Determination and validation of medicinal plants used by farmers to control internal and external parasites in goats in the Eastern Cape Province, South Africa

By

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DECLARATION

I, Marcia Sanhokwe, hereby declare that this work has not previously been submitted at this or any other university, and it is my own work in design and execution and that all references contained herein have been duly acknowledged.

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Prof J. Mupangwa…… **Prof V. Muchenje**……..

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ABSTRACT

The broad objective of the study was to determine and validate medicinal plants used by resource-limited farmers to control internal and external parasites in goats in the Eastern Cape Province, South Africa. A survey was conducted among 50 farmers and three herbalists to determine medicinal plants used to control parasites in goats. The survey revealed nine plant species belonging to eight families that were used. Among the identified plant species, *Aloe ferox, Acokanthera oppositifolia* and *Elephantorrhiza elephantina* were the plants having the highest Fidelity Level for their use, each scored 100.00%, followed by *Albuca setosa* (83.33%). These plants were then selected for validation studies.

Gas-Chromatography-Mass-Spectrometry (GC-MS) revealed 7, 33, 26 and 32 bioactive phytochemicals in *A. ferox, E. elephantina, A. oppositifolia* and *A. setosa,* respectively. Terpenes and fatty acids were present, oxygenated terpenes being the most abundant hydrocarbons present in all the four plant species.

The effect of acetone, methanol and ethanol extracts of leaves of *Aloe ferox* and *Acokanthera oppositifolia* on tick repellency and acaricidal activity were investigated on blood engorged *Ambylomma hebraeum* and *Rhipicephalus decoloratus* ticks at concentration 15, 30 and 50%. The 30 and 50% acetone extract of *A. ferox* and Dazzel dip had the highest acaricidal properties of 100%. The 50% methanol extract of *A. oppositifolia* and 50% acetone extract of *A. ferox* had the highest repellency activity of 89% and 85.33%, respectively. Results from this study revealed that the efficacy of medicinal plants used by farmers to control ticks vary with the type of solvent used for extracting the bioactive compounds. Furthermore, it revealed that *Aloe ferox* and *A. opppositifolia* plant extracts possess repellent and acaricidal activities.

In a study to investigate the anthelminthic effect of crude extracts of *Elephantorrhiza elephantina* and *Albuca setosa* plants, significant anthelminthic effect on nematodes was observed in both plants. In this study, all *E. elephantina* and *A. setosa* extracts caused paralysis and mortality. Methanol was the most effective solvent in extracting bioactive compounds and methanol extract showed the best anthelminthic effects among the crude extracts investigated in both plants. The least time taken for the worms to be paralysed was 8.33 mins and 14.33mins in 100mg/ml methanol extracts of *E. elephantina* and *A. setosa*, resepectively. Methanol extract of *E. elephantina* and *A. setosa* (100mg/ml) had the highest anthelminthic activity and mortality was recorded after 18mins and 20mins, respectively. Results from this study revealed that these two plants possess anthelminthic activities.

The study revealed that resource-limited farmers use medicinal plants to control internal and external parasites in goats. Gas-Chromatography-Mass-Spectrometry analysis showed that these plants contain bioactive compounds that have a potential in controlling parasites. Validation studies showed that *A. ferox* and *A. oppositifolia* possess repellent and acaricidal activities whereas *A. setosa* and *E. elephantina* possess anthelminthic activities.

Keywords: efficacy, medicinal plants, parasites

DEDICATION

This dissertation is dedicated with love and respect to my parents Mr and Mrs S.G. Sanhokwe, my siblings; MaryGold, Tapiwa and Tadiwa and my granny, Ms M. Majazi for supporting me in all my endeavors.

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List of Abbreviations

EVM	Ethnoveterinary medicine
DAFF	Department of Agriculture, Forestry and Fisheries
FL	Fidelity Level
NIST	National Institute of Standard and Technology
ICSW	Instiute for Soil Climate and Water

CHAPTER 1: INTRODUCTION

1.1 Background

South Africa has approximately 3% of Africa's goats and less than 1% of the world's goat population (DAFF, 2012). Goats play a major role in Africa particularly for remote and poor communities (Peacock, 2005). They help in alleviating poverty by providing food and in income generation. They are slaughtered for traditional purposes (DAFF, 2012). Their waste products serve as organic fertilizer (Beevi *et al*., 2002). Some of the farmers also use goats to control bush encroachment (Peacock, 2005).

Most of the goats in the Eastern Cape are found in the communal areas where internal and external parasites are a problem (Peacock, 2005; Mwale and Masika, 2009). Gastro-intestinal tract parasites pose a huge health problem in small ruminants (Vatta and Lindberg, 2006). These parasites are more prevalent in places where there is poor grazing management (Masika and Mafu, 2004). Infestation results in poor animal products such as milk and meat (Perry and Randolph, 1999; Njoroge and Bussmann, 2006). The most prevalent internal parasites in small ruminants are lung worms, stomach worms, coccidian and liver flukes (Molefe *et al*., 2012). They disrupt nutrient utilization resulting in significant weight loss, affect growth rate and may also cause mortality (Molefe *et al*., 2012). External parasites such as ticks, fleas, mites and lice are a threat to small ruminant production (Kubkomawa *et al.,* 2013). Ticks transmit protozoan, viral and rickettsial diseases. Diseases such as babesiosis, anaplasmosis and heartwater are a major constraint in the production and development of livestock and cause great losses to the livestock industry (Kettle, 1995). External parasites cause discomfort and irritation. Ticks are an example of external parasites that feed on the body tissue causing wounds and thereby

predisposing animals to secondary bacterial infection (Colebrook and Wall, 2004). Mites feed on the skin surface and secrete toxins which cause intense irritation and itching (Mullen and Connor, 2002). Skin problems that are caused by ticks and mites result in huge economic losses in the tanning industry (Mekonnen, 1998). It is therefore important to control these parasites so as to increase livestock productivity.

Acaricides and anthelmintics have been used to control parasites for many years but now most resource-limited farmers have resorted to alternative measures which are cheap (Okello-Onen *et al*., 1997). The need to expand organic agriculture is also paving a way for use of medicinal plants which leave no residues in food, are cheap and easily accessible (Maphosa and Masika, 2012). Most of the livestock owners in communal areas receive inadequate veterinary services and are experiencing high mortality of goats as a result of parasites (Shale *et al*., 1999). These farmers cannot afford to purchase acaricides due to their high costs resulting in animals being infested by parasites and having reduced production potential (Shale *et al*., 1999). Use of alternative methods such as ethno-veterinary practices could provide alternatives options (Gueye, 1999).

Ethno-veterinary medicine involves the use of traditional knowledge and practices to treat diseases so as to maintain a healthy herd (Mathias-Mundy and McCorkle, 1989; Tabuti *et al*., 2003). Information on the use of medicinal plants is passed on orally from one generation to another (Wanzala *et al*., 2005) and may disappear due to rapid changes in the socioeconomic, cultural, technological and environmental changes (Nfi *et al*., 2001). Ethno-veterinary knowledge can be passed on through family members, interaction with peers and through traditional healers or herbalists to researchers (Philander *et al*., 2008). South Africa has more than 23 000 plant species that have been reported as traditional medicine (Hutchings *et al*.,

1996). These plants possess bioactive phytochemicals that enable them to treat diseases or eliminate a certain phase of parasite's life cycle thereby preventing animal loss (Nguyen *et al*., 2005). These medicinal plants have a huge potential in therapeutic programs and maintaining good animal health (Kubkomawa *et al*., 2013). There is a danger that knowledge of these plants may disappear after the death of older people as it is usually stored in the memory of a few entrusted older people within communities (Wanzala *et al*., 2005). Therefore, the aim of the study was to determine and document medicinal plants used to control internal and external parasites of goats in Chris Hani district Municipality, South Africa. Further investigation was done to determine the efficacies of these plants on some of the parasites for which they are used by farmers to improve health and goat production in South Africa.

1.2 Problem statement

Goats are mainly kept by resource-limited farmers and their production is very low as a result of parasites (Akingbade *et al*., 2001). Farmers lose goats to mortality attributed to parasites (Rooyen and Homann-Kee Tui, 2009). Goats infested with parasites have low production potential and death can even occur. Diagnostic or therapeutic programs in trying to control parasites or diseases can result in huge financial burdens on the farmer (Masika and Mafu, 2004). Commercial drugs which are used in control of parasites are expensive and most resource-limited farmers in Eastern Cape cannot afford them. Commercial drugs are not user friendly and more so, many of these parasites have developed resistance to them. This has led to the reappearance of interest in use of ethno-veterinary medicine to control parasites (Masika and Afolayan, 2003). The use of medicinal plants in the control of parasites by livestock owners in Eastern Cape was reported by Dold and Cocks (2001) and Lagu and Kayanja (2010). However, there is poor documentation of these medicinal plants in Chris Hani municipality in order to

preserve the indigenous knowledge. Also, few studies have been conducted on the efficacy of some of these medicinal plants.

1.3 Justification

The use of commercial drugs to control parasites is expensive and most livestock owners in the Eastern Cape cannot afford to purchase them. Knowledge on the use of medicinal plants is confined to a few individuals and efficacy of some of these plants is not known (Maphosa and Masika, 2012). This study sought to help validate plants used to control internal and external parasites in Chris Hani district municipality. Documenting medicinal plants can help to avoid the disappearance of ethno-veterinary knowledge in these areas and in ensuring their continual use (Opiro *et al*., 2010). The study will provide information that will confirm efficacy of the plants so as to encourage or discourage their use and increase the knowledge of ethno-veterinary medicine. The use of medicinal plants which are cost effective, readily accessible, having no residue problem and no eminent resistance (Lagu and Kayanja, 2010) can help to discover remedies that could be used in organic animal production.

1.4 Main objective

To determine and validate medicinal plants used by farmers to control internal and external parasites in goats in Eastern Cape Province.

1.5 Specific objectives

- To determine medicinal plants used by farmers to control internal and external parasites in goats.
- To determine the phytochemical constituents in the plant extracts.
- To validate the efficacy of medicinal plants against external parasites.
- To validate the efficacy of medicinal plants against internal parasites.

1.6 Null hypotheses

- Farmers do not use medicinal plants to control internal and external parasites.
- Medicinal plants are not effective against external parasites.
- Medicinal plants are not effective against internal parasites.

1.7 Alternate hypotheses

- Farmers use medicinal plants to control internal and external parasites.
- Medicinal plants are effective against external parasites.
- Medicinal plants are effective against internal parasites.

1.8 References

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Goats are a valuable asset in many African countries. Most resource-limited farmers in Africa rely on livestock production for food and income as they are situated in harsh agro-ecological zones (Islam and Jabbar, 2005). However, production levels of goats in many African countries are low as a result of diseases, poor management practices and parasites (Salem and Smith, 2008). Most poor resource-limited farmers use plants to treat ailments and control parasites in livestock. Therefore, the aim of this study was to document and validate medicinal plants used by farmers to control internal and external parasites in goats in Eastern Cape Province, South Africa. This review therefore seeks to provide knowledge on internal and external parasites of goats, medicinal plants used and the chemical composition of plants.

2.2 Importance of goats

The goat, also known as *Capra aegagrus hircus* belongs to the family Bovidae and subfamily Caprinae. Goats fulfill many roles in communal areas. Their importance differs with culture, agro-ecological zones and countries (Gwaze *et al*., 2009a). They provide animal products such as milk and meat. These products are important as they are highly digestible; provide high quality protein, energy and other essential micronutrients needed by the consumers (Kitalyi *et al*., 2005). Skins, cashmere and mohair are some of the products from goats (Haenlein and Ramirez, 2007). Goat's skin can be used to make traditional mats, clothes for ladies, tents and drums (Peacock, 2005). In other countries, goats are used for draught power (Saico and Abul, 2007).

Some of the areas that are not suitable for crop production on a farm can be grazed by goats thereby adding value to households (Kayongo *et al*., 1992). Goats are used to pay bride price and in performing cultural rituals (McMilin *et al*., 2012).Goat manure and urine are used as organic

fertilizer. It is cheap and readily available. This is important as there is a global move towards organic agriculture (Hansson and Fredricksson, 2004).

2.3 Internal parasites

Parasites are organisms that benefit at the expense of their host (Oberem and Schroder, 1993). Internal parasitism is a major problem that does not cause economic losses only but also production loss (Waller, 2006). Most of the goats that are raised on natural pastures are exposed to parasites. Most of these parasites live in places close to the ground and goats that are raised on natural pastures are usually infested during feeding and watering. Internal parasites are also a major threat to humans. Usually poor people in developing countries are usually infested with gastro-intestinal tract parasites. Internal parasite infestations result in inflammation of the mucous membrane of the gastro-intestinal tract, fever, pain, irritation, loss of body mass; diarrhea and death can even occur (Waller, 2006). Table 2.1 shows the common internal parasites affecting goats.

Table 2. 1 Common internal parasites in goats

Parasite name	Common name	Location	Ideal Conditions	Clinical signs
Haemonchus contortus	Stomach worm, barber worm, wire worm	Abomasum	Warm, moist	Oedema, anemia, reduced feed intake, weakness, sudden death
Trichostronglus spp	Black scours worm, stomach hair worm, bankrupt worm	Abomasum,	Warm, moist	Reduced appetite, production loss, black scours, death
Coopera spp	Small intestinal worm	Small intestines	Cool, wet	Loss of appetite, diarrhea, weight loss
Eimeria spp	Coccidian	Small intestines	Cool,wet, overcrowding	Diarrhoea, weight loss, dehydration
Moniezia spp	Common tapeworm	Small intestines	Wet	Gastro-intestinal disturbances, unthriftness
Fasciola hepatica	Liver fluke	Bile duct of liver	Wet	Condemnation of organ (liver), production loss, death

Source: (Schoenian, 2012)

2.4 External parasites

External parasites are organisms that live on the surface of their host's epidermis, to shelter and feed (Colebrook and Waller, 2004). They damage the skin and other subcutaneous tissue. Moreover, they feed on body tissue such as blood causing wounds and discomfort to the animal. Skin damage as a result of ectoparasites cause huge losses to the tanning industry (Tadesse *et al*., 2011). Parasites limit goat production and it is important to control them. Examples of external parasites affecting goats are ticks, mites, lice and fleas. Their description is shown in Table 2.2.

Table 2. 2 Description of external parasites affecting goats

PARASITE NAME	***DESCRIPTION***	***COMMON SPECIES***	***EFFECT ON HOST***	***COMMENTS***	***REFERENCES***
Ticks	-Obligate ectoparasites -Have long mouth parts - divided into 2 main families:Argasidae and Ixodidae -Argasids(soft ticks) do not have hard chitinous plates on the dorsal surface of their bodies -Ixodes(hard ticks) have dorsal plates -Have peripheral sensory organs on legs, body, mouthparts which enable them to communicate with other ticks	-*Demodex caprae* -*Ixodes holocyclus* -*Rhipecephalus sanguineus* -*Rhipicepalus microplus* -*Boophilus decoloratus*	-Transmit tick borne diseases eg theileriosis, babesiosis, anaplasmosis, heartwater -skin,udder and ear damage -reduced growth -low milk production -predispose animals to secondary attacks from other parasites	-more prevalent when temperature and rainfall is high -cause greatest economic loss -about 35 tick species are found in Southern Africa -belong to phylum arthropoda -soft ticks usually feed on one host -bites from hard ticks are usually painless -Ixodid's life cycle has 4 stages:egg, larvae, nymph, adult -have an instant response to chemical eg CO_2 and NH_3 which indicate presence of host	Maphosa and Masika, 2012 Papadopoulos, 1996 Plumb, 2008 Parola and Raoult, 2001
Lice	-small, wingless, dorsally flat -have stout legs and claws for clinging -2 types exist:chewing (*Mallophaga*)and sucking lice (*Anoplura*) -Sucking lice have narrow heads	-African goat louse*(Linogathus Africanus*) - goat sucking louse(*Linogathus stenopsis* -sheep foot louse	-Dull, matted coat -Irritation -Distress -Biting at self -Hides damage -Scratching -Suck blood-	-spread by contact -Spread more in overcrowded places -More prevalent in winter and early spring - Need one month to complete life cycle	Talley and Dave Sparks, 2010 Zeryehun and Atomsa, 2012 Wall and Shearer, 2001 Lund and Algers 2003 Sargison *et al*., 2000

	with piercing mouth -Chewing lice have large heads and mouth parts -Live on the skin of their host -Chewing lice feed on hair,scabs, skin, skin exudations -Sucking lice draw blood -Stressed animals are more susceptible	(*Linogathus pedalis*) -goat biting louse (*Bovicola caprae*) -Angora goat biting louse(*Bovicola crassipes*) - (*Bovila limbata)*	anaemia -Reduced milk production -listlessness -weight loss	-spend their life on one host	
Mites	-very tiny -only seen with a microscope -Burrow beneath the skin	-*Psoroptes cuniculi* -*Raillietia caprae* -*Chorioptes bovis*	-irritation -scratching -weight loss -damage goats skin -result in pruritus over the face, neck -formation of crusts on skin	-spread by direct contact -more prevalent when temperature, sunlight and humidity is high -life cycle completed within one month -life cycle has 4 stages-egg, larvae, nymph,adult	Sargison *et al*., 2000 Curtis, 2004

Fleas	-are laterally flattened -adult fleas are approximately 1.5-4mm in length -are wingless, dark brown -have long legs adapted for jumping -body is hard, polished and covered with hair -their eggs are smooth, oval and tiny -infest their host and move to another of the same kind or different	-*Ctenocephalides felis* -*Ctenocephalides canis*	-severe blood loss leading to anaemia -mortality -restlessness -weakness -their salivary allergens cause allegic reaction eg urticaria	-severe in summer and winter -high temperatures and humidity favours proliferation of fleas -100 flea species are known to paratize domestic animals in South Africa -life cycle involves 4 stages:egg, larvae, pupa and adult	Plumb, 2008 Pugh, 2002 Colebrook and Wall, 2004 Rahbari *et al*., 2008

2.5 Parasite control

Parasites are controlled by mainly two ways which include the use of ethno-veterinary medicine and commercial drugs which are described below.

2.5.1 Use of commercial drugs

Internal parasites are controlled by drugs which belong to three families namely benzimidazoles, nicotinics and macrolytic lactones (Schoenian, 2009). Benzimidazoles such as Albendazole and Oxybendazole are also known as white dewormers and are active against tapeworms, round worms (*Trichostrongyles)* and liver flukes (*Fasciola hepatica)* (Mekonnen, 1998). Macrolytic lactones are chemical derivatives of soil microorganisms of the genus *Streptomyces* which are active against immature nematodes and arthropods (Zajac, 2006). Nicotinics are effective against cestodes and trematodes (Pugh, 2002). Some of these drugs are specific to particular parasites, whereas some have a broad spectrum effect. Table 2.3 below shows the common three families of drugs used to control internal parasites. Drenching of goats using these drugs is usually done on a monthly basis to cut the life cycle of worms, which takes an average of 21 days (Axford *et al.*, 2000). Continuous use of these drugs has resulted in the development of resistance by some internal parasites.

