

Brine shrimp (*Artemia salina* Leach) eggs (*Artemia salina* sanders^{TM} Great Salt Lake, Brine Shrimp Company L.C., USA) were hatched in simulated seawater prepared from sea salt and acted as culture medium. (Sea salt is usually prepared by boiling seawater to evaporation). The simulated seawater was prepared by dissolving 3.8 g sea salt in 1 litre of distilled water. Rectangular glass chamber divided into two unequal compartments with holes on the divider was used for hatching. The eggs were sprinkled into the large compartment that is darkened, while small chamber was illuminated. After 24 hours incubation at room temperature, nauplii (larvae) were collected by Pasteur pipette from the lighted chamber, while their shells were left in the darkened chamber.

3.9.2 The bioassay

The brine shrimp lethality test was carried out using the standard procedure as described by Meyer *et al*. (1982) and McLaughlin (1991) with slight modifications. The stock solution of the study plants extracts were prepared by dissolving 160 mg of the dry extract in 4 ml of DMSO to get a concentration of 40 $mgml^{-1}$. Taking 30, 15, 10, 5, 3 and 1µl of the stock solutions the final concentrations of 240, 120, 80, 40, 24 and 8 μgml^{-1} were obtained by dilution with 5 ml of the sea salt solution in vials. Each concentration was tested in duplicate making a total of 12 vials using DMSO as a negative control as previously described by Moshi and Mbwambo (2005) and Moshi *et al*. (2006). Ten larvae of brine shrimps suspended in a small amount of the culture medium (sea salt solution) were transferred, using Pasteur pipettes, into each of the vials containing test extract, followed immediately with adjusting the volume of the sea salt solution to 5 ml mark. The vials were left on the laboratory bench, at room temperature (25ºC), for 24 hours in order to determine survival rate of the larvae at the different concentrations of *T. diversifolia* extract. Survivors were counted after 24 h and from these the percentage death at each concentration was determined according to Meyer *et al*. (1982) and (McLaughlin, 1991) (Appendix 4).

3.10 Experimental Design

The parasite free goats described in section 3.4 were divided into three groups, Group 1, 2 and 3, consisting of five goats each. All animals were then administered 1250 *Haemonchus contortus* larvae orally. On Day 21 after administration of larvae,

faecal samples were collected from each goat and analysed for epg count to establish presence and magnitude of worm burden.

Goats in Group 2 were treated with extracts of *Tithonia diversifolia* leaf extract at an oral dose of 50 mg/kg body weight, while goats in Group 3 were treated with albendazole (Albandazole®) at a dose of 8 mg/kg body weight orally. Goats in Group 1 were left untreated and acted as negative control. Following treatment, faecal samples were collected from each goat on Day 0, Day 4, Day 7, Day 10 and Day 14 and analysed for epg. On day 14 all animals were sacrificed and *H. contortus* worms isolated from the abomasa and transferred into a petri-dish where the total worm counts were carried out and recorded for each goat.

The epgs were used to calculate faecal egg count reduction (FECR) according to Coles *et al.* (1992). FECR%=100 (1- $\bar{X}_t/\bar{X}_c$) where $\bar{X}_c$ and $\bar{X}_t$ represent the arithmetic means of the control and treated groups respectively. From the same data the lower confidence limit (95% CI) was determined using the formula: 100(1- $\bar{X}_t/\bar{X}_c$ exp (+2.048√Y^2) where $\bar{X}_c$ and $\bar{X}_t$ represent the arithmetic means of the control and treated groups respectively and Y^2 represents variance of reduction.

The percentage efficacy of the drug against the worms was calculated by using the formula:

$$\% \text{ efficacy} = \frac{C-T}{C} \quad \text{.....(1)}$$

Where C is the arithmetic mean of the worms in the control group and T is the arithmetic mean of worms in the treated group.

