IN VITRO DETERMINATION OF EFFICACY OF INDIGENOUS PLANT EXTRACTS USED FOR INTERNAL PARASITES CONTROL BY SMALL-HOLDER LIVESTOCK FARMERS IN CHIEF ALBERT LUTHULI LOCAL MUNICIPALITY, MPUMALANGA PROVINCE, SOUTH AFRICA

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BY

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A FULL-DISSERTATION SUBMITTED FOR THE DEGREE OF MASTER OF AGRICULTURAL MANAGEMENT (ANIMAL PRODUCTION), DEPARMENT OF AGRICULTURAL ECONOMICS AND ANIMAL PRODUCTION, SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo for the degree of Master of Agricultural Management (Animal Production) has not previously been submitted by me for examination at this or any other university. This work is my own in design, execution and that all material contained herein and help from people and other institutions have been duly acknowledged.

Signature……... Date……

Shiba Mlungisi Richard

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DEDICATION

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ABSTRACT

Ethno veterinary medicine practices are popular among the resource constrained small-holder farmers. Nonetheless, the effectiveness of traditional remedies particularly the indigenous plants has not been extensively documented. Hence, this study was conducted to determine efficacy of indigenous plant extracts used by small-holder farmers in Chief Albert Luthuli Local Municipality, Mpumalanga Province, South Africa for internal parasites control. Information on indigenous plants used for the control of internal parasites of livestock by local farmers in the study area was gathered through a questionnaire survey. A total of 188 livestock farmers (both males and females) of mixed ages were interviewed. Thirteen different plants were frequently mentioned by the respondents as remedies for livestock internal parasites. Of these, seven plants could be identified up to their families and species. Plant species collected were *Dicerocaryum* sp (50%), *Pappeacapensis* (61%), *Aloe ferox* (90%), *Helichrysum* sp (56%), *Senecio congestus* (83%), *Senecio barbertonicus* (67%) and *Gardenia* sp (73%). These plants were extracted using distilled water and analysed to determine their efficacy through *in vitro* assays; Egg hatch, larval development and larval mortality assays. All the assays were performed at different concentrations of 2.5mg/ml, 5.0mg/ml and 7.5mg/ml. The nematode third stage larvae were incubated for 24hr, 48hr and 72hr during the larval mortality assay. The present study showed that all the seven-plant species under investigation possessed some anthelmintic activities of varying strength. The highest egg hatch inhibition was observed from the extracts of *Senecio barbertonicus* with 100 % and the lowest from *Dicerocaryum eriocarpum* with 2.25 %, for larval development the highest was *Gardenia* sp 100 % and the lowest *Helichrysum* sp 26 % at concentration 7.5 mg/mL respectively. Whereas, the highest in larval mortality assay was *Senecio barbertonicus* and *Gardenia* sp achieved 100 % after 48hrs and the lowest was *Dicerocaryum eriocarpum* with49.89 % after 72hrs at concentration 7.5 mg/mL respectively. The use of other different forms of extraction media is recommended because different results can observe and be compared with the results of the present study. Toxicity studies on the indigenous plants observed to have stronger anthelmintic activities would assist in the future recommendation of these remedies for large scale or commercial use as anthelmintic drugs.

Keywords: ethno veterinary medicine, gastrointestinal parasites, anthelmintic

CHAPTER 1

BACKGROUND

1.1 BACKGROUND

In South Africa, a large proportion of the population relies on traditional remedies to treat themselves and their animals for common diseases. It has been estimated that up to 60% of South Africans consult traditional healers, usually in addition to making use of orthodox medical services (Van Wyk *et al*., 1997). Treatment of animal diseases developed in parallel with the treatment of human diseases (McGraw and Eloff, 2008). However, in the case of animal diseases, it appears that livestock owners will generally treat their animals using medicinal plant knowledge that they themselves possess, rather than consulting traditional healers (McGraw and Eloff, 2008). The knowledge base of ethno veterinary medicine differs not only from region to region but also amongst and within communities and households. Ethno veterinary medicine has been developed through trial and error and deliberate experimentation (Jabbar *et al*., 2006). Hence, it is less systematic, less formalized, and not universally recognized as a valid method of disease control in animals. The practice of ethno veterinary medicine is of specific value in developing countries where allopathic veterinary medicines are often not accessible to livestock producers (Matekaire and Bwakura, 2004). Livestock production and productivity is impeded by various constraints chief among them being persistent diseases outbreaks (Louw, 1999). Infections by gastrointestinal nematodes remain the most common and a major constraint to economic productivity of grazing livestock throughout the world *Haemonchus* and *Trichostrongylus* nematode generally remain the main parasites responsible for disease-related production losses arising from stock mortality, severe weight loss and poor production, especially in small ruminants (Chiejina, 2001; Perry and Randolph, 1999; Van Wyk *et al*., 1997). Ethno veterinary medicine is accessible, cheap and effective, especially in rural areas where modern veterinary services are absent or irregular and expensive. To keep animals healthy, traditional healing practices have been applied for centuries and have been passed down orally from generation to generation, largely undocumented (Matekaire and Bwakura, 2004). This situation places ethno veterinary medicine in danger of extinction (Matekaire and Bwakura, 2004).

Gastrointestinal nematodes are commonly controlled by the use of synthetic commercial anthelmintic drugs that are sold at the agricultural offices for safety purposes (Cala *et al*., 2012; Tsotetsi and Mbati, 2003). The limitations of this reliance on chemotherapy are the threat of parasites developing resistance to drug treatment (Sargison, 2012; Sutherland and Leathwick, 2011). Different worm species worldwide have already developed anthelmintic resistance which severely impairs the control of parasites (Jackson and Coop, 2000). The limited knowledge of most traditional farmers of using modern medicines among other factors has promoted the development of drug resistance (Vatta and Lindberg, 2006). In South Africa, anthelmintic resistance in the commercial sheep farming sector has been described as being the worst in the word (Van Wyk *et al*., 1999). In resource-poor systems in South Africa, resistance has been reported in sheep (Van Wyk *et al*., 1999) and in goats (Bankunzi, 2003; Vatta *et al*., 2001). Besides the development of resistant populations, gastrointestinal parasites present challenges which include high cost of commercial drugs, risk of environmental pollution and reduction of animal production and death. There has been increasing interest in medicinal plants use as an alternative source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of parasites and as anthelmintic drugs (Mole *et al*., 2003; Vieira *et al*., 1999; Waller, 1997; Herd, 1996). Use of indigenous plant preparations as livestock dewormers is gaining ground as one of the alternative and sustainable methods readily adaptable to rural farming communities (Danøe and Bøgh, 1999; Hammond *et al*., 1997). Reports by McCorkle *et al*. (1996) and Plotkin. (1992) indicate that 80% of people in developing countries rely on phytomedicine for primary healthcare in both humans and animals. Options like biological control, vaccine and traditional medicinal plants are being examined in different parts of the world (Eguale *et al.,* 2007). The practice of ethno veterinary medicine includes the use of diagnostic procedures, animal husbandry practices, surgical methods and traditional veterinary theory in addition to the use of ethno veterinary plants to prevent and control diseases (Van der Merwe *et al*., 2001; Schillhorn van Veen, 1996). Screening and proper evaluation of the claimed medicinal plants could offer the possible alternatives that may both be sustainable and environmentally acceptable if proper harvesting practices are applied. It is therefore important to keep in mind that laboratory-based *in vitro* screening system

do not always give the true reflection of efficacy level of a traditional medication (McGraw and Eloff, 2008).

Several studies have been carried out on the ethno veterinary practices of the rural communities (Setlalekgomo and Setlalekgomo, 2013; Luseba and Tshikhawe, 2013). However, very few of these studies paid attention to the effectiveness of the traditional remedies particularly, anti-parasitic effects of indigenous plants. As far as it can be ascertained there is no record on the use of ethno veterinary remedies and their scientific validation for Chief Albert Luthuli municipality. Hence, this study aimed at determining plant species used by small-holder farmers against gastrointestinal nematode infections in Chief Albert Luthuli municipality and to determine their anthelmintic properties *in vitro*.