Ticks and mites are usually controlled by acaricides which are applied in different ways. Acaricides can be applied by dipping, pour on and spraying (Rajput *et al*., 2006). Fleas are controlled by insecticides which are formulated as dust sprays or fine sprays (Boone, 2001). Anti-tick vaccines have also been developed, and are environmentally friendly (Uilenberg, 2005). Ivermectin can be used to control parasites such as ticks, fleas and mites. Parasites have developed resistance against some of the acaricides which makes tick control difficult. For example *Boophilus* ticks are resistant to

Table 2. 3 Anti-parasitic drug classes and their actions

Class	Active ingredient/Drug	Action
Macrocylic lactones	Moxidectin, Ivermectin, Doramectin	Effective against all helminthes Interfere with the GABA-mediated neurotransmission thereby killing and paralyzing parasites
Benzimidazole	Thiabendazole, Albendazole, Mebendazole, Fenbendazole, Oxfendazole	Effective against tape worms, round worms and flukes Interfere with the worm's metabolism by binding to the block (beta tubulin) thereby preventing its incorporation into microtubules.
Nicotinics	Levamisole, Morantel, Pyrantel	Effective against cestodes and trematodes. Mimic the activity of acetylcholine that initiate muscle contraction and the worm will not be able to feed.

Source: (Pugh, 2002; Schoenian 2010).

organophosphate carbonates (Mekonnen, 1998). Another problem is that commercial insecticides are expensive, and most resource-limited farmers cannot afford to buy them. Commercial drugs tend to indiscriminately kill beneficial insects and birds thereby affecting the food chain (Uilenberg, 2005).

2.5.2 Use of medicinal plants to control internal parasites

Medicinal plants are believed to have compounds in their leaves, seeds, fruits and inflorescence for medicinal purposes (DAFF, 2012). They possess a range of pharmacologically active phytochemical compounds which include alkaloids, glycosides, resins, volatile oils and tannins (Kumar *et al*., 2011). In developing countries, most communal people are now using plants due to their low cost and effectiveness. This has led researchers validate these medicinal plants reported in various countries. Research into medicinal plants is usually done as part of a community based approach with the aim of improving animal health (Lans *et al*., 2008). Across the world, many plants with anthelmintic activity have been discovered and there are many published reports on these plants (Akhtar *et al*., 2000; Al-shaibani *et al*., 2009). Tables 2.4 and 2.5 show some of the medicinal plants used to control internal and external parasites in livestock, respectively.

Table 2. 4 Plants used to control internal parasites

PLANT NAME	FAMILY NAME	USES	REFERENCE
Chenopodium album L.	Chenopodiaceae	Anthelminthic-cause mortality and inhibit eggs from hatching	(Jabbar *et al*., 2007)
Calotropis procera	Apocynaceae	Anthelmintic	(Iqbal *et al*., 2010)
Azadirachta indica A	Meliaceae	Effective against *Haemonchus spp.* and *Trichostrongylus spp*.	(Iqbal *et al*., 2010).
Leonotis leonurus	Lamiaceaea	Effective against gastrointestinal nematodes	Maphosa *et al*., 2010
Elephantorrhiza elephantina	Fabaceae	Effective against gastrointestinal nematodes	Maphosa *et al*., 2010
Aloe ferox Mill	Asphodelaceae	Effective against gastrointestinal nematodes	Maphosa *et al*., 2010
Manihot esculenta	Euphorbiacaeae	Effective against nematodes	Al-Rofaai *et al*., 2012
Citrullus vulgaris	Cucurbitaceae	Effective against tapeworms	Nguyen *et al*., 2005
Gliricidia sepium	Fabaceae	Effective against intestinal worms	Nguyen *et al*., 2005

Albizia schimperiana	Fabaceae	Anthelminthic	Githiori *et al*., 2002
Lotus pedunculatus	Fabaceae	Reduce larval developmental and egg hatching of gastrointestinal parasites	Kahn and Diaz-Hernandez, 2000
Lotus corniculatus	Fabaceae	Reduce larval developmental and egg hatching of gastrointestinal parasites	Kahn and Diaz-Hernandez, 2000
Onobrychis virciifolia	Fabaceae	Reduce larval developmental and egg hatching of gastrointestinal parasites	Kahn and Diaz-Hernandez, 2000
Myrsine Africana	Myrsinaceae	Anthelminthic	Githiori *et al*., 2002
Trianthema portulacastrum	Azioaceae	Effective against gastro-intestinal nematodes	Hussain *et al.*, 2011
Rapanea melanophloeos	Myrsinaceae	Anthelminthic	Githiori *et al.*, 2006
Acokanthera oppositifolia	Apocynaceae	Effective against tapeworms	Dold and Cocks, 2001
Viscum verrucosum	Viscaeae	Effective against nematodes	Tibe *et al*., 2013
Caesalpinia arista L.	Fabaceae	Anthelminthic	Jabbar *et al*., 2007

Table 2. 5 Medicinal plants used to control external parasites

PLANT NAME	FAMILY NAME	USES	REFERENCE
Aneilema hockii	Fabaceae	Effective against fleas	Matekaire and Bwakura, 2004
Thamnosma africana	Burseraceae	Effective against fleas	Matekaire and Bwakura, 2004
Nicotiana tabacum (smoke)	Solanaceae	Effective against ticks and fleas	Alawa *et al*., 2002
Lavandula officinalis	Lamiceae	Effective against fleas	Alawa *et al*., 2002
Tagetes minuta	Asteraceae	Effective against ticks and fleas	Njoroge and Bussmann 2006
Tithonia diversifolia	Asteraceae	Effective against ticks and fleas	Njoroge and Bussmann 2006
Lippia javanica	Verbenaceae	Effective against ticks	Madzimure *et al.*, 2011
Tephrosia vogelli	Fabaceae	Acaricidal effect	Njoroge and Bussmann, 2006
Vernonia amygdalian	Asteraceae	Acaricidal effect	Njoroge and Bussmann, 2006
Ageratum houstonium	Asteraceae	Acaricidal effect	Pamo *et al*., 2005

Medicinal plants face a major threat due to urbanization and high deforestation. It is therefore important to document. There are known to be a rich source of beneficial bioactive compounds such as flavonoids, terpenoids and phenolics that are effective in controlling ticks than most commercial acaricidal chemicals (Douglas and Soejarto, 2002). The use of medicinal plants has merits and demerits which are discussed below.

2.5.3 Advantages of using medicinal plants

The use of medicinal plants is important particularly to resource-limited farmers who cannot afford to purchase commercial drugs and communal people who can not access these. Ethno-veterinary usage also help to empower farmers by enhancing the use of their own knowledge and resources (Iqbal *et al*., 2005). Farmers have resorted to the use of plants as there are easy to cultivate and be easily formulated for effective control of parasites (Scantlebury *et al*., 2013). There are also believed to be generally safer and more favorable with biological systems (Erasto, 2003 cited in Maphosa and Masika 2010). Administering of these drugs is easy, it can be done orally or applied to the skin (Lagu and Kayanja, 2010). Medicinal plants do not leave residues in food products such as meat or milk.

2.5.4 Drawbacks of using medicinal plants

Medicinal plants are not available at certain times of the year (Moyo and Masika, 2009). The process of harvesting and preparation of the herbal concoctions is laborious. Due to the laborious process of harvesting plant materials, use of these concoctions on a large scale becomes impractical (Mathias-Mundy and McCorkle, 1989). Also, there is a possibility that some medicinal plants are toxic (Moreki, 2012). The efficacy of the plants depends on the environmental condition, season and preparation method (Wickens, 2004). There is also a report that most reported efficacy of medicinal plants is anecdotal and has not been validated (Maphosa

and Masika, 2012). Storing and preserving the herbal concoctions is very difficult. Most of them are not standardized and are of unknown dosages which might result in toxicity (Martin, *et al*., 2001). Furthermore, some diseases exhibit similar clinical signs and traditional diagnosis can be very difficult which can result in improper treatment. Under dosage can also lead to the ineffective use of the remedy (Fielding, 2001). Some of the plants are now extinct which makes preparation of ethno-veterinary medicine difficult (Mathias-Mundy and McCorkle, 1989). Plants can have negative effects on the growth and productivity of infested host (Githiori *et al*., 2006).

2.6 Phytochemicals

Plants are known to produce chemical constituents, mainly secondary metabolites which have pharmacological activities. The discovery of these chemical constituents from medicinal plants is important as it forms the basis for the development of therapeutic drugs (Muhit *et al*., 2010). Plants synthesize many classes of natural products during root and shoot development which have defense mechanisms against predators (Wink, 1999). A number of bioactive constituents have been reported in many medicinal plants. These constituents include terpenes, monoterpenes, sesquiterpenes, diterpenes, napthoquinones, alkaloids, fatty acids, lipids (Muhit *et al*., 2010). Sesquiterpenes and diterpenes have phytoalexins which are involved in the direct defense against pathogens (Dudareva *et al*., 2004). Monoterpenes are colourless, lipoptic compounds which comprise the majority of volatile compounds in plants and have defenses against pathogens (Langenheim, 1994). Chemical components of a plant depends on several factors which include season of plant collected, environmental conditions, storage conditions and drying procedures, microbial contamination and developmental stage are also factors that affect the chemical profile of plants (Kokkini *et al*., 1997).

2.7 Summary

Goats play a vital role in generation of income and provision of food (Peacock, 2005). The productivity of goats is however, undermined by parasite infestations. This results in high animal losses. Parasites are usually controlled through the use of commercial drugs. However the latter are expensive, less accessible and not environmental friendly. This has resulted in farmers resorting to cheap, easily accessible and culturally accepted ethno-veterinary medicines. Knowledge on ethno-veterinary medicine is transferred orally from one generation to another and there is danger of its extinction. Therefore, there is need to determine ethno-veterinary medicines used by farmers to control parasites in order to preserve the knowledge.

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CHAPTER 3

A survey of medicinal plants used to control internal and external parasites in goats in the Eastern Cape Province, South Africa

Abstract

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A survey was conducted to determine medicinal plants used to control parasites in goats in Kwezi and Ntambethemba villages in the Eastern Cape Province, South Africa. Information from 50 farmers and three herbalists was obtained through the use of a structured questionnaire and snowball sampling technique was used to identify key informants. The obtained data were analysed using PROC FREQ of SAS (2003) and Fidelity level (FL) values were determined to estimate the healing potential of the mentioned plants. The survey revealed nine plant species belonging to eight families that were used to control parasites in goats. Asphodelaceae (22.22%) was the most frequently used plant family. Leaves were the most used plant parts constituting 60.38%. Leaves were prepared either as infusions or decoctions of single plants or in mixtures. *Aloe ferox, Acokanthera oppositifolia* and *Elephantorrhiza elephantina* were the plants having the highest FL for their use to control parasites, each scoring 100.00%, followed by *Albuca setosa* (83.33%). The study showed that there was limited knowledge on ethno-veterinary medicine (EVM) in the study area. It also revealed that information of EVM in this area is mostly confined to older people and there is danger that this knowledge can be lost before being passed on to younger generations. There is need to therefore document information on these plant species so that future generations can benefit. Further investigation should be done to validate the efficacy and safety of the mentioned plants so as to provide alternative ways of controlling parasites.

Keywords: ethno-veterinary practices, small ruminant, traditional medicine

3.1 Introduction

Goats play an important role in the socio-economic activities of people especially in developing countries by providing food and income (Peacock, 2005). However, parasites limit goat productivity as they reduce fertility, cause skin irritation, and suck blood ultimately leading to death (Molefe *et al*., 2012). Gastro-intestinal tract parasites such as *Haemonchus contortus* and *Fasciola hepatica* are a major health problem in small ruminants (Vatta and Lindberg 2006). External parasites such as ticks, lice and mites have also been reported in goats (Bekele *et al.*, 2011). Most of these parasites are more prevalent in developing countries due to inappropriate housing and lack of adequate veterinary services (Mungube *et al*., 2006). Commercial drugs are mostly used to control parasites. However, these are expensive and are out of reach for many resource-limited farmers. Some of the parasites have also developed resistance against commercial drugs (Clark *et al*., 1996). Therefore, they pollute the environment (Wall, 2007). This has led farmers to resort to the use of medicinal plants to treat and control livestock parasites. There is also a belief that natural products are safe to use and are in harmonious with the environment (Erasto, 2003). Knowledge on the use of ethno-veterinary medicine is passed orally and there is danger that this information might disappear due to the socio-cultural changes. This study, was therefore, conducted to document medicinal plants used to control internal and external parasites in goats in Chris Hani District Municipality, South Africa.

3.2 Materials and method

3.2.1 Study site

The survey was conducted in Chris Hani district municipality in two local municipalities namely; Emalahleni and Tsolwana where Kwezi and Ntambethemba villages were selected, respectively.

The area lies within latitude 31º70’63-32º31’34S and longitude 27º23’41-27º51’17E. It receives an average annual rainfall of 483mm, with most rain occurring in summer. The study area has an average minimum and maximum temperature of 7 ºC and 22 ºC respectively (Institute for Soil, Climate and Water, 2008). The area is covered by Eastern Mixed Nama Karoo, sub arid Thorn Bushveld, South-Eastern Mountain Grassland and Moist Bushland.

3.2.2 Sampling procedure

Villages were randomly selected and farmers who keep goats were identified using the snowball sampling procedure. This sampling technique which involved approaching goat farmers and herbalists who have more knowledge on the plants used. These people in in turn direct to other potential respondents (Patton, 1990). Interviews were conducted amongst 50 farmers and three herbalists.

3.2.3 Data collection

Structured questionnaires were used to collect data. The data collected included demographic information such as gender, age, source of information and employment status. Information gathered included: local name of plant used, condition of plant used (dry or fresh), plant parts used, method of preparation, adverse effects, source of knowledge, parasites affecting livestock and other ways they use to control parasites. This survey was done in accordance with the University of Fort Hare Research and Ethics Policy (Ethical certificate number MAP011SSAN01). Plants were collected with the help of herbalists and were authenticated by a botanist, Professor Grierson at the University of Fort Hare.

3.3 Data analysis

Descriptive statistics were carried out using PROC FREQ of SAS (2003). Fidelity level (FL) values were determined to estimate the healing potential of the reported plants. This was calculated for plant species which had been reported more than three times. FL values were determined so as to estimate the medicinal use values and the relative preference of species by the local communities in this study area. Fidelity level [FL: the percentage of respondents who use a certain plant for the same main function (Sofowara, 1982)]was calculated as:

$(N_a / N) \times 100$

where: N_a is the number of respondents who claim a use of a plant species to treat a particular ailment

N is the number of informants that use the plant as medicine for any ailment (Alexiades, 1996)

3.4 Results

3.4.1 Demographic information

The demographic data of respondents are shown in Table 3.1. The majority of the households were male headed (73.58%). The most dominant age group within these heads were above 51 years (84.91%) followed by those in 31-50 years (13.21%). Most of the respondents (43.40%) never went to school. It was found that most of the respondents were unemployed (71.70%) but depended on government grants (39.62%) and selling livestock (35.85%) as their source of income.

TABLE 3. 1 DEMOGRAPHIC DATA OF RESPONDENTS

Gender	Number n[1](%)	Age	Proportion	Level of education	Proportion	Employment status	Proportion	Source of income	Proportion
Male	39 (73.58%)	**20-30**	1 (1.89%)	**Primary**	19 (35.85%)	**Employed**	3 (5.66%)	**Salary**	10 (18.87%)
Female	14 (26.42%)	**31-50**	7 (13.21%)	**Secondary**	9 (16.98%)	**Unemployed**	38 (71.70%)	**Livestock**	19 (35.85%)
		≥51	45 (84.91%)	**Tertiary**	2 (3.77%)	**Self-employed**	2 (3.77%)	**Crop farming**	3 (5.66%)
				Never went to school	23 (43.40%)	**Retired**	10 (18.87%)	**Grant**	21 (39.62%)
TOTAL	53 (100%)	**TOTAL**	53 (100%)	**TOTAL**	53 (100%)	**TOTAL**	53 (100%)	**TOTAL**	53 (100%)

n[1]- number in gender class

3.4.2 Livestock inventory

About 92.45% of the respondents reported that they owned owned cattle, (71.70%) sheep, (100.00%) goats and (75.47%) chickens. Farmers' kept goats for multiple purposes such as sources of meat and milk, income and for cultural reasons. Most of the farmers kept goats for cultural reasons (58.49%). Diseases, parasites, stock theft and poor rangelands were reported as the main challenges faced by the farmers. Parasites (47.17%) were reported as the most problematic challenge that the farmers are facing. Most of the farmers had more than 40 sheep whilst cattle, goats and chicken were kept in smaller numbers ranging from one to 10.

3.4.3 Prevalence of diseases and parasites

Gall sickness, heart water, red water, diarrhoea and bloating are some of the diseases that were reported to affect goats in the area. The most prevalent tick borne diseases in the area were gall sickness (60.02%), heart water (10.44%) and red water (11.54%). These diseases were more prevalent in summer. Government veterinary officers helped the farmers to identify the diseases and this helped in providing the right treatment. Most of the farmers (88.68%) treated their goats when suffering from these diseases.

All respondents acknowledged both internal and external parasites caused huge problems. Common parasites in the study area were mites (75.38%), ticks (75.38%), lice (65.38), fleas (30.77%) and helminthes (100%). These were reported to be more prevalent in summer. Figure 3.1 shows the prevalence of parasites in the study area. Farmers were able to tell that a goat was been infested by parasites through loss in body condition (32.08%), loss of appetite (33.96%) and rubbing against poles (33.96%).

