Indigenous Knowledge on the uses and factors that determine the use of *Strychnos spinosa* Lam. found in Bushbuckridge, Mpumalanga, South Africa

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Dissertation submitted in fulfilment of the requirement for the degree Master of Science at the University of Mpumalanga

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DECLARATION

I declare that the dissertation titled "Indigenous Knowledge on the uses and factors that determine the use of *Strychnos spinosa* Lam. found in Bushbuckridge, Mpumalanga, South Africa", was conducted at the University of Mpumalanga under the joint supervision of Dr Tshepiso Ndhlovu, Dr Elizabeth Kola and Dr Mwazvita Dalu. I further declare that the study is my original research efforts and has not been submitted for a degree or diploma at any other institution. All references to work conducted by others or materials obtained from external sources, have been appropriately cited and referenced.

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DEDICATION

To the young woman who dares to dream of having it all! I dedicate this study to every young girl, running her race at her own pace, towards a life of purpose. To every woman navigating life's many roles, this is an ode to your dreams forever being valid. To Milani and Leilani.

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ABSTRACT

In sub-Saharan Africa, Indigenous knowledge (IK) is a vital source of information about the local environment, including the identification and use of various plant species. One of the plant species that has attracted much attention in the area is *Strychnos spinosa* Lam. (*S. spinosa*), commonly known in English as 'spiny monkey orange' or 'umKwakwa' in isiZulu and isiXhosa, and used by local communities for medicinal, nutritional, and cultural purposes. The aim of this study was to document the ethnobotanical uses of *Strychnos spinosa* among selected communities in Bushbuckridge, Mpumalanga Province, South Africa, and to identify the taxonomic identity of market-sold *Strychnos* products using DNA barcoding. This study investigates the ethnobotanical uses and taxonomic authenticity of *S. spinosa* in Bushbuckridge, Mpumalanga Province, South Africa. An ethnobotanical survey was conducted through in-depth interviews with 108 participants selected using purposive and snowball sampling techniques across six settlements in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa. The sites were selected based on their high reliance on indigenous plant resources and the presence of active informal medicinal plant markets, while participants were selected for their knowledge and experience in use of *S. spinosa*. Through qualitative and quantitative analyses, the research revealed the diverse uses of the plant, including its nutritional, medicinal and cultural significance. The plant's average Use Value (UV) and Relative Frequency of Citation (RFC) were determined and found to be 1.9 and 0.4, respectively. The findings revealed that 93% of participants depended on wild harvesting, while concerns about its declining availability due to urbanisation, agriculture and climate change were commonly reported. Market participation was high, with 99% of respondents selling *S. spinosa* products. However, conservation efforts were deemed inadequate, with 46% of participants pointing out lack of formal protective measures. DNA barcoding was used to verify the authenticity of fruit, seed and powdered samples of *Strychnos spp*. sourced from Bushbuckridge Local Municipality, Mpumalanga Province, South Africa. DNA extraction and amplification were performed using two plastid markers, *rbcLa* and *matK*, followed by Sanger sequencing. Sequences obtained were aligned and analysed using BLAST against GenBank. DNA barcoding results revealed that none of the analysed market samples matched *S. spinosa*. Instead, samples showed a high degree of sequence identity (≥99% similarity) with other *Strychnos* species, particularly *S. madagascariensis, S. innocua,* and *S. xanthoclada*. Results confirm species identity through DNA barcoding analysis and suggest possible misidentification or substitution within local markets, despite the ethnobotanical focus on *S. spinosa*. The results confirm that molecular identification is feasible even for degraded and processed material. The phylogenetic placement of all Bushbuckridge Local Municipality samples within a well-supported *S. madagascariensis* clade further validated BLAST identifications and showed no evidence of adulteration or substitution. Collectively, the study underscores the importance of integrating Indigenous Knowledge with molecular techniques to enhance the safety of medicinal plants, conserve biodiversity, and inform evidence-based policies and regulations aligned with the Nagoya Protocol and South African biodiversity frameworks. This work provides novel molecular data on southern African *Strychnos*, supports pharmacovigilance in traditional medicine markets. This study also provides evidence-based insights into the mismatch between ethnobotanical knowledge and molecular identification, revealing that market-sold products may not correspond to *S. spinosa*. This highlights potential misidentification or substitution within local medicinal plant markets and underscores the critical role of DNA barcoding in ensuring species authenticity and protecting indigenous knowledge systems.

