IN VITRO ANTHELMINTIC ACTIVITY OF METHANOL EXTRACTS OF SELECTED KENYAN MEDICINAL PLANTS AGAINST *Haemonchus contortus*

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DECLARATION

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

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DEDICATION

I dedicate this work to my mother Evelyn for the moral support, financial assistance and encouragement through the entire period of conducting the study.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF APPENDICES

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
EHA	Egg Hatch Assay
FAO	Food and Agriculture Organization of the United Nations
G.D.P	Gross Domestic Product
GC-MS	Gas chromatography-Mass spectrophotometry
KALRO	Kenya Agricultural and Livestock Research Organization
L3	Third-stage larvae
W.A.A.V.P	World Association For The Advancement Of Veterinary Parasitology

ABSTRACT

Agriculture contributes to a quarter of the gross domestic product in our beloved country Kenya with the livestock sector contributing 45% of its total. There are many constraints that hamper livestock production. Among them, helminthiasis is associated with deaths among sheep, weight loss and reduced production. Anthelmintic drugs are used in treating and control of helminth infections. The current emergence of resistance to anthelmintic drugs added to their high cost has necessitated developing regimens that are more effective, cheap, and eco-friendly compounds in battling the war against helminths. Plants which are abundant and cheap offer a promising alternative to circumvent resistance and spiralling cost of drugs. In Kenya plants have been traditionally used for treating helminthiasis, however there lacks scientific prove of their efficacy. This study evaluated the efficacy of *Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physalis peruviana* and *Caesalpinia volkensii* traditionally used by the Meru and Tharaka Nithi community for parasitic worm infections, against *Haemonchus contortus*. The study entailed screening of the methanolic extracts of the above plants against *Haemonchus contortus*, both drug susceptible and isolates resistant to albendazole from sheep. Efficacy of these plants extracts was tested in an *in vitro* system using eggs and larvae of *Haemonchus contortus*. Egg hatchability was determined after 48-hour incubation with extracts while with larvae survival was determined after six days incubation. Physiological saline was used as negative control while positive controls used included albendazole and levamisole for susceptible and resistant isolates respectively. The experiments were carried out in triplicates. One-way ANOVA was used for analysis followed by Tukey's post hoc test. *P. peruviana* and *R. apetalus,* inhibition percentages of 95.24±0.54, 90.00±1.00, 88.24±0.66 and 96.55±0.45, 85.71±0.79, 82.14±0.76 at 50mg/ml, 25mg/ml and 12.5 mg/ml respectively with no significant difference ($P<0.05$) in the egg hatch assay for susceptible isolates with the positive control. For the egg hatch assay with the resistant isolates, the highest mean inhibition percentages were observed with dilutions of 50 mg/ml achieving 93.42±0.46, 91.67±0.47, 94.56±0.36 and 91.80±0.59 with *R. apetalus*, *B. micrantha, C. volkensii* and *H. abyssinica* respectively with no significant difference ($P<0.05$) between them and levamisole. In the larvae developmental test for the susceptible isolates the highest mean percentage larvicidal activity of 100.00±0.00 was achieved with extracts from *R. apetalus* and *H. abyssinica* across the three dosages with no significant difference between the two and albendazole. For the L3 larvae from the resistant isolates reduced larvicidal activity was recorded with 78.38±0.48, 58.33±0.37 and 52.00±0.16 at 50mg/ml for *R. apetalus, C. volkensii* and *T. garckeana* respectively which had a statistically significant difference compared to levamisole. Synergism between the extracts with albendazole was conducted against the resistant L3 isolates where increased larvicidal activity was achieved with 98.46±0.32 for *R. apetalus* at 50mg/ml which had no significant difference at $P<0.05$. The MIC_{50} was determined where *R. apetalus* had 3.37mg/ml. Phytochemical analysis through GC-MS was also conducted on the plant extracts where compounds such as terpenoids were conspicuously present in the extracts which could account for some of the activity observed. However, from this study, supplementary studies are recommended to elucidate the phytochemicals accountable for the anthelmintic activity demonstrated by the plants in this study.

CHAPTER ONE

1. INTRODUCTION

1.1 Background information

Agriculture in Kenya remains the backbone of the economy playing crucial roles, especially in the rural economy. It contributes up to 26% of the total GDP (Gross Domestic Product) of our country Kenya ("Kenya at a glance | FAO in Kenya | Food and Agriculture Organization of the United Nations," 2017). It also plays a very integral part in providing employment opportunities to citizens employing more than 40% of the country's populace with the bulk being Kenyans living in the rural areas where more than 70% of them rely on agriculture as a source of income ("Kenya at a glance | Kenya | Food and Agriculture Organization of the United Nations," 2017).

The agricultural sector contributes to 65% of the total exports earnings made by the country ("Kenya at a glance | Kenya | Food and Agriculture Organization of the United Nations," 2017). The livestock industry accounts for 45% in the agricultural industry, and comprises of cattle, goats, sheep, and camels among others. Infestation by gastrointestinal nematodes has become a major factor limiting productivity in livestock in Kenya. This is further compounded by resistance to currently used anthelmintic drugs (Shalaby, 2013).

Helminthiasis which is as a result of infestation by helminths continues to be a major challenge in the productivity of farm animals all around the planet. Anthelmintic agents remain to be mainstay in the control of helminthiasis. These agents also have challenges associated with their use such as toxicity, increased cost of production, existence of drug remnants in the products got from animals and their byproducts in the ecosystem, cases of the drugs being not adaptable and available in the remote parts of the nation. This calls for the

search on alternative control measures to deal with challenges of helminth infestation in livestock (Rajeswari, 2014).

Haemonchus contortus also known as barber's pole worm is one of the most prevalent and pathogenic nematodes affecting small ruminants such as sheep and goats. These parasites adversely affect production due to high rates of mortality in the animals especially during the rainy seasons (Rajeswari, 2014). Parasitism by the gastrointestinal nematodes in ruminants and the emergence of resistance among these parasites has been documented across the globe (Kamaraj *et al.,* 2011). This has resulted in the increased interest in the search for alternative anthelmintic drugs. This is because the emergence of resistance to the existing anthelmintics has been associated with increased costs in farming, the occurrence of their residues in the environment and food and this has in turn awakened the need for the search of alternative anthelmintic regimens from plant origins (Kamaraj *et al.*, 2011).

In the developing countries, 80% of the populace rely on phytochemicals for their primary healthcare needs for both animals and plants alike (Kamaraj *et al.,* 2011). *Haemonchus contortus* when compared with the other nematodes are pathogens that are highly parasitic affecting the small ruminants and the parasite has the ability of resulting in acute disease and also have a high mortality rate in the stock (Kamaraj *et al.,* 2011). *Haemonchus contortus* is among the single most predominant nematodes affecting the gastrointestinal tract in farm animals such as goats and sheep since it sucks the blood from the host resulting in the loss of plasma in blood and proteins as well as causing severe anaemia.

In Kenya multiple resistance to albendazole, levamisole, thiophanate and ivermectin that is orally administered has been documented in *Haemonchus contortus* isolated from a farm where resistance to benzimidazole had earlier been identified (Waruiru *et al.,* 1997). Gastrointestinal nematodes affecting both goats and sheep that are resistance to ivermectin

and febendazole have been identified in the coastal region of Kenya (Mwamachi *et al.*, 1995). Low efficacies of 44%, 77%, 66%, and 42% in the faecal egg count reduction percentages for the drugs ivermectin, levamisole, a combination of levamisole and rafoxanide and albendazole respectively for gastrointestinal nematodes in sheep on a farm in Kabete Kenya have been documented (Gakuya *et al.*, 2008).

Utilizing medicinal herbs to manage helmintic parasites has been in use since time immemorial due to numerous advantages such as the sustainability of supply and their biodegradability capabilities hence making them environmentally friendly (Chen *et al.*, 2016). For ages, plants have offered a constant source of remedy for numerous diseases. Traditional medicine practices have been as old as the human civilization itself. Plants have been documented to be richly endowed with antibacterials, antifungals, antihelminthics and insecticidal compounds (Iqbal *et al.*, 2001).

Among the Maasai community 289 plant species have been documented to be used in indigenous medicine with the majority being used as a remedy to gastrointestinal and respiratory ailments (Nankaya *et al.*, 2019). In Migori county 21 plants have been documented to harbour anthelmintic properties. Among the identified plants *Vernonia amygdalina* was identified to have a mortality of 20-33.3% at 6.25 mg/ml, 23.3-46.7% at 12.5 mg/ml and 26.7-56.7% 25mg.ml against adult *Haemonchus contortus* (Yusuf *et al.*, 2015). Tannins, saponins and cardiac glycosides in the plant have been attributed to its anthelmintic effect.

In Kirinyaga County, the aqueous extracts of *Aspillia pluriseta, Vernonia lasiopus, Entada leptostachya* and *Erythrina abyssinica* have been screened for anthelmintic properties against *Haemonchus, Mecistocirrus, Ostertagia, Trichostrogylus, Cooperia, Bunostomum* and *Oesophagostomum stronyles* (Njonge *et al.*, 2013). These plants have been used with success by farmers in the treatment of gastrointestinal nematodes in cattle.

Traditionally among the Meru and Tharaka Nithi community plants have been employed to treat numerous ailments afflicting the human populace and the livestock as well. This knowledge has been inherited from one generation to another. However, only a handful of scientists have since documented this knowledge with no single study documenting the effectiveness of these plants against helminths. Since there is no scientific information to validate this assertion this study demonstrated effectiveness of the study plants against *Haemonchus sp,.* and provided insights that are valuable in the advancement of modern medicine.

This study focused on evaluating the *in vitro* anthelmintic properties of selected plant materials that have been commonly used in the treatment of suspected helminthic infestations among the Meru and Tharaka Nithi community. No prior studies have systemically evaluated the anthelmintic efficacy of these plants in Meru and Tharaka Nithi hence the study addressed a critical gap in ethnopharmacological knowledge.

1.2 Statement of the problem

Helminths encompass a variety of parasitic worms that include trematodes, nematodes and cestodes. They pose a considerable health risk to humans and animals globally. Despite the fact that the diseases caused by helminths can be drastically reduced through control measures such as improved pasture management in the grazing fields for domestic animals, such measures are not adequate to mitigate negative effects of these parasites.

With the absence of a vaccine, the control of helminths is entirely reliant on the use of anthelmintics to reduce morbidity and mortality and minimize their transmission. This has resulted in the concerted use of anthelmintic agents consequently leading to the emerging of widespread anthelmintic resistance to all the available medications.

The understanding of the emergence and development of resistance is crucial in a bid to extend the efficacy of the available drugs (Laxminarayan*et al.,* 2006), in addition to formulation of novel therapeutic agents to overcome and prevent resistance among these parasites. Resistance to anthelmintic drugs by nematodes is already a serious problem among small ruminants such as sheep and goats (Várady *et al*., 2011).

Use of traditional medicine in the treatment and control of helminths has been reported in some parts of the globe, Africa and even in Kenya. Several plants in Meru and Tharaka Nithi are reputed to treat helminths by herbalist but no scientific information on their use is available. This study was designed to plug this gap. This study investigated the efficacy of *Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physalis peruviana* and *Caesalpinia volkensii* which are herbal plants in Meru and Tharaka Nithi that are traditionally employed to treat suspected helminth infestations.

1.3 Justification

In the developing countries like Kenya, the incidence and severity of resistance pose a major menace to the profitability of the entire livestock industry. Occurrence of resistance has increased to all the common anthelmintic drugs that are broad spectrum such as levamisole and benzimidazoles as well as in ivermectins and milbemycins (Geerts and Gryseels, 2000). In addition to the development of resistance the anthelmintic drugs are expensive and their improper use leaves drug residues in the products derived from the animals which include milk and meat. Development of herbal therapies would resolve the problems associated with the conventional drugs as mentioned above. Herbal drugs are easily available and methods of preparation are also easy and cheap. The study provided deeper insight into the available options for treating helmintic infections and contributed to the expansion of the number of regimens in the anthelmintic armament. The active compounds from these plants were also be

elucidated by determining the secondary metabolites which provided lead compounds for further drug development.

1.4 Null Hypothesis

Medicinal plants used in Meru and Tharaka Nithi for treating helmintic infections have no anthelmintic activity against *Haemonchus contortus.*

1.5 Objectives

1.5.1 General objective

To determine the effect of methanol extracts of *Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physalis peruviana and Caesalpinia volkensii* against *Haemonchus contortus.*

1.5.2 Specific objectives

i. To determine the phytochemical constitution of methanol extracts of *Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physalis peruviana* and *Caesalpinia volkensii*.

ii. To determine the *in-vitro* activity of methanolic extracts of *Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physalis peruviana* and *Caesalpinia volkensii* against *Haemonchus contortus*.

iii. To determine the minimum *in-vitro* inhibitory concentrations of methanol extracts of *Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physali speruviana* and *Caesalpinia volkensii* against *Haemonchus contortus*.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Helminthiasis in small ruminants

Livestock farming is an important part of agricultural practices. It provides a crucial source of food for humans whose populace is ever on the rise. It also contributes greatly to the economy of our great nation Kenya (Bahta *et al.*, 2022). Regardless of the remarkable milestones in the animal rearing practices, challenges are still face the sector. These challenges need to be overcome so as to better the general animal welfare and productivity in order to realize increased earnings by the farmers.