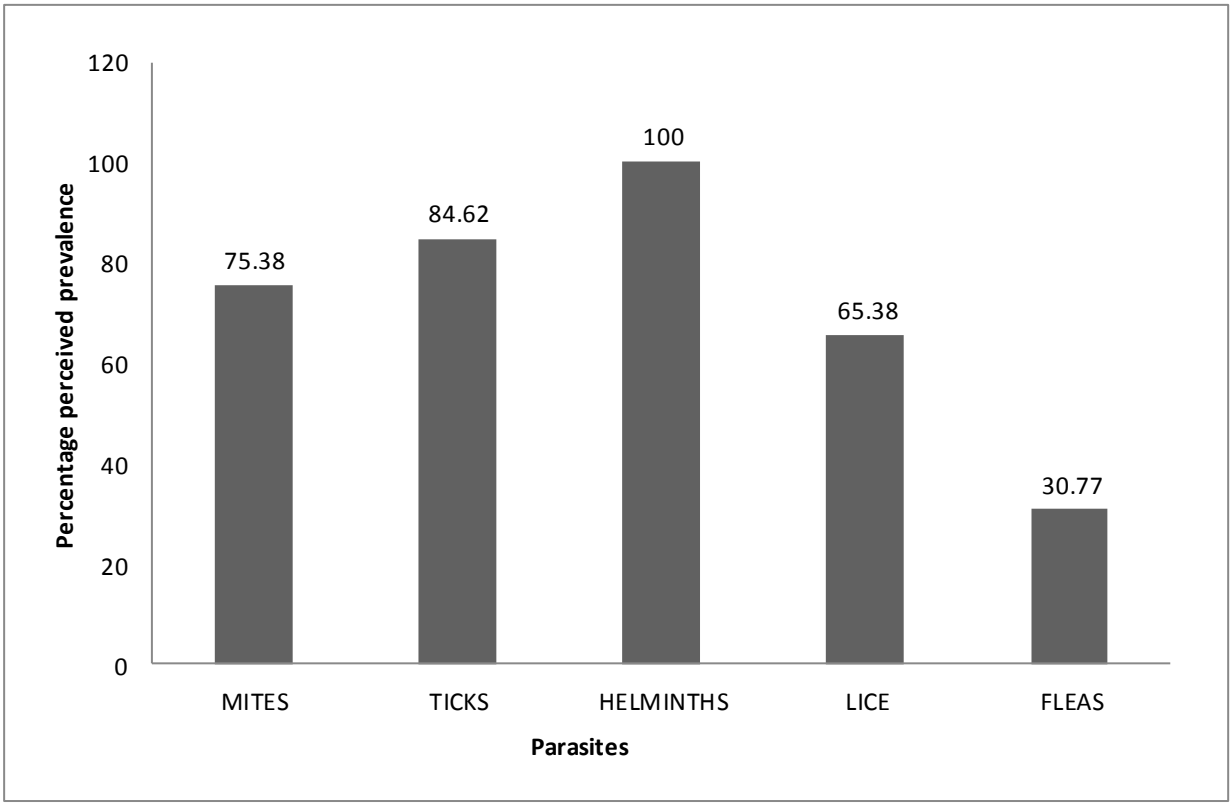

Figure 3. 1 Perceived prevalence of parasites in Chris Hani District Municipality in Eastern Cape Province, South Africa

3.4.4 Parasite control

The study revealed that most of the farmers (96.23%) dipped their goats once a month. The government provided dipping commercial chemicals for use in spray race or dip tanks. Dazzle dip (Diazinon 30%) was one of the commercial chemicals that they used to control external parasites in the area. Those farmers that did not dip (3.77%) felt that there is no need since they believe that goats are resistant to parasites. The majority of the respondents (69.23%) used medicinal plants, a few used commercial chemicals (11.54%) and a proportion of (19.23%) used both medicinal plants and commercial chemicals to control parasites in their herd (Figure 3.2). Farmers used medicinal plants for various reasons: they are effective (69.81%), cheap (1.89%), easily accessible (13.21%) and easy to use (15.09%). While others (11.54%) indicated that they do not have knowledge about the plants.

Overall, nine plants belonging to eight families used to control parasites in goats were reported (Table 3.2). Asphodelaceae was the most frequently mentioned plant family (22.22%). *Aloe ferox* was the most used plant in the area (43.40%). Different plant parts such as leaves, roots, tuber and barks were used in preparation of the remedies. Most of the farmers used leaves the most (60.38%) in preparing the medicine. A total of (60.38%) respondents used decoctions and only (39.62%) used infusions to prepare their remedies. No side effects were reported by the respondents. Some of the respondents combined more than one plant in the preparation of medicines. Others also mixed plant extracts with non-plant materials such as Epson salt, flour, butter, potassium permanganate, rock salt and oil cakes. FL values were determined so as to estimate the medicinal use values and the relative preference of species by the local communities in this study area. *Aloe ferox, Elephantorrhiza elephantina and Acokanthera oppositifolia* were the plants having the highest fidelity level values for their use to control

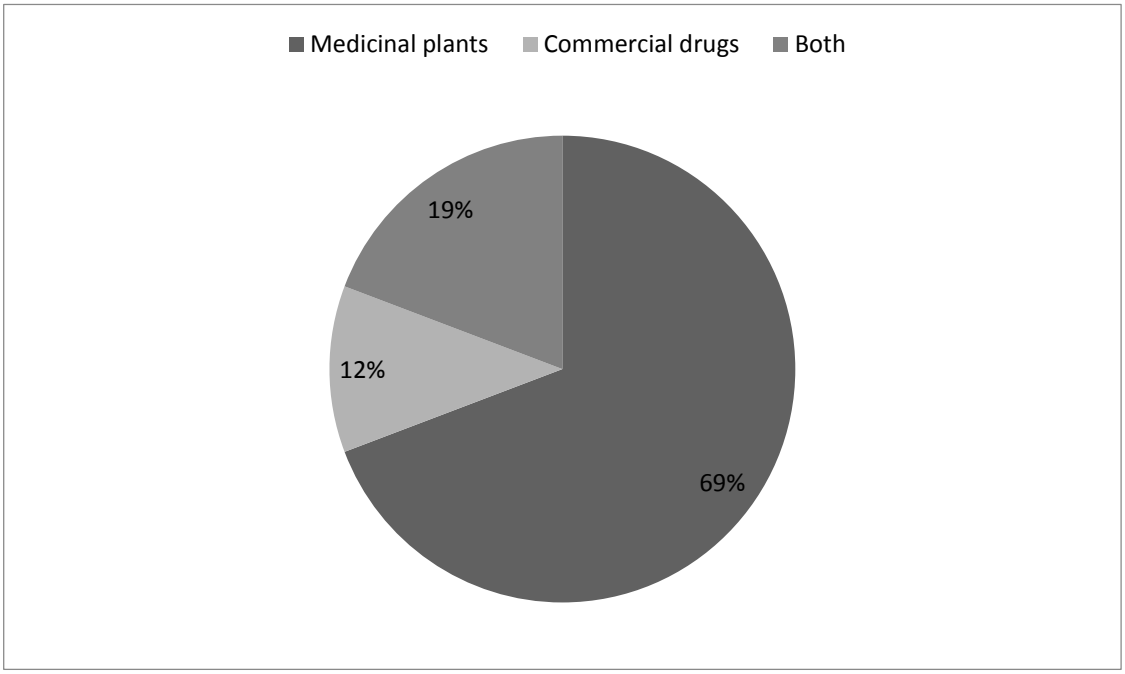

Figure 3. 2 The proportion of various methods used to control parasites in goats

Table 3. 2 Plants used to control parasites in Chris Hani District municipality

Family	Scientific name	Local name	Plant part used	Preparation method	Type of parasite controlled
Asphodelaceae	*Aloe ferox*	Ikhala elikhulu	Leaves	Decoction	helminths, ticks, mites
Fabaceae	*Elephantorrhiza elephantina*	Intolwane	Roots	Decoction	helminths, mites, ticks
Hyacinthaceae	*Albuca setosa*	Ingwebeba	Tuber	Decoction	helminths
Apocynaceae	*Acokanthera oppositifolia*	Intlungunyemba	Leaves	Decoction	helminths, ticks
Apiaceae	*Centella coriacea*	Inyongwana	Bark	Decoction	helminths
Araliaceae	*Cussonia spicata*	Umsenge	Bark	Infusion	helminths
Gunneraceae	*Gunnera perpensa*	Iphuzi	Tuber	Decoction	helminths
Agapanthaceae	*Agapanthus praecox*	Umkondo	Leaves	Infusion	helminths
Asphodelaceae	*Bulbine latifolia*	Ingcelwana	Leaves	Decoction	ticks, helminths

Decoction-boil plant material in water Infusion-soaking in ambient water temperature overnight

parasites, each scoring 100.00%, followed by *Albuca setosa* (83.33%) (Table 3.3). Most of the respondents (75.47%) reported that they acquired their knowledge orally from their parents and from other farmers. About 88.68% of respondents did not put any conservation methods in place. The reasons were that there is not enough land to cultivate the plants (50.94%) and 49.06% of the respondents believed the plants are abundant in the wild and there is no need to conserve them. Those who practiced conservation, cultivated the plants in their gardens and used plant parts such as leaves which do not destroy the whole plant. Close to 19% of the respondents think conventional drugs are the best for controlling parasites.

Table 3. 3 Fidelity level (FL) indices of plant species used to control parasites in the study area

Species	Parasite controlled	N_a	N	Fidelity level (%)
Aloe ferox	helminths, ticks, mites	23	23	100.00
Acokanthera oppositifolia	helminths, ticks	8	8	100.00
Elephantorrhiza elephantina	helminths, mites, ticks	6	6	100.00
Albuca setosa	helminths	5	6	83.33
Gunnera perpensa	helminths	3	9	33.33
Centella coriacea	helminths	1	4	25.00
Cussonia spicata	helminths	1	6	16.67

N^a- number of respondents who claim a use of a plant species to treat a particular ailment

N-number of informants that use the plant as medicine for any ailment

3.5 Discussion

In this study the demographic characteristics of the farmers were similar to those reported by Mwale and Masika (2009) in the Eastern Cape Province by Luseba and Van Der Merwe (2006) and in Limpopo Province of South Africa. The majority of the households were male headed while the most dominant age group consisted of older generation. Most of the respondents who were using medicinal plants and had essential knowledge were generally older than 51 years. The findings of this study are in agreement with those of Wanzala *et al*. (2005) who mentioned that information on medicinal plants is mostly stored in the memory of a few entrusted older people within communities. Most of these older people are unemployed and rely on grants for survival. This concurs with findings by Masika *et al*., (2000) who reported that most of the farmers rely on grants. The reason could be that most of them are old and poor so grants are their major source of income.

The reasons for keeping goats were similar to those cited by Bester *et al*. (2009) and Masika and Mafu (2004). Most of the farmers were keeping goats for cultural reasons and for cash income. Furthermore most of the farmers mentioned parasites as the most problematic challenge that they are facing and this concurs with studies by Mwale and Masika (2009). Parasites might be a problem in this area because the animals are raised on poorly managed grazing pastures. The infestation of internal and external parasite was reported to be more prevalent in the summer season and this could be due to the climatic conditions in the tropics (Phiri *et al*., 2007) which are favourable for parasite development, inappropriate housing (Mungube *et al*., 2006) and poor management of grazing pastures (Masika and Mafu, 2004).

Findings from this study revealed that farmers usually dipped their goats to control parasites. However, this contradicts findings by Kunene and Fossey (2010), who observed that most goats are hardly dipped in rural communities. The farmers are not provided with commercial drugs to control internal parasites in the study area. A majority of the respondents used medicinal plants, while others bought their own commercial drugs and a smaller proportion used both medicinal plants and commercial drugs to control parasites in their herd. Farmers gave reasons why medicinal plants are still in use, which included the efficacy of the plants, no side effects, cheap and easily accessible. Moreki *et al*. (2010) attributed the wide use of ethno-veterinary medicine in villages to the high price of commercial drugs and lack of knowledge in their use. However, Gueye (1999) argued that the use of ethno-veterinary medicine is the only option for most resource-limited farmers in Africa because of poor veterinary services in the rural areas. Farmers reported that they were able to define diseases using clinical signs but it should be kept in mind that some diseases exhibit similar signs (differential diagnosis) and that may affect the accuracy of the diagnosis. Wrong diagnosis of disease will result in incorrect treatment.

Aloe ferox and *Bulbine latifolia* were the reported plant species which belong to the most frequently mentioned plant family, Asphodelaceae. Maphosa and Masika (2010) reported similar findings. This could be as a result of its vast natural distribution, with 13 genera (Treutlein *et al*., 2003). The use of leaves the most in this study, concurs with studies by Masika and Afolayan (2003), Maphosa and Masika (2010), Setlalekgomo and Setlalekgomo (2013), Mohammed and Seyoum (2013) and Gebrezgabiher *et al*. (2013). The use of leaves in preparation of herbal remedies reduces loss of plants from the natural habitats as it does not destroy whole plant. Our findings contradicts findings by Cheikhyoussef *et al*. (2011), who reported that roots were the

most commonly used plant part. The use of roots in preparation of herbal remedies is not advised as this results in loss of medicinal plants from their habitats.

Some of the respondents combined more than one plant in preparation of medicines. The use of combined plant materials was also reported by Masika *et al.* (2000) and Maphosa and Masika (2010) but contradicts findings by Van der Merwe *et al*. (2001) who reported use of a single plant. Other respondents mixed plant materials with non-plant substances such as Epsom salt, rock salt, butter, oil cake, flour and potassium permanganate. These findings were similar to those by Maphosa and Masika (2010), Djoueche *et al*. (2011), Mohammed and Seyoum (2013) and Mohammed and Berhanu (2011). Mixing of plant materials with non-plant substances influence the absorption of compounds contained in the plants (Djoueche *et al*., 2011). Epsom salt is known to have a laxative effect. Oil cakes increase bile secretion which promotes the solubilisation of non-water soluble compounds (Djoueche *et al*., 2011). Rock salt has emulsifying properties which forms stable emulsions in the gastro-intestinal tract and therefore increase the solubilisation of alkaline compounds in plant extracts thus increasing their absorption (Djoueche *et al*., 2011). Addition of butter is known to improve the flavor and reduce the chances of animals vomiting (Mohammed and Seyoum, 2013).

Farmers in the Eastern Cape Province are aware of toxicity of some plants such as *Acokanthera oppositifolia* and therefore add more water to the dilute herbal preparations and boil the plant material before administering to the animals. *A. oppositifolia* is toxic due to the cardiac glycosides it contains. They also mixed *A. oppositifolia* with other plant materials such as *Aloe ferox*. This is in consonance with studies by Maphosa and Masika (2010), who reported the awareness of poisonous plants by farmers in the Eastern Cape Province. Addition of large quantities of water before boiling results in the extract becoming more dilute and therefore the

toxicity of the plant preparation will be reduced. Boiling of the plant extract also reduce toxicity by evaporating aromatic poisonous compounds.

Out of the nine plants species that are used to control parasites in goats in Chris Hani district, *Aloe ferox* was the most frequently used plant and this is similar to findings by Setlalekgomo and Setlalekgomo (2013), Maphosa and Masika (2010) and Moyo and Masika (2009). The plant *Aloe ferox* has a laxative effect due to the presence of glycoside aloin (Eloff and McGaw, 2014). It is also known as an insect repellent and is used to treat heartwater and gall sickness (Van Wyk *et al.,* 2002), poultry diseases, sheep scab and control ticks in cattle (Moyo and Masika, 2009). *Elephantorrhiza elephantina* is used to treat heartwater (Eloff and McGaw 2014; Luseba and Van der Merwe 2006; Van der Merwe *et al*., 2001), used in goats to control helminthes (Katerere and Luseba 2010; Maphosa and Masika 2010) and in humans for high blood pressure (Mathias-Mundy and McCorkle, 1989). *E.elephantina* possesses antibiotic properties (Wyk and Wink 2004; Van Wyk *et al*., 2002). It relieves inflammation in animals and is also used as a purgative (Cocks, 2006). *Centella coriacea* contains triterpenoids which have antibiotic and purgative effects. *Agapanthus praecox* has been reported to contain saponins which have antibiotic, analgesic, laxative, anti-edema, anti-inflammatory and immunoregulatory effects (Van Wyk *et al*., 2002). *Albuca setosa* is used in the management of diabetes mellitus (Oyedemi *et al*., 2011). *Acokanthera oppositifolia* has been reported to treat anthrax and tapeworms (Dold and Cocks, 2001). FL values of *Aloe ferox, Acokanthera oppositifolia, Albuca setosa* and *Elephantorrhiza elephantine* were high showing that most people in the area prefer these plants and constantly use them in controlling parasites. In this study, the most cited plants had the highest fidelity level and this contradict findings by Njoroge (2012). Trotter and Logan (1986) reported that plants which are constantly used by people in a certain area are more likely to contain bioactive

substances. Validation of these plants is important so as to isolate the active compounds and produce drugs.

Plants with low FL values did not have known preparation methods and dosages. Most of the informants attained their knowledge from their elders and this is similar to findings by Mwale *et al.* (2005) and Mwale and Masika (2009). Ethno-veterinary knowledge is not documented, it is passed on orally from elders and this is analogous with studies by Giday *et al*. (2009) and Farooq *et al*. (2008). Lack of documentation can result in loss of ethno-veterinary knowledge. It is therefore, important to document ethno-veterinary knowledge for community benefit. Farmers should use plant parts such as leaves rather than the whole plant to avoid plants from becoming extinct (Maroyi, 2012). Other respondents cultivate the plants in their gardens and this is not recommended as it is believed that monoculture conditions do not trigger the production of secondary metabolites (Schippmann *et al*., 2002). Plants that are cultivated are believed not to possess healing power as compared to wild plants. It is therefore advisable to use wild plants which grow under stress conditions and competition as they possess secondary metabolites. This would mean that ethno-veterinary medicine as a field will disappear soon as human population expand.

3.6 Conclusion and recommendation

The study revealed nine plant species which are used to control parasites in goats in Chris Hani district municipality. It was also revealed that information of ethno-veterinary medicine in this area is mostly confined to older people and there is danger that this knowledge can be lost before being passed on. Therefore, there is an urgent need to document these medicinal plants before the death of knowledgeable people in the study area. Further research should be done to access the efficacy and safety of the mentioned plants especially those with the highest fidelity level.

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CHAPTER 4

Phytochemical components *of Aloe ferox, Elephantorrhiza elephantina, Albuca setosa* and *Acokanthera oppositifolia* plants used to control parasites in goats in Eastern Cape Province, South Africa

Abstract

The objective of the study was to determine chemical constituents in leaves of *Aloe ferox, Albuca setosa, Acokanthera oppositifolia* and roots of *Elephantorrhiza elephantina* so as to add more knowledge on the medicinal value of these plants. Acetone extracts of *A. ferox, A. setosa, E. elephantina* and *A. oppositifolia* were analyzed using Gas Chromatography Mass Spectrometry (GC-MS). Terpenes and fatty acids esters were present, oxygenated terpenes were the most abundant hydrocarbons present in the four plant species. A total of 7, 32, 33 and 26 chemical constituents were revealed in *Aloe ferox, Albuca setosa, Elephantorrhiza elephantina* and *Acokanthera oppositifolia*, respectively. The mass spectra of the compounds present in each of the plants were matched with National Institute of Standards and Technology (NIST) library.These compounds have been reported to have biological activities against parasites, this could have potential in the development of drugs. Further investigation should be done to determine the pharmacological activites of these plants.

