Medicinal plants used by subsistence farmers for the treatment of cattle diseases in Nkomazi local Municipality, Mpumalanga, South Africa

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TABLE OF CONTENTS

ABSTRACT

Farmers frequently use a combination of ethnoveterinary knowledge and contemporary veterinary medicine to treat their cattle. The sustainability of livestock production in local communities is continuously threatened by distinct types of diseases. The current study documented the medicinal plants used to treat and manage various cattle diseases in Nkomazi local municipality, Mpumalanga province, South Africa. The ethnoveterinary data were collected using semi-structured interviews (face-to-face). Descriptive data and ethnobotanical indices such as frequency of citation (FC) and informant consensus factor (ICF) were used to present the collected data. In the study area, 28 plant species from 19 families are used for medicinal purposes. *Cissus quadrangularis* L., *Synadenium grantii* Hook.f., *Phragmites australis* (Cav.) Trin. Ex Steud. *Vernonia abbreviata* DC., *Vachellia sieberiana* var. woodii (Burtt Davy) Kyal. & Boatwr. had the highest FC. Eighteen cattle diseases were classified into six categories in the study area. Dermatological diseases, diarrhoea, bloating, intestinal worms, and allergic reactions, scored the highest ICF value, whereas musculoskeletal diseases, such as fractured bones, received the second highest ICF value. Maggot infestations (30%) were the most frequently cited cattle disease treated by *Cissus quadrangularis.* The plant parts commonly preferred in the preparation of remedies for various cattle diseases were leaves (45%), followed by bark (28%), and seeds (3%) being the least used plant part. Poultice (39%) and decoction (36%) were the preferred methods of preparation. The frequent methods of administration were orally (54%) and topically (46%). These findings indicate that a wide range of plant-based remedies is used by farmers and herders for the treatment of health complications associated with subsistence cattle farming. Furthermore, the UPLC-qTOF-MS analysis tentatively revealed the presence of metabolites, 20 of which were identified in acetone extracts and another 20 in the ethanol extracts of *V. abbreviata*. The identified metabolites included compound classes such as terpenoids which were highest in the ethanol extracts (27, 77%) and Alkaloids which were highest in the acetone extracts (15.79%). The results showed significant chemical differences between both extracts of the plant in the profiles. *V. abbreviata*. leaf extracts were reported to possess compounds capable of providing healing abilities to various cattle ailments such as wounds and diarrhoea.

Keywords: Ethno-veterinary, biodiversity, indigenous Knowledge, cattle diseases, UPLC, Metabolites, compounds, chromatographs, medicinal plants.

DECLARATION

Student number: 201602849

I, **Antoinnette Tracy Mbebe**, hereby, declare that this study on Medicinal plants used by subsistence farmers for the treatment of cattle diseases in Nkomazi Local Municipality, Mpumalanga, South Africa is my original work and has not been submitted to any institution or university for any degree.

30/05/2023

AT Mbebe (Candidate) Date

30/05/2023

Prof W Otang Mbeng (*Supervisor*) Date

30/05/2023

Dr. Terence Suinyuy (*Co-supervisor*) Date

DEDICATION

I dedicate this work to the almighty God and ancestors who rightfully guided, and gave me strength knowledge, wisdom and understanding throughout this study. I also dedicate this work to my family for the love and support they have given me.

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LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS AND LIST OF ABBREVIATIONS

EVM	Ethnoveterinary medicine
PHC	Primary health care
IKS	Indigenous knowledge systems
NLM	Nkomazi Local Municipality
BSE	Bovine spongiform encephalopathy
UNEP	United Nations Environment Programme
WCMC	World Conservation Monitoring Centre
SANBI	South African National Biodiversity Institute
MAP	Medicinal and aromatic plants
UPLC	Using ultra performance liquid chromatography
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-pressure liquid chromatography
LC-Ms/Ms	Liquid chromatography-mass spectrometry/mass spectrometry
TLC	Thin layer chromatography
^{1}H-NMR	Proton-Nuclear magnetic resonance
ICF	Informant consent factor
SPSS	Statistics package for the social sciences
FC	Frequency of citation

CONFERENCES

Mbebe, A.T., Ndhlovu, P.T., Suinyuy, T., Otang-Mbeng, W., 2022. Ethnoveterinary study of medicinal plants used for treating and managing cattle diseases in Nkomazi Local Municipality, South Africa, 47th Annual Conference South African Association of Botanists (17-20 January 2022). North-West University, Mahikeng campus, South Africa). Zoom Virtual Conference (**Oral presentation).**

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CHAPTER 1: INTRODUCTION

1.1 Background

Plants have not only played a salient role in ameliorating human health conditions, but animals as well. This research is an ethnoveterinary study aimed at documenting medicinal plant species that play a role in treating various livestock diseases in Nkomazi local municipality. According to Gidey et al., (2012), ethnoveterinary medicine (EVM) also known as veterinary anthropology is broadly defined as "the holistic, interdisciplinary study of local knowledge and its associated skills, practices, beliefs, practitioners, and social structures. Ethnoveternary medicine entails the healthcare and healthful husbandry of food, work, and other income-producing animals, with an ultimate goal of increasing human well-being through increased benefits from stock raising. EVM is recognized as one of the most important parts of animal health care in underdeveloped countries (Cunningham & Zondi, 1991). Traditional veterinary philosophy, medications, surgical methods, animal husbandry practices, and diagnostic procedures all fall under the umbrella of ethnoveterinary research (McCorkle, 1986). Traditional medicine is part of the Indigenous Knowledge Systems (IKS) of people globally, however, (Mathias-Mundy & McCorkle, 1995) stated that the traditional practices used to keep animals productive and healthy, as well as controlling the treatment of diseases makeup EVM. Across the globe, traditional medicine is constantly the only readily available alternative to the expensive orthodox health care for subsistence farmers in the rural areas for the treatment of animal diseases (Martin & Mathias-Mundy, 2006). Furthermore, the use of non-conventional medicines is due to the lack of access to veterinary services and the high cost of pharmaceutical products.

Medicinal plants are regarded as rich resources of ingredients that can be used in the synthesis and development of drugs (Tibi, 2012). Globally, medicinal plants form the backbone of traditional medicine systems (Bukuluki et al., 2013). Unfortunately, medicinal plants and the supported traditional medicinal systems are at risk of extinction not only because of land uses, population growth, rural to urban migration, but also due to conservation practices and compromised harvesting practices. This scenario raises concerns given that herbal traditional medicine is used by 75-95% of the world's rural population for their Primary Health Care (PHC) (Bukuluki et al., 2013). Several plants have been reported to have curative properties for livestock diseases, particularly cattle in different parts of the world.

Despite the widespread practices of medicinal plant use, none of their usage has been documented in Nkomazi local municipality for the treatment of cattle diseases. Several factors such as the use of orthodox medicine , urbanization, and modernization have been reported as primary causes of the gradual disappearance of indigenous knowledge on the use of medicinal plants (Gürbüz et al., 2003). The use of medicinal plants to treat cattle diseases plays a large role in the livelihoods of disadvantaged farmers for sustainable livestock production. Hence the current study aims at documenting medicinal plants used for the treatment of cattle diseases in the Nkomazi local municipality.

1.2 Problem statement and justification

According to Karunamoorthi et al, (2013), the popularity of medicinal plants is attributed to their accessibility, affordable, and culturally acceptance. Njoroge and Bussmann (2006), stated that in South Africa, the increase in the price of orthodox medicine and diseases' resistance to conventional medicine makes it difficult for subsistence farmers in rural areas to treat livestock diseases with consequent increase in livestock mortality. However, there is an increase in cultural practices that utilises medicinal plants by rural farmers for the management of cattle diseases. The success of this practices using medicinal plants are attracting researchers as evident by the volumes of ethnoveterinary surveys being published in South Africa. Current information reveals that there has been no such survey and documentation of ethnoveterinary practices and medicinal plants used in Nkomazi local municipality for the treatment of cattle diseases, creating a knowledge gap. Given that there is no documentation thus far, it is presumably correct to conclude that there is little, or no evidence of the biological activity and phytochemical profile of the medicinal plants used further widening the knowledge gap.

1.3 Aim and Objectives of the study

Aim

To identify, document and determine phytochemical profiles of medicinal plants used to treat cattle diseases in Nkomazi local municipality**.**

Objectives

- To identify and document cattle diseases treated by medicinal plants in Nkomazi local municipality.
- To explore the ethno-botanical practices used for the management of cattle diseases.

- To identify the phytochemical composition of the selected plants used in the treatment of cattle diseases in Nkomazi Local Municipality.

1.4 Research questions

1. Which cattle diseases are treated by medicinal plants in Nkomazi local municipality?
2. Which plant species are used by subsistence farmers for the treatment of cattle diseases in the Nkomazi local municipality?
3. What are the ethnobotanical practices used for the management of cattle diseases in Nkomazi Local Municipality?
4. What is the phytochemical chemistry of the plant used to treat cattle diseases in the Nkomazi local municipality?

1.5 Limitations of the study

1. Limitations based on geographical scope.

The study only focuses on a specific geographic area: Nkomazi Local Municipality in Mpumalanga Province. This came as a limitation because the findings of the study cannot be considered as a general representation of the medicinal plants used to treat cattle disease by subsistence farmers of the Nkomazi Local Municipality in the whole of Mpumalanga province and South Africa.

2. Limited sample size

During data collection, the study focused mostly on knowledgeable cattle owners in Nkomazi Local Municipality due to time constraints. A larger sample could have provided enough data for the study but only 100 participants from 5 different villages were identified. The majority of the participants interviewed were demanding to be paid for obtaining knowledge from them however, some traditional health practitioners were overprotective of the information they hide about how they prepare drugs from medicinal plants. Lastly, due to COVID-19 restrictions, some participants could not keep the interview going any longer. All these became challenges and limitations as the potential knowledge experts in medicinal plants used by subsistence farmers to treat cattle disease were selected through purposive sampling

CHAPTER 2: ORIENTATION OF THE STUDY

2.1 Introduction

The general orientation of the study provides a guide to help produce the end results through a systematized scientific process. According to Williams (2007), the research process is systematic in that defining the objective, managing the data, and communicating the findings occur within established frameworks and in accordance with existing guidelines. The three common approaches to conducting research are quantitative, qualitative, and mixed methods (Creswell, 2002). This chapter focuses on clarification of the research approach, research design, study area, target population as well as the sampling technique and criteria followed in the study.

2.2 Research approach

Qualitative-quantitative type of research approach was employed to conduct this study, through the administration of questionnaires to the participants. Scientists extensively use qualitative research design in studies where the behavior of a human and their habits are being investigated. Crucial information is obtained from research conducted through qualitative approach and forms as lead to formulate a realistic and testable hypothesis. However, the formulated hypothesis can then be mathematically analyzed and comprehensively tested through quantitative research, this, therefore, justifies that qualitative research is normally regarded as an antecedent to the quantitative research method (Thani, 2009).

Moreover, a qualitative research design entails a detailed encounter with the object of study, important features such as openness to multiple sources of data, and the selection of a small number of cases to be studied. This study will therefore be conducted under a qualitative research design. Qualitative research will help outline and describe the most vital information on the medicinal plants used in the treatment of different cattle ailments. A qualitative research approach will give effective information on prevalent cattle diseases, their causal agents, and medicinal plants used to treat those ailments, as well as their availability and dominance in the Nkomazi local municipality. Throughout the study, the researcher will interact with the participants to acquire quality and trustworthy data on the usage of medicinal plants to treat various cattle ailments. However, when gathering data, a multi-method approach will be considered. For example, quality and trustworthy data will be collected using research methodologies such as in-depth interviews, and observations.

The goal of a quantitative research design, according to Thani (2009), is to measure the features of phenomena and objects, such as people's attitudes toward certain research topics, by assigning numbers to observed aspects of things. A quantitative paradigm was employed in this study to measure the number of medicinal plants used to treat various cattle diseases, the number of participants or subsistent farmers that rely on medicinal plants to keep their cattle healthy and producing as well as those that use medicinal plants as a supplement to western medicine to prevent resistance.

2.3 Research design

To properly address the research questions of a research study, a research design is employed to organize and display key parts of the project such as actions, samples, and procedures to follow. A research design is a structured approach that gives the researcher guidance on how to obtain answers to study questions (Hamal & Sapkota, 2020; Thani, 2009), moreover, a research design moves underlying philosophical assumptions to specify the selection of the participants. When conducting a research project, a plan is important to help achieve results.

The design of this study was descriptive (survey research), (Hamal & Sapkota, 2020) stated that descriptive research is all about describing participants who are involved in a study in possibly three ways: observational, case study, or survey. An ethnobotanical survey is a detailed discussion with a research participant concerning a precise matter, making it appropriate for this study.

2.4 Study area

The study was conducted across 4 communities (KaMandulo, Khombaso, Tsambokhulu, and Masibekela) in Nkomazi Local Municipality, Mpumalanga Province. Nkomazi local municipality is located in the eastern part of Ehlanzeni District, Mpumalanga Province (Fig 1). The Nkomazi Municipality covers 3240.42 km^2, or 4.07 % and 23 % of the landmass of the Mpumalanga Province and the Ehlanzeni District Municipality, respectively (Adeola et al., 2016). The Municipality is bordered on the east by Mozambique, on the south by Swaziland, on the north by Kruger National Park, on the west by Umjindi Local Municipality, and the Northwest by Mbombela Local Municipality with Malalane, Hectorspruit, Marloth Park, and Komatipoort being the main urban centres. Nkomazi has a subtropical climate with an average annual temperature of 28°C and 775 mm of rainfall (Adeola et al., 2016). July is the driest month, with only 9 mm of rain, while January has the most rain, with 127 mm on average with temperatures of 26.2 °C. June experiences temperatures of about 18.4 °C. Nkomazi Local Municipality is dominated by Swaziland Sour bushveld in the south and west and by the sensitive Barberton Montane Grassland in the northeast. Nkomazi is diverse in heritage and is dominated by Siswati (Mostly) and Tsonga language-speaking communities. Furthermore, the Nkomazi Local Municipality is made up of several villages and communities that are governed by traditional authorities.

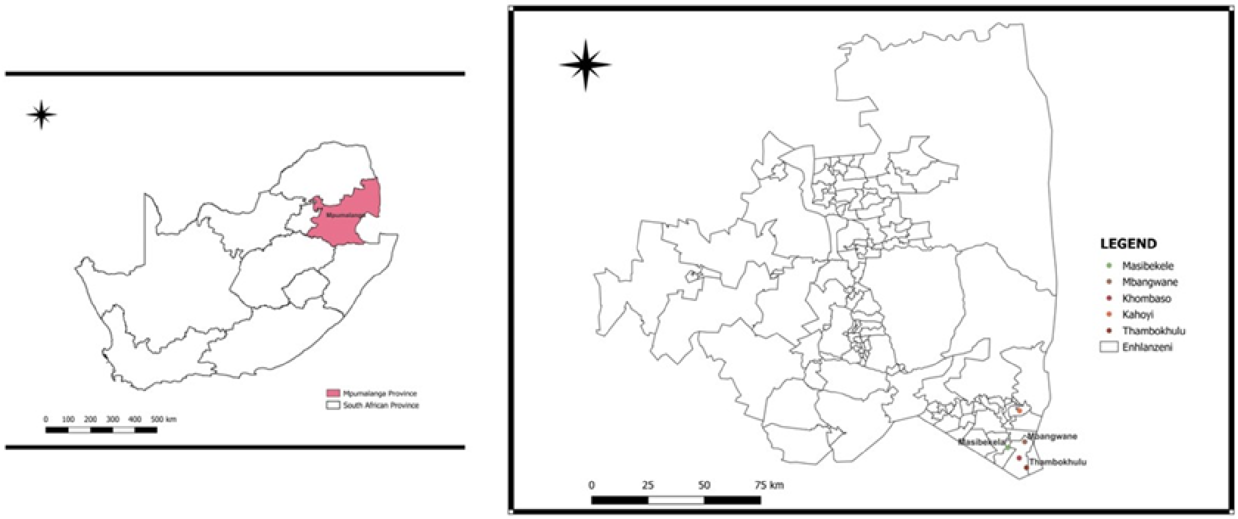


Figure 2.1: Map of Nkomazi Local Municipality, Mpumalanga Province, South Africa.