Group mean for the treated group was calculated by using the formula:

$\overline{X} = \frac{\sum fX}{\sum f}$ ..... (2)

Where

$\overline{X}$ is the mean

f is the number of occurrences

$\sum fX$ is sum of products fX

$\sum f$ is the total number of occurrences

Procedures for total worm count

This was done according to Tritschler (2008). The abomasum was opened along its greater curvature and the contents were spilled into a black flat tray. The abomasum was then spread on another flat tray. Worms attached to mucosal folds of the abomasum were removed manually and placed on the tray. The abomasal contents were transferred into a 50 µm sieve and then washed using gentle jet of tap water to leave *H. contortus* worms on the sieve, with dirt and fine particles removed. The washed contents were then taken and poured into another illuminated black flat tray. The worms were collected using forceps and then counted manually. The procedure was done to all abomasa from the experimental goats.

Data analysis

Data were entered in Microsoft Excel and the Means±SD of total worm count in each group was calculated. For the determination of LC_{50}, data were analyzed by using single-factor ANOVA and the regression line was plotted using the Microsoft Excel Program. The faecal egg count reduction percentage was obtained by using the formula described by Scoles *et al*., (1992).

CHAPTER FOUR

4.0 RESULTS

Informal interviews which were conducted in five villages (located in three wards in Mbinga District) revealed that 69% of the villagers were aware that *T. diversifolia* is used as anthelmintic in goats. They said that the plant parts which are commonly used are the leaves and it is given to the animals as decoction preparation. Seventy six (76%) of the respondents revealed that the plant is used as anthelmintic in human beings while 11% knew nothing about the uses of the plant. The plant was identified by a botanist at SUA as *Tithonia diversifolia* (Plate 1).

Plate 1:*Tithonia diversifolia* plant showing branches, leaves and flowers.

Twenty nine days after experimental infection of 1250 larvae to the goats the epg was determined. It was found that the epg in all animals was high. This shows that all animals were successfully experimentally infected. On the same day of counting the animals were treated with either albendazole or *T. diversifolia* extracts (day 0).The epg on day 7 (post-treatment) were higher in the negative control group as compared to the *Tithonia diversifolia* treated group. On the other hand the epg in albendazole treated group (positive control group) was 0 on Days 7 and 14 (post-treatment), showing that albendazole was 100% effective. The epg in the negative control group on Day 14 was higher compared to the treated group (Table 1).

Table 1: Mean± SD egg counts in faeces after infecting goats with 1250 larvae of *Haemonchus contortus*

	Faecal egg counts per gram of faeces (epg)		
	Control Group (Untreated)	***T. diversifolia* treated Group**	**Albendazole treated Group**
Day 0	2260±2145	1140± 680	2680 ±1530
Day 7	3420±3282	1140 ±764	0
Day 14	4780±2926	3420 ±1432	0

On Day 14 all animals were sacrificed (Plate 2) and epg analysis was carried out in all groups of animals. The percentage reduction in epg in the *Tithonia diversifolia* treated group was 29%, while the percentage reduction in the albendazole treated (positive control) group was 100% (Table 2).

Plate 2: Sacrificed goatsshowing abomasum (bottom picture with red arrow) where the *Haemonchus contortus* worms were recovered.

Table 2: **Post-treatment faecal egg-count on Day 14**

	Faecal egg counts per gram of faeces (epg)		
	Control Group (Untreated)	***T. diversifolia* treated Group**	**Albendazole treated Group**
Number in group	5	5	5
Arithmetic mean	4780	3420.00	0.00
variance of counts	8567000.00	2052000.00	0.00
Percentage reduction		29	100.00
Variance of reduction (log scale)		0.11	Undefined
Approximate 95% confidence Limits	-	-	-
Lower confidence limit		80	Undefined

With respect to the total worm count, results show that on Day 14 the mean of the total worm count in the untreated group (negative control) was 155 worms while that of the treated group was 158 worms (Figure 3). The percentage efficacy in the *T. diversifolia* group was 0 while that in albendazole treated group was 100% (Table 3).