Keywords: compounds, herbal remedies, medicinal plants, ticks

4.1 Introduction

Most livestock owners in communal areas use plants to control parasites due to their accessibility and low cost. Phytochemicals are natural bioactive compounds found in plants and act as a defense system against diseases and parasites (Krishnaiah *et al*., 2009). Medicinal plants are known to contain bioactive substances such as flavonoids, terpenoids, steroids, alkaloids and phenolics that are more effective in controlling parasites (ticks) than most commercial acaricidal chemicals (Douglas and Soejarto, 2002; Edoga *et al*., 2005). Traditionally, medicinal plants possess different secondary metabolites such as antimicrobial, antifungal, antibacterial and antiviral agents. It is therefore important to know the phytochemical constituents of plants potential for the development of therapeutic drugs (Milne *et al*., 1993). Identification of chemical compounds in plants adds more knowledge on the medicinal value of plants as reported by Olubunmi and Afolayan (2011).

Gas chromatography has been used to determine phytochemical constituents in plants for a long time but has recently been replaced by gas chromatography mass spectrometry (GC-MS). The combination of the Gas Chromatography and Mass Spectrometry (GC-MS) is the most preferable tool for the analysis of chemical constituents in plants because of its simplicity, sensitivity and effectiveness (Krishnaiah *et al*., 2009). It has very good separation ability, produces chemical fingerprints of high quality and is able to give qualitative and quantitative composition information of the plant being investigated. The search for chemical compounds in medicinal plants is of great importance because it gives information about the quality of the plant. Medicinal plants have been reported to control parasites. Given that medicinal plants are used to control parasites, this study was therefore conducted to determine the chemical constituents present in *Aloe ferox, Acokanthera oppositifolia*, *Albuca setosa* and *Elephantorrhiza*

elephantina. To our knowledge, this is the first study to identify chemical compounds in acetone extracts of these medicinal plants.

4.2 Materials and methods

4.2.1 Description of site where assays were done

The study was conducted in the Department of Botany laboratory, University of Fort Hare. It is situated 520m above sea level and is located 26.9° longitude and 32.8° latitude.

4.2.2 Plant material collection and preparation

Fresh leaves of *Aloe ferox, Acokanthera oppositifolia,* roots of *Elephantorrhiza elephantina* and tubers of *Albuca setosa* were collected in Chris Hani District Municipality (Kwezi village) in Eastern Cape Province, South Africa. The area lies within latitude 32º31'34S and longitude 27º51'17E. It receives an average annual rainfall of 483mm, with most rain occurring in summer. The area has an average minimum temperature of 7ºC and maximum temperature of 22ºC (Institute for Climate, Soil and Water, 2008).

After collection, plant materials were washed with distilled water and cut into small pieces, air dried and then milled into powder using a grinder with 1mm pore size sieve. For the extraction process, 10g of each powdered plant material was mixed with 100ml of acetone, left on an orbital shaker for 24 hours and thereafter filtered through Whatmann filter paper using a Buchner funnel. The filtered material was transferred into a round bottomed flask and then condensed using a rotary evaporator at 56ºC. The condensed extracts were left to dry in a perforated chamber.

4.2.3 GC-MS analysis of bioactive components

The extracts were diluted at a ratio of 1:50 per volume using acetone and only 2µl of each acetone plant extract was used for GC-MS analysis. The extract was analyzed using Agilent 7890B GC system coupled to an Agilent 5977A MSD with a Zebron-5MS column (ZB-5MS 30 m x 0.25 mm x 0.025 µm) (5 %-phenylmethylpolysiloxane). GC-grade helium was used as the carrier gas at a flow rate of 2 mL/min. Sample injection was achieved through an auto-sampler. The temperature was set at 50^0C with an increment of 10^0C per minute until a final temperature of 250^0C. The time taken for the GC-MS analysis was calculated automatically as (18.09 mins). The eluted constituents were detected by a flame ionization detector and the Gas chromatogram of each plant extract was recorded.

Chemical components present in the 4 plant species was done based on molecular weight and molecular structure. Identification of the components of the plants was made by matching their recorded mass spectra in the computer library (NIST 11 MS library version 2005 software, Turbomas 5.2). This was done to determine whether these plant species contain one compound or a group of compounds.

4.3 Plant materials

Plant materials were identified by a botanist, Professor Grierson (South Africa) in January 2015. These specimens have been deposited in the University of Fort Hare herbarium, *A. ferox* (MSAN01/2015), *A.oppositifolia* (MSAN04/2015), *A. setosa* (MSAN03/2015) and *E. elephantina* (MSAN02/2015). Figures 4.1, 4.2, 4.3 and 4.4 show the plants which were used in this study.

FIGURE 4. 1 *ALOE FEROX (MILL)*

Family name: Asphodelaceae

Source:www.zimbabweflora.co.zw

Aloe ferox is commonly known as Red aloe or Bitter aloe. It is a tall, single stemmed aloe with thick fleshy leaves arranged in rosettes. The leaves have reddish brown spines on the margins with smaller spines on the upper and lower surfaces. The inflorescence is a candelabra of tubular orange red flowers which stand above the leaves. *Aloe ferox* is characterized by the presence of anthranoids, polysaccharides and anthraglycosides in the leaves (Harborne *et al*., 1997). Herbal preparations from the leaves of *A. ferox* are used as a purgative, to treat redwater, sores and to control ticks (Roberts, 1997).

Figure 4. 2 *Acokanthera oppositifolia*

Family name: Apocynaceae

Source*:* www.plantinfo.co.za

Acokanthera oppositifolia is commonly known as bushman poison. *A.oppositofila* is a small evergreen woody shrub with white latex. The latex contains toxic cardiac glycosides which are used to treat various ailments. *A. oppositifolia* is used in the treatment of tapeworms and anthrax (Hutchings and Staden, 1994). The leaves have white or tinged pink sweet scented flowers. Fruits are round greenish red when unripe and then turn purplish on maturity. All parts of the plant are poisonous but seeds have the highest concentration of poisonous compounds.

Figure 4. 3 ***Albuca setosa***

Family name: Hyacinthaceae

Source*:* www.ecoman.co.za

Albuca setosa is a plant with narrow, fleshy dark green leaves that become broader at the base. The flowers are white or yellow with broad green to brownish central stripes on their tepals. The flowers are erect and grow on long pedicels in a flat topped raceme. The upper part of the bulb has a tough fibrous tunic covering. *A. setosa* is used to kill parasitic worms, relieves inflammation and induces vomiting (Cocks, 2006).

FIGURE 4. 4 *ELEPHANTORRHIZA ELEPHANTINA*

Source:www.biodiversityexplorer.org

E. elephantina is commonly known as Elephant root. It is a low shrub that produces ground level annual stems. It has dull green leaves which are bipinnately compound with opposite or sub-opposite pairs of pinnae. The bark is dark reddish brown. It bears dark reddish brown fruits which are in compressed pods. The flowers are golden yellow to pale yellowish white and are arranged in solitary, axillary or clustered. *E.elephantina* possesses antibiotic properties (Van Wyk and Wink, 2004). It relieves inflammation in animals and is also used as a purgative (Cocks and Dold, 2006). Root decoctions are used for treating diarrhea whilst the infusion of the roots is used for dysentery (Hutchings *et al*., 1997). It is also used to treat tick borne diseases, and

pneumonia (McGaw and Eloff, 2008). In cows it is used to control mange (Dold and Cocks, 2001).

4.4 Results

4.4.1 Gas Chromatography Mass Spectrometry analysis of *A. ferox*

Gas Chromatography Mass Spectrometer analysis of *A. ferox* leaf acetone extracts showed the presence of seven chemical constituents. The major compounds found were 2H-Cyclopropa[a]naphthalen-2-one,1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1a.alpha.,7.alpha.,7a.alpha.,7b.alpha) followed by diacetone alcohol, 2,4-dimethylbenzo[h]quinoline, Tris(tert-butyldimethylsilyloxy)arsane and hexamethylcyclotrisiloxane. Tris(tert-butyldimethylsilyloxy)arsane had the highest retention time and diaectone alcohol had the lowest retention (Table 4.1). The leaf of *A. ferox* contained four different types of terpenes, with oxygenated sesquiterpenes being the major hydrocarbons followed by oxygenated hemiterpenes.

Table 4. 1 Chemical components in acetone leaf extract of *A. ferox*

No.	RT	Name of compound	Molecular formula	MW	Peak Area (%)
		Oxygenated hemiterpenes			
1	3.250	Diacetone alcohol	$C_6H_{12}O_2$	116	33.95
2	13.410	Hexamethylcyclotrisiloxane	$C_6H_{18}O_3Si_3$	222	3.57
		Oxygenated monoterpenes			
3	5.975	Terpinen-4-ol	$C_{10}H_{18}O$	154	2.40
4	14.604	Methyltris(trimethylsiloxy)silane	$C_{10}H_{30}O_3Si_4$	310	1.81
		Sesquiterpenes			
5	14.378	2,4-dimethylbenzo[h]quinolone	$C_{15}H_{13}N$	207	10.49
		Oxygenated sesquiterpenes			
6	9.898	2H-Cyclopropa[a]naphthalen-2-one,1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)	$C_{15}H_{22}O$	218	39.47
7	13.795	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	468	8.32

RT-retention time MW- molecular weight

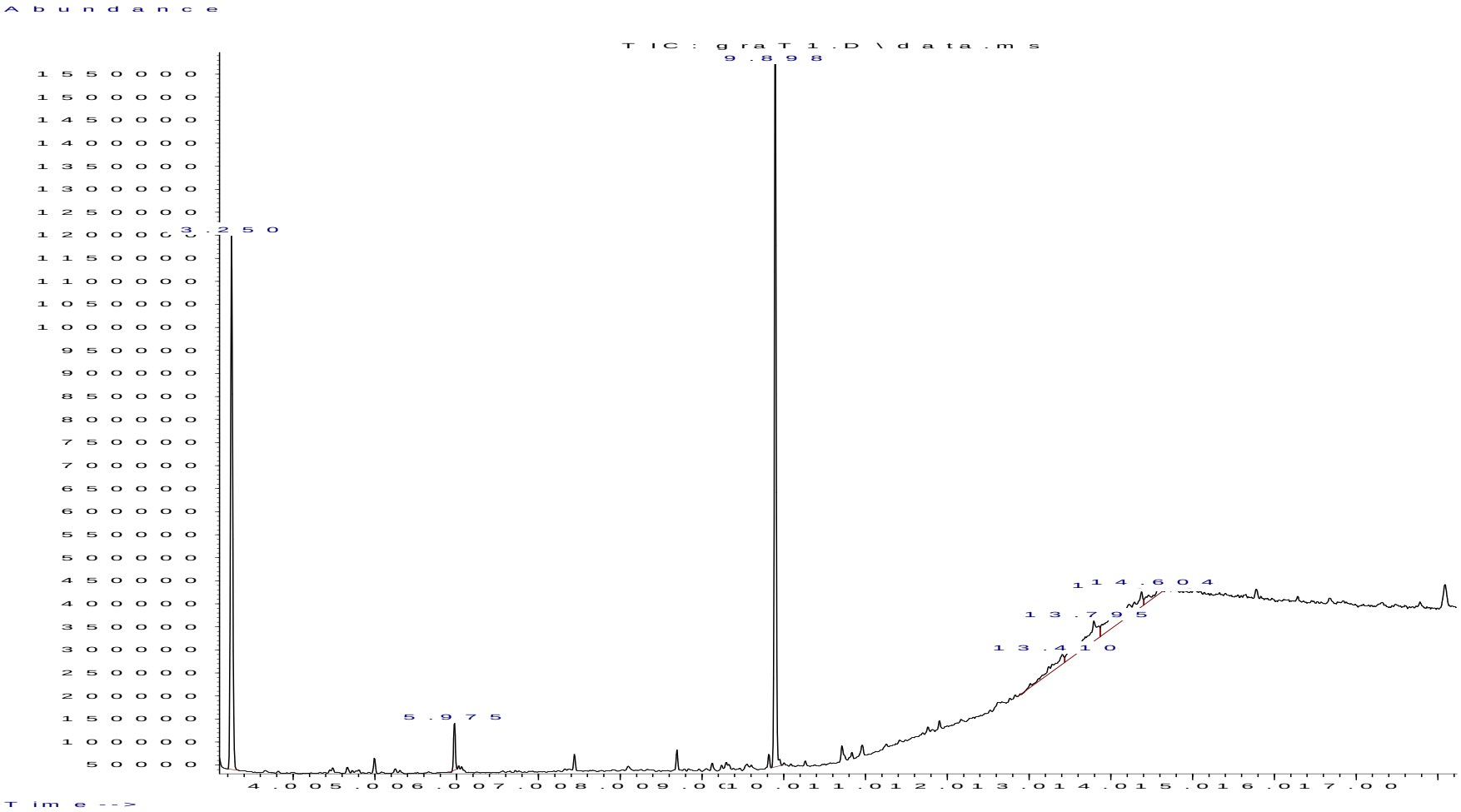

Figure 4. 5 Chromatogram showing chemical compounds of *A. ferox*

4.4.2 Gas Chromatography Mass Spectrometry analysis of *E. elephantina*

Gas Chromatography Mass Spectrometer analysis of *Elephantorrhiza elephantina* root acetone extracts revealed the presence of 33 chemical constituents as shown in Table 4.2. The major compound present were diaectone alcohol, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R). hydrocinnamic acid, benzyldimethyl silyl ester had the highest retention time and diacetone alcohol had the lowest retention time (Figure 4.6). The plant contained terpenes and fatty acids with terpenes being the most abundant. The different types of terpenes present in *E. elephantina* were oxygenated hemiterpenes, hemiterpenes, oxygenated monoterpenes, monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, diterpenes and tetraterpenes. Oxygenated hemiterpenes were the most abundant hydrocarbons present in the roots of *E. elephantina.*

4.4.3 Gas Chromatography Mass Spectrometry analysis of *A. oppositifolia*

The leaves of *A. oppostifolia* were found to contain 26 chemical constituents as shown in Table 4.3. The leaves contained terpenes and fatty acids. Oxygenated hemiterpenes were the major constituents in the leaves of *A.oppositifolia*. Decamethyl tetrasiloane was the major hydrocarbon in the plant followed by diacetone alcohol (Figure 4.7). Tris[dimethyl(2-methyl-2-propanyl)silyl] arsenite had the highest retention time and diacetone alcohol had the lowest retention time (Table 4.3).

Table 4. 2 Chemical components in acetone root extract of *E. elephantina*

No.	RT	Name of compound	Molecular formula	Molecular weight	Peak Area (%)
		Oxygenated hemiterpenes			
1	7.636	DL-Alanine, N-acetyl	$C_5H_9NO_3$	131	2.02
2	3.253	Diaectone alcohol	$C_6H_{12}O_2$	116	25.63
3	13.801	Cyclotrisiloxane,hexamethyl	$C_6H_{18}O_3Si_3$	222	5.85
4	14.279	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222	0.68
5	9.673	Benzaldehyde, 3-hydroxy-, oxime	$C_7H_7NO_2$	137	6.04
6	13.281	1,2-Benzisothiazol-3-amine tbdms	C_7H_5NOS	151	0.90
		Monoterpenes			
7	4.667	(+)-4-Carene	$C_{10}H_{16}$	136	2.50
8	4.997	Gamma-Terpinene	$C_{10}H_{16}$	136	4.16
9	6.086	Naphthalene	$C_{10}H_8$	128	2.68
10	12.833	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222	0.96
11	7.443	Tetradecane	$C_{14}H_{30}$	198	1.78
		Oxygenated monoterpenes			
12	5.975	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	$C_{10}H_{18}O$	154	8.56
13	14.321	Methyltris(trimethylsiloxy)silane	$C_{10}H_{30}Si_4$	310	0.67
14	10.714	Diphenyl sulfone	$C_{12}H_{10}O_2S$	218	3.89
15	11.701	5-Methyl-2-trimethylsilyloxy-acetophenone	$C_{12}H_{18}O_2Si$	222	1.32
16	11.800	5-Methyl-2-trimethylsilyloxy-acetophenone	$C_{12}H_{18}O_2Si$	222	0.97
17	11.444	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9 ,11,11,13,13-tetradecamethyl	$C_{14}H_{42}O_6Si_7$	503	0.86
18	11.768	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	$C_{14}H_{42}O_6Si_7$	503	0.66

19	10.952	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	$C_{14}H_{42}O_6Si_7$	503	1.37
		Sesquiterpenes			
20	13.407	2,4-dimethylBenzo[h]quinoline,	$C_{15}H_{13}N$	207	1.11
21	8.696	Octadecane	$C_{18}H_{38}$	254	2.16
22	10.837	Nonadecane	$C_{19}H_{40}$	268	0.89
23	9.820	Eicosane	$C_{20}H_{42}$	282	1.41
		Oxygenated sesquiterpenes			
24	8.844	Caryophyllene oxide	$C_{15}H_{24}O$	220	2.94
25	11.237	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{48}O_7Si_8$	577	0.08
26	10.626	Phellopterin	$C_{17}H_{16}O_5$	300	2.84
27	11.508	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	468	0.29
28	9.319	Folic Acid	$C_{19}H_{19}N_7O_6$	441	2.60
		Diterpenes			
29	14.229	1,2,4-Benzenetricarboxylic acid,-dodecyl dimethyl ester	$C_{23}H_{34}O_6$	406	7.27
		Tetraterpenes			
30	14.439	Tris(tert-butyldimethylsilyloxy)arsane	$C_{48}H_{66}O_9Si_3$	871	1.66
		Fatty acids			
31	14.554	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342	0.19
32	13.861	bis(trimethylsilyl) ester diethyl silicate	$C_{10}H_{26}O_3Si_3$	278	0.86
33	18.091	Hydrocinnamic acid, benzyldimethyl silyl ester	$C_{18}H_{22}O_2Si$	298	4.35