2.5 Target population

According to (Thani, 2009), a population is a set of individuals to be interviewed for viable findings. In this study, in Nkomazi local municipality to collect valid and reliable data. To explore a wider perspective of Nkomazi local municipality's ethnoveterinary knowledge, the collected data was not compared within the villages rather a larger population sample was considered.

2.6 Sampling technique and criteria

The first two participants were selected from the identified villages using a purposive sampling technique. While snowball sampling method was used to select the rest of the participants after the two purposively selected participants per village. According to (Cotton, 1996), study participants should have tangible information based on their understanding of the ethnoveterinary phenomenon being studied. Participants should have a thorough understanding of the study's domain. A total number of five subsistence cattle farmers, herders, traditional health practitioners, and elders will be purposively selected and interviewed per village. They then assisted in identifying subsequent informants and the cycle continued until the end of the data collection process.

Traditional health Practitioners (THP): The inclusion of (THP) was meant to assist in picking and identifying medicinal plants from the wild, as some medicinal plants are used for human diseases as well. Traditional health practitioners were also providing valuable information in terms of the methods of drug preparation. Preferably, traditional health practitioners who are in a possession of a legal permit were interviewed as the main health care providers.

Elderly: The use of medicinal plants for the treatment of cattle diseases has been practiced by great elders of different communities and has been taught and passed down to the next generation from ancient times. The inclusion of elders was meant to provide valuable and reliable information on where to pick or find medicinal plants around the area/village.

Subsistence cattle farmers: Farmers will provide more insight into the common cattle diseases that affect cattle in the selected villages as well as the medicinal plants used to treat the diseases. Data was collected through questionnaires and interviews at the grazing spots.

Herders: Cattle illnesses can easily be spotted by herders because they spend most of the time with the herd before and after grazing. The inclusion of herders helped in the identification of diseases, illnesses, and any injuries that cattle commonly suffer from. The herders also provided other medicinal plant remedies used during grazing periods such as pastes. Data was collected through questionnaires, interviews, and observations at the grazing spots.

Subsistence cattle farmers: Farmers will provide more insight into the common cattle diseases that affect cattle in the selected villages as well as the medicinal plants used to treat the diseases. Data was collected through questionnaires and interviews with kraals and households.

2.7 Ethical clearance

Names and other crucial information provided by the participants were kept confidential and anonymous. Principles of respect were applied whereby all participants were treated with respect and dignity. Participants were granted their privacy and were given the opportunity to withdraw from the study at any given time. Covid-19 guideline pronounced by the World health organization and South Africa was followed, including social distancing and limited participants, sanitization, and wearing of masks. Subsistence farmers were given a brief explanation of what the study was about, therefore information obtained was confidential and used for research purposes. The ethical clearance was issued by the faculty of Agriculture and Natural Sciences.

CHAPTER 3: LITERATURE REVIEW

3.1 Introduction

This chapter outlines the review related to the significance of cattle farming and ethnobotanical practices to sustain rural livelihoods. It also presents literature related to various cattle diseases prominent in rural areas. Further on outline the literature in identifying the phytochemical chemistry of medicinal plants.

3.1.1 Cattle farming

Cattle (*Bos taurus*) are large herbivores with domesticated cloven hooves. They are the most common species in the genus - and a prominent member of the modern subfamily Bovinae. Adult females are referred to as cows, while adult males are referred to as bulls (Edwards et al., 2011). Cattle were initially divided into three species: *Bos taurus*, the European or "taurine" cattle (which included similar types from Africa and Asia); *Bos indicus*, the Indicine or "zebu" cattle; and the extinct *Bos primigenius*, the aurochs (Beja-Pereira et al., 2006). Aurochs cattle are related to zebu and taurine cattle. Later, they were reclassified as a single species, *Bos taurus*, with subspecies such as aurochs, zebu, and taurine cattle. According to Beja-Pereira et al. (2006), different types of cattle are found in different geographical areas. Taurine cattle are mostly found in Europe, temperate Asia, the Americas, and Australia. Zebus are primarily found in India and tropical Asia, America, and Australia. Sanga cattle are mostly found in Africa.

These types are subdivided further into over 1000 recognized breeds. There are approximately 14 million cattle in South Africa, with 1.6 million dairy cattle (604,781 cows in milk) and 12.5 million beef cattle. Furthermore, commercial and subsistence systems account for 53% and 47%, respectively (Chakale et al., 2021). Cattle farming serves a variety of socio-cultural functions. Cattle in rural areas, on the other hand, are frequently susceptible to a variety of diseases. Every year, cattle diseases cause loss of millions of Rands. They result in death, loss of production, and, in many cases, loss of body condition. Animals that are unhealthy require more food and grow at a slower rate than healthy animals (Kahrs, 2001). In general, animals are born free of disease and parasites. However, they are usually infected by infected animals or by poor sanitation, feeding, care, and management (Mahmud et al., 2021).

Dairy cattle are susceptible to a variety of diseases, so they must be protected. Because many diseases can be transmitted to humans through milk, understanding cattle diseases is also important for public health (Bellows et al., 2002). Limiting purchases to healthy herds, using proper quarantine when bringing in new animals, employing sound sanitation, management, and feeding principles, and using appropriate and dependable vaccines are all practical and cost-effective ways to avoid disease losses (Andrews et al., 2008). By using proper management and feeding, the dairy farmer can greatly reduce disease outbreaks. Good housing promotes herd health, whereas prudent feeding not only increases body resistance to disease but also speeds recovery in the event of a disease attack. Healthy, well-cared-for, and productive cattle contribute to the communities' future livelihood being sustainable, healthier, and more inclusive (Kock, 2005).

Cattle farming is the rural sector's backbone (Chakale et al., 2021). Cattle, in particular, are a component of livestock farming and a catalyst for improving household food security and alleviating poverty in small-scale cooperative farming areas (Bellows et al., 2002). Furthermore, the major literary themes identified as the framework for the research study include the socioeconomic value of medicinal plants to herbalists in South Africa, indigenous practices, types of cattle diseases in South Africa, the most prevalent cattle diseases, conventional management of cattle diseases, traditional management of cattle diseases, economic losses caused by cattle diseases, and methods used to identify the phytochemical chemistry in medicinal plants. The literature review includes several empirical studies on the subject and investigates the implications for socio-economic impact.

3.2 Importance of cattle in rural areas

Cattle are commonly raised as livestock for meat (beef or veal, see beef cattle), milk (see dairy cattle), and hides (see leather cattle). They are used as draft animals and as riding animals (oxen or bullocks, which pull carts, plows, and other implements). Dairy cows, also known as milking cows, are specific breeds of cows kept for their milk (Edwards et al., 2011). Most dairy cows' young male offspring are sold for veal and are sometimes referred to as veal calves. Cattle dung is another product that can be used to make manure or fuel (Lovarelli et al., 2020). Cattle have religious significance in some areas, such as parts of India. Cattle farming in South Africa contributes to social and cultural values such as lobola negotiations (bridal), cleansing, ancestral rituals, and long-term rural livelihoods. Cattle, mostly small breeds like the Miniature Zebu, are kept as pets as well (Pinto et al., 2008).

3.3 Climatic factors affecting the cattle production

Cattle become dehydrated due to heat and stress. When the weather turns hot and humid, some cattles may become ill. Summer temperatures in India can reach 40 degrees Celsius, which is unbearably hot for even humans (Deshler, 1963). Livestock can withstand temperatures as high as 38°C. They will become dehydrated and may faint after a certain point (Rust & Rust, 2013). Fever, concentrated or yellow urine, concave eyes, and coat tent are some symptoms or indicators of dehydration. Shade structures and provision of water are usually used as primary controllers of hot temperature and protection of cows from direct sunlight (Pinto et al., 2008).

3.4 Cattle diseases

3.4.1 Disease categories in cattle

Cattle are afflicted with several diseases. Diseases are more prevalent in herds kept in close quarters, such as feedlots, or large herds kept on too few acres (Masika & Afolayan, 2002). Diseases are also more common in stressed animals, such as calves being weaned and shipped to new locations (Kahrs, 2001). Keeping new animals quarantined until you are certain they are disease-free is a simple herd management practice that can help keep the majority of livestock to remain healthy by reducing the number of potentially transmitted diseases (Masika & Afolayan, 2002; Masika et al., 2000). Beef cattle diseases are classified as follows:

Respiratory: These diseases are caused by microorganisms that are spread through coughing, sneezing, eye discharge, and mucous discharge (Masika & Afolayan, 2002).

Enteric: Enteric diseases manifest themselves in the gastrointestinal tract. They are frequently caused by parasites consumed while feeding (Dashtdar et al., 2016).

Neurological: Bacteria and viruses transmitted through insect bites cause neurological diseases. Cattle may stumble or have difficulty walking as a result of these (Masika & Afolayan, 2002).

3.4.2 Most prevalent cattle diseases

Cattle diseases dominated headlines in the 1980s and 1990s, when Bovine spongiform encephalopathy (BSE), also known as mad cow disease, was a major concern (Casalone & Hope, 2018). Other diseases that cattle may contract and develop include blackleg, bluetongue, and foot rot. In most states, because cattle health is a public health issue, as well as a veterinarian issue, public health and food safety standards and farming regulations, have a direct impact on the daily work of cattle farmers. However, the rules change frequently and are frequently contested. In the United Kingdom, for example, it was proposed in 2011 that milk from tuberculosis-infected cattle be allowed to enter the food chain. Internal food safety regulations may also have an impact on a country's trade policy (Mukherjee et al., 2019). For example, the United States has recently revised its beef import rules following "mad cow standards," whereas Mexico prohibits the entry of cattle older than 30 months (Mukherjee et al., 2019).

Foot and Mouth Disease in cattle is distinguished by a high fever and blisters in the mouth, back, and between the toes and feet. It is spread through contact with soil, manure, infected grains, or contact with infected cattle are most diseases on this list. Insects, rodents, and other animals can also carry the disease. Foot and mouth disease is one of the most common cattle diseases frequently seen on dairy farms. In India, cow urine is commonly used for internal medical purposes (Mohanty et al., 2014). Patients seeking treatment for a wide range of illnesses consume it after it has been distilled. There is currently no conclusive medical evidence that this has any effect. Indian medicine containing cow urine, on the other hand, has already received US patents (Kock, 2005).

The bacteria species of the genus *Treponema* cause digital dermatitis. It differs from foot rot in that it can appear as a result of unsanitary conditions such as poor hygiene or insufficient hoof trimming, among other things (Wilson-Welder et al., 2015). It primarily affects dairy cattle and has been shown to reduce milk production while leaving milk quality unaffected. Cattle are also susceptible to ringworm, which is caused by the fungus *Trichophyton verrucosum* and is a contagious skin disease that can be transmitted to humans who come into contact with infected cows (Ming et al., 2006). Cattle are prone to rabies, which causes them to become agitated and aroused. They may bite themselves and spit saliva out of their mouth. The most important indicator in cattle is that the animal breathes frequently and with an unusual sound. As a result, the livestock will become immobile and die (Syakalima et al., 2018).

3.4.3 Types of cattle diseases in South Africa

The farmers blamed red water for drinking contaminated water (*Bovine babesiosis*). Farmers suspect heartwater when an overly excited animal runs and collapses dead with froth from the mouth. It was thought to be caused by an excess of blood in the head (Syakalima et al., 2018). This is why this condition was traditionally treated by cutting a small edge of the animal's ear to allow blood flow (Luseba & Van der Merwe, 2006). Tick saliva was also thought to spread tick-borne diseases such as heartwater and redwater. Farmers identified tick bites as the leading cause of wounds. Wounds were well-defined and irritant plants were used to treat them.

When the animal did not wake up, limping, and occasionally showed swellings on the legs, black quarter illness was suspected (Luseba & Tshisikhawe, 2013). These clinical signs have been reported to appear in the spring or after a frost in the winter. Internal parasite infection was strongly associated with diarrhea and loss of appetite, but distinguishing nematode infections from tapeworms or flukes was frequently non-specific and difficult. Newcastle disease in chickens was linked to symptoms of greenish diarrhea and paralysis that occur as the seasons' change. No supernatural causes of animal diseases were mentioned, and no rituals were observed when diagnosing or treating livestock (Syakalima et al., 2018).

3.4.4 Economic losses caused by cattle diseases

The consequences of livestock diseases are commonly viewed as having only direct consequences, but in reality, they can be quite complex. Diseases reduce animal productivity and deprive farmers of dairy earnings. Disease morbidity causes both short-term and long-term product losses. These losses are more economically significant than mortality (Husnain and Usmani, 2006). Livestock diseases also result in a loss of income from other activities that rely on animals, such as farming, transportation, and tourism. Animal welfare losses are caused by the inefficient use of scarce resources in the presence of diseases (Ashfaq et al., 2015). Other difficulties with *Fasciola* infection include the high cost of treatment in the high prevalence and endemic areas, as well as the risk of drug residues in food and parasites resistant to the frontline drug (triclabendazole). In South Africa, the average cost of Fasciolosis treatment per animal is ZAR 15-20. The disease burden and treatment costs affect sustainable livestock production (Quayle et al., 2010).

Bovine fasciolosis could have far-reaching and disastrous consequences for animal health, the South African economy, and food security (Elliott et al., 2015; Greter et al., 2014; Van Wyk and Boomker, 2011). According to the findings of the study done by Jaja et al. (2017), conducted in the Eastern Cape province in South Africa, infection with *Fasciola* spp. causes economic loss due to liver condemnation. The magnitude of such loss can only be understood considering the province's high poverty index, hunger, and food insecurity. The province is the second largest in terms of land area, has the most livestock, and has a high poverty rate. The scarcity of animal health professionals, such as veterinarians, indicates a gap in the primary animal health care system and a failure to maintain an effective herd health program. As a result, the prevalence of Fasciola infection in cattle may remain persistently high (Jaja et al., 2017).

3.5. Therapeutic approaches used to manage cattle diseases

3.5.1 Conventional management of cattle diseases

Conventional medicine is a system in which healthcare professionals use drugs, radiation, or surgery to treat symptoms and diseases. According to medical literature, while conventional care manages disease symptoms, the underlying cause is frequently overlooked. This method is said to focus on treating symptoms rather than the individual as a whole (Stanford et al., 2020). This method incorporates the veterinary practice, which is the branch of medicine concerned with the prevention, management, diagnosis, and treatment of disease, disorder, and injury in livestock. It also deals with animal rearing, husbandry, breeding, nutrition research, and product development (Hardeng & Edge, 2001).

3.5.2 Ethno-botanical knowledge

Within a defined microsystem, ethnobotanical knowledge is primarily represented by experiential knowledge shared among community members who identify with a specific culture (Quave & Pieroni, 2015). The majority of indigenous diagnostic and ethnobotanical knowledge methods used in cattle healthcare have been passed down through generations, primarily through word of mouth and apprenticeship. Such indigenous knowledge is currently held by the community's elders and the few young members who are interested in learning how to use it (Daya & Vink, 2006). Furthermore, due to differences in disease epidemiology, culture, and biodiversity, ethnoveterinary practices are frequently local and culturally specific. As a result, if not documented, the vast knowledge, skills, and experience accumulated over generations in developing countries may become extinct as a result of migrations, urbanization, and technological development (Houessou et al., 2012).