Table 3: Percentage efficacy on Day 14

Groups	**Percentage efficacy**
T.diversifolia treated group	0
Albendazole treated group	100

When the brine shrimp lethality test was carried out, results show that the LC_{50} was 58.5µg/ml (Figure 2). Therefore the extract from the study plant is considered to be safe when used as medicinal drug. In addition the drug is considered to be cytotoxic if the value of LC_{50} is less than 20 µg/ml and it is bioactive if it is less than 100 µg/ml.

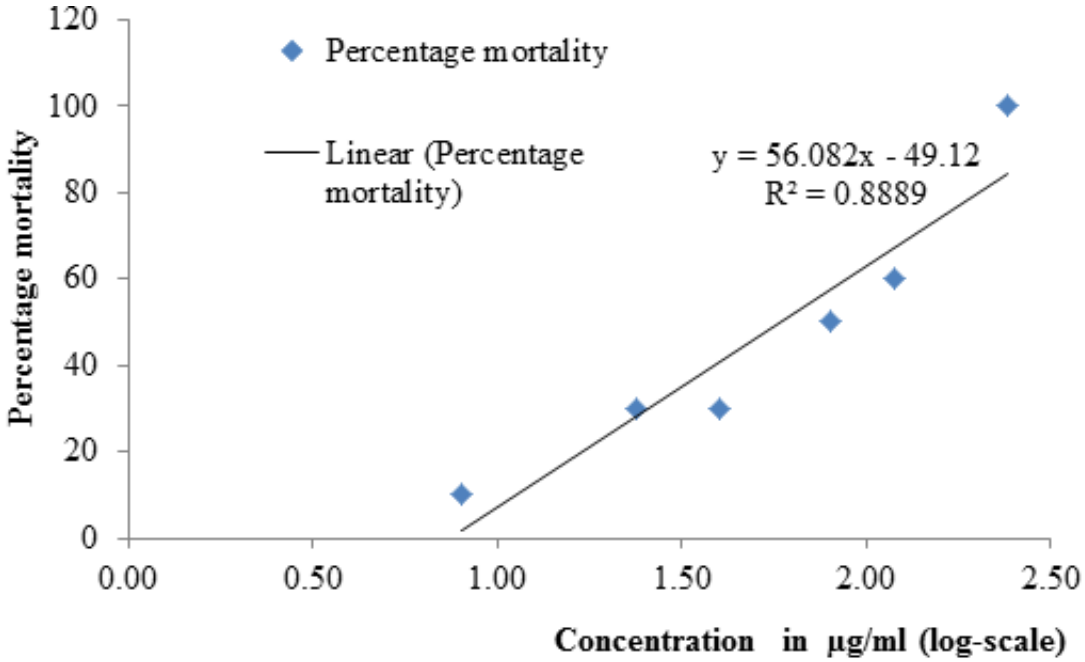

Figure 2: Percentage of larvae mortality against *T. diversifolia* concentration in µg/ml (log-scale).