Keywords: Biodiversity conservation; Bushbuckridge Local Municipality; DNA barcoding; Ethnobotany; Indigenous knowledge; *Strychnos spinosa.*

DEFINITION OF KEY CONCEPTS

Indigenous Knowledge (IK) – Knowledge, practices, and beliefs developed by local communities through experience and passed down across generations. It reflects an intimate relationship between people and their environment and is critical for survival, resource use and cultural identity.

Indigenous Knowledge Systems (IKS) - The broader framework within which IK is situated, encompassing cultural norms, social institutions, and practices that guide how communities generate, transmit and apply indigenous knowledge. Indigenous Knowledge Systems are dynamic, adaptive, and essential for biodiversity conservation and sustainable resource management.

Traditional Ecological Knowledge (TEK) - A subset of IKS that focuses specifically on ecological processes, species and ecosystems. TEK includes the cumulative body of observations, practices, and beliefs regarding interactions between living beings and their environment.

Ethnobotany - The scientific study of relationships between people and plants, including how plants are used for food, medicine, rituals, shelter and other cultural practices. It often integrates both qualitative and quantitative approaches to capture local knowledge and practices.

Use Value (UV) - A quantitative ethnobotanical index that measures the relative importance of a plant species to a community. It is calculated based on the number of uses cited by informants and reflects the versatility and cultural significance of the species.

Relative Frequency of Citation (RFC). - An ethnobotanical index that indicates the proportion of participants who mention a particular species. RFC provides insights into the popularity and prominence of a plant within community knowledge.

DNA Barcoding - A molecular technique used to identify species by analysing short, standardised regions of DNA. It is especially valuable for authenticating medicinal plant materials, resolving taxonomic uncertainties and detecting adulteration in herbal markets.

Plastid Markers (*rbcLa* and *matK*) -Standard plant DNA barcode regions located in the chloroplast genome. The *rbcLa* (ribulose-1,5-bisphosphate carboxylase large subunit) and *matK* (maturase K) markers are widely used for identification of species in plants due to their high universality and discriminatory power.

BLAST (Basic Local Alignment Search Tool) - A bioinformatics algorithm used to compare nucleotide or protein sequences against sequence databases, such as GenBank. It provides similarity scores to help identify unknown samples by matching them with reference sequences.

GenBank - An open-access genetic sequence database maintained by the National Center for Biotechnology Information (NCBI). It serves as a reference library for DNA barcoding and other molecular studies.

Conservation Assessment and Management Plan (CAMP) - A scientific process used to evaluate the conservation status of species and recommend management actions. CAMP assessments inform Red List categorisations and guide conservation priorities.

Other Effective Area-Based Conservation Measures (OECM) - Geographically defined areas that are not formally protected areas but managed in ways that achieve long-term biodiversity conservation outcomes, while also delivering cultural, spiritual and socio-economic benefits to local communities.

Bayesian Inference - a systematic method for inferring the posterior distribution of model parameters by applying Bayes' rule, which incorporates prior and likelihood distributions.

Maximum Parsimony - the state in which a phylogenetic tree features the fewest possible number of common ancestors, evolutionary steps, and branches between the organisms it features.

PUBLICATIONS

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CONFERENCE PRESENTATIONS

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LIST OF ABBREVIATIONS AND ACRONYMS

ACDB	African Centre for DNA Barcoding
ANOVA	Analysis of Variance
BI	Bayesian Inference
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Data System
BSA	Bovine Serum Albumin
CAMP	Conservation Assessment and Management Plan
CTAB	Cetyl Trimethylammonium Bromide
DNA	Deoxyribonucleic Acid
EIA	Environmental Impact Assessment
ESIA	Environmental and Social Impact Assessment
FANS	Faculty of Agriculture and Natural Sciences
IA	Impact Assessment
IK	Indigenous Knowledge
IKS	Indigenous Knowledge Systems
IPUF	Indigenous Plant Use Forum
IUCN	International Union for Conservation of Nature
MP	Maximum Parsimony
NCBI	National Centre for Biotechnology Information
OECM	Other Effective Area-based Conservation Measures
PAUP	Phylogenetic Analysis Using Parsimony
PCR	Polymerase Chain Reaction
PP	Posterior probability
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RFC	Relative Frequency of Citation
SAAB	South African Association of Botanists
SBES	School of Biology and Environmental Sciences
SEIA	Social–Ecological Impact Assessment
SIA	Social Impact Assessment
SPSS	Statistical Package for the Social Sciences
TEK	Traditional Ecological Knowledge

UMP University of Mpumalanga

UV Use Value

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Chapter 1: General introduction

1.1 Introduction

Conservation efforts over recent decades have highlighted the challenges of managing biodiversity in human-dominated landscapes, where many species exist outside formally protected areas (Persha et al., 2010). In developing countries, communities often rely heavily on natural resources for their livelihoods, creating a complex relationship between biodiversity use and conservation (Gidebo, 2022; Vira and Kontoleon, 2010). These realities underscore the importance of locally embedded knowledge systems, particularly Indigenous Knowledge (IK), in understanding how natural resources are used and managed within communities. While conservation provides important context, this study primarily focuses on ethnobotanical knowledge and species authentication within local markets.