RT-retention time

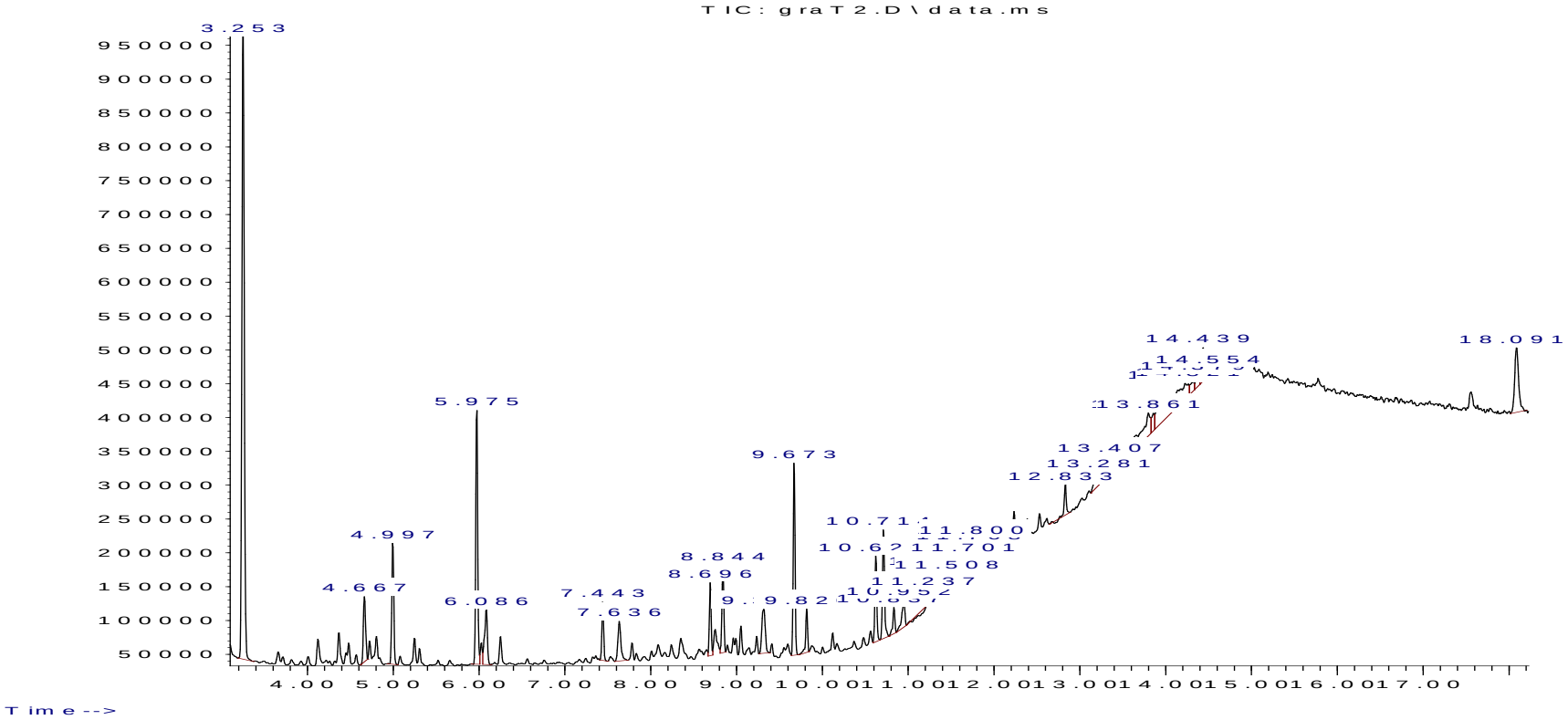

Figure 4. 6 Chromatogram showing chemical compounds of *E. elephantina*

Table 4. 3 Chemical components in leaf acetone extract of *A. oppositifolia*

No.	RT	Name of compound	Molecular formula	Molecular weight	Peak Area (%)
		Hermiterpenes			
1	7.443	Tetradecane	C_4H_{13}	198	0.89
2	12.518	Piperazine	$C_4H_{10}N_2$	86	0.99
		Oxygenated hermiterpenes			
3	7.660	N-aetyl-DL-Alanine	$C_5H_9NO_3$	131	0.84
4	7.711	Pentanamide	$C_5H_{11}NO$	101	4.44
5	3.255	Diacetone alcohol	$C_5H_{10}O$	86	12.32
6	0.925	1,6- anhdro-propanedioic acid	$C_6H_{12}O_6$	180	2.49
7	9.148	2,6-Di-O-methyl-d-galactopyranose	$C_8H_{16}O_6$	208	1.94
8	14.571	Hexamethylcyclotrisiloxane	$C_6H_{18}O_3Si_3$	222	1.42
9	14.396	Decamethyl tetrasiloxane	$[(CH_3)_3SiOSi(CH_3)_2]_2O$	310	22.26
		Monoterpenes			
10	6.088	Naphthalene	$C_{10}H_8$	128	1.02
		Oxygenated monoterpenes			
11	12.611	Decamethyltetrasiloxane	$C_{10}H_{30}O_3Si_4$	310	0.17
12	9.600	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	1.39
13	10.714	Diphenyl sulfone	$C_{12}H_{10}O_{2S}$	218	2.20
14	9.422	2-(2-(2-(2-Butoxyethoxy)ethoxy) ethoxy)ethyl acetate	$C_{12}H_{24}O_5$	248	17.35
15	14.683	4-Methyl-2-trimethylsilyloxy-acetophenone	$C_{12}H_{18}O_2Si$	222	5.33
16	14.434	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	$C_{14}H_{42}O_6Si_7$	503	2.14
17	14.771	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O_2$	222	4.03
		Sesquiterpenes			
18	8.697	Hexadecane	$C_{16}H_{34}$	226	0.95
19	9.821	Octadecane	$C_{18}H_{38}$	254	0.38

20	13.406	2,4-dimethylbenzo[h]quinoline,	$C_{15}H_{13}N$	207	0.09
21	10.087	6,10,14-trimethylpentacedan-2-one	$C_{18}H_{36}O$	268	0.77
		Oxygenated sesquiterpenes			
22	14.462	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{48}O_7Si_8$	577	4.04
23	15.773	Tris[dimethyl(2-methyl-2-propanyl)silyl] arsenite	$C_{18}H_{45}AsO_3Si_3$	468	1.56
		Diterpenes			
24	11.402	Kaur-16-ene	$C_{20}H_{32}$	272	1.53
		Fatty acids			
25	13.876	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342	8.90
26	14.880	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342	0.75

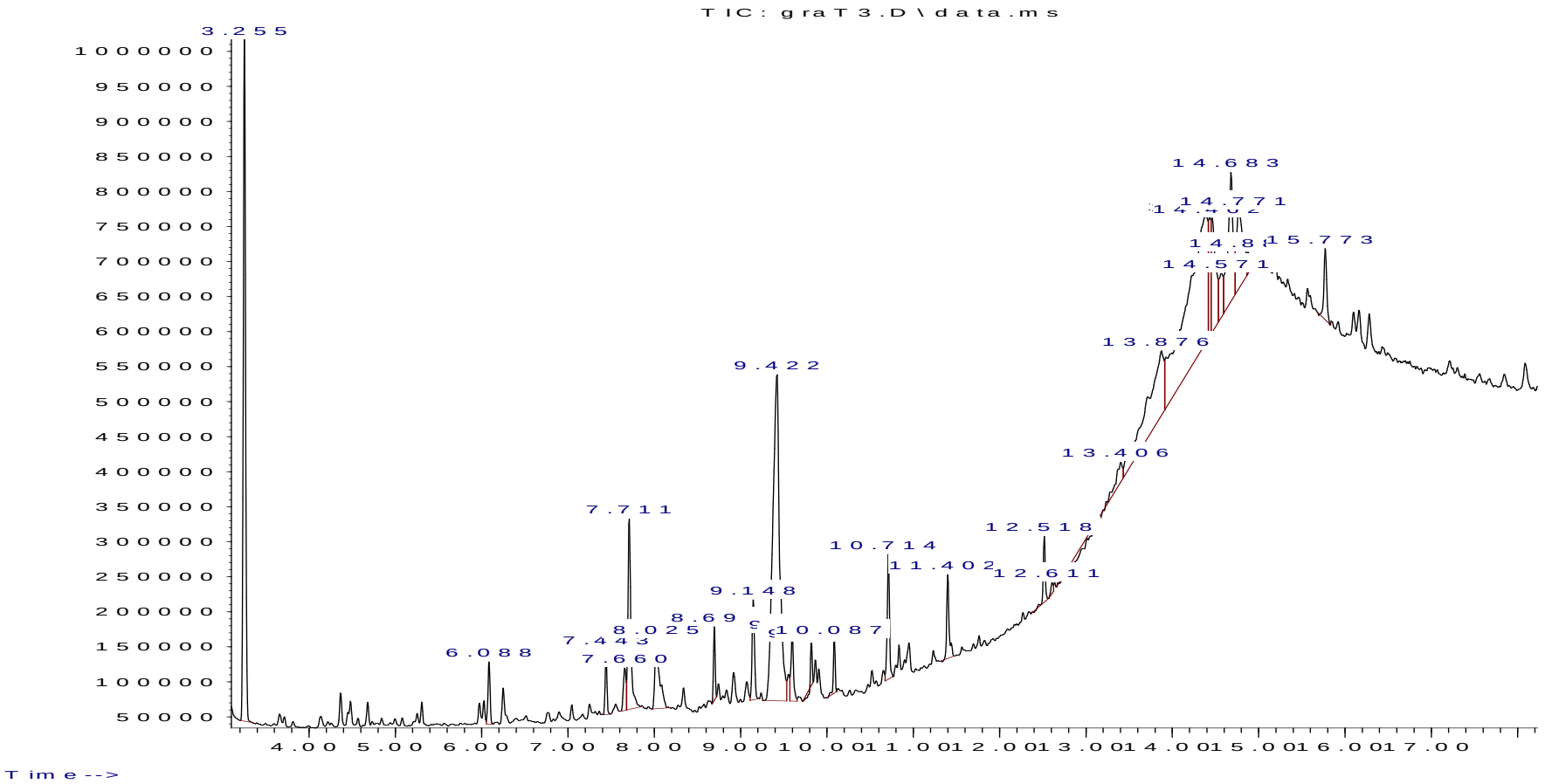

Figure 4. 7 Chromatogram showing chemical compounds of *A. oppositifolia*

4.4.4 Gas Chromatography Mass Spectrometry analysis of A. setosa

Albuca setosa revealed the presence of 32 phytochemical constituents which included terpenes and fatty acids (Table 4.4). The major hydrocarbon was alpha.-Amyrin followed by 4-Methyl-2-trimethylsilyloxy-acetophenone and 1,4-Bis(trimethylsilyl)benzene (Figure 4.8). Diacetone alcohol was found to have the least retention time whereas 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- had the highest retention time.

Table 4. 4 Chemical components in leaf acetone extract of *Albuca setosa*

No.	RT	Name of compound	Molecular formula	Molecular weight	Peak Area (%)
		Oxygenated hermiterpenes			
1	3.254	Diaectone alcohol	$C_6H_{12}O_2$	116	7.23
2	4.368	1,2-Ethanediol, diacetate	$C_6H_{10}O_4$	146	0.31
3	4.483	Ethanol, 2-(2-ethoxyethoxy)-	$C_6H_{14}O_3$	134	0.24
4	12.574	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222	0.50
5	13.018	1,2-Benzisothiazol-3-amine tbdms	C_7H_5NOS	151	0.89
6	9.673	Benzaldehyde, 3-hydroxy-, oxime	$C_7H_7NO_2$	137	0.23
7	13.802	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	222	3.62
8	4.682	2-ethylhexanol	$C_8H_{18}O$	130	0.32
9	6.252	Ethanol, 2-phenoxy	$C_8H_{10}O_2$	138	0.29
10	5.309	Nonanal	$C_9H_{18}0$	142	0.28
11	10.948	1,2-Benzenediol, 4-(2-amino-1-hydroxypropyl)-	$C_9H_{13}NO_3$	183	0.39
		Monoterpenes			
12	6.088	Naphthalene	$C_{10}H_8$	128	0.57
13	12.273	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222	0.40
14	14.684	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222	9.82
15	7.443	Tetradecane	$C_{14}H_{30}$	198	0.24
		Oxygenated monoterpenes			
16	5.976	3-Cyclohexen-1-ol, 4-methyl-1-methylethyl)-, (R)-	$C_{10}H_{18}O$	154	0.52

17	10.714	Diphenyl sulfone	$C_{12}H_{10}O_2S$	218	4.40
18	13.616	Brallobarbital	$C_{10}H_{11}BrN_2O_3$	287	2.72
19	14.145	4-Methyl-2-trimethylsilyloxy-acetophenone	$C_{12}H_{18}O_2Si$	222	11.57
20	15.834	4-Methyl-2-trimethylsilyloxy-acetophenone	$C_{12}H_{18}O_2Si$	222	0.76
21	17.435	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	$C_{13}H_{22}OSi_2$	250	1.08
22	15.095	4,6-di-tert-Butylresorcinol	$C_{14}H_{22}O_2$	222	6.48
		Sesquiterpenes			
23	11.443	2-Ethylacridine	$C_{15}H_{13}N$	207	0.71
24	8.697	Octadecane	$C_{18}H_{38}$	254	0.35
		Oxygenated sesquiterpenes			
25	15.187	Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	$C_{15}H_{24}O_2Si$	264	1.26
26	11.766	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{48}O_7Si_8$	577	0.36
27	15.773	Tris(tert-butyldimethylsilyloxy)arsane	$C_{18}H_{45}AsO_3Si_3$	468	0.91
		Oxygenated diterpenes			
28	9.312	Pregnan-20-one, 3-(acetyloxy)-5,6-epoxy-, cyclic 20-(1,2-ethanediyl acetal), (3.beta.,5.alpha.,6.alpha .)-	$C_{21}H_{34}O_3$	334	0.68
		Diterpenes			
29	9.820	Eicosane	$C_{20}H_{42}$	282	0.16
		Triterpenes			

30	12.001	.alpha.-Amyrin	$C_{30}H_{50}O$	426	35.25
		Fatty acids			
31	15.438	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{26}O_3Si_3$	278	1.83
32	13.506	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	$C_{15}H_{23}NO_3$	265	5.62

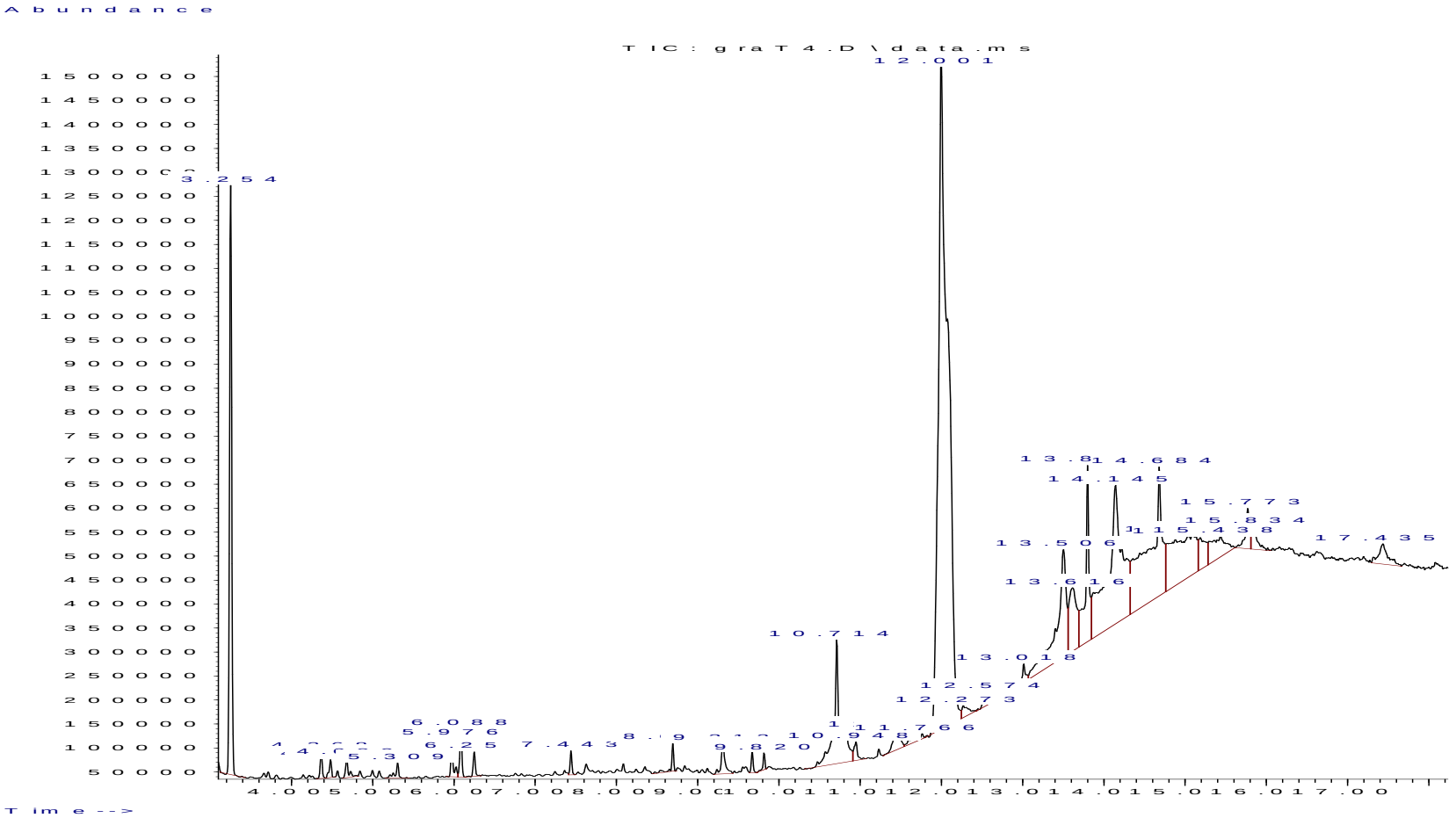

Figure 4. 8 Chromatogram showing chemical compounds of *Albuca setosa*

4.5 Discussion

Gas Chromatography-Mass Spectrometry analysis revealed the presence of compounds in the four plant species investigated. Some of the compounds found in these plants have been reported to have biological activities. *A. ferox* was found to contain terpinen-4-ol and 2,4-dimethylbenzo[h]quinolone. Terpinen-4-ol is a monoterpenoid alcohol known for its anti-inflammatory, antiviral, antifungal, antibacterial and antiprotozan properties (Carson and Riley, 1995). Terpinen-4-ol is a component of essential oils of many plants such as *Melaleuca alternifolia* (Dewick, 2001), *Tanacetum cadmeum* (Ozek *et al*., 2007). It is used as an insect repellent against lice and ticks (Morris, 2003). Compounds such as 2,4-dimethylbenzo[h]quinolone whose names end with quinolone belong to the family of synthetic broad spectrum antibacterial drugs called quinolone (Anderson and MacGowan, 2003). Quinolones are oxygen containing compounds found in various plants and are known to possess medicinal properties. Quinolone alkaloids have also been found in *Balfourodendon* as well as in *Lunasia* spp. and have been used in the development of drugs (Heeb *et al*., 2011).