3.5.3 Ethno-veterinary practices

Ethnoveterinary practices in animal healthcare are a century-old practice that can be traced back to the domestication of various livestock species (Mwale & Masika, 2009). It entails the application of domestic knowledge, beliefs, practices, and skills related to human, animal, and bird healthcare and management (Mc Corkle, 1986). It makes use of natural products such as medicinal plants, edible earth and minerals, animal parts and products, and other ingredients

(Balaji and Chakravarthi, 2010). Review reports detail various aspects of indigenous medicinal practices used around South Africa for the treatment and control of diseases affecting both humans and animals. Traditional methods of classifying, diagnosing, preventing, and treating common animal ailments are typically used by livestock raisers and locals with a strong knowledge of veterinary medicine (Krishana et al., 2005). Traditional medicines, which are commonly used in animal healthcare, significantly reduce costs especially where they are easily accessible to the average farmer (Pradhan & Mishra, 2018).

3.5.4 Trends and present state of medicinal plants

According to Awas and Demissew (2009) for the national biodiversity strategy and action plan, the current account of medicinal plants in Ethiopia shows that about 887 plant species were recorded to be used in the preparation of traditional medicine and approximately 26 endemic species are facing danger of extinction. Ejobi and Olila (2004) documented a total of 182 plants for traditional use in the treatment of cattle diseases in Uganda's Teso sub-region, as cited by (Lagu & Kayanja, 2010). Medicinal plants are the key ingredients of Traditional health systems utilised by an estimated 70-80% of the population in Africa, India, and other developing countries for primary healthcare (Rao & Rajput, 2010). About 77000 plants (18.2% of total plant species) are being used for medicinal purposes globally, according to R Rajeswara et al. (2012). The United Nations Environment Programme's (UNEP), World Conservation Monitoring Centre (WCMC) has identified 17 mega-diverse countries that are home to the majority of the world's species, including medicinal plants: Australia, Brazil, China, the Democratic Republic of the Congo, Ecuador, India, Indonesia, Madagascar, Malaysia, Mexico, Papua New Guinea, Peru, Philippines, South Africa, United States of America, and Venezuela (RAOBR Rajeswara et al., 2012). Conservation International has identified 34 biodiversity hotspots with high levels of species endemism (>1500) and biodiversity loss (70% habitat destruction), two of which are in India (Eastern Himalayas and the Western Ghats). Currently, 900-1100 species are used for medicinal purposes in various Indian systems of medicine (Ramawat & Goyal, 2008).

3.6 The uses of medicinal plants in Africa

Traditional medicine is still used to treat livestock diseases in emerging economies, whereas orthodox medicine is more commonly used in developed countries. Because of their affordability and accessibility, African medicinal plants play an important role in PHC, particularly in rural areas. Only a few African phytomedicines are available on the international market. (El-Kamali, 2009) identified the following African plant species as medicinal plants in global trade: *Cassia acutifolia*, *Jateorhiza palmate*, Harpagophytum species, *Pygeum africanum, Phystostigma venenosum, Rauvolfia vomitoria, Tamarindus indica, Warbugia ugandensis,* and *Withnia somnifera.*

Tanzanian Maasai pastoralists have been reported to use medicinal plants to treat livestock ailments such as foot and mouth disease and brucellosis (Kamoga, 2010). Kenya's Turkana and Samburu people use medicinal plants to treat cattle ailments such as cough and diarrhoea (Nanyingi et al., 2008). Herbalists play an important role in the health care system for both humans and animals in East African countries where resources are scarce. After the herbalist has failed to treat the sick animal, a veterinarian is called. It is a common trend that runs through most African traditional medical systems. Only two of the 32 medicinal plant species recorded by Liengme (1981), namely *Pterocarpus angolensis* (Fabaceae) and *Sarcostemma viminale* (Asclepiadaceae), were reported as EVM remedies in that study.

According to McGaw and Eloff (2008b), plant-based remedies are mostly used by non-commercial, rural livestock keepers who prepare and administer the medication on their own. South Africa has a diverse range of plant species that could play an important role in drug preparation. South Africa has approximately 24 000 plant species, accounting for more than 10 % of the world's vascular plant flora, according to Germishuizen and Meyer (2003). Although over 200 plants were known to have been used in South African EVM until 2008, only about 13 % of these species have been studied for *in-vitro* bioactivity in veterinary assays.

This suggests that more research is required to examine these plants for bioactivities that could be beneficial. An oral format is used to transmit traditional information gained and imparted by their ancestors, community members, and elders (McGaw & Eloff, 2008b).The EVM practices, on the other hand, vary by ethnicity and location. Whereas some areas failed to keep records of practices, this resulted in gaps in information being passed down to the next generation, which favoured ethnic groups using different traditional medicines to treat the same disease, therefore, generalizing results from one area to another will cause biasness (Khunoana et al., 2019).

The study by (Luseba & Van der Merwe, 2006) reported 19 species from 12 plant families, with the most common families being Euphorbiaceae and Fabaceae, each with four plant species. Medicinal plants such as *Balanites mau ghamii* (Balanitaceae), *Combretum paniculatum* (Combretaceae), *Diospyros mespiliformis* (Ebeneceae), *Dombeya rotundifolia*

(Sterculiaceae), and *Elephantorrhiza elephantina* (Fabaceae) were the most used plants that were used either as single plant decoctions or infusions for dosing animals or crushed and applied topically to treat wounds. In contrast, traditional healers frequently employ complex mixtures.

This agrees with findings in Madikwe, Northwest Province (Van der Merwe et al. 2001), but contradicts with the findings in the Eastern Cape Province (Masika et al., 2000). Farmers cited a variety of reasons for the continued use of ethnoveterinary medicines, including the absence of side effects or being more effective than western medicine. Traditional medicines are also preferred by farmers because there are no restrictions or waiting periods for eating meat from treated animals. Traditional medicines were generally used as a last resort after pharmaceutical medicines in chronic cases and when pharmaceutical medicines were ineffective.

Tsonga people use phytotherapy less than other ethnic groups, such as the Zulus, who use more than 40 species (Cunningham & Zondi 1991), the Xhosa, use 53 species (Dold & Cocks 2001), and the Batswana, use approximately 50 species (Van der Merwe et al., 2001). Tsongas are an ethnic minority group in South Africa, and it is assumed that loss of tradition is greater in a minority group than in a larger group. Young people are hesitant to use EVM, most likely due to a lack of information and interest, or to rural exodus, as Van der Merwe and co-workers (2001) suggested.

3.7 The market value of medicinal plants

The term "High-Value Minor Crops" refers to plants that contribute only a small amount to a country's agricultural output. Herbs and spices are divided into two categories, which are medicinal and aromatic plants. Despite being minor contributors to output, the importance of these plants in aggregate commercially was estimated at US$ 60 billion in 2006 (Sher et al., 2014). Europe alone imports about $1 billion in medicinal and aromatic plants from Africa and Asia each year. Because of the growing popularity of herbal medicines, this trade is expected to grow significantly by 2050 (Sher et al., 2014).

Medicinal and aromatic plants make a small contribution to national agriculture output, but their value per weight is among the highest among traded plants. Pharmaceutical cash crops hold enormous promise for remote communities that rely on subsistence agriculture and have limited access to the regional economy (Ahvazi et al., 2012). For centuries, MAPs have been collected, cultivated, and traded in many parts of South and Western Asia. Traditional medicine trade is part of a multi-million rand 'hidden economy' in southern Africa, fuelled by rapid urbanization, unemployment, and the high cultural value of traditional medicines. As a result, the trade in traditional medicines is now greater than ever before, and it is without doubt the most complex resource management issue confronting conservation agencies, healthcare professionals, and resource users in South Africa today. According to Mander et al. (2007), there are 27 million indigenous medicine consumers in South Africa, with a sizable supporting industry. As a result, the use and trade of plants for medicine is no longer limited to traditional healers but has spread to both the informal and formal entrepreneurial sectors of the South African economy, increasing the number of herbal gatherers and traders.

The South African market for indigenous plants was valued at R270 million annually due to the demand for plant-derived medications. Throughout the nation, it is known that over 700 plant species are actively traded for their medical properties, and extensive wild material collection is acknowledged as a severe danger to biodiversity in the area (Dold & Cocks, 2002).

3.8 Cattle diseases treated by medicinal plants

The quantity and quality of meat, manure output, and milk production are all declining as a result of increased parasite and disease levels in cattle, which frequently result in mortality (Cabaret et al., 2002). According to (Bahaman et al., 1987), the seriousness of cattle disease incidences is mostly revealed in tropical areas. However, because of their chronic and insidious nature, parasitic diseases, along with vectors, endemic pathogens, and diseases, are the least attended to in developing countries, particularly in areas where extensive grazing is practiced (Magona et al., 2008). Nalule et al. (2011) conducted a study that found a prevalence of Trypanosomiases (20.9%) in the dry lands of Uganda at Nakasongola, as well as East coast fever (15.5%) and Helminthiasis (12.8%). These three diseases were found to be the most prevalent among the twenty-five diseases affecting cattle in the area, with the others being Brucellosis, Foot and Mouth disease, Ringworm, Lumpy skin disease, Placental retention, Mastitis, Bloat, Gall sickness, internal parasites, and Heartwater (Gitonga et al., 2021).

Heartwater, also known as cowdriosis, is a noncontagious tick-borne disease found in both wild ruminants and domesticated livestock such as cattle, goats, and buffalo. Heartwater symptoms include loss of appetite, rigid posture, ascites, respiratory discomfort, edema of the lungs, and hydropericardium (fluid build-up in the sac surrounding the heart), from which the name derives. *Drimia delagoansis* (previously *Urgenia lydenburgensis*) is a medicinal herb used by some Limpopo farmers to treat heartwater disease in goats and cattle.

Small-scale farmers in South Africa's Eastern Cape Province, according to reports, use a variety of plants to treat heartwater, red water, and anaplasmosis (Dold & Cocks, 2001). These plants include *Arctotis arctotoides* (L.f.) O.Hoffm., *Cussonia spicata* Thunb. , *Ledebouria revoluta* (L.f.) Jessop., *Heteromorpha trifoliate* (H.L. Wendl.) Eckl. & Zeyh. *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb., and *Verninia mespilifolia* Hochst. ex A. DC. In Limpopo Province, the shrub *Petalidium oblongifolium* is used to treat cattle, goats, and game. When grazing becomes scarce in the autumn and winter, livestock will browse on the shrub. Farmers believe that the nutritional properties of the plant are what allow the animal to thrive while also curing it as it browses on the plant (Masika & Afolayan, 2003).

3.9 Medicinal plants parts used for the treatment of various ailments in cattle

Almost all parts of medicinal plants are used by different farmers in different areas to treat various ailments. Kamoga (2010) discovered that leaves account for approximately 62.7% of the plant part that is mostly used as or for medication to treat cattle disease in Kabira Sub County, Rakai District. According to Nalule et al. (2011), the popularity of stems, roots, and whole plants is detrimental to the ecosystem and conservation practices.

Medicinal preparations containing bulbs, stems, barks, roots, rhizomes, or the entire plant have a significant impact on the mother plant's survival (Dawit & Ahadu, 1993). According to Kumar (2014), *Tamarindus indica* L. plant roots were used to treat worms, cough, and wounds in cattle in the Serere district. The stem of *Euphorbia candelabrum* Tremaux ex Kotschy was used to treat anaplasmosis, ECF, and tick control in cattle in the same district. According to Tesfaye (2009), there are cases where different parts of the same plant are used to treat the same ailment, as well as cases where more than one medicinal plant is used to treat the same ailment.

The aqueous leaf extract of *Tephrosia vogelii* Hook. f. has also been observed in the Teso region for the treatment of mange(Gibson, 1963). The Fulani, in the North West region of Cameroon, complement their livestock health care using medicinal plants namely; *Kigelia africana* (Lam.) Benth. for brucellosis and mastitis, *Entada abyssinica* for foot and mouth disease, babesiosis and worms, *Khaya anthotheca* for black quarter, brucellosis and worms, *Solanum aculeastrum* for worms, *Cassia occidentalis* for worms, black quarter and babesiosis (Toyang et al., 1995). In East Africa, unidentified parts of *Aerva persica* and *Clerodendrum myricoides* are used in the treatment of East Coast Fever, while *Erythrococca bongensis* and *Monsonia anguistifolia* are used for black quarter disease (Kokwaro, 1976). In Tanzania, the use of traditional medicine by the Maasai pastoralists was reported for the treatment of livestock diseases such as foot and mouth disease, brucellosis, and rinderpest (Marecik, 1998). The bark extract of *Albizia anthelminthica* locally used by pastoral farmers in Kenya to treat worms was found to decrease faecal egg counts of *Haemonchus contortus* in sheep by 34% (Githiori et al., 2003).

3.10 Routes of administration of the medicine.

Scholars such as Nalubega (2007) have documented the use of the oral route for the administration of drugs for the treatment of livestock disease, whereas Nalule et al. (2011) reported that oral drenching was documented as the primary route of administration of approximately 34 medicinal plant preparations in Uganda at pastoral communities. Medicinal plant remedies are easily administered orally and require little skill, which is why most farmers use them the most (Nalubega 2007). Furthermore, Harun et al., (2010) discovered that in other regions, the oral route of administration was mostly used in the application of medicinal plant preparation in addition to topical application to control external parasites. The route of administration of herbal preparations is usually determined by the perceived diagnosis served. In South Africa, topical application is commonly used in the treatment of skin diseases, wound care, and parasite control (Masika et al., 2000).

3.11 Plant extracts preparation

3.11.1 Extraction

Plant extracts are thought to be the most important sources of biomolecules that can be extracted from plant parts. Traditional medicine, which includes the use of medicinal herbs and cures, has recently gained popularity in developed countries (Abdullahi et al., 2022). According to Farnsworth (1994), the European Union now uses over 1400 herbal preparations. Plant parts (bark, leaves, roots, seed, and so on) are used to make medications for a variety of health conditions. This is usually determined by the unique qualities of the plant as well as the desired extract (Farnsworth, 1994). Extraction of such biomolecules from medicinal plants is accomplished using a variety of solvents and extraction methods (Redfern et al., 2014).

The two most common methods for drug preparation are decoction and infusion. According to (Raza et al., 2014), the other common preparation methods were ball drench and jaggery; however, decoction and oral drug administration were very common modes of administration. A reasonable quantity of extracts and biomolecules with sufficient and excellent antimicrobial results must be extracted successfully using various solvents of different polarities, including polar to nonpolar. According to studies, the best solvent systems for extracting plant extracts are ethyl-acetate, hexane, dichloro-methane, chloroform, acetone, butanol, and/or their correct ratio combinations (Gupta et al., 2020).

3.11.2 Decoction

Decoction involves the simmering of the thicker and less permeable parts of the plant such as the bark, roots, seed, and fruit for easy extraction of their medicinal constituents as reported by Nwachukwu, Umeh, Kalu, Okere, and Nwoko (2010). The plant material is cut into smaller pieces, the simmering pot is covered to prevent the volatile components of the decoction from escaping, and the solids and liquids are separated (Sasidharan et al., 2011).

3.11.3 Infusion

The active medicinal constituent of plants is extracted using hot water in this method. This method enables the extraction of volatile components from aerial parts such as flowers, leaves, fruits, roots, and so on. Other remedies can be made in powdered form by grinding dried or roasted plant material. Fresh plant parts are mashed to make pastes (Sasidharan et al., 2011). The remaining medicine is obtained by pressing the sap from freshly picked stems, leaves, flowers, and other plant parts. According to Farnsworth (1994), combining plants increases the active ingredients, so most remedies contain material from more than one plant species (Gupta et al., 2012).