Indigenous Knowledge Systems (IKS) provide a detailed, experience-based understanding of local ecosystems, including the identification, use, and management of plant species (Gautam, 2024). Within many rural communities, IKS underpins the use of plants for food, medicine, and cultural practices, and is transmitted across generations (Gautam, 2024; Vira and Kontoleon, 2010). Such knowledge is particularly valuable in ethnobotanical research, as it offers insight into how plant resources are utilised and perceived within specific socio-cultural contexts. However, IK is increasingly threatened by modernisation and globalisation, which contribute to its rapid erosion and potential loss (Gómez-Baggethun, 2021). This highlights the urgency of documenting and scientifically engaging with IKS before they are lost.

Human actions, such as deforestation, cultivating on steep terrain, large and small-scale mining, overgrazing, and rapid population growth, are significantly impacting the environment, leading to unprecedented land degradation and depletion of natural resources (Gebru et al., 2019). It becomes imperative to identify and evaluate these impacts. According to Dendena and Corsi (2015), different forms of Impact Assessments (IA) have emerged from debates about human-ecosystem interactions. Environmental Impact Evaluation (EIA) is the most widely applied, while Environmental and Social Impact Assessment (ESIA), extends EIA by integrating social dimensions (Zimmermann and Neu, 2022). These frameworks highlight the need to assess not only environmental trends but also the intertwining of human well-being and natural resource use.

Strychnos spinosa Lam., commonly known as the spiny monkey orange, is an indigenous fruit tree widely distributed across sub-Saharan Africa and valued for its nutritional, medicinal and cultural significance (Omotayo and Aremu, 2021; Tittikpina et al., 2020). The species is predominantly harvested from the wild, as attempts at cultivation have yielded limited success (Leakey and Akinnifesi, 2017; Thiong'o et al., 2002), making natural populations the primary source for local communities (Omotayo and Aremu, 2021). *S. spinosa* is recognised for its high nutritional value, including essential vitamins and bioactive compounds with antioxidant and anti-inflammatory properties across the species' different plant parts (Brielmann et al., 2006; Ngadze et al., 2016; Omotayo and Aremu, 2021; Shahidi and Ambigaipalan, 2015; Sitrit et al., 2003), supporting the species' widespread use in traditional medicine and food systems (Aremu and Moyo, 2022; Ngadze et al., 2016). These attributes highlight the plant's importance in supporting local livelihoods, food security and healthcare practices.

Given the extensive reliance on *S. spinosa* for food and medicine, it becomes essential to assess not only the plant's uses but also the accuracy of its identification in local markets. Despite the species' widespread use, there is limited empirical evidence confirming whether plant materials sold as S. spinosa are taxonomically accurate. This raises concerns regarding species misidentification, substitution, and the reliability of plant-based products used by communities. Accurate identification of medicinal plant species is critical, as misidentification or substitution may compromise the safety, efficacy and reliability of plant-based products used by communities (Yu et al., 2021)

To address these challenges, molecular techniques such as DNA barcoding provide a reliable approach for species identification, particularly for processed or degraded plant materials where morphological identification is not possible. DNA barcoding has emerged as a reliable molecular tool for species authentication, particularly in cases where plant materials are processed or lack distinguishing morphological features (Hebert et al., 2003; Hollingsworth et al., 2011). By integrating ethnobotanical knowledge with molecular authentication, it becomes possible to evaluate the consistency between locally recognised plant use and scientifically verified species identity.

This study contributes to the documentation and scientific validation of IK by examining the ethnobotanical uses, harvesting practices and cultural significance of *S. spinosa* within Bushbuckridge Local Municipality communities. In addition, DNA barcoding is used to authenticate market-sold *S. spinosa* products, providing insight into species identity and potential discrepancies between traditional knowledge and traded materials. This integrated approach strengthens the reliability of ethnobotanical data and supports a more evidence-based understanding of plant use within local communities.