A previous study by Magwa *et al*. (2006) of the hexane extract of *Aloe ferox* reported 21 chemical constituents compared to seven found in the current study. The difference might be due to environmental factors and the solvent used. Different solvents are known to affect the percentage composition of these compounds in plant extracts. Wintola and Afolayan (2011) reported that ethanol and methanol extracts of *A. ferox* had higher level of essential compounds as compared to aqueous and acetone extracts. This might be the reason why there were only seven compounds in this study. Acetone has been shown to be effective in extracting volatile compounds and hexane is effective against non-polar compounds (Mawela, 2008). The current study could imply that most of the compounds present in *A. ferox* were non-polar compounds.

Ahuja *et al*. (2010) reported that agronomic practices and climatic conditions influence the chemical constituents present in a plant. The season the plant was collected, environmental conditions, storage conditions, dehydration procedures, microbial contamination and developmental stage are also factors that affect the chemical profile of plants (Kokkini *et al*., 1997). One of these factors could have affected the chemical profile of the plants.

Elephantorrhiza elephantina was found to have 33 chemical constituents. The plant contained terpenes and fatty acids with terpenes being the most abundant. However a previous study on hexane extract of *E. elephantina* reported that fatty acids were the most abundant compounds in the plant (Msimanga *et al*., 2013). Little work has been done on the chemical profile of *E. elephantina, A. setosa* and *A. oppositifolia*. Chemical compounds which were found in *E.elephantina* plant extract such as naphthalene, folic acid, carophyllene oxide, phellopterin and hydrocinnamic acid have been reported to have biological potential in the development of therapeutic drugs (Yang *et al*., 2007) . Naphthalene is an aromatic hydrocarbon that is used as a repellent and an insecticide (Bogen *et al*., 2008). Carophyllene oxide, an oxygenated trerpenoid is used as a preservative in food and drugs (Chavan *et al*., 2010). The compound is also known to have inflammatory and antifungal activities (Yang *et al*., 2007). Carophyllene oxide is also found in guava (*Psidium guajava*) and in the bark of *Annona squamosa* (Chavan *et al*., 2010). Phellopterin has also been reported to produce strong chemical defenses against predators such as insects (Kakar *et al*., 2004). Hydrocinammic acid has been reported to be effective against internal parasites in domestic animals (Sharma and Singh, 2012). The compound Piperazine which was present in *E. elephantina* is used as a narrow spectrum anthelminthic drug in pigs and chicken (Jacela *et al*., 2009). One of the compound which was abundant in *A. oppositifolia* plant was 2-(2-(2-(2-Butoxyethoxy)ethoxy) ethoxy)ethyl acetate and is mostly found in anthelmintic

drugs such as Levamisole (Lewis, 2007). This compound is used to control endoparasites in domestic animals such as goats, cattle and sheep.

A majority of the volatile constituents in these plants investigated belonged to a group known as terpene. The results are similar to studies by Omuroyi *et al*. (2014) who also reported terpenes to be the most abundant in *Mesembryanthemum edule*. Terpenes are natural products from micro-organisms, plants and animals which are known to have strong biological activities against parasites (Cheng *et al*., 2014). Monoterpenes have been reported to have healing properties (Kamal *et al*., 2011). Monoterpenes are colourless, lipoptic compounds which comprise the majority of volatile compounds in plants and have defense mechanisms against parasites (Langenheim, 1994). Sesquiterpenes and diterpenes have phytoalexins which are involved in the direct defense against pathogens (Dudareva *et al*., 2004). In this study oxygenated hemiterpenes were the most abundant compound in the four plant species. Oxygenated terpenes are more valuable in plants as they produce fragrance that has defense mechanisms against pathogens compared to other terpenes (Kessler and Baldwin, 2001). This study showed that these plants have a great potential in controlling parasites.

4.6 Conclusion

The results obtained from the GC-MS analysis revealed 7, 33, 26 and 32 chemical constituents in *A. ferox, E. elephantina, A. oppositifolia* and *A. setosa*, respectively. The chemical constituents present in the plants belonged to hemiterpenes, sesquiterpenes, monoterpenes, diterpenes and fatty acids esters. This study confirmed the presence of chemical compounds in the plants which have a great therapeutic potential. Further investigation should be done to test the efficacy of these plant extracts in controlling parasites.

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CHAPTER 5

In vitro repellency and contact bioassay of crude extracts of *Aloe ferox* and *Acokanthera oppositifolia* plants against *Amblyomma hebraeum* and *Rhipicephalus decoloratus* ticks

Abstract

The objective of the study was to validate the medicinal properties of *A. ferox* and *A. oppositifolia* plant extracts used by farmers to control ticks. The effect of acetone, methanol and ethanol extracts of leaves of *Aloe ferox* and *Acokanthera oppositifolia* on tick repellency and acaricidal activity were investigated on blood engorged *Ambylomma hebraeum* and *Rhipicephalus decoloratus* ticks at concentration 15, 30 and 50%. Acaricidal and repellency activity of *A. ferox* and *A.oppositifolia* increased significantly ($P<0.05$) with concentration. The 30 and 50% *A. ferox* acetone extracts were more effective in causing *R. decoloratus* and *A. hebraeum* tick mortality compared to the other treatments. The highest tick repellency (83.33%) was observed at 50% concentration in *A. ferox* acetone extract while in the *A. oppositifolia* extract, the 50% methanol extract caused 89% repellency. Although both plant extracts demonstrated repellency activity, the acetone extracts provided 30mins compared to 2hours repellency for methanol and ethanol extracts. The 30mins repellency period is not enough to protect livestock from ticks. Results from this study showed that acetone, ethanol and acetone extracts of *A. ferox* and *A. oppositifolia* plants have acaricidal and repellency activities. This also confirmed the efficacy of these plants.

Keywords: *Acokanthera oppositifolia*, *Aloe ferox*, efficacy, herbal remedies, plants, ticks

5.1 Introduction

Ticks are the most prevalent external parasites of goats in tropics and subtropics (Callow, 1978). *Rhipicephalus decoloratus* is an African blue tick which causes dermatophilosis in heavy infestations (Nyangiwe *et al*., 2013). *Amblyomma hebraeum*, a hard tick is an agent of heartwater and African tick-bite fever (Boomker *et al*., 1994). *Rhipicephalus decoloratus* and *A. hebraeum* are major contributors to animal health problems and impede goat production. However, these are expensive, leave residues in animal products and are not readily available to rural farmers (Okello-Onen and Rutangwenda, 1997). Furthermore, some of the ticks have developed resistance against some of the commercial drugs. Therefore, there is need to develop management strategies to reduce the development of resistance to commercial drugs by parasites (Rust, 2005).

The use of plant derived products that have repellency and acaricidal activities can be suitable alternatives to address this problem (Magwa *et al.,* 2006). Medicinal plants with acaricidal activities have a major potential especially in developing countries as they are easily biodegradable and less toxic to the environment (Nchu *et al*., 2005). These plants have defense strategies against pathogens such as the production of secondary compounds (Futyuyma and Agrawal, 2009). The secondary compounds can be toxic (Frenandez-Ruvalcaba *et al*., 1999) or repellent to parasites (Stjernberg and Berglund, 2000). Repellency is one of the protective mechanisms, which plants have developed to combat predator and pathogen attack (Prajapati *et al*., 2005). Reports have shown that some of the resource- limited farmers in Eastern Cape Province, South Africa are already using *A. ferox* and *A. oppositifolia* to control parasites (Moyo and Masika, 2009). Although studies have been conducted on some of the plants such as *A. ferox,* no study has been done on the acaricidal and repellency properties of extracts of *E.*

elephantina, A. oppositifolia and *A. setosa* plants using acetone, methanol and ethanol solvents. This study sought to validate the medicinal properties of selected plants used by farmers in the control of ticks using acetone, methanol and ethanol.

5.2 Materials and Methods

5.2.1 Study site

The study was conducted in the Animal Science laboratory, University of Fort Hare. It is situated 520m above sea level and is located 26.9° longitude and 32.8° latitude.

5.2.2 Description of material used in the study

5.2.2.1 Plant material

Description of plant materials used in the study is as described in Chapter 4 section 4.3.

5.2.2.2 Description of reference dip

Dazzel dip (Diazinon 30% m/v) is a registered commercial, non-systematic organosphosphate insecticide used by farmers to control external parasites in the study area. It was used as a reference (positive control). It is effective against ticks, fleas and mites. The product is available in wettable powder, dust, granules and emulsifiable solution formulations ranging between 25-60% concentration.

5.2.3 Plant material collection and preparation

Fresh leaves of *Aloe ferox* and *Acokanthera oppositifolia,* were collected in Chris Hani District municipality (Kwezi village) in Eastern Cape Province, South Africa. Description of study site is given in Chapter 3. After collection, plant materials were cut into small pieces, air dried for 10 weeks under ambient temperature and then milled into powder using a grinder through a 1mm sieve. The powder was stored in airtight plastic containers till their use. The different extracts per

plant were generated. Acetone, ethanol and methanol extracts were prepared from each of the two plant powders.The extracts were left on an orbital shaker for 24 hours and then filtered through Whatman filter paper using a Buchner funnel. A total of 75g of plant powder was mixed with 750ml of the solvent and this was done twice. The filtered material were transferred into a round bottomed flask and then condensed using a rotary evaporator. The condensed extracts were left to dry in a perforated chamber. They were later stored in tightly closed sterile containers and kept in the refrigerator until use. For the extraction process, weight of plant materials, solvents used and yield are shown in Table 4.1.

TABLE 5. 1 WEIGHTS OF RAW MATERIAL AND EXTRACTION YIELDS FROM 2 PLANT SPECIES

Plant species	Level of extraction	Acetone		Ethanol		Methanol	
		Raw plant material used (g)	**Yield (g)**	**Raw plant material used (g)**	**Yield (g)**	**Raw plant material used (g)**	**Yield (g)**
Aloe ferox	1st extraction	75.00	1.15	75.00	1.86	75.00	2.70
	2nd extraction	75.00	2.06	75.00	2.33	75.00	2.31
	Total	**150.00**	**3.21**	**150.00**	**4.19**	**150.00**	**5.01**
Acokanthera	1st extraction	75.00	4.83	75.00	5.82	75.00	8.50
oppositifolia	2nd extraction	75.00	4.92	75.00	6.16	75.00	8.86
	Total	**150.00**	**9.75**	**150.00**	**11.98**	**150.00**	**17.36**

5.2.4 Ticks

Repellency method described by Thorsell *et al*. (2005) was used in the bioassay study. Solutions from the two plants were applied at the edge of two filter papers which were then air dried for two minutes and placed in a petri dish with an inner diameter of 9.5cm. Different concentrations for each plant extract (acetone, methanol and ethanol) were used and (15% (150mg/ml), 30% (300mg/ml) and 50% (500mg/ml)). Distilled cold water was used as a negative control and Dazzel dip (Diazinon 15% and 30%) was used as a positive control. The treatments were replicated thrice. The petri dishes were left open and the behavior of six ticks per replicate was observed. Ticks were observed to see if they avoided the treated area. If the ticks avoided the treated area it meant that they have been repelled and if they continued their motion beyond the treated area then they were considered non-repelled. The number of ticks avoiding the area on each occasion was recorded. The tests were repeated after 30mins, 1,2,3,4, 5 and 6hrs. The average repellency was calculated from the values obtained in the three replicates, using the formula by Thorsell *et al*. (2005)

$R = p/n \times 100\ \%$

where R= repellency;

p = number of ticks avoiding the treated area;

n= total number of ticks placed on the filter paper

5.2.5 Contact bioassay

The dipping method for the contact bioassay as described by Pirali-Kheirabadi *et al*. (2007) was used. Plant extracts from acetone, methanol and ethanol with different concentrations (15%, 30% and 50%) were used. The positive control was Dazzel dip (Diazinon 30% m/v) and negative

control was distilled water. A total of 10 ticks were immersed in a specific treatment for a minute. Ticks were removed from the test tubes and placed in a petri dish with an inner diameter of 9.5cm and a lid. This was replicated thrice and ticks were incubated at 25^{o}C and relative humidity of 85%. The percentage mortality was recorded after every 24 hours for seven days (Pamo *et al*., 2005). The numbers of ticks motionless after 24 hours were considered dead. Live ticks were considered to exhibit normal behavior when physically moved by a stick or breathed upon. Ticks which were incapable of moving, coordinating legs or showing any signs of life were considered dead (Panella *et al*., 2005). The number of dead ticks was recorded and tick mortality was calculated using the formula by Chungsamarnyart *et al*. (2003):

Corrected mortality (%) = $(1-{}^{T}/_{C})$ X 100 %

Where T= number of ticks alive after being exposed to test material

C= number of ticks in the control (distilled water).

5.2.6 Statistical analysis

5.2.6.1 The *in vitro* repellency bioassay and contact bioassay

The data were tested for normality and was not transformed. The collected data on repellency bioassay were analyzed using PROC GLM for repeated measures (SAS, 2003). Data on contact bioassay were analysed using PROC GLM of SAS (2003). Turkey test was used to compare differences between treatment means. A probability value of less than 5% was used to denote a significant difference.

The statistical model used for this analysis was as follows:

$Y_{ijkl} = \mu + P_i + C_j + X_k + (P_i \times C_j) + (P_i \times X_k) + (C_j \times X_k) + (P_i \times C_j \times X_k) + E_{ijkl}$

Y_{ijkl} = response effect due to treatment (mortality and repellency)

μ = overall mean

P_i = effect due to plant

C_j=effect due to concentration

X_k=effect due to extract

$(P_i \times C_j)$= interaction between plant and concentration

$(P_i \times X_k)$= interaction between plant and extract

$(C_j \times X_k)$= interaction between concentration and extract

$(P_i \times C_j \times X_k)$= interaction between plant, concentration and extract

E_{ijkl}= random error

5.3 Results

5.3.1 *In vitro* repellency bioassay

Tick repellency increased significantly ($P<0.05$) with concentration when tested on acetone, methanol and ethanol extracts of *A. ferox* and *A. oppositifolia* plants (Table 5.2). The highest tick repellency (83.33%) was observed at 50% concentration in *A. ferox* acetone extract while 50% methanol extract of *A. oppositifolia,* had the highest mortality of 89%. No repellency was observed for 15% methanol extract of *A. ferox,* 15% acetone extract of *A. oppositifolia* and for distilled water. Generally there was a decrease in tick repellency with time in acetone extract of

A. ferox and *A. oppositifolia*. Diazinon at 15% had the same acaricidal activity as 50% acetone extract of *A. ferox* and 50% ethanol and methanol extracts of *A. oppositifolia*.

TABLE 5. 2 LEAST SQUARE MEANS ± STANDARD ERROR SHOWING PERCENTAGE TICK REPELLENCY OF *A. FEROX*, *A. OPPOSITIFOLIA* AND DAZZEL DIP AT DIFFERENT CONCENTRATIONS

Material	**Extract**	**Concentration**	**Time**						
			30mins	**1hr**	**2hr**	**3hr**	**4hr**	**5hr**	**6hr**
			n=6	**n=6**	**n=6**	**n=6**	**n=6**	**n=6**	**n=6**
A.ferox	Acetone	15	22.00^{g}	15.00^{h}	6.00^{i}	nil	nil	nil	nil
		30	67.00^{d}	63.00^{c}	46.00^{e}	61.00^{d}	39.00^{f}	46.00^{e}	50.00^{e}
		50	85.33^{b}	81.67^{b}	63.00^{c}	65.00^{c}	65.00^{c}	63.00^{c}	61.00^{d}
	Ethanol	15	13.00^{h}	6.00^{i}	nil	nil	nil	nil	nil
		30	46.00^{e}	40.67^{e}	46.00^{e}	42.33^{f}	39.00^{f}	44.00^{e}	44.00^{f}
		50	65.00^{d}	67.00^{c}	67.00^{b}	61.00^{d}	65.00^{c}	63.00^{c}	67.00^{c}
	Methanol	15	Nil	Nil	nil	nil	nil	nil	nil
		30	13.00^{h}	17.00^{g}	15.00^{h}	20.33^{h}	26.00^{g}	24.00^{f}	31.33^{g}
		50	31.33^{f}	28.00^{f}	37.00^{f}	35.00^{g}	42.33^{f}	48.00^{e}	50.00^{e}
A.oppositifolia	Acetone	15	Nil	Nil	nil	nil	nil	nil	nil
		30	44.00^{e}	40.67^{e}	28.00^{g}	35.33^{g}	37.00^{f}	20.67^{f}	17.00^{h}
		50	65.00^{d}	65.00^{c}	61.00^{c}	50.00^{e}	50.00^{e}	57.33^{d}	61.00^{d}
	Ethanol	15	6.00^{i}	Nil	nil	nil	nil	nil	nil
		30	48.00^{e}	50.00^{d}	57.33^{d}	63.00^{d}	61.00^{d}	63.00^{c}	67.00^{c}
		50	85.00^{b}	79.67^{b}	67.00^{c}	67.00^{b}	67.00^{d}	67.00^{c}	67.00^{c}
	Methanol	15	11.00^{i}	11.00^{i}	nil	nil	nil	nil	nil
		30	78.00^{c}	78.00^{b}	67.00^{c}	65.00^{c}	67.00^{d}	67.00^{c}	61.00^{d}
		50	83.00^{b}	89.00^{a}	83.00^{a}	83.00^{a}	83.00^{a}	83.00^{a}	83.00^{a}
Diazinon		15	83.00^{b}	83.00^{b}	83.00^{a}	78.00^{a}	67.00^{d}	65.00^{c}	55.67^{e}
		30	100.00^{a}	83.00^{b}	83.00^{a}	78.00^{a}	78.00^{b}	78.00^{b}	78.00^{b}
SE			1.49	1.30	1.29	1.32	0.97	1.66	1.32

^{a,b,c,d,e,f,g,h,i}Means in the same column with different superscripts are significantly different at, $P<0.05$

5.3.2 Contact bioassay

Acaricidal activity of *A. ferox* and *A. oppositifolia* increased ($P<0.05$) with increase in extra concentration (Table 5.3). All tested materials showed acaricidal activity with the exception of 15 and 30% acetone extract of *A. oppositifolia.* Th highest tick mortality of 100% in *A. ferox* extract was observed in 30 and 50% acetone extract while 50% ethanol extract of *A. oppositifolia* had the highest tick mortality of 83.33%. The tick mortality was similar ($P>0.05$) to the positive control (Diazinon) at these concentrations. There was no mortality observed in distilled water, 15 and 30% concentration of acetone extract of *A. oppositifolia*. *Aloe ferox* exhibited better contact activity as compared to *A. oppositifolia* when compared at the same concentration and solvent extract.