3.11.4 Analytical techniques used in the phytochemical studies

High-pressure liquid chromatography (HPLC) is an analytical technique for the separation and detection of active biomolecules that are commonly used in conjunction with other chromatographic systems such as gas chromatography-mass spectrometry (GC-Ms) and liquid chromatography-mass spectrometry/mass spectrometry (LC-Ms/Ms) (Otang-Mbeng & Sagbo, 2019; Sarais et al., 2010). The GC-MS have a high capacity for isolating and classifying volatile compounds in complex mixtures. The LC-Ms can also analyse and classify a large number of non-volatile compounds in small amounts (Choma & Grzelak, 2011). Thin layer chromatography (TLC) and nuclear magnetic resonance (1H NMR) are two other methods used in the characterization and confirmation of biomolecules present in plant extracts.

CHAPTER 4: MEDICINAL PLANTS USED FOR THE MANAGEMENT OF CATTLE DISEASES IN NKOMAZI LOCAL MUNICIPALITY, MPUMALANGA, SOUTH AFRICA

4.1 Introduction

Since time immemorial, medicinal plants have always been used globally for treating and managing cattle diseases (Chaachouay et al., 2022; Nortje & Van Wyk, 2015). Ethno-veterinary medicine (EVM) is made up of a complex system of knowledge, skills, practices, and beliefs that are closely associated with animal husbandry and general animal care (McGaw & Eloff, 2008b). The uses of traditional medicine during the last decade have expanded globally and are gaining popularity (Masoko, 2013). The World Health Organization WHO (2013), stated that herbal medicine serves the health needs of about 80% of the world's population, especially for the vast majority of the populations in different rural areas of both developed and developing countries. A previous study has shown that almost 75% of resource-limited livestock owners use traditional medicine to treat animal ailments (Masika et al., 2000).

Over the last decade, ethnoveterinary investigations have increased in developing and developed countries such as India (Mondal, 2012), Pakistan (Mirani et al., 2014), Nepal (Dhital & Pudasaini, 2019), Ethiopia (Kebede et al., 2016) Botswana (Gabalebatse et al., 2013), South Africa (Chakale et al., 2021; Maphosa & Masika, 2010; McGaw & Eloff, 2008a), Kenya (Njoroge & Bussmann, 2006), and China (Shen et al., 2010). Despite its economic revenue, livestock plays an integral part in cultural identity (Moichwanetse et al., 2020). Amongst the indigenous communities, a person's wealth is measured by the number of livestock that they have. According to Peters (1994), cattle in Botswana are a symbol of social and political power and not only a crucial source of food and monetary value. Livestock contributes up to 80% of agricultural Gross Domestic Product GDP in emerging economies, particularly those in Africa, and 600 million people rely heavily on livestock for their subsistence 21q. Rural farmers in South Africa from many ethnic groups, such as the Batswana, Tsonga, Xhosa, and Zulu, have persisted in using traditional methods to maintain the health and productivity of their cattle (Khunoana et al., 2019).

Subsistence farmers in rural areas are suffering from a high incidence of cattle diseases such as retained placenta, diarrhoea, eyes sight problems, inflammations, general wellness, and fertility problems. Low income and lack of access to viable veterinary services are partly to blame for the high livestock mortalities (Luseba & Tshisikhawe, 2013; Masika & Afolayan, 2003). Cattle diseases and parasites affect meat, milk, and manure output, as well as asset value due to increased mortality, particularly in calves (Gabriel, 2015). Livestock diseases negatively affect the income and farming activities of subsistence farmers in rural areas, which in turn pose negative implications on their livelihood and society in general. Mthi et al. (2020) also stated that cattle diseases affect not only the production but also the import and export of animal products.

According to Ernst (2005), the majority of synthetic drugs used currently originated from the plant kingdom, yet only a small proportion of pharmacopeia is dominated by herbal medicines. Traditional methods of animal husbandry are increasingly being recognized for their potential usefulness in contributing to animal well-being, particularly at the level of primary animal healthcare (Abo-EL-Sooud, 2018; Luseba & Tshisikhawe, 2013). Hence, there has been more interest in documenting EVM compared to the previous decade in South Africa (Chakale et al., 2021; Maphosa & Masika, 2010). Mpumalanga is one of the provinces with a high reliance on indigenous medicine. However, it is no exception that cattle in the rural settlements of Nkomazi Local municipality are also affected by various conditions such as calf diarrhoea, intestinal worms, paleness, wounds, maggots' infestation, poor eyesight, bloating, bone fracture, dehorning, snakebites, foot, and mouth disease, retained placenta, dystocia and lumpy skin which are treated using EVM. Indigenous traditions have been replaced by Western-derived traditions (Luseba & Tshisikhawe, 2013). Hence, this study is aimed at identifying and documenting the different cattle diseases treated by EVM plants through indigenous practices in Nkomazi Local Municipality (NLM).

4.2 Materials and methods

4.2.1 Data collection

Ethnoveterinary data was collected from October 2021 to April 2022 through face-to-face semi-structured interviews. Interviews were conducted in SiSwati, the indigenous language of the people in the study area. A total number of 100 participants were selected amongst the five villages (20 participants per village) purposely for the study. Local names of the medicinal plants, the various cattle ailments they treat, preparation methods, administration, and dosage were recorded. For each reported medicinal plant species, a specimen was collected, pressed, prepared, and deposited at the herbarium of the South African National Biodiversity Institute

(SANBI) in Pretoria. The botanical names of the collected plants' specimens were identified in the herbarium using the dichotomous key (Leistner, 2000). All scientific plant names were validated through the "The world flora online (http://www.worldfloraonline.org/) and Plant Africa (pza. sanbi.org).

4.2.2 Data analysis

The data were analysed using descriptive statistics such as mean, percentage, and ethnobotanical indices such as frequency of citation (FC) and informant consensus factor, which were calculated using the formula described by Tardío and Pardo-de-Santayana (2008) and Heinrich et al. (1998).

$$FC = \frac{N_p}{N} \times 100$$

Where Np = number of times a specific plant species is indicated for use. N = total number of times that all plant species were mentioned.

The informant consensus factor (ICF) value of each disease category was calculated as follows: The number of use citations in each category (Nur) was divided by the number of species used (nt) (Heinrich et al., 1998).

$$\mathbf{ICF} = \frac{\boldsymbol{N_{ur} - N_t}}{\boldsymbol{N_{ur} - 1}}$$

Where Nur = number of use citations in each category; Nt = number of plants used. All citations were placed into one of the six categories: dermatological diseases, eye-related diseases, gastrointestinal-related diseases, reproductive-related systems, musculoskeletal and respiratory-related diseases.

4.3 Results and discussions

4.3.1 Socio-demographic characteristics of participants

Table 4.1 shows the socio-demographic characteristics that were considered during the study. These characteristics included the age of participants (years), gender of participants, marital status, and educational status. Participants included farmers (herders and cattle owners) and traditional health practitioners. One hundred participants were interviewed, and a substantial proportion of the participants were males 75% and 25% were females. The high number of male participants indicates that cattle raring in rural areas is dominated by men in Nkomazi Local Municipality. Most men own cattle as they received it as part of *"lobola"* when their daughters get married. According to the participants the culture allows men to graze cattle given the amount of energy and muscle input required moving from place to place while women are allowed to plough the farm land closer to settlement area. A similar study done by Gabriel (2015) reported 66.2% males and 33.8% females. Khunoana et al. (2019) and Chinsembu et al. (2014) also reported that, in most rural cultures, males possess a greater knowledge of EVM than females since they were often responsible for the welfare of the cattle.

The majority of the participants' age ranged from 41–60 years (35%), followed by 20–40 years (30%), while the least age ranged between 81-100 years old. This implies that EMV knowledge is more in the hands of the older population (41-60). Above 70 years, most of the men do not have the physical strength of taking cattle around the fields for grazing and normally hand the cattle to the next male in the family who still has the strength. Most participants in the majority age group stated that urbanization is imparting negatively on the younger population. Furthermore, Fajardo et al. (2017) revealed that the older generation is more likely to be more knowledgeable about the uses of different medicinal plants compared to the younger generation. There is a positive correlation between the process of knowledge acquisition and that aging of individuals and experience according to the works of (Chinsembu et al., 2014; Fajardo et al., 2017). Therefore, the age distribution of the participants who possess EVM knowledge in NLM is like the number of years of practice as knowledge holders. In addition, a similar study by Beltrán-Rodríguez et al. (2014) has shown that age seems to be the only factor associated with knowledge, although some studies have found no link between age and ethnobotanical knowledge (Byg & Balslev, 2004). There is a lack of interest in EVM due to advanced access to modern services (e.g. veterinary clinics) decreased indigenous knowledge acquisition, changed values, and greater orthodox treatment opportunities (Hopkins et al., 2015).

Sixty-three percent (63%) of the participants were married, and such preferences for using medicinal plants may be explained by the fact that cattle farmers and healers with family members seek to reduce conventional medicine costs to cover food and other basic household needs (Makbli et al., 2016). Furthermore, the educational status for both male and female participants was exceptionally low, with the post-secondary level being the highest earned (37%), followed by the matric level at 25%. These findings were because as people advance with education, they tend to leave behind traditional knowledge and adopt conventional medicine (such as veterinary antibiotics). Kisangau et al. (2007), indicated that there was a low literacy rate among traditional health practitioners, and this poses a serious challenge in the documentation and preservation of culture in the form of research.

Table 4.1: Socio-demographic information of participants. No. representing number of participants and the percentage.

Variables	No.	Percentage (%)
Gender of the participants		
Male	75	75
Female	25	25
Age of the participants		
20-40	33	33
41-60	35	35
61-80	30	30
81-100	2	2
Marital status of the participants		
Single	23	23
Married	63	63
Divorced	5	5
Widow/widower	9	9
Education status of the participants		
Formal education	8	8
Secondary	37	37
Degree	3	3
Diploma	5	5
Matric	25	25
Informal education	22	22

4.3.2 Medicinal plant diversity

The current study documented twenty-eight (28) plant species used traditionally to treat and manage different cattle ailments (Table 4.2). These medicinal plants were distributed among 19 families (Figure 4.1). The largest proportion of medicinal plants documented belonged to the Fabaceae (4 species), followed by Poaceae and Asteraceae (3 species each), Euphorbiaceae, and Solanaceae (2 species each). Fabaceae family has been mentioned as one of the plant families consisting of several plant species used in EVM amongst different indigenous communities in Southern Africa (Eiki et al., 2022; Luseba & Tshisikhawe, 2013). Furthermore, this is one of the largest family of plants in South Africa and consists of 567 species and 156 genera of plants (Van Wyk, 2020). A study by Tshikalange et al. (2016) reported that the Fabaceae family consists of a diversity of phytochemicals, with flavonoids and tannins as the most common polyphenols. The existing literature supports the popularity of Fabaceae for their therapeutic efficacy; according to an exhaustive assessment of plant groups utilized in African traditional medicine (Lawal et al., 2022; Van Wyk, 2020). The most cited plant species were from the Fabaceae family, *Vachellia sieberiana* var. woodii (Burtt Davy) Kyal. & Boatwr. mentioned by 12% of the participants for the same disease (Table 4.3). *Vachellia sieberiana* is a pantropical and subtropical genus with species abundant throughout Australia, Asia, Africa, and America (Olajuyigbe & Afolayan, 2012). *Vachellia sieberiana* used in EVM as foliage amongst livestock has also been reported to treat stomach aches, tapeworms, bilharzia, haemorrhage, orchitis, colds, diarrhoea, gonorrhoea, kidney problems, syphilis, ophthalmia, rheumatism, and circulatory system disorders (Coates Palgrave, 2002; Palmer & Pitman, 1972).

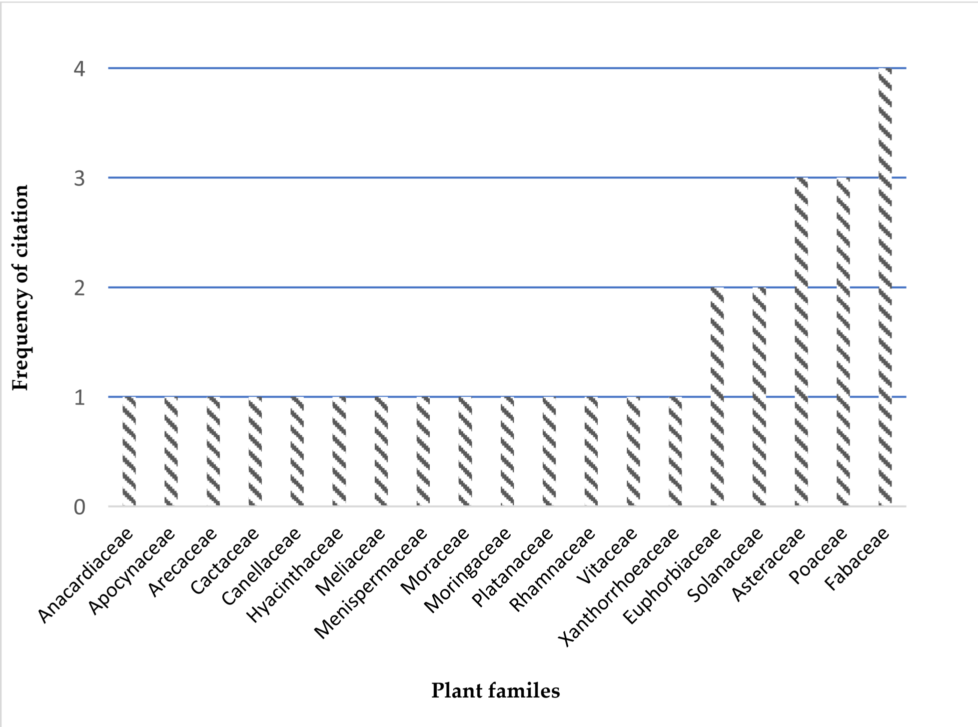


Figure 4.1: Distribution of plant families used to treat and manage cattle diseases in Nkomazi Local municipality, Mpumalanga (n=19).

4.3.3 Frequency of citation

Based on their FC values that ranged from approximately 1-76%, the most frequently mentioned medicinal plants were *Vernonia abbreviata* DC. (76%), *Synadenium grantii* Hook.f

(42%), *Cissus quadrangularis* L. (50%), *Phragmites australis* (Cav.) Trin., (18%), and *Vachellia sieberiana* (12%) (Table 4.3). *Synadenium grantii* and *C. quadrangularis* being the second and third species with the highest FC value have been supported by a studies in Giyani and the Nkomazi local Municipality who both used *C. quandrangularis* (Nyangala) for treating wounds and *S. grantii* used to treat diarrhoea (Luseba & Tshisikhawe, 2013). There is increasing evidence from various studies that suggests that the population living in rural areas is often aware of the ethnoveterinary and the value of their local plants amongst their livestock (Chakale et al., 2021; Chinsembu & Cheikhyoussef, 2016). *Cissus quadrangularis* plays a huge role in treating different cattle ailments such as open wounds caused mostly by maggot infestation as well as *Synadenium grantii* to treat poor eyesight. Maggot infestation and poor eyesight are considered the most threatening cattle ailments in NLM (Table 4.2).

The majority of ethnoveterinary knowledge is passed down orally from one generation to the next and is exclusive to a particular culture. However, the locality also influences the plants used to prepare the remedies since some plants may not be found in certain regions. This finding is in line with that other scholars including Khunoana et al. (2019). More often than not, farmers considered using plants that were recommended by family members or better yet, other farmers of the same ethnic group in the same locality which is also an indication that there is more trust in the use of medicinal when the doctor is a family member or close relative or friend. Luseba and Tshisikhawe (2013) conducted a similar study with the Tsonga people in the Greater Giyani municipality and it was found that the farmers used 38 plant species from 19 families. However, this was different from the situation in the Nkomazi local municipality, where few plants were used when compared to the previous study, perhaps because the area is smaller compared to Giyani and both studies were done amongst Tsonga and Swati people. The compared results showed that there is a relationship between plant use and locality.