1.2 Problem statement

Studies by Omotayo and Aremu (2021) and van Rayne et al. (2023) have shown that lack of comprehensive understanding and documentation of indigenous knowledge of *S. spinosa* hinders the preservation and utilisation of its valuable traditional knowledge. Hoffmann (2021) also highlight that 'other effective area-based conservation measures' (OECM) can be managed and governed to achieve positive and long-lasting results for conserving biodiversity in its original location, along with the related functions and benefits of the ecosystem, and, if relevant, cultural, spiritual, socio-economic and other important values. This reinforces the need to systematically document IK associated with *S. spinosa* to inform both cultural preservation and conservation planning. Despite increasing awareness of the importance of integrating IK into biodiversity management, challenges persist in conserving species that occur in human-dominated landscapes, where land use pressures, limited formal protection and livelihood dependence can intensify threats to wild populations. These pressures underline the urgency of understanding how communities interact with, use and manage culturally important species such as *S. spinosa*. Furthermore, there is currently a lack of empirical evidence verifying whether market-sold and community-used *Strychnos* products are taxonomically authentic. The absence of such evidence raises concerns regarding product reliability, potential adulteration, loss of consumer trust and reduced market value.

1.3 Rationale and justification for the study

Despite the well-documented ethnobotanical importance of *S. spinosa,* significant gaps remain in verifying the taxonomic identity of plant materials used by communities and sold in local markets (Omotayo and Aremu, 2021; Tittikpina et al., 2020). Most existing studies rely on morphological identification and do not account for processed or traded plant materials, where distinguishing features are often absent.

The lack of molecular validation creates uncertainty regarding the authenticity of plant products, raising concerns about species misidentification, substitution, and potential risks to users (Yu et al., 2021). Furthermore, limited integration of IKS with molecular approaches restricts the ability to assess the consistency between locally recognised plant use and scientifically verified species identity. In addition, there is limited research examining harvesting practices and market dynamics associated with *S. spinosa*, particularly within localised contexts (Omotayo and Aremu, 2021), such as Bushbuckridge. Existing studies largely focus on ethnobotanical uses and phytochemical properties, with comparatively less attention given to how the species is sourced, traded and accessed within informal medicinal plant markets.

Therefore, this study is justified in its integration of ethnobotanical documentation with DNA barcoding to authenticate *Strychnos* species in local medicinal plant markets. This approach strengthens the scientific validation of IK and provides a more reliable understanding of plant use within community and trade contexts.

1.4 Aim of the study

The aim of the study was to document the ethnobotanical uses of *Strychnos spinosa* among selected communities in Bushbuckridge, Mpumalanga Province, South Africa, and to authenticate the taxonomic identity of market-sold *Strychnos* products using DNA barcoding.

1.5 Objectives of the study

The objectives of the study were to:

- Document medicinal, nutritional and cultural uses of *S. spinosa* among selected communities in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa;

- Examine prevailing harvesting practices and market dynamics associated with *S. spinosa* in Bushbuckridge Local Municipality;
- Authenticate the taxonomic identity of market-sold *Strychnos* species using DNA barcoding techniques.

1.6 Research questions

The following research questions were asked in the study:

- What are the ethnobotanical uses of *S. spinosa* among communities in Bushbuckridge Local Municipality?

- What are the prevailing harvesting practices, market dynamics and conservation perceptions surrounding *S. spinosa* in Bushbuckridge Local Municipality?

- What does DNA barcoding reveal about the authenticity and taxonomic integrity of processed *Strychnos* samples sold in Bushbuckridge Local Municipality?

1.7 Scope and limitations of the study

This study was conducted within selected areas in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa. The research focused on *S. spinosa* Lam., an indigenous fruit tree valued for its cultural, medicinal and economic roles.

To complement the ethnobotanical results, DNA barcoding (using the *rbcLa* and *matK* plastid markers) was used to verify *Strychnos* samples from herbal markets in the region due to the impracticality of correctly identifying processed market samples. This helped in assessing and identifying accuracy of species in the local medicinal plant trade, addressing issues of substitution and adulteration.

While the ethnobotanical component of this study focused specifically on *S. spinosa,* the molecular analysis included broader *Strychnos* species to account for potential species misidentification or substitution in market-sold materials.

This study focuses on ethnobotany, conservation science, molecular taxonomy and Indigenous Knowledge research, with the aim of promoting the sustainable use and preservation of *S. spinosa*, by combining community knowledge with molecular identification methods.