1.2 Problem statement

The high prevalence of livestock diseases in South Africa presents a major challenge particularly to the resource constrained small-holder farmers in the communal areas of the country. Several studies were carried out on identifying ethno veterinary medicine used by small-holder farmers in the rural communities of South Africa (Luseba and Van der Merwe 2006; Van der Merwe *etal*., 2000). However, the efficacy of most of the traditional claimed plant species has not been scientifically determined. Since a significant proportion of small-holder farmers often resort to the use of indigenous remedies because of poor availability of veterinary assistance and high cost of commercial drugs, the effectiveness of these remedies need to be verified so as to provide a scientific backing to indigenous knowledge and further promote those plants as important tools in the treatment of several gastrointestinal nematodes (Eguale *et al*., 2011).

Furthermore, the extensive system of livestock production practiced by the communal farmers exposes livestock to internal parasites in contaminated pastures thereby making internal parasitism one of the greatest challenges among small scale livestock farmers (Dreyer *et al*., 1999). An integrated approach to control internal parasites which encompasses the use of plants with anti-parasitic properties is needed. This study will address the problem of the lack of documentation of the

efficacy of medicinal plants in the control of gastrointestinal parasites by smallholder farmers.

1.3 Aim of the study

The study was carried out in order to determine the anthelmintic effects of different indigenous plant species used by small-holder farmers and their effectiveness against gastrointestinal nematodes of livestock.

1.4 Objectives

The objectives of this study were to:

i. Identify indigenous plant species used by small-holder livestock farmers to control internal parasites in the Chief Albert Luthuli Municipality.
ii. Compare the anthelmintic effectiveness of some of these plants to a commercial anthelmintic drug.

1.5 Research questions

The study attempted to answer the following research questions:

i. Which indigenous plants are used by small scale livestock farmers to control internal parasites in the Chief Albert Luthuli Municipality?
ii. What is the anthelmintic effectiveness of the indigenous plants as compared to a commercial anthelmintic drug?

1.6 Definition of terms

1. **Ethno veterinary medicine** (EVM), is the scientific term for traditional animal health care, encompassing the knowledge, skills, methods, practices, and beliefs about animal health care found among the members of a community (McCorkle, 1986).
2. **Ethnobotany** is the biological, economic, cultural inter-relationship studies between people and plants in the environment in which they exist.

1.7 Significance of the study

The present study will provide information on the effectiveness of the plants which are used by small scale livestock farmers in the Chief Albert Luthuli local municipality as remedies for internal parasites. This will promote the use of indigenous animal health care which is often cheap and readily available to the resource constrained livestock farming communities of the Mpumalanga province. The information gained from this study will also be beneficial to the relevant stake holders who are directly tasked to assist small scale holder farmers with their animal production activities. Furthermore, this study will provide useful information upon which future research may be based.

1.8 Limitations of the study

Since ethno veterinary medicine is a complex system of practices involving more than just the application of plant based remedies to sick animals, it may give rise to misleading expectations about the degree of efficacy of a single plant used as part of a cure. Inadequate diagnosis of disease symptoms limits the performance of the plants since they are often prescribed for the wrong disease. Seasonal availability of indigenous vegetation and some of the materials used by small-holder farmers for the treatment of livestock disadvantages ethno veterinary medicine. Also, environmental effect influence vegetation and soil types from one region to the other, so that limits the use of ethno veterinary medicine. It is often not possible to assume efficacy *in vivo* after achieving good results with *in vitro* tests. Since it may not be practical to extrapolate the dose from that which is active *in vitro* to that which would be required to reach adequate plasma concentrations in the target species (Houghton *et al*., 2007). Other factors to be considered include bioavailability, absorption and metabolism which may be responsible for discrepancies between *in vitro* and *in vivo* tests (Houghton *et al*., 2007).

1.9 Outline of dissertation

1. A review of both the background and the introduction pertinent to the topic of this thesis is presented in Chapter 1.
2. Chapter 2 reviews the theoretical and the empirical literature pertinent to the topic of this dissertation.
3. Chapter 3 describes the research methodology, including a brief description of the research setting, data collection procedures and analytical techniques.
4. Chapter 4 reports the results and discussion of the study.
5. Chapter 5 focuses on the conclusions and recommendations drawn from the study.

CHAPTER 2

LITERATURE REVIEW

2.1 Gastrointestinal parasite occurrence in livestock production systems

Gastrointestinal nematodes limit livestock production in many areas and countries (Keyyu *et al*., 2005). The losses caused by these parasites are attributed to acute illness and death, premature slaughter and rejection of some parts during meat inspection. In several cases, death due to gastrointestinal nematodes has been recorded in ruminants (Agyei *et al*., 2004). Indirect losses include the diminution of productive potential such as decreased growth rate, weight loss in young animals and late maturity of slaughter stock (Tembely *et al*., 1997; Hansen and Perry, 1994). The infections are either clinical or sub clinical (Makundi *et al*., 1998; Msanga, 1985). Understanding of the parasite epidemiology and the factors that affect parasite growth are crucial for controlling and management of internal parasites (Tembely *et al*., 1997; Sissay *et al*., 2007; Waller & Thamsborg, 2004).

The excessive use of anthelmintic drugs contributes to the emergence of resistant strains and increases the contamination of water supplies and foodstuffs. According to Mirkena *et al.* (2010), gastrointestinal nematodes are a problem not only for young stock, but also for breeding females especially during late pregnancy and early lactation. During this period, the animals are immunologically vulnerable.

There is strong evidence to suggest that climate and environmental factors have an influence in a host-parasite relationship in a livestock production system (Martinez-Gonzalez *et al.,* 1998). In addition, seasonal dynamics are also essential for parasite growth and development (Teel *et al*., 1996). The seasonal dynamics of nematodes infection are the consequence of complex inter-relationships between the livestock, their husbandry and the prevailing climate (Viassoff *et al*., 2001).

2.2 Control and treatment of gastrointestinal nematodes

Gastrointestinal nematodes are a major factor that limits livestock production worldwide (Cala *et al*., 2012). Commercial drugs have been used effectively to control the infection by curing clinical symptoms and /or diseases and basically to reduce mortality rates (Molefe *et al*., 2012; Roets and Kirsten, 2005). However,

commercial drugs are unaffordable and unavailable to farmers with poor socio-economic status in most developing countries since, in many cases, drugs are imported (Amin *et al*., 2009). Locally available medicinal plants have been used by small-holder farmers to manage various livestock gastrointestinal nematodes (Eguale *et al*., 2011; Masimba *et al*., 2011; Mathias *et al*., 1999). Medicinal plants typically contain several different pharmacologically active compounds that could be responsible for anti-inflammatory and anti-oxidative activity (Torras *et al*., 2005; Afolayan *et al*., 2007; Diouf *et al*., 2009)

2.3 Anthelmintic resistance development and control

Traditional farmers have several misconceptions that contribute to drug resistance. These may include drug adulteration, mixing two or more different drugs, use of expired drugs, effective for one is effective for other diseases and a drug that is good for humans is also used for animals (Fielding, 1998). In addition to that, repeated administration of the drug on the host provides a suitable medium for nematodes to build up a wide range of resistance (Carvalho *et al*., 2012; Hernandez-Villegas *et al*., 2012). Investigation of indigenous plant extracts against gastrointestinal nematodes of livestock is the alternative to control anthelmintic resistance of nematodes (Maphosa *et al*., 2009; Mphahlele *et al*. 2016).

