# MEDICINAL PROPERTIES OF PLANTS PREDOMINANTLY USED FOR ETHNO-VETERINARY PURPOSES IN THE HIGHLAND GRASSLANDS OF THE FREE STATE PROVINCE, SOUTH AFRICA, AND LESOTHO

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Central University of Technology, Free State

# DECLARATION OF INDEPENDENT WORK

## DECLARATION OF INDEPENDENT WORK

I, **TANKISO MOTS'OARI**, passport number _____ and student number _____, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Master of Health Sciences in Environmental Health Sciences, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself of any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

_____ _____

**SIGNATURE OF STUDENT** **DATE**

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## ABBREVIATIONS

| | |
|---|---|
| ATCC | American Type Culture Collection |
| CSIR | Council for Scientific and Industrial Research |
| CTM | Chinese Traditional Medicine |
| DMEM | Dulbecco's Modified Eagle's Medium |
| EVM | Ethnoveterinary medicine |
| FBS | Foetal bovine serum |
| INT | *p*-iodonitrotetrazolium violet |
| *Mbovis* | *Mycobacterium bovis* |
| MH broth | Mueller Hinton broth |
| M.tb | *Mycobacterium tuberculosis* |
| NCD | Newcastle disease |
| PBS | Phosphate buffer solution |
| RIF | Rifampicin |
| SANBI | South African National Botanical Institute |
| StatsSA | Statistics South Africa |
| TTBD | Ticks and tick-borne disease |
| WAAVP | World Associations for Advancement of Veterinary Parasitology |
| WHO | World Health Organization |
| WOAH | World Organization for Animal Health |

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## ABSTRACT

The use of medicinal plants to maintain animal health in most parts of the world is well-known. It is also common for ethnoveterinary knowledge to be orally passed from generation to generation. However, this knowledge can easily be lost or distorted if not properly documented. The present study, therefore, aimed to identify and document the commonly used medicinal plants for ethnoveterinary practices in the QwaQwa and Thaba 'Nchu areas of the Free State province in South Africa and Maseru and Mokhotlong towns of Lesotho. Furthermore, the most predominantly used plants were tested for phytochemical constituents, antimicrobial, cytotoxicity, and anthelmintic activities. Semi-structured interviews for ethnobotanical information were held with 69 randomly selected respondents, consisting of subsistence livestock farmers, traditional healers and other traditional knowledge holders. Data on the plants used, their common names, methods of preparation and administration and livestock ailments treated were collected. The relative citation frequency (RFC) index determined the most predominantly used plant species. The most frequently reared livestock in the study areas were cattle (62) > sheep (37) >goats (25)> horses (10) > pigs (6) > donkeys (6) > poultry (2). Fifty-one plant species belonging to 35 families were reported to have ethnoveterinary uses. The RFC values indicate that *Rhamnus prinoides* (0.45), *Aloe striatula* (0.38), *Monsonia burkeana* (0.29) and *Leucosidea sericea* (0.25) were the most frequently mentioned and important plants of the study. Digestive anomalies or liver malfunction (excess bile) was the most mentioned (56.5%) disease. Black quarter (15%) and intestinal parasites (8%) were also frequently reported. Tannins, saponins, flavonoids, cardiac glycosides, phenols, and steroids were detected in *R. prinoides, A. striatula, M. burkeana* and *L. sericea* while alkaloids were absent. Total phenolic content of *R. prinoides was* the highest at 5.102 mg/ml, followed by the aqueous extract of *M. burkeana* at 3.991 mg/ml, while the lowest (1.846 mg/ml) was observed from the aqueous extract of *L. sericea*. The total tannin content was highest in the aqueous extract of *A. striatula* (2.242 mg/ml) followed by the acetone extract of *R. prinoides* (1.47 mg/ml), while the lowest (0.242 mg/ml) was observed from the methanol extract of *R. prinoides*.

The ethanolic extract of *L. sericea* had antibacterial activity with MIC ranging from ≤0.098 mg/ml to 0.78 mg/ml, and the MBC ranged from 0.195 mg/ml to 1.56 mg/ml.

However, the aqueous extract of *L. sericea* was not active against all tested fungal strains. The methanolic extract of *M. burkeana* MIC ranged from ≤0.098 mg/ml to 1.56 mg/ml, while the MBC ranged from 1.56 mg/ml to 3.125 mg/ml. The acetone extract of *A. striatula* displayed high MIC values ranging from 0.78 mg/ml to >12.5 mg/ml and a high MBC ranging from 6.25 mg/ml to >12.5 mg/ml, but was inactive against the bacterial strains. The acetone extract of *A. striatula* was also fungicidal at 0.049 mg/ml.

The acetone extract of *L. sericea* and the water extract of *M. burkeana* had some toxicity at 200 µg/mL. The methanol extract of *M. burkeana* was the most cytotoxic against Vero cells, exhibiting nearly 50 % cell death at 100 µg/mL. In contrast, the water extract of *L. sericea*) had no cytotoxic effects.

All the extracts showed some level of larval development inhibition. Total inhibition (100 %) was recorded for the positive control at 50 µg/ml and *L. sericea acetone* extract at 25 µg/ml. The least inhibition was 22.95 µg/ml for the *M. burkeana* methanol extract, which showed generally lower inhibition than the rest of the other treatments. *L. sericea* water extract and *L. sericea* acetone extract showed higher egg hatch inhibition than *M. burkeana* methanol extract, water extract and positive control. *M. burkeana* methanol extract recorded the least (9.57 µg/ml) under higher concentrations.

The present study observed that most plants used in the highland grasslands of the Free State province of South Africa and Lesotho can treat multiple animal diseases thus validating the use of medicinal plants in traditional medicine. Further comprehensive studies are recommended to investigate their bioactive compounds and potential development of plant-derived medicinal products.

# CHAPTER 1 :
# INTRODUCTION AND LITERATURE REVIEW

## 1.1 General Introduction

From the onset of the Neolithic revolution, when animals were first domesticated (between 10,000 and 15,000 years ago), reared animals have become a critical element of human culture and play a crucial role in societal traditions and norms across the world (Chitura *et al.,* 2018). Rearing animals commenced with domesticating them from the wild. Animals wandered the wilderness and adapted to the prevailing climatic conditions for survival. However, changing environmental conditions forced those who could not adapt to these changes into extinction. Domesticating some animals ensured their survival, while humans also began commercialising this domestication for their own good (Larson and Fuller, 2014; Zeder, 2012).

Today, livestock has become an essential and vital source of income for over 70% of the world's resource-poor population that lives in marginalised rural areas (Nyahangare *et al.,* 2015). Rearing livestock in rural areas contributes to livelihoods. It enhances social status because of livestock's role in some societies during important occasions such as marriages and funerals, especially in Africa and Asia (Chitura *et al.,* 2018). Thus, animals increase household income and well-being by assisting with food and nutrition security. Across the spectrum, animal products have been employed raw or modified to improve the nutritional value of meals consumed globally (Bettencourt et al., 2013).

Animal products are getting increasingly popular, and demand for them is predicted to double by 2050 because of their economic, social, and cultural significance in rural, peri-urban, and urban households (Notenbaert *et al*., 2017). This projection is supported, among other things, by the likelihood of a rapid increase in the global population and the continual increase of edible and non-edible animal products meant to improve human well-being. Superfood consumption has been increasingly popular worldwide despite the anticipated increase in animal product demand (Notenbaert *et al.,* 2017). Demand has increased for various products, including chia seeds, blueberries, wheatgrass, avocados, and green tea. This is a result of people's increasing desire to be healthy and substitute "bad fats" for animal products; as a

result, the herbal business has grown. South African animal husbandry continues to use ethnoveterinary medicine (EVM) techniques, especially in rural livestock healthcare (McGaw et al., 2020).

Although people raise animals for various reasons, such as social, economic, or religious ones, both people and domesticated animals are susceptible to illness (Ahmad et al., 2017). Both humans and animals can contract diseases, some of which are transmissible between humans and animals (zooanthroponosis). Some illnesses can spread from animals to people (zoonotic). Before the 20th century, people predominantly depended on medicinal herbs to treat ailments in humans and animals. The 20$^{th}$ and 21$^{st}$ centuries witnessed an increase in the use of pharmaceutical treatments and immunisations for all diseases (Moreki, 2013). Pharmaceutical companies have come under fire for prioritising profit over treating illness. As a result, there is a growing need for herbal remedies (Maroyi, 2017).

For several reasons, including lowering the risk of drug residue from conventional drugs, the herbal medicine sector has recently attracted a lot of attention throughout the globe (Luseba and Van der Merwe, 2006). Additionally, it is affordable for those who cannot afford expensive veterinary formulae. Compared to more than half of the population in established nations, over 75% of the population in emerging countries receives herbal medical care, particularly for lifestyle-connected illnesses and conditions (Daniyal & Akram, 2015). The modern usage of plants for ethnoveterinary purposes can also be attributed to factors like the accessibility of veterinary clinics and socioeconomic constraints, such as financial constraints. Moreover, although most families only have a small flock, most pharmaceutical vaccines are supplied in 1,000-dose packs (Moreki, 2013). Ethno-veterinary procedures are excellent substitutes for conventional animal healthcare because they are typically less expensive, safe, and time-tested. They also draw on local resources and capabilities. Compared to conventional therapy, they are frequently more environmentally friendly (Ahmad *et al.,* 2017).

Over 3.5 billion people in developing nations depend on medicinal plants for their healthcare, according to the World Health Organization (WHO) (Phondani, 2011). The drawbacks of conventional medical practices include the seasonal unavailability of plants, the possibility of hazardous or ineffective therapies, ambiguous dosages, and a lack of guidelines (McGaw *et al.,* 2007). Changing socioeconomic and cultural values

and unlawful harvesting from the wild are wreaking havoc on the vast knowledge-based information accessible on these precious plants. Due to insufficient access to the preservation of flora in the herbal industry, medicinal plants have been endangered, and some have led to rapid extinction. Without being documented, knowledge of traditional medicine is gradually becoming restricted to a small segment of society, particularly for most people.

These rapid changes result in the loss of traditional knowledge within societies and the extinction of species, negatively impacting the environmental health and options for diagnosis of animal health (Phondani, 2011). Medicinal plants play a crucial role in rural areas where they are the primary source of treatment and cure for various diseases. The loss of this vital traditional knowledge, therefore, diminishes the ability of society to combat diseases. The oldest known medical medicines in the world are derived from medicinal plants. Additionally, they are essential components of numerous formulations used in conventional medical systems across the globe (Tandon & Yadav, 2017). Plants are believed to be a rich source of phytochemical substances with medicinal properties (Shakya, 2016). Among the several phytochemicals that fall under this group are flavonoids, phenols, phytosterols, proanthocyanidins, tannins, and terpenes (Maroyi, 2017). Medicinal plants are the most significant source of medicines, biopharmaceuticals, and chemical entities for synthesised therapeutics, which also serve as a bioresource for traditional medicine (Shrisha *et al.,* 2011).

In addition to being used as traditional medicines in many cultures, these medicinal plants are valuable as trade goods that meet the demands of often-distant marketplaces (Schippmann & Cunningham, 2006). In addition to treating human illnesses, plants are useful for preventing and treating cattle-related diseases (Shakya, 2016). According to Moreki (2013), ethnoveterinary medicine (EVM) describes conventional animal health care that incorporates community members' knowledge, abilities, techniques, practices, and beliefs about animal care. According to Sibanda and Chiuta (2018), EVMs and treatments are affordable, efficient, and simple to use. Farmers and traditional healers use local plants since they are readily available materials. They result from millennia of trial-and-error application and learning (Sansoucy & Jabbar, 1995). Fewer veterinarians operate in rural African areas; therefore, EVM is the best alternative for most locals (Guèye, 1999). In Southern

Africa, the usage of EVMs is not well documented; however, some authors have documented the local use of medicinal plants for ethnoveterinary purposes (Luseba & Van der Merwe, 2006; Van der Merwe *et al.,* 2001; McGaw & Eloff, 2008; McGaw *et al.,* 2020; Maphosa & Masika, 2010; Motsoari *et al.,* 2022; Nyahangare *et al.,* 2015; Magwede *et al.,* 2018).

According to some reports, ethnic communities utilise ethnoveterinary plants such as *Combretum molle, Aloe marlothii, Ziziphus mucronata, Dalbergia obovata,* among others, to cure livestock diseases such as Heartwater, Newcastle disease (NCD), Black quarter, Rift Valley Fever, Wounds, Lumpy skin disease, mastitis, and retained placenta (Chitura *et al.,* 2018; Mthi *et al.,* 2018; Luseba & Tshisikhawe, 2013; Luseba & Van der Merwe, 2006). Ethno-pharmacologists, botanists, microbiologists, and natural-product chemists are desperately searching the globe for phyto-constituents which could be developed for the treatment of infectious diseases, particularly considering the urgent need to develop more efficient antimicrobial, antiviral, anthelmintic, antifungal, inflammatory and antioxidant agents (Ncube *et al.,* 2008; Shrisha & An, 2011). This has led to the discovery that some plants, such as those belonging to the *Piper* species, possess bioactive compounds like amides and alkaloids that can inhibit the proliferation of some cancers (Mgbeahuruike *et al.,* 2017). Therefore, the nexus between medicinal plants and new drug discovery cannot be overemphasised.

## 1.2 Literature Review

### 1.2.1 Epidemiology of veterinary diseases

An estimated 60% of human pathogens are microorganisms transferred from vertebrate animals, including livestock, to people (Klouse*t al.,* 2016). Communicable or infectious diseases are illnesses caused by microorganisms such as viruses, bacteria, and fungi that people or animals spread directly or indirectly between one another through contact with contaminated surfaces, insect bites, and air (Wang *et al.,* 2019). Zoonotic diseases such as anthrax and brucellosis are the most common diseases imposing significant economic impacts on livestock production and debilitating, as well as chronic disease in humans worldwide (Goodwin & Pascual, 2016). One million estimated cases of severe human leptospirosis, while 58,900

estimated deaths are recorded annually, including significant economic losses globally due to veterinary diseases, and 80% of the world's cattle population is at risk from ticks and tick-borne diseases (TTBD) (Nyahangare *et al.,* 2015). Ticks cause skin damage and open wounds, leaving the cattle vulnerable to secondary infections, causing toxicosis and paralysis in some cases.

Fatal diseases caused by ticks, such as babesiosis and theileriosis, can infect humans through contact with infected animals or water and soil that infected animals have contaminated. Inter-species transmission of antimicrobial-resistant strains may be caused by the isolation of *Mycobacterium bovis* from humans, while *Mycobacterium tuberculosis* (M. tb) from livestock may pose a risk to humans. One of the significant veterinary diseases and zoonotic threats in developing countries is *M.* bovis. About 37.7 and 31.6% cases of TB are reportedly caused by this parasite in Africa and Mexico, respectively (Layton *et al.,* 2017). The World Organization for Animal Health (WOAH), (2019) lists several illnesses, such as anthrax, infection with Brucella abortus, Heartwater, equine influenza, anaplasmosis in cattle, babesiosis in cattle, equine infectious anemia, Rift Valley virus, ovine epididymitis (Brucellaovis), and salmonellosis (*S. abortusovis*). These zoonotic diseases negatively affect the agricultural and health sectors because they are transmissible between animals and/or humans. Most of these diseases end in animal death due to the inability to timeously treat these diseases. This is because the costs of vaccines are very high in developing countries, discouraging many farmers from investing in the health of their animals.

### 1.2.2 Ethnoveterinary medicinal plants

The tropics of South America have the highest number of species of flora, estimated to be about 6400, followed by Asia and Africa with 4000 and 1500 species, respectively (Chinsamy *et al.,* 2011). Medicinal plants are widely used for the treatment of numerous human and livestock diseases in different parts of the world, including Asian countries like India, Thailand, and China, as well as Southern African countries such as Botswana, Lesotho, Zimbabwe, and South Africa, among others (Bekalo *et al.,* 2009; Panmei *et al.,* 2019; Assefa & Bahiru, 2018; Brooks-pollock *et al.,* 2015). Southern Africa is rich in plant biodiversity and home to about 24,000 flowering plant species. This accounts for one-tenth of the world's higher plants, and 80% of these

species are endemic to the region (Fennell *et al.,* 2004). Herbal medicines have always played a significant role in livestock therapy among small-scale, resource-poor farmers. These farmers use plants extensively to treat livestock diseases (Luseba & Van der Merwe, 2006). However, because many academics and medical professionals consider these methods outdated, there is little evidence of the use of ethno-veterinary medications in developing countries (Mwale & Chimonyo, 2015).

### 1.2.3 Ethnoveterinary landscape in Lesotho and South Africa

To maintain the health and productivity of their livestock as well as to prevent and control diseases like retained placenta, diarrhoea, gall sickness, fractures, eye inflammation, general illness, fertility issues, gastrointestinal ailments, heart water, helminthiasis, coughing, red water, and tick reduction, farmers from various ethnic groups in South Africa, such as Tswana, Tsonga, Xhosa and Zulu, Sotho, Venda, Ndebele, among others employ traditional methods including the use of medicinal plants (Khunoana et al., 2019). Although the government provides clinics free of charge in South Africa, several studies have demonstrated that medicinal plant use still plays a major role in treating various diseases in rural areas (De Wet *et al.,* 2016).

A study conducted in the Eastern Cape province of South Africa revealed that the percentage of livestock lungs condemned due to various diseases in 2010, 2011, and 2012 was 4.04%, 6.03% and 3.58%, respectively (Jaja *et al.,* 2014). This demonstrates that livestock diseases can potentially affect the nation's economy negatively. Diseases such as African swine fever, the Nipah virus, and contagious *bovine pleuropneumonia* have previously cost Cote d'Ivoire, Malaysia, and Botswana about $9.2,114 billion and $300 million, respectively. When human consumes diseased meat or livestock products, they become liable to many zoonotic diseases such as Anthrax, Ebola, Middle East Respiratory Syndrome (MERS), gall sickness (anaplasmosis), Heart water, Newcastle disease (NCD), Black quarter, Rift Valley Fever, Wounds, Lumpy skin disease and Warts (Luseba & Van der Merwe, 2006).

In Africa and many other world regions, ethnoveterinary knowledge is passed from generation to generation by oral tradition. Without proper documentation, this information can easily be lost or distorted (Eshetu *et al.,* 2015; Seleteng-Kose *et al.,* 2015). This also applies to the present study area. Because ethnobotanical or

ethnoveterinary knowledge has been orally passed from generation to generation, this knowledge has not been well documented in the study area. However, a small cohort of studies have previously attempted to document some medicinal plants that are used in the treatment of various livestock diseases (Maliehe, 1997; Shale *et al.,* 1999; Moteetee & Van Wyk, 2011; Seleteng-Kose *et al.,* 2015; Moffett, 2010). Shale *et al.* (1999) reported that some medicinal plants, such *as Malva parviflora, Rumex acetosella* and *Euphorbia clavarioide*s in Lesotho, were used for antibacterial and anti-inflammatory activities. In Lesotho, traditional and Western medicine are used collaboratively. As a developing country, and because of a lack of resources, most of the population heavily relies on traditional medicine. This plays a vital role towards the well-being of the rural population, particularly where there is limited accessibility to clinics or healthcare facilities (Seleteng-Kose *et al.,* 2015).

### 1.2.4 Secondary metabolites in medicinal plants

Phytochemicals ubiquitous in plants can be classified as primary and secondary metabolites. Metabolites are products and intermediates of cellular metabolism, in which primary metabolites include proteins, sugars, and chlorophylls among others, while secondary metabolites can be classified into alkaloids, carotenoids, phenolics, organosulfur, and nitrogen-containing compounds (Bvenura *et al.,* 2018; Raghuveer *et al.,* 2015). There is a qualitative and quantitative distribution of these metabolites among different species, members of the same species, and various plant parts. Alkaloids found in low concentrations relative to the phenolic compounds are offset by their high biological potency in vegetative tissues (Khan *et al.,* 2011). This feature is key as a base for antimicrobial effects, meaning it is noteworthy against most microbes (Othman *et al*., 2019).

Plants' secondary metabolites are rich sources of free radical scavengers such as alkaloids, tannins, saponins, flavonoids, anthraquinones and terpenoids, among others, which have therapeutic activity (Chingwaru *et al.,* 2019; Gracelin *et al.,* 2012). According to the previous authors, these components promote wound healing through mopping up reactive oxygen species from wounded tissues, inhibiting effects against infections that delay wound healing, anti-inflammatory activities, and modulation of pathways in the endocannabinoid system. Therefore, the present study also profiled

several ethnoveterinary medicinal plants from both South Africa (Free State highland grasslands) and Lesotho, intending to profile the secondary metabolites.

### 1.2.5 Antimicrobial properties of medicinal plants

Antibiotic-resistant bacteria are responsible for about 90% of infection-related deaths globally (Al-judaibi, 2014). As a result, modern medications are finding it more and more challenging to prevent or treat infectious diseases. This is because different microbes are becoming resistant to antimicrobial agents. Antimicrobials lose their efficacy when they are unnecessarily consumed or overused by patients, or when they are widely used in agriculture as growing substances and to avoid infections (Khunoana *et al.,* 2019; Van Vuuren & Holl, 2017). As most disease-causing germs mutate to produce a particular chemical, synthetic drugs do not eliminate them and their effects (Taylor, 2005).