1.8 Ethical considerations

Ethical approval was requested and obtained from the Faculty of Agriculture and Natural Sciences (FANS) Research Ethics Committee, University of Mpumalanga (Protocol Reference Number: UMP/Thothela230351360/SBES/MSC/2024/01) before collecting data for the study. Before proceeding with the ethnobotanical survey, the researcher ensured all participants gave consent to participated in the interviews. Participants were fully informed of the objectives of the study, and expectations from both the researcher and each participant. The principle of voluntary participation and the right to withdraw from the study at any time were emphasised. Ethical principles were strictly followed, including the principle of anonymity to ensure the identity of participants remained confidential. The principle of autonomy was also adhered to, with the researcher obtaining permission from local chiefs and knowledge holders. Lastly, the principle of respect was applied, and all participants treated with respect and dignity. Participants were also encouraged to respect each other's opinions.

1.9 Outline of the study

This dissertation is divided into five chapters. Each chapter addresses a key component of the research, building towards a comprehensive understanding of the ethnobotanical importance, trade dynamics and taxonomic authenticity of *S. spinosa* in Bushbuckridge, Mpumalanga Province, South Africa.

Chapter 1- Introduction: This chapter provides the introduction and background, problem statement, objectives of the study, research questions and significance of the research. It also provides the rationale for the study and explains the research design, which is a combination of ethnobotanical documentation with molecular authentication through DNA barcoding. The chapter concludes with the scope and limitations of the study and the outline of the study.

Chapter 2 - Provides a systematic review of ethnobotanical uses of *S. spinosa*: The chapter presents a systematic review of literature from 2000 to 2025, focusing on the ethnobotanical uses of *S. spinosa* across Africa. It provides a synthesis of data on the medicinal, nutritional, and cultural applications of species, plant parts used, methods of preparation and conservation concerns. The chapter also focuses on knowledge gaps identified and sets the contextual foundation for the field and molecular studies that follow.

Chapter 3 - Ethnobotanical study of *S. spinosa* in Bushbuckridge: This chapter provides a report on the qualitative and quantitative field study conducted in Bushbuckridge, Mpumalanga Province, South Africa, using semi-structured interviews. It explores local knowledge, cultural significance, and practical uses of *S. spinosa*, including harvesting practices, market trade and intergenerational knowledge transfer. Statistical analyses are presented to examine how demographic variables (such as age, sex and education) influence plant knowledge and use.

Chapter 4 - Molecular authentication of traded *Strychnos* samples using DNA Barcoding: This chapter focuses on the presentation of findings of the molecular analysis conducted to authenticate *Strychnos* species sold in local markets. DNA was extracted from six degraded or processed herbal samples, and *rbcLa* and *matK* barcode regions amplified and sequenced. The results are compared with reference sequences in GenBank using BLAST to determine species identity. To strengthen authentication, this chapter incorporates Bayesian inference (BI) and Maximum Parsimony (MP) phylogenetic analyses based on a combined *rbcLa* and *matK* dataset. The chapter highlights the utility of mutlilocus barcoding for identifying poorly preserved or processed herbal products, and addresses safety, conservation and Indigenous knowledge systems implications.

Chapter 5 - General discussion, synthesis and conclusion: The final chapter integrates the findings from the preceding chapters and discusses their broader implications for biodiversity conservation, the preservation of Indigenous Knowledge, and the regulation of medicinal plants. The chapter provides a consolidated reflection on species use patterns, market fidelity, and the importance of molecular verification in complementing ethnobotanical data. The chapter further examines the methodological strengths and limitations of the study and proposes directions for future research and policy development. The chapter concludes by summarising the scientific and societal contributions of the study to ethnobotany, molecular authentication and conservation policy in South Africa.

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Chapter 2: Medicinal, nutritional and cultural significance of *Strychnos spinosa Lam*. in sub-Saharan Africa

2.1. Introduction

The world is currently confronted with urgent environmental issues, including climate change, deforestation, habitat loss and biodiversity depletion (Rowe et al., 1999). It is becoming increasingly clear that Indigenous Knowledge Systems (IKS) can play a critical role in environmental conservation (Omotayo and Aremu, 2021). These systems contain a wealth of knowledge on local ecosystems, agroecosystems and sustainable resource management (Mpofu and Miruka, 2009; Omotayo and Aremu, 2021). Indigenous people, who have lived in harmony with their natural surroundings for generations, possess invaluable knowledge that can contribute to the protection and preservation of the environment (Aremu and Moyo, 2022; Le Roux, 2005). However, the older generation is predominantly the custodian of information (Mpofu and Miruka, 2009).