TABLE 5. 3 LEAST SQUARE MEANS ± STANDARD ERROR SHOWING TICK MORTALITY OF CRUDE EXTRACTS OF *A. FEROX*, *A. OPPOSITIFOLIA* AND DAZZEL DIP AT DIFFERENT CONCENTRATIONS

		Tick mortality %	n=10					
	Material Concentration	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Total mortality (%)**
Acetone	*A.ferox 15%*	20	10	30	0	0	0	20	83.33[b]
	A.ferox 30%	70	0	0	20	10			100.00[a]
	A.ferox 50%	100							100.00[a]
Methanol	*A.ferox 15%*	10	20	10	10	0	0	10	63.33[d]
	A.ferox 30%	20	20	0	0	30	0	0	73.33[c]
	A. ferox50%	30	20	30	0	0	0	10	73.33[c]
Ethanol	*A.ferox 15%*	20	20	20	10	0	0	0	83.33[b]
	A.ferox 30%	30	20	10	10	0	0	10	70.00[c]
	A.ferox 50%	50	30	10	0	0	0	10	80.00[b]
Acetone	*A.oppositifolia 50%*	20	0	0	0	0	0	0	16.67[g]
Methanol	*A.oppositifolia15%*	10	0	0	40	0	0	0	43.33[f]
	A.oppositifolia 30%	20	0	50	0	0	0	0	66.67[d]
	A.oppositifolia 50%	20	30	0	0	0	10	0	63.33[d]
Ethanol	*A.oppositifolia15%*	10	10	0	0	0	0	0	20.00[g]
	A.oppositifolia 30%	20	40	0	0	0	10	0	53.33[e]
	A.oppositifolia 50%	20	30	10	10	0	0	0	73.33[c]
Dazzel dip 15%		80	0	20					100.00[a]
Dazzel dip 30%		100							100.00[a]
Distilled Water		0	0	0	0	0	0	0	0[h]
SE									2.53

[a,b,c,d,e,f,g]Means with different superscripts are significantly different at, P<0.05 SE=standard error

5.4 Discussion

5.4.1 Repellency activity of plants

Many plants have demonstrated acaricidal and repellency activity against ticks (Jbilou *et al*., 2006). The acetone extract of *A. ferox* showed the highest tick repellency although it had a short-live protection. This study is in agreement with findings by Mawela (2008) who reported that the highest tick repellency was found in acetone extract of *A. ferox* at 30% concentration when tested on *Rhipicephalus appendiculatus* ticks as compared to methanol and dichloromethane. However this study is not in sync with Fourie *et al*. (2001), who found that powdered *A. ferox* juice did not kill ticks when tested on *R. decoloratus* ticks on dogs. Moyo and Masika (2013) also reported that *A. ferox* showed no tick repellency at 20 and 40% concentration using water as a solvent. The use of different solvents could explain the anomaly in tick repellency. Goli *et al*. (2005) reported that the number of active compounds extracted depend on the solvent and method of extraction used. In this study, the use of different solvents could have caused the difference in repellency and acaricidal activity. Different factors such as the age of the plant, environmental and storage conditions may have alos potentially affected the results (Kokkini *et al*., 1997). This is because the age of the plant determines its medicinal potency as it governs the relative proportions of the active principles (Roy *et al.,* 1989). It has also been reported that improper storage of these plants harbor mycotoxin producing fungi which compromise the bioactive compounds in the plants (Horie *et al*., 1979). These factors should therefore be taken into consideration when preparing ethno-veterinary medicine otherwise their efficacy will be compromised.

Although both plants demonstrated repellency activity, acetone extract provided a short lasting repellency period of 30mins in *A. ferox*. Acetone has been shown to be effective in extracting

volatile compounds (Mawela, 2008) and this may suggest that these types of compounds were present in the *A. ferox* plant since it had short-lived repellency. Repellency activities of plants are usually as a result of volatile hydrocarbons which comprise mostly of sesquiterpenes and monoterpenes (Nerio *et al*., 2010). Volatile hydrocarbons act at a vapor phase and that is why the acetone extract of the plant was effective for a short period of time (Zhu *et al*., 2001). The high volatility of these hydrocarbons decreases their activity and this will not be enough to protect livestock against ticks. In this study, *A. ferox* and *A. oppositifolia* showed significant repellency activity against ticks and this could be attributed to the pungent smell which repels ticks.

Repellency activity of *A. ferox* showed that usually non polar compounds are responsible for the repellency activity of plants as they require a degree of volatility for ticks to sense their presence (Nema *et al*., 2013). Although repellency is mostly attributed to a particular compound in a plant, it has been reported that at times compounds work in synergy and this increases the bioactivity of the plant (Omolo *et al*., 2004). Methanol extract of *A. oppositifolia* showed repellency activity against ticks higher than *A. ferox.* These results showed that less polar compounds were present in the *A. oppositifolia* plant and were responsible for the repellency activity. The efficacy of *A. oppositifolia* plant could be due to the presence of terpenes. Terpenes are natural products from micro-organisms, plants and animals which are known to have strong biological activities against parasites (Cheng *et al*., 2014). *Acokanthera oppositifolia* was found to contain oxygenated hemiterpenes when subjected to GC-MS. Oxygenated terpenes are more valuable in plants as they produce a strong scent that has defense mechanisms against parasites as compared to other terpenes (Kessler and Baldwin, 2001). There is lack of information of previous use against ticks and this is the first study to report the tick repellency and acaricidal activity of *A. oppositifolia*.

Previous studies reported that *A. oppositifolia* is used in the treatment of tapeworms and anthrax (Dold and Cocks, 2001).

5.4.2 Acaricidal activity of plants

The 30 and 50% acetone extract and 50% ethanol extract of *A. ferox* caused the highest mortality in the two ticks' species. There was no difference (P>0.05) in tick mortality between Diazinon and 50% ethanol extract of *A. oppositifolia* and acetone extract of *A. ferox* (30 and 50%). This shows that these extracts contain bioactive compounds which have acaricidal effects. The acaricidal activity in these plants could be as a result of terpenes present in the plants. When *A. ferox* was subjected to the GC-MS (Chapter 4) , it was found to contain terpenes, with oxygenated sesquiterpenes being the most abundant followed by oxygenated hemiterpenes. Sesquiterpenes have phytoalexins which are involved in the direct defense against pathogens (Dudareva *et al*., 2004). Terpenes are known to produce smell that has defense mechanisms against parasites (Tawatsin *et al*., 2001). Terpinen-4-ol was also found in the *A. ferox* plant and this compound is used as an insect repellent against ticks and mites (Dudareva *et al*., 2004). The presence of terpinen-4-ol in *A. ferox* may suggest that this compound could also be responsible for the repellency effect. *Aloe ferox* has been reported to possess pharmacologically active substances. It is also known as an insect repellent, used to treat heartwater and gallsickness (Van Wyk *et al*., 2002), sheep scab and in the control of ticks in cattle (Moyo and Masika, 2009). Tick mortality could be as a result of anthranoids and anthraglycosides present in the leaves of *A. ferox* which have defense mechanism against parasites (Van Wyk and Wink, 2004). *Aloe ferox* has also been found to have anti-inflammatory effects due to the presence of three malic acid acylated carbohydrates (Steenkamp and Stewart, 2007). Therefore *A. ferox* can also be used as an acaricide to relieve inflammation and pain in livestock caused by tick infestations

5.5 Conclusion

Results from this study revealed that *A. ferox* and *A. oppositifolia* plants have repellency and acaricidal activities. The 30 and 50% acetone extract of *A. ferox* and 50% ethanol extract of *A. oppositifolia* and Dazzel dip had the highest acaricidal properties. Methanol extract (50%) of *A. oppositifolia* and acetone extract (50%) of *A. ferox* had the highest repellency activity. *Aloe ferox* and *A. opppositifolia* plants have a greater potential as acaricides and repellents. Further investigation should be done to evaluate the safety of these plants before assessing their efficacy *in vivo* so as to develop remedies for commercial purpose.

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CHAPTER 6

Anthelmintic activity of *Elephantorrhiza elephantina* and *Albuca setosa* plant extracts in the control of gastro-intestinal nematodes

Abstract

(Submitted to *Tropical Animal Health and Production)*

The objective of the study was to screen *Elephantorrhiza elephantina* and *Albuca setosa* plant extracts as potential anthelminthics. The study sought to determine the potential of *E. elephantina* and *A. setosa* extracts as anthelminthics. Paralysis and helminthiasis effect of *E. elephantina* and *A. setosa* extracts at various concentrations of (20, 50, 100mg/ml) was investigated. Albendazole was used as a positive control. Crude extracts of *E. elephantina* and *A. setosa* showed significant anthelminthic effect but time of death and paralysis was different between them. In this study, E. *elephantina* and *A. setosa* caused paralysis and mortality in all extracts even at low concentrations. Methanol was the most effective solvent in extracting bioactive compounds and showed the best anthelminthic effects among the crude extracts investigated in both plants. The least time taken for the worms to be paralysed was 8.33 mins and 14.33mins in 100mg/ml methanol extracts of *E. elephantina* and *A. setosa*, resepectively. Methanol extract of *E. elephantina* and *A. setosa* (100mg/ml) had the highest anthelminthic activity and the nematodes died after 18mins and 20mins, respectively. Results from this study gives 100mg/ml methanol extract of *E.elephantina* and *A. setosa* the credence in its ethnoveterinary use against gastro-intestinal parasites. However further research should be done to evaluate the safety of these plants before conducting *in vivo* experiment so as to recommend appropriate dosages and avoid toxicity.

Keywords: efficacy, extract, helminthes, medicinal plants

6.1 Introduction

Gastro-intestinal tract parasites pose a huge health problem in small ruminants (Vatta and Lindberg, 2006). They are more prevalent in places where there is poor management of grazing pastures (Masika and Mafu, 2004). Infestation results in poor productivity and poor quality products (Perry and Randolph, 1999; Njoroge and Bussmann, 2006). Farmers use anthelminthic drugs to control endo-parasites but their effectiveness is compromised due to the development of resistance against some of the drugs (Sargison, 2011). Due to the huge economic impact of nematodes and a number of disadvantages presented by commercial drugs, there is now interest in using indigenous plants to control gastro-intestinal parasites.

Plants play an important role in protecting livestock from pathogens. They are known to possess defense mechanisms against pathogens and this has led scientists to look for bioactive compounds from plants which are effective against these parasites (Sheludko, 2010). *Elephantorrhiza elephantina* and *Albuca setosa* are used to control gastro-intestinal parasites by resource-limited farmers in the Eastern Cape Province, South Africa. Although farmers are using these plants to control parasites, their pharmacological preparations and dosages lack scientific evidence. Moreover, no study has been done to validate their efficacy on nematodes. This study was therefore conducted to determine the efficacy of *E. elephantina* and *A. setosa* plant extracts on gastro-intestinal nematodes.

6.2 Materials and Methods

6.2.1 Study site

The study was conducted in the Animal Science laboratory, University of Fort Hare.

6.2.2 Description of material used in the study

Description of plant materials used in the study is as described in Chapter 4 section 4.3.

6.2.2.1 Plant collection

Description of plant material and collection is as described in Chapter 5.2.3.

6.2.2.2 Description of reference anthelminthic drug

Valbazen is a broad spectrum anthelmintic drug which belong to the benzimidazole class and is effective against roundworms, flukes and tapeworms. The active ingredient in the drug is albendazole.

6.2.3 Worms

Adult nematodes were collected from the gastro-intestinal tracts of goats slaughtered in Adelaide abattoir. Small, large intestines and abomasum were brought to the lab were the contents were washed in tap water and adult nematodes collected. The worms were placed in a petri dish with phosphate buffer saline and validation of acetone, methanol and ethanol extracts of *Elephantorrhiza elephantina* and *Albuca setosa* on nematodes was then performed.

6.2.4 *In vitro* screening of nematodes

Acetone, ethanol and methanol extracts of *E. elephantina* and *A. setosa* were tested at different concentrations (20mg/ml, 50mg/ml and 100mg/ml). Albendazole was used as a positive control. A total of 10 worms per treatment were used for each concentration per extract and control. Worms were considered alive if they just moved or if they wriggled after being touched. If they exhibited very minimal movement, they were considered to be in a state of paralysis. Otherwise, they were considered dead if they did not exhibit motility. The treatment was replicated thrice.

The time was recorded when the nematodes were considered paralysed and when there were considered dead.

6.2.5 Statistical Analysis

The collected data were then analyzed using PROC GLM of SAS (2003). A probability value of less than 5% was used to denote a significant difference. Tukey's test was used to compare differences between treatment means.

6.3 Results

The different crude extracts showed variable times of paralysis and death at different concentrations. All the crude extracts of *E. elephantina* and *A. setosa* showed significant ($P<0.05$) anthelminthic effect but time of paralysis and death was different in each case. There was a linear relationship between different concentrations of crude extracts of *E. elephantina* and *A. setosa* and the time taken for the nematodes to die (Figures 6.1, 6.2 and 6.3). An increase in the concentration resulted in stronger anthelminthic effect on the nematodes. Higher concentration of crude extracts produced anthelminthic and paralytic effects much earlier than lower concentrations. The time taken for the worms to be paralysed was the same in 50mg/ml methanol extract of *A. setosa* and that Albendazole. In terms of mortality, the time taken for all the worms to die was the same with 100mg/ml methanol extract of *E.elephantina*. Generally, methanol extract showed the best anthelminthic effects among the crude extracts investigated in both plants compared to acetone and ethanol extracts. The least time taken for the worms to be paralysed was 8.33 mins and 14.33mins in 100mg/ml methanol extracts of *E. elephantina* and *A. setosa*, respectively. Methanol extract of *E. elephantina* and *A. setosa* (100mg/ml) had the highest anthelminthic activity and this was noted after 18mins and 20mins, respectively. Effect

of crude extracts on paralysis and helminthiasis according to Table 6.1 may be shown as methanol>ethanol>acetone.

Table 6.1 Least Square Means ± Standard Error of anthelmintic activity of *E. elephantina, A. setosa* and positive control (Albendazole)

Material	Extract	Concentration (mg/ml)	Paralysis (mins)	Mortality (mins)
			n=10	**n=10**
E. elephantine	Acetone	20	51.00^{c}	62.33^{b}
		50	45.67^{d}	55.33^{c}
		100	28.33^{g}	37.67^{e}
	Ethanol	20	32.67^{f}	47.67^{d}
		50	26.33^{g}	38.33^{e}
		100	17.33^{i}	25.00^{g}
	Methanol	20	21.67^{h}	30.00^{f}
		50	18.00^{i}	24.67^{g}
		100	8.33^{k}	18.00^{h}
A. Setosa	Acetone	20	59.00^{a}	69.00^{a}
		50	56.00^{b}	63.00^{b}
		100	33.00^{f}	46.00^{d}
	Ethanol	20	40.00^{e}	51.67^{c}
		50	29.00^{g}	39.67^{e}
		100	20.33^{h}	31.33^{f}
	Methanol	20	25.33^{g}	37.00^{e}
		50	21.00^{h}	31.33^{f}
		100	14.33^{j}	25.00^{g}
Albendazole		20	16.33^{i}	20.00^{h}
Standard Error			0.46	0.46

^{a,b,c} Means in the column with different superscripts are significantly different at $P<0.05$