2.4 The efficacy of plant extracts against gastrointestinal parasites of livestock.

Plants have developed biochemical mechanisms to defend themselves from biological antagonists that act as their natural enemies (Ryan and Jagendorft, 1995). This principle has led scientists to search for bio-active compounds produced by plants against pathogens (Sheludko, 2010). A wide range of plants and their products around the world are being explored to look for their possible anthelmintic effects on gastrointestinal parasites (Abdel-Ghaffaretal *et al,* 2012; Datsu *et al.,* 2011). Traditionally, some plants around the world are well known as anti-parasitic plants because they contain substances with anthelmintic effects (De Jesús-Gabino *et al*., 2010; Galicia-Aguilar *et al*., 2008; López-Aroche *et al*., 2008). Modern pharmacopoeias still contain in the order of 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants (Danøe and Bøgh, 1999).

In South Africa, numerous plants are commonly used by rural communities as remedys to cure numerals of diseases caused by parasitic nematodes (Shai *et al*., 2009; Mphahlele *et al*., 2016). Some forage has been evaluated searching for potential bio-active compounds against sheep and goat parasitic nematodes with variable results.

2.5 Ethno veterinary medicine in the treatment of gastrointestinal nematodes

A relatively high number of the population in developing countries still use medicinal plants in livestock healthcare as first line of action before veterinary consultation takes place. Surveys of plants used in ethno veterinary medicine worldwide against ruminants intestinal helminthes have been publicized (Kaboré *et a*l., 2007). Medicinal plants play an important role in the discovery and isolation of new drugs (Gurib-Fakim, 2006; Balunas and Kinghorn, 2005). Ethno veterinary medicine preparations are widely used by small holder farmers for treatment of their livestock against helminth parasites (Masimba *et al.,* 2011). Traditional uses of some South African plants have been documented in several books (Van Wyk *et al*., 1997; Hutchings *et*

al., 1996; Watt and Breyer-Brandwijk, 1962). *Pappea capensis* was reported to be effective in treating diarrhea (Cunningham, 1988). *Aloe ferox* species have been used for centuries to control gastrointestinal disorders and internal parasites in livestock (Jia *et al*., 2008; Grierson and Afolayan, 1999). Small holder farmers have been using *Helichrysum* sp in livestock for constipation and worms (Quer, 1993). *Senecio congestus* and *Senecio barbertonicus* species are known to treat both diarrhea and gastrointestinal parasites (Hirschhorn, 1983). The use of *Gardenia* species was reported by Nsekuye in 1994 for the treatment of livestock intestinal nematodes.

Novel approaches to internal parasite control are needed to counteract the problem of anthelmintic resistance (Waller, 2003). Resistance is becoming widespread because relatively few chemically dissimilar groups of anthelmintics have been introduced over the past decades. Also misuse and poor formulations of these products have led to the development of anthelmintic resistance (Lans and Brown, 1998).

CHAPTER 3

METHODOLOGY AND ANALYTICAL PROCEDURES

3.1 Research setting

3.1.1 Description of the study area

Figure 3.1.1(a): Map of Mpumalanga showing Gert Sibande District in red.

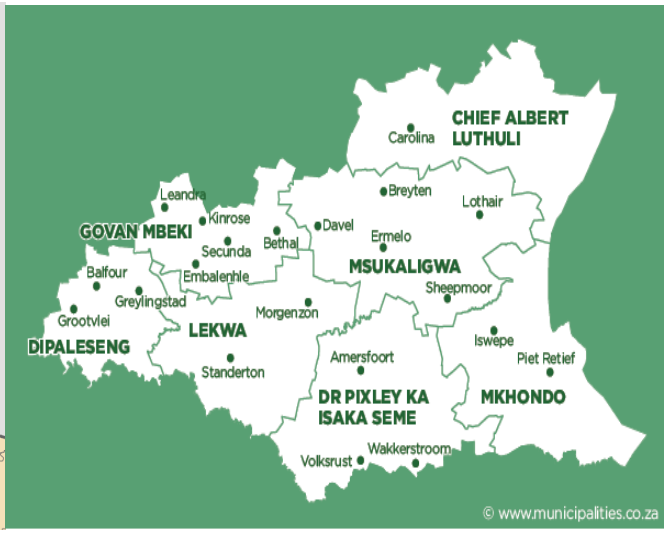

Figure 3.1.1(b): map of Gert Sibande District showing Chief Albert Luthuli Municipality top right.

The site identified for primary data collection was Chief Albert Luthuli Municipality which is situated in Gert Sibande District of Mpumalanga Province (25.4236° S latitude and 29.4724° E longitudes by 25.3224° S latitude and 31.0824° E longitudes). The majority of rural settlements occur in the eastern part of the municipality with forestry area in the central as well as the river system, Vygeboom dams and edge of a greater wetland region. Economic activities that are dominant spatially in the municipality include agriculture and forestry.

3.1.2 Questionnaire survey

Field work on plant specimen collection was undertaken during September and October 2014. Purposive sampling method was used to select farmers in the study area. The questionnaire design was based on identification of indigenous plant species used by small-holder farmers for the treatment of gastrointestinal nematodes of their livestock. The questions were formulated to extract key information on local names, identification of indigenous species, as well as preparation and application procedures.One hundred and fifty (150) individual participants who had some knowledge of ethno veterinary medicine were interviewed of which 106 were males

and 44 were females. Points of discussion were guided by descriptions such as farmer's age, gender, animal husbandry and indigenous knowledge in animal healthcare. A small number of youth participated; this might be a reflection of the culture and a consequence of modernization and its consequence on youth beliefs. Young people do not accept outdated information (Van der Merwe *et al*., 2001).

3.1.2 Plant specimen collection

Plants species were selected as identified through group interviews of 188 young and elderly small-holder farmers that are using indigenous knowledge in the treatment of gastrointestinal nematodes. However, 38 of those farmers declined to share their ethno veterinary medicine knowledge with us. As noted for Tsonga speaking people in South Africa, traditional healers do not share their knowledge with farmers (Luseba and Van der Merwe, 2006).

During the interviews and focus group discussions, plants that were repeatedly mentioned by the small-holder farmers for the treatment of gastrointestinal nematodes were subsequently collected for further identification and laboratory testing. The species were identified by their vernacular names by farmers. Information on methods of traditional preparation of these plants for deworming livestock was gathered. All specimens were then taken to the University of Limpopo where they were identified by Dr T.G. Mandiwana-Neudani (Department of Biodiversity) using the University's Larry Leach Herbarium (UNIN) resources. Specimens were identified as *Dicerocaryum eriocarpum*, *Pappea capensis*, *Aloe ferox*, *Helichrysum* sp, *Senecio congestus*, *Senecio barbertonicus* and *Gardenia* sp.

3.1.3 Sub-sampling from specimens

From the same specimens, sub-samples were prepared, dried, pulverized and extracted at the University of Limpopo, Animal Production Laboratory. Matured plant parts were sub-sampled as follows: *Dicerocaryum eriocarpum* (leaves), *Pappea capensis* (bark), *Aloe ferox* (leaves), *Helichrysum* sp (leaves and stalk), *Senecio congestus* (roots), *Senecio barbertonicus* (leaves) and *Gardenia* sp (fruits).

3.2 Preparation of plant extracts

Specific plant parts were dried in an oven at a temperature of 50^{o}C to a constant weight, pulverized and ground. A 25-mesh diameter sieve was used to obtain fine dust that was then preserved in airtight plastic containers, till their use for extract preparation. Ten grams of each powdered material was extracted in 10 mL/g of distilled water overnight. All extracts were filtered using filter paper (Whatman No. 1). The water extracts were freeze- dried. The yields were 1.1, 1.5, 1.4, 0.3, 1.2, 0.7 and 1.5 g of *Dicerocaryum eriocarpum* (leaves), *Pappea capensis* (bark), *Aloe ferox* (leaves), *Helichrysum* sp (leaves and stalk), *Senecio congestus* (roots), *Senecio barbertonicus* (leaves) and *Gardenia* sp (fruits) respectively. Individual extracts were reconstituted in their respective solvent to give a stock solution of 50 mg/ml (Ashafa and Afolayan, 2009). These were diluted to the required series of concentration of 2.5, 5.0 and 7.5 mg/ml for the bioassay analysis, using tocris dilution calculation formula presented below.

$$C_1V_1=C_2V_2$$

Where: C_1= Concentration 1

V_1= Volume 1

C_2= Concentration 2

V_2= Volume 2

In vitro data collection was done at Agricultural Research Council in Onderstepoort Veterinary Research institute. The area is located at 25.6513° S latitude and 28.1844° E longitudes in Pretoria, South Africa.