Traditional medicines are intended to elicit complex reactions and their adverse effects or to treat symptoms of a particular disease and aid in ameliorating drug resistance (Karimi *et al.,* 2015). Medicinal plants are consequently used to prevent the resistance of pathogenic organisms to antibiotics by utilising new compounds that are not based on existing synthetic antimicrobial agents (Rojas *et al.,* 2006). Therefore, they can potentially ameliorate multi-drug resistance and increase the gene pool of drugs for present and future use. The active antimicrobial, antifungal, anti-inflammatory, anthelmintic, antiviral and antioxidant activities of medicinal plants have been shown in most ethnobotanical or ethno-veterinary studies (Jamshidi-kia *et al.,* 2018; Mabaleha *et al.,* 2019; Kobisi *et al.,* 2019; Moteete *et al.,* 2019; Buwa-Komoreng *et al.,* 2019; Tasneem *et al.,* 2019; Omara *et al.,* 2020).

These phytochemicals such as alkaloids, saponins, tannins and phenols, not only protect plants from microbial pathogens, insects and herbivores but also exert physiological effects on animals, and they are of importance in the production of conventional drugs (Mahesh & Satish, 2008; Prohp & Onoagbe, 2012). These compounds found in herbs function synergistically to achieve polyvalency including saponins which has the potential to combat infections caused by parasites, viruses, and bacteria (Ajiboye *et al.,* 2013). Some plants, such as *Abrus precatorus* and

*Jatropha curcas,* continue to be used in traditional medicine to treat bacterial and fungal infections, although they are reportedly toxic (Ndhlala *et al.,* 2013).

Researchers are constantly studying plant secondary metabolites with unknown pharmacological activities to elucidate their pharmacological activities (Gracelin *et al.,* 2012) and to scientifically validate various such medicinal plants to elucidate their mechanism of action and how they cannot cause harm to livestock despite their toxicity (Taylor *et al.,* 2001).

### 1.2.6 Safety of medicinal plants

The safety of traditional herbal medicines is becoming increasingly important. Since traditional healers utilise several herbs in varying doses, often in combinations, many therapeutic cures are dubious (Krishnaraju *et al.,* 2006). Although there is a common misconception that medicinal plants are natural and therefore safe for consumption, such plants may be therapeutic at one dose and potentially toxic at another (Mongalo *et al.,* 2017). Most medicinal plants are toxic if taken in large quantities; hence, the dosages should be prepared with great care and accuracy. Unless chemical analysis is conducted, it may not be possible to estimate the quantity of the extract that can be used as a correct dose. Any additional amount may cause deleterious effects, such as the oxalates of *Dieffenbachia picta,* which can cause severe inflammation and necrosis of the epithelium of the tongue and oral cavity intravascular (Loretti *et al.,* 2003). Some plants' toxicity may cause methemoglobinemia, salivation, dehydration, severe gastroenteritis and haemolysis in cattle, sheep, and horses or even death (Woteko *et al.,* 2014).

Moreover, the build-up of specific toxicants in the system, such as cardiac glycosides, pyrrolizidine alkaloids (Pas), lantadenes A and B, steroidal saponins, and excessive oxalates, which cause liver damage can result in genetic interference with prolonged use, necessitating further research to verify safety (Whittamore & Hatch, 2014; Ramulondi*et al.,* 2018).

## 1.3 Problem statement

The high cost of pharmaceutical products and access to veterinary services are significant valid reasons for farmers to use non-conventional medicines (Luseba & Merwe, 2006). This puts a strain on most small-scale farmers. These farmers cannot afford such services, and the veterinary clinics are not accessible, especially for people in the mountainous parts of Lesotho, the grassland and the highlands of the Free State. Some Lesotho and South African Free State province farmers allow dogs to roam with livestock, likely aiding in infestation with *E. granulosus*, as it facilitates the interface between dogs and domestic livestock (Jaja et al., 2014).

The villagers or community often consume and sell the sick or, at times, dead livestock products, such as meat. Not only does this act put their lives in danger, but it also causes unwanted food poisoning cases and deaths. When humans consume diseased meat or livestock products, they become liable to many zoonotic diseases such as gall sickness (anaplasmosis), heartwater, Newcastle disease (NCD), black quarter, wounds, lumpy skin disease, and warts (Luseba & Merwe, 2006). The environmental health practitioners ought to isolate and condemn spoiled or contaminated carcasses. Affordable and yet effective means to prevent the distribution of contaminated meat and to assist in detecting and eradicating certain livestock diseases and potentially zoonotic infections need to be identified (Jaja *et al.*, 2014). Therefore, the present study aims to document and chemically profile ethnoveterinary medicinal plants used by resource poor farmers in treating zoonotic and non-zoonotic diseases in livestock in the highland grasslands of the Free State province of South Africa and Lesotho.

## 1.4 Main aim

This study aimed to document the ethnoveterinary use of plants to treat livestock diseases in the Highland Grasslands of the Free State province of South Africa and Lesotho and evaluate the medicinal properties of selected species.

### 1.4.1 Specific objectives

1. To investigate the ethnoveterinary use of plants in the highland Grasslands of the Free State province of South Africa and Lesotho,

2. To determine the secondary metabolite constituents of the most popular ethnoveterinary plants based on the RFC index,
3. To evaluate the antibacterial activity, cytotoxicity potential and anthelmintic activity of the plants identified in objective two.

Figure 1.1 shows the study process indicating the ethnoveterinary survey, selection of medicinal plants used in the study and the activities carried out.

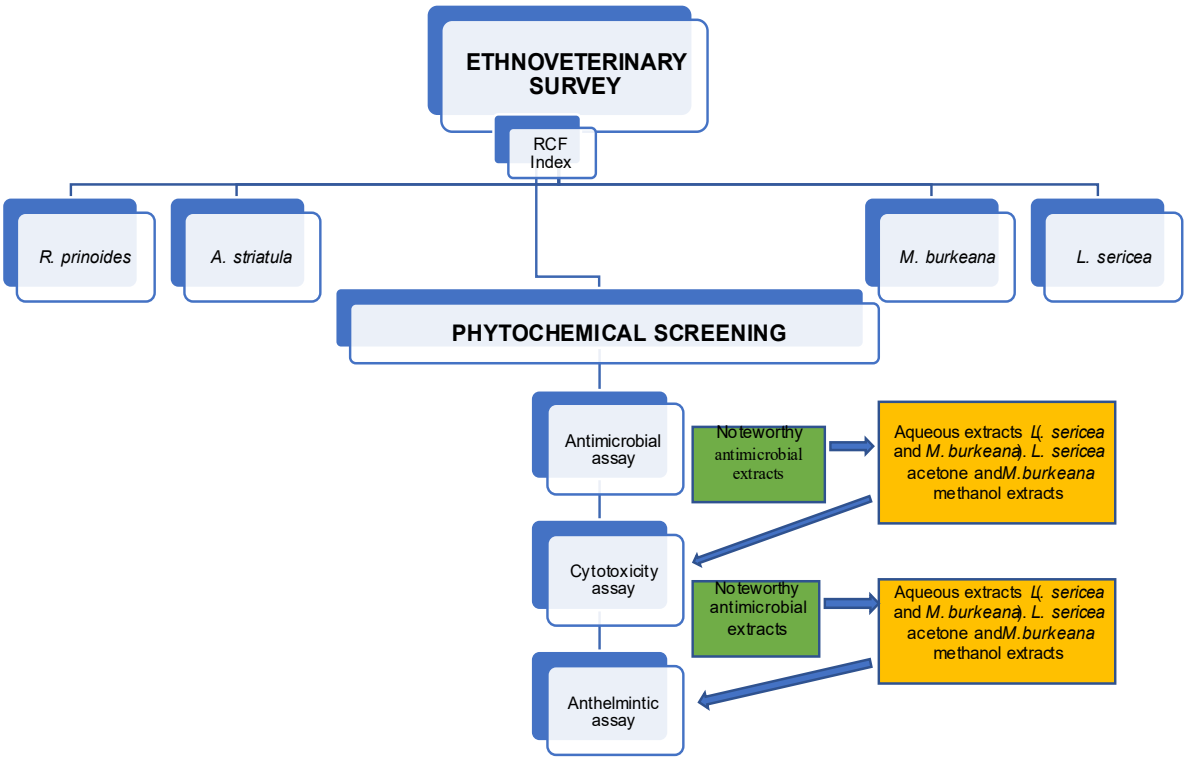


***Figure 1.1: The study process***

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# CHAPTER 2 :
# ETHNOVETERINARY SURVEY OF MEDICINAL PLANTS IN THE HIGHLAND GRASSLANDS OF SOUTH AFRICA AND LESOTHO

## 2.1 Introduction

Livestock, including cattle, poultry, pigs, sheep, and goats, are paramount in Africa, more so in the Southern African countries of South Africa and Lesotho. They provide food and transport, improve livelihoods and are of significant cultural value, especially in rural settings. However, the high prevalence of diseases experienced in communal livestock production systems presents a serious setback to profitability, food security, nutrition, and sustainability due to increased morbidity and mortality (Mthi *et al.,* 2018). Additionally, the high costs of modern drugs, lack of access to veterinary facilities, and increasing resistance of pathogens to pharmaceutical medicines are some of the productivity challenges. The options available to mitigate these problems are limited in mostly rural and peri-urban areas. Therefore, resource-poor smallholder farmers often turn to traditional methods such as using medicinal plants for disease management. In fact, the World Health Organization (2010) estimates that 80% of people in developing countries use ethno-methods to monitor livestock-related diseases. It has also been reported that people utilise medicinal plants to treat various livestock diseases because they possess the knowledge of these plants' medicinal properties (Phumthum *et al.,* 2018).

In South Africa and Lesotho, livestock farmers from different ethnic groups also utilise specific medicinal plants such as *Aloe ferox*, *Aloe maculata* and *Lycium horridum* spp for ethnoveterinary purposes. Health conditions such as diarrhoea, fractures, eye inflammations, reproductive problems, ecto- and endoparasitism, black quarter and digestive difficulties are some of the diseases that have been treated with these medicinal plants (McGaw & Eloff, 2008; Khunoana *et al.,* 2019). However, traditional medicinal knowledge and practices within may differ and may be influenced by environmental origin, ethnicity, religion, age, and gender (Luseba & Tshisikhawe, 2013). In most African backgrounds, ethno-knowledge is transmitted by oral tradition from generation to generation. Without adequate documentation, information from traditional knowledge holders can be easily lost or distorted, and insufficient

knowledge may be passed on to younger generations (Maphosa & Masika, 2010; Eshetu *et al.*, 2015; Khunoana *et al.*, 2019). Consequently, the documentation of traditional medicinal knowledge becomes important as it preserves vital information and creates possibilities for discovering new drugs (Lu *et al.*, 2011; Khan & Razzaq, 2018). Therefore, this research was conducted to reveal and document various medicinal plants used to treat livestock diseases in the highland grasslands of the Free State province of South Africa and some parts of Lesotho.

## 2.2 Materials and methods

### 2.2.1 Description of the study areas

The present study was conducted in the highland grasslands of the Free State, South Africa (QwaQwa and Thaba 'Nchu) and Lesotho (Maseru and Mokhotlong). These areas were selected because of their high concentration of smallholder livestock farmers, accessibility of farmers and the willingness of the informants to participate in the study.

Qwaqwa is situated at 28.5530°S, 28.8247°E, and 1 646 m above sea level (a.s.l.), with a mean annual rainfall of 687.8 mm and a mean annual temperature of 15°C. Thaba 'Nchu is situated at 29.22°S, 26.83°E at 1 532m a.s.l. As defined by the Köppen climate classification, the province's climate is semi-arid, primarily, except for the eastern and north-eastern parts, where a humid subtropical climate prevails. An average rainfall of 300 mm to over 900 mm per year, with about 70% falling from September/October to April/May, has been reported (Moeletsi, 2010). The topography of the Free State is a succession of flat plains, sprinkled with pasture lands (Low & Rebelo, 1996). The topography in the province is variable, with elevations as high as 1,200 m a.s.l. in the western and southern parts, and as high as 1,800 m a.s.l. in the eastern and central parts of the province (Moeletsi *et al.,* 2011). In these areas, frosty conditions inhibit tree development and aid in maintaining grass dominance (Low & Rebelo, 1996). The natural grassland vegetation boasts wild grasses, flowers, and various wild animals and insects (FS-SANBI, 2021). However, the Free State climate can generally be classified as arid in the west and semiarid in the east (PCU-UCT, 2021).

On the other hand, the mountain kingdom of Lesotho is surrounded by South Africa. Maseru, the capital of Lesotho, is situated at 29.3151°S, 27.4869°E and 1600 m a.s.l with an annual average precipitation of 691mm and a mean annual temperature of 15.1°C. Mokhotlong is situated at 29.2573°S, 28.9529°E at 2512m a.s.l. and has a mean annual precipitation of 601mm and a mean annual temperature of 12.5°C (weather-atlas, 2020). The two study towns in Lesotho are characterised by mountainous topography and grassland vegetation (Seleteng*et al.*, 2015). The main vegetation of this zone consists of typical grass species like *Hyparrhenia hirta* and *Themeda triandra* (Department of Environment, 2009). In both the Free State and Lesotho, *Sesotho,* the language of the *Basotho* people, is widely spoken (StatsSA, 2021). The majority of *Basotho* gain their livelihood from subsistence farming and animal husbandry (Government of Lesotho, 2018).

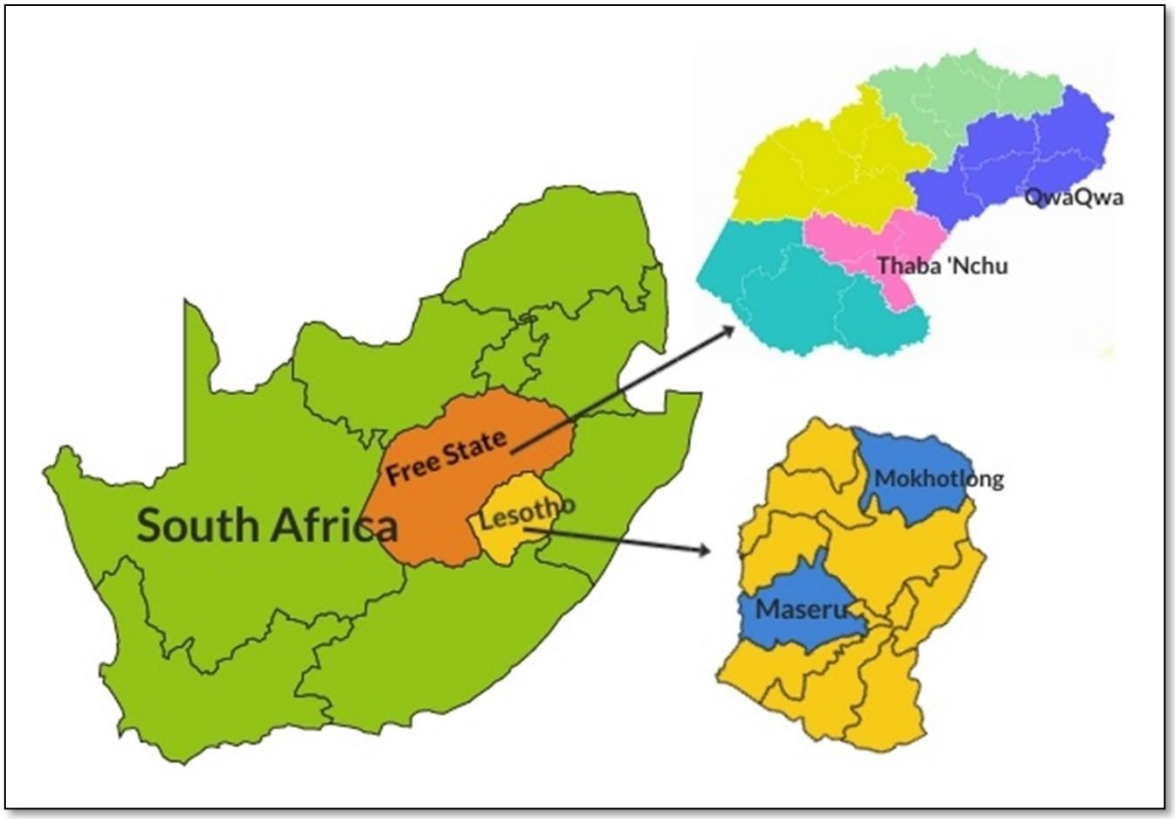


***Figure 2.1: Map of South Africa and Lesotho showing the study areas.***

### 2.2.2 Collection of information

The University Research and Innovation Committee (FRIC) of the Central University of Technology approved the study. Semi-structured interviews were subsequently

conducted with participants who had been fully informed and provided written consent (Informed consent form and interview guide provided in the Annexure). The research followed the methodological framework described by Bvenura and Afolayan (2014), with minor modifications to suit the study context. In addition, all procedures were undertaken in accordance with the Nagoya Protocol on Access and Benefit-Sharing. Compliance with this international agreement was essential to ensure the ethical use of genetic resources and associated traditional knowledge, safeguard the rights of local communities, and promote equitable sharing of any potential benefits arising from the research. The interviews were conducted with 69 respondents. The interviewees comprised livestock farmers who engage in ethnoveterinary practices, traditional healers, and/or traditional knowledge holders in the study regions. The respondents were selected purposefully with the assistance of community elders and local authorities based on their traditional knowledge of medicinal plants and willingness to participate in the interview. Participants were interviewed in Sesotho and/or English. Questions such as “What plants do you use on your farm?”, “the local names of the plants”, “health indications for plant use”, “plant parts used”, “methods of preparation and administration” and “types of diseases cured”, among others, were asked. The interviews were held either on the farm, homestead, or communal locations in the selected study areas. The ethnoveterinary survey was conducted from November 2020 to March 2021.

### 2.2.3 Collection of plant specimens and identification

The mentioned medicinal plants were collected from their different wild habitats and identified with the assistance of the knowledge holders (pre-harvesting), published literature and Botanists (post-harvesting). Environmental laws were considered during plant collection, and the harvested plants were tagged with their local names. These plants were freshly collected during different seasons. Thereafter, voucher specimens were deposited in the herbarium of the Centre for Applied Food Sustainability and Biotechnology of the Central University of Technology, Free State, after positive identification with assistance from scientists from the Free State National Botanical Garden. The specimens were then assigned voucher specimen numbers as shown in Table 2.3.

### 2.2.4 Data analysis

Results were validated using the relative frequency of citation (RFC) according to the procedures of Samaha *et al. (*2019). This procedure describes the relative importance of a plant based on its frequency of mention by respondents using this formula:

$$RFC_s = FC_s / N$$

Where:

$FC_s$ is the frequency of citation and N is the number of respondents.

**NB:** The index varies from 0.0 to 1.0; the closer the values are to 1.0, the higher the consensus among the informants.

## 2.3 Results

### 2.3.1 Demography of respondents

Results of the current study revealed that the majority (28%) of respondents were between the ages of 66 and 75 years. Male respondents were higher (94%) than female respondents. Primary school education was the highest (64%) educational level attained by most respondents. As seen in Table 2.1, more respondents were from the Maseru region than from any other study locality.

***Table 2.1: Demographic characteristics of respondents***

| Characteristics | Gender | | Frequency | Percentage (%) |
|---|---|---|---|---|
| | Male | Female | | |
| ***Age (years)*** | | | | |
| **< 15** | 0 | 0 | 0 | 0 |
| **16-25** | 3 | 0 | 3 | 4 |
| **26-35** | 6 | 0 | 6 | 9 |
| **36-45** | 13 | 0 | 13 | 19 |
| **46-55** | 11 | 3 | 14 | 20 |
| **56-65** | 13 | 1 | 14 | 20 |
| **66-75** | 19 | 0 | 19 | 28 |
| **Total** | 65 | 4 | 69 | 100 |

| | | | | |
|---|---|---|---|---|
| ***Level of education*** | | | | |
| **Primary education** | 40 | 4 | 44 | 64 |
| **Secondary education** | 4 | 0 | 4 | 6 |
| **High school education** | 1 | 0 | 1 | 1 |
| **No education** | 20 | 0 | 20 | 29 |
| **Total** | 65 | 4 | 69 | 100 |
| ***Study areas*** | | | | |
| **Maseru** | 19 | 3 | 22 | 32 |
| **Mokhotlong** | 16 | 1 | 17 | 25 |
| **QwaQwa** | 16 | 0 | 16 | 23 |
| **Thaba 'Nchu** | 14 | 0 | 14 | 20 |
| **Total** | 65 | 4 | 69 | 100 |

### 2.3.2 Livestock reared in the study areas

The different types of livestock reared and treated with medicinal plants in the various study areas are shown in Table 2.2. Cattle were the most dominant livestock, followed by sheep, goats, and horses, while chickens were the least reared in the study areas.