Among the leading constraints negatively impacting production in animals is the prevalence of parasites in the livestock and this in turn negatively impacts food security globally (Rehman & Abidi, 2022). Among the various parasitic infections affecting livestock, helminths result in severe pathology which results in subsequent deterioration in the health of the animals. This critically affects the productivity of the animals hence causing enormous losses economically (Rehman & Abidi, 2022).

The hunt for a vaccine that is efficacious against the helminths is still a work in progress and anthelmintic medications remain the mainstay treatment in treating and controlling helminthic infections (Vercruysse *et al.*, 2018). This however can't be relied on as the exclusive alternative for a sustainable developmental approach to safeguard the economy. This as a consequence calls for more efficacious integrated measures meant to control the threat posed by the parasites (Vercruysse *et al.*, 2018).

In the recent past, several strategies such as management of pasture, anthelmintic agents and diagnosis that is disease-specific among others have been embraced in an attempt to realize

better handling of the health and production of the animals but the results from successfully implementing them on the farms are yet to be realized (Rehman & Abidi, 2022).

In Kenya, key gastrointestinal nematodes in sheep and goats *include Haemonchus contortus, Trichostrongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp., *Nematodirus* spp., and *Trichuris* spp. *Haemonchus contortus* is the most critical due to its blood-feeding, causing severe anaemia, weight loss, and mortality. Infestation by *Trichostrongylus* and *Oesophagostomum* spp. lead to diarrhoea and reduced growth. These effects cause significant economic losses, necessitating control through anthelmintics, pasture management, and nutritional support to ensure animal health and sustainable production.

2.2 Treatment and control of helminths

Helminthic infections in livestock largely impact negatively on production success in ruminants across the world and their effective control is crucial for livestock production to increase and meet the future need by the human populace.

The management of helminthic infections is heavily reliant on the periodic application of anthelmintic medications but this line of attack is becoming unsustainable due to the emergence of resistance which is increasing and widespread (Rehman & Abidi, 2022). Furthermore, infection patterns are getting altered by the ever-changing climate, land use and the farming practices.

Anthelmintic drugs which are the mainstay of helminthic infections treatment available rely on a limited number of chemical classes of drugs (Nixon *et al*., 2020), as summarised in Table 1.1 below. This poses a number of shortcomings besides resistance which include the side effects, diminished efficacy, presence of drug remnants in products derived from animals and in the ecosystem which poses health threats to humans and the costs-effectiveness of their use (Nixon *et al.*, 2020).

This hence drives the need for alternative control measures which include the development of vaccines against helminths and the use of natural products that are effective against the helminths.

Table 1.1: Anthelmintic drug classes, mechanisms of action, and reported resistance in Kenya.

Drug Class	**Drug Name**	**Mode of Action**	**Resistance Reported in Kenya?**	**References for Resistance**
Benzimidazoles	Albendazole	Binds to β-tubulin, inhibiting microtubule formation, disrupting parasite metabolism	Yes, Widespread.	(Mwamachi *et al*., 1995; Waruiru *et al.*, 1997)
	Fenbendazole	Same as albendazole, affects nematode energy metabolism	Yes, Widespread.	(Waruiru *et al.*, 1998)
	Thiabendazole	Similar to albendazole, disrupts microtubule assembly	Yes, less common due to reduced use.	(Waruiru *et al.*, 1998)
Imidazothiazoles	Levamisole	Acts as a nicotinic acetylcholine receptor agonist, causing parasite paralysis	Yes, moderate to high.	(Wanyangu *et al*., 1996; Waruiru *et al.*, 1998)
Macrocyclic Lactones	Ivermectin	Binds to glutamate-gated chloride channels, causing paralysis and death	Yes, Widespread.	(Mwamachi *et al.*, 1995)
	Moxidectin	Similar to ivermectin, with longer persistence	Yes, Emerging.	Limited specific Kenyan studies, but global trends noted in (Coles *et al*., 2006)
Salicylanilides	Closantel	Uncouples oxidative phosphorylation, effective against blood-feeding	Yes, Variable.	(Waruiru *et al.*, 1998)

		nematodes		
Other	Parbendazole	Similar to benzimidazoles, inhibits microtubule formation	Yes, limited data	No specific Kenyan studies; global resistance noted in (Coles et al., 2006)
	Netobimin	Metabolizes to albendazole, same mode of action	Yes, linked to benzimidazole resistance	Limited Kenyan data; resistance inferred from benzimidazole studies (Waruiru *et al*., 1998)

2.3 Emergence of anthelmintic resistance

Resistance to anthelmintic drugs refers to the inherited lack of sensitivity of a helminth to a drug in a parasitic populace that was previously sensitive to the same medicine. Anthelmintic resistance is said to have occurred when a higher proportion of the parasitic individuals in a populace have the ability to lose susceptibility to doses of a drug than that in an ordinary populace belonging to the same family and it is propagated from one generation to the other (Fissiha & Kinde, 2021).

There are three types of anthelmintic resistance which include multiple resistance, the cross-resistance and side resistance (Nipane *et al.*, 2008). Cross resistance occurs when a parasite has the ability to tolerate doses of anthelmintic drugs that are therapeutic and which are not related chemically or harbouring different modes of action. Side resistance refers to the phenomenon where resistance to an anthelmintic is caused by selection by another drug which has a related mode of action. The resistance to benzimidazoles is deemed to be a case of side resistance. It has been documented that helminths that are resistant to levamisole also develop side resistance to morantel (Nipane *et al.*, 2008). Multiple resistance occurs when resistance to two or more drugs which have either related or differing mode of actions as a result of selection independently by each group or by side resistance.

The threat caused by anthelmintic resistance can be minimised through delaying the inception of resistance itself or by utilisation of alternative strategies for instance by employing integrated parasite management. Diseases caused by helminths are treated with a group of medication such as praziquantel, benzimidazoles, imidazothiazoles and macrocyclic lactones. Resistance to the anthelmintics seems to be a phenomenon that is inherited and pre-adaptive with the genes that confer resistance in these parasites being present in the helminth populace even before the advent of the use of anthelmintic drugs for the first time (Silvestre & Humbert, 2002). In this condition, resistance arises after the occurrence of selection through exposing of the parasitic populace to an anthelmintic agent. When the host is exposed to the drug only those worms that are harbouring resistance gene survive the exposure, and subsequently transfer these genes to their offspring, and this gives rise to a new generation of resistant parasites.

2.3.1 Factors that promote anthelmintic drug resistance

2.3.1.1 The frequency of treatment

Use of a similar type of anthelmintic agent frequently can bring about the evolution of resistance. It has been observed that in areas where deworming of animals is done regularly resistance develops faster (Elsheikha & Khan, 2011).

The number of times a treatment is administered is a very crucial deciding factor of the speed with which development of anthelmintic resistance occurs (Fissiha & Kinde, 2021). When an anthelmintic drug is given more regularly, the emergence of resistant parasites is hastened. This is so because the principle of selection of resistant strains following treatment gives the parasites that survive an advantage of reproducing and replicating over the parasites that are susceptible for around fourteen to twenty-one days after treatment is administered (Fissiha & Kinde, 2021).

Resistance to anthelmintics can also be selected when lower treatment frequencies are administered. This is so particularly when a similar treatment option is administered repeatedly for several years (Shalaby, 2013). It has also been documented that anthelmintic resistance developing even when only two or three treatments are administered annually (Shalaby, 2013).

2.3.1.2 Under-dosing of anthelmintics

Under-dosing plays a significant role in the evolution of resistance to anthelmintics (Shalaby, 2013). This is because doses that are sub-therapeutic have the potential of allowing heterozygous resistant worms to survive and give rise to an entirely resistant generation or strains that are tolerant (Geerts & Gryseels, 2000).

Over and above this, variations in the bioavailability of a drug in different host animals are an important factor to consider when administering treatment. For instance in goats the bioavailability of benzimidazoles and levamisole is much lower when compared to sheep (Shalaby, 2013). Hence the goats should be given treatment that is 1.5 to 2 times higher than that administered to sheep. Anthelmintic resistance is more prevalent in goats and this is postulated to be a consequence of the differences in drug metabolism.

In the developing countries, use of lower than recommended dosage in the treatment of anthelmintic infections is most likely to happen in an attempt to reduce the cost of anthelmintic treatment (Shalaby, 2013). Such practices should be highly discouraged. Currently most of the administered treatments are sub-therapeutic in at least part of the population. Moreover, the different nematode species that are present in mixed infections in livestock globally respond differently to the various anthelmintic drugs available. This transpires as a consequence of the difference in the susceptibility of these species to the administered anthelmintic drug (Shalaby, 2013). This is only considered allowable during

morbidity control, but such a practice is highly discouraged since it may result in the development of anthelmintic resistance eventually.

2.3.1.3 Mass treatment

Mass treatment in animals that is prophylactic in nature results in the development of resistance in the helminths. Serious and dramatic levels of resistance have been recorded where this strategy has been employed mostly in ruminants (Kennedy & Harnett, 2013).

2.3.1.4 Single drug regimen

The use of a single anthelmintic agent frequently and continuously often results in the development of resistance (Shoop, 1993). For instance, the usage of ivermectin frequently unaccompanied by changing to other agents has been documented as a major cause for the accelerated emergence of resistance in *Haemonchus contortus* in countries such as South Africa and New Zealand (Shalaby, 2013).

2.3.1.5 Treatment when there is low refugia

Low refugia, where few gastrointestinal nematodes remain unexposed to anthelmintics, heighten the risk of resistance, particularly in intensive or dry-season treatment regimes. (Kaplan, 2020) highlights that maintaining refugia preserves susceptible worms to dilute resistant genotypes. Targeted selective treatment, treating only animals with high fecal egg counts or clinical signs, is effective in sustaining susceptible populations. Integrating non-chemical methods, like rotational grazing and tannin-rich forages, as shown in Kenyan studies (Gakige *et al.,* 2023), further reduces anthelmintic reliance, ensuring long-term parasite control efficacy in low-refugia settings.

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2.3.2 Prevention of development of resistance to anthelmintic drugs

In order to reduce the losses incurred by helminthic infections and increase productivity of the livestock rearing ventures, proper helminth infestation control measures should be put in place so as to mitigate the threat caused by these parasites. Several strategies have been employed in an attempt to address the menace that is caused by the parasites and they include:

i. Delaying the inception of anthelmintic resistance through refugia
ii. Adoption of strict quarantine measures
iii. Use of combined drug therapy
iv. Genetic improvement
v. Nutrition
vi. Pasture management
vii. Biological control agents
viii. Vaccines
ix. Use of dewormers that are botanical in nature

2.3.2.1 Delaying the inception of anthelmintic resistance through refugia

Refugia is a subpopulation that has not been exposed to drug treatment. They are parasite stages in the environment, for instance, the larvae on the pasture or inhibited larvae that are not vulnerable to the influence of drugs. They are critical because the greater the proportion of the populace that is in refugia the lower the selection for resistance (Elsheikha & Khan, 2011). Refugia is the most significant factor that influences the selection for parasites that are resistant to drugs.

The populace of parasites unexposed to anthelmintic drugs delays resistance by maintaining susceptible genes, reducing selection pressure from treatments. In the tropics, where the

parasite burdens and year-round transmission prevail, refugia can be applied through targeted selective treatment for instance using FAMACHA to treat only symptomatic livestock, rotational grazing to preserve free-living larvae, reduced treatment frequency timed with seasonal peaks, and combination therapies, while educating communities and monitoring resistance via faecal egg count tests. These strategies balance effective parasite control with preserving drug efficacy in resource constrained tropical regions.

2.3.2.2 Adopting strict quarantine measures

To mitigate the spread of anthelmintic resistance, stringent quarantine measures are essential for breeding herds introduced to a farm. It has been documented that certain high-quality breeding herds exhibit tolerance to conventional anthelmintics, such as benzimidazoles and moxidectin (Shalaby, 2013). The introduction of gastrointestinal nematodes, particularly those carrying resistant genes, poses a significant risk for disseminating anthelmintic resistance within a farm. Implementing robust quarantine protocols, including thorough parasite screening and targeted treatment of incoming animals, prevents the introduction of resistant nematodes, thereby safeguarding the efficacy of anthelmintic treatments and supporting sustainable parasite management.

2.3.2.3 Use of combined drug therapy

The use of two drugs simultaneously which belong to different classes of anthelmintics is one of the different ways to preclude development of resistance among helminths. Unlike single drug effect, when different classes of anthelmintics are used together they produce a synergistic effect, and this results in increased efficacy in the treatment process.

2.3.2.4 Genetic improvement

There is evidence suggesting that some differences in resistance by hosts to parasitic infection lies in their genetic makeup. Resistance, in this case, is entirely reliant on inheritance of genes

that regulate the immunity of the host animal. Some sheep have been documented to be resistant to infection by worms. Use of breed with this trait wholly or in crossbreeding strategies would lead to enhanced resistance by the off-springs to parasitic infection (McManus *et al.*, 2014).