4.3.4 Informant consent factor

Following the Ruminant Veterinary Association of South Africa (Van Jaarsveld et al., 2015) and the classification in previous studies (Chakale et al., 2021; McGaw & Khunoana, 2018), 18 cattle diseases were classified into six categories in the study area (Table 4.2 and Table 4.3). Dermatological diseases, such as diarrhoea, bloating, intestinal worms, and allergic reactions, scored the highest Informant consent factor (ICF) value (Table 4.2), whereas musculoskeletal diseases, such as fractured bones, received the second highest ICF value. Reproductive and respiratory-related diseases were cited as the categories with the third highest ICF, with 8 to 5 species recorded, indicating a high level of agreement among participants for this specific usage. The fourth ICF value was assigned to gastrointestinal disorders, which were followed by eye-related diseases, which were rated fifth. The lower ICF reported in this category might be ascribed to the sociocultural trend of society and the inclination of people in these contemporary communities that identify diseases to follow traditional medicine. High ICF values can be used to pinpoint interesting species in search of bioactive compounds (Canales et al., 2005; Chaachouay et al., 2022).

Table 4. 1: Cattle diseases based on categories and informant consensus factor (ICF).

Categories of cattle diseases	No. of used reports (Nur)	No. of plant taxa (Nt)	ICF
Eye disease	27	1	0.03
Gastrointestinal diseases	22	15	0.33
Respiratory related diseases	9	5	0.5
Reproductive systems	16	8	0.5
Musculoskeletal	3	5	0.75
Dermatological diseases	173	28	0.84

Table 4. 2: Ethno-botanical information of plants used for the treatment of cattle diseases Ethno-botanical information of plants used for the treatment of cattle diseases in Nkomazi Local Municipality.

Scientific names, Families & Voucher no.	Vernacular Name (Siswati)	Plant part & methods of preparation	Cattle diseases/conditions	N[a]	Plant Form	FC (%)	Cs
Albertisia delagoensis (N.E.Br.) Forman. **Menispermaceae** **[ATM 011]**	Umthunduluka	Leaves, Poultice	Dystocia	1	S	1	LC
Aloe ferox Mill. **Xanthorrhoeaceae** **[ATM 010]**	Inhlaba	Leaves, Poultice	Dehorning	2	T	2	LC
Artemisia afra Jacq. Ex Willd. **Asteraceae** **[ATM 020]**	Umhlonyane	Leaves, Maceration	Cough	3	S	3	LC
Physalis barbadensis Herb.Wight ex Wall. **Solanaceae** **[ATM 006]**	Inhlaba	Leaves, Poultice	Cough and diarrhea	3	S	3	LC
Ceratonia siliqua L. **Fabaceae** **[ATM 008]**	Vovovo	Leaves, Decoction	Diarrhea, weak and pale	6	T	6	NE
Cissus quadrangularis L. **Vitaceae** **[ATM 003]**	Umhlohlamphetfu	Stem, Poultice	Maggot infestation and wounds	50	C	50	LC
Cocos nucifera L. **Arecaceae** **[ATM 021]**	Inkumba	Bark, Infusion	Poor eyesight	1	T	1	LC
Cynara cardunculus L. **Asteraceae** **[ATM 022]**	Silulwane	Bulb, Decoction	Pale and weak	4	H	4	IA
Datura ferox L. **Solanaceae** **[ATM 002]**	Zaba-zaba	Leaves, Poultice	Wounds	2	H	2	IA
Dipcadi brevifolium (Thunb.) Fourc. **Hyacinthaceae** **[ATM 023]**	Curly-curly	Roots Poultice	Wounds	1	H	1	LC

Scientific names, Families & Voucher no.	Vernacular Name (Siswati)	Plant part & methods of preparation	Cattle diseases/conditions	N[a]	Plant Form	FC (%)	Cs
Elephantorrhiza elephantina (Burch.) Skeels. **Fabaceae** **[ATM 024]**	Intfolwane	Bulb, Decoction	Calf diarrhea	5	S	5	LC
Euphorbia ingens E. Mey. ex Boiss. **Euphorbiaceae** **[ATM 014]**	Umphahla	Leaves, Maceration	Bloating	2	T	2	LC
Ficus glumosa D. **Moraceae** **[ATM 004]**	Inkokhokho	Bark & roots, Poultice	Broken bone	3	T	3	LC
Maringa oleifera Lam. **Moringaceae** **[ATM 015**]	Maringa	Leaves, Decoction	Cough	3	T	3	LC
Opuntia ficus-indica (L.) Mill. **Cactaceae** **[ATM 005]**	Lidolofiya	Leaves, Maceration	Bloating	4	S	4	NE
Phragmites australis (Cav.) Trin. Ex Steud. **Poaceae** **[ATM 018]**	Lihlanga	Leaves, Decoction	Retained placenta, dystocia and wounds	18	G	18	LC
Platanus occidentalis L. **Platanaceae** **[ATM 025]**	Emasiti	Bark, Poultice	Snake bite and diarrhoea	4	T	4	LC
Saccharum officinarum L. **Poaceae** **[ATM 026**]	Umoba	Stem, Poultice	Eye problem	1	G	1	LC
Sclerocarya birrea (A. Rich.) Hochst. **Anacardiaceae** **[ATM 013]**	Umganu	Bark, Decoction	Diarrhoea, weak and pale	6	T	6	LC
Senegalia nigrescens (Oliv.) P.J.H. Hurter. **Fabaceae** **[ATM 007]**	Umkhaya	Bark, Decoction	Weak and pale	3	T	3	LC
Synadenium grantii Hook.f **Euphorbiaceae** **[ATM 001]**	Umdlebe	Stem, Poultice	Poor eyesight	42	S	42	IA

Scientific names, Families & Voucher no.	Vernacular Name (Siswati)	Plant part & methods of preparation	Cattle diseases/conditions	N[a]	Plant Form	FC (%)	Cs
Tabernaemontana elegans Stapf **Apocynaceae** **[ATM 019]**	UmKhahlwana	Stem, Infusion	Infertility in bulls	4	T	4	LC
Trichilia dregeana Sond. **Meliaceae** **[ATM 016]**	Umkhuhlu	Bark, Decoction	Intestinal worms	6	T	6	LC
Vachellia sieberiana var. woodii (Burtt Davy) Kyal. & Boatwr. **Fabaceae** **[ATM 012]**	Mashubane	Leaves, Decoction	Calf diarrhoea, intestinal worms, diarrhoea, weak and pale	12	T	12	LC
Vernonia abbreviata DC. **[ATM 027]** **Asteraceae**		Leaves, Infusion	Cough, diarrhoea, skin problem, gastro-intestinal parasites and maggot infestation	76	H	76	LC
Warburgia salutaris (G. Bertol. Chiov. **Canellaceae** **[ATM 028]**	Isibhaha	Bark, Maceration	Foot and mouth	2	T	2	EN
Zea Mays L. **Poaceae** **[ATM 017]**	Umbila	Seeds, Decoction	Snake bite	3	T	3	LC
Ziziphus mucronata Willd. **Rhamnaceae** **[ATM 009]**	Umhlonhlo wehlathi	Leaves, Poultice	Maggot infestation	1	T	1	LC

[a]N=Number of participants; [b] Plant form: T=Tree, S=Shrub, and H=Herb. Ethnobotanical Index used, FC= Frequency of Citation; Cs=Conservation status: NE=Not Evaluated; LC= Least concern; IA= Invasive alien species and EN=Endangered. The conservation status was verified using the South African Red data list (http://redlist.sanbi.org/specices)

4.3.5 Types of cattle diseases/conditions

Fig.4.2 shows that maggot infestation (30%), eyesight (27%), and calf diarrhoea (16%), are the most prevalent disease within the study area. Similar diseases were also identified in a study by Gabalebatse et al. (2013), whereby diarrhoea constituted 67%, followed by eye diseases (49%). During the interviews, a number of farmers indicated that untreated-open wounds are the main cause of high maggot infestations. According to Hunter (1990), adult flies lay eggs in a wool moistened from various causes like skin wounds or the lesions of foot-rot leading to a secondary bacterial infection. Several factors, including calves consuming excessive amounts of milk, consuming worm-infested grass, and drinking tainted water, were also mentioned as potential causes of diarrhoea in calves (Gabalebatse et al., 2013). In addition, poor blood circulation, high temperatures, and overfeeding are common causes of eye diseases (Dashtdar et al., 2016). Furthermore, the selected study area is close to Kruger National Park, which might contribute to several livestock diseases. Gabriel (2015), stated that livestock, particularly cattle, are constantly under threat of contracting various diseases due to their proximity to wildlife.

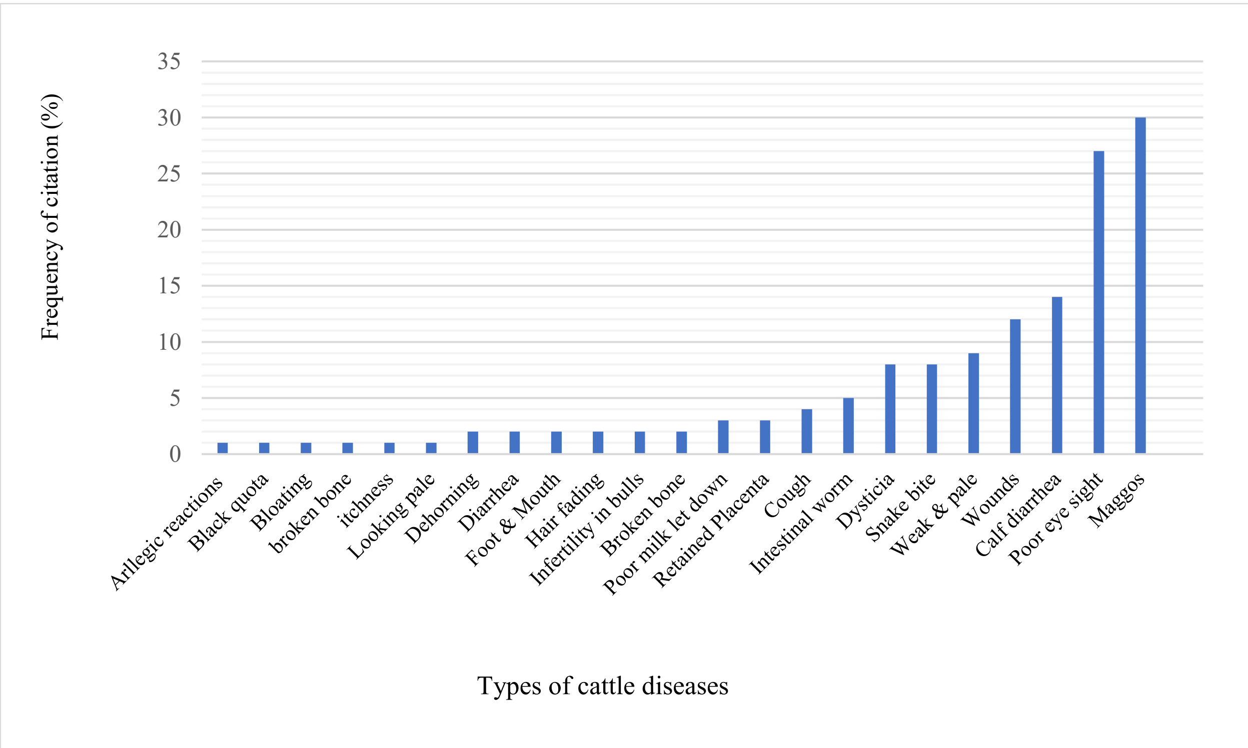


Figure 4.2: Types of diseases affecting the cattle in Nkomazi local Municipality, South Africa.

4.3.6 Distribution of plant parts used to treat cattle diseases

In this study, the most frequently used plant part was leaves, followed by bark, while the stem and other parts ranged from 14% to 1% (Fig. 4.3). The leaves according to the participants are easily accessible, easy to process due to high-water content between 70% and 80% and soft texture. It also contains chemical compounds that act as antioxidants (Affandi & Batubara, 2019). Similar findings were also reported by Luseba and Tshisikhawe (2013) and Kamoga (2010) who articulated that leaves were the most used plant part due to their relative abundance compared to other plant parts. Furthermore, some farmers mentioned the use of non-plant material together with other medicinal plants to treat animal infections. For example, salt was mixed with *A. marlothii* to treat diseases such as gastrointestinal diseases. The use of salt by the Venda people was also reported by Luseba and Tshisikhawe (2013). Several rural farmers reported using Jeyes fluid to ward off ticks and gasoline to clean wounds after applying *C. quadrangulari.* Previous studies have also revealed that *C. quadrangularis* has been used to treat retained placenta, wounds, and dermatophilosis in cattle (Chitura et al., 2018; Ndhlovu, 2014).

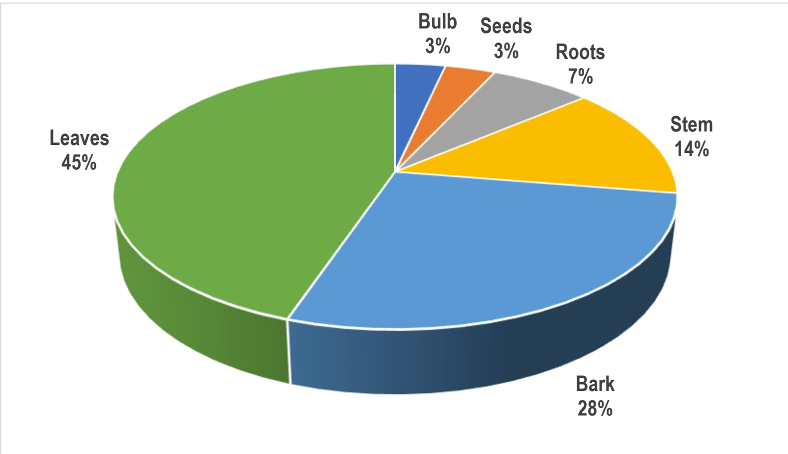


Figure 2.3: Plant parts used to produce plant remedies as described by the participants in Nkomazi Local municipality, South Africa (n=29).

4.3.7 Methods of preparation and administration

Different methods of preparation, including powder, paste, ointment, lotion, poultice, plant juice, decoctions, concoctions, or infusions, have been recorded in different ethnoveterinary surveys (Ahmad et al., 2015; Akerreta et al., 2010; Ali-Shtayeh et al., 2016; Eshetu et al., 2015). In this study, poultice (39%) and decoction (36%) were identified as the most common methods of preparation for ethnoveterinary remedies used to treat cattle diseases in NLM. The oral route (54%) and topical (46%) were the most preferred method of administration. Skin conditions such as wounds, maggot infestation, sores, and cuts were treated topically by applying paste of

C. quadrangularis on the infected area as a poultice, and this was also reported in a study by Masika et al. (2000). According to Luseba and Tshisikhawe (2013), the Tsonga people of Greater Giyani also used *C. quadrangularis* for wound treatment, as a tick repellent, and for lumpy skin disease. The poultice is a common method of preparation and is justified by its strong and immediate effect on disease conditions in cattle. For eye infections, *Synadenium grantii* was cut open and squeezed to release milky sap, which is then applied topically to the area that was referred to as the "nerve" located outside the eye. “The sap must be carefully applied so that it does not enter the eye, as it may be poisonous and cause damage to the eye” a THP emphasized. Moreover, in instances where cattle are suffering from diarrhoea, weakness, and paleness, *Sclerocarya birrea*, *Senegalia nigrescens*, and *Ceratonia siliqua* (Table 4.3) are prepared through decoction and administered to the cattle orally. In addition, spiritually perceived causes of illness were treated by drying a bark of *Warburgia salutaris* and sprinkling it into a fire, and the affected animal was kept nearby to inhale the smoke. The Vha-Venda (Luseba & Tshisikhawe, 2013), Batswana (Gabalebatse et al., 2013; Moichwanetse et al., 2020; Van der Merwe et al., 2001), Xhosa (Maphosa & Masika, 2010; Masika et al., 2000), Zulu (McGaw & Eloff, 2008b) and Tsonga (Luseba & Van der Merwe, 2006) used similar preparation methods. Water-based remedies were administered orally with the aid of beverage bottles with a capacity of 1L for adults and 500mL for calves.