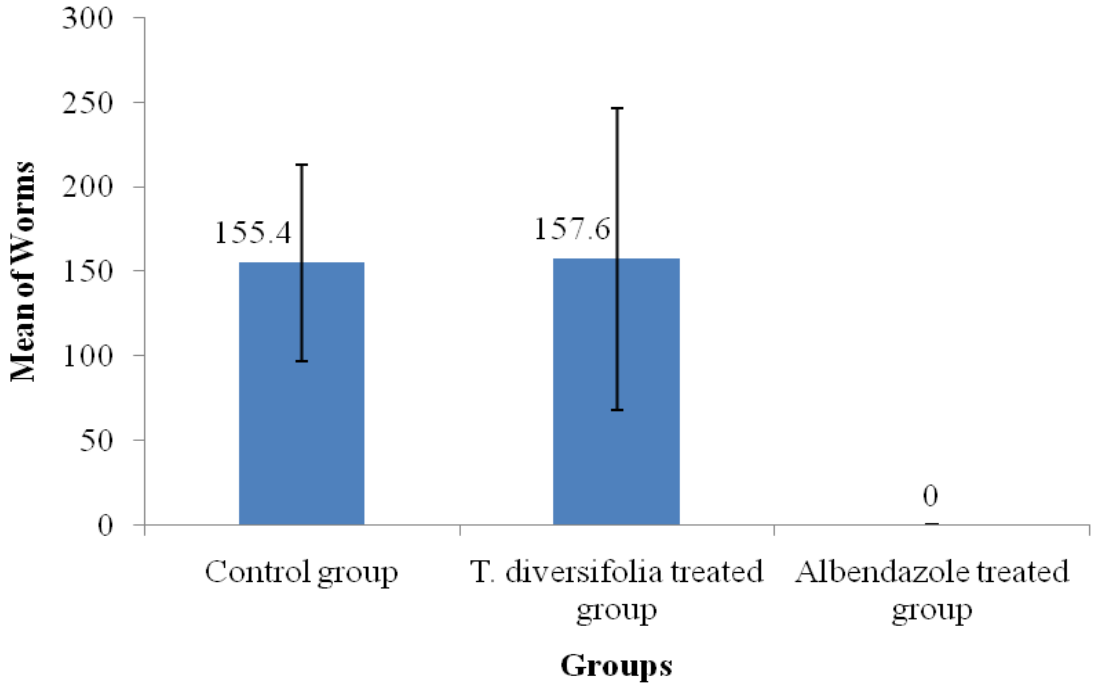

Figure 3: Means and standard deviations of the total worm count on Day 14.

CHAPTER FIVE

5.0 DISCUSSION

Informal interviews conducted in five villages in Mbinga District revealed that 69% of the villagers were aware that *T. diversifolia* is used as anthelmintic in goats. Most of these respondents had been using the plant for the regular deworming of their goats. The commonly used plant parts used are leaves. Upon the interviews most of the people said that they use the leaves simply because they are easy to boil and are given to the animals as decoction preparation. The same plant was observed to be used as anthelmintic in human. The villagers said that they use it due to its availability and affordability. These reasons also agree with Githiori (2004) who reported the uses of plants as anthelmintics.

The goats were dewormed before administration of the larvae. This was done so as to clear gastro-intestinal helminths. After the deworming the goats were analysed for epg and all animals had zero epg. After 22 days all goats were infected with infective larvae of *Haemonchus contortus*. On Day 21 epg was done before the administration of the drugs in the two groups (*T. diversifolia* and albendazole treated groups) and it was found that all animals had significant levels of epgs. This shows that the animals in all groups had acquired infection successfully.

The percentage reduction in epg after deworming with albendazole shows that the drug is 100% effective against *Haemonchus contortus*. Similar results were obtained by Ahmad and others (2010) when they used albendazole as anthelmintic against

Haemonchus contortus. Seven days after treatment there was a significant difference between the *T. diversifolia* treated group and albendazole treated group. The albendazole treated group had 0 epg showing that the drug was more effective than the extracts of *T. diversifolia* plant. The difference which was observed on Day 14 was not due to pharmacological activity of the drug since the differences were not statistically significant (P=0.964).

The results of the total worm count done on Day 14 after treatment of the experimentally infected goats show that the difference between the means from untreated and *T.diversifolia* treated groups was not statistically significant (P= 0.987). This shows that the plant had no pharmacological activity against adult worms. The mean worm count in the albendazole treated group (positive control) was 0 showing that albendazole is very effective in controlling the *Haemonchus contortus* worms as compared to *T.diversifolia.* The results shown by albendazole agree with those reported by Kumsa and Wossene (2006).

The results from this study show that the aqueous extract of *T. diversifolia* plant was not effective against *H. contortus*. Although the people in Mbinga district have been using this plant for deworming animals, it is for certain that it is not effective against *H. Contortus* based on the results of the present study.