3.3 Faecal sample collection

Faecal samples were collected from 9 adult ewes that were naturally infected in the grazing field by nematodes field strains. The sheep belonged to a farmer around Pretoria North (Mr Harmse) that needed Department of agriculture veterinary science assistance after seeing symptoms of internal parasites. Samples were collected directly from the recta of animals (Reinecke, 1983) and immediately transported to

the Helminthology Laboratory of the Agricultural Research Council, Onderstepoort Veterinary Research in a cooler box. Faecal samples were analysed using the McMaster technique (Soulsby, 1982). The method described by Reinecke (1973) was used to prepare faecal cultures and to confirm the presence of nematode eggs while the method outlined by Van Wyk *et al*. (2004) was used to confirm the nematode genera.

3.4 Diagnostic methods

The McMaster technique (Soulsby, 1982) was used for nematode egg diagnosis in this study. Briefly, two grams were weighed, and 58 mL of 40% sugar solution was added to the sheep faeces as a floatation medium. Samples were thoroughly crushed and dissolved using a blender. Two chambers of the McMaster slide were filled using a pasture pipette. The slides were allowed to stand for about 2 minutes so that the eggs could float on the surface of the floatation medium and to lie in contact with the upper glass of the chamber. A dissecting microscope (Olympus Japan) was used for egg detection and egg per gram counts of faeces were done by counting all the eggs in the chambers of the slides and multiplied by 100 for sheep.

3.5 Egg recovery assay

Egg recovery assay was conducted as published by Maphosa *et al*. (2009) with some minor modifications. Four grams of collected faecal sheep pellets were weighed, water was added and the mixture blended. The slurry was filtered through sieves of 110 µm, 70 µm and 25 µm.The contents of 25 µm sieve were back washed with tap water and transferred into a 500 mL beaker. The suspension was allowed to stand for 30 minutes, the supernatant was decanted and the sediments were suspended in a 40% sugar solution and the suspension was allowed to stand for another 30 minutes. The supernatant was washed through a 25 µm pore mesh sieve using tap water. The eggs were washed off from the 25 µm with tap water and transferred back into the 50 ml tubes and allowed to stand for 2 hours, and then the supernatant was decanted. The concentration of the eggs was estimated by counting

the number of eggs in 3 aliquots of 0.5 mL of the suspension in a microscope slide repeatedly, then the mean number of eggs per 0.5 mL was determined.

3.6 Egg hatch assay

The egg hatch assay was conducted as published by McGaw *et al.* (2007) and Bizimenyera *et al.* (2006). Using a dissecting microscope, (Wild Heerbrugg) approximately 80 eggs of nematodes parasites were pipetted into a 96 well microtitre plate; each tube contained 0.5 mL of egg suspension and 0.5 mL of *Dicerocaryumeriocarpum*, *Pappea capensis*, *Aloe ferox*, *Helichrysum* sp, *Senecio congestus*, *Senecio barbertonicus*, *Gardenia* sp extracts at increasing concentrations (2.5, 5 and 7.5 mg/mL) reconstituted in their respective solvents. In addition, a positive control of Thiabendazole® at the same concentrations as the plant extracts and negative control of distilled water was tested. All tests were replicated three times. The plates were covered and incubated under humidified conditions for 48 hours at 27°C thereafter a drop of Lugol's iodine solution was added to each well to stop further hatching. The number of unhatched eggs and the first stage larvae (L_1) present per well was counted using a dissecting microscope. Inhibition percentages were calculated using a formula as described by Cala *et al.* (2012).

$$E = \frac{(Eggs + L1) - L1}{Eggs + L1} \times 100$$

Where: E= Egg hatch percentage

L_1= first stage larvae

3.7 Larval development assay

The method described by Bizimenyera *et al. (*2006) was used for larval development assays. The counted number of eggs in a 0.5 mL pipetted suspension was put in each well in a 96-microtitre plate. The contents of the wells were then mixed, and the plates placed in an incubator under humidified conditions at 27º C for 48 hours for incubation of the eggs. After 48 hours, 0.5 mL of distilled water plant extracts of *Dicerocaryumeriocarpum*, *Pappea capensis*, *Aloe ferox*, *Helichrysum* sp, *Senecio congestus*, *Senecio barbertonicus*, *Gardenia* sp as well as Thiabendazole as a

positive control at 2.5, 5 and 7.5 mg/mL was added to the respective plates. The negative control plate had 0.5 mL of distilled water. All experiments were replicated three times. Incubation of the plates was continued for 5 days, after which all the plates were examined to determine the survival of the larvae at different concentrations. All the L_3 stage larvae in each well were counted and a percentage inhibition of larval development was calculated using the formula (Cala *et al.,* 2012):

$$E = \frac{(L1+L2+L3)-L3}{L1+L2+L3} \text{ X } 100$$

Where: E= Egg hatch percentage

L_1= First stage larvae

: L_2= Second stage larvae

: L_3=Third stage larvae

3.8 Larval mortality assay

Larval mortality assay was conducted according to the method described by McGaw *et al.* (2000) and Zafar *et al.* (2006) with some minor modifications. *In vitro* cultures were prepared from microscopically positive faecal samples of sheep. The cultures were incubated for 7 days under humidified conditions of 27˚C and on the seventh day the L_3 larvae were harvested from the *in vitro* cultures prepared and poured into a single petri dish. The 0.5 mL L_3 were pipetted into the 96 well microtitre plate and crude extracts of the same volume were added at three different levels of concentrations (2.5, 5 and 7.5 mg/mL). Thiabendazole® was used as a positive control at the same concentrations as the plant extracts and a negative control of distilled water was tested. After the addition of the extracts, larval counts were done firstly after two hours and then daily for three days. All live and dead L_3 stage larvae in each well were counted and mortality was expressed as a percentage. All tests were replicated three times.

3.9 Data analysis

General Linear Model (GLM) procedures of the Statistical Analysis System (SAS, 2012) package was used to analyse the egg hatch, larval development and larval mortality assays in order to determine if there was significant difference in the hatched eggs, larvae developed and larval mortality from the different indigenous plant extract treatments with different concentrations. The differences between the means were considered significant at $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

Distilled water without plant extract was used as a negative control in all the in vitro tests and this yielded 0% inhibition in all the assays that were performed. This result was expected since distilled water has no effect on egg hatch inhibition, larval development inhibition and larval mortality regardless of the time of incubation. The positive control in the form of Thiabendazole produced 100% mortality within the first 2 hours of incubation and all the concentration levels yielded the same results.

4.1 Field data

The surveys made it possible to identify a total of seven potentially anthelmintic plants species used as remedies for gastrointestinal nematodes of livestock in Chief Albert Luthuli Municipality. All seven plants species were *in* vitro tested against sheep nematodes. For each plant mentioned, we indicated the family, local name, botanical/ scientific name, the plant part(s) used, the administered form and method of administration to the animal (Tables 4.1.1).