4. Concluding remarks

The present study documented 28 medicinal plant species used by traditional health practitioners and farmers of the Nkomazi local municipality, Mpumalanga Province, in treating cattle diseases. This study provides a report as well as knowledge of medicinal plant availability among the five communities in the Nkomazi municipality. The data generated from this study forms part of an inventory that may be utilized by conservation authorities as a source of information to allow the effective management documentation biodiversity and resources. Further studies are still needed to educate more local farmers on the sustainable utilization of indigenous natural resources and opens a platform for further studies of the plants used for cattle disease management such as profiling the chemical composition of the plants.

CHAPTER 5: UPLC-QTOF/MS ANALYSIS OF *VERNONIA ABBREVIATA* DC. - A MEDICINAL PLANT USED FOR THE TRADITIONAL MANAGEMENT OF CATTLE DISEASES IN NKOMAZI LOCAL MUNICIPALITY, SOUTH AFRICA.

Summary

Vernonia abbreviate has a wide range of medicinal applications including antioxidant, antibacterial, anti-inflammatory, anti-yeast, and antidiuretic properties. The main aim of the present study was to profile bioactive secondary metabolites from the *V. abbreviata*. Acetone and ethanol leaf extracts using the UPLC-QTOF/MS to validate their use for the management of cattle diseases in Nkomazi Local Municipality of Mpumalanga Province, South Africa. Secondary metabolites from the acetone and ethanol extracts of *V. abbreviate* leaves prepared by Soxhlet extraction technique were identified by UPLC-QTOF/MS. A total of 20 secondary metabolites were identified from the acetone extract in both negative and positive ionization modes, and 18 were tentatively identified in ethanol extract between the retention time of 6.28 and 17.17 min. The major classes of compounds identified were terpenoids, alkaloids, flavonoids, terpenes, phenols, esters, glycosides, lipids, and toluene. The findings highlighted that the leaves extract of *V. abbreviate* could be used as one of the ethnoveterinary medicine.

5.1 Introduction

Ethnoveterinary medicine is easier to prepare and administer at little or no cost to farmers (Iqbal et al., 2005). Moreover, due to the lack of access to a modern veterinarian in rural areas and the high cost of modern medicines, farmers have been relying on traditional veterinary practitioners to treat livestock ailments which is rooted in their customs which they believe it's more effective than- orthodox medicine (Tanzin et al., 2010). However, there is inadequate documentation to validate the valuable knowledge of different ethnic groups that have been traditionally using plants to treat various livestock ailments (Tolossa et al., 2013). As a result, documentation of ethnoveterinary medicine from regions with a rich ethnographic and biodiversity setting would be extremely valuable. The use of traditional knowledge of ethnoveterinary medicinal plants by indigenous cultures is important for the preservation of cultural traditions and biodiversity as well as for drug development in the present and future (Sheng-Ji, 2001).

According to (Farnsworth & Morris, 1976; Olila, 1993), rural farmers in Africa still rely on traditional medicine for the primary health care of their livestock, despite the advancements seen in modern medicine. In the eastern district of Uganda, the use of medicinal plants for the treatment of cattle diseases has been documented (Ejobi et al., 2004; Tabuti et al., 2003). Some of the species documented in literature include; *Carissa edulis* whose roots are used for East coast fever, Leaves of *Leonitis nepetifolia* are used for mastitis and cramps in the udder, while the leaves of *Clerodendrum rotundifolia* are commonly used by farmers for internal parasites in goats and cattle, *Senna occidentalis* for diarrhea and worms (Neuwinger Dieter, 1994). Additionally, the same authors cited the use of *S. occidentalis* for worms in Uganda and other African countries.

V. abbreviate was used in this study as a medicinal plant. This plant was chosen based on four criteria: First, ethnopharmacological data show that the plant has been used traditionally to treat cattle diseases. Second, there is no research on these plants in Mpumalanga, South Africa, in the field of cattle disease management. The third and fourth criteria were based on the therapeutic properties of the plant materials and their availability, respectively. *V. abbreviate* **(Fig. 5.1)** was chosen for further phytochemical analysis based on its relatively higher antimicrobial activity and plant material availability.

Figure 5. 1: *Vernonia abbreviata* DC. *(Erasto et al., 2007).*

V. abbreviate is probably the most widely used plant in tropical Africa for both food and medicine. It is a small tree that grows between 1 and 3 meters tall. The plant is well-known for its astringent bitter flavour, which is attributed to non-nutritional phytochemical constituents (Igile, Oleszek, Burda, & Jurzysta, 1995). Despite its bitter taste, *V. abbreviata* is cultivated as a vegetable in West and Central Africa (Ajebesone & Aina, 2004; Igile et al., 1995). The plant is used to treat malaria, diabetes, diarrhoea, venereal disease, hepatitis, gastrointestinal problems, skin diseases, and wounds in addition to its use as a vegetable (Erasto et al., 2007). Several phytochemical studies on *V. abbreviata* have been conducted due to their medicinal and nutritional uses. Nutritional analysis revealed a high concentration of crude protein, fibre, vitamins A and C, minerals, and resin (Ajebesone & Aina, 2004; Igile et al., 1995). Flavonoids, sesquiterpene lactones, and steroidal saponins are among the phytochemicals reported from *V. abbreviata* DC. (Igile et al., 1994; Igile et al., 1995; Jisaka, Ohigashi, Takegawa, Huffman, & Koshimizu, 1993; Koshimizu, Ohigashi, & Huffman, 1994; Ohigashi et al., 1991).

Using ultra-performance liquid chromatography (UPLC) coupled with quadrupole time of flight mass spectrometry (QTOF/MS) to investigate natural products allows the versatility to identify the fragmentation processes of metabolites, effective separation, and good sensitivity by applying newer mass spectrometry $^{\text{Elevated energy}}$ (MS^E) methods to acquire MS/MS (without specific precursor ion selection) data at both low and high energy from a single injection (Li et al., 2016). Not only is UPLC-QTOF-MS^E vital in metabolomics, but it is also a very crucial technique. However, the metabolite profiling of *V. abbreviata* remains not fully clarified and unclassified, despite its active medicinal value in treating and managing cattle ailments. Moreover, because of the rapid socioeconomic and cultural changes that are occurring across traditional community, documentation of indigenous knowledge and evaluation of the use of plants for a variety of purposes is becoming increasingly important, not only to retain it but also to keep it alive and available for future use. Keeping this in view, the present study was initiated to profile secondary metabolites from the *V. abbreviata* with known biological activities, using acetone and ethanol leaf extracts using the UPLC-QTOF/MS systems.

5.2 Material and methods

5.2.1 Plant collection, identification, and extraction

Fresh plant leaves of *V. abbreviate* were collected in May 2021 from the White River Township of the Mpumalanga province, South Africa. Plant specimens were taxonomically identified and voucher specimens (ATM 027) of *V. abbreviata* were prepared and authenticated through the South African National Biodiversity Institute (SANBI) herbarium in Pretoria. Leaves of *V. abbreviata* were removed from the entire plant and cleaned off debris by washing with tap water followed by distilled water. Furthermore, the leaves were oven dried at 40°C for 24 hours. Pulverization of the leaves into a homogenous powder was done using a sterile electric blender (Commercial Blender type GB27, Hamilton Beach Brands, Inc. China). The powdered sample was then stored in airtight containers to prevent contamination.

To identify the active phytocompounds from *V. abbreviate*, the leaves were sequentially extracted with acetone and ethanol as described by (Redfern et al., 2014) using Soxhlet extraction technique. The choice of solvents used was based on their availability. Approximately 50 g of the powdered leaves was extracted separately in 300 ml of 70% ethanol and acetone (99.99%) on an orbital shaker (Labcon laboratory service [Pty], South Africa) for 24 hours. The extracts were thereafter filtered using through vacuum using a Whatman No. 1 filter paper, and the filtrate was concentrated to dryness using a rotary evaporator (Heidolph Laborata 4000, Heidolph instruments, GmbH and Co, Germany) at 40°C (Otang et al., 2012). Finally, the extracts were dried and stored at room temperature until further taken for phytochemical analysis.

5.2.2 UPLC-Q-TOF-MS Profiling

A 0.22-µm polytetrafluoroethylene filter was used to filter the supernatants. A Quadrupole 120 time-of-flight (QTOF) mass spectrometer UPLC-QTOF/MS (Waters, Milford, MA, USA) was used to identify and quantify predominant secondary metabolites. An ACQUITY UPLC BEH C18 column (2.1 × 100 mm i.d., 1.7 × 10−6 m; Waters) was used for all analyses. The mobile phase was composed of acetonitrile (A) and 0.1% formic acid, v/v (B), with the following gradient elution: 0–8 min, 95–80% A; 8–12 min, 80–70% A; 12–15 min, 70–65 A; 15–18 min, 65% A; 18–21 min, 65–20% A; 21–23 min, 20–5% A; 23–24 min, 5% A; 25–30 min, 95% A. The flow rate of the mobile phase was 0.4 mL/min and the temperatures of the column and auto sampler were maintained at 30 °C and 10°C, respectively. Data were analysed in both negative and positive ionization modes. Data were processed using MSDIAL and MSFINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan) (Liu et al., 2018; Tsugawa et al., 2015). Functions 1 (unfragmented channel) and 2 (fragmented channel) of the Waters MSe data were processed by MSDial to produce MS1 and MS2 spectra as well as extracted ion chromatograms with associated peak height intensity data. Since calibration standards are not available for most of these compounds, the peak height intensity was converted to concentration in a semi-quantitative manner by interpolation of a calibration curve for catechin acquired under the same instrumental conditions. Each deconvoluted feature (alignment in MSDial), together with its associated MS1 and MS spectra was exported from MSDial to MSFinder. Based on the accurate mass elemental compositions, possible compounds were identified from the listed databases and then subjected to in-silico fragmentation. According to the spectral match between the in-silico and measured spectra, a score (out of 10) is assigned to each of the possible compound matches with the highest score being accepted as the most likely (assuming a score of at least 4).

5.2.3 Data Analysis

Using the Markerlynx V4.1., alignment and peak detection and raw data filtering were conducted. A mass range of 100–1000 Da, 5–21 min retention time as well as 50 mDa tolerance time were used as parameters. In addition, 0.4 min retention time tolerance, a 500-intensity threshold/ counts of collection parameters, and a noise elimination level of 1.00 were all set. SIMCA P+ (13.0) software (Umetrics, Umeå, Sweden) was used to determine *m/z* data pair and retention time for each peak.

5.3 Results and discussion

5.3.1 UHPLC-QTOF-MS identification and characterization of the bioactive metabolites

A total of 18 secondary metabolites were identified in the *V. abbreviata* ethanol extracts between the retention time limit of 6.28 and 17.17 min (Table 5.1). While a total of 20 metabolites were also identified between 4.03 and 15.33 min in acetone plant extracts (Table 5.2) of *V. abbreviata* in both negative and positive ionization modes. Compounds were tentatively identified according to their retention time, molecular formula, and fragment ions compared with literature data. The chromatograms displaying the peaks in Tables 5.1 and 5.2 are shown in Fig 5.1. The results from the ethanol leaf extract identified secondary metabolites such as terpenoids (11.75%), Lignans (29.41%), Sitosterol (5.88%), Cinnamic acid (5.88%), terpenes (5.88%), phenols (11.75%), Glycosides (17.65%) and electrochemical transporter (5.88%). The acetone leaf extract consisted of Terpenoid (6.25%), alkaloids (12.5%), flavonoids (6.25%), sulfones (6.25%), terpenes (12.5), Phenols (12.5%), Pyrrolidines (6.25%), Esthers (6.25%), Alcohol (6.25%), Glycosides (6.25%), Naphthalenes (6.25%), Benzoquinones (6.25%) and Toluenes (6.25%). The main reason behind using ethanol and acetone extracts is that acetone is very potent and can dissolve both organic and inorganic substances. This is due to its ability to evaporate and dissolve quickly and is a safe and effective extractant (Eloff, 1998). With its capacity to extract phenolic compounds, ethanol appears to be the optimum solvent for ultrasonic extraction of compounds with antibacterial activity from olive leaves. Hence, the current study found that acetone showed more effect and produced more metabolites than ethanol (Padmalochana & Rajan, 2014).

5.3.2 Major classes of metabolites identified

5.3.2.1 Terpenoids

The terpenoid identified in the acetone leaf extract of the current study is 1,1,6-Trimethyl-1,2-dihydronaphthalene, 1,6-O, O-Diacetyl-britannilsactone and, 12-Ursene-3,28-diol (peak 4, 7 and 12 respectively) (Table 5.1). Whilst in the ethanol leaf extract of *V. abbreviata* peak 2, 4, 8, 9, and 13 of Borneyl ferulate, Denudatin B, Hypoglaucine A, (-)-Istanbulin A, and (+)-Bakuchiol respectively were identified. Terpenoids (also called "isoprenoids") constitute one of the largest families of natural products accounting for more than 40,000 individual compounds of both primary and secondary metabolites (Goto et al., 2010). Most of them are of plant origin, and hundreds of new structures are reported every year (Sacchettini & Poulter, 1997). Peak 12 was identified as 12-Ursene-3, 28-diol with an iron mass of 442.4 m/z at 10.25 min on the acetone extract. Al-Warhi et al. (2022) reported the compound 12-Ursene-3, 28-diol on the wound-healing potential of *Olea europaea* L. Cv. Arbequina leaves extract. Some terpenoids play a role in the interaction between plants and the environment, such as participating in plant defence systems in the form of phytoalexins and interspecies competition as interspecific sensing compounds (Yang et al., 2020).

5.3.2.2 Alkaloids

Alkaloids are widely distributed in higher plants belonging to Apocynaceae, Ranunculaceae, Papaveraceae, Solanaceae, and Rutaceae occurring in Africa (Roberts, 2013). Alkaloids display an array of pharmacological effects and are found in various traditional medications used to treat livestock diseases, as recreational drugs, or in entheogenic rituals (Kuete, 2014). Peak 13 (Table 5.2) from the acetone leaf extract of *V. abbreviate* was identified as 13, 14-Dehydrosophoridine with the mass of the charge parent 246.2 m/z at 12.57 min. According to Wang et al. (2020) compound, 13, 14-Dehydrosophoridine is not only an excellent sand-fixing plant but also an important medicinal plant resource. Ji et al. (2022) reported the potential usefulness of 13, 14-Dehydrosophoridine on pharmacological effects such as plants of *Artemisia* used as the traditional Chinese medicine "Kushen" for the treatment of livestock diseases.

5.3.2.3 Flavonoids

As depicted in Table 5.2, UHPLC-QTOF-MS analysis allowed for the detection and identification of two quercetin derivatives such as (S)-4-Methoxy-dalbergione (peak 2) and Catechin-(4α→8)-catechin (peak 19) in acetone leaf extracts of *V. abbreviata*. The compound (+)-Catechin-pentaacetate peak 14 is the only flavonoid identified in the ethanol leaf extract. Many studies have demonstrated the antioxidant actions of flavonoids on animal health and different mechanisms are known by which flavonoids can directly or indirectly exert antioxidant action (Juc et al., 2020). The chemical constituents from the heartwood of the *Dalbergia cultrata* plant identified (S)-4-Methoxy-dalbergione with the potential to cure livestock diseases such as diarrhoea (Aye et al., 2019). Catechin-pentaacetate has been highlighted by various studies of its important role in protecting against degenerative livestock diseases as well as its inverse reaction between its intake and the risk of cardiovascular diseases, particularly in cattle (Ide et al., 2018).