Genetically improved breeds of small ruminants, such as Santa Inês (Brazil), Gulf Coast Native and Katahdin (United States), Creole (Caribbean), Gaddi (India), and selectively bred Romney and Perendale lines (New Zealand), exhibit enhanced resistance to gastrointestinal nematode infections, particularly *Haemonchus contortus*, through lower fecal egg counts (FEC), higher packed cell volume (PCV), and favorable FAMACHA scores (Joan & James, 2020; McRae *et al.*, 2015; Singh *et al.*, 2014). These breeds, adapted to tropical or humid environments, reduce parasite burdens and pasture contamination, with heritability estimates (0.1–0.4 for FEC) supporting selective breeding and crossbreeding (e.g., Santa Inês x Texel) to enhance resistance while maintaining productivity (Amarante *et al*., 2009; McRae *et al.*, 2015). In tropical regions, these breeds complement refugia based strategies, reducing anthelmintic reliance and delaying resistance, making them vital for sustainable livestock management in parasite prone areas (Kaplan, 2020).

2.3.2.5 Nutrition

Nutrition is a key factor in parasitism where there is an established link between protein intake and resistance to gastrointestinal nematode infections. This is because immunity is strongly related to protein supply. Supplementing the diet with phosphorus, for instance, has been documented to thwart the establishment of the worms (Shalaby, 2013). Copper also influences the immunity of the host to gastrointestinal nematodes. There is also evidence that treating lambs orally with copper oxide wires reduce the burden in the host of *Haemonchus contortus.*

However, this practice is not encouraged due to toxic effects of copper associated with it. Supplementation with molybdenum at concentrations of 6-7 mg/d reduced worm infestations in lambs (Sykes & Coop, 2001).

2.3.2.6 Pasture management

This is meant to provide safe grazing ground for the animals. A pasture that is safe is the one that has not been grazed upon for a specified period. This practice ensures that minimal exposure of susceptible animals to large populations of larvae that are infective. During this practice pasture should be subdivided into smaller portions to facilitate longer times before regrazing occurs (Shalaby, 2013).

2.3.2.7 Use of biological control agents

These include the nematode-trapping fungi. It entails the use of natural enemies of the nematodes. Their use results in the reduction of infection levels on the pasture to the extent that the animals that are grazing avoid both the clinical and subclinical symptoms of parasitism by nematodes. Such fungi are found to be existing in soil everywhere and they devour soil nematode larvae. Such fungi trap the nematode larvae by producing sticky traps on their hyphae that is growing (Sharma *et al.,* 2015). Examples of fungi with these capabilities include the *Duddingtonia flagrans* which have the capacity to thrive in the digestive tract of the ruminants.

2.3.2.8 Vaccines

With the increase in cases of resistance, efforts have been made in the development of functional vaccines. This has been facilitated by the discovery of new technologies such as identification of antigens, their characterization and subsequent production. However, only one such drug is available in the market at present which is a vaccine against *Dictyocaulus*

viviparus. This worm causes parasitic bronchitis in cattle. The vaccine consists of L3 larvae that are irradiated and cannot progress or mature into the adult worm (Sharma *et al.,* 2015).

2.3.2.9 Use of dewormers that are botanical in nature

There has been a renaissance in the significance of the traditional medicine in the recent years the world over. This is because the plant kingdom has been documented to harbour a wide range of antibacterial agents, insecticides and botanical anthelmintics. For instance, the most common plants that have been documented to be having anthelmintic properties include; pumpkin seeds, turmeric, garlic, Artemisia species, and the Acacia species (Tariq, 2018).

In Loitoktok plants such as *Albizia anthelmintica, Rapanea melanophloeos, Olea africana, Clausena anisata, Rumex usambarensis* and *Salvadora persica* have been used by herbalists as anthelmintic remedies (Muthee *et al*., 2011). The active secondary metabolites in these plants include sesquiterpenes, benzoquinones, essential oils and anthraquinones which have been attributed to the anthelmintic properties in these plants.

Myrsine africana, Albizia anthelmintica and *Hilderbrantia sepalosa* have been used as traditional medicine against mixed natural sheep helmintic infections by the local Turkana and Samburu communities. It was determined that these plants were 77%, 89% and 90% effective against nematodes which comprised of *Haemonchus* species, *Trichostrogylus* species and *Oesophagostomum* species when compared to albendazole which was 100% effective (Gathuma *et al.,* 2004). These herbal remedies were more effective against *Monezia* species where they were 100% effective when compared to albendazole which was 63% effective.

Kareru *et al* (2012) reported that in Mbeere County, Kenya eight plants which include: *Albizia anthelmintica, Senna didymobotrya, Leonotis mollissima, Entada leptostachya, Rapanea rhododendroides, Terminalia brownii, Amaranthus hybridus, Psidium guajava* and

Vangueria madagascariensis were used by herbalists in the community to treat suspected helminth infections. The methanolic extracts of *E. leptostachya,* and *R. rhododendroides* were the most potent exhibiting 77% mortality by *E. leptostachya* and *R. rhododendroides* exhibiting a mortality rate of 54% against the *H. contortus* adult worms (Kareru *et al.*, 2012).

2.4 Commonly used indigenous medicinal plants in Kenya

Use of herbal medicine has been practised by the Meru and Tharaka Nithi community since time immemorial. Herbalists have used various plants in the treatment of different ailments. Treatment of suspected helminth infections has been commonly practiced but no single study yet has evaluated the efficacy of the used plants.

This study has singled out seven plant species in Meru and Tharaka Nithi which have been utilised in treating helminthiasis. They comprise: *Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physalis peruviana* and *Caesalpinia volkensii.* The plants used in this study were identified with the help from botanists at the Department of Botany at University of Nairobi and specimens deposited at the University of Nairobi herbarium.

2.4.1 *Bridelia micrantha*

The plant commonly known as Mukwego among the Tharaka Nithi community is a tree that is semi-deciduous to deciduous with a height of approximately 20 metres. It grows in the savanna, in swampy forests, along forest edges and river-line woodland. Its bark decoction has been used for treatment of diarrhoea in children and stomach-aches. Herbalists in the Tharaka Nithi community have used bark and the leaves of *Bridelia micrantha* in the treatment of suspected worm infestations (Kokwaro, 1993).

Figure 2.1 A picture of *Bridelia micrantha* taken in Chogoria in Tharaka Nithi County (Source author)

2.4.2 *Aframomum zambesiacum*

The plant commonly known as Meenyua among the Tharaka Nithi community. It is also called “grains of paradise” and is a perennial herb that produces tufted stems with a height of 1 to 4 metres from a rhizomatous stock. It belongs to the ginger family and has been utilized as a condiment and as a herbal remedy to infections for over two thousand years. It is well known in Rome and was introduced for cultivation in Africa during the slave trade era (Lock, 1980).

Figure 2.2 A picture of *Aframomum zambesiacum* taken in Chogoria in Tharaka Nithi County (Source author)

2.4.3 *Rubus apetalus*

It is a deciduous tree commonly known as Ntaratare in Kimeru and grows to a height of approximately 30 m. It has numerous uses that include providing both food and a source of medicine. Its different parts have been used in decoctions, and massages being utilised in the treatment of rheumatism, intercostal pain and diarrhoea (Ramesh *et al.*, 2014).

Figure 2.3 A picture of *Rubus apetalus* taken in Chogoria in Tharaka Nithi County (Source author)

2.4.4 *Thespesia garckeana*

This plant is commonly known as Mutoo in the local Meru dialect and is a tree that is deciduous with a shape that is umbrella-like. It can attain a height approximately 7 to 24 metres. A decoction from the bark of the tree has been used in the treatment of indigestion and its roots are used for relieving chest pains and colds (Amugune *et al.,* 2014). Other plant parts including the stalk and leaves have been documented to have various biological effects which include anticancer and hepatoprotective properties (Amugune *et al.,* 2014).

Figure 2.4 A picture of *Thespesia garckeana* taken in Magutuni in Tharaka Nithi County (Source author)

2.4.5 *Physalis peruviana*

The plant commonly known as Mukabakabu in Kimeru is a flowering plant belonging to the nightshade family. It grows in warm temperate and the subtropical areas. It has been used by people as a diuretic, sedative, antispasmodic, in the treatment of infections of the throat and in eliminating amoeba (Perk *et al.,* 2013). Other plant parts including the stalk and leaves have been documented to have various physiological activities such as anticancer and hepatoprotective properties (Perk *et al.,* 2013).

Figure 2.5 A picture of *Physalis peruviana* taken in Chogoria in Tharaka Nithi County (Source author)

2.4.6 *Caesalpinia volkensii*

This plant commonly known in Kimeru as Mujuthi is a climber that is woody and can grow to an elevation of 1.8 to 4 metres. The stem has deflexed pricklets and has bipinnate leaves. It has been used to treat malaria in Kenya and Tanzania where the herbalists prescribe a decoction made from the leaves of the plant (Kokwaro, 1993).

Figure 2.6 A picture of *Caesalpinia volkensii* taken in Magutuni in Tharaka Nithi County (Source author)

2.4.7 *Hagenia abyssinica*

This dioecious tree commonly known in Kimeru as Mujoga is slender and open crowned and grows to a height of 5 to 25 metres. It is a highly valuable tree that grows in the wild and is used for medicinal purposes. It has traditionally been utilised in the treatment of tapeworms, malaria, diarrhoea and stomach ache (Simion, 2018). The dried female flowers of this plant have been traditionally used to treat helminthic infections in Ethiopia (Thomsen *et al.*, 2012).

Figure 2.7 A picture of *Caesalpinia volkensii* taken Kithaene in Mt Kenya forest Meru County (Source author)

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study design

This was a completely randomized experimental design which was based on testing the efficacy of the plants as anthelmintic agents in an *in vitro* system using *Haemonchus contortus* eggs and larvae. It was conducted on two batches of the helminth entailing the strains that were known to be susceptible to the common anthelmintic drugs which included albendazole and those that were known to be resistant to ivermectin, albendazole and closantel. Albendazole was used as the positive control for susceptible strains and levamisole for the resistant strains while normal saline was used as the negative control. The study was performed at the Veterinary Research Institute helminthology laboratories at the Kenya Agricultural and Livestock Research Organization in Muguga.

3.2 Collection and identification of the plant specimen

Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physalis peruviana and *Caesalpinia volkensii* used in this experiment were collected from Tharaka Nithi and Meru County with assistance from the local traditional medicine practitioners. The collected parts included the leaves of *Bridelia micrantha* from Chogoria in Tharaka Nithi County with the geographical coordinates "-0.23000419, 37.6264786", seeds from the fruit of *Aframomum zambesiacum* from Chogoria in Tharaka Nithi County with the geographical coordinates "-0.23000419, 37.6264786*"*, leaves of *Rubus apetalus* from Chogoria in Tharaka Nithi County with the geographical coordinates "-0.23000419, 37.6264786", leaves of *Thespesia garckeana* from Magutuni in Tharaka Nithi County with the geographical coordinates "-0.2186937, 37.7338683", leaves of *Physalis peruviana* from Chogoria in Tharaka Nithi County with the geographical coordinates –

"0.23000419, 37.6264786", flowers of *Hagenia abyssinica* from Kithaene in Meru County with the geographical coordinates "-0.0937685, 37.5645875" and leaves of *Caesalpinia volkensii* from Magutuni in Tharaka Nithi County with the geographical coordinates "-0.2186937, 37.7338683". They were collected in the month of March where the temperatures ranges were 26ºC – 27ºC and an average annual precipitation of 147.98 mm. The plants were identified and authenticated with the help of Botanists at University of Nairobi Botany Department and voucher specimens deposited at the University of Nairobi herbarium with voucher specimen KM2019/UoN001 for *Bridelia micrantha,* KM2019/UoN006 for *Aframomum zambesiacum,* KM2019/UoN007 for *Hagenia abyssinica,* KM2019/UoN002 for *Rubus apetalus,* KM2019/UoN005 for *Thespesia garckeana,* KM2019/UoN003 for *Physalis peruviana,* and KM2019/UoN004 for *Caesalpinia volkensii*

3.2.1 Preparation of plant extracts

The plant materials that included leaves, flowers and seeds were collected and dried in the shade at room temperature. They were then ground into a fine powder using an electric grinder. One hundred (100) grams of the powdered material was soaked in 500 millilitres absolute methanol at 25ºC for three days. The mixture was then shaken for two hours at 200 rpm followed by centrifugation for 20 minutes at 4000 rpm at 4ºC. The resultant supernatant was filtered through the Whatman filter paper 25 mm and then the methanol evaporated using the rotary evaporator (Nor Azwanida Abdul Aziz, 2015). A stock solution was used to prepare the desired concentrations of 12.5mg/ml, 25mg/ml and 50 mg/ml by diluting it with a calculated volume of normal saline.

The volumes required to prepare the working concentrations of 12.5mg/ml, 25mg/ml and 50 mg/ml were calculated using the below dilution formula:

$$C_1V_1 = C_2V_2$$

Where:

C_1: Concentration of the stock solution

V_1: Volume of stock solution needed

C_2: Desired final concentration

V_2: Final volume required for the assay

3.3 *Haemonchus contortus* eggs and larvae

The eggs used in this study were extracted from faecal samples collected from two sources: one batch, comprising susceptible *Haemonchus contortus* eggs, was obtained from farmers' sheep, and another batch, comprising resistant *Haemonchus contortus* eggs was obtained from sheep at KALRO. The susceptibility or resistance of the *Haemonchus contortus* strains was determined based on prior resistance screening conducted at the department of helminthology at KALRO in Muguga. After collection, faecal samples were stored in polythene bags and transported to the helminthology laboratory at the KALRO for egg extraction. Egg extraction was done using the McMaster technique. The extracted eggs were used in the egg hatch assay and the larvae were obtained following egg incubation.