Indigenous Knowledge (IK) refers to knowledge, practices and beliefs accumulated and passed down through generations within specific cultural groups (Avakoudjo et al., 2019). The use of IK has been a critical aspect of natural resource management, especially in rural communities across Africa, as most developing countries rely on local remedies from traditional medicines for their healthcare needs (Harvey-Brown, 2024; Madzimure et al., 2013; Mahmoud et al., 2024). Studies by Bhatta (2024) and Mpofu and Miruka (2009) have shown that IK is practised mostly by the elderly than the younger generation. This is largely due to urbanisation and globalisation (Mpofu and Miruka, 2009).

Mpofu and Miruka (2009) argue that young people often lack interest in Indigenous Knowledge Systems because the wisdom, skills, and practices developed by indigenous peoples over generations, are frequently overlooked in their activities, such as the school curriculum. Modern school subjects are often valued more highly than indigenous knowledge. Thus, IK is losing ground globally. The potential for finding new solutions to today's most challenging health and environmental issues is at risk of being lost (Perrino et al., 2024). In sub-Saharan Africa, IK is an essential repository of information about the local environment, including identifying and using various plant species for medicinal purposes, culinary uses, ecological understanding and conservation efforts (Georgiadis, 2022).

One such plant species that has garnered significant interest in the African region is *Strychnos spinosa* Lam. This flowering plant belongs to the family Loganiaceae, within the order Gentianales, commonly known as ‘monkey orange' or umKwakwa' (Le Roux, 2005). This tree is endemic to Africa (Moher et al., 2009; UNESCO, 2023). In South Africa, it is densely distributed in 44.5% of provinces (Mbhele et al., 2022; Moher et al., 2009). *S. spinosa* has acclimatised to drought and low soil fertility(Ali et al., 2024; Bisso et al., 2022). Local communities have used *S. spinosa* for medicinal, nutritional and cultural purposes (Tittikpina et al., 2020). However, there is a need to study the botanical uses of *S. spinosa* and factors that determine its utilisation within different communities (Bero et al., 2009; Mahmoud et al., 2024; Mbhele et al., 2022; UNESCO, 2023).

Studies by Aremu and Moyo (2022); Omotayo and Aremu (2021) highlight the excellent potential for *S. spinosa* to add value to sustainable food systems and health benefits. These studies also highlight the high nutritional content of *S. spinosa*, which includes essential vitamins, such as vitamin C, minerals like iron and zinc, and phytochemical compounds with antioxidant qualities. The fruit is drought tolerant and grows well in soils with low fertility, making it a good option for addressing food and nutrition insecurity, especially in rural and arid areas of sub-Saharan Africa. The potential of *S. spinosa* to improve diet quality and serve as an economic resource for marginalised communities, further reinforces its significance (Omotayo and Aremu, 2021).

The studies also highlight the urgent need for sustainable harvesting practices and effective monitoring systems to ensure the long-term existence of *S. spinosa*. Agricultural expansion, urbanisation and related habitat destruction are major threats to its natural populations (Aremu and Moyo, 2022). Furthermore, absence of formal cultivation practices and reliance on wild harvesting, increases the risk of depletion. Establishing guidelines for sustainable use, promoting agroforestry initiatives, and integrating local communities into conservation efforts, are critical steps to mitigate these risks (Aremu and Moyo, 2022; Avakoudjo et al., 2019; Omotayo and Aremu, 2021).

While *S. spinosa* is currently classified as “Least Concern” on the IUCN Red List (Harvey-Brown, 2024), its widespread utilisation by local communities necessitates a reassessment of its conservation status. Ongoing usage patterns and environmental challenges suggest that its long-term availability may be at risk (Omotayo and Aremu, 2021). For instance, studies have documented its application in traditional medicine, where the roots, bark and fruits, are extensively harvested for the treatment of different ailments. If these practices are not monitored, they could result in localised overexploitation (Aremu and Moyo, 2022; Avakoudjo et al., 2019). Additionally, research indicating its importance as a renewable food supply and its beneficial bioactive characteristics, calls for greater emphasis on its preservation (Madzimure et al., 2013).

This study is crucial for biodiversity conservation and sustainable natural resource management (Mahmoud et al., 2024); an initiative that has already begun worldwide, particularly in regions with higher plant diversity (Georgiadis, 2022; Perrino et al., 2024; UNESCO, 2023). The study examines the traditional uses of *S. spinosa*, providing insights into sustainable harvesting practices. The findings can also benefit environmental policymakers by incorporating Indigenous Knowledge into conservation and resource management policies. The study aims to close the gap in understanding traditional uses of *S. spinosa* in African communities, by reviewing existing knowledge, examining the socio-ecological impact of harvesting and use, and screening phytochemicals.