***Table 2.2: Characteristics of livestock reared in the study area***

| **Characteristics** | **Study Area** | | | |
|---|---|---|---|---|
| | **Maseru** | **Mokhotlong** | **QwaQwa** | **Thaba 'Nchu** |
| **Livestock** | | | | |
| **Pig** | 2 | 2 | 1 | **1** |
| **Cattle** | 20 | 13 | 15 | **14** |
| **Goat** | 9 | 8 | 6 | **2** |
| **Poultry** | 1 | 1 | 0 | **0** |
| **Sheep** | 9 | 15 | 7 | **6** |
| **Donkey** | 3 | 3 | 0 | **0** |
| **Horses** | 2 | 7 | 1 | **0** |
| **Total** | 46 | 49 | 30 | **23** |
| **Gender** | | | | |
| **Male** | 19 | 16 | 15 | **15** |
| **Female** | 3 | 1 | 0 | **0** |
| **Total** | 22 | 17 | 15 | **15** |

### 2.3.3 Livestock diseases and conditions treated with herbal remedies

The livestock diseases commonly treated with medicinal plants in the study areas are shown in Figure 2.2. The most reared livestock in the two study areas generally decreased in the order cattle (62) > sheep (37) goats (25) horses (10) > pigs (6) > donkeys (6) > poultry (2). Digestive anomalies or liver malfunction which presents as excess bile acid circulation in the animal's body (bile acid malabsorption) was the most mentioned (39%) disease. This condition in animals is known as *nyoko* in the study areas. Other significant diseases were black quarter (15%), intestinal parasites (8), diarrhea (5%), external parasites (5%) and liver disease (2%) among others.

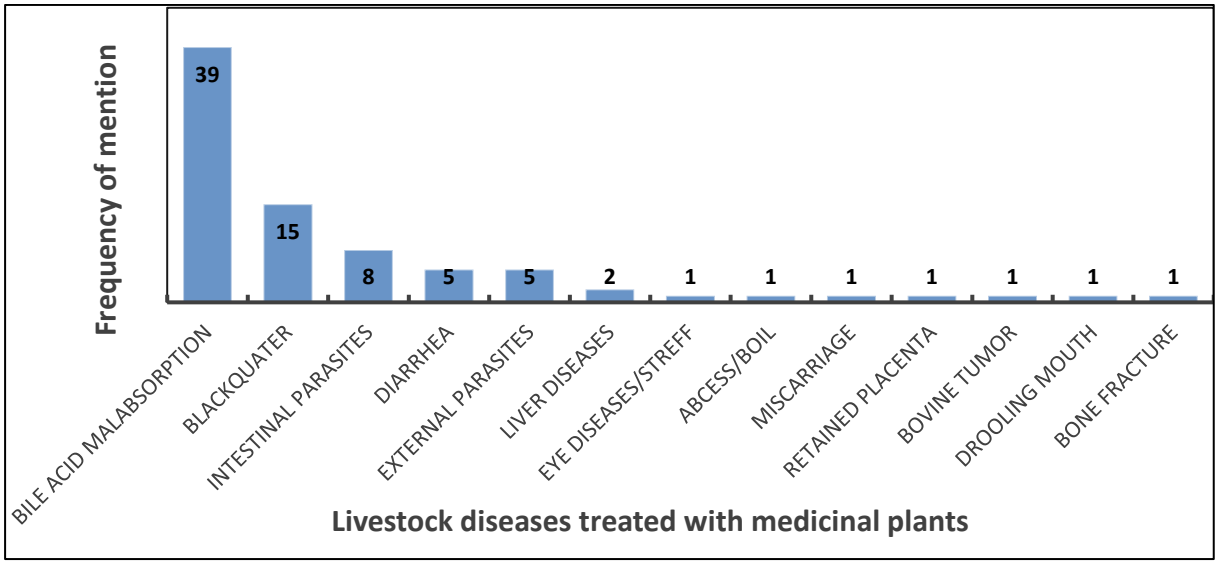


***Figure 2.2: Frequency of mention of livestock diseases treated with medicinal plants***

### 2.3.4 Diversity of medicinal plant species used in the study area

A total of 51 medicinal plants belonging to 35 families, including trees, shrubs, perennials, and grasses, were documented in the present study (Table 2.3). The total number of species reported per family decreased in the order Asteraceae (8) > Asphodelaceae (4) > Anacardiaceae (2) > Fabaceae (2) > Hyacinthaceae (2) > Lamiaceae > (2) Rosaceae (2) > Thymelaeaceae (2). However, the remaining families recorded one (1) species each. These plants were collected from different locations such as mountains, valleys, homes (as weeds or cultivated in gardens), and the wild. Plant roots (45%) and leaves (43%) were the most used parts for herbal remedies, while oral administration was the most predominant route of administration (84%).

The number of plant species reported per location and the number of species shared between locations is presented in Figure 2.3. More plants (32) were mentioned in Thaba ‘Nchu than in any other study locality, with 15 only documented for that place. Four plants, *Aloe striatula*, *Leucosidea sericea*, *Monsonia burkeana* and *Dicoma anomala* were mentioned in all study areas, depicting their common usage in these areas.

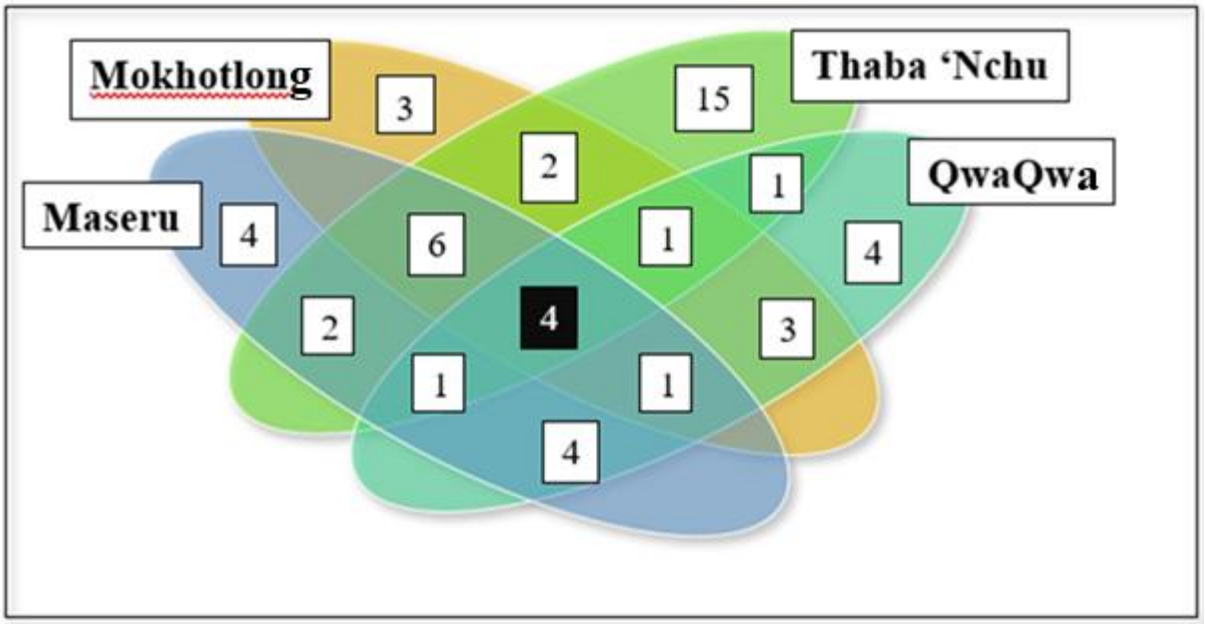


***Figure 2.3: Array of medicinal plants used for ethnoveterinary purposes in the study areas. Numbers represent the count of plant species unique to or shared among the four study areas.***

***Table 2.3: Plants used for ethnoveterinary purposes in the highland grasslands of the Free State, South Africa, and Lesotho***

| Botanical name | Family name | Voucher number | Local name | RFC | Part used | Ethnoveterinary use | Preparation and administration | Study areas |
|---|---|---|---|---|---|---|---|---|
| *Rhamnus prinoides* L'Hérit | Rhamnaceae | 01/2019 | Mofifi | 0.45 | L | Acts against bile acid malabsorption | Prepared as a decoction, or the leaves are ground and mixed with salt. It is administered orally | Maseru, Thaba 'Nchu, QwaQwa |
| *Aloe striatula* Haw. var. *striatula* | Asphodelaceae | 02/2019 | Mohalakane | 0.38 | L | Acts against bile acid malabsorption | Decoction of *Aloe striatula* Haw. var. striatula *and Rumex lanceolatus* is prepared and administered orally | Maseru, Mokhotlong, Thaba 'Nchu, QwaQwa |
| *Monsonia burkeana* Planch ex Harv. | Geraniacea | 03/2019 | Khoara/ Makorotsoane | 0.29 | R | Diarrhoea, intestinal parasites and acts against bile acid malabsorption | Decoctions are prepared or roots are ground and mixed with salt. It is administered orally | Maseru, Mokhotlong, Thaba 'Nchu, QwaQwa |
| *Leucosidea sericea* Eckl. &Zeyh. | Rosaceae | 04/2019 | Cheche | 0.25 | L | Used to treat black quarter/ blackleg disease, Papisi (small parasites) | Decoction of the leaves are made and administered orally or used as enema for horses | Maseru, Mokhotlong, Thaba 'Nchu, QwaQwa |
| *Rumex acetosella* L. subsp. *angiocarpus* (Murb.) Murb. | Polygonaceae | 05/2019 | Khamane | 0.22 | R | Acts against bile acid malabsorption and intestinal parasites | Ground roots are mixed with water and administered orally | Mokhotlong, QwaQwa |
| *Gomphocarpus fruticosus* subsp. *Fruticosus* | Apocynaceae | 06/2019 | Moithimolo/ Lebejane/ Matalenyane | 0.22 | L | Acts against bile acid malabsorption | Prepared as a decoction, or the leaves are ground and | Maseru, Thaba 'Nchu, Mokhotlong |

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|---|---|---|---|---|---|---|---|---|
| | | | | | | | mixed with salt and administered orally | |
| *Phytolacca heptandra* Retz. | Phytolaccaceae | 07/2019 | Monatja/ Monyela- ntja | 0.19 | R | To treat Kokoana (small parasites), stomach pains caused by parasites (Papisi) and Streff syndrome | Mix ground roots in hay or with salt and administer orally | Mokhotlong, Thaba 'Nchu, QwaQwa |
| *Dicoma anomala* (Sond.) subsp. *Anomala* | Asteraceae | 08/2019 | Hloenya | 0.16 | R | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | Maseru, Mokhotlong, Thaba 'Nchu, QwaQwa |
| *Eriocephalus tenuifolius* DC. | Asteraceae | 09/2019 | Sehala-halasamatlaka | 0.16 | L | Treats Kokoana (small parasites) and acts against bile acid malabsorption | Decoction is prepared, and leaves are ground and mixed with salt. It is administered orally | Maseru, Thaba 'Nchu, Mokhotlong |
| *Gunnerabperpensa* L. | Gunneraceae | 010/2019 | Qobo | 0.13 | R | Treats diarrhea | Decoctions of the roots are made and administered orally | Mokhotlong, QwaQwa |
| *Buddleja salviifolia* (L.) Lam. | Buddlejaceae | 011/2019 | Lelothoane | 0.10 | L | Acts against bile acid malabsorption | Decoctions of the leaves are made and administered orally | Maseru, Thaba 'Nchu, Mokhotlong |
| *Aloe ferox* Mill. | Asphodelaceae | 012/2019 | Lekhala la Quthing | 0.10 | L | Acts against bile acid malabsorption | Decoctions of the leaves are made and administered orally | Maseru, QwaQwa |
| **Agave Americana* L. subsp. *Americana* | Agavaceae | 013/2019 | Lekhala le leputsoa | 0.09 | L | Tick repellant | Ground leaves are mixed with water and administered topically | Maseru, Thaba 'Nchu, Mokhotlong |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Senecio discodregeanus* Hilliard & B.L. Burtt | Asteraceae | 014/2019 | Lehlomane le lenyenyane | 0.09 | R | Acts against bile acid malabsorption and Papisi (small parasites) | Decoctions of the roots are made and administered orally | Mokhotlong, Thaba 'Nchu |
| *Bulbine narcissifolia* Salm. Dyck | Asphodelaceae | 015/2019 | Khomoeabalisa | 0.09 | R | Acts against bile acid malabsorption, diarrhoea and abscess | Prepared as a decoction/ground roots are mixed with salt. It is orally administered | Maseru, Thaba 'Nchu, Mokhotlong |
| *Elephantorrhiza elephantina* (Burch.) Skeels | Fabaceae | 016/2019 | Mositsane | 0.09 | R | Acts against bile acid malabsorption | Grind whole plant and mix with salt, then administer orally | Maseru, Thaba 'Nchu |
| *Euphorbia cericoides* Lam. | Euphorbiaceae | 017/2019 | Malebesana | 0.04 | R | Acts against bile acid malabsorption | Decoctions of Malebesana and *Rumex lanceolatus* are made and administered orally | Mokhotlong, QwaQwa |
| **Populus* sp. | Salicaceae | 018/2019 | PopulerieaThaba | 0.04 | R | Acts against bile acid malabsorption | Decoctions of Populiri and peach tree are prepared and administered orally | Maseru, Thaba 'Nchu |
| *Aloe maculate* All. | Asphodelaceae | 019/2019 | Lekhala la thaba \ bafu | 0.04 | L | Acts against bile acid malabsorption | Ground fresh leaves are mixed with water and administered orally | Maseru, QwaQwa |
| *Cussonia paniculata* EckL. & Zeyh. subsp. *Sinuate* (Reyneke&Kok) De Winter | Araliaceae | 020/2019 | Mots'ets'e | 0.04 | L | Acts against bile acid malabsorption | Decoctions of leaves are prepared and administered orally | Thaba 'Nchu |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Asparagus microraphis* (Kunth) Baker | Asparagaceae | 021/2019 | Lerara-tau/ lehonyeli | 0.04 | R | Black quarter/ blackleg disease and acts against bile acid malabsorption | Grind roots and mix with water, then administer orally | Maseru, Mokhotlong, QwaQwa |
| **Malva parviflora*L.var. *parviflora* | Malvaceae | 022/2019 | Mosala suping/ Tika-motse | 0.03 | WP | Used as a lotion for bruised or broken limbs and acts against bile acid malabsorption | Grind whole plant and mix with water, then administer orally/topically | Thaba 'Nchu, Mokhotlong |
| *Ornithogalum shawii* Baker | Hyacinthaceae | 023/2019 | Sesepasalinoh a | 0.03 | L | Intestinal parasites and gastrointestinal diseases in sheep | Macerate leaves and add water. Administer orally | Mokhotlong |
| *Othonna natalensis*Sch. Bip. | Asteraceae | 024/2019 | Phela/ Naka | 0.03 | WP | Anthelmintic for calves | Decoctions are prepared and administered orally | Thaba 'Nchu, QwaQwa |
| *Salvia repens* Burch. ex Benth. var. *repens* | Lamiaceae | 025/2019 | Mosisili/ Mosisili oa loti | 0.03 | L | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | Maseru, QwaQwa |
| *Pentanisia prunelloides*(Klotzsch ex Eckl. &Zeyh.) Walp. | Rubiaceae | 026/2019 | Setima-mollo | 0.03 | L | Used for external cuts and acts against bile acid malabsorption | Decoctions are prepared and administered orally/ as enema/topically | Maseru, QwaQwa |
| *Artemisia afra* Jacq. ex Willd | Asteraceae | 027/2019 | Lengana | 0.03 | L | Used to treat stomach ailments and intestinal worms | Decoctions are prepared and administered orally | QwaQwa |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Gnidia kraussiana* (Meisn.) var. *kraussiana* | Thymelaeaceae | 028/2019 | Thobeha/ th'opa e nyenyane | 0.03 | L | Acts against bile acid malabsorption/ heart water, anthrax and as tick repellent | Prepare decoctions and administer orally | Thaba 'Nchu |
| *Hermannia depressa*N.E.Br. | Sterculiaceae | 029/2019 | Seletjane | 0.01 | L & R | To treat or prevent miscarriage | Decoctions are prepared and administered orally | Mokhotlong |
| *Euphorbia clavarioides* (Boiss.) *var. clavarioides* | Poaceae | 030/2019 | Sehloko | 0.01 | L & R | Acts against colic | Decoctions are prepared and administered orally | Thaba 'Nchu |
| **Eucalyptus* sp. | Myrtaceae | 031/2019 | Boloukomo | 0.01 | L | Eliminates excessive drooling of mucus in sheep | Prepared as a decoction and administered orally | Maseru |
| *Eucomis autumnalis* (Mill) Chitt. *subsp. clavate* (Baker) Reyneke | Hyacinthaceae | 032/2019 | Khapumpu | 0.01 | R | Used to treat black quarter/ blackleg disease | Prepared as a decoction and administered orally | Thaba 'Nchu |
| *Podaxis pistillaris* (L. ex Pers.) Fr. | Agaricaceae | 033/2019 | Ts'upaneealih olo | 0.01 | WP | Used to treat black quarter/ blackleg disease | Grind whole plant and mix with salt, then administer orally | Thaba 'Nchu |
| *Halleria lucida* L. | Stilbaceae | 034/2019 | Lebetsa | 0.01 | L & S | Used in the treatment of Streff syndrome | Dry and burn the plant parts, and administer the smoke through inhalation | QwaQwa |
| *Calpurnia sericea* Harv. | Fabaceae | 035/2019 | Tloele/ Mot'sohlo | 0.01 | L & S | Used as a tick repellent | Shred and mix plant parts with water and administer topically | Thaba 'Nchu |
| *Gazania krebsiana* (Less.) subsp. | Asteraceae | 036/2019 | Tsikitlana | 0.01 | R | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | Thaba 'Nchu |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *serrulata* (DC.) Roessler | | | | | | | | |
| *Morella serrata* (Lam.) Killick | Myriaceae | 037/2019 | Monna mots'o | 0.01 | R | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | Maseru |
| *Clematis brachiate* Thunb. | Ranunculaceae | 038/2019 | Moraraoathaba | 0.01 | R | Acts against bile acid malabsorption | Infusions are made and administered orally | Thaba 'Nchu |
| *Cyathula uncinulata* (Schrad.) Schinz | Amaranthaceae | 039/2019 | Bohomebalipoli/ Bohomeboboholo | 0.01 | R | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | Thaba 'Nchu |
| *Allium sativum* L. | Alliaceae | 040/2019 | Konofolo | 0.01 | L | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | Thaba 'Nchu |
| *Searsia aeroa* (Thunb.) | Anacardiaceae | 041/2019 | Ts'inabelo\ Ts'ilabele | 0.01 | R | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | Thaba 'Nchu |
| *Xysmalobium undulatum*(L). Aiton f. var.*undulatum* | Apocynaceae | 042/2019 | Poho-ts'ehla/ Leshokhoa | 0.01 | R | Diarrhea | Grind the roots, mix with salt and administer orally | Thaba 'Nchu |
| *Senecio asperulus* DC. | Solanaceae | 043/2019 | Moferefere/ Letapisoana | 0.01 | L | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | Maseru |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Senecio serratuloides* DC. | Asteraceae | 044/2019 | Khotoliaeanok a | 0.01 | L | Acts against bile acid malabsorption and is used for external cuts | Burn or grind leaves and apply topically. Or decoctions are prepared and administered orally | Maseru |
| *Olea europaea* subsp. *Africana* (Mill) P.S. Green | Oleaceae | 045/2019 | Mohloare | 0.01 | L | Acts against bile acid malabsorption | Infusions are prepared and administered orally | Maseru |
| *Erica maesta* Bolus | Ericaceae | 046/2019 | Chalebeke/ Sekikitlela | 0.01 | L | Acts against bile acid malabsorption | Decoctions are prepared and administered orally | QwaQwa |
| *Searsia divaricate* (Eckl. & Zeyh.) | Anacardiaceae | 047/2019 | Kolits'ana | 0.01 | R | Used to treat blurry eyes; acts against bile acid malabsorption | Decoctions are prepared and administered orally | QwaQwa |
| *Dierama robustum*N.E.Br. | Iridaceae | 048/2019 | Lethepu | 0.01 | R | Used for constipation | Crush and mix the roots with water. Administer as an enema | Mokhotlong |
| *Plectranthus ciliatus* E.Mey. | Lamiaceae | 049/2019 | Lephele-phele | 0.01 | WP | Used for diarrhoea | Decoctions are prepared and administered orally | Thaba 'Nchu |
| **Prunuspersica* (L.) Batsch var. *persica* | Rosaceae | 050/2019 | Sefatesapereki si | 0.01 | L | Acts against bile acid malabsorption | Decoctions of Papoleri and peach tree are prepared and administered orally | Thaba 'Nchu |
| *Melolobium microphyllum* (L.f.) Eckl. & Zeyh. | Asteraceae | 051/2019 | Mofahla-toeba | 0.01 | R | To treat inflammation/ swelling in the mouth | Decoctions are prepared and administered orally | Maseru, Mokhotlong, Thaba 'Nchu |

RFC = Relative Frequency Citation; Asterisk (*) indicates naturalised alien species; L = leaves, R = roots, WP = whole plant, S = stem.