3.4 Experimental procedures

This study's procedures entailed the egg hatch assay test (EHA), the larval development assay and qualitative screening of phytochemicals.

3.4.1 Egg hatch assay test

The procedure for the test adhered to recommendations by "World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P., 1992)". Samples that were fresh and which contained at least one hundred eggs per gram from sheep were subjected to

homogenization in tap water. They were then used to completely fill 100 ml bottles hence rendering them anaerobic (von Samson-Himmelstjerna *et al*., 2009).

Eggs were then extracted through sieving, centrifugation, and flotation in 4 Molar sodium chloride according to protocol by Stevo & Cagan (2012). The eggs were then washed and suspended in tap water at a concentration of 100 eggs per ml (von Samson-Himmelstjerna *et al*., 2009). The suspension containing the eggs was subsequently inspected microscopically to ascertain commencement of embryonation. Each sample was tested in triplicate containing 12.5mg/ml, 25mg/ml and 50mg/ml of the plant extracts and albendazole was used as the positive control for susceptible strains and levamisole for the resistant strains while normal saline was used as the negative control.

The egg suspension was then placed in wells in a 24 well culture plate and then 10 micrograms of the extract from the different plants added. The plates were sealed to inhibit their drying out then incubated for 48 hours at 25^{o} C. This was followed by the addition of a drop of Gram's iodine to stop the assay (von Samson-Himmelstjerna *et al*., 2009). At least one hundred eggs and larvae were counted from each well and one-way ANOVA conducted followed by Tukey pairwise comparison to analyse the data on the percentage inhibition in hatching that occurred.

3.4.2 *In vitro* larval development assay

The eggs were extracted and incubated in a 24-well microtiter plate for 7 days at 25^{o} C. A wet sponge was placed under the plate and the system covered with a pouch in order to prevent dehydration (Holdsworth *et al.,* 2006). After hatching, the wells were supplemented with growth nutritive medium containing 20 microlitres of yeast extract and normal saline was added to the negative control well (Heim *et al.,* 2015). The plant extracts in triplicate of 12.5mg/ml, 25mg/ml and 50mg/ml were introduced into the wells of a microtiter plate after

which at least one hundred living *Haemonchus contortus* larvae were introduced to each well and the plates were then returned to the incubator for six days. On the seventh day, all the larvae were counted under a microscope using an eel chamber as either living third stage larvae (L3) or dead larvae (Coles *et al.*, 1992). Albendazole was used as the positive control for susceptible strains and levamisole for the resistant strains while normal saline was used as the negative control. One-way ANOVA conducted followed by Tuksey's pairwise comparison to analyse the data.

3.4.3 Qualitative screening of phytochemicals

Screening for the major phytochemicals was conducted using the standard qualitative methods. The plant extracts were screened for alkaloids, steroids, tannins, anthraquinones and flavonoids. The presence of phytochemicals or their absence was confirmed through changes in the test reagents.

3.4.3.1 Test for saponins

To half a gram of the crude extract 5 ml of distilled water was added, shaken and then heated until it boiled. The existence of saponins in the crude extract was affirmed by the occurrence of frothing that is the emergence of small bubbles (Egwaikhide & Gimba, 2007).

3.4.3.2 Test for alkaloids

Two grams of the crude extract were hydrolysed using 2 mls of 2% hydrochloric acid by warming in a heated bath for ten minutes. A few drops of Mayer's reagent were then added to 5 ml of the filtrate. The presence of alkaloids was confirmed through the emergence of turbidity (Usman *et al.*, 2009).

3.4.3.3 Test for tannins

Half a gram of the crude extract was boiled in 10 ml of water in a test tube and then filtered thereafter. A few drops of ferric chloride were then added. The emergence of a blue-black colour affirmed the existence of gall tannins and green-black colour affirmed the existence of catechol tannins (Ayoola & Salako, 2008).

3.4.3.4 Test for anthraquinones

To half a gram of the crude extract, 10 ml of an immiscible organic solvent which was benzene was added, shaken and then filtered. 0.5 g of 10% ammonium solution was added and the mixture was shaken well thereafter. The presence of anthraquinones was confirmed through the appearance of a violet colour in the layer phase (Auwal *et al.*, 2014).

3.4.3.5 Test for steroids and triterpenes

Two grams of the crude extract was defatted using n-hexane and the residue then extracted using chloroform. To 0.5 ml of the chloroform extract, 0.5 ml of acetic acid was added and then two drops of concentrated H_2SO_4 added thereafter (Sofowora, 1996). The existence of the two phytochemicals was confirmed through the emergence of a gradual colour change to green-blue (Sofowora, 1996).

3.4.3.6 Test for flavonoids

10 ml of ethyl-acetate was added to 0.5 g of the crude extract and the mixture heated in a steam bath for three minutes. The mixture was then filtered and 4 ml of the filtrate shaken with 1 ml of diluted ammonia solution after which a yellow colouration was then examined (Sofowora, 1996).

3.4.3.7 Test for phenols

0.5 g of the crude extract was put in a test tube after which it was then treated with a few drops of 2% of Ferric chloride. The existence of phenols in the extract was affirmed by the emergence of blue-green or black colour (Harborne, 1998).

3.4.3.8 Test for glycosides

Separately half a gram of the crude extract was dissolved in 5 ml of methanol after which 10 ml of 50% hydrochloric chloric was then added to 2 ml of each extract in the test tubes. The mixtures were then heated in a water bath that was boiling for 30 minutes. 5 ml of Fehling's solution was added and the mixtures boiled for 5 minutes. The existence of glycosides in the extract was confirmed by the emergence of a brick red precipitate (Harborne, 1998).

3.4.3.9 Fractionation of the plant extracts

The seven plan extracts were subjected the gas chromatography-mass spectrophotometry (GC:MS) analysis at the Government Chemist to determine their phytochemical composition. Derivatization of the samples was first conducted and then subjected to analysis by GC: MS.

3.5 Determination of ED_{50} and ED_{90}

The ED_{50} and ED_{90} was determined using probit analysis. This collected data which entailed the percentage egg hatch inhibitions and the percentage larvicidal activity of the plants at the various concentrations of methanol plant extracts was then normalized using the arcsine square-root transformations to stabilize the variances and meet the assumptions of normality required for probit analysis (Ritz & Streibig, 2005). The transformed data was then subjected to probit regression analysis using Minitab version 17.0 and the ED_{50} and ED_{90} determined at 95% confidence interval (Collett, 2002).

3.6 Statistical data analysis

One-way analysis of variance (ANOVA) and Tukey's as the post hoc test was used to establish whether there was a significant difference in plant extracts and the controls in egg hatching and the larval development test. The analysis was conducted at a significance level of $P < 0.05$. Minitab version 17.0 was used in the analysis.

CHAPTER FOUR

4. RESULTS

4.1 Effects of the plant extracts on *H. contortus* eggs hatching

Methanol extracts of *P. peruviana* and *R. apetalus* achieved the highest mean hatch inhibition of drug susceptible *H. contortus* eggs of 95.24±0.54, 90.00±1.00, 88.24±0.66 and 96.55±0.45, 85.71±0.79, 82.14±0.76 at 50mg/ml, 25mg/ml and 12.5 mg/ml respectively. Similarly, *H. abyssinica* had high mean inhibition percentages of 94.12±0.52 and 82.14±0.76 at 50 mg/ml and 25 mg/ml respectively. *Caesalpinia volkensii* also achieved a mean egg hatch inhibition of 95.00±1.53 but only at the highest concentration of 50mg/ml. There was no significant difference ($P<0.05$) in the mean percentage inhibition between the positive control (albendazole) and *P. peruviana, R. apetalus, C. volkensii and H. abyssinica* at 50 mg/ml.

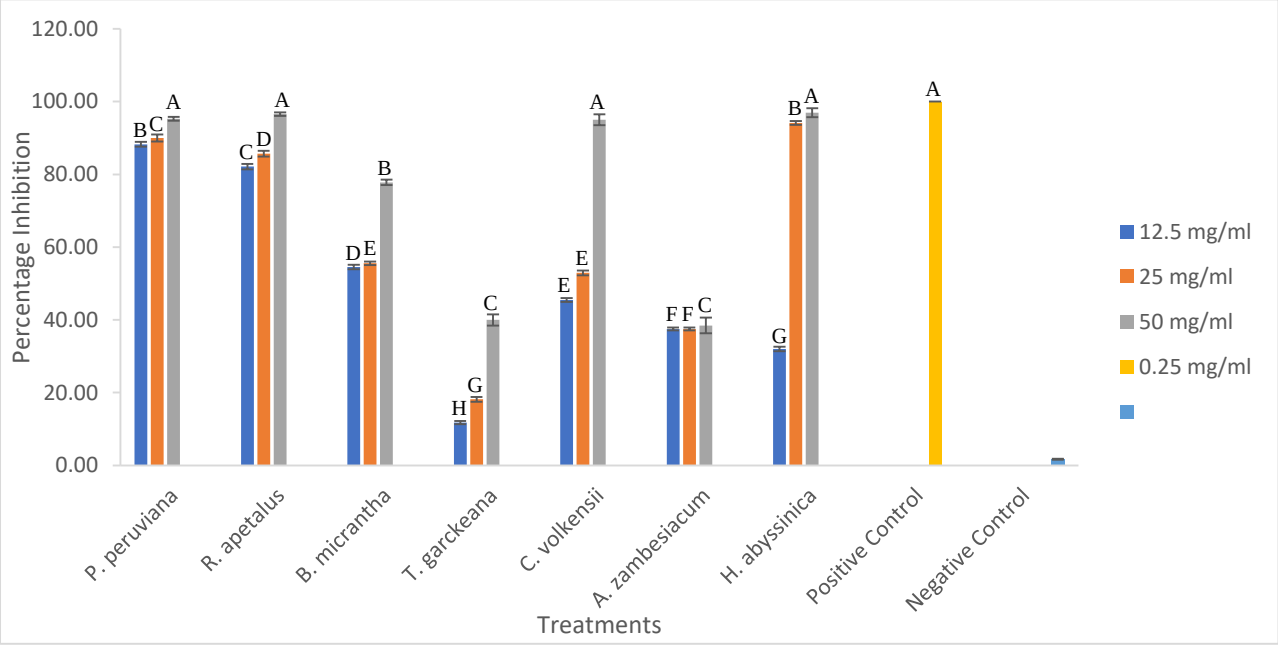

Figure 4.1.1: Mean percentage hatch inhibition of *H. contortus* eggs from the susceptible helminths by varying methanolic plant extract concentrations. Means that do not share a letter are significantly different

With the resistant *H. contortus* eggs at 50mg/ml *C. volkensii*, *R. apetalus*, *H. abyssinica* and *B. micrantha* achieved mean egg hatch inhibition of 94.56±0.36, 93.42±0.46, 91.80±0.59 and 91.67±0.47 respectively. There was no significant difference statistically (P>0.05) between the plant extracts and the positive control and between the extract themselves at this dilution. At lower doses the extracts from these plants achieved lower percentage inhibitions as well as all the other plant extracts under trial (Fig. 4.1.2).

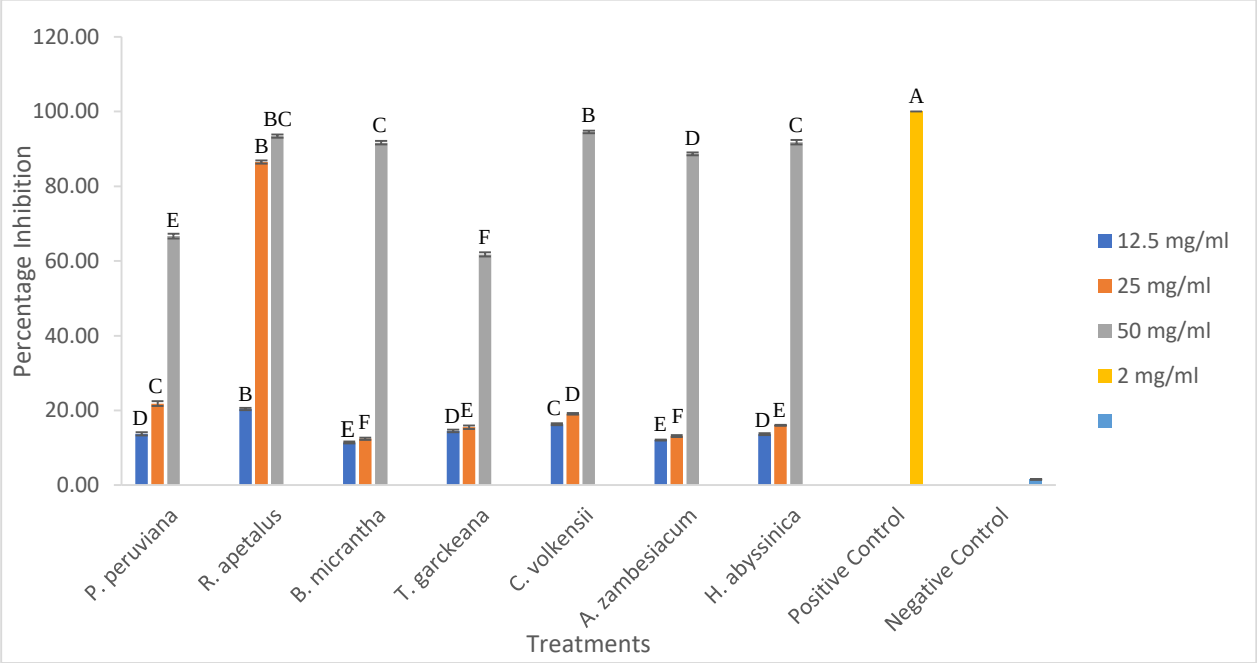

Figure 4.1.2: Mean percentage hatch inhibition of *H. contortus* eggs from the resistant helminths by varying methanolic plant extract concentrations. Means that do not share a letter are significantly different

4.2 Effects of the plant extracts on *H. contortus* larval development

Methanolic extracts of *R. apetalus* and *H. abyssinica* had highest mean percentage larvicidal activity of 100.00±0.00 across the three dosages for the drug susceptible *H. contortus* larvae. There was no statistically significant difference between the two extracts and the positive control and between the plant extracts at this dilution. It was also recorded that at 50 mg/ml, *P. peruviana* and *C. volkensii* both had a mean percentage larvicidal activity of 100.00±0.00 hence there was also no significant difference between the two and the positive control. At higher dilutions the plant extracts above exhibited lower larvicidal activity as well as the other plant extracts as shown in the figure 4.2.1 below.