2.2. Materials and methods

The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (2020) statement developed by Moher et al. (2009), was used in this study. A comprehensive electronic search of databases, such as JSTOR, ScienceDirect, PubMed, and Google Scholar, was conducted between April 2024 and January 2025. This search included research reports, theses and dissertations. Some of the leading search terms used included “Indigenous Knowledge”, “socio-ecological impact”, or “traditional ecological knowledge”, and “phytochemical analysis” in conjunction with “*S. spinosa*”. In this study, the inclusion criteria focused on studies explicitly conducted in Africa. Studies that met the inclusion criteria were selected for in-depth evaluation (Figure 2.1).

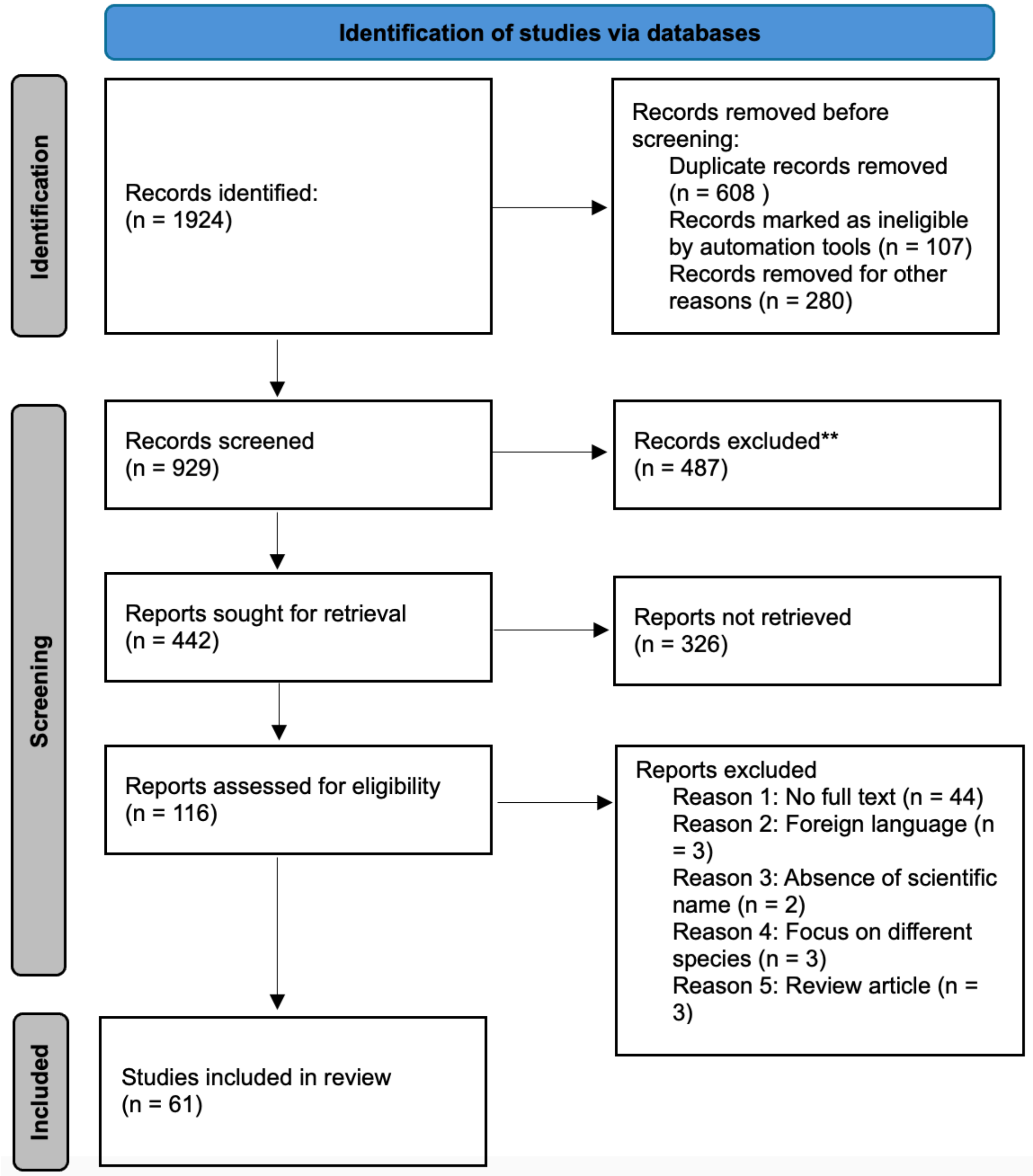

Figure 2.1: Process used to select articles related to traditional knowledge of *S. spinosa* in sub-Saharan Africa (n is the total number of studies)