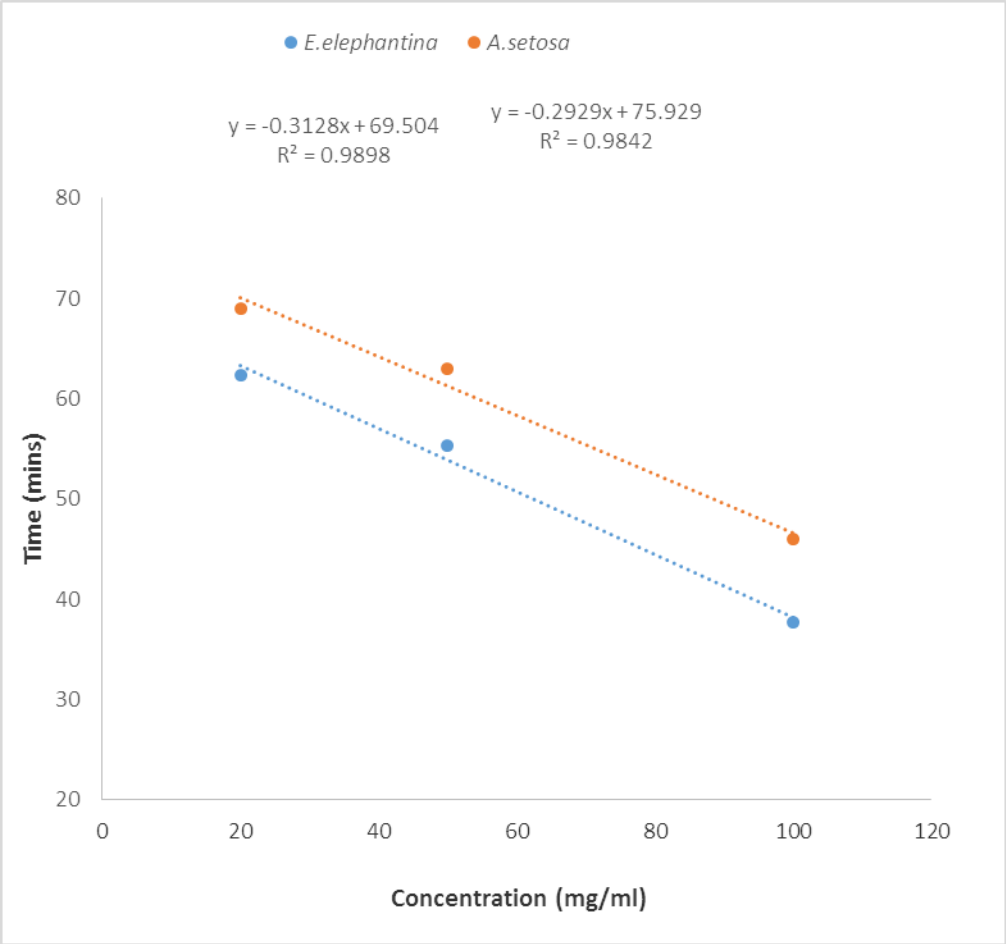

FIGURE 6. 1 CORRELATION OF ACETONE EXTRACT OF E. ELEPHANTINA AND A. SETOSA AND TIME TAKEN FOR NEMATODES TO DIE

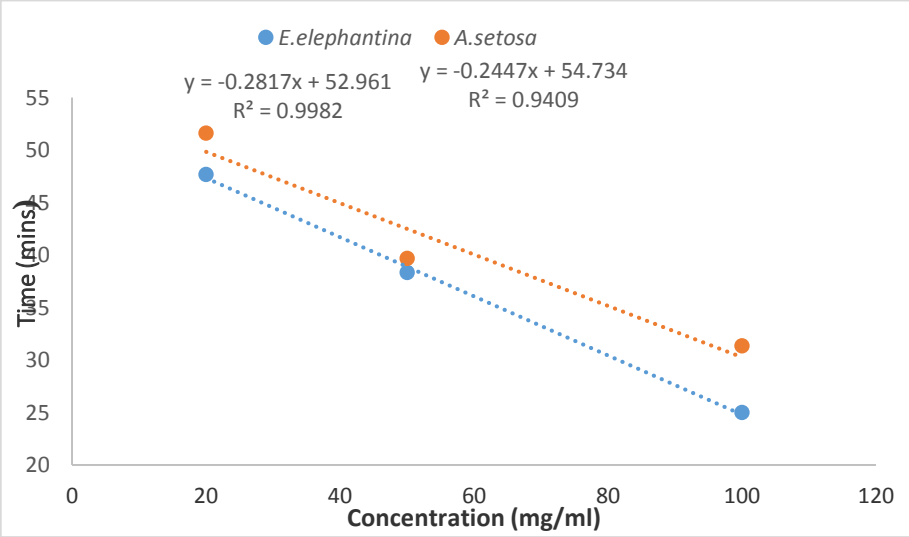

FIGURE 6. 2 CORRELATION OF ETHANOL EXTRACT OF *E. ELEPHANTINA* AND *A. SETOSA* AND TIME TAKEN FOR NEMATODES TO DIE

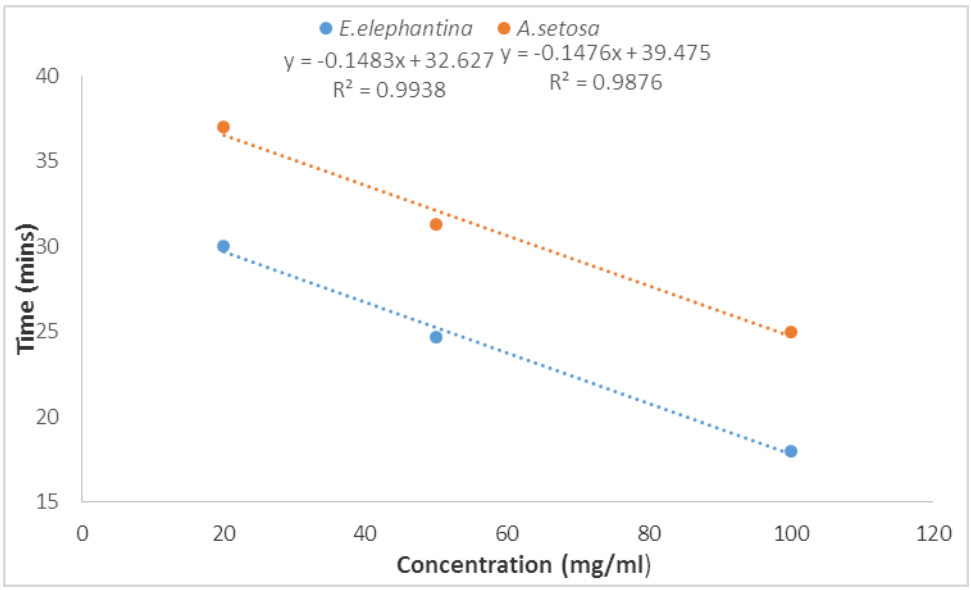

FIGURE 6. 3 CORRELATION OF METHANOL EXTRACT OF *E. ELEPHANTINA* AND *A. SETOSA* AND TIME TAKEN FOR NEMATODES TO DIE

6.4 Discussion

In vitro screening of crude extracts of *E. elephantina* and *A. setosa* demonstrated anthelmintic effects on gastro-intestinal nematodes of goats. The findings of this study are in agreement with Maphosa *et al*. (2010), who reported anthelmintic activity of aqueous extract of *E.elephantina* against *Haemonhus contortus*. *Elephantorrhiza elephantina* has also been found to be effective against coccidiosis in goats (Tyasi *et al*., 2015). In this study, *E. elephantina* and *A. setosa* extracts caused paralysis and mortality at all concentrations investigated. This shows that *E. elephantina* and *A. setosa* plant extracts are very effective against nematodes. *A. setosa* has been reported to control gastro-intestinal parasites in livestock (Maphosa and Masika, 2012). *Elephantorrhiza elephantina* plant can be used as a broad spectrum drug as it has also been reported to have anthelminthic, antirickettsial (Naidoo *et al*., 2006), antibacterial and antifungal activities (Aaku *et al.,* 1998).

There was a linear releationship between all the concentrations of three crude extracts of *E. elephantina* and *A. setosa* and the time taken for paralysis and mortality of nematodes to occur though there were variations. The good correlation between concentrations and anthelminthic activity of the plants may suggest that the higher concentrations dissolved successfully (Nchu *et al*., 2005). The difference in the degree of helminthiasis between variable extracts might be due to the level of tannins extracted in the compounds. Ncube *et al*. (2008) reported that aqueous methanol was effective in extracting tannins whereas aqueous acetone was effective in extracting phenolics. Many factors such as the age of leaf, season of plant collection and storage conditions could have affected the active compounds extracted (Kokkini *et al*., 1997). Goli *et al*. (2005) reported that active compounds extracted depend on the solvent used and this could have also caused the difference in anthelminthic activity of *E. elephantina* and *A. setosa* plant extracts.

In this study, methanol proved to be the best extraction solvent as its extract had the highest anthelminthic activity. The least time taken for the plant extracts to cause paralysis and mortality in nematodes was recorded in 100mg/ml methanol extract of *A. setosa* and *E. elephantina* where it also exhibited same anthelmintic activity as that of reference (Albendazole). Albendazole showed anthelminthic activity and this could be due to binding effect of the drug to the protein tubulin of the parasite (Lalchhandama, 2009). Results from this study gives 100mg/ml methanol extract of *E.elephantina* and *A. setosa* the credence in ethnoveterinary use against gastro-intestinal parasites. However, it is important to evaluate the toxicity of these plant extracts *in vivo*

Plants which have anthelminthic effects have been reported to have alkaloids, saponins, flavonoids and phenolic compounds (Jackson and Miller, 2006). Phytochemical analysis of *E. elephantina* showed that this plant contain flavonoids (Palombo, 2006) while the rhizomes contained tannins (Naidoo *et al*., 2005). Anthelminthic activity of *E. elephantina* plant could be as a result of these compounds. Tannins are polyphenolic compounds that have chemical defenses against predators (Bernays *et al*., 1989; Athnasiadou *et al*., 2001). Molan *et al*. (1999) reported that condensed tannins reduce egg viability and nematodes larval development. They also interact with proteins of oesophagus, cloaca, cuticle, oral cavity and vulva of nematodes (de Oliveira *et al*., 2009). Tannins also damage important processes such as reproduction and feeding of the parasite (Chanda and Dave, 2009). Other studies which were conducted using different tannin containing forages reported a significant decrease in Fecal egg reduction (FER) and worm burdens using quebracho in sheep infested with *Trichostronglus colubriformis*. Other bioactive compounds such as polyphenols and flavonoids also have anthelminthic effects. Polyphenols affect oxidative phosphorylation in helminths (Martin, 1997). Flavonoids are used in controlling

human and parasitic diseases such as redwater and heartwater (Kerboeuf et al., 2008). Alkaloids have also been reported to affect the nervous system of nematodes (Lateef *et al*., 2003). Saponins are known to disrupt cell membrane and they change the morphology of the cells (Geidam *et al*., 2007). The anthelminthic activity of these plants could be attributed to the presence of these bioactive compounds.

6.5 Conclusion

This study showed that *E. elephantina* and *A. setosa* have anthelminthic activities. Crude extracts of *E. elephantina* and *A. setosa* caused paralysis and mortality in nematodes. The least time taken for the worms to be paralysed was 8.33 mins and 14.33mins. This was noted in 100mg/ml methanol extracts of *E. elephantina* and *A. setosa*, resepectively. Methanol extract of *E. elephantina* and *A. setosa* (100mg/ml) had the highest anthelminthic activity and this was recorded after 18mins and 20mins, respectively. Methanol was the best solvent in extracting bioactive compounds responsible for anthelminthic activity. The use of these plants in controlling gastro-intestinal parasites could promote good animal health by serving as potential sources of compounds in the development of new herbal anthelminthics. Further research should be done to evaluate the efficacy of these plants *in vivo* and to determine their pharmacological action.

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Chapter 7: General discussion, conclusions and recommendations

7.1 General Discussion

The Eastern Cape Province, South Africa has the largest agricultural sub-sector with significant numbers of goats and other livestock (Eastern Cape Development Corporation, 2011). Goats play a major role in Africa particularly for remote and poor communities (Peacock, 2005). However, their production is affected by several challenges, the major one being the prevalence of internal and external parasites. Acaricides and anthelmintics have been used to control parasites for many years but their effectiveness is compromised due to a number of reasons. Some of the reasons are that most of the parasites have developed resistance against some of the drugs, conventional drugs are expensive, leave residues in meat and milk and are toxic to the environment (Sargison, 2011). Due to the huge economic impact of parasites and a number of disadvantages offered by commercial drugs, there is now interest in using indigenous plants to control parasites (Maphosa and Masika, 2012). The objective of the study was to determine and validate medicinal plants used to control internal and external parasites in goats.

As one of the key objectives of the current study, a survey was conducted to document medicinal plants used to control internal and external parasites in goats (Chapter 3). A total of nine plant species were identified. Plant parts used and preparation methods were also determined. Parasites were reported as the most problematic challenge that the farmers are facing. Ticks and helminths were the most prevalent parasites in the area. Fidelity Level (FL) was calculated for all the plants and those with the highest FL were used for the other experiments; *Aloe ferox*, *Acokanthera oppositifolia, Albuca setosa* and *Elephantorrhiza elephantina*. Trotter and Logan (1986) reported that plants which are constantly used by people in a certain area are more likely to contain bioactive substances.

As a result the chemical profile of the four acetone plant extracts with the highest FL was determined using GC-MS (Chapter 4). Gas Chromatography Mass Spectrometry revealed the presence of 7, 33, 26 and 32 chemical compounds in *A. ferox, E. elephantina, A. oppositifolia* and *A. setosa,* respectively. Most of the compounds present in the plants were terpenes and fatty acids esters. According to Cheng *et al*. (2014), terpenes are natural products from micro-organisms, plants and animals which have strong biological activities against parasites. The outcome of the study suggested that these compounds have biological activities against parasites and this led to the screening of these plants as potential acaricides and anthelminthics.

In Chapter 5, crude extracts of *A. ferox* and *A. oppositifolia* were then validated for their efficacy *in vitro* against *Ambylomma hebraeum* and *Rhipicephalus decoloratus* ticks. *In vitro* repellency and contact bioassay revealed that the two plants possess repellent and acaricidal activities. The results showed that 30 and 50% acetone extracts of *A. ferox* were a stronger treatment in causing mortality in the two ticks' species. The highest tick repellency (83.33%) was observed at 50% concentration in *A. ferox* acetone extract while in the *A. oppositifolia* plant, the 50% methanol extract caused 89% mortality. Repellency activity of *A. ferox* has been reported in earlier work by Mawela (2008) in acetone extract of *A. ferox* at 30% concentration when tested on *Rhipicephalus appendiculatus* ticks. However studies conducted by Fourie *et al*. (2001) and Moyo and Masika (2013) did not show tick repellency when tested on ticks using water as a solvent. The different solvents used for extracting bioactive compounds could have caused the difference. The outcome of the study suggested that the efficacy of the plants could be attributed to the compounds reported in Chapter 4.

Helminthiasis and paralysis effect of crude extracts of *E. elephantina and A. setosa* against nematodes was investigated in Chapter 6. In this study, E. *elephantina* and *A. setosa* caused

paralysis and mortality in all extracts even at low concentrations. Methanol was the most effective solvent in extracting bioactive compounds and showed the best anthelminthic effects among the crude extracts investigated in both plants. The findings of this study are in agreement with Maphosa *et al*. (2010) who reported anthelmintic activity of aqueous extract of *E.elephantina* against *H. contortus*. *Elephantorrhiza elephantina* has also been found to be effective against coccidiosis in goats (Tyasi *et al*., 2015). *Albuca setosa* has been reported to control gastro-intestinal parasites in livestock (Maphosa and Masika, 2010). There was a linear releationship between all the concentrations of three crude extracts of *E. elephantina* and *A. setosa* and the time taken for paralysis and mortality of nematodes to occur though there were variations. The good correlation between concentration and mortality may suggests that the higher concentration dissolved effectively (Nchu *et al*., 2005).

7.2 Conclusions

The study revealed that resource-limited farmers in the Eastern Cape Province, South Africa use medicinal plants to control internal and external parasites. Chemical analysis of *A. ferox*, *A. oppositifolia, A. setosa* and *E. elephantina* plants used by the farmers revealed that they contain bioactive compounds which have a huge potential in controlling parasites. Furthermore, in the *in vitro* studies, *A. ferox* and *A. oppositifolia* exhibited both repellency and acaricidal activities against ticks. The study also revealed that efficacy of medicinal plants used by farmers to control ticks vary with the type of solvent used for extracting bioactive compounds. Crude extracts of *E. elephantina* and *A. setosa* also caused significant helminthiasis and paralysis effect in nematodes.

7.3 Recommendations

Although findings of this study have shown that *A. ferox and A. oppositifolia* have acaricidal and repellent activities whilst *E. elephantina* and *A. setosa* showed anthelminthic activity, it is

imperative to evaluate the toxicity of these plants and the potential side effects. Some of the plants such as *A. oppositifolia* and *E. elephantina* should be used with extra caution since they are poisonous.

Further research should focus on validating the efficacy of these plants *in vivo.* It is also crucial to assess the safety of these plants and recommend appropriate dosages. In addition it is imperative to determine the mechanism of action of these plant materials against ticks and helminths. An attempt to isolate the bioactive compounds with anthelminthic and acaricidal activity from the extract fractions of the plants is important in developing herbal remedies.

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8.1 Appendix

Determination of medicinal plants used to control external parasites in goats by farmers in Eastern Cape Province, South Africa

Date ---------- Questionnaire number ----------

Enumerator's name ---------- Household name ----------

Village ----------

SECTION A: DEMOGRAPHIC INFORMATION OF RESPONDENT

1. Gender Male ☐ Female ☐

2. Age ≤19 ☐ 20- 30 ☐ 31-50 ☐ ≥51

3. Marital status Single ☐ Married ☐ Widowed ☐ Divorced ☐

4. Level of education Primary ☐ Secondary ☐ Tertiary ☐ Never went to school ☐

5. Employment status Employed ☐ Unemployed ☐ Self-employed ☐ Retired ☐

6. Size of household Male ☐ Female ☐

7. What is your source of income? Salary ☐ Livestock ☐ Crop farming

Other (specify) ☐----------

8. Total monthly income ≤1000 ☐ 1100-3000 ☐ 3000-8000 ☐ ≥8000 ☐

SECTION B: LIVESTOCK INVENTORY

9. Do you own any form of livestock? Yes ☐ No ☐

10. Which form of livestock do you own and provide the numbers?

Livestock	**Numbers**
Cattle	
Sheep	
Goats	
Pigs	
Chicken	
Other (specify)	

11. Why do you keep the mentioned livestock above? Meat ☐ Milk ☐

Income ☐ Cultural/Religious ☐ Other (specify) .☐----------

12. Challenges faced in livestock rearing Diseases ☐ Parasites ☐ Stock theft ☐ Poor rangelands ☐ Other (specify) ----------

13. Which is the most problematic challenge that you face? ----------

SECTION B: PREVALENCE OF DISEASES AND PARASITES

14. What are the major diseases and parasites in your area? External Parasites☐ Internal ☐ parasites ☐ Heartwater ☐ East coast fever ☐ Tick borne fever☐ Anaplasmosis ☐ Ehrlichiosis ☐ Redwater ☐ Other (specify) ----------

15. Prevalent diseases in your herd. Provide local name/symptoms, animals affected, if treated when sick and rank the most common disease first

Local name/symptoms	Animal affected	Treated if sick (yes /no)

16. Are your livestock affected by external parasites in your area? Yes☐ No☐

17. Which parasites are more common in your area and which animals are affected? Rank according to prevalence

1. More prevalent 2. Moderate 3. Less prevalent 4. Not prevalent

External parasite	**Time most prevalent**	**Degree of prevalence**	**Animals affected**
Mites			
Ticks			
Lice			
Fleas			
Other (specify)			

18. How do you tell that a goat is infested by parasites? Explain ----------

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SECTION C: DISEASE CONTROL

19. How do you treat diseases in your herd? Medicinal plants ☐ Acaricides ☐ Both ☐

Other (specify) ☐ ----------

20. Give reason (s) why you use that method ----------

---

21. If animals are sick whose advice do you seek? Government veterinarian ☐ Private veterinarian ☐ Veterinary drug suppliers ☐ Extension officers ☐ None ☐

Others(specify)----------

22. Name the acaricides that you usually use in your area to treat diseases and how you use them.

Name of Acaricide	**Method of use**

SECTION D: PARASITE CONTROL

23. Do you dip your goats? Yes ☐ No ☐

24. If yes, how often? ----------

25. If no, why? ----------

---

26. What dipping method do you use? ----------

27. How do you control external parasites in your herd? Medicinal plants ☐ Acaricides ☐

Both ☐ Other (specify) ☐ ----------

28. Give reason (s) why you use that method ----------

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29. Name the acaricides that you usually use in your family to control parasites and how you use them.

Name of Acaricide	**Method of use**

30. Fill in the table below (**for those who use plants)**

Name of plant used	Type of parasite controlled	Plant parts used	Condition used (fresh/ dry)	Preparation method	Method of application	Side effects noticed

31. Rank the plants**.** **5 MOST EFFECTIVE PLANTS**

PLANT NAME

32. Who has more knowledge on medicinal plants in your family? ----------

33. How was that knowledge acquired? ----------

34. How is that knowledge passed on to others? ----------

35. For how long have you been using these plants to control parasites? ----------

36. Are there any conservation methods you put in place to avoid plants from becoming extinct?

Yes ☐ No ☐

37. If yes, explain how? ----------

38. If no, why? ----------

39. In your opinion, do you think plants should be used more to control parasites? Yes ☐ No ☐

40. If yes, why? ----------

41. If no, why and what do you think is the best method for controlling external parasites ---------

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