Table 4.1.1: List of medicinal plants used by livestock farmers of Chief Albert Luthuli Municipality for the treatment of gastrointestinal nematodes of livestock

No	Local Name (siSwati)	Botanical/ Scientific Name	Family name	Preparation	Parts extracted	Application
1	Inkundzana	*Dicerocaryum eriocarpum*	Pedaliaceae	Crushed and mixed with water	Leaves	**Oral**
2	Imfuce	*Pappea capensis*	Sapindaceae	Crushed bark and mixed with water	Bark	**Oral**
3	Inhlaba	*Aloeferox*	Xanthorrhoeaceae	Crushed leaves and mixed with water	Leaves	**Oral**
4	Muhlomantsetse	*Helichrysum* sp	Asteraceae	Crushed leaves and mixed with fresh milk	Leaves and Stalk	**Oral**
5	Lichama	*Senecio congestus*	Asteraceae	Boil crushed roots with water	Roots	**Oral**
6	Intseleti	*Senecio barbertonicus*	Asteraceae	Crushed leaves and mixed with water	Leaves	**Oral**
7	Umtfuma/Itfuma	*Gardenia* sp	Rubiaceae	Crushed fruits boiled with water and filtered	Fruits	**Oral**

During the discussions with the farmers, it was evident that some of the plant species were more popular than others. Figure 4.1.2 below shows the popularity of the mentioned plants as remedies against internal parasites among the farmers interviewed.

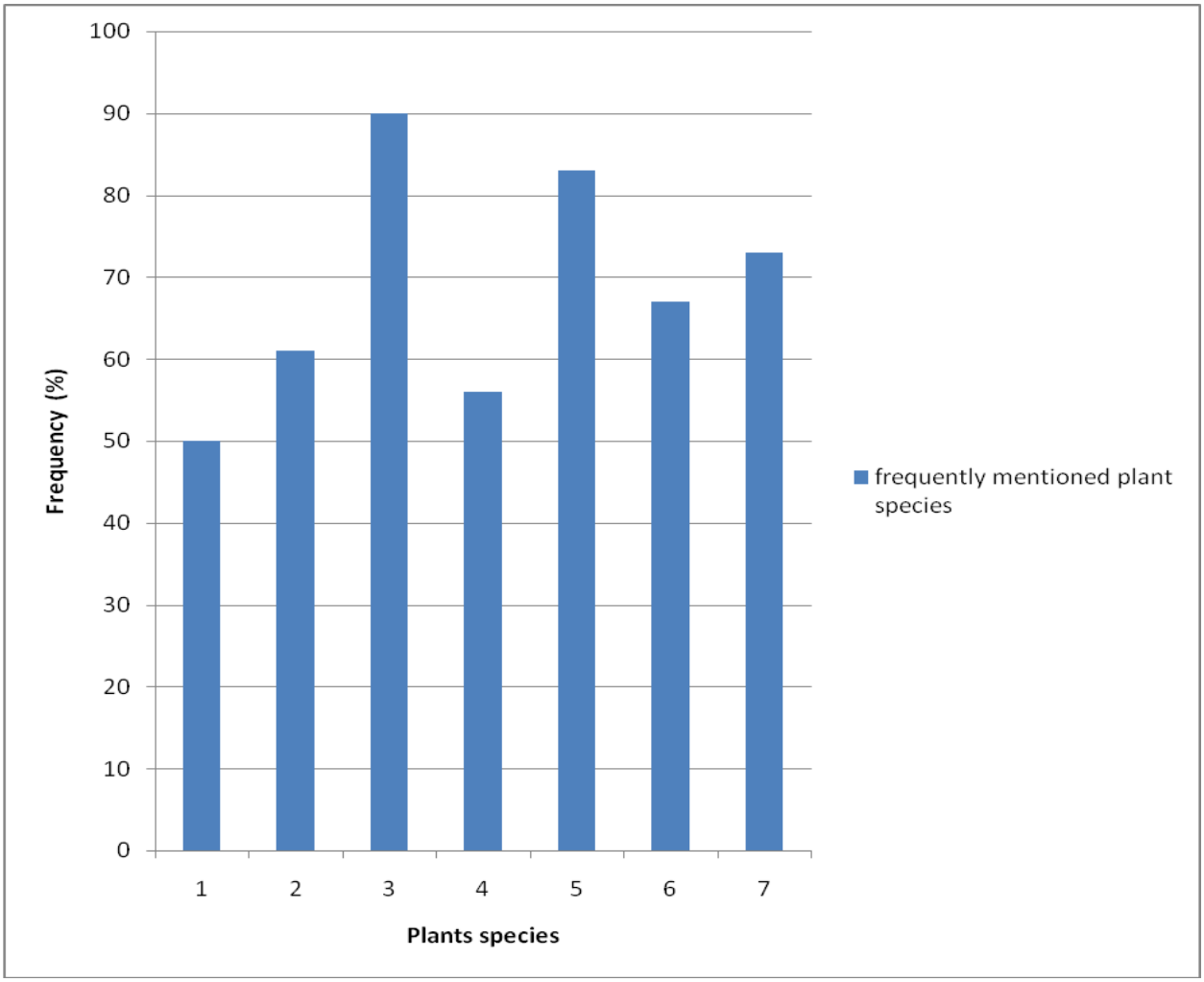

Figure 4.1.2: Popularity of the collected plant species as remedies for livestock internal parasites in the study area

Key: plants species represented, 1= *Dicerocaryumeriocarpum*, 2= *Pappea capensis*, 3= *Aloe ferox*, 4= *Helichrysum* sp, 5= *Senecio congestus*, 6= *Senecio barbertonicus*, 7= *Gardenia* sp.

4.2 Laboratory data

4.2.1 Egg hatch assays

The anthelmintic activities with respect to egg hatch inhibition (%) of the different plant extracts in distilled water as the extraction medium were investigated at varying concentrations. Table 4.2.1 below is a summary of the findings.

Table 4.2.1 Egg hatch inhibition (%) of the different plant extracts at varying concentrations (mg/mL) with distilled water as extraction medium

	Egg hatch			
	Concentrations (mg/mL)			
Treatments	2.5	5.0	7.5	SEM
Dicerocaryum eriocarpum	0.00^{b}	0.00^{b}	2.27^{a}	0.315
Pappea capensis	18.33^{b}	27.50^{a}	33.37^{a}	3.897
Aloe ferox	34.60^{b}	37.53^{b}	44.97^{a}	5.273
Helichrysum sp	2.93^{c}	8.37^{b}	23.77^{a}	2.829
Senecio congestus	51.70^{c}	67.13^{b}	79.60^{a}	9.169
Senecio barbertonicus	87.00^{b}	97.50^{a}	100.00^{a}	12.482
Gardenia sp	84.60^{c}	92.00^{b}	98.77^{a}	12.096
Thiabendazole	100.00^{a}	100.00^{a}	100.00^{a}	13.056

^{a,b,c} : Means in the row not sharing a common superscript are significantly different ($P<0.05$)

SEM: Standard error of the means

In this study, the *in vitro* model demonstrated ovicidal effect of distilled water plant extracts of *Dicerocaryumeriocarpum*, *Pappea capensis*, *Aloe ferox*, *Helichrysum* sp, *Senecio congestus*, *Senecio barbertonicus*, *Gardenia* sp against gastrointestinal nematodes of livestock, with Thiabendazole® as a positive control and distilled water as negative control. Distilled water plant extracts of *Dicerocaryumeriocarpum* and *Helichrysum* sp demonstrated weaker anthelmintic activities with respect to egg hatch inhibition (%) at concentrations 2.5mg/ml, 5.0 mg/ml and 7.5 mg/ml with less than 25% egg hatch inhibition observed for the highest concentration. The low

anthelmintic activities observed could be attributed to the extraction medium that was used as a different extraction medium would have yielded different results. In a study by Luseba (2007) farmers crushedthe plant parts and used tap water or spring water as an extraction medium. Luseba *et al*. (2007) reported antibacterial and anti-inflammatory properties of *Dicerocaryum* sp. Also, according to a review by Samuelsen. (2000) the seed coats of *Dicerocaryum* sp are removed, dried and ground to a fine powder. The powder absorbs water in the intestinal tract producing a bulky mass that is unaffected by bacteria as it moves through the intestines. It provides relief from constipation and chronic diarrhea.