According to WAAVP an anthelmintic is considered to be effective if it has percentage faecal egg count reduction of 95% and above, with lower confidence limit

of 90% and above (Coles *et al*., 1992). Basing on this fact, the plant studied did not meet the criteria of being considered effective against *H.contortus.*

In previous studies, it has been noted that not all cases of alleged anthelmintic activity of certain plant extracts reported was confirmed by controlled experimentation. For example, the administration of *Myrsine africana* and *Rapanea melanophloeos* extracts to parasitized sheep in Kenya did not result in reduction of the level of parasitism in controlled experimental studies (Githiori *et al.*, 2002).

There are a number of factors which could have led to the observed ineffectiveness of the anthelmintic plants. These factors include the process of collection/ harvesting and storage. The dose of the drug given to the animals, may be was small thus failing to kill the worms in the group treated with *Tithonia diversifolia*. The physical and chemical properties of anthelmintic may be affected by season at which the plant is harvested. This is due to the fact that the concentration of the active ingredients may vary with the season.

If the plant is stored for a long time, there are changes in plant availability in nutrients and metabolites which affect the reproducibility of anthelmintic activity. In the study conducted by Chandrawathani *et al*. (2006) on the neem tree, the fresh leaves were collected and given to the animals on daily basis while in another study done by Githiori *et al*. (2004) the animals were fed with conserved leaves. The results were inconsistent. The inconsistency could be attributed to the method of preservation which may have affected the plant properties.

In most of the medicinal plants the doses and duration of the treatment are not well known and established. For example some plants become effective anthelmintics only if they are consumed regularly by animals (Githiori, 2004; Athanasiadou *et al.,* 2007).

On the other hand, the livestock keepers may claim anthelmintic effect of the different plants if they observe parts of the worms expelled with faeces, but it becomes difficult to appreciate the effect when the species of worms are nematodes whose identification needs specialized techniques and equipments.

In this study we observed FECR percentage of 29% which is very low for *T. diversifolia*. Similar (low FECR percentage) was documented by Iqbal *et al.*, (2004) who demonstrated that the FECR percentage of 62% of abomasal nematode *H. contortus* egg counts after the animals were fed with whole plant preparation.
The observed low FECR percentage could also have been contributed by small sample size per test group since the recommended sample size is 6 animals per group.

Although this plant did not show significant anthelmintic activity against *H. contortus*, other plants have been reported recently to have anthelmintic activity against nematodes. These include *Artemisia herba-alba* (Sonibare *et al*., 2011), *Anarcadium occidentale* (Ademola and Eloff, 2011), *Nauclea latifora* (Mallik *et al*., 2012), *Musa paradisiaca*, *Anogeissus leiocarpus,* and *Danielliaoliveri* (Bouberte *et al*., 2010).

The brine shrimp toxicity results show that, the plant is not acutely toxic due to its high LC_{50} value of 58.5µg/ml. A substance is regarded as acutelytoxic to biological systems, if it has an LC_{50} value of not more than 20 μgml^{-1} (McLaughlin *et al*., 1991; Yoga Latha *et al*., 2007; Mbwambo *et al*., 2007). Furthermore, it has been established that, for a compound to be considered completely safe, it should have LC_{50} value greater than 100 μgml^{-1} (Carballo *et al*., 2002; Chowdhury *et al*., 2005; Yoga Latha *et al*., 2007). Therefore more elaborate toxicity studies are needed to establish the safety of the extract from *T. diversifolia*.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study it can be concluded that, based on the dosage and formulation used it appears that *Tithonia diversifolia* did not show effectiveness on both faecal egg count and post-mortem worm count reduction tests carried out in this study as compared to conventional drugs.

6.2 Recommendations

From the study it can be recommended that more studies should be carried out so as to validate the anthelmintic effects of the plant by investigating other specific species of the nematodes. Since the understanding of most livestock keepers of the word 'worms' refers to macroscopic gastrointestinal parasites (tapeworm segments); it would be worthwhile to test the effects of this plant against cestodes and trematodes. The studies using laboratory animals should be carried out so as to demonstrate the efficacy of the plant.