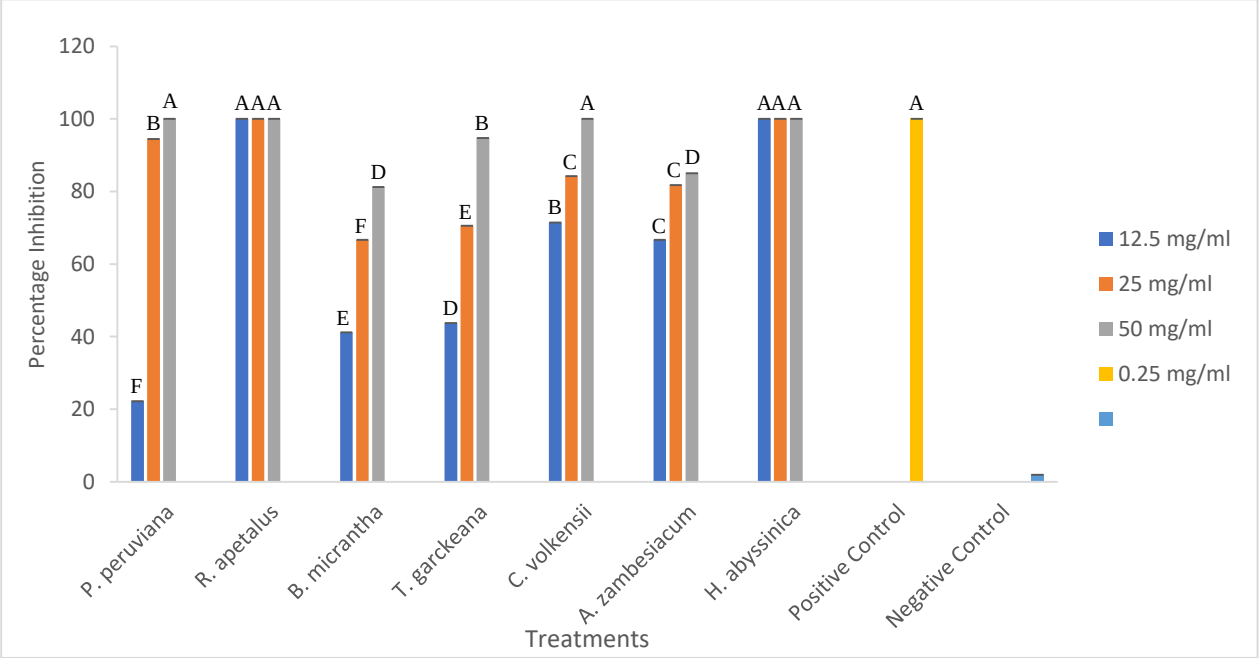

Figure 4.2.1: Mean percentage larvicidal activity against *H. contortus* larvae from susceptible helminths at varying methanolic plant extract concentrations. Means that do not share a letter are significantly different

With the L1 from the resistant *H. contortus* only *R. apetalus* displayed 100.00±0.00 larvicidal activity across the three dilutions, while *H. abyssinica* achieved the same level at 50mg/ml and 25mg/ml and *P. peruviana* at 50mg/ml only with no statistically significant difference between the extracts and also the positive control (Fig. 4.2.2).

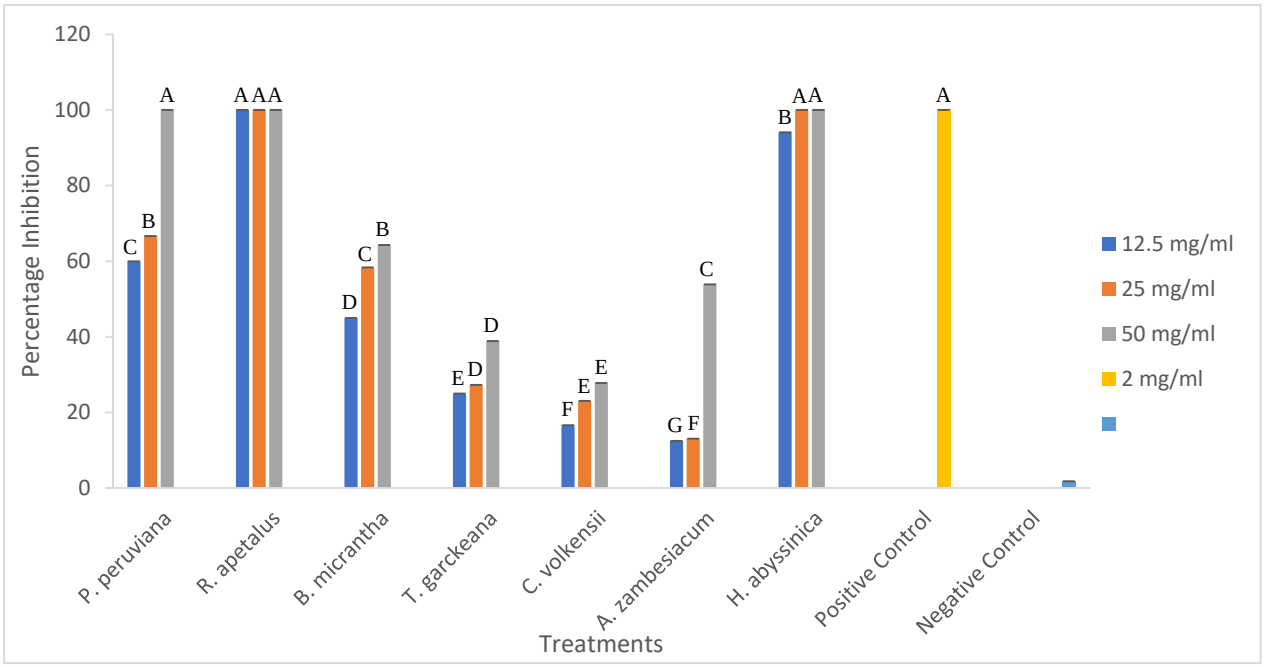

Figure 4.2.2: Mean percentage larvicidal activity against *H. contortus* larvae (L1) from the resistant helminths by varying methanolic plant extract concentrations. Means that do not share a letter are significantly different

For resistant *H. contortus* L3 larvae the plant extracts had diminished larvicidal activity achieving 78.38±0.48, 58.33±0.37 and 52.00±0.16 at 50mg/ml for *R. apetalus, C. volkensii* and *T. garckeana* respectively as compared to 94.71±0.31 from the positive control (levamisole). There was a statistically significant difference between the plant extracts and the positive control. The other plant extracts obtained larvicidal activity lower than 50%.

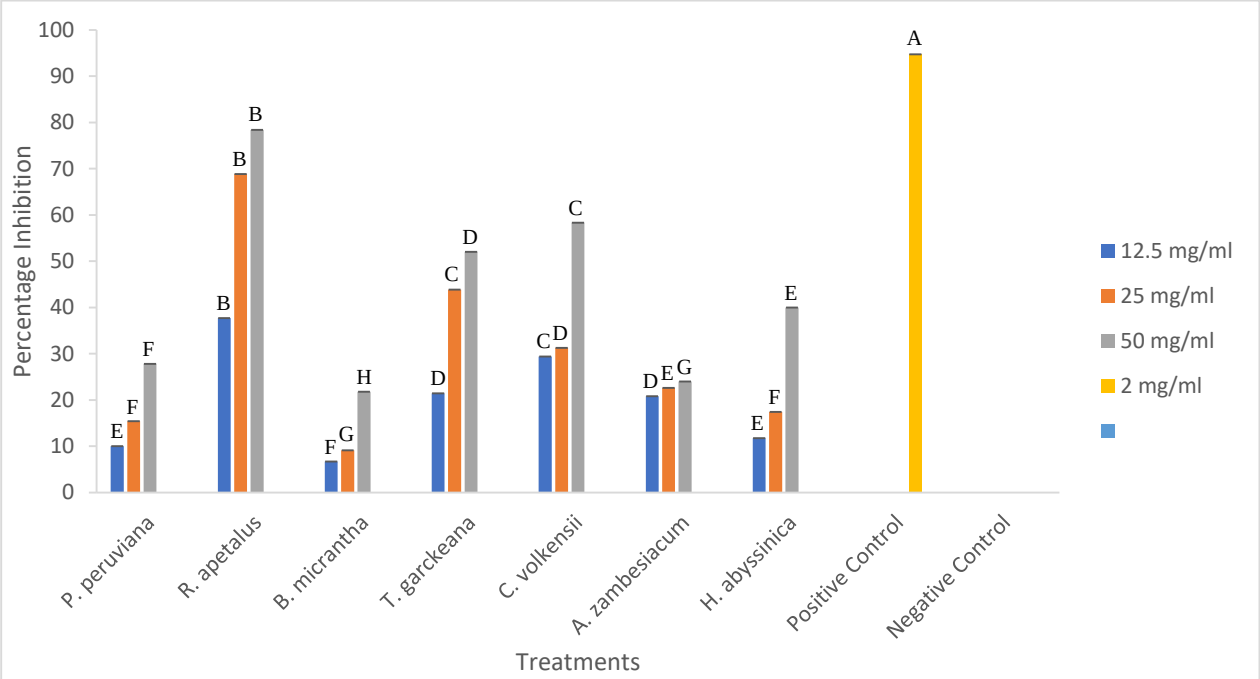

Figure 4.2.3: Mean percentage larvicidal activity against resistant *H. contortus* larval (L3) by varying methanolic plant extract concentrations. Means that do not share a letter are significantly different

Synergism between albendazole and the plant extracts in the ratio of 1:1 was determined on resistant *H. contortus* L3 larvae and the larvicidal activity for *R. apetalus* increased to 98.46±0.32, 97.28±0.64 and 96.43±0.35 at 50 mg/ml, 25 mg/ml and 12.5 mg/ml respectively. The same effect was observed for *C. volkensi* where activity increased to 94.44±0.47 at 50mg/ml and 88.89±0.38 at 25 mg/ml. There was no significant difference between *R. apetalus* plus albendazole at 50 mg/ml when compared to the positive control. The other extracts did not receive meaningful increment (Figure 4.2.4).

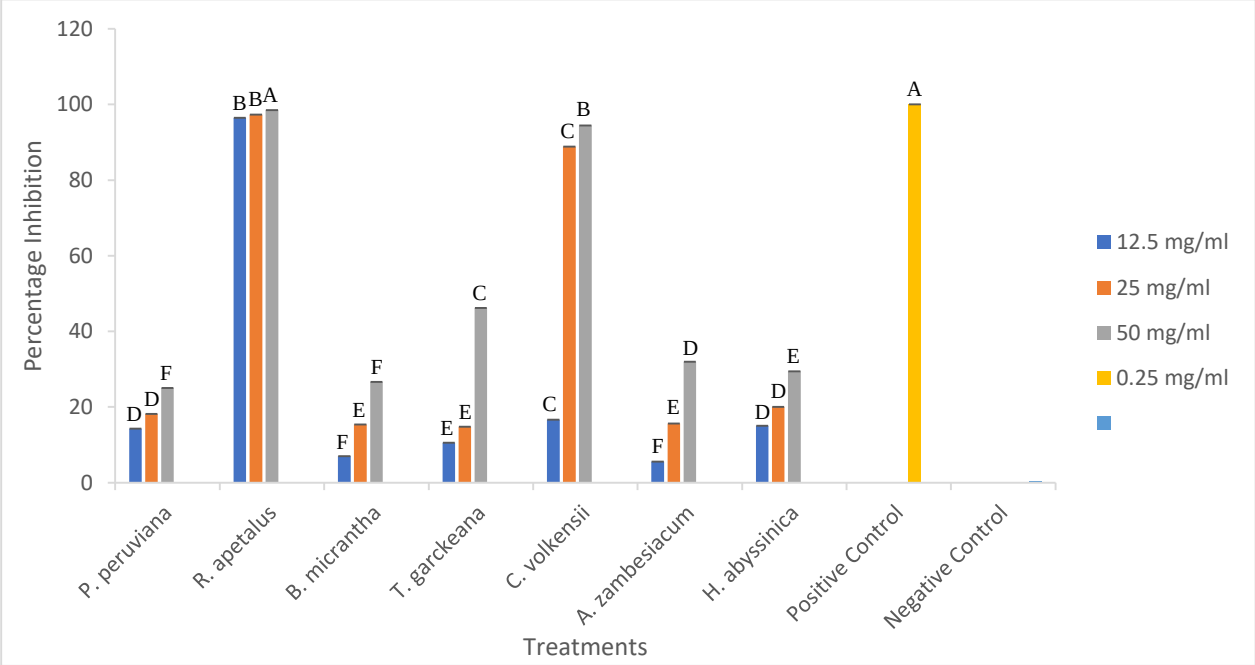

Figure 4.2.4: Mean percentage larvicidal activity against resistant *H. contortus* larvae (L3) by varying dilutions of methanolic plant extracts with albendazole at ratio of 1:1. Means that do not share a letter are significantly different

4.3 Phytochemical screening for each crude extracts

Phytochemical screening for each of the seven crude extracts for secondary metabolites was conducted and yielded the following.