The weak anthelminthic activities demonstrated by extracts of the *Helichrysum* sp in distilled water may be due to diferent extraction medium used in this study. The interviewed farmers indicated that they use fresh milk as extraction medium. However, antihelmintic properties of *Helichrysum* sp were reported in other studies (Ahtarov *et al.,* 1939 and Georgiev, 2013). According to Maphosa *et al.* (2009) antihelmnitic activities of medicinal plants vary because of different conditions in the gastrointestinial tract and under *in vitro* conditions.

Pappea capensis and *Aloe ferox* plant extracts demonstrated stronger anthelmintic activities than those of *Dicerocaryumeriocarpum* and *Helichrysum sp* at the same concentrations (Table 4.2.1). Medicinal properties of *Pappea capensis* and *Aloe ferox* including the use of the barks and roots of these plant species for the treatment of stomach problems and diarrhea in human and animals have been document from earlier studies (Cunningham, 1988 and Gerstner, 1939). However, *Aloe ferox* was once reported as having weak anthelmintic activities against egg hatch inhibition of gastrointestinal nematodes of livestock.

According to Eshun and He (2004) *Aloe ferox* posses weak anthelminthic activities possibly because of the presence of the gel in the extract which does not contain plant secondary compounds with biological activity as those are found in the sap and outer leaf part and not in the inner gel of the *Aloe* plant species. Therefore, the results in (Table 4.2.1) confirm that *Aloe ferox* contain weak anthemintics activies. Furthermore, *Aloe ferox* achieved less than 50 % egg hatch inhibition even at the highest concentration of 7.5 mg/mL, the same was observed with *Pappea capensis*

plant species.*Pappea capensis* was also reported by Mphahlele *et al*. (2016) as possessing low anthelmintics effect against nematodes egg hatch.

Senecio plant species is popularly known for its anti-oxidant activities. The crude extracts and some *flavonoids* from these plants were screened for their potential of having anti-oxidants (Ghisalberti, 1998). Senecio species are used for the treatment of gastrointestinal nematodes of livestock in many parts of the world because they posse's anthelmintic effects (Minja, 1989).*Senecio congestus*, *Senecio barbertonicus* and *Gardenia* sp demonstrated strong anthelmintic activities with respect to egg hatch inhibition of gastrointestinal nematodes of livestock by achieving a minimum of 51.7 % at the lowest concentration of 2.5 mg/ml. In addition, the distilled water plant extracts of *Senecio congestus*, *Senecio barbertonicus* and *Gardeniasp* at a concentration 7.5 mg/ml, had significantly high anti-helminthic activities (70-100 %). According to Perez and Anesini (1994) *Senecio barbertonicus* possess strong anthelmintic activities against gastrointestinal nematodes of livestock. *Senecio barbertonicus* is the only plant species that yielded 100 % egg hatch inhibition at a concentration of 7.5 mg/ml, demonstrating the same strength as the positive control at the same concentration. According to Perez and Anesini, (1994) and Hirschmann and Jakupovic, (1988) *Senecio barbertonicus* has anthelmintic effects against gastrointestinal nematodes of livestock. It is further used for the treatment of diarrhea and constipation in livestock.

Thiabendazole® achieved 100 % egg hatch inhibition in all the concentrations, while negative control inhibited 0.00 %. *Gardenia* sp *in vitro* test against egg hatch inhibition of gastrointestinal nematodes of livestock confirmed that the plant has a significantly high anthelmintic activity. Gastrointestinal nematodes of ruminants are treated by the use of*Gardenia* sp (Djoueche *et al*., 2011; Nsekuye, 1994).Leaves of *Gardenia* sp have anthelmintic effects against goats and sheep gastrointestinal nematodes (Kaboré *et al*., 2007). All the concetrations of the seven plant species yielded significatlly different egg hatch inhibition with the highest results being observed at the highest concentration of 7.5 mg/mL and least results at the lowest concentration of 2.5 mg/mL. It was observed that *Pappea capensis* and *Senecio barbertonicus* further increasing the concentration of the plant extract beyond 5.0 mg/mL did not yield a significant difference in the egg hatch inhibition potential.

4.2.2. Larval development assay

Tests were carried out to determine the effects of the various plant extracts in distilled water on the inhibition of larval development at various concentrations. The table below summarizes the findings.

Table 4.2.2. Larval development inhibition (%) of the different plant extracts at varying concentrations (mg/mL) with distilled water as extraction medium

	Larval Development			
	Concentrations (mg/mL)			
Treatments	2.5	5.0	7.5	SEM
Dicerocaryum eriocarpum	19.33^{c}	27.33^{b}	38.67^{a}	4.33
Pappea capensis	27.33^{c}	32.67^{b}	45.33^{a}	5.032
Aloe ferox	68.67^{c}	81.33^{b}	97.33^{a}	11.242
Helichrysum sp	10.00^{c}	17.33^{b}	26.00^{a}	2.968
Senecio congestus	70.67^{c}	81.33^{b}	98.67^{a}	11.33
Senecio barbertonicus	73.33^{b}	93.33^{a}	99.33^{a}	12.023
Gardenia sp	83.33^{b}	99.33^{a}	100.00^{a}	12.476
Thiabendazole	100.00^{a}	100.00^{a}	100.00^{a}	13.056

^{a,b,c} : Means in the row not sharing a common superscript are significantly different ($P<0.05$)

SEM: Standard error of the means

Distilled water plant extracts were tested *in vitro* for their efficacy levels against larval development. All the plant extracts demonstrated varying levels of anthelmintic activity. *Helichrysum* sp and *Dicerocaryumeriocarpum* yielded the weakest anthelmintic activities on larval development assay as observed for egg hatch assay at the same concentrations of 2.5, 5.0 and 7.5 mg/ml. Less than 50 % larval development inhibition was achieved even at the highest concentration demonstrating the weak anthelmintic activities of these two-plant species. However, there was significant difference ($P<0.05$) in all the three concentrations 2.5, 5.0 and

7.5 mg/mL in both plant species. Amongst many treatments of *Helichrysum* species all over the world digestive disorder treatment was reported (Rivera *et al*., 2008; Aslan *et al*., 2007; Peris *et al*., 2001; Peris *et al*., 1995; Quer, 1993; Mulet, 1991; González-Tejero, 1989; Scarborough, 1978). *Dicerocaryum senecioides* was reported to possess anthelmintic, antibacterial effect as well as brine shrimp toxicity (McGaw *et al*., 2007).

The weak anthelmintic activities of *Pappea capensis* observed during egg hatch inhibition *in vitro* assays were also observed during larval development inhibition assays (Table 4.2.2.). However, in the study done by Mphahlele *et* al. (2016) *Pappea capensis* showed the lowest inhibition percentage at the lowest concentration of 2.5 mg/mL for larval development assay. The mean larval development inhibition percentages for the extracts of *Pappea capensis* were significantly different ($P<0.05$) at the different concentrations, showing that anthelmintic activities of these plants increased with concentration. According to the report of Masika and Afolayan (2002)extracts of *Pappea capensis* plant species can be used for the treatment of livestock diseases related to intestinal disorders, such as worms, constipation and diarrhea related sickness. The seed oil is extracted from the seed and is used medicinally against baldness, ringworm, nosebleeds, chest complaints, eye infections, and venereal disease (Hutchings *et al*., 1996; Pooley, 1993).

Aloe ferox plant extracts demonstrated strong anthelmintic activities in larval development assay than in egg hatch assay, even at the lowest concentration (Table 4.2.2). This can be because larvae are more susceptible at the pre-infective stage (Molan *et al*., 2003). However, extracts of the *Aloe ferox* powder yielded good inhibitory effects on two roundworm species in laboratory tests, affecting both the hatching of eggs and the development of larval stages. This indicates that the *Aloe* powder is effective in combating roundworm infestations in livestock without having an undesired effect on the animals (Githiori, 2006). *Aloe ferox* also showed good results at a concentration of 2.5 mg/mL in an *in vitro* test of larval development and egg hatch assay (Maphosa *et al*, 2010).