## 2.4 Discussion

The use of medicinal plants to treat livestock diseases is a long-standing practice, especially among rural farmers. In the present study, the majority (94%) of respondents were males. Similar results were reported by Moteetee and Van Wyk (2011), Yirga *et al. (*2012), Landau *et al. (*2014), Seleteng *et al. (*2015) and Mthi *et al. (*2018). According to these authors, most traditional healers and subsistence farmers are men. Women are traditionally believed or expected to perform home duties and care for their families. Also, existing taboos exclude women from tending livestock or handling strong medicines (Moteetee & Van Wyk, 2011; Seleteng *et al.*, 2015). This may explain the poor representation of women in this study since they are only allowed to rear small animals like pigs and poultry. Morris and Msonthi (1996) averred that women are interested and knowledgeable in the general use of medicinal plants and healing. Therefore, women should also be considered knowledge holders in traditional healing activities, which males have since dominated.

The reared livestock in this study are considered very important in the African community as they play crucial roles in the socio-cultural and socio-economic wellbeing of marginalised rural populations. These animals are used for traditional rituals and ceremonies, the provision of milk, meat, manure for agricultural activities, and raw materials (Dovie *et al.*, 2006; Ndebele *et al.*, 2007; Bettencourt *et al.*, 2013; Nyahangare *et al.*, 2015). According to the same authors, they also serve as social security/store of wealth in many cultural settings. The majority of the respondents were between the ages of 66 - 75, and this concurred with the submissions of Luseba and Tshisikhawe (2013) who reported that the younger age group are less keen on traditional matters due to modernisation and its impact on the youths of today. As a result, the younger generation possesses limited knowledge of agricultural activities and traditional medicines (Van der Merwe *et al.*, 2001).

Fifty-one medicinal plant species from thirty-five families used for ethnoveterinary purposes were recorded in this study. Previous studies have documented various medicinal plant species used for livestock in the Southern African region. For instance, in 2006, Luseba and Van der Merwe documented 19 plants species from 12 families among the Tsonga speaking people of South Africa, Moteetee *et al. (*2019) reported

437 plant species in both Lesotho and the Free State province of South Africa, while in 2020, McGaw *et al.* recorded 139 plant species in South Africa. Like the present study, Nyahangare *et al*. (2015) reported 51 plant species in Zimbabwe, while Bruschi *et al*. (2017) documented 39 Angolan plant species used for ethnoveterinary purposes.

Most (27%) of the medicinal plants we report belong to the Asteraceae family. This portrays their high frequency of use and availability in the study areas. *Rhamnus prinoides*, *Aloe striatula, Leucosidea sericea* and *Monsonia burkeana* were the most mentioned medicinal plants. This may be due to the antimicrobial (Luseba & Van der Merwe, 2006; Tshivhandekano *et al.*, 2014), anthelmintic (Aremu *et al.*, 2010), anti-inflammatory and high antioxidant activities of these plants (Mamphiswana *et al.*, 2010; Mafole *et al.*, 2017). Additionally, Burhanu (2014) and Molla *et al. (*2016) showed that *Rhamnus prinoides* possesses purgative and antimicrobial activity, while Chen *et al., (*2020) also showed that *R. prinoides* is a good source of polyphenols and flavonoids. According to Aremu *et al.* (2010), *Aloe striatula* and *Leucosidea sericea* possess antimicrobial and anthelmintic activities. Likewise, Berhanu (2014) and Molla *et al. (*2016) reported that people can easily access it due to the greater frequency of use of Rhamnus prinoides for ethnoveterinary purposes. Other researchers, including Olajuyigbe and Afolayan (2012), have reported the use of *Erythrina caffra* extract against bacteria associated with diarrhoea, while Birhan *et al. (*2018) reported the use of *Withania somnifera* for the treatment of blackleg (black quarter) disease. A study by Shlar *et al. (*2019) showed the use of *Momordica charantia* against parasites. Yipel *et al. (*2017) and Khan *et al. (*2018) also reported the treatment of diseases and health conditions such as dysentery and retained placenta using the same plant.

The most frequently used plant parts in herbal remedies in this study were roots (45%) and leaves (43%). These results agree with those of other researchers who stated that most plant bioactive compounds are manufactured in the roots (Teklay, 2015; Eshetu et al., 2015; Assefa & Bahiru, 2018). Therefore, herdsmen and traditional healers continue utilising these plant parts to formulate their traditional medicine. However, the harvesting of some plant parts such as bark and roots, coupled with the high local and international market demand for medicinal plants, can result in the extinction of such plant species and damage the environment (Ngarivhume *et al.*, 2015; Mandal & Rahaman, 2016). Hence, the practice of sustainable harvesting of these natural

resources within their limits to regenerate is encouraged since this promotes conservation and enhances the sustainability of medicinal plants.

Local farmers and livestock herdsmen employ different methods to prepare remedies for their animals. Decoctions and infusions were the most predominant aqueous preparations recorded in the present study. This concurs with an earlier report by Luseba and Tshisikhawe (2013), who also documented the same preparation method. Earlier studies by Masika *et al.* (1997) averred that most livestock farmers diagnose their livestock based on the signs and symptoms the animals exhibit or even by postmortem findings. Based on their diagnosis, appropriate medicinal plants are collected, prepared, and administered to the ailing animal. Problems such as parasitism (*kokoana, papisi*), excessive salivation in sheep due to bacterial infections, bovine tumours, miscarriages, bone fractures, retained placentas, eye problems, gall bladder abnormalities and liver issues are combated with several plants, either individually or in combination with other plants or non-plant materials. In their study of 2017, Yipel and co-workers reported similar observations where the most predominant livestock diseases treated with medicinal plants were gastrointestinal disorders. It has been noted that these diseases and parasites cause significant economic losses to the farmers and consequently the local economies, which are constantly under stress (Rajput et al., 2006; Nwafor et al., 2019).

## 2.5 Conclusions

The use of medicinal plants for ethnoveterinary purposes has remained a common and important practice amongst the rural resource-poor subsistence farmers in the present study areas. Although the survey showed some possible decline in knowledge of these plants, this study revealed that the region is endowed with a rich plant biodiversity of medicinal plant species used to treat various animal diseases. Some plants, such as *Rhamnus prinoides* and *Monsonia burkeana,* used in treating bile acid malabsorption, have been identified by the RFC index in this study as some of the most useful and effective species against internal parasites and diarrhoea. These medicinal plants indicate efficacy and may be priority plants for further investigation with the potential for new ethnoveterinary drug development. Therefore, traditional knowledge should be conserved through documentation to safeguard the use of plants

as medicines. Documentation of medicinal plants will complement the oral tradition of dissemination of knowledge and generate comprehensive baseline data, which will be used in future studies and references. The youth should also be encouraged to actively participate in knowledge acquisition, propagation, and practice using these beneficial plants to ensure continuity. Further investigations to validate medicinal claims, ascertain safety, establish mechanisms of action and effective dosage of administration of these medicinal plants are still required.

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# CHAPTER 3 :
# QUALITATIVE AND QUANTITATIVE ANALYSIS OF SECONDARY METABOLITES IN SOME ETHNOVETERINARY HERBAL MEDICINES FROM HIGHLAND GRASSLANDS OF THE FREE STATE, SOUTH AFRICA, AND LESOTHO

## 3.1 Introduction

The pharmaceutical industry is shifting its focus towards medicinal plants because they are rich sources of therapeutic compounds with diverse and tremendous applications (Akhtar & Mirza, 2018). Herbal medicines are increasingly becoming popular as they are believed to be safe, easily accessible and have fewer side effects (Morankar & Jain, 2019). Furthermore, reports indicate that approximately 80% of the population in developing countries relies on traditional medicine for healthcare (Beyene *et al.,* 2016). Medicinal plants possess bioactive compounds that serve as defence mechanisms against predators such as microorganisms, insects, and herbivores. These compounds play an important role in curing and healing different diseases (Morankar & Jain, 2019).

Phytochemicals, including secondary metabolites, are rich in free radical scavengers such as vitamins, terpenoids, phenolics, lignins, stilbene, tannins, flavonoids, quinones, alkaloids, amines, and other metabolites (Gracelin *et al.,* 2012; Jimenez-Garcia *et al.,* 2013; Ajiboye *et al.,* 2013). These secondary metabolites can act as antioxidants, antiseptics, antibacterials, antifungals, anti-inflammatory, anthelmintic, anti-tumour, anti-carcinogenic and antiviral agents (Edziri *et al.,* 2020; Sala *et al.,* 2003; Ajiboye *et al.,* 2013).

Some reports indicate that plants such as *Aloe striatula* are toxic (Wink, 2008; Van Wyk, 2008), but most traditional herbalists or subsistence farmers use these medicinal plants without adequate knowledge of their properties or the active ingredients and therefore potential dangers. Therefore, the plant's authenticity, quality, and quantity do not guarantee its safety (Rukangira, 2001; Buwa & Van Staden, 2006). This chapter, therefore, focuses on the phytochemical study of water, methanol, and acetone extracts on *R. prinoides, L. sericea, M. burkeana* and *A. striatula.* These plants were selected based on the RFC values indicated in Chapter 2. The knowledge generated

in this study will further assist in elucidating the phytochemical constituents that can be linked to these plants' pharmacological values and determine the toxicity of some compounds.

### 3.1.1 *Leucosidea sericea* Eckl. & Zeyh

*L. sericea* (Figure 3.1) is a tree/ large shrub known as old wood in English or Cheche in the southern Sotho language (Moteetee & Van Wyk, 2011). It falls under the Rosaceae family and is an evergreen plant that tolerates harsh, cold conditions and is primarily found in the eastern and southern parts of South Africa (Wink, 2008). This plant is traditionally used to treat high blood pressure, coughs, HIV, and herpes in humans (Seleteng-Kose *et al.,* 2015; Moffett, 2010). In animals, it is used to cure diseases or conditions such as vermifuge, eye problems and excessive bile secretion. Farmers/ herdsmen use the leaves and stems to prepare the medicine by decoction (Motsoari *et al.,* 2022).

***Figure 3.1: Leucosedia sericea Eckl. & Zeyh***

### 3.1.2 *Rhamnus prinoides* L 'Herit

*R. prinoides* (Figure 3.2) is a tree belonging to the Rhamnaceae family and known as dogwood in English and mofifi in the southern Sotho language (SANBI, 2020). This

tree is widely spread in most parts of South Africa. It has light, young green leaves which turn dark and shiny as the plant matures (Suntrees, 2020). *R. prinoides* grows on mountains, stream banks and among rocks. Sotho-speaking people traditionally use this tree as a protective charm against evil spirits and lightning. In addition, it is used to treat respiratory infections, pneumonia and tonsillitis (Possa & Khotso, 2015; Moteetee & Van Wyk, 2011).

***Figure 3.2: Rhamnus prinoides L. 'Herit***

### 3.1.3 *Monsonia burkeana* Planch ex Harv

*M. burkeana* (Figure 3.3) is a Southern African herb belonging to the Geraniaceae family and known as Special tea in English and Khoara by the southern Sotho-speaking people of Lesotho and South Africa (Woldesemayat *et al.,* 2016; Touloumenidou *et al.,* 2007). This herb is widely distributed in the Southern African countries such as Lesotho, South Africa, Botswana, and Swaziland (Nnzeru *et al.,* 2016). This herb is one of the most used plants to cure diarrhoea and excessive bile secretion (Motsoari *et al.,* 2022).

***Figure 3.3: Monsonia burkeana Planch ex Harv***

### 3.1.4 *Aloe striatula* Haw. var. *striatula*

*A. striatula* (Figure 3.4) is a herb widely spread in the mountainous areas of the Eastern Cape, Free State and Lesotho (van Wyk & Smith, 1996). This herb belongs to the family Asphodelaceae, and the Southern Sotho people call it mohalakane, while some common English names include climbing aloes, rambling aloes, and scrambling aloes. In humans, *A. striatula* is traditionally used to treat upset stomach, bad digestion, constipation, HIV, high blood pressure, to heal wounds and excessive secretion of bile in animals (Moteetee & Van Wyk, 2011; Moffette, 2010; Seleteng-Kose *et al.,* 2015; Motsoari *et al.,* 2022).

***Figure 3.4: Aloe striatula Haw. var. striatula***

## 3.2 Materials and methods

### 3.2.1 Plant collection

Plant collection and identification was carried out as described in section 2.2.3 and as shown in Table 3.1. Fresh plant material was collected.

***Table 3.1: Plant collection locations and plant parts collected***

| Plant name | Plant part | Collection season | Location |
|---|---|---|---|
| *Aloe striatula* | Leaves | Late spring to early summer | Maseru |
| *Leucosidea sericea* | Leaves and stems | Spring to early summer | Qwaqwa |
| *Monsonia burkeana* | Roots | Late autumn to winter | Qwaqwa |
| *Rhamnus prinoides* | Leaves | Late spring to midsummer | Qwaqwa |

### 3.2.2 Preparation of plant extracts

Plant material was washed with distilled water and dried at room temperature. The dried plant material of each plant species was then ground into a fine powder and stored in the refrigerator at 4°C until further use (Mostafa *et al.,* 2018). The plant extracts were prepared by dissolving 20 g of the powdered plant material separately

in 200 mL of distilled water, 99.9% methanol and 99.9% acetone. The mixtures were then placed on a rotary shaker for 24 hours for maximum extraction. The resultant mixture was then filtered using an oil-free vacuum pump and dried using a fan in an incubator at 37°C. The initial and final masses of the extracts were all recorded. All extracts were stored in the fridge at 4°C until required.

### 3.2.3 Qualitative analysis of secondary metabolites

Secondary metabolites in the extracts were identified using standard phytochemical analysis methods for alkaloids, steroids, phenols, cardiac glycosides, tannins, flavonoids and saponins (Njoku & Obi, 2009). The test for the secondary metabolites was based on visual observation of colour changes or through the formation of a precipitate after adding the specified reagent(s).

#### *3.2.3.1 Tannin test*

After stirring the mixture, a small amount of iron (III) chloride ($FeCl_3$) solution was added along with 2 mL of extract and 2 mL of distilled water. The development of a green precipitate signalled the presence of tannins.

#### *3.2.3.2 Saponin test*

A test tube containing 5 mL of the extract and 5 mL of distilled water was forcefully shaken to create a stable, long-lasting froth. Three drops of extra virgin olive oil were added to the foam, which was violently mixed before being checked for the creation of an emulsion, a sign that saponins were present.

#### *3.2.3.3 Test for Flavonoids*

Five millilitres of each extract were combined with 3 mL of an aluminium chloride solution. A yellow precipitate that formed indicated the presence of flavonoids.

#### *3.2.3.4 Test for Steroids*

When 2 mL of the organic extract was dissolved in 2 mL of chloroform and then subjected to acetic and sulphuric acid treatment, a greenish tint resulted, indicating the presence of steroids.

#### *3.2.3.5 Test for alkaloids*

In a steam bath, 3 mL of the extract and 3 mL of 1 per cent HCl were combined. A few drops of Mayer's reagents were added to the combination (1,36 g of mercuric chloride and 5 g of potassium iodide in 100 mL of distilled water). The production of a cream colour indicated a positive test for alkaloids.

#### *3.2.3.6 Tests for cardiac glycosides and cardenolides (Killer-Killian test)*

About 5 mL of the extract was treated with 2 mL of glacial acetic acid, including one drop of ferric chloride. About 1ml of concentrated sulfuric acid downplayed this. The presence of cardenolide was positively confirmed by a brown ring at the interface, which shows cardenolide deoxy sugar properties. A violet-green ring that appeared beneath the brown ring in the acetic acid sheet denoted the presence of glycosides.

#### *3.2.3.7 Test for phenols*

A 30 mL test tube was filled with 5 mL of the extract and 10 mL of distilled water. The 5 mL of concentrated amyl alcohol and 2 mL of ammonium hydroxide solution were also added, and the mixture was allowed to react for 30 minutes. The development of a blue-green colour indicated the presence of phenols.

### 3.2.4 Quantitative analysis of phytochemicals

#### *3.2.4.1 Determination of total Phenolic content*

The total phenolic content was determined by utilising the approach of Oluwaseun (2011) and Shad *et al.* (2012). Specifically, 1mg/ml of extract was diluted with water at a ratio of 1:10 before combining with 4 mL (75 g/l) sodium carbonate and 5 mL of

Folin-Ciocalteu reagent. The test tubes were vortexed for 15 seconds, then left to stand at 40°C for 30 min to check for colour change. A spectrophotometer was then used to measure the absorbance at 765 nm in triplicate. The total phenolic content was consequently calculated using an equation derived from the gallic acid calibration curve and reported as mg/g gallic acid equivalent. The concentration of the phenolic compounds in the samples was determined using an equation obtained from a standard gallic acid curve.

The concentration of the total phenol compounds in the samples was determined as milligrams of gallic acid equivalent by using the following equation:

A = (cxv)/ m

Where:

A = total phenol content (mg gallic acid equivalents)

C = X / 1000 = concentration of gallic acid in mg/ml

V = volume of extract

M = mass of extract

#### *3.2.4.2 Determination of total Tannins*

Tannins were measured by mixing 0.1 mL of the extract (1mg/ml) with 7.5 mL of distilled water. The test tubes holding the diluted solution extract received 0.5 mL of Follin-Ciocalteu phenol reagent. The liquid was then given a 1 mL addition of 35 % sodium carbonate before being adjusted to 10 mL with distilled water. After shaking, the mixture was left to stand at room temperature for 30 min. The absorbance at 510nm was measured against a blank using a spectrophotometer and compared to a standard curve of produced gallic acid solution made in distilled water. Three duplicates of each concentration were tested. Tannins were then quantified as mg of gallic acid equivalent per gram (mg/g) (Masiphephethu, 2019; Komape, 2019). The total content of tannins in the plant extracts in tannic acid equivalent was calculated by the following formula equation:

C = (c x V)/ m

Where:

C = Total content of tannin compounds, mg/g plant extract, in tannic acid

c = the concentration of tannic acid established from the calibration curve, mg/ml

V = the volume of the extract, mL

m = the weight of pure plant extracts, gm.

## 3.3 Results

The results of the present study indicate that the four plants have some bioactive compounds. Six phytochemicals (tannins, saponins, flavonoids, cardiac glycosides, phenols, and steroids) which were extracted using three solvents; methanol, acetone and distilled water were present in *R.prinoides, M. burkeana, L. sericea* and *A. striatula* (Table 3.1). However, all the tested plant extracts showed negative results for alkaloids. *A. striatula* showed the presence of tannins, flavonoids, and cardiac glycosides. At the same time, the other four metabolites could not be detected. *M. burkeana* extracts tested positive for saponins, cardiac glycosides, phenols, and steroids, but tested negative for the other compounds. *R. prinoides* and *L. sericea* tested positive for six phytochemicals, excluding alkaloids.

***Table 3.2: Qualitative analysis of secondary metabolites in medicinal herbs used for ethnoveterinary purposes in the highland grasslands of the Free State province, South Africa, and Lesotho***

| Medicinal plants | Extract | Tannins | Saponins | Flavonoids | Cardiac glycosides | Alkaloids | Phenols | Steroids |
|---|---|---|---|---|---|---|---|---|
| ***R. prinoides*** | Methanol | + | - | + | + | - | + | + |
| | Acetone | + | + | - | + | - | + | + |
| | Water (D) | + | + | + | + | - | + | + |
| ***A. striatula*** | Methanol | - | - | + | + | - | - | - |
| | Acetone | + | - | - | + | - | - | - |
| | Water (D) | - | - | + | - | - | - | - |
| ***M. burkeana*** | Methanol | - | - | - | + | - | + | + |
| | Acetone | - | - | - | + | - | + | + |
| | Water (D) | - | + | - | + | - | + | + |
| ***L. sericea*** | Methanol | + | - | + | - | - | + | + |
| | Acetone | + | + | - | + | - | + | + |
| | Water (D) | - | - | + | + | - | + | + |

+ = Present, - = Absent, D- Distilled

### 3.3.1 Total phenolic content (TPC)

The phenolic content of the plant extracts was measured using the Folin-Ciocalteu reagent. The results were derived from a calibration curve (y = 0.0048x+0.0187, $R^2$=0.9966) (Figure 3.1) of gallic acid (0-500 µg/ml) and expressed in gallic acid equivalents (GAE) per gram dry extract weight (Table 3.2). The total phenolic content from all plant extracts varied from 5.102 to 1.846 GAE/g of dry weight (Table 3.2). The methanol extract of *R. prinoides* was observed to be the highest (5.102 GAE/g dried extract), followed by the aqueous extract of *M. burkeana* (3.991 GAE/g dried extract), while the lowest (1.846 GAE/g dried extract) was observed from the aqueous extract of *L. sericea*.