S/N	Plant sample (botanical name)	Saponins	Alkaloids	Tannins	Anthraquinones	Steroids and triterpenes	Flavonoids	Phenols	Glycosides
1	*Bridelia micrantha*	+	+	+	-	+	+	+	+
2	*Aframomum zambesiacum*	+	+	-	-	+	+	+	-
3	*Hagenia abyssinica*	+	+	+	-	+	+	+	-
4	*Rubus apetalus*	+	+	+	-	+	+	+	-
5	*Thespesia garckeana*	+	-	+	-	+	-	+	+
6	*Physalis peruviana*	+	+	+	-	+	-	+	-
7	*Caesalpinia volkensii*	+	+	+	-	+	+	+	-

+ = presence; − = absent

Figure 4.3.1: Phytochemical screening for the methanolic plant extracts

4.4 Phytochemical screening for the plant extracts by GC-MS

GC-MS analysis on the methanolic extracts of the seven plants revealed the presence of monoterpenoid phenols such as thymol, menthol, carvacrol, Borneol, α pinene, camphor, 3-carene, eucalyptol, β pinene and terpinen-4-ol which could be contributing to the anthelmintic activity observed among the plants in this study. Also contained in the extracts are other classes of secondary metabolites which include sesquiterpenes, triterpenes, fatty acids, sterols, straight chain alkanes, norterpenes, 1-benzofurans, phenols, aromatic ketones, aromatic alcohols, aromatic hydrocarbons, monoterpenes, monohydroxyacetophenone and vitamin E. Details on specific secondary metabolites in the seven plant extracts are in Appendix I to VII.

CHAPTER FIVE

5. DISCUSSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In this study the effects of *Bridelia micrantha, Aframomum zambesiacum, Hagenia abyssinica, Rubus apetalus, Thespesia garckeana, Physalis peruviana* and *Caesalpinia volkensii* against *Haemonchus contortus* were investigated. Overall, methanolic extracts of the plants exhibited varying inhibitions on egg hatching and also larval development.

In particular, the methanolic extracts of *P. peruviana, R. apetalus, B. micrantha, C. volkensii* and *H. abyssinica* exhibited high activity while *T. garckeana* and *A zambesiacum* exhibited moderate activity on egg hatch assay as well as larval development for both drug susceptible and resistant *H. contortus* helminth parasites. There was no statistically significant difference ($P>0.05$) with the conventional drugs. The activity observed was dose related and was comparatively less in drug resistant *H. contortus* eggs and also larvae.

All the above-mentioned plants were determined to contain saponins, alkaloids, tannins, terpenes and phenols. These secondary metabolites could account in part the anthelmintic activity observed among the plants used in this study.

In particular alpha,alpha,4-trimethyl-3-cyclohexene-1-methanol, 4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol are terpineols that were identified in the extract of *Aframomum zambesiacum.* Terpineols have been shown to have anthelmintic properties and are known to inhibit hatching of helminth eggs (Grando *et al., 2016*; Mirza *et al*., 2020). Terpineols have also been documented to inhibit hatching and migration of *Haemonchus contortus* larvae (Grando *et al*., 2016). However, the mode of action by which these secondary metabolites act is yet to be elucidated.

Terpenoids have been reported to harbour insecticidal activity where they have been associated with the inhibition of growth, maturation damage and decreased reproductive ability (Carvalho *et al.*, 2012), which result in mortality in the insects.

Bridelia micrantha, Physalis peruviana, Aframomum zambesiacum and *Thespesia garckeana* were all determined to contain Phenol, 2-methyl-5-(1-methylethyl)- which has been documented to be 100% effective in the egg hatch assay involving *Haemonchus contortus* at a dose of 2mg/ml (Andre *et al*., 2016).

Thymol a monoterpenoid phenol and a derivative of cymene which was present in *P, peruviana, A. zambesiacum, T. garckeana* and *C, volkensii* is known to possess various properties which include being an antioxidant, scavenging for free radicals, anti-inflammatory, antispasmodic, antibacterial, antifungal, antiseptic and analgesic (Nagoor Meeran *et al.*, 2017). Thymol at a dose of 4mg/ml was observed by André *et al.* (2017) to inhibit hatching of *Haemonchus contortus* eggs by 98%. The ovicidal activity of thymol is attributed to the presence in its structure of the hydroxyl radical. It has been hypothesized that its mechanism of action against hatching entails either preventing changes in the permeability of the egg shell by binding to the lipoproteins of the membranes of the egg (Perry, 2002) or through binding competitively to hatching factors in the egg shell resulting in the altering of the process of hatching (André *et al*., 2017).

Carvacrol contained in *Physalis peruviana* is a monoterpenoid that has been found to harbour several pharmacological actions among them anthelmintic (Zhu *et al*., 2013), acaricidal (Costa-Júnior *et al*., 2016), harbour activity against *Leishmania infantum* and also *Trypanosoma cruzi* (Escobar *et al*., 2010). It has been established that at a concentration of 2mg/ml carvacrol was 100% effective against hatching of *Haemonchus contortus* eggs

(Andre *et al*., 2016). The ovicidal activity of carvacrol is attributed to the presence of the phenolic group in its structure. This radical has been postulated to inhibit enzymes such as the proteases, lipases, chitinases, beta glucosidase and leucine aminopeptidase which are responsible for hatching of the eggs (Andre *et al*., 2016). This mode of action is similar to that of polyphenols and tannins that contain in their structure the hydroxyl radical (Vargas-Magaña *et al*., 2014).

Hagenia abyssinica and *Rubus apetalus* yielded statistically significant ($P<0.05$) inhibition of *Haemonchus contortus* larval development from drug susceptible helminths across the three concentrations tested. The most effective plant extracts were consistently found to contain different essential monoterpenoid oils which could perhaps explain the activity as these secondary metabolites have been shown to have different and unique targets and mode of action which include acting on proteins responsible for various functions in the parasite such as receptors.

The inhibition of hatching and also larvicidal activity of the plant extracts was lower in drug resistant *Haemonchus contortus* strains. The reduced action could be attributed to the development of resistance in the mature L3 form of the parasite. The plant extracts exhibiting larvicidal activity were all observed to contain thymol which is known to harbour larvicidal properties. Its larvicidal action is attributed to its interaction with SER-2 tyramine receptor (André *et al*., 2017) and this monoterpene has been documented to act on this receptor in *Caenorhabditis elegans* (Lei *et al*., 2009). This receptor is responsible for modulating a number of key processes in nematodes such as pharyngeal pumping, locomotion and egg laying (André *et al*., 2017). It is expressed in all phases of the life cycle of *H. contortus* and this justifies the possible interaction of thymol with the SER-2 tyramine receptor and hence being responsible for its larvicidal action.

Thymol has also been documented to affect the organization and the electrostatic properties of the membrane surface and this in turn changes the permeability and inhibits the activity of the membrane proteins such as the ATPases (Nagoor Meeran *et al*., 2017). It has also been established to penetrate the cuticle of the helminths and cause internal ultrastructural lesions (André *et al*., 2017).

Physalis peruviana was determined to contain monoterpenoid essential oil carvacrol which just like thymol is effective against nematodes and it has been documented by (Kong *et al*., 2007) to have very strong nematocidal activity. It was found to be very effective against sheep gastrointestinal nematodes, *Haemonchus contortus* and *Bursaphelenchus xylophilus* nematode found in pine wilt (Kong *et al*., 2007). The activity of thymol and carvacol is attributed to their ability to trigger a signalling cascade that leads to eventual demise of the nematodes through interaction with a TyrR like SER-2 receptors (Lei *et al*., 2009). The interaction of thymol and carvacrol with the SER-2 results in the altering of the functions of the receptor.

Carvacrol results in changes in the cuticle and the intestine of the L3 larvae form of *Anisakis simplex* and its efficacy against helminths is credited to the presence in its structure of the phenolic group which causes helminth excretory cell lysis and alterations to the cuticle. (Hierro *et al*., 2004). This monoterpenoid has effects on the membrane of the bacteria, consisting of the phenol group connection with the amine and hydroxyl groups of the membrane proteins in bacteria. The interaction between the hydroxyl radical and the membrane proteins affect the lipid layer stability and this increases the passive flow of the protons across the membrane resulting in the altering of the permeability and eventual cell death (Andre *et al*., 2016).

Thymol and carvacrol have been established to result in the damage of the cuticle and the digestive apparatus in the larvae of *Anisakis* (Giarratana *et al*., 2014). Besides the changes to the cuticle, carvacrol is known to be neurotoxic to the nematode *Caenorhabditis elegans* where it interacts with the SER-2 tyramine (Lei *et al*., 2010). The changes in the cuticle and neurotoxicity caused by carvacrol have the possibility of interfering with the permeability of the cuticle and motility hence impeding maintaining of homeostasis within the parasite (Andre *et al*., 2016). The cuticle forms a barrier that protects the parasite and is also involved in the metabolic exchanges.

In addition to cuticular changes carvacrol also results in changes in the reproductive tract of parasites. The structural alterations in the external reproductive organs of the female parasite also affects reproduction in the parasite and reduces the production of eggs by the parasite (Andre *et al*., 2016). Carvacrol in acidic pH values has been established to have antifungal activity (Chavan & Tupe, 2014) and has also been documented to be effective against different stages of the *Haemonchus contortus* (Andre *et al*., 2016).

There was increased larvicidal activity when *R. apetalus* and *C. volkensii* were combined with albendazole pointing to possible synergism between the two plants with albendazole against the L3 *H. contortus* from the resistant helminths. However, there was reduced larvicidal activity observed when *T. garckeana* was combined with albendazole suggesting possible antagonism in the extract and albendazole. These observations give insight to uncertainty of treatment results from blind drug combinations in control of helminth parasites.

On combination of the most effective of the plants used and albendazole there was increased egg hatch inhibition and arrest of larval development of the resistant isolates possibly due to

the difference in the drug targets in the parasites. This is due to the unravelling of targets by the monoterpenoid oils that are different from that of albendazole.

The effects on egg hatch and larval development were found lower in resistant isolates than the susceptible ones possibly due to the alterations of the drug targets in structure and function. A study demonstrated for instance that two SER-2 serine residues which interact with phenyl ring bonded the hydroxyl radical present in thymol and carvacrol exerting their anthelmintic activity. When mutations occur, the cells were observed to stop responding to thymol and carvacrol and produced none or remarkably reduced receptor translocation in the mutated cell lowering their anthelmintic activity remarkedly (Lei *et al*., 2010).

5.2 Conclusions

From the observations made in this study it is concluded that:

i. Methanolic extracts of the studied plants exhibited anthelmintic activity against *Haemonchus contortus*, with *P. peruviana*, *R. apetalus*, *C. volkensii*, and *H. abyssinica* producing effects comparable to the conventional drug, while combinations of *R. apetalus* and *C. volkensii* with albendazole showed synergistic effects in drug-resistant helminth strains, but the combination of *T. garckeana* with albendazole displayed antagonistic effects.

ii. Methanolic extracts of the studied plants demonstrated effective inhibitory concentrations against *Haemonchus contortus*, with ED_{50} values ranging from 2.35 to 5.08 mg/mL and ED_{90} values from 7.11 to 12.27 mg/mL across egg hatch and larval development assays. Notably, *Hagenia abyssinica* exhibited the lowest inhibitory concentrations in both assays, highlighting its superior anthelmintic potential, while *Thespesia garckeana* showed the highest values, indicating relatively lower potency.

iii. The plant extracts that exhibited significant anthelmintic activity contained alpha,alpha,4-trimethyl-3-cyclohexene-1-methanol, 4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol, Phenol, 2-methyl-5-(1-methylethyl)-, thymol and carvacrol which most likely were responsible for the activity.

5.3 Recommendations

5.3.1 Recommendations from the study

From the findings of this study, the following recommendations are made:

i. The plants that were studied continue to be used for control of helminth parasites and their use promoted as an adjunct to conventional anthelmintic control.
ii. Combination of conventional anthelmintic drugs and the plant extracts be promoted for the control of resistant *H. contortus* parasites.
iii. It is recommended that guided combination of the extracts and conventional drugs be applied as some combinations may produce antagonistic effects.

5.3.2 Recommendations for further research

i. I recommend that further studies are carried out to elucidate the individual anthelmintic activity of the various secondary metabolites contained in the plants used in this study.
ii. The modes of action by these metabolites should also be investigated for use as potential novel anthelmintic drugs.
iii. It is recommended that similar plants as was used in this study but from a different geographical location be studied to determine any differential effects.
iv. Toxicity studies on the most effective plants of this study be conducted so as to develop confidence in the use of the herbal remedies.