Senecio congestus showed good results in the larval development assay with more than 70 % larval inhibition at a lowest concentration of 2.5 mg/mL. Further increasing

the concentration of the plant extract beyond 2.5 mg/mL significantly improved larval development inhibition ($P<0.05$) in all the three concentrations.

Senecio barbertonicus and *Gardenia* sp has achieved good results at 2.5 mg/mL concentration. In another case fruits of *Gardenia* sp have been reported in traditional medicine amongst other treatments gastric disorders were mentioned (Tseng *et al*., 1995). However, there was no significant difference at a concentration of 5.0 and 7.5 mg/mL for both plant species while Thiabendazole® positive control had maximum larval development inhibition in all the concentrations.In another study of *Senecio* sp, it was confirmed that the plant extracts of *Senecio barbertonicus* are used as a tranquilizer for gastritis (Bastien, 1983). Therefore, it possesses anthelmics effect for livestock parasites.

4.2.3 Egg hatch assay and larval development assay

The extracts of seven plant species were compared in a column for inhibition of egg hatch and larval development at concentrations. Table 4.2.3 below summarizes the findings.

Table 4.2.3. Egg hatch (EH) and larval development (LD) inhibition (%) of different plant extracts varying concentrations (mg/mL)with distilled water as extraction medium

	Egg hatch and larval development					
	Concentration 2.5		Concentration 5.0		Concentration 7.5	
Treatment	EH	LD	EH	LD	EH	LD
Dicerocaryum eriocarpum	0.00^{f}	19.33^{ed}	0.00^{f}	27.33^{dc}	2.25^{e}	38.67^{b}
Pappea capensis	18.33^{e}	27.33^{d}	27.50^{d}	32.67^{c}	33.37^{d}	45.33^{b}
Aloe ferox	34.60^{c}	68.66^{c}	37.53^{c}	81.33^{b}	44.97^{c}	97.33^{a}
Helichrysum sp	2.93^{f}	10.00^{e}	8.37^{e}	17.33^{d}	23.77^{e}	26.00^{c}
Senecio congestus	51.70^{c}	70.67^{c}	67.13^{b}	81.33^{b}	79.60^{d}	98.67^{a}
Senecio barbertonicus	87.00^{b}	73.33^{cb}	97.50^{a}	92.33^{a}	100.00^{a}	99.33^{a}
Gardenia sp	84.60^{b}	83.33^{b}	92.00^{a}	99.33^{a}	98.77^{a}	100.00^{a}
Thiabendazole	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
SEM	1.459	2.55	1.67	2.285	1.809	1.801

^{a,b,c,d,e,f} : Means in the column not sharing a common superscript are significantly different ($P<0.05$)

SEM: Standard error of the means

EH: Egg hatch

LD: Larval development

In this study, the *in vitro* model demonstrated ovicidal and larvicidal effect of distilled water plant extracts of *Dicerocaryumeriocarpum*, *Pappea capensis*, *Aloe ferox*,

Helichrysum sp, *Senecio congestus*, *Senecio barbertonicus*, *Gardenia*sp and positive control Thiabendazole®. The distilled water plant extracts of *Senecio congestus*, *Senecio barbertonicus* and *Gardenia*sp achieved good egg hatch inhibition of 51.70, 87.00 and 84.60% respectively, at a low concentration of 2.5 mg/mL. *Pappea capensis* and *Aloe ferox* showed weak anthelmintic activity of less than 40% at a concentration of 2.5 mg/mL. Maphosa *et al.* (2009) observed *in vitro* test of an anthelmintic activity of *Aloe ferox* against *H. contortus*, poorresults were achieved. *Dicerocaryumeriocarpum*and *Helichrysum* sp *in vitro* test in the current study also showed weak anthelmintic activities of 0.00 and 2.93% respectively, at the same concentration of 2.5 mg/mL. *Aloe ferox and Senecio congestus* at concentration 2.5 mg/mL in egg hatch inhibition there is no significant difference. Thiabendazole® as a positive control achieved 100% egg hatchability inhibition at a concentration of 2.5 mg/mL. Table 4.2.3 shows that there's no significant different between *Senecio barbertonicus* and *Gardenia*sp for egg hatch inhibition at all the levels of concentration.

The distilled water plant extracts revealed the similar results in concentration 5.0 mg/mL in terms of anthelmintic strength. However, *Senecio barbertonicus*achieved 100% egg hatch inhibition at a concentration 7.5 mg/mL, even though there's no significantdifference between *Senecio barbertonicus* and *Gardenia*sp. Other than *Senecio barbertonicus* and *Gardenia*sp there is a significant difference ($P<0.05$) in all the other plant species table 4.2.3.

On larval development assay, the *in vitro* test of plant extracts and Thiabendazole® as a positive control results are shown in the table 4.2.3 above.Thiabendazole® as a positive control achieved 100% larval mortality at all the concentration levels. The highest larval development inhibition of the plant extracts at 2.5 mg/mL concentration was achieved by *Gardenia*sp with 83.33% larval development inhibited. Lowest larval development inhibition was observed in *Helichrysum* sp with 10% inhibition. There's a mean significant difference ($P<0.05$) in all the plants species on 2.5 mg/mL concentration including Thiabendazole® positive control.

In concentration 5.0 mg/mL there's no significant difference between *Senecio barbertonicus*, *Gardenia*sp and Thiabendazole® positive control. This simple means*Senecio barbertonicus* and *Gardenia*sp have the same anthelmintic efficacy

level with Thiabendazole® positive control against larval development inhibition. Also, there's no significant difference *Senecio congestus*and *Aloe ferox* this simple means both plant species contain the equal anthelmintic efficacy level against larval development inhibition.

Aloe ferox, *Senecio congestus*, *Senecio barbertonicus*, *Gardenia*sp and positive control Thiabendazole® confirmed the same results against larval development at a concentration of 7.5 mg/mL see table 4.2.3. These results further prove that the four plant extracts possess similar anthelmintic potential as the Thiabendazole® positive control. *Helichrysum* sp demonstrated weak anthelmintic effect against larval development in all the concentrations level by inhibiting the smallest percentages 10.00, 17.33 and 26.00% (Table 4.2.3). It means more than 70% of L1 developed to L3's in all the concentrations level.

Based on the table above we can conclude by say *Senecio barbertonicus* and *Gardenia*sp were best in all the plant species and both have achieved highest inhibition percentages in all the levels of egg hatch and larval development assays. Furthermore, both plant species have achieved analogous results with Thiabendazole® positive control in egg hatch and larval development at concentration 5.0 and 7.5 mg/mL respectively.

4.2.4 Larval mortality assay

In vitro tests were carried out to determine the anthelmintic activity of the different plant extracts in distilled water as demonstrated by their ability to cause larval mortality at varying concentrations and different incubation periods. Table 4.2.4 highlights the findings.