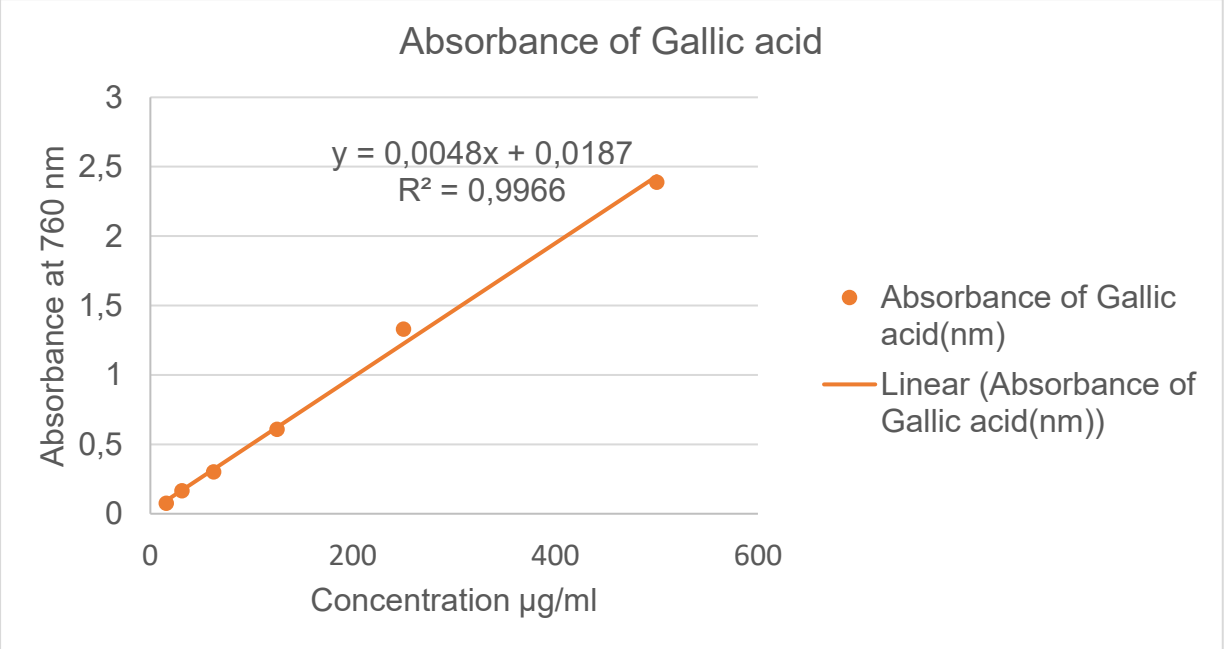


***Figure 3.5: Calibration curve of Gallic acid for total phenol content***

***Table 3.3: The total phenolic and tannin contents of L. sericea, M. burkeana, R. prinoides and A. striatula extracts***

| Extracts | TPC (mg GAE/g dry extract wt) | TTC (mg TAE/g dry extract wt) |
|---|---|---|
| L.s $dH_20$ | 1.846 | ND |
| M.b $dH_20$ | 3.991 | ND |
| L.s Ace | 2.491 | 0.671 |
| R.p Ace | 2.739 | 1.47 |
| M.b Ace | 2.744+ | ND |
| L.S MeOH | 3.955 | 0.346 |
| M.b MeOH | 3.477 | ND |
| R.p MeOH | 5.102 | 0.344 |
| A.s $dH_20$ | ND | 2.242 |
| R.p $dH_20$ | ND | 1.207 |

**Where:** *Ls = *L. sericea*; M.b = *M. burkeana*; R.p = *R. prinoides*; *$dH_20$ = distilled water; Ace = acetone; MeOH = methanol, As = *A. striatula,*

### 3.3.2 Total Tannin content (TTC)

The total tannin content (TTC) results were derived from a calibration curve (y=0.0005x-0.0277, $R^2$= 0.9992) (Figure 3.6) and expressed in tannic acid equivalents (TAE) per gram dry extract weight (Table 3.2). The total tannin content of plant extracts obtained from the three different solvents varied from 1.47 to 0.242 (TAE/g dried extract) (Table 3.2). The aqueous extract of *A. striatula* was the highest (2.242 TAE/g dried extract), followed by the acetone extract of *R. prinoides* (1.47 TAE/g dried extract), while the lowest (0.242 TAE/g dried extract) was observed from the methanol extract of *R. prinoides*.

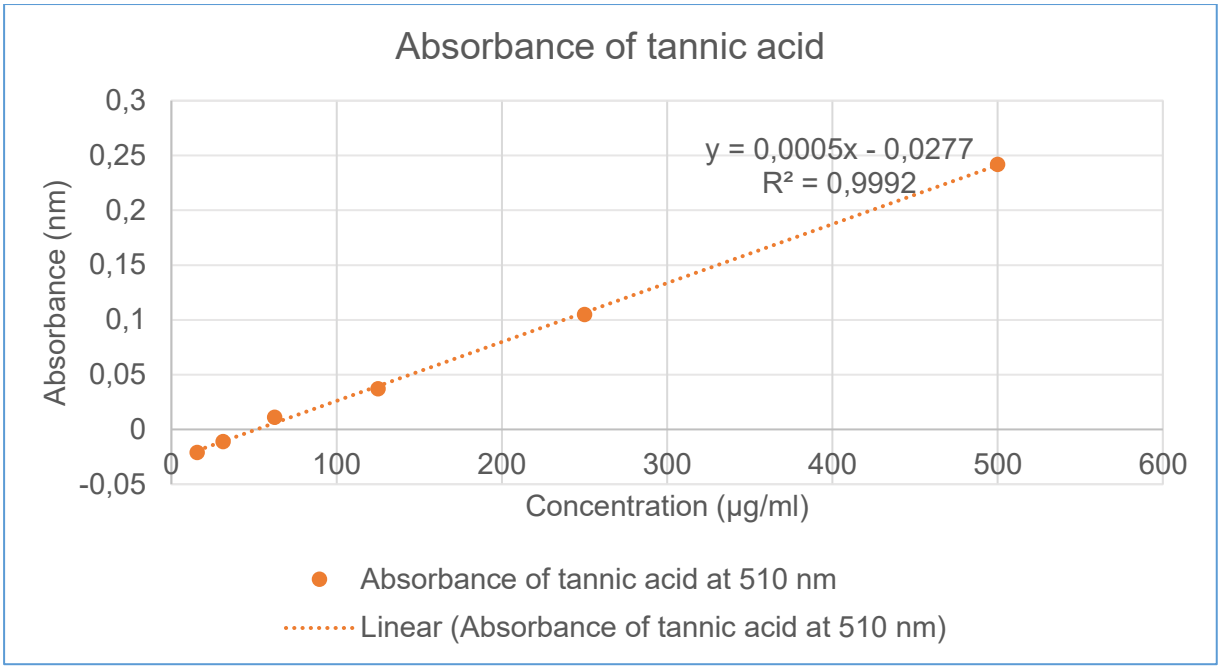


***Figure 3.6: Calibration curve of tannic acid for total tannin content***

## 3.4 Discussion

All the tested plant materials showed negative results for alkaloids. This could be due to various reasons, including climatic and environmental factors of the locations where the plants were collected. Environmental, climatic, season of collection and plant species, among other factors, have been shown to influence the development of secondary metabolites in medicinal plants (Pant *et al.,* 2021). Some of these phytochemicals are both beneficial and toxic, such as cardiac glycosides, which assist in wound healing and are also used by hunters as arrow poisons (Borgia *et al.,* 2017; Figueiredo *et al.,* 2008; Chaboo *et al.,* 2019).

Alkaloids are also known to be toxic (Buwa & Van Jaden, 2006; Ngobeni *et al.,* 2015) while saponins act as defence mechanisms against pathogens and herbivores in addition to their antimicrobial, antiparasitic and anti-inflammatory activities (Augustin *et al.,* 2011; Elekofehinti, 2015). Tannins protect against biotic and abiotic stress and possess free radical scavenging activity and antimicrobial and anti-nutritional properties (Ngobeni *et al.,* 2016). Different studies indicate that the intake of tannins may prevent the onset of several chronic diseases for both animals and humans (Wang *et al.,* 2014; Smeriglio *et al.,* 2017).

Phenols are ubiquitous in the environment and play a role in determining colours. They are polymers and constitute natural substances and drugs (Michalowicz & Duda, 2007). They are said to be the most prominent family of phytochemicals that have dynamic standards of plant-derived nutraceuticals, homegrown therapeutic items and possess antimicrobial properties against a wide range of pathogenic microorganisms such as *Escherichia coli* and *Staphylococcus epidermidis* (Espín *et al.,* 2017; Shahzad *et al.,* 2016). Hosu *et al. (*2014) reported that quantitative phytochemical analysis can determine the existence and abundance of phenols and tannins in plants. Tannins are important markers of a plant's medicinal potential (Tshivhandekano *et al.,* 2014). Phenolic compounds play a crucial role in the physiological value and quality of the plant (Mohammed & Manan, 2015).

The study's findings also agree with Reyaba *et al. (*2015), who found that extraction values strongly depend on solvent polarity, and that different contents of dissolved phenols and total tannin contents correspond to various concentrations. Iloki-Assanga *et al.* (2015) and Nawaz *et al.* (2020) reported that solvents rarely extract all Phyto-constituents in a plant; hence, using different solvents with different polarities. As a result, different researchers found that solvent polarity significantly impacts the extract yield of phenolic compounds in plant materials (Barchan *et al.,* 2014; Ghasemzadeh *et al.,* 2015). Other researchers have suggested that the existence of the total phenolic content and total tannic content in plants is influenced by variables such as plant tissues, species, temperature, water stress and light conditions (Rana *et al.,* 2019; Alam *et al.,* 2019; Ng *et al.,* 2020; Tan & Kassim, 2011; Achakzai *et al.,* 2009). The results of our study also concur with those of Mamphiswana et *al.* (2010) and Tshivhandekano *et al.* (2014), who reported similar total phenol constituents in *M. burkeana.* The study of Badeggi *et al.* (2020) and Pendota *et al.* (2018) revealed that *L. sericea* possesses some phenolic contents, while Chen *et al*. (2020) and Amabye *et al.* (2015) reported that *R. prinoides* possesses polyphenols and consequently some outstanding antioxidants and anti-inflammatory activities. The studies by these authors are well in agreement with the present report.

## 3.5 Conclusion

*R. prinoides*, *A. striatula, M. burkeana,* and *L. sericea* showed that they possess phytochemical compounds that confer plants their therapeutic properties. However, although *Aloe striatula* was the most mentioned plant with the highest RFC index, this plant tested negative for all methanol extracts for all phytochemicals**.** The current results indicate that these plants can potentially serve as sources of new antimicrobial drugs with the potential to be effective against drug-resistant strains. In conclusion, conducting phytochemical studies on animal medicinal plants is of great importance as it provides insight into the chemical compounds present in the plants and their potential medicinal benefits. These studies can help identify bioactive compounds that may be useful in developing new drugs or supplements for animal health. Additionally, phytochemical studies can potentially help to determine optimal methods for plant cultivation and extraction to maximise the yield of bioactive compounds. Therefore, phytochemical studies are crucial for the continued exploration and development of animal medicinal plants, including, but not limited to, synergistic effects, chemical compositions, and antimicrobial activities, as sources of natural remedies for various animal ailments.

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# CHAPTER 4 :
# ANTIMICROBIAL ACTIVITY OF ALOE STRIATULA, LEUCOSIDEA SERICEA, MONSONIA BURKEANA AND RHAMNUS PRINOIDES FROM THE HIGHLAND GRASSLANDS OF THE FREE STATE PROVINCE, SOUTH AFRICA, AND LESOTHO

## 4.1 Introduction

Due to the incorrect application of synthetic fungicides and bactericides, resistance of fungal and bacterial pathogens is increasing. These chemicals are hazardous to the environment, animals, and people (Tariq *et al.,* 2019; Paul *et al.,* 2019). The impact of antimicrobial resistance on public health is significant. Despite actions taken to ameliorate this problem, it continues to cause tremendous economic damage, forcing the world to challenge the loss of new therapeutics to prevent and treat various diseases in both animals and humans (Dadgostar, 2019; Hoelzer *et al.,* 2017; Cheesman *et al.,* 2017; Famuyide *et al.,* 2019). A growing problem of resistance against current antimicrobials has necessitated the discovery of new antimicrobial compounds/extracts to address this problem (Eloff, 2019).

Herbal medicines have continued to thrive in some societies despite the availability of modern medicine, owing to historical and cultural reasons as well as their efficacy and lower costs (Anyanwu & Okoye, 2017). Tshivhandekano *et al. (*2014) demonstrated that plants have long inspired sources for diverse compounds with antioxidant properties and antimicrobial effectiveness. Evidence shows that medicinal plants provide potential habitats for endophytic microbes due to their active compounds (Egamberdieva *et al.,* 2017). As a result, these plants are the foundation of traditional medicine, which explains why medicinal plants are used by over 3.3 million people in less-developed countries (Duchesne *et al.,* 2000). There are a variety of medicinal plants that are rich sources of ingredients that can be used for the manufacture and decoding of medications, and they have also played a key role in the development of ancient cultures around the globe (Singh, 2015).

Extracts of phytochemicals from leaves, roots, bark, seeds, and flowers contain various naturally occurring pharmaceuticals, which contribute to the plant's medicinal properties (Shankar *et al.,* 2016; Folashade & Omoregie, 2012). *In-vitro* studies have

shown that medicinal plants are abundant in phytochemicals with antimicrobial properties, which include tannins, terpenoids, alkaloids and flavonoids (Tshivhandekano *et al.,* 2014; Sapkota *et al.,* 2012). Naturally occurring antimicrobials are in great demand in many parts of the world, and plant-derived products are safer and more biodegradable than their chemically synthesised counterparts (Singh *et al.,* 2012). As an alternative to chemical preservatives, food companies are turning to plant extracts for fighting food spoilage and food poisoning, since they are healthy, safe, and natural (Singh *et al.,* 2021; Malaysia *et al.,* 2020). Medicinal plants are used as alternative medicine to improve human and animal life while also being used to eradicate antibiotic residual effects in animal products (Dhama *et al.,* 2014; Lillehoj *et al.,* 2018). Therefore, this chapter focuses on determining the minimum concentration of extracts required to inhibit the growth of bacteria and fungi using the microdilution method.

## 4.2 Materials and methods

### 4.2.1 Collection of plant samples

The plant materials were collected from different study area locations as described in Chapter 3.

### 4.2.2 Preparation of plant extracts

The plant materials were dried at 50°C in an oven and ground into fine powder using a blender. To prepare the extracts, 20 g of the powdered plant material was dissolved separately in 200 mL of 99.9% methanol, 99.9% acetone, and distilled water. The mixtures were then placed on a rotary shaker for 24 hr for maximum extraction using the maceration method. The plant extracts were filtered into glass jars through Whatman No. 1 filter paper and an oil-free vacuum pump. The filtrates were then dried in an incubator at 37°C. The initial and final masses of the extracts were recorded and stored until needed in a refrigerator at 4˚C.

### 4.2.3 Antibacterial activity of the plant extracts

#### *4.2.3.1 Bacterial strains*

The following bacterial strains were used to test the antimicrobial potency of each plant extract: *Bacillus pumilis* (ATCC 14884), *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (ATCC 25922), *Pseudomonas aeruginosa* (laboratory isolate), *Proteus vulgaris* (ATCC 6830), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermis* (laboratory isolate), *and Shigella flexneri* (laboratory isolate). The bacterial strains were obtained from the Department of Botany, University of Free State, Qwaqwa campus.

Eloff's (1998) microplate dilution method was used to determine plant extracts' minimal inhibitory concentration (MIC) values with antibacterial activity. Overnight cultures of the bacteria to be tested were prepared by inoculating 100 µl of the bacteria into 10 mL of Mueller-Hinton (MH) Broth medium and incubating for 24 hr at 20°C in a shaking water bath at 80 rpm. Subsequently, the overnight subculture stock was diluted with MH Broth medium at a concentration of 1:100 (200 µl bacteria: 19.8 mL MH broth) to ensure that the bacterial cells are at the start of the log phase at the beginning of the experiment.

Residues of the plant extracts were dissolved at 50 mg/ml with the corresponding extracting solvents in the case of water, acetone, and methanol. About 100 µl of sterile $dH_2O$ was pipetted into all the wells, and 100 µl of the plant extract was added to all wells in row A. This was followed by mixing with a pipette and serially diluting two-fold by pipetting 100 µl from well A down to well H, then discarding 100 µl from well H. All extracts were initially tested at 12.5 mg/ml in 96-well microplates and serially diluted two-fold to 0.098 mg/ml, after which 100 µl of bacterial subculture was added to each well properly and the microplates were covered with parafilm.

The antibiotic neomycin at a concentration of 256 to 0.5 µg/mL was included as a reference in each assay. An extract-free solution was used as a blank control. The microplates were incubated overnight at 37°C. As an indicator of bacterial growth, 40 µl of *p*-iodonitrotetrazolium violet (INT) was dissolved in water, added to the wells, and incubated at 37°C for 30 min. Minimal inhibitory concentration (MIC) values were then recorded as the lowest concentration of the extract that completely inhibited bacterial growth, i.e., a clear well.

The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms (Eloff, 1998). Where bacterial growth was inhibited, the solution in the well remained clear after incubation with INT. The microplates were then re-incubated for an extra 24 hours to determine the extract's minimal bactericidal concentrations (MBC), and the MBC values were recorded. MBC is "the lowest/ least concentration of the antimicrobial agent required to kill microorganisms after subculture onto antibiotic-free media" (Andrews, 2001; Owuama, 2017).

If 100 µl of 50 mg/ml plant extract is added to well A, then the final concentrations are as follows (Table 4.1):

***Table 4.1: Concentrations of each plant extract in wells A to I***

| Well | Concentration (mg/ml) |
|---|---|
| A | 12.5 |
| B | 6.25 |
| C | 3.125 |
| D | 1.56 |
| E | 0.78 |
| F | 0.39 |
| G | 0.195 |
| H | 0.098 |
| I | 0.049 |

#### *4.2.3.2 Fungal strains*

The following fungal strains were used to test the antimicrobial potency of each plant extract: A standard strain of *Candida albicans* (Ca), *Candida vulgaris* (Cv) and *Trichophyton mucoides* (Tm). The fungal strains were obtained from the Department of Botany, University of Free State, Qwaqwa campus.

The water extract residues were redissolved in water, and the organic solvent extract residues were dissolved in dimethyl sulfoxide (DMSO). All extracts were dissolved to a concentration of 100 mg/ml. The NCCLS proposed method (M27-P) broth microdilution test was modified (Espinel-Ingroff *et al.,* 1995). Four millilitres of sterile saline were added to approximately 400 µl of 24 hr old tested fungal strains. The absorbance was read at 530 nm and adjusted with sterile saline to match that of a 0.5

McFarland standard solution, and from the prepared stock yeast culture, a 1:1000 dilution with broth (e.g., 10µl stock yeast culture: 10 mL broth) was prepared. For aqueous extracts, 100 µl of broth was added to each well of a 96-well microplate and 100 µl of the water extract was added to well A and serially diluted from well A by taking 100 µl into B. This two-fold dilution was continued down the plate, and 100µl from the last well (H) was discarded. In the case of organic solvent extracts, 25 µl of the extracts were added to 175 µl broth and serially diluted. Three replicates were prepared for each extract. All the wells were then filled with 100 µl of stock yeast culture. Amphotericin B with the concentration of 0.03 to 16 µg/ml was used as a reference for this experiment, and the following controls were prepared: wells containing broth only, fungal strain with no extract, and serial dilutions of Amphotericin B with the fungi at the recommended inhibitory concentrations. The plates were then read at 630 nm on an ELISA reader, covered with parafilm and incubated at 33°C overnight, where their absorbance was reread.