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APPENDICES

Appendix I: The GM-MS profile of compounds In *Physalis peruviana*

RT(min	Chemical Name	Compound
10.7078	Thymol (ID#:89-83-8)	Monoterpenoid phenol
14.6738	Benzophenone (ID#:119-61-9)	
18.3589	1.2-Benzenedicarboxylic acid, diphenyl ester (ID#:84-62-8)	
31.4155	Vitamin E (ID#:59-02-9)	
4.2858	o-Xylene (ID#95-47-6)	
6.5004	1.3-Benzenediamine, 4-methoxy- (ID#:615-05-4)	
6.6589	Phenol, m-tert-butyl-(ID#:585-34-2)	
6.6589	Phenol, 3-methyl-5-(1-methylethyl)- methylcarbamate (ID# 2631-37-0)	
6.7791	2-Cyclohexen-1-one, 3.5,5-trimethy - (ID#:78-59-1)	
7.8559	Cyclohexane, ethyl-(ID#:1678-91-7)	
8.6843	Acetophenone, 2-chloro- (ID# 532-27-4)	
9.0093	Benzene, tert-butyl-(ID# 98-06-6)	Aromatic hydrocarbon
10.3565	Nonanoic acid (ID#:112-05-0)	Straight-chain saturated fatty acid
10.7078	Phenol, 2-methyl-5-(1-methylethyl)-(ID# 499-75-2)	Monoterpenoid phenol
10.8229	2-Pyrrolidinone, 1-methyl-(ID#:872-50-4)	
13.1971	Dodecanoic acid, methyl ester (ID#:111-82-0)	Saturated fatty acid
14.0582	Diethyl Phthalate (ID#:84-66-2)	
14.5225	Methane, iodo- (ID#:74-88-4)	
14.6738	Benzophenone (ID#:119-61-9)	
15.5132	Methyl tetradecanoate (ID#:124-10-7)	Saturated fatty acid
15.7972	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)- (ID#:2478-38-8)	Aromatic ketone
16.1879	1-Tridecene (ID#:2437-56-1)	Acyclic olefin
16.3153	Octadecane (ID#:593-45-3)	Straight-chain alkane
16.7164	Menthol (ID#:1490-04-6)	Monoterpenoid
17.6356	Hexadecanoic acid, methyl ester (ID#:112-39-0)	Fatty acid methyl ester
18.1517	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (ID#:84-69-5)	Aromatic dicarboxylic acid
18.2336	n-Hexadecanoic acid (ID#:57-10-3)	Saturated long-chain fatty acid
18.3517	Eicosane (ID#:112-95-8)	Straight chain alkane
18.6035	Dimethyl sulfone (ID#:67-71-0)	Organosulfur compound
19.2782	9,12-Octadecadienoic acid (Z.Z)-, methyl ester (ID#:112-63-0)	Fatty acid methyl ester
19.3859	9-Octadecenoic acid (Z)-, methyl ester (ID#:112-62-9)	Fatty acid methyl ester
19.5776	Octadecanoic acid, methyl ester (ID#:112-61-8)	Fatty acid methyl ester
19.6440	Benzene, 1,3,5-trimethyl-(ID#:108-67-8)	
21.8503	Hexanedioic acid, bis(2-ethylhexyl) ester (ID#:103-23-1)	
25.7562	Squalene (ID#7683-64-9)	Triterpene
31.1958	Cholesterol (ID# 57-88-5)	Sterol
10.7078	Thymol (ID#:89-83-8)	Monoterpenoid phenol
16.7164	Cyclohexanol, 5-methyl-2-(1-methylethyl)- (1.alpha. 2.beta.,5.alpha.)-.+/-)-(ID#:15356-70-4)	Monoterpenoid

Appendix II:The GM-MS profile of compounds in Aframomum zambesiacum

RT(min)	Chemical Name	Compound
4.2620	Styrene (ID#:100-42-5)	
4.4827	Ethanol, 2-butoxy- (ID#:111-76-2)	Alcohol
4.6859	Benzene, 1-ethyl-2-methyl- (ID#:611-14-3)	
4.7988	alpha.-Pinene (ID#:80-56-8)	Monoterpene
5.1253	Benzene, propyl-(ID#:103-65-1)	Alkylbenzene
5.4040	3-Carene (ID#:13466-78-9)	Monoterpene
5.4848	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (15)-(ID#:18172-67-3)	Monoterpene
5.9844	Benzene, 1-methyl-2-propyl-(ID#:1074-17-5)	
6.0911	Ethanone, 1-(2-hydroxyphenyl)- (ID#:118-93-4)	Monohydroxyacetophenone
6.2258	Benzene, 1-methyl-3-(1-methylethyl)-(ID#:535-77-3)	Aromatic hydrocarbon
6.3232	Eucalyptol (ID#:470-82-6)	Monoterpenoid
6.8227	Benzyl Alcohol (ID#:100-51-6)	Aromatic alcohol
6.9917	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol - (ID#562-74-3)	Terpineol
8.5617	Camphor (ID#:76-22-2)	Cyclic monoterpene ketone
8.5793	Borneol (ID#:507-70-0)	Bornane monoterpenoid
8.9296	alpha,alpha,4-trimethyl-3-cyclohexene-1-methanol (ID#98-55-5)	Terpineol
9.9566	Benzofuran, 2,3-dihydro- (ID#:496-16-2)	1-benzofurans
10.3244	Nonanoic acid (ID#:112-05-0)	Straight-chain saturated fatty acid
10.5970	Decanoic acid, methyl ester (ID#:110-42-9)	Fatty acid methyl ester
10.6602	Phenol, 2-methyl-5-(1-methylethyl)- (ID#:499-75-2)	Monoterpenoid phenol
10.6633	Phenol,-tert-butyl-(ID# 585-34-2)	Alkylbenzene
13.7329	1,6,10-Dodecatrien-3-ol, 3.7.11-trimethyl-, [S-(Z)]- (ID#:142-50-7)	Sesquiterpene alcohol
17.6844	Hexadecanoic acid, methyl ester (ID#:112-39-0)	Fatty acid methyl ester
19.6098	Octadecanoic acid, methyl ester (ID#:112-61-8)	Fatty acid methyl ester
21.1436	Tricasane (ID#:638-67-5)	Straight chain alkane
22.0670	Hexanedioic acid, bis(2-ethylhexyl) ester (ID#:103-23-1)	
22.8131	Pentacosane (ID#:629-99-2)	
25.8640	Squalene (ID#:7683-64-9)	Triterpene
27.0599	Triacontane (ID#:638-68-6)	Straight chain alkane
30.8239	Heneicosane (ID#:629-94-7)	Straight chain alkane
6.8227	Benzyl Alcohol (ID#:100-51-6)	Aromatic alcohol
8.5617	Camphor (ID#:76-22-2)	Cyclic monoterpene ketone
10.6602	Thymol (ID#:89-83-8)	Monoterpenoid phenol
8.5617	Camphor (ID#:76-22-2)	Cyclic monoterpene ketone
10.0871	Isobornyl acetate (ID#:125-12-2)	
10.6602	Thymol (ID#:89-83-8)	Monoterpenoid phenol
31.5213	Vitamin E (ID#:59-02-9)	

Appendix III:The GM-MS profile of compounds in *Thespesia garckeana*

RT(min)	Chemical Name	Compound
14.7058	Benzophenone (ID#:119-61-9)	
31.4040	Vitamin E (ID#:59-02-9)	
4.2826	o-Xylene (ID#:95-47-6)	

10.8497 Phenol, 2-methyl-5-(1-methylethyl-(ID#:499-75-2) Monoterpenoid phenol
10.8860 Phenol, m-tert-butyl-(ID#:585-34-2) Alkylbenzene
12.5607 Phenol, 2-methoxy-4-(1-propenyl)-(ID#:97-54-1) Phenylpropanoid
14.7058 Benzophenone (ID#:119-61-9)
15.2499 Heptadecane (ID#629-78-7) Straight chain alkane
15.5339 Methyl tetradecanoate (ID#:124-10-7) Fatty acid methyl ester
16.3287 Octadecane (ID#:593-45-3) Straight chain alkane
17.0220 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (ID#:84-69-5) Aromatic dicarboxylic acid
17.6324 Hexadecanoic acid, methyl ester (ID#:112-39-0) Fatty acid methyl ester
18.1620 n-Hexadecanoic acid (ID#:57-10-3) Saturated long-chain fatty acid
18.3568 Eicosane (ID#:112-95-8) Straight chain alkane
19.2636 9,12-Octadecadienoic acid (Z.Z), methyl ester (ID#:112-63-0) Fatty acid methyl ester
19.3268 9-Octadecenoic acid (Z)-methyl ester (ID#:112-62-9) Fatty acid methyl ester
19.5610 Octadecanoic acid, methyl ester (ID#:112-61-8) Fatty acid methyl ester
19.7216 7H-Benz[delanthracen-7-one (ID#:82-05-3)
20.2128 Docosane (ID#:629-97-0) Straight chain alkane
21.8399 Hexanedioic acid, bis(2-ethylhexyl) ester (ID#:103-23-1)
21.9248 Tetracosane (ID#:646-31-1)
10.8860 Thymol (ID# 89-83-8) Monoterpenoid phenol
16.7101 Cyclohexanol, 5-methyl-2-1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-)-(ID#:15356-70-4) Monoterpenoid

Appendix IV:The GM-MS profile of compounds in *Hagenia abyssinica*

RT(min) Chemical Name Compound
8.1501 Camphor (ID#:76-22-2) Cyclic monoterpene ketone
16.7039 Cyclohexanol, 5-methyl-2-(1-methylethyl)-. (1.alpha. 2.beta.,5.alpha.-.+/-)-(ID#:15356-70-4) Monoterpenoid
4.2888 Propanoic acid (ID#:79-09-4)
4.8153 alpha-Pinene (ID#:80-56-8) Monoterpene
4.8153 3-Carene (ID# 13466-78-9) Bicyclic monoterpene
5.4951 Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- (15)-(ID#:18172-67-3) Monoterpene
6.2412 Benzene, 1-methyl-3-1-methylethyl)-(ID#:535-77-3) Aromatic hydrocarbon
6.3345 Eucalyptol (ID#:) Monoterpenoid
8.1501 Camphor (ID#:76-22-2) Cyclic monoterpene ketone
8.5854 Borneol (ID#:507-70-0) Bornane monoterpenoid
12.9752 Butylated Hydroxytoluene (ID# 128-37-0)
14.3276 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-(S-(Z)1- (ID#: 142-50-7) Sesquiterpene Alcohol
15.5194 Methyl tetradecanoate (ID#:124-10-7) Saturated fatty acid
16.3246 Octadecane (ID#:593-45-3) Straight chain alkane
17.6324 Hexadecanoic acid, methyl ester (ID# 112-39-0) Fatty acid methyl ester
17.9941 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl ester (ID#84-69-5) Aromatic dicarboxylic acid
19.2657 9.12-Octadecadienoic acid (Z.Z)-, methyl ester (ID#:112-63-0) Fatty acid methyl ester
19.3258 9-Octadecenoic acid (Z)- methyl ester (ID#:112-62-9) Fatty acid methyl ester

19.5621	Octadecanoic acid, methyl ester (ID#:112-61-8) Fatty acid methyl ester	
21.0896	Tricosane (ID#:638-67-5)	
21.0896	Eicosane (ID#:112-95-8)	Straight chain alkane
21.8482	Hexanedioic acid, bis(2-ethylhexyl) ester (ID#:103-23-1)	
21.9280	Tetracosane (ID#:646-31-1)	
24.0244	Benzene, 1,2,3,5-tetramethyl-(ID#:527-53-7)	
8.1501	Camphor (ID#76-22-2)	Cyclic monoterpene ketone
10.0849	Isobornyl acetate (ID#:125-12-2)	
31.4237	Vitamin E (ID#:59-02-9)	