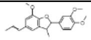
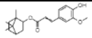
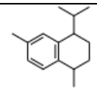
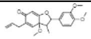
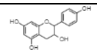
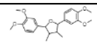
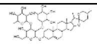
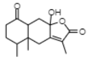
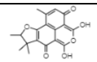
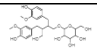
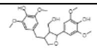
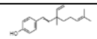
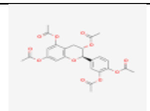
5.3.2.4 Glycosides

The current study discovered glycosides such as Acuminatin, (-)-Secoisolariciresinol-4-O-β-D-glucopyranoside, (+)-Pinoresinol-β-D-glucopyranoside, and (+)-Suspensaside A [peak 1, 11, 16, and 17], with ion mass of 340.2, 524.2, 730.2, and 622.2 m/z, at 6.28, 8.95, 12.87, and 15.81 min (respectively) from ethanol leaf extracts of *V. abbreviata* which are widely used in traditional medicine to treat various livestock diseases. In acetone leaf extract, peak 15 and 20 were identified as Actinidioionoside and Chikusetsusaponin IV, with ion mass 406.2 and 926.5 m/z, at 13.39 and 15.33 min (respectively). According to Minic (2008) the majority of these plants glycoside are involved in cell wall polysaccharides as well as participation in the biosynthesis and remodulation of glycans, mobilization of energy, defence, symbiosis, signalling, secondary plant metabolism and metabolism of glycolipids. Acuminatin was identified in the leaves of *Magnolia Ovata* (A.St.-Hil.) Spreng. which is a medicinal plant that is widely distributed in Brazil (Barros et al., 2009).

5.3.2.5 Phenolics

The benefits that phenolic compounds have on medicinal plants is that they improve their beneficial healing effects (Djeridane et al., 2007). The current study discovered phenols such as (+)-Syringaresinol [peak 18], with mass of 418.2 m/z, at 15.87 min from ethanol leaf extract of *V. abbreviata* which are widely used by farmers in traditional medicine to treat various cattle ailments. From the acetone extract, compounds identified included 1,4-Dihydroxy-2-methyl-anthraquinone and 1,7-Bis(4-hydroxyphenyl)-hepta-4E,6E-dien-3-one [Peak 6 and 8] with a mass of 254.1 and 294,1 m/z, at 7.11 and 8.00 min, and was also found to have potential medicinal properties in plants such as *Catharanthus roseus* (Mbaveng et al., 2014).

Table 5. 1: Tentative identification of 20 secondary metabolites in Ethanol extracts of *V. abbreviata* DC.

Peak No	Component name	Ontology	Observed RT (min)	Mass error (ppm)	Chemical formula	Chemical structure	Observed neutral Mass (Da)	Es Mode	Adducts	Product ion m/z
1	-Acuminatin	Glycosides	6,28	-7,4	$C_{21}H_{24}O_4$		6	-	+Na	155.47, 169.87, 177.07, 191.47 195.15
2	-Borneyl ferulate	Terpenoids	7,10	-2,7	$C_{20}H_{26}O_4$		330,2	-	+H	30.43, 143.87, 153.53, 162.51,222.96,300.23
3	-Calamenene	Terpenes	7,16	8,4	$C_{15}H_{22}$		202,2	-	+H	176.49, 181.36, 182.68, 185.89, 194.13, 200.36
4	-Denudatin B	Terpenoids	7,28	-5,1	$C_{21}H_{24}O_5$		356,2	-	+H	145.64, 145.90, 153.10, 192.36,
6	(-)- epi-Afzelechin	flavonoid	7,29	0,4	$C_{15}H_{14}O_5$		274,1	-	+H	141.40, 196.60, 175.27, 189.31, 184.29, 192.83
7	Galbelgin	Lignans	7,31	-6,3	$C_{22}H_{28}O_5$		372,2	-	+K	223.88, 227.55, 377.33
8	Hypoglaucine A	Terpenoids	7,74	-9,6	$C_{46}H_{74}O_{15}$		868,5	-	+H	116.47, 121.34, 122.43, 125.01, 133.81 134.90 221.53 385.43 456.20 463.40 477.39 480.70 488.48 489.24
9	(-)-Istanbulin A	Terpenoid	8,18	5,9	$C_{15}H_{20}O_4$		264,1	-	+H, +K, +Na, +NH4	199.4, 203.56, 228.85, 283.32,
10	(-)Sclerodin	Polyketides	8,54	1,6	$C_{18}H_{16}O_6$		328,1	-	+Na	_
11	(-)-Secoisolariciresinol-4-O-β-D-glucopyranoside	Glycosides	8,95	-8,3	$C_{32}H_{46}O_{16}$		524,2	-	+K	468.51, 480.98, 484.87,512
12	(+)-5,5′-Dimethoxylariciresinol	Lignans	10,72	4,2	$C_{22}H_{28}O_8$		420,2	+	+CH3COO	_
13	(+)-Bakuchiol	Terpenoids	10,74	9,5	$C_{18}H_{24}O$		256,2	+	+CH3COO	180.40, 187.60, 182.73, 187.60, 191.27, 194.80, 212.87,
14	(+)-Catechin-pentaacetate	Flavonoids	11,23	-8,8	$C_{25}H_{24}O_{11}$		500,1	+	-H	466.38, 470.27, 474.16, 482.74, 504.31, 554.60

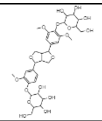
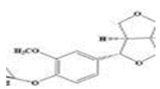
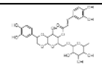
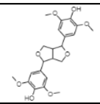
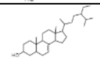
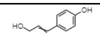
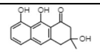
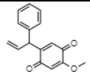
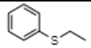
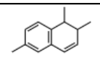
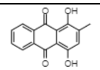
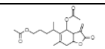
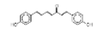
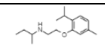
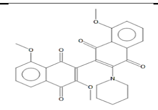
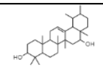
Peak No	Component name	Ontology	Observed RT (min)	Mass error (ppm)	Chemical formula	Chemical structure	Observed neutral Mass (Da)	Es Mode	Adducts	Product ion m/z
15	(+)-Medioresinol di-O-β-D-glucopyranoside	Lignans	11,26	5,2	$C_{33}H_{44}O_{17}$		712,3	+	+CH3COO	_
16	(+)-Pinoresinol-β-D-glucopyranoside	Glycoside	12,87	3,1	$C_{32}H_{42}O_{16}$		730,2	+	+CH3COO	_
17	(+)-Suspensaside A	Glycosides	15,81	5,6	$C_{29}H_{34}O_{15}$		622,2	+	+CH3COO	125.47, 175.83, 178.82, 192.52, 195.51, 500.12
18	(+)-Syringaresinol	Phenols	15,87	3,7	$C_{22}H_{26}O_8$		418,2	+	+HCOO	18,63 133.46, 146.62, 159.43, 189.4, 203.56, 228.75, 383.32, 394.58, 396.60, 399.59 400.44, 401.15
19	(24R)-Stigmast-7-en-3β-ol	Sterols	15,93	1,2	$C_{29}H_{50}O$		414,4	+	+CH3COO	119.16 136.31 141.18 144.65 152.83 218.84
20	11-O-p-Coumarylnepeticin	-	17,17	4,0	$C_{13}H_{19}ClN_2O_2$		588,4	+	+CH3COO	125.67, 149.86, 154.73, 158.40, 169.13, 176.33, 190.73, 193.06, 197.93, 201.60, 212.33, 375.58

Table 5. 2: Tentative identification of 20 secondary metabolites in acetone extracts of *V. abbreviata* DC.

Peak No	Component name	Ontology	RT (min)	Mass error (ppm)	Chemical formula	Chemical structure	mass (Da)	Es Mode	Adducts	Product ion m/z
1	(R)-Prechrysophanol	Lipids	4,03	5,8	$C_{15}H_{14}O_4$		258,1	-	+H	111.12,236,45
2	(S)-4-Methoxy-dalbergione	Flavonoids	4,14	5,9	$C_{16}H_{14}O_3$		254,1	-	+H	153.93 173.20 180.40 184.07
3	1-(Ethylthio)-2-methylbenzene	Toluenes	5,49	-4,4	C_9H_{12}		152,1	-	+H	136.43, 158.37, 165.57, 179.97, 201.57
4	1,1,6-Trimethyl-1,2-dihydronaphthalene	Terpenoids	7,11	8,5	$C_{13}H_{16}$		172,1	-	+H	_
5	1,1-Diethoxy-n-tetradecane	Ethers	7,11	5,4	$C_{18}H_{38}O_2$		286,3	-	+Na	92.75, 123.16, 136.69, 139.19, 203.03, 211.57, 245.25, 380.34, 394.57, 399.50, 413.68, 477.99, 485.77, 494.33
6	1,4-Dihydroxy-2-methyl-anthraquinone	Phenols	7,11	1,4	$C_{15}H_{10}O_4$		254,1	-	+H	142.85, 143.40, 148.27, 151.95, 240
7	1,6-O, O-Diacetyl-britannilsactone	Terpenoids	7,78	-0,9	$C_{19}H_{26}O_6$		350,2	-	+K	213.11, 332.69, 398.96, 413.36, 417.81, 452.15, 459.93, 541.97
8	1,7-Bis(4-hydroxyphenyl)-hepta-4E,6E-dien-3-one	Phenols	8,00	5,0	$C_{19}H_{18}O_3$		294,1	-	+Na	109.25, 133.46, 146.62, 159.43 189.47, 203.56, 228.75, 383.32 394.58, 396.60, 399.59, 400.44, 401.15
9	1-[(2E,4E)-2,4-Dodeca-dienoyl]pyrrolidine	Alkaloids	8,67	-3,6	$C_{16}H_{26}NO$		249,2	-	+H	244.41, 259.41,
10	10-Eicosanol	Fatty Alcohol	8,82	6,6	$C_{20}H_{42}O$		298,3	-	+Na	199.03, 200.36,300,12
11	11-O-p-Coumarylnepeticin	phenols	9,38	8,9	$C_{13}H_{19}ClN_2O_2$		588,4	+	+K	382.25 388.12 395.89 492.42
12	12-Ursene-3,28-diol	Terpenoids	10,25	4,8	$C_{30}H_{50}O_2$		442,4	+	+H	92.75, 123.16, 136.69, 147.72, 245.25

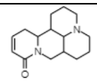
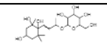
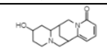
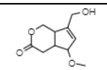
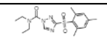
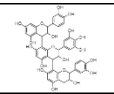
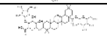
13	13,14-Dehydrosophoridine	Alkaloids	12,57	8,2	$C_{15}H_{22}ON_2$		246,2	+	+K	107.49, 205.24, 230.51,358.62,358.77371.24,383.71,396.18 516.05
14	13-Hydroxy-9,11-hexadecadienoic acid	Terpenes	12,68	4,1	$C_{16}H_{26}O_3$		268,2	+	+H	_
15	Actinidioionoside	Glycoside	13,39	2,2	$C_{19}H_{34}O_9$		406,2	+	+HCOO	300.5,111.5,200.5,189.9
16	Baptifoline	Alkaloids	13,50	-8,1	$C_{15}H_{20}N_2O_2$		260,1	+	+CH3COO	191.69, 210.26 216.18 224.72 246.31
17	Buergeriside C1	Esters	14,08	-7,6	$C_{16}H_{20}O_7$		324,1	+	+CH3COO	_
18	Cafenstrole	Sulfones	14,28	-0,9	$C_{16}H_{22}N_4O_3S$		350,1	+	+CH3COO	205.67,303,45,331.18,
19	Catechin-(4α→8)-catechin	Flavonoid	15,07	-8,0	$C_{30}H_{26}O_{11}$		578,1	+	-H	522.28,526.97,534.75,543.48
20	Chikusetsusaponin IV	Glycosides	15,33	0,9	$C_{47}H_{74}O_{18}$		926,5	+	+HCOO	99.23, 399.93, 409.06, 415.73,701.78, 899.09

Table 5. 3: A summary in percentage of bioactive chemical classes identified in acetone and ethanolic (n=18) extracts of V. abbreviata DC.

Class	***Acetone Percentage***	***Ethanol Percentage***
Terpenoid	15.79%	27.77%
Lignans	-	16.66%
Alkaloids	15.79%	-
Flavonoids	10.52%	-
Sulfones	5.26%	-
Sitosterols	-	5.55%
Terpenes	5.26%	5.55%
Phenols	10.52%	11.11%
Esters	10.52%	-
Glycosides	5.26%	22.22%
Toluenes	5.26%	-
Polyketides	-	5.55%
Fatty Alcohol	5.26%	-
Lipids	10.52%	-
Total	**100%**	**100%**

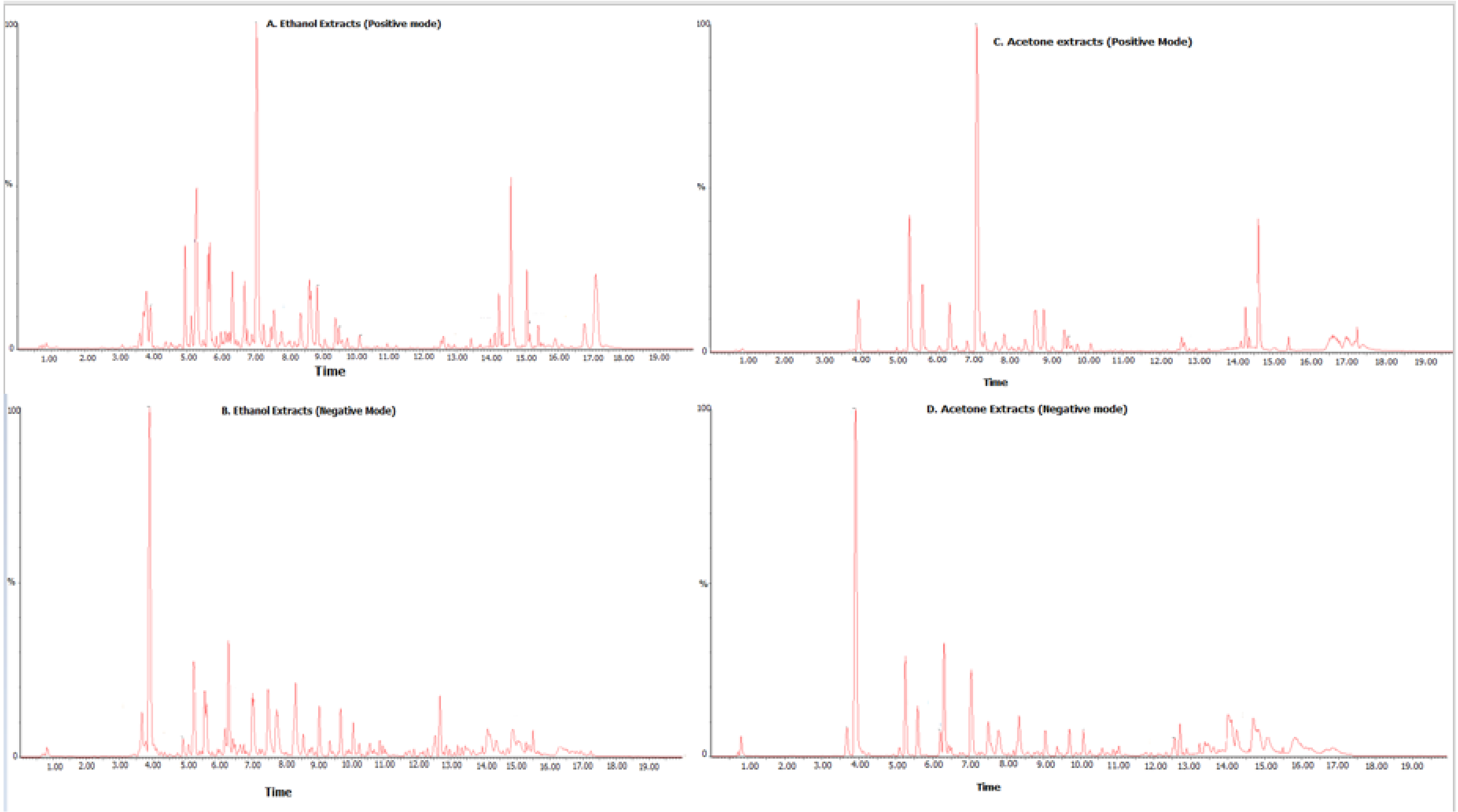


Figure 5. 2: Chromatographs of ethanol and acetone leaf extracts on positive and negative modes.