## 4.3 Results

***Table 4.2: Antibacterial activity of some ethnoveterinary medicinal plant extracts from highland grasslands of the Free State province, South Africa, and Lesotho***

| Plant name/Scientific name | Extract | Extract yield (g) | Bacterial strains | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *B.p* | *E.c* | *K.p* | *P.a* | *P.v* | *S.a* | *S.e* | *S.f* |
| | | | MIC/ **MBC** (mg/ml) | MIC/ **MBC** (mg/ml) | MIC/ **MBC** (mg/ml) | MIC/ **MBC** (mg/ml) | MIC/ **MBC** (mg/ml) | MIC/ **MBC** (mg/ml) | MIC/ **MBC** (mg/ml) | MIC/ **MBC** (mg/ml) |
| ***Aloe striatula*** | Water | 1.11g | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** |
| | Acetone | 3.1g | > 12.5/ **N/A** | > 12.5/ **N/A** | > 12.5/ **N/A** | > 12.5/ **N/A** | 3.125/ **6.25** | 1.56/ **12.5** | 0.78/ **12.5** | 3.125/ **12.5** |
| | Methanol | 2.16g | 1.56/ **6.25** | 1.56/ **3.125** | 1.56/ **6.25** | 1.56/ **N/A** | 1.56/ **0.78** | 1.56/**6.25** | 1.56/**6.25** | 1.56/ **1.56** |
| ***Leucosidea sericea*** | Water | 3.54g | 0.78/**>12.5** | 1.56/ **>12.5** | 1.56/ **>12.5** | 1.56/ **>12.5** | 0.78/ **>12.5** | 1.56/ **>12.5** | 0.78/ **>12.5** | 0.78/ **>12.5** |
| | Acetone | 11.49g | 0.78/ **1.56** | ≤0.098/ **1.56** | ≤0.098/ **0.195** | 0.39/ **1.56** | 0.39/ **0.78** | 0.195/ **0.78** | ≤0.098/ **0.78** | ≤0.098/ **0.78** |
| | Methanol | 2.65g | 0.78/ **1.56** | 0.78/ **1.56** | 0.78/ **1.56** | 0.78/ **1.56** | 0.78/ **1.56** | 0.78/ **1.56** | 0.78/ **1.56** | 0.78/ **1.56** |
| ***Monsoniaburkeana*** | Water | 5.24g | 0.195/ **>12.5** | 0.39/ **>12.5** | 0.39/ **>12.5** | 0.39/ **>12.5** | 0.195/ **>12.5** | 0.195/**>12.5** | 0.195/**>12.5** | 0.39/**>12.5** |
| | Acetone | 8.02g | 0.39/**>12.5** | 0.098/**>12.5** | 0.098/**>12.5** | 0.098/**>12.5** | 0.039/**>12.5** | 0.195/**>12.5** | 0.195/**>12.5** | 0.195/**>12.5** |
| | Methanol | 5.78g | 1.56/ **1.56** | 0.78/ **1.56** | 0.78/ **1.56** | 0.78/ **1.56** | ≤0.098/ **3.125** | 0.195/ **3.125** | ≤0.098/ **3.125** | 0.195/ **3.125** |
| ***Rhamnus prinoides*** | Water | 3.54g | 6.25/ **1.56** | 6.25/ **0.39** | 6.25/ **0.195** | 6.25/ **0.39** | 6.25/ **1.56** | 6.25/ **1.56** | 6.25/ **0.39** | 6.25/ **0.39** |
| | Acetone | 11.49g | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **1.56** | 1.56/ **1.56** | 1.56/ **1.56** | 1.56/ **1.56** |
| | Methanol | 2.65g | 1.56/ **3.125** | 1.56/ **3.125** | 1.56/ **3.125** | 0.78/ **3.125** | 0.78/ **0.78** | 0.78/ **0.78** | 0.78/ **0.78** | 0.78/ **0.78** |
| Neomycin (µg/mL) | | | 3.125/ **3.125** | 0.39/ **0.39** | 3.125/ **3.125** | 0.78/ **0.78** | 12.5/ **12.5** | 0.39/ **0.39** | 0.78/ **0.78** | 3.125/ **3.125** |

**Where:** B.p = *B. pumulis;* E.c = *E. coli;* K.p = *K. pneumonia;* P.a = *P. aeruginosa;* P.v = *P. vulgari;* S.a = *S. aureus;* S.e = *S. epidermis* S.f = *S. flexneri.* NA= Not Active.

***Table 4.3: Antifungal activity of some ethnoveterinary medicinal plant extracts from highland grasslands of the Free State province, South Africa, and Lesotho***

| Plant name | Extract | Fungal strains and MIC/ MBC values in mg/ml | | |
|---|---|---|---|---|
| | | C.a | C.v | T.m |
| | | MIC/ **MBC** | MIC/ **MBC** | MIC/ **MBC** |
| ***Aloe striatula*** | Water | **0.78** | **1.56** | **1.56** |
| | Acetone | **0.049** | **3.125** | **3.125** |
| | Methanol | **3.125** | **1.56** | **3.125** |
| ***Leucosidea sericea*** | Water | **N/A** | **N/A** | **N/A** |
| | Acetone | **1.56** | **3.125** | **1.56** |
| | Methanol | **0.78** | **3.125** | **3.125** |
| ***Monsonia burkeana*** | Water | **0.78** | **0.39** | **0.78** |
| | Acetone | **1.56** | **3.125** | **3.125** |
| | Methanol | **0.39** | **0.39** | **0.39** |
| ***Rhamnus prinoides*** | Water | **0.78** | **1.56** | **6.25** |
| | Acetone | **0.78** | **0.39** | **0.195** |
| | Methanol | **0.195** | **0.78** | **1.56** |
| Amphotericin B (control) | | 0.39/**0.39** | 0.39/**0.39** | 0.39/**0.39** |

Where: C.a = *Candida albicans;* C.v = *Candida vulgaris;* T.m = *Trichophyton mucoides;* NA = Not Active

The results of the antibacterial activity of ethnoveterinary medicinal plant extracts from highland grasslands of the Free State province, South Africa, and Lesotho are shown in Table 4.2 and Table 4.3. A total of three different solvents (Acetone, Methanol, and distilled water) were used to extract compounds in 4 separate plant species each (*R. prinoides*, *A. striatula*, *M. burkeana* and *L. sericea*), and were tested against eight bacterial (Table 4.2) and three fungal (Table 4.3) species. The extract yields were recorded, and the acetone extracts of the four plants obtained the highest yields. These results show acetone was a better solvent than distilled water and methanol.

The ethanolic extract of *L. sericea* was active with MIC ranging from ≤0.098 mg/ml to 0.78 mg/ml, and the MBC ranged from 0.195 mg/ml to 1.56 mg/ml. However, the aqueous extract of *L. sericea* was not active against all tested fungal strains. The methanolic extract of *M. burkeana* MIC ranged from ≤0.098 mg/ml to 1.56 mg/ml, while the MBC ranged from 1.56 mg/ml to 3.125 mg/ml. The acetone extract of *A. striatula*

displayed high MIC values ranging from 0.78 mg/ml to >12.5 mg/ml and a high MBC ranging from 6.25 mg/ml to >12.5 mg/ml, but was inactive against the bacterial strains. This shows that a high concentration is needed to kill the pathogens. When the acetone extract of *A. striatula* was tested against fungal strains, it displayed a good MBC of 0.049 mg/ml.

## 4.4 Discussion

The results of the antibacterial activity are variable; however, acetone is usually used to isolate compounds that display antimicrobial activity (Loru *et al.,* 2000; Alberti *et al.,* 2014). The study of Rafajlovska *et al. (*2007), Meela *et al. (*2019) and de Oliveira *et al.* (2013) further proved that acetone is a better solvent; hence, a greater yield was obtained from the present study. The performance of acetone extracts against both bacterial and fungal strains in our study concurs with the work of Dzoyem *et al. (*2015), who also reported that acetone is the best solvent for most plant species and is suitable for antimicrobial activity.

However, methanol and distilled water had lower yields than acetone, and in most cases, distilled water gave the least yield, possibly because of the poor solubility of the chemicals. Solvent polarity affects the extraction of phytochemical compounds (Dhawan & Gupta, 2017; Elfalleh *et al.,* 2019). Traditional healers and farmers usually use aqueous extracts of medicinal plants. However, it has been noted that the use of organic solvents produces more potent and consistent antimicrobial activities than water extracts. In addition, time, temperature, and solvent are some of the factors to be considered during extraction, as these also determine the extraction yield (Anyanwu & Okoye, 2017; Nasir *et al.,* 2015).

Previous studies concur with the results of our study, which revealed that *L. sericea* is endowed with some secondary metabolites. For example, the study of Pendota *et al.* (2018) showed antibacterial activity (1.5 – 12.5 µg/ml) and noteworthy antifungal activity (3.9 - 250 µg/ml) of *L. sericea*. Pitso and Ashafa (2015) observed good inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with an MIC of 0.78 mg/ml. Adamu *et al., (*2012) also observed noteworthy antifungal activity with an MIC of 0.08 to 0.31 mg/ml against *Cryptococcus neoformans* and *Candida albicans.* Mafole *et al., (*2017) also found that *L. sericea* possessed different

antimicrobial, anti-inflammatory, anti-parasitic and antioxidant activity, and some noteworthy inhibitory antimicrobial activity.

Some researchers have successfully used aqueous plant extracts to produce metal nanoparticles which inhibit the growth of bacterial and fungal pathogens. For example, the *M. burkeana* aqueous extract has previously been used to make Zinc oxide nanoparticles (Ngoepe *et al.,* 2018). In addition, some authors have shown that this plant possesses some noteworthy antimicrobial activity, and these studies agree well with the results of the present study (Tshivhandekano *et al.,* 2014; Nnzeru *et al*., 2019; Ngoepe *et al.,* 2018; Mathivha *et al.,* 2019).

Eloff *et al. (*2005) showed that aqueous extracts rarely have any antimicrobial activity; however, in our study, the aqueous extract of *R. prinoides* showed a high MIC against bacterial strains with 6.25 mg/ml and a low MBC range of 1.56 to 0.195 mg/ml. The methanol and ethanol extracts of *R. prinoides* displayed effective results of 0.195 mg/ml against *Candida albicans* and *Trichophyton mucoides,* respectively. *R. prinoides* has been used for different purposes, such as in the treatment of scabies (Gebeyehu, 2016), brucellosis (Kiringe, 2006), and malaria (Cock *et al*., 2019). Other studies also corroborate the current study, which showed that *R. prinoides* possesses active compounds with antimicrobial, antimalarial, anti-inflammatory, and anti-biofilm activity (Campbell *et al*., 2019). Also, previous studies concur with the current study, revealing that *A. striatula* possesses some antimicrobial activity (Bisi-Johnson *et al.*, 2012). Grace *et al., (*2008) also showed that the Aloe species are traditionally recommended for treating different ailments, such as digestive ailments and internal parasites. The high MIC or poor activity against tested pathogens could be influenced by factors such as storage conditions of the plant material or extract, as this may affect the microbial efficacy (Anyanwe & Okoye, 2017). It has also been reported that due to factors such as incubation period, inoculum preparation method, inoculum size and assay medium, the antimicrobial activity of any plant extract may be negatively affected (Anyanwe & Okoye, 2017; Balouri *et al*., 2016; Gomez-Lopez *et al*., 2005).

## 4.5 Conclusion

The majority of the sampled ethnomedicinal plants from the present study have shown some antimicrobial activity against the pathogenic microorganisms. Acetone was the

most effective solvent for the extraction of the phytochemicals as compared to distilled water and methanol. *L. sericea* and *M. burkeana* plant extracts will be studied further for cytotoxicity and anti-anthelmintic activity. The tremendous amount of research that has been undertaken to elucidate the antimicrobial potential of medicinal plants would be better understood if studies on the antimicrobial activity of medicinal plants were compiled and made accessible to the public. Such studies could stir the public's interest in medicinal plants with antimicrobial activity, potentially leading to the discovery of novel antimicrobial compounds.

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# CHAPTER 5 :
# *IN-VITRO* CYTOTOXICITY SCREENING OF MONSONIA BURKEANA AND LEUCOSIDEA SERICEA AGAINST VERO CELLS

## 5.1 Introduction

Determining the bioactivity of plant extracts or natural products and synthetic compounds, such as antioxidant, antimicrobial, cytotoxic, among others, is crucial in discovering new and improved therapeutic entities (Olaru *et al*., 2015). Historically, natural products have proved to be the most prolific and diverse sources of antibiotics and other drugs. There is urgent need to develop new safe and efficacious drugs to reduce the global disease burden (Nguta *et al.,* 2016).

Although there is a common misunderstanding that medicinal plants are natural and therefore nontoxic for consumption, such plants may be beneficial at one dose and potentially poisonous at another (Mongalo *et al.,* 2017). Plant-related poisoning, in some cases, is not necessarily the result of the toxicity of the plants themselves but instead of the prescribed dosage, misidentification of the plant, or similar responses in an individual case, among other factors (Colegate *et al.,* 2015; Ndhlala *et al.,* 2013). The misidentification of herbal medications can come at a high cost, making phylogenetics an essential aspect of medicinal plant sciences (Mapfumo et al., 2023). Herbal drugs are typically regarded as nontoxic by consumers due to a misconception about their phytochemistry. Therefore, safety and toxicological approaches should be considered since the perception that herbal medicines are safe can lead to lethal complications (Gothai *et al.,* 2019; Mapfumo et al., 2023).

Most medicinal plants are poisonous if taken in large quantities; hence, the dosages should be prepared with great care and accuracy. Unless cytotoxicity assays are conducted on plant extracts, it may not be possible to estimate the quantity of the extract that can be used without toxic effects. Any additional amount above the recommended dosage may cause deleterious effects, for example, the oxalates of *Dieffenbachia picta,* which can cause severe inflammation and necrosis of the epithelium of the tongue and oral cavity intravascular (Loretti *et al.,* 2003). Many traditional healers and farmers use medicinal plants for different purposes. However, there is a lack of verifiable data pertaining to measures of safety, quality and efficacy

of the plant extracts used for ethnoveterinary and human purposes (Ngezahayo *et al.,* 2015; Mahomoodally, 2013).

An important initial step when investigating possible new therapies or developing new compounds for the treatment of an ailment is the determination of their cytotoxic potential and the determination of their harmful effects (Aslantürk *et al*., 2018; Adan *et al*., 2016). *Leucosidea sericea* and *Monsonia burkeana* displayed notable antimicrobial activity (Chapter 4) and were therefore chosen for further bioactivity studies including cytotoxicity. *L. sericea* is one of the important high-economical tree species in traditional medicine due to its variety of medicinal properties and consequent uses (Mafole *et al.,* 2017). *L. sericea* is primarily found in Southern Africa and used by different ethnic groups for its therapeutic properties, such as antimicrobial (Seleteng-Kose *et al.,* 2015; Pendota *et al.,* 2018) and anti-inflammatory activities (Mafole *et al.,* 2017). *M. burkeana* is an indigenous plant in Southern Africa, including Angola, Namibia, Zimbabwe, South Africa, and Lesotho (Ngoepe *et al.,* 2018). This plant's extracts are of high value due to the elevated phenolic, tannin, and antioxidant contents, which are desirable substances in biological applications. It has also been found to be significantly effective against several bacterial strains such as *E. coli*, *K. pneumoniae,* and *S. aureus* (Nnzeru *et al.,* 2017). In this study, extracts from *L. sericea* and *M. burkeana* were tested for cytotoxicity using the African green monkey kidney cell line (Vero cells).

## 5.2 Materials and methods

### 5.2.1 Sample preparation

The prepared plant extracts described in Chapter 4 were reconstituted in dimethyl sulfoxide (DMSO) to give a final concentration of 100 mg/mL. In case of solubility problems, the samples were sonicated and stored at 4°C until further use.

### 5.2.2 Treatment protocol

Vero, the African green monkey kidney cell line, was used for cytotoxicity screening (Senthilraja and Kathiresan, 2015). Cells were maintained at 37°C in a humidified incubator with 5 % $CO_2$ in 10 cm culture dishes. Complete growth medium consisted

of DMEM supplemented with 10 % FBS and 10 % penicillin-streptomycin. Cells were seeded into 96-well microtiter plates at a density of 4000 cells/well using a volume of 100 µl in each well. The microtiter plates were incubated at 37°C, 5% $CO_2$, and 100% relative humidity for 24 hours before adding test compounds to allow for cell attachment.

Cells were treated with 50, 100 and 200 µg/mL of each extract and 10, 20 and 40 µm melphalan as the positive control, diluted in culture medium. One hundred microliters of aliquots of the diluted extract in fresh medium were used to treat the cells. The cells were then incubated for a further 48 hours.

Cell viability was determined using the Hoechst 33342/PI dual staining method. Treatment medium was aspirated from all wells and replaced with 100 µL of Hoechst 33342 nuclear dye (5 µg/mL) and incubated for 20 min at room temperature. Thereafter, cells were stained with propidium iodide (PI) at 100 µg/mL to enumerate the proportion of dead cells within the population. Cells were imaged immediately after adding PI using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices) with a 10X Plan Fluor objective and DAPI and Texas Red filter cubes. Nine image sites were acquired per well, representing about 75 % of the surface area of the well (Pringle *et al*., 2018).

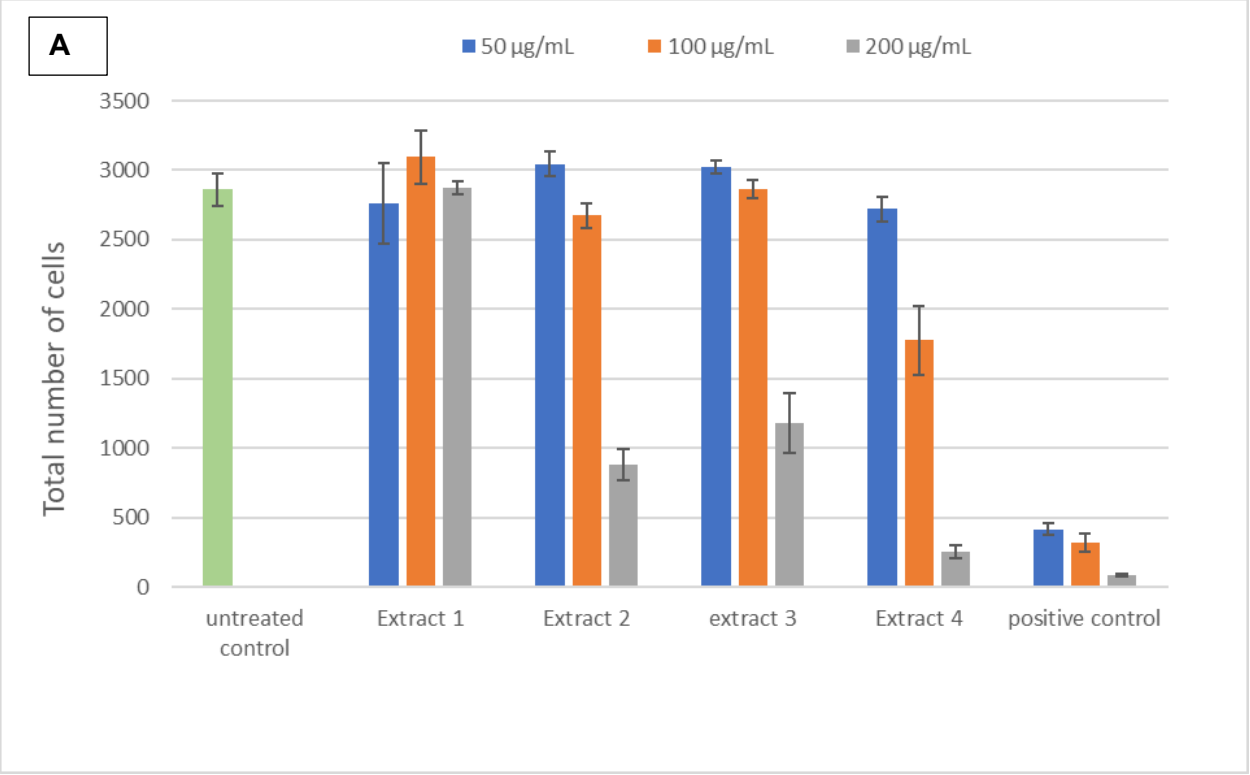
### 5.2.3 Data quantification

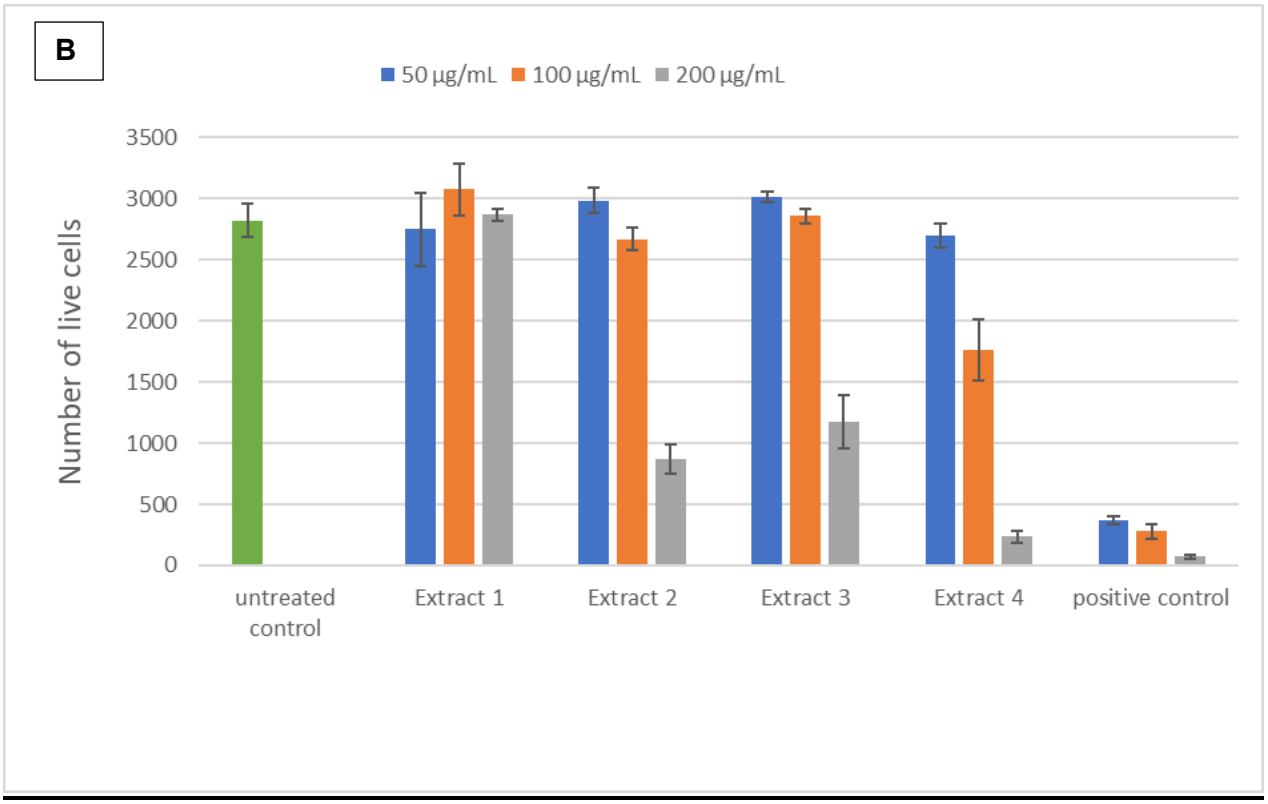
Quantifying live and dead cells for the screening assay was performed using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices). The acquired images were analysed using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module. Acquired data was transferred to an EXCEL spreadsheet and the data were analysed and processed.