Table 4.2.4. Larval mortality (%) of different plant extracts in distilled water at varying concentrations and different incubation periods

	Larval mortality time (hours)								
	Concentration 2.5			Concentration 5.0			Concentration 7.5		
Plant species	24	48	72	24	48	72	24	48	72
Dicerocaryum eriocarpum	4.00^{f}	11.11^{g}	22.11^{d}	9.78^{f}	15.55^{e}	37.55^{d}	13.78^{g}	35.56^{d}	49.89^{c}
Pappea capensis	14.00^{e}	43.33^{e}	76.33^{b}	17.11^{fe}	48.78^{d}	90.44^{b}	23.11^{f}	74.45^{b}	100.00^{a}
Aloe ferox	35.55^{c}	59.11^{c}	99.11^{a}	47.26^{c}	72.00^{cb}	100.00^{a}	59.33^{c}	81.78^{b}	100.00^{a}
Helichrysum sp	10.00^{fe}	31.78^{f}	42.78^{c}	19.78^{ed}	39.55^{d}	51.11^{c}	32.00^{e}	52.22^{c}	61.78^{b}
Senecio congestus	26.11^{d}	50.00^{e}	100.00^{a}	52.89^{c}	70.67^{c}	100.00^{a}	51.33^{d}	86.45^{ba}	100.00^{a}
Senecio barbertonicus	52.00^{b}	70.67^{b}	100.00^{a}	64.33^{b}	82.44^{b}	100.00^{a}	74.89^{b}	100.00^{a}	100.00^{a}
Gardenia sp	14.56^{e}	55.33^{dc}	100.00^{a}	25.33^{d}	66.22^{c}	100.00^{a}	47.78^{d}	100.00^{a}	100.00^{a}
Thiabendazole	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
SEM	1.407	1.440	1.185	1.669	2.348	1.831	1.242	3.207	0.726

^{a,b,c,d,e,f,g} : Means in the column not sharing a common superscript are significantly different ($P<0.05$)

SEM: Standard error of the means

Larval mortality assays using different plant extracts at different concentrations against time periods of 24 hours, 48 hours and 72 hours, showed that larval mortality increased with time and concentration ($P<0.05$). *Dicerocaryum eriocarpum* plant extracts gave the lowest larval mortality percentage after 72 hours of incubation at 7.5 mg/ ml (Table 4.2.4). This could explain why this plant species is seldom used as a remedy for treatment of gastrointestinal parasites. Theseed coats of *Dicerocaryum eriocarpum* are removed, dried and ground to a fine powder. The powder absorbs water in the intestinal tract, producing a bulky mass that is unaffected by bacteria as it moves through the intestines. It provides relief from constipation and chronic diarrhea (Samuelsen, 2000). At concentrations of 2. 5 mg/ml and 72 hours of incubation *Pappae capensis* plant extracts achieved 76. 3 % larval mortality. The barks and roots of *Pappea capensis* were reported to be used as preliminary checklist of Zulu names of plants with short notes to treat gastrointestinal nematodes and diarrhea in livestock, and also stomach pain in humans (Gerstner, 1939). It was also reported by Cunningham (1998) for the treatment of intestinal worm infestation, diarrhea and other signs related to worm infestations. During the counting of larval mortalities, it was observed that *Pappae capensis* solution formed a mesh that holds the larvae until they have low motility and eventually they die. The low anthelmintic activities of the plant extracts of *Dicerocaryum eriocarpum* and *Helichrysum* spwere also observed with respect to larval mortality (Table 4.2.4). However, the uses of Helichrysum species in South Africa was also reported by Lourens *et al.* (2008) for the treatment of stomach disorders and related diseases.

Plant extracts of *Senecio congestus*, *Senecio barbetonicas* and *Gardenia* spdemonstrated similar anthelmintic strength as the positive control by achieving 100 % larval mortality at a concentration of 2. 5 mg/ml after 72 hours of incubation (Table 4.2.4). According to Nsekuye. (1994) *Gardenia ternifolia* species is used for the treatment of gastrointestinal nematodes of livestock. *Gardenia* sp was also reported for treatment of round worm in goats (Jangde and Bansod, 2004). *Senecio* plants species have been known for the anthelmintic effect that they possess against nematodes parasites all over the world (Jain *et al*., 1994). It was reported by Harborne and Williams, (2000) as having anti-oxidant and antiseptic activities. *Senecio* sp was further reported for the anthelmintic effect against gastrointestinal nematodes of goats and sheep (Minja, 1989). *Senecio congestus* has been reporting

to be used for the treatment of mites' parasites on the skin of livestock (Hutchings *et al*., 1996).

Aloe ferox plant extracts in the current study yielded the same results with Thiabendazole® positive control at concentration 2.5, 5.5 and 7.5 mg/mL after 72 hours.In another study by Ahmed *et al.* (2012) of larval mortality assay *Aloe ferox* was one of the plant species extracted and *in vitro* tested for anthelmintic effects against nematode parasite. However, *Aloe ferox* is not used for intestinal parasites in the rural areas of South Africa. *Aloe ferox* was reported as a tick control remedy in some South African villages (Mogotsi *et al.,* 2013). For example, poultry are protected from ticks and lice infestation as well as intestinal disorders such as coccidiosis by using fresh *Aloe* leaves in drinking water (Dold and Cocks, 2001).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The use of indigenous plant extract has the potential to be a complementary control option which may reduce reliance on drug treatment, and slow the development of resistance. Here we have carried out a comprehensive *in vitro* assessment of the effects of seven different indigenous plant extracts against gastrointestinal parasites of livestock. In light of the research findings it can be concluded that the egg hatch assay, larval development assay and larval mortality assay *in vitro* tests for the anthelmintic effect of the seven plants against livestock gastrointestinal nematodes confirm that some of these plants possess anthelmintic effects.

The conclusions reached for the specific research questions are presented as follows:

- Which indigenous plants are used by small scale livestock farmers to control internal parasites in the Albert Luthuli Municipality?

In conclusion these are (*Dicerocaryumeriocarpum*, *Pappea capensis*, *Aloe ferox*, *Helichrysum* sp, *Senecio congestus*, *Senecio barbertonicus*, *Gardenia* sp).

- How do the anthelmintic effects of indigenous plants compare to thoseof a commercial anthelmintic drug?

In conclusion, all indigenous plants in the present study possess anthelmintic activities. The mode of action of these plants could be similar to that of commercial anthelmintic although more work needs to be done before rational conclusions can be drawn.

The relevance of *in vitro* studies to *in vivo* efficacy, with regard to anthelmintic efficacy is greatly influenced by the differences in the physiology and the bioavailability of plant preparations within the animal host (Githiori *et al.,* 2006). According to Molan *et al*. (2003) larvae's most susceptible stage is the pre-infective stage, the feeding stage in order to develop to the infective stage. It is therefore much exposed to medicinal components than the egg, hence 100% larval development inhibition rates. However, the current study archived larval mortality in different growing stages. According to Tembley, (1998) eggs and larvae of the

gastrointestinal nematodes depend on the species and developmental stage of the parasite as well as the geo-ecological regions. Chemical constituents can vary considerably between individual plants species due to genetic or environmental differences, age or developmental stage at harvesting, method of plant material drying, the storage technique and the type of solvent (Ononuju and Nzenwa., 2011; Hördegen *et al*., 2003).

5.2 Recommendations

Based on the results of the present study, *Senecio barbertonicus* and *Gardenia sp* inhibited highest percentage of eggs from hatching at the lowest concentration of 2.5 mg/mL. Whereas on the other hand in larval development assay *Aloe ferox, Senecio congestus, Senecio barbertonicus* and *Gardenia sp* exhibited high inhibition percentages of larvae. Those extracts also revealed the highest larval mortality rates in the larval mortality assay. Therefore, we recommend indigenous people of Chief Albert Luthuli Municipality to continue using those plant species for the treatment of livestock gastrointestinal nematodes since they demonstrated anthelmintic activities against these parasites.

5.3 Future research

Concentrations of potentially active substances used *in vitro* do not always correspond to *in vivo* bioavailability. Therefore, *in* vivo tests still need to be conducted since *in vivo* is influenced by physiological and bioavailability factors in the animal body (Githiori *et al.,* 2006). Hence, further *in vivo* studies are required to enlighten the therapeutic potential of plant extracts in treating helminthes infection. The *in vivo* assay such as faecal egg count reduction test is suitable for the evaluation of all types of anthelmintics (Verma *et al*., 2006).

Furthermore, toxicity studies need to be conducted to determine the toxicity of the plant samples used in the current study. Other forms of extraction method using the same plant species as the current study need to be conducted to determine the efficacy levels of the plants in different extraction medium.

CHAPTER 6

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