5.4 Concluding remarks

In the current study, a novel HPLC-QTOF-MS based approach was used to identify active components from acetone and ethanol extracts of *V. abbreviata*, and it proved to be a practical method for identifying fragmentation pathways of metabolites in leaf extracts, as well as being more sensitive than previously reported methods. The findings from this study revealed that *Vernonia abbreviata* acetone leaf extracts contain more bioactive components than ethanol leaf extracts. Major compounds such as flavonoids, terpenoids and alkaloids were extracted and identified from *Vernonia abbreviata* were reported to contain properties for treating and managing various cattle ailments such as diarrhoea, poor eyesight, gastro-intestinal parasites and maggot infestation. Nevertheless, more in vitro studies are needed to validate its efficiency.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

Several scholars have indicated that many livestock owners in rural areas are faced with relatively few veterinarians and shortages of other facilities, and traditional medicinal plants are the only choice to treat many ailments. There are estimated to be 250 000 plant species in the world, and only 5-15% of these species have been tested for potentially useful biologically active compounds (Pieters & Vlietinck, 2005). However, this is not researched well because of lack of awareness on the reliance and importance of medicinal plants usage by subsistence farmers.

6.2 Highlights of the research findings

The study reported a lesser number of females compared to males who were knowledgeable about EVM in the Nkomazi Local Municipality, this is due to that male were often responsible for the welfare of the cattle. Which limits the prominence of the knowledge and application of the practice. The older generation of the participants had more knowledge compared to the youth. As such, fewer participants with EVM knowledge will remain unless the younger population is willing to learn. The men from NLM who were knowledgeable about EVM confirmed that the medicinal plants used to treat cattle diseases contributed to their socio-economic status within their villages since the veterinary facilities and services were far. Based on the results from participants, it is evident that some of the NLM men are still largely reliant on medicinal plants for the wellbeing of their cattle in various villages in Nkomazi Local Municipality district municipality. However, some participants confirmed that medicinal plants did not contribute to their welfare.

The current study documented twenty-eight (28) plant species as useful in traditionally treating and managing different cattle ailments. These medicinal plants were distributed among 19 families. On the medicinal plants' diversity, the study reported that Fabaceae family was one of the plant families consisting of several plant species used in EVM amongst different indigenous communities in Mpumalanga. Moreover, 18 cattle diseases were classified into six categories in the study area. Dermatological diseases, such as diarrhoea, bloating, intestinal worms, and allergic reactions, scored the highest Informant consensus factor (ICF) value with the selected villages showing that they were largely affected by maggot infestation.

The plant part distribution used in this study to treat cattle diseases revealed that the most frequently used plant part was leaves, followed by bark. This was due to the easy accessibility of the leaves, secondly, it is simple to process because of its high-water content (between 70% and

80%) and soft texture. The study also explored the methods of preparation and administration which reported that poultice was the commonly preferred method of preparation with the medicine mostly administered topically. The identified metabolites included compound classes such as terpenoids which were highest in the ethanol extracts (27, 77%) and alkaloids and terpenoids were highest in the acetone extracts (15.79%). The results showed significant chemical differences between both extracts of the plant in the profiles. *V. abbreviata* extracts were reported to possess compounds capable of providing healing abilities to various cattle ailments.

6.3 Recommendations from the study

These results can serve as inputs for the evidence-based policy interventions to promote the use of medicinal plants by subsistence farmer to sustain their livelihoods and radical socio-economic transformation, particularly in the rural areas of Nkomazi Local Municipality, in Mpumalanga Province. The NLM men who were Knowledgeable about the skills and practices of EVM should be recognized by local authorities to be provided with full access to harvest and utilize the plants to sustain their livelihoods. Awareness programmes to educate the community about the importance of the various medicinal plants used to treat cattle diseases. This will help keep the knowledge alive within the communities and households for generations to come. Based on the outcomes of this study, the following should be considered in the future;

- There is need to further document the ethnoveterinary findings from NLM before the knowledge disappears either because of endangerment of the plant species in the wild or because of loss of knowledge among the new generation of the ethnoveterinary practitioners.
- There is need to conduct agronomic research into growing medicinal plant gardens, especially on those extensively used in the study area and other parts, this could reduce pressure on plant populations that remain in the wild.
- Introduction of medicinal plants in degraded government and common lands in the municipality could be another option for promoting the rural economy together with environmental conservation that has not received attention in the land rehabilitation programs in this district.
- Efficacy of documented medicinal plants against the most prevalent cattle diseases should be evaluated, to recommend effective preparations and treatments to subsistence farmers.
- Proper ethno-diagnosis of cattle diseases/ill-health conditions are required with a lot of experience and expertise greatly based on the knowledge of the disease causes, symptoms

and signs, knowledge of known vectors, history of the environment and seasonality of disease outbreaks in addition to the knowledge of medicinal plant species used in treatment.

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APPENDICES

Appendix 1: Questionnaire

Questionnaire number: …....
Please Fill and Tick (√) in the appropriate place where necessary.

SECTION (A): SOCIO-ECONOMIC CHARACTERISTICS OF PARTICIPANTS

(A1) District and ward...

(A2) Type of location

Village	0	Township	1	Urban area	2

(A3) Age of the participant (Years):...

(A4) Gender of the participant

Male	0
Female	1

(A5) Marital Status

(0) Married	(1) Single	(2) Divorced	(3) Widowed	(4) Other

(A 6) Educational status

(0) Informal	(1) Formal	(2) Standard	(3) Matric	(4) Diploma	(5) Degree	(6) Other

(A7) Race

(0) Black	(1) White	(2) Coloured	(3) Indian	(4) Others

(A8) Religion

(0) Christianity	(1) Islam	(2) Traditionalist	(3) Others

(A9) Household size

(0) Less than 5	(1) More than 5

(A10) Type of practice:

(0) Diviner	(1) Herbalist	(2) Herbalist & diviner	(3) Herbal vendor	(4) TBA

(A11) Source of ethnobotanical knowledge:

(0) Parents	(1) Grandparents	(2) Mentor	(3) Ancestors	(4) Dream

(A12) Number of year(s) of experienced in ethnobotanical practice...

(A 13) Do you have any other source of income?

(0) No	(1) Yes

Objective 1: Cattle diseases treated by subsistence farmers in Nkomazi Local Municipality, Mpumalanga province, South Africa

In the table below, please tick the diseases which you frequently treat.

		Cattle's gender		Cattle age	
Categories of cattle diseases	**Local name**	**Male**	**Female**	**Mature**	**immature**
Skin diseases/conditions					
1. Ringworms					
2. Lumpy skin disease					
3. Rain scald					
4. Warts					
5. Skin parasites					
6. Cow pox					
7. Hair loss/fading					
8. Lumpy jaw					
Respiratory related diseases					
1. Cough					
2. Bovine Respiratory Disease Complex					
3. Malignant Catarrhal fever					
Gastro-intestinal related diseases					
1. Heart water					
2. Bloat					
3. Intestinal worms					
Urinary genital disorders					
1. Urolithiasis					

2. Poor milk let down					
3. Retained placenta					
Oral related diseases					
1. Food and Mouth disease					
2. Wounds					
3. Snake bite					
4. Ulcerations in the mouth					
Other common cattle diseases and wellbeing					
1. Dystocia					
2. Infertility in bulls					
3. Ticks					
Pain and inflammation					
Mastitis					
Pneumonia					
Calf Diarrhoea					
Dehorning					
Eye problems 'silage eye' and 'New Forest eye"					

Willshire J A, Bell, N J (2009) An Economic Review of Cattle Lameness. Cattle Practice 2009 Vol. 17 No. 2 pp. 136-141

Objective 2: Ethnobotanical survey of medicinal plants used for the treatment of cattles diseases in Nkomazi Local Municipality Mpumalanga province, South Africa

	Plant	**Disease**	**Local name**	**Part used** ((Leaves=0), (Stem=1), (Bark =2), (Seeds=5), (Roots=3), (Flower=4) (Whole plant =6) (Rhizome/bulb=7)	**Preparation** (Maceration = 0); Poultice=1; Decoction 2. Enema=3; infusion=4	**Administration** (Topical=0), (Orally=1), (Bathing=2), (Enema=4)	**Dosage** (0=1x/day), (1=1-3x/day), (2=As needed and 4= other	**Abundance** (Common=0), (Rare=1), (Abundant=2)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								

Objective 3: Indigenous knowledge and practices of high utilized medicinal plants used for cattle diseases among the subsistence farmers.

(3.1) Where do you often harvest this medicinal plant used for cattle diseases?

(0) Home	(1) Backyard	(2) Forest	(3) Wild

(3.2) Is there sufficient awareness regarding environmental regulations concerning collection/harvesting of certain medicinal plants among subsistence farmers? --

----

(3.3) Do you have any knowledge about Red data/protected plant species or permit for plant collection? ------------------------------

----

(3.4) Have you experience a decline in any of the medicinal plants that you prescribed?--

----

(3.5) Which challenges often occur when dealing or using with medicinal plants used for treatment and management of cattle diseases?

(3.6) What indigenous methods do you apply to conserve medicinal plants used for managing cattle diseases?------------------------

----

Appendix 2: South African National Biodiversity Institute field label for plant collection.

Collector: .... No.: .... Date: ....

Provisional name:

Region: Grid: lt: m

GPS ° ' S ° ' E

Locality

Biome Fynbos Grassland Savanna Nama Karoo Succulent karoo
Forest Indian Ocean Coastal Belt Albany Thicket Desert Azonal

Vegetation type

Habitat mountain peak mountain slope hilltop hill slope ridge cliff face ravine/kloof/gorge
talus/scree plateau valley floodplain waterfall river/stream bank river/stream
dry streambed donga/gulley/ditch pan depression marsh swamp wetland
seepage dune (desert) dune (coastal) estuary littoral lagoon sea
lake dam pond plain other:

Substrate soil stony soil rocky soil gravel bare rock in water termite mound
bark leaf leaf litter roots other:

Soil type gravel sand loam black turf humus clay salt/brack baserock

Lithology sandstone shale granite quartzite calcrete dolomite dolerite

Moisture regime well-drained seasonally waterlogged free standing water tidal mist/fog
moist/damp permanently waterlogged running water other:

Exposure shade partial shade full sun Slope none gentle

Aspect N S W E NE NW SE SW moderate steep

Biotic effect abandoned land cultivated land pasture recently burned garden roadside
plantation grazed disturbed none seen other:

Life form tree shrub dwarf shrub herb graminoid geophyte epiphyte
climber parasite succulent hydrophyte bryophyte lichen scrambler
saprophyte lithophyte other:

Plant features (underground parts, bark, leaves, flowers, fruit, seeds, aroma)

Flowers: present absent Fruit: present absent Plant height: m

Notes (local abundance, phenology, pollinators, herbivory, economic & ethnobotanical factors, voucher specimen)

Permit Number: Issuing authority:

Voucher: photo ecology cytology anatomy seed spirit

Plant name: ....

....

Genspec: .... / .... Det.: .... Date: .... No. of labels: ....

Appendix 3: Consent form

I ___________ hereby willingly agree to contribute to the following research: **Medicinal plants used by subsistence farmers for the treatment of cattle diseases in Nkomazi local Municipality, Mpumalanga, South Africa.** I have been fully briefed about the research and the steps to be followed when participating in the research have been explained to me fully. I am aware that I should only agree to participate in this study only if I have information about the use of different medicinal plants to treat livestock diseases. I understand that I must answer questions relating medicinal plants used to treat livestock diseases in Nkomazi Local Municipality. I know that I am not forced to be part of this research and that I can withdraw at any time. I also understand that I will not receive any payment after participating in this research. With the understanding of the above information, I am willing to contribute to this research.

Informant signature……………………………….

Appendix 4: Ethical clearance

UNIVERSITY OF MPUMALANGA

Creating Opportunities

FESC/SITHOLE/001/2022

Faculty of Agriculture and Natural Sciences

Faculty Ethics Committee

Date: 05 August 2022

Dear Miss Antoinnette T. Mbebe

Student no. 201602849

RESEARCH PROJECT ETHICAL CLEARANCE CERTIFICATE – FESC/MBEBE/0002

The Faculty of Agriculture and Natural Sciences Ethics Sub-Committee received and reviewed your research proposal entitles: **Medicinal plants used by subsistence farmers for the treatment of cattle diseases in Nkomazi local municipality, Mpumalanga, South African**. In your objectives and methodology, the research shall involve interviewing and an interaction with people during collection of data. In section 3.5 of the proposal, you indicated ethical awareness and requirements for the proposal, which are consent, confidentiality, and anonymity. In addition to these, we request that the researcher treat participants with respect and kindness, and uphold ethics principles of the University of Mpumalanga.

With respect to the above assessment, the current proposal is grant ethical clearance by UMP Faculty of Agriculture and Natural Sciences Ethics Sub-Committee. This ethical clearance certificate expires on the 31st of December 2022.

For any further enquire regarding the matter, never hesitate to contact me.

Please Note

Any alteration/s to the approved research protocol i.e. Questionnaire/Interviews Schedule, Informed Consent form, Title of the project, Location of the study, Research Approach and methods must be reviewed and approved through the amendment/ modification prior to its implementation.

Appendix 5: Student Declaration upon Submission

UNIVERSITY OF MPUMALANGA

FACULTY OF AGRICULTURE AND NATURAL SCIENCES

Student Declaration upon Submission of a Dissertation/ Treatise/Thesis for Examination

[This form accompanies the Dissertation/ Treatise/Thesis]

1	Student Name:	Mbebe Antoinnette Tracy
2	Student Number:	201602849
3	School & Faculty	School of Agricultural Sciences Faculty of Agriculture and Natural Sciences
4	Degree Registered for:	Master of Science degree in Agriculture
5	Date of First Registration:	February, 2021
6	Approved & Registered Title:	Medicinal plants used by subsistence farmers for the treatment of cattle diseases in Nkomazi Local Municipality, Mpumalanga, South Africa.
7	Full-time/Part-time:	Full-time
8	Supervisor/Promoter:	Prof. W Otang Mbeng
9	Co-supervisor(s)/Co-promoter(s) (if applicable):	Dr. Terrence Suinyuy
10	**About the Submission:**	
	Does it comply with the academic rules, technical requirements, and policy on ethics in research as provided for in the University and Faculty rules?	Yes

	Has a satisfactory plagiarism report been obtained? Please attach the summative pages of the report.	
13	Has clearance been obtained from the relevant Ethics Committee? If, yes, please attach the Clearance Certificate. If, no, please give reasons.	Yes

Student's Signature: **Date: 01 March 2023**