Further studies should be conducted in the study area so as to investigate at which season the plant should be harvested, and how the plants should be stored before being processed for treatment. More work need to be done in the discovery, documentation and validation of local ethnoveterinary practices in Africa. More researches should be carried out so as to improve livelihood of many poor families.

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APPENDICES

Appendix 1: Questionnaire on Evaluation of Anthelmintic Effects of *Tithonia Diversifolia*

Date: .....

Village name: .....

Ward name: .....

Name of respondent: .....

Sex: M/F

Age: .....yrs

Species of animals treated

(1) Human being

(2) Goats

(3) Sheep

(4) Cattle

(5) Chickens

Plant name.....

Uses

(1) Deworming

(2) Other uses.....

Parts of the plant used

(1) Leaves

(2) Root barks

(3) Stem barks

Preparation

(1) Decoction

(2) Poultice

(3) Others.....

Appendix 2: Goats housed in a slatted floor

Appendix 3: Larvae incubated in a conical flask

Appendix 4: Protocol for Brine Shrimp Toxicity (BST) Assay

1. Introduction

The BST assay is based on the ability of plant extract to kill laboratory cultured *Artemia nauplii* brine shrimp.

- The assay is a useful tool for a preliminary assessment of toxicity of plant extract.
- LC_{50} of <20 µg/ml is suggestive of possible cytotoxicity (anti-cancer activity). LC_{50}>100 µg/ml is regarded to be non-toxic.

2. Procedure:

2.1 Preparation of Brine Shrimp Salt Water as follows

(i) Weigh 3.8 g of brine shrimp salt and put it into a 100 ml beaker

(ii) Add distilled water and stir well

(iii) Filter the dissolved salt through filter paper into a measuring cylinder with a capacity of 1 litre.

(iv) Add some more water into the beaker. Stir well and filter again.

(v) Repeat step (iv) above until all the salt has dissolved

(vi) Filter more distilled water until you get 1L solution

2.2 Hatching the Nauplii

- Put the Brine shrimp salt water into hatching tank. (at least prepare 2L solution of brine shrimp salt water since more than 1L solution will be used for hatching brine shrimps).
- Take a maximum of 1g of brine shrimp eggs and put it into the dark side of the hatching tank.

- Switch on the light to allow the hatching tank to have two sides: light and dark sides. This will allow the hatched nauplii to move from the dark side to the light side, therefore making it easier to collect the nauplii.
- Leave the eggs for overnight to allow hatching.

2.3 Preparation of Extract Stock Solution

- Dissolve 40 mg of the crude extract into 1 ml DMSO or water (depending on the nature of extract)

2.4 Experimental setup

- Arrange vials in duplicates. Mark them as 1:1, 1:2, 2:1, 2:2, 3:1, 3:2, 4:1, 4:2, 5:1, 5:2 and 6:1, 6:2.
- Put 2 ml of Brine Shrimp salt water into each vial
- Take ten nauplii from the light side of the hatching tank by using Pasteur Pipette and put them into each of the of the 12 vials (Note: each extract will be tested by using a set of 12 vials) with more than 5 ml capacity
- Draw 30 µl of the extract stock solution and put it into the 1:1 labelled vial. Take another 30 µl of the extract stock solution into the 1:2 labelled vial. This will complete the first set.
- Draw 15 µl for the 2^{nd} set, 10 µl for the 3^{rd} set, 5 µl for the 4^{th} set, 3 µl for the 5^{th} set, and 1µl for the 6^{th} set.
- Adjust the volume to 5 ml once you have added the stock solution to each of the vials.
- Leave the nauplii in the vials for up to 24 hours for the extracts to exert their effects.
- Record the number of survivors for each vial after overnight incubation by using this table.

Set No.	No. of survivors	No. of dead	Percentage mortality	Average Percentage mortality
1:01				
1:02				
2:01				
2:02				
3:01				
3:02				
4:01				
4:02				
5:01				
5:02				
6:01				
6:02				

Appendix 5: *Haemonchus contortus* worms in a petri dish