2.3. Results and discussion

2.3.1 Literature findings

The search, through various scientific databases, yielded a total of 1924 studies. After removing duplicates, 929 studies were retained. In addition, data from five sources, including theses, dissertations and books, were obtained from the Thoko Mayekiso

Library and Information Centre, University of Mpumalanga, South Africa. The full texts of the 116 studies were reviewed in detail. About 49 studies were excluded because the full texts were either not available, or in foreign languages and without scientific names. Furthermore, studies that focused on different *Strychnos* species or those that did not examine traditional uses of medicinal plants as their main subjects, were also excluded from the study. Sixty-one studies were thus, used the study as indicated in Figure 2.1.

Table 2.1 shows that the studies considered represented 37% of African countries. More studies have been conducted in Southern Africa, with seven in South Africa, KwaZulu Natal Province and Limpopo provinces. Notwithstanding, studies conducted by Avakoudjo et al. (2019) and Mbhele et al. (2022), which focused on documenting Indigenous Knowledge on the uses of *S. spinosa* and the study by Madzimure et al. (2013), which focused on the efficacy of *S. spinosa* extracts on cattle ticks, other records were part of broader ethnobotanical surveys by different researchers. Generally, semi-structured interviews or questionnaires were the predominant tools used to collect data, with only a few having *In-vitro* analysis. The number of participants involved in the interviews was not reported in approximately 20% of these studies.

A comprehensive assessment of nutritional and bioactive compounds found in *S. spinosa* was not analysed in over 70% of these studies. Authenticating the phytochemical composition of *S. spinosa* within different African communities, is a crucial step towards understanding its pharmacological and nutritional effects (Ali et al., 2024; Bisso et al., 2022). A study conducted by Tittikpina et al. (2020), provides insights into the chemical compounds present in *S. spinosa*, including alkaloids, terpenes, sterols, flavonoids, saponins and other bioactive components. By identifying and characterising these phytochemicals, researchers can further investigate the potential therapeutic properties of *S. spinosa* and assess its efficacy in addressing various health conditions traditionally treated with this plant. The literature reviewed on the phytochemical composition and bioactivity of *S. spinosa* is limited, with seven studies focusing on the plant's antimicrobial and antioxidant properties(Bero et al., 2009; Bizimana et al., 2006; Lawal et al., 2019; Madzimure et al., 2013; Magassouba et al., 2007; Molander et al., 2014; Waterman et al., 2010). In-depth research into the phytochemical composition of products made from parts of the *S. spinosa* plant, could yield significant benefits.

Table 2.1: In-depth botanical review of S. spinosa across sub-Saharan Africa, examining its diverse ecological range, traditional medicinal uses, ethnobotanical significance, and potential for nutritional and pharmacological applications

Author	**Study title**	**Location**	**Participant-type**	**Interviewee number**	**Methodological framework**	**Nutritional, Phytochemical, and/ or biological signature**
Asase et al. (2005)	Ethnobotanical study of some Ghanaian anti-malarial plants	Wechiau Community Hippopotamus Sanctuary, **Ghana**	Local community members, herbalists	20	Field interviews, house-to-house interviews, focus group	None
Avakoudjo et al. (2019)	Local knowledge, uses, and factors determining the use of Strychnos spinosa organs in Benin (West Africa)	Guinean, Sudano-Guinean and Guinean climatic zones, **Benin**	Farmers, THPs, cattle herdsmen, artisans, and students	733	Semi-structured questionnaires	None
Bero et al. (2009)	In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria	South of **Benin**	N/A	N/A	In vitro testing	B
Bizimana et al. (2006)	Evaluation of medicinal plants from Mali for their in vitro and in vivo trypanocidal activity	Southern part of **Mali**	N/A	N/A	In vitro testing	B
Bruschi et al. (2011)	Traditional healers and laypeople: A qualitative and quantitative approach to local	Muda, **Mozambique**	Local leaders and laypeople THPs, Independent	67	Semi-structured interviews	None

Author	Study title	Location	Participant-type	Interviewee number	