## 5.3 Results

Cytotoxicity was determined using the dual staining procedure with a nuclear dye, Hoechst 33342 and propidium iodide (PI). All live cells stained positive with Hoechst 33342, but only dead/dying cells stained positive with PI.

Four extracts were screened against Vero cells (Figure 5.1). The red line in Figure 5.1B represents half the live cells relative to the untreated control (UT) population and is used to indicate potential cytotoxic extracts. Extracts exhibiting cytotoxic potential need a live cell number below this red line. The raw data was then processed and is presented in the “Averages” table, and the standard deviations are shown in the “STDEVS” table. The “Averages” are represented as a heat map; the redder the treatment, the more cytotoxic it is. This is then processed into a bar graph represented as Figure 5.1.





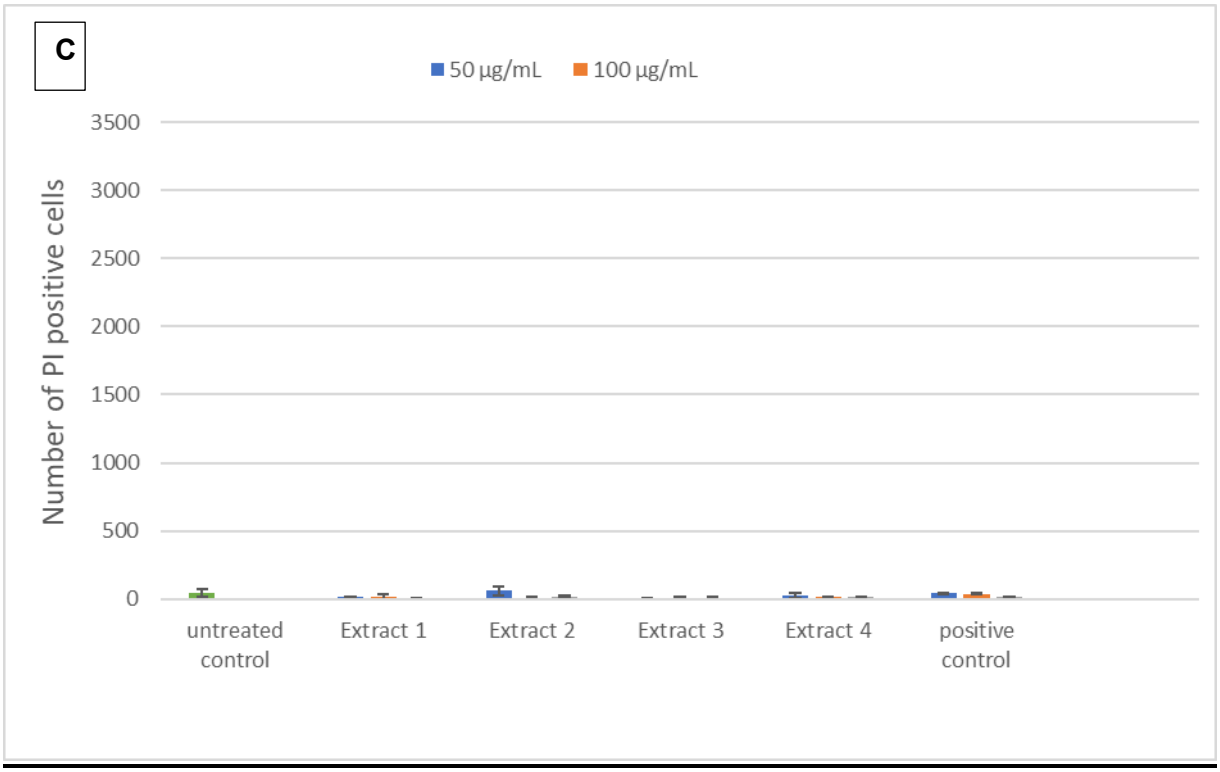




***Figure 5.1: Cytotoxicity of four extracts against Vero cells after 48 hours of exposure (A, B, C)***

**Where:** *Leucosidea sericea* - Water extract = Extract 1; Acetone extract = Extract 2; *Monsonia burkeana* - Water extract = Extract 3; Methanol extract = Extract 4

Results displayed as figure 5.1A (total number of cells), 5.1B (number of cells stained either with Hoechst 33342 only (Live)) or 5.1C (Hoechst 33342 and PI (Dead)). Error bars indicate the SD of quadruplicate values from a single experiment. In the present study, extract 2 (acetone extract of *L. sericea*) and extract 3 (water extract of *M. burkeana*) exhibited some toxicity at 200 µg/mL concentrations. Extract 4 (methanol extract of *M. burkeana*) demonstrated the most significant cytotoxic potential when tested against Vero cells, exhibiting nearly 50 % cell death at 100 µg/mL concentration.

In contrast, extract 1 (water extract of *L. sericea*) exhibited no cytotoxic effects despite high concentrations. As the concentration of plant extract increases, more cell death occurs, indicating how toxic the plant extract is to humans or animals. The toxic substance has an $LC_{50}$ less than 100 µg/mL, and the nontoxic substance is defined as having a concentration greater than 100 µg/mL. The results of the present study agree with this observation since the toxic extracts below or at 100 µg/mL were found to be toxic or mildly toxic. In contrast, the aqueous extract of *L. sericea* displayed non-cytotoxic potential regardless of the high concentration. The number of compounds in water extracts is lower, making them less effective against most infections.

## 5.4 Discussion

Methanol extracts of *Monsonia burkeana* and acetone extracts of *Leucosidea sericea* showed potential antimicrobial effects in Chapters 3 and 4. To test for cytotoxicity, two extracts from each plant were tested. It is crucial to perform cytotoxicity studies on plants before consuming them as medicine (Makhafola *et al.,* 2019). Due to the different solvents used in the current study, different polarities and solubilities of the compounds potentially caused different levels of cytotoxicity (Wang *et al.,* 2011; Ferreira-Santos *et al.,* 2020). Therefore, the current study concurs with the studies of Ferreira-Santos *et al., (*2020) and Wang *et al., (*2011) as extract 1 (aqueous *L. sericea*) displayed no cytotoxic potential and did not kill the Vero cells within the expected time of 48 hr. However, the study also indicated the mild cytotoxic potential of extract 2 (acetone extract of *L. sericea*) against the Vero cells. Previous studies of Nair *et al.,* (2012) found that *L. sericea* possessed phytochemicals such as phenols, tannins, and alkaloids, which naturally have cytotoxic effects. Adamu *et al.,* (2013) further revealed that *L. sericea* has highly toxic substances known as condensed alkaloids and

apsidiole. Nonetheless, *Leucosidea sericea* essential oils contain β-thujone, and it is known to be genotoxic, neurotoxic, reproductively toxic, and chronically toxic (Pitso & Ashafa, 2015; Mafole *et al.,* 2017). *Monsonia burkeana* has been used to synthesise NiO and ZnO nanoparticles (Kganyago *et al.,* 2018; Ngoepe *et al.,* 2018) as photocatalysts, antibacterial, and anticancer agents. The plant has also been studied for its cytotoxic properties. Tshivhandekano *et al.,* (2014) also reported this plant's chemical and antibacterial activity, although they did not assess its cytotoxicity. Present results showed that extracts 4 (methanol extract) and 3 (aqueous extract) of *Monsonia burkeana* exerted cytotoxicity and mild cytotoxicity, respectively. Our findings agree with those of other authors who concluded that this cytotoxicity is either because of phytochemicals found in these plants (Kganyogo *et al.,* 2018) or because of the solvent used (Mafole *et al.,* 2017; Eloff, 1998).

## 5.5 Conclusion

In conclusion, this chapter tested the in vitro cytotoxicity of *Leucosidea sericea* and *Monsonia burkeana* against Vero cells. These native plants of Southern Africa, which were previously known to possess antibacterial qualities, were investigated for possible cytotoxic effects. The chapter acknowledged that medicinal plants can be harmful at specific dosages but beneficial at others, underscoring the critical significance of cytotoxicity screening given the complex nature of natural products. It is emphasised how necessary these tests are for determining appropriate dosages and avoiding adverse effects from overdose. The chapter clarified the myth that medicinal plants are inherently safe and highlighted the significance of safety and toxicological considerations. It emphasised that a thorough grasp of phytochemistry and potential toxicity was required before promoting plant extracts in conventional medicine.

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# CHAPTER 6 :
# ANTHELMINTIC ACTIVITY OF PLANT EXTRACTS AGAINST EGGS (OOCYTES) AND LARVAL STAGES OF HAEMONCHUS CONTORTUS

## 6.1 Introduction

The socio-economic activities of rural areas, such as the highland grasslands of Free State and Lesotho, are profoundly influenced by livestock. Particularly in rural areas where poverty and unemployment are high, livestock is a source of food and extra income (Chitura *et al.,* 2019; Djoueche *et al.,* 2011; Moreki *et al.,* 2010; Peacock, 2005). It is also a significant challenge for resource-limited smallholder farmers in communal areas to deal with the prevalence of livestock diseases, and these diseases present a serious threat to profitability and the sustainability of communal livestock production systems because of the high morbidity and mortality rates (Chitura *et al.,* 2019; Mthi *et al.,* 2018). Animals suffer because of insufficient quality and quantity of feed, a wide array of diseases, and a failure to develop their genetic potential (Duguma & Debsu, 2019). Livestock productivity is also restricted by internal parasites that impair fertility, cause skin irritation and suck animal blood, leading to more fatalities (Chitura *et al.,* 2019; Molefe *et al.,* 2012). The *Haemonchus contortus* causes *Haemonchosis* disease in small ruminants, and this gastrointestinal parasite is one of the most economically important parasites (Nwosu *et al.,* 2022; Štrbac *et al.,* 2022).

The global impacts of gastrointestinal nematodes have caused enormous economic and productivity losses, and though these losses are difficult to assess, they account for 17.94 % of animal economic costs (Abbas *et al.,* 2020; Štrbac *et al.,* 2022). Synthetic commercial anthelmintic drugs have been used for ages. Parasitic nematodes have since developed resistance to these drugs, and the residues of anthelmintic drugs in meat and dairy products pose a health risk to the public (Abbas *et al.,* 2020). A lack of availability of anthelmintic or veterinary services, or the high cost of these services, keeps smallholder farmers from seeking commercially available anthelmintic and veterinary services. Thus, most farmers are forced to rely on ethnoveterinary medicine, as in most underdeveloped countries, including Lesotho and much of southern Africa (Eguale *et al.,* 2011).

Traditional medicine among the Basotho, especially within the South Sotho-speaking populations of Lesotho and the Free State, is profoundly rooted in cultural customs and indigenous knowledge systems. Medicinal plants are extensively utilised to address diseases in both humans and livestock, particularly in rural regions with little access to conventional healthcare and veterinary services. Ethnobotanical surveys have recorded numerous plant species utilised by Basotho healers (lingaka) for the treatment of gastrointestinal infections, parasitic diseases, and various livestock ailments (Seleteng et al., 2015; Moteetee & Van Wyk, 2011; Moteetee et al., 2019). These remedies are often prepared as decoctions, infusions, or powders and administered either orally or topically. It is crucial to validate these techniques through scientific screening, especially for anthelmintic efficacy, as evidenced by the persistent dependence on traditional medicine. The quest for cheaper anthelmintics has been investigated for ages. Plant extracts are some alternatives worth exploring (Zaman *et al*., 2020). These plant extracts potentially exhibit a wide range of pharmacological effects, including antibacterial, antiviral, and antiparasitic (Khater *et al.,* 2018; Abbas *et al.,* 2018; Salman *et al.,* 2020; Ragusa *et al.,* 2022). Eugale *et al., (*2011) showed that people have turned to using plants to treat several ailments, including gastrointestinal problems of livestock.

In this study, two bioassays have been used to screen four different plant extracts from two plants. These assays have been developed and recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles *et al.,* 1992). The assays have been tested on fresh nematode eggs for the egg hatch test and the third larval stage ($L_3$) for the larval development assay. These tests were conducted with the assumption that any substance having anthelmintic activity can potentially be expected to possess ovicidal properties on nematode eggs. The substances with anthelmintic activities also have larvicidal properties to immobilise and eventually kill the larval stages of helminths. The egg hatch assay is employed to detect the egg's ability to develop to the larval stage under test substances. The larval development assay is used to determine the ability of parasitic eggs to hatch and expand to the infective $L_3$ under test substances. In their study, Jackson and Hoste (2010) reported that these two assays have been successfully used to screen for anthelmintic properties of plant extracts from two plant species. Consequently, the purpose of the present study was to evaluate the anthelmintic activity of *Leucosidea*

*sericea* and *Monsonia burkeana* against parasitic infections of livestock used by Sotho-speaking communities of the highland grasslands of the Free State province, South Africa, as well as in Lesotho.

## 6.2 Materials and methods

### 6.2.1 Preparation of plant extracts

Plant materials (*L. sericea* leaves and *M. burkeana* roots) were collected from the study areas described in Chapter 2, washed with distilled water, and dried at ambient temperature. The dried plant material of each plant species was then ground into fine powder and stored at 4°C in the refrigerator till further use (Mostafa *et al.,* 2018). The plant samples were extracted separately using three different solvents: distilled water, 99.9% methanol, and 99.9% acetone. The plant extracts were prepared by dissolving 20 g of the powdered plant material in 200 mL of each of the three solvents. Using the maceration method, the mixtures were then placed on a rotary shaker for 24 hr for maximum extraction. The samples were then filtered using the oil-free vacuum pump and dried using a fan in an incubator at 37°C. The initial and final masses of the extracts were recorded. The acetone and aqueous extracts of *L. sericea* and the methanol and aqueous extracts of *M. burkeana* were used for this study because of their elevated bioactivities (antimicrobial activities) or better yields than other extracts. These extracts were prepared with concentrations of 1, 0.5, 0.25, 0.125, 0.0625 and 0.0313 mg/ml in a total volume of 0.3 mL in Phosphate Buffered Saline (PBS) for the egg hatch test and the larval development assay.

### *6.2.2 Egg Hatch Assay*

The egg hatch test was conducted following the guidelines of the WAAVP (Coles *et al.,* 1992) with some minor modifications. The sheep used were under the traditional owner's care, and they permitted faeces to be collected from their sheep. Farmers were not interviewed about their animal management regimes; therefore, all experimental fresh eggs were sampled from Qhoalinyane and Mount Moorosi in Quthing and Ha-Sekake in Qacha's neck districts of Lesotho, and all samples were pooled, purified, and concentrated. The faecal samples were only collected in Lesotho. Faecal materials were taken directly from the sheep's rectum using the gloved-hand

technique, stored in cooler bags at 4°C, then transported to the laboratory for egg isolation and concentration. Faecal materials were then mixed with water in an electrical homogeniser and run through a series of sieves (300, 150, 75, 53, and 37 microns) and combined with the centrifugation technique. To remove debris, 5 mL of the mixture from 75 microns was transferred to several 15 mL tubes, topped with 40 % NaCl solution and centrifuged at 700 rpm for 2 min. After every 2 min, 2 mL of the top mixture was transferred to a new tube 3 times. The resultant mix for eggs was observed under the compound microscope.

Eggs were then washed with sterile distilled water five times, and the retentate was collected on a 37-micron sieve. The eggs were then concentrated to give about 50 - 100 eggs per 200 µl. An Albendazole drug group was used as a positive control, while untreated eggs in PBS were used as a negative control. A suspension of 200 µL with approximately 50-100 eggs was aliquoted into a 96-microtiter plate. The Albendazole group drug was diluted in PBS with concentrations of 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml. The 96 microplates were then covered with their lids and incubated at 27°C for 48 hr above 80 % humidity. Each concentration was done in triplicate. A drop of Lugol's Iodine was added to each well to stop further egg development. Hatched eggs (larvae dead or alive) and unhatched eggs were then counted in a McMaster chamber on a compound microscope at 100X magnification.

### *6.2.3 Larval Development Assay*

The larval development assay was conducted following the methods of Hubert and Kerboeuf (1992) and Kamaraj *et al., (*2011) with minor modifications. Specifically, fresh eggs were isolated from faecal matter as indicated in the previous egg hatch test. Each egg sample was incubated for 24 hr to develop into the first larval stage. The mixtures of yeast and antifungal agents (Ketoconazole) of 50 µl were then introduced to 200 µl of larval suspension containing ±50 larvae. Larvae were then incubated at 25°C for 7 days. At the end of the seventh day, $L_3$ in suspension were observed for signs of life. Each $L_3$ was stared at for 30 seconds for no movement. Dead and alive $L_3$ were counted and recorded. Parasitology (WAAVP) with minor modifications (Coles *et al.,* 1992; Jackson & Hoste, 2010).

Samples were stored in cooler boxes before being transferred to the laboratories for further processing and analysis. Upon arrival at the laboratory, the faecal samples were pulled together, mixed with water, and thoroughly mixed in a food mixer for 5 minutes at moderate speed. About 2 g of samples were identified in a McMaster slide. The faecal mixer was then run through a series of sieves measuring 300, 150, 100, and 75 µm. The mixer was then put in concentrated salt solution and centrifuged at 700 rpm for one minute in 15 mL centrifuge tubes. About 2 mL of the top liquid was removed and transferred to a new tube. The procedure was repeated 3 times. The concentrate was then washed 5 times and run through a 35 µm sieve, and the retentate collected and diluted to obtain about 100 larvae/200 µl.

## 6.3 Results

### 6.3.1 Egg Hatch Assay

The egg hatch assay results were variable, as shown in Table 6.1. *L. sericea* water extract and *L. sericea* acetone extract showed higher egg inhibition than *M. burkeana* methanol extract, water extract and positive control. In contrast, *M. burkeana* methanol extract recorded the least inhibition (9.57 µg/ml) at a higher concentration.

***Table 6.1: Egg hatch test for Haemonchus contortus with some ethnoveterinary medicinal plant extracts***

| Treatment µg/ml | *L. sericea* water extract | *L. sericea* acetone extract | *M. burkeana* methanol extract | *M. burkeana* water extract | Positive Control |
|---|---|---|---|---|---|
| **50** | 66.67± 57.74 | 100± 0 | 38.71± 9.86 | 9.57± 8.35 | 50.77± 17.51 |
| **25** | 100± 0 | 100± 0 | 30.07± 4.94 | 16.51± 2.91 | 41.87± 9.59 |
| **12.5** | 100± 0 | 100± 0 | 17.12± 14.37 | 20.45± 13.98 | 30.97± 7.06 |
| **6.25** | 92.59± 12.83 | 54.67± 14.05 | 24.49± 3.76 | 17.84± 10.39 | 32.73± 1.95 |
| **3.13** | 91.87± 3.96 | 86.4± 12.64 | 14.58± 4.57 | 20.32± 11.73 | 13.74± 3.82 |

*Values shown are Mean ± SD.*

### 6.3.2 Larval Development Assay

The results of the larval development assay showed that all the extracts possess some level of larval development inhibition, although this varied from one treatment to the following (Table 6.2). The highest larval development inhibition of 100 % was recorded for the positive control at 50 µg/ml and *L. sericea acetone* extract at 25 µg/ml. The least inhibition was 22.95 µg/ml for the *M. burkeana* methanol extract, which showed lower inhibition than the rest of the other treatments. The less the inhibition, the stronger the anthelmintic activity.