Appendix V: The GM-MS profile of compounds in *Caesalpinia volkensii*

RT(min)	Chemical Name	Compound
6.7988	Benzyl Alcohol (ID#:100-51-6)	Aromatic alcohol
8.9367	Methyl Salicylate (ID#:119-36-8)	
10.6590	Thymol (ID#:89-83-8)	Monoterpenoid phenol
16.7132	Cyclohexanol, 5-methyl-2-(1-methylethyl)-(1.alpha.,2.beta.,5.alpha.H.+/-.- ID#:15356-70-4)	Monoterpenoid
4.1219	Phenol, 2-methoxy- (ID#:90-05-1)	Phenol
4.1219	Mequinol (ID#:150-76-5)	Phenol
4.8298	Phenol, 3,5-dimethyl-(ID#:108-68-9)	
6.7988	Benzyl Alcohol (ID#:100-51-6)	Aromatic alcohol
7.9211	Phenylethyl Alcohol (ID#:60-12-8)	
8.5574	1-Propanone, 1-phenyl-(ID#:93-55-0)	Aromatic ketone
8.8838	Benzene, tert-butyl-(ID#:98-06-6)	Aromatic hydrocarbon
9.9678	Benzofuran, 2,3-dihydro- (ID#:496-16-2)	1-benzofurans
11.3907	Phenol, 3-methyl-(ID#:108-39-4)	
13.1752	Dodecanoic acid, methyl ester (ID#:111-82-0) Saturated fatty acid	
14.6582	Benzophenone (ID# 119-61-9)	
15.2261	Heptadecane (ID# 629-78-7)	Straight chain alkane
15.5069	Methyl tetradecanoate (ID#:124-10-7)	Saturated fatty acid
16.3142	Octadecane (ID#:593-45-3)	Straight chain alkane
16.7132	Menthol (ID#:1490-04-6)	Monoterpenoid phenol
17.0272	1.2 Benzenedicarboxylic acid, bis(2-methylpropyl) ester (ID# 84-69-5) Aromatic dicarboxylic acid	
17.6407	Hexadecanoic acid, methyl ester (ID# 112-39-0) Fatty acid methyl ester	
18.3547	Eicosane (ID#:112-95-8)	Straight chain alkane
18.3941	n-Hexadecanoic acid (ID#:57-10-3)	Saturated long-chain fatty acid
19.2750	9,12-Octadecadienoic acid (Z.Z).. methyl ester (ID#:112-63-0) Fatty acid methyl ester	
19.3413	9-Octadecenoic acid (Z), methyl ester (ID# 112-62-9) Fatty acid methyl ester	
19.5879	Octadecanoic acid, methyl ester (ID#:112-61-8) Fatty acid methyl ester	
20.2232	Docosane (ID#629-97-0)	Straight chain alkane
21.8761	Hexanedioic acid, bis(2-ethylhexyl ester (ID# 103-23-1)	
25.8027	Squalene (ID#7683-64-9)	Triterpene
8.9367	Methyl Salicylate (ID#:119-36-8)	
10.6590	Thymol (ID#:89-83-8)	Monoterpenoid phenol
14.6582	Benzophenone (ID#:119-61-9)	
31.4714	Vitamin E acetate (ID# 58-95-7)	
31.4714	Vitamin E (ID#:59-02-9)	

Appendix VI: The GM-MS profile of compounds in *Bridelia micrantha*

RT(min)	Chemical Name	Compound
13.5474	Nonanedioic acid, dimethyl ester (ID#:1732-10-1)	
16.7113	Cyclohexanol, 5-methyl-2-11-methylethyl)- (1.alpha. 2.beta. 5.alpha)-(.+/-)-(ID#:15356-70-4)	Monoterpenoid
10.4892	Nonanoic acid (ID#:112-05-0)	Straight-chain saturated fatty acid
10.8633	Phenol, 2-methyl-5-(1-methylethyl)-(ID#:499-75-2)	Monoterpenoid phenol
13.2365	Dodecanoic acid, methyl ester (ID#: 111-82-0)	Saturated fatty acid
15.2521	Heptadecane (ID#:629-78-7)	Straight chain alkane
15.5361	Methyl tetradecanoate (ID#:124-10-7)	Saturated fatty acid
16.3320	Octadecane (ID#593-45-3)	Straight chain alkane
16.7113	Menthol (ID#1490-04-6)	Monoterpenoid
17.0273	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (ID#:84-69-5)	Aromatic dicarboxylic acid
17.6346	Hexadecanoic acid, methyl ester (ID#:112-39-0)	Fatty acid methyl ester
18.3590	Eicosane (ID#:112-95-8)	Straight chain alkane
19.2637	9,12-Octadecadienoic acid (Z.Z)-, methyl ester (ID# 112-63-0)	Fatty acid methyl ester
19.3238	9-Octadecenoic acid (Z)-, methyl ester (ID#:112-62-9)	Fatty acid methyl ester
19.5601	Octadecanoic acid, methyl ester (ID#:112-61-8)	Fatty acid methyl ester
20.2140	Docosane (ID#:629-97-0)	Straight chain alkane
21.8400	Hexanedioic acid, bis(2-ethylhexyl) ester (ID# 103-23-1)	
22.2110	Tetracosane (ID# 646-31-1)	
31.4135	Vitamin E (ID#:59-02-9)	
32.4301	Vitamin E acetate (ID#:58-95-7)	

Appendix VII: The GM-MS profile of compounds in *Rubus apetalus*

RT (min)	Chemical Name	Compound
4.2816	o-Xylene (ID#:95-47-6)	
15.2562	Pentadecane, 2,6,10,14-tetramethyl-(ID#:1921-70-6)	Norterpene
16.7102	Menthol (ID#:1490-04-6)	Monoterpenoid
17.0294	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (ID#:84-69-5)	Aromatic dicarboxylic acid
17.6377	Hexadecanoic acid, methyl ester (ID#:112-39-0)	Fatty acid methyl ester
19.2658	9.12-Octadecadienoic acid (Z.Z), methyl ester (ID#:112-63-0)	Fatty acid methyl ester
19.3248	9-Octadecenoic acid (2)-, methyl ester (ID#:112-62-9)	Fatty acid methyl ester
19.5611	Octadecanoic acid, methyl ester (ID#:112-61-8)	Fatty acid methyl ester
21.8431	Hexanedioic acid, dioctyl ester (ID#:123-79-5)	
22.2120	Tetracosane (ID#:646-31-1)	
16.7102	Cyclohexanol, 5-methyl-2-(1-methylethyl)- (1.alpha. 2.beta.,5.alpha.)-(+/-)-(ID#:15356-70-4)	Monoterpenoid

Appendix VIII: Mean percentage hatch inhibition of H. contortus eggs from the susceptible helminths by varying methanolic plant extract concentrations.

	12.5 mg/ml	25 mg/ml	50 mg/ml
P. peruviana	88.24±0.66	90.00±1.00	95.24±0.54
R. apetalus	82.14±0.76	85.71±0.79	96.55±0.45
B. micrantha	54.55±0.60	55.56±0.47	77.78±0.75
T. garckeana	11.76±0.42	18.18±0.66	40.00±1.53
C. volkensii	45.45±0.54	52.94±0.66	95.00±1.53
A. zambesiacum	37.50±0.38	37.50±0.42	38.46±2.15
H. abyssinica	32.00±0.65	94.12±0.52	96.96±1.21
Positive Control	100.00±0.00	100.00±0.00	100.00±0.00
Negative Control	1.6900±0.10	1.6900±0.10	1.6900±0.10

Appendix IX: Appendix IV: Mean percentage hatch inhibition of *H. contortus* eggs from the resistant helminths by varying methanolic plant extract concentrations.

	12.5 mg/ml	25 mg/ml	50 mg/ml
P. peruviana	13.76±0.44	21.82±0.64	66.66±0.67
R. apetalus	20.39±0.31	86.47±0.44	93.42±0.46
B. micrantha	11.42±0.23	12.41±0.33	91.67±0.47
T. garckeana	14.50±0.32	15.50±0.47	61.76±0.56
C. volkensii	16.31±0.28	19.08±0.19	94.56±0.36
A. zambesiacum	12.09±0.14	13.12±0.25	88.68±0.40
H. abyssinica	13.65±0.25	16.02±0.11	91.80±0.59
Positive Control	100.00±0.00	100.00±0.00	100.00±0.00
Negative Control	1.50±0.12	1.50±0.12	1.50±0.12

Appendix X: Mean percentage larvicidal activity against *H. contortus* larvae from susceptible helminths at varying methanolic plant extract concentrations.

	12.5 mg/ml	25 mg/ml	50 mg/ml
P. peruviana	22.22±0.33	94.44±0.35	100.00±0.00
R. apetalus	100.00±0.00	100.00±0.00	100.00±0.00
B. micrantha	41.18±0.55	66.67±0.39	81.25±0.23
T. garckeana	43.75±0.47	70.59±0.64	94.74±0.35
C. volkensii	71.43±0.44	84.21±0.18	100.00±0.00
A. zambesiacum	66.67±0.44	81.81±0.51	85.00±0.37
H. abyssinica	100.00±0.00	100.00±0.00	100.00±0.00
Positive Control	100.00±0.00	100.00±0.00	100.00±0.00
Negative Control	1.89±0.07	2.46±0.18	3.22±0.22

Appendix XI: Mean percentage larvicidal activity against *H. contortus* larvae (L1) from the resistant helminths by varying methanolic plant extract concentrations.

	12.5 mg/ml	25 mg/ml	50 mg/ml
P. peruviana	60.00±0.24	66.67±0.42	100.00±0.00
R. apetalus	100.00±0.00	100.00±0.00	100.00±0.00
B. micrantha	45.00±0.19	58.33±0.24	64.29±0.35
T. garckeana	25.00±0.34	27.27±0.37	38.89±0.35
C. volkensii	16.67±0.19	23.08±0.14	27.78±0.36
A. zambesiacum	12.50±0.42	13.04±0.18	53.85±0.39
H. abyssinica	94.12±0.39	100.00±0.00	100.00±0.00
Positive Control	100.00±0.00	100.00±0.00	100.00±0.00
Negative Control	2.94±0.14	1.78±0.07	2.13±0.14

Appendix XII: Mean percentage larvicidal activity against resistant *H. contortus* larval (L3) by varying methanolic plant extract concentrations.

	12.5 mg/ml	25 mg/ml	50 mg/ml
P. peruviana	10.00±0.10	15.38±0.25	27.78±0.44
R. apetalus	37.66±0.37	68.85±0.56	78.38±0.48
B. micrantha	6.67±0.39	9.09±0.17	21.74±0.50
T. garckeana	21.43±0.24	43.86±0.51	52.00±0.16
C. volkensii	29.41±0.29	31.25±0.88	58.33±0.37
A. zambesiacum	20.83±0.51	22.58±0.35	24.00±0.09
H. abyssinica	11.76±0.59	17.39±0.27	40.00±0.13
Positive Control	94.71±0.31	94.71±0.31	94.71±0.31
Negative Control	0.61±0.10	0.08±0.02	1.46±0.06

Appendix X111: Mean percentage larvicidal activity against resistant *H. contortus* larvae (L3) by varying dilutions of methanolic plant extracts with albendazole at ratio of 1:1.

	12.5 mg/ml	25 mg/ml	50 mg/ml
P. peruviana	14.29±0.24	18.18±0.14	25.00±0.21
R. apetalus	96.43±0.35	97.28±0.64	98.46±0.32
B. micrantha	6.98±0.44	15.38±0.29	26.67±0.64
T. garckeana	10.53±0.49	14.81±0.24	46.15±0.14
C. volkensii	16.67±0.50	88.89±0.38	94.44±0.47
A. zambesiacum	5.56±0.32	15.63±0.36	32.00±0.19
H. abyssinica	15.00±0.17	20.00±0.64	29.41±0.51
Positive Control	100.00±0.00	100.00±0.00	100.00±0.00
Negative Control	0.49±0.08	1.58±0.09	1.97±0.09

Appendix XIV: ED_{50} and ED_{90} of egg hatch assay for the seven plant extracts (methanol) on *H. contortus* eggs.

Plant extract	values	Concentration (mg/ml)	95% CL	
			Lower	upper
P. peruviana	ED_{50}	3.21	2.76	3.59
	ED_{90}	9.94	7.71	10.45
R. apetalus	ED_{50}	3.77	3.02	4.58
	ED_{90}	10.53	8.73	12.17
B. micrantha	ED_{50}	3.43	3.22	3.69
	ED_{90}	10.39	9.44	13.26
T. garckeana	ED_{50}	5.08	3.86	5.77
	ED_{90}	12.27	10.95	14.43
C. volkensii	ED_{50}	3.85	2.72	4.04
	ED_{90}	10.87	6.76	10.25
A. zambesiacum	ED_{50}	4.17	3.48	4.83
	ED_{90}	11.78	9.94	12.88
H. abyssinica	ED_{50}	2.57	2.20	3.58
	ED_{90}	8.46	6.75	10.06

ED_{50} dose that causes 50% egg hatching inhibition, ED_{90} dose that causes 90% egg hatching inhibition, CL, 95% confidence limit, significant at $p<0.05$ level

Appendix XV: ED_{50} and ED_{90} of larval development assay for the seven plant extracts (methanol) *H. contortus* larvae (L3).

Plant extract	values	Concentration (mg/ml)	95% CL	
			Lower	upper
P. peruviana	ED_{50}	3.21	2.87	3.56
	ED_{90}	9.67	7.94	12.09
R. apetalus	ED_{50}	2.84	2.33	3.21

	ED_{90}	9.31	8.73	10.95
B. micrantha	ED_{50}	3.40	2.72	4.19
	ED_{90}	10.88	8.64	11.61
T. garckeana	ED_{50}	4.68	3.85	5.47
	ED_{90}	11.53	9.88	14.06
C. volkensii	ED_{50}	2.76	2.45	3.57
	ED_{90}	8.85	6.38	10.13
A. zambesiacum	ED_{50}	4.22	3.59	4.86
	ED_{90}	11.42	8.72	13.33
H. abyssinica	ED_{50}	2.35	2.06	3.37
	ED_{90}	7.11	5.21	8.76

ED_{50} dose that causes 50% larval development inhibition, ED_{90} dose that causes 90% larval development inhibition, CL, 95% confidence limit, significant at $p<0.05$ level

Appendix XVI: National Commission for Science Technology and Innovation Approval Letter.

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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This is to Certify that Mr.. Kelvin Mawira of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: In vitro anthelmintic activity against Haemonchus contortus of methanolic extracts of selected medicinal plants from Meru county, Kenya. for the period ending : 29/October/2022.

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