***Table 6.2: Anthelmintic activity of medicinal plant extracts against larval development inhibition of Haemonchus contortus***

| Treatment (µg/ml) | *L. sericea* water extract | *L. sericea* acetone extract | *M. burkeana* methanol extract | *M. burkeana* water extract | Positive Control |
|---|---|---|---|---|---|
| **50** | 98.28 ± 2.18 | 97.22 ± 4.81 | 52.92 ± 31.53 | 35.6 1 ± 2.58 | 100 ± 0 |
| **25** | 88.12 ± 10.67 | 100 ± 0 | 83.72 ± 7 | 52.29 ± 30.34 | 97.89 ± 1.87 |
| **12.5** | 84.37 ± 6.66 | 80.94 ± 8.68 | 22.95 ± 13.63 | 93.07 ± 7.43 | 96.53 ± 3.38 |
| **6.25** | 89.86 ± 4.07 | 94.12 ± 0.19 | 48.25 ± 37.81 | 98.77 ± 2.14 | 99.59 ± 0.7 |
| **3.13** | 70.75 ± 12.76 | 84.26 ± 10.5 | 50.48 ± 29.37 | 98.87 ± 1.96 | 99.82 ± 0.32 |

*Values shown are Mean ± SD.*

## 6.4 Discussion

The acetone and aqueous extract of *L. Sericea* and the methanol and, water extract of *M. burkeana* displayed noteworthy anthelmintic properties. Like the present study, Vargas-Magana *et al.* (2014) used water and acetone extracts of *Pennisetum purpureum* grass and *Lysiloma latisiliquum* to investigate their anthelmintic properties, and they found positive results on all tested plants. Acetone is non-toxic for bacteria and fungi and is a better extractant than water and methanol (Eloff, 1998; Adamu *et al.,* 2013). On the other hand, Singh *et al.,* (2016) used aqueous extracts to determine the anthelmintic properties of *Zanthoxylum armatum* DC. seeds showed complete mortality of the *H. contortus parasite.* In this study, the extracts from *L. sericea* (aqueous and acetone) *and M. burkeana* (aqueous and methanol) for all treatments revealed some degree of anthelmintic activity ranging from 14.58-100% against

*Haemonchus contortus* (Table 6.1). Since the eggs were allowed to develop to the first larval stage ($L_1$) before introducing the extracts, this implies that larval stages were exposed to the treatment, which affected the larvae and killed some of them. The egg hatch test ended with some morulation (embryonic development) of most eggs. Some had progressed to characteristics of the first larval stage but could not proceed to eclosion (emergence of larva from egg). Fewer eggs had developed to the second larval stage ($L_2$), i.e., the short, stumpy and immobile stage of *H. contortus*. A comparison of the $LD_{50}$ revealed *L. sericea* with lower values than *M. burkeana*. This may be an indication that small doses of the former are capable of both ovicidal and larvicidal activities.

Contrary to the present study, the study of Kamaraj *et al., (*2011) discovered that methanol extracts showed a remarkable inhibition effect on egg and larval development while working on *A. paniculata, A. malabarica, A. squamosa, D. metel and S. torvum*. Although the aqueous extracts of *L. sericea* in this study had impressive anthelmintic effects, the acetone extract was the strongest of all other solvents and showed anthelmintic properties at higher concentrations. The present study, therefore, concurs with the study of Mafole *et al.* (2017), who reported that *L. sericea* possessed anthelmintic properties. There was a similar pattern of activity of the plant extracts in both assays, except for the positive control. Albendazole seemed to have less ovicidal properties than *L. sericea,* but was very effective on the larvae. *M. burkeana* showed lower efficacy on both the aqueous and the methanolic extracts, and this was the first time such tests were conducted on *M. burkeana* for anthelmintic properties.

## 6.5 Conclusion

The results of the present study revealed that *L. sericea* possesses impressive anthelmintic properties compared to *M. burkeana*. *L. sericea* demonstrated the presence of both ovicidal and larvicidal properties. Comparatively, acetone extracts were of higher relative inhibition for both the egg hatch and larval development assays. The methanol extract of *M. burkeana* was the least effective of all extracts. There is a need to isolate active compounds for further investigation of *L. sericea*. This study further reports the first *M. burkeana* anthelmintic test. In conclusion, just as China has popularised and mainstreamed Chinese Traditional Medicine (CTM) to cure human

diseases, the same can be applied in southern Africa with potentially outstanding results. For Basotho, traditional medicines remain an alternative to scientific pharmaceutical products, which could be tried and tested to ensure they receive treatment similar to CTM. Ignoring the impact of Basotho medicines has led to unregulated and potentially hazardous practices by unlicensed members of the public selling and reselling medicinal products. The government must curtail such activities as their effects can be dire if left unchecked.

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# CHAPTER 7 :
# GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

## 7.1 Introduction

Livestock is crucial for several developing countries' economies. The livestock sub-sector significantly contributes to the national economy of most African countries including in Lesotho and South Africa; however, its growth is constrained by various factors (Perry & Grace, 2009). These factors consist of widespread infectious diseases that reduce market opportunities, harm human welfare, and cause livestock and agricultural productivity loss. Poor communities that primarily rely on livestock yet have little access to resources such as modern veterinary care are severely affected by animal diseases (Eiki et *al.,* 2021). These diseases have a significant economic impact on people. The most important causes of these diseases, which annually result in considerable livestock losses that impact the economy, animal variety, and ecological balance, are poor nutrition, breeding, hygiene, and management systems (Fatima & Amina, 2022).

Ethnoveterinary knowledge is the use of local people's habits, traditions, and beliefs to preserve the health and welfare of their domestic animals and treat livestock ailments. Ethnoveterinary research aims to investigate and apply traditional veterinary knowledge, theory, and practice (Erarslan & Kültür, 2019; Yigezu *et al.,* 2014). Most of this traditional knowledge of medicinal plants is either no longer used or completely lost, owing to the quick socio-economic, ecological, and technical changes in people's lifestyles over the years (Abraha, 2016). The issue, according to some authors, is worsened by beliefs that traditional activities are wicked, evil, and hence ungodly (Odongo *et al.,* 2018). When some religious groups hold such beliefs, the loss of this knowledge is guaranteed since religion plays a huge role in decision-making processes in most cultures.

Furthermore, some young people find it difficult to uphold their traditions and culture. At the same time, governments refuse to acknowledge the role played by ethnoveterinary practitioners in the prevention, management, and treatment of livestock diseases in various nations (Eika *et al.,* 2021). Madisha and McGaw (2023) also confirmed that many rural areas in Africa, especially in places with limited access

to western veterinary medicine, use ethnoveterinary medicine to treat livestock ailments, although this is poorly documented.

Indigenous herbal remedies are the only option for treating many livestock illnesses in rural areas because there is a shortage of veterinarians per capita, including essential services. The availability of current veterinary medications is restricted in many impoverished nations due to pricing, supply uncertainty, and shortages (McGaw & Eloff, 2008; Tolossa *et al.,* 2013). The vast variety of plant species and cultural groupings found in South Africa and Lesotho make it an ideal place to research traditional uses of plants for human and animal health (Eika *et al.,* 2021). The adoption of EVM by western-trained veterinary professionals and the relevant government agencies charged with promoting primary animal healthcare will only be able to advance once conventional methods for caring for animals and plant-based remedies have been adequately documented and evaluated for efficacy and toxicity (McGaw *et al.,* 2020.

## 7.2 General discussion

The ethnoveterinary survey findings were published in 'The Journal of Medicinal Plants and By-products' as shown in Table 7.1. Some findings were further analysed for phytochemical constituents, antimicrobial activity, *in vitro* cytotoxicity and anthelmintic properties.

***Table 7.1: An overview of the results obtained from the current study***

| Ethnoveterinary survey | Most mentioned plants | RFC index | Phytochemical screening | Antimicrobial assay | Invitro cytotoxicity | Anthelmintic activity |
|---|---|---|---|---|---|---|
| 51 ethnoveterinary medicinal plant species | *R. prinoides* | 0.45 | Standard method: Alkaloids, steroids, phenols, cardiac glycosides, tannins, flavonoids and saponins | + | Not done | Not done |
| | *A. striatula* | 0.38 | Standard method: Alkaloids, steroids, phenols, cardiac glycosides, tannins, flavonoids and saponins | + | Not done | Not done |
| | *M. burkeana* | 0.29 | Standard method: Alkaloids, steroids, phenols, cardiac glycosides, tannins, flavonoids and saponins | ++ | Highly toxic | Least active + |
| | *L. sericea* | 0.25 | Standard method: Alkaloids, steroids, phenols, cardiac glycosides, tannins, flavonoids and saponins | ++ | Mildly toxic | Highly active ++ |

The use of medicinal plants to cure animal-related conditions and diseases is well known, especially in poor rural and marginalised South African and Lesotho communities. However, the elderly, especially men, are the repositories of this knowledge. There are fears that this knowledge may be lost in time if it is not passed on to younger generations or not properly documented using modern methods. Documenting this knowledge for future reference and posterity is critical since most subsistence farmers and traditional healers rely on it. A study was conducted to document ethnoveterinary medicinal plant uses in the Free State province of South Africa and Lesotho. The results revealed 51 medicinal plant species belonging to 35 different families from 69 respondents. These medicinal plants are used to treat other diseases in the study areas, and the most mentioned or predominantly used plants were calculated using the relative frequency citation index. Four medicinal plants, including Rhamnus *prinoides, Aloe striatula, Leucosidea sericea* and *Monsonia burkeana,* were then selected to test their phytochemical properties qualitatively and quantitatively. Although alkaloids were absent, these ethnoveterinary medicinal plants possessed some important secondary metabolites. Plants that showed the presence of tannins and phenols were further subjected to quantitative analysis for total phenolic and tannin contents. *A. striatula* aqueous extract showed the highest presence of total tannins (2.242 mg/ml), and *R. prinoides* methanol extract showed the maximum phenolic contents (5.102 mg/ml). Chen *et al.* (2020), Amabye (2015), and Abebe (2023) showed that phenols as part of *R. prinoides* had noteworthy antioxidant, anti-inflammatory activities, and antimicrobial activities, justifying their use in its folkloric use to treat ringworm infections. Tannins in *A. striatula* potentially enable the plant to act against antimicrobial infections, gastrointestinal and inflammatory conditions (Cock, 2015).

The four medicinal plants were further investigated for antimicrobial activity. These plants were tested against eight bacterial strains and three fungal strains. *R. prinoides* showed some antibacterial activity, although *L. sericea* and *M. burkeana* showed more noteworthy results. *L. sericea* acetone extracts showed an MIC of ≤0.098 mg/ml against four bacterial strains with an MBC of 0.098 mg/ml, and its aqueous extract showed an MIC of 0.78 mg/ml and an MBC of 12.5 mg/ml against half of the bacterial strains tested. *M. burkeana* methanol extract displayed ≤0.098 mg/ml MIC against two bacterial strains, with an MBC of 1.56 mg/ml, while the aqueous extract showed an

MIC of 6.25 mg/ml and MBC of >12.5 mg/ml for all bacterial strains. The assay carried out against the fungal strains showed *A. striatula acetone* and *R. prinoides* acetone extracts to have obtained 0.049 mg/ml and 0.195 mg/ml against *Candida albicans* and *Trichophyton mucoides,* respectively. *M. burkeana* did not show much activity against the tested fungal strains, and *L. sericea* extracts showed no activity against all tested strains. These results confirmed other researchers' findings who reported that some extractants such as water are not effective and this is not in the best interest of subsistence farmers and traditional healers as they mostly or entirely use aqueous extracts (Dhawan & Gupta, 2017; Elfalleh *et al.,* 2019; Anyanwe & Okoye, 2017).

Following the antimicrobial activities of the selected medicinal plants, the noteworthy plant extracts, i.e., *L. sericea* (water and acetone) and *M. burkeana* (methanol and water extracts), were further screened for both cytotoxic and anthelmintic properties. Cytotoxicity tests were conducted to investigate the effects of the extract on cell viability in livestock. The assay was determined using four extracts against monkey Vero cells. The assay results displayed mild toxicity of L. sericea acetone extract; L. sericea has Phyto-constituents such as phenols, tannins, and alkaloids, which in their nature have cytotoxic effects. This medicinal plant showed that its aqueous extract is not toxic to the Vero cells. This may be due to the solubility of the extractant used.

*M. burkeana* results showed that its aqueous extract has low cytotoxicity, while the methanol extract has mild cytotoxicity. After being tested for cytotoxicity, these medicinal plant extracts were further screened for anthelmintic activity. Two tests were conducted: the egg hatch and the larval development assays. The $L_1$, $L_2$ and $L_3$ stages of *Haemonchus* were exposed to different extracts of the tested medicinal plants. Both aqueous and acetone extracts of *L. sericea* showed the highest inhibition of larvae at 50 µg/ml with 98 % and 97 % respectively. All extracts showed less inhibition at 12 µg/ml, especially *M. burkeana* methanol extract, with 22.95 % inhibition of larvae. *L. sericea* further showed activity against the egg hatch assay as it inhibited 100 % at 25 µg/ml and 12.5 µg/ml for both acetone and water extract, respectively. However, *M. burkeana* methanol extract was the least recorded with 9.57 % at the highest 50 µg/ml concentration. These assays confirm that the two medicinal plants both possess ovicidal and larvicidal properties.

To extrapolate the cytotoxicity data to a recommended safe dose for animals, the $LC_{50}$ values based on Vero cells were scaled using standard interspecies scaling factors.

As a rule of thumb, a safety factor between 100-1000 is used to estimate a tolerable in vivo dose (mg/kg body weight) from the in vitro LC50 according to OECD or WHO recommendation (OECD, 2009; WHO, 2005). The aqueous extract of *Monsonia burkeana* which had low cytotoxicity was assigned a conservative extrapolation factor of 1000 and an estimated safe dose of about 10 mg/kg if the $LC_{50}$ was 10,000 µg/ml. However, an additional cytotoxicity test showed cytotoxic effects with a dose of 100 ug/mL, and the methanol extract was scaled down 500 times to a tentative safe dose of 2 – 5 mg/kg based on which the actual $LC_{50}$ concentration values. The estimates offer a translational platform for future in vivo studies of therapeutics, to ensure prescribed doses are kept under cytotoxic levels, whilst also maintaining anthelmintic effectiveness. More crucially, these figures should be checked with acute and sub-chronic toxicity studies in the target animal species, prior to the extension of these findings into the field (OECD, 2010; WHO, 2005).

## 7.3 Conclusion

Animal ailments are frequently treated using conventional herbal remedies. The present study observed that most plants can be used to treat multiple animal diseases and are used in both highlands and grasslands of the Free State province of South Africa and Lesotho. The study further showed that four selected medicinal plants with the highest RFC indices from the ethnoveterinary survey were rich in secondary metabolites and active against bacteria and fungi. The two medicinal plants (*M. burkeana* and *L. sericea*) that displayed the highest antimicrobial activity were further tested for anthelmintic properties against livestock parasitic infections. They displayed impressive results, especially *L. sericea* and *M. burkeana*. The water extract indicated no cytotoxicity, and this justifies the farmers' use of water for extraction. Extract toxicity is very negligible. The present study thus validated the use of medicinal plants in traditional medicine. However, more work must be done to document these species for posterity. Suppose these species are not properly documented using modern methods. In that case, they may be lost in time and driven to extinction in the face of globalisation, urbanisation, monoculture, and climate change. The younger generations' interest in these plants also needs to be encouraged to keep local traditions of oral transfer of knowledge and information alive and to preserve their culture. However, further comprehensive studies are required to investigate their

phytochemistry and validate these species. Not much has been done in this regard. The solutions to drug resistance problems potentially lie in some compounds that have yet to be discovered in these plants. Although the road ahead is long, it looks promising.

## 7.4 Recommendations

The indigenous population continues to employ plants in ethnoveterinary medicine despite modern advancements and the creation of synthetic medications. Animal ailments are frequently treated using conventional herbal remedies. Some plants have poisonous elements that are harmful to animal health. These plants' adverse effects or overdose might be detrimental; to avoid overdose, harmful side effects, or even death, special care should be taken when using them, and dose adjustments ought to be done carefully. Further comprehensive studies are needed to investigate the phytochemistry of these medicinal plants and validate these species. These may include extensive in vitro, in vivo, and clinical trials.

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## ANNEXURES

## INFORMED CONSENT FORM FOR LIVESTOCK FARMERS AND HERD BOYS

**PROJECT TITLE: Medicinal properties of plants used for ethnoveterinary purposes in the Highland Grasslands the Free State and Lesotho**

**INTRODUCTION:**

You are invited to participate in a research study on **Medicinal properties of plants used for ethnoveterinary purposes in the Highland Grasslands of the Free State and Lesotho**. Please take your time to discuss the study with your family and friends, or anyone else you wish to. The decision to join or not to join is based on you. I am investigating on the treatment of animal diseases used by different ethnic groups using medicinal plants in the Highland Grasslands of Free State and Lesotho. Findings will be documented through catalogues and publishing in accredited journals which will be written in Sesotho for community members and the public at large. Aspects such as determining the phytochemical contents, evaluating medicinal properties of plants most commonly used in the study area and finally attempt to isolate, purify and identify the bioactive compounds of medicinal plants will be carried out.

### What is involved in the study?

If you decide to participate you will be asked to share your knowledge of plants, their uses and methods of preparation. This project will take at least 3 years. You can stop participating at any time. If you stop, you will not lose any benefits.

### Risks

There may be risks that we cannot predict.

### Benefits to taking part in the study

It is reasonable to expect cultural, economic and environmental benefits from the research. However, we cannot guarantee that you will personally experience them from participating in this study. Others may benefit in future from the information we find in the study.

**Your rights as a participant**

Participation in the study is voluntary. You have the right not to participate at all or to leave the study at any time. Deciding not to participate or choosing to leave the study will not result in any penalty or loss of benefits to which you are entitled, and it will not harm your relationship with the Central University of Technology.

**Confidentiality**

Confidentiality will be provided to the fullest extent possible by law. We will write down the information you give us on interview sheets. We will keep information about you confidential and protect it from unauthorized disclosure, tampering, or damage. We will not identify you by name in any reports using information obtained from this interview. Subsequent uses of records and data will be subject to standard data use policies which protect the anonymity of individuals and institutions.

**Contacts for questions or problems**

If you have questions about the study, any problems, or think that something unusual or unexpected is happening you can contact:

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If you have any questions or concerns about your rights as a research participant, contact:

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Consent of participant

---

Name Signature Date

Person taking consent

---

Name Signature Date

**INTERVIEW GUIDE FOR LIVESTOCK FARMERS AND HERD BOYS**

## Demographic details

Name (Lebitso):

_____

Gender(Boleng):

_____

Age(Lilemo):

_____

Race(Mohlobo):

_____

Religion(Tumelo):

_____

Marital status (Moemo ba lenyalo):

_____

Level of education (Boemo ba thuto):

_____

When did you start practicing as a livestock farmer/ herd boy? (U qalile neng ho rua/ho lisa liphoofolo?)

_____

_____

What kind of livestock do you keep? (U ruile liphoofolo lifeng)

_____

_____

How did you become a livestock farmer/ herd boy? (Ho tlile joang hore u be morui/molisana oa liphoofolo)

_____

_____

What other type of occupation are you involved in? (Ke ofeng mosebetsi o mong ntle le ho sebetsana le liphoofolo?)

_____

_____

Where did you work before you became a farmer/ herd boy? (U ne u sebetsa kae pele u sebetsana le liphoofolo)

_____

## PUBLISHED ARTICLE FORM THE STUDY

Motsoari, T., Manduna, I., Buwa-Komoreng, L., Ngobeni, B. and Nwafor, I., 2023. Predominantly Used Medicinal Plants for Ethnoveterinary Purposes in the Highland Grasslands of South Africa and Lesotho: An Ethnobotanical Survey. Journal of Medicinal plants and By-product, 12(3), pp.293-303.

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IMPS

**Original Article**

# Predominantly Used Medicinal Plants for Ethnoveterinary Purposes in the Highland Grasslands of South Africa and Lesotho: An Ethnobotanical Survey

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**ABSTRACT**

Medicinal plants have been used for the maintenance of animal health in most parts of the world. However, ethnoveterinary knowledge is verbally passed on from generation to generation and can easily be lost or distorted if not documented for future reference. This study therefore seeks to ascertain the mostly used medicinal plants for ethnoveterinary practices in the research area. An ethnobotanical survey was carried out with 69 respondents consisting of subsistence livestock farmers, traditional healers and other traditional knowledge holders from four (4) towns in the study region. Interviews were conducted using semi-structured questionnaires designed to collect data on the plants used, their common names, methods of preparation and administration and livestock ailments treated. The relative frequency of citation (RFC) index was calculated in order to determine the most predominantly used plant species. Fifty-one (51) plant species were mentioned by respondents. *Rhamnus prinoides* L'Hér., *Aloe striatula* var. *striatula*, *Monsonia burkeana* Planch. ex Harv. and *Leucosidea sericea* Eckl. & Zeyh. were the frequently mentioned plants mostly from the Asteraceae family. Roots (45%) and leaves (43%) were the most frequently used plant parts. Approximately 84% of Medicines were prepared in the form of decoctions and administered orally. Thirteen (13) health conditions of livestock were treated with medicinal plants and bile acid malabsorption was the most predominant (39%). The study region is endowed with a rich biodiversity of medicinal plant species which are used for the treatment of various animal diseases. Therefore, it is imperative to establish the salient medicinal plant species used in this area for possible drug development.

## INTRODUCTION

Livestock such as cattle, poultry, pigs, sheep and goats are of paramount importance in Africa. They provide food and transport, improve livelihoods and are of cultural value especially in rural settings. However, the high prevalence of diseases experienced in communal livestock production systems present a serious setback to profitability, food security and sustainability due to increased morbidity and mortality [1]. Additionally, the high cost of modern drugs, lack of access to veterinary facilities and increasing resistance of pathogens to pharmaceutical medicines present numerous challenges to productivity [2]. In mostly rural and peri-urban areas, the options available to mitigate these problems are limited. Therefore, resource-poor smallholder farmers often turn to traditional methods such as the use of medicinal plants for disease management. In fact, the World Health Organization [3] estimates that 80% of people in developing countries use ethno-methods to monitor livestock-related diseases. It has also been reported that people utilize medicinal plants to treat various livestock diseases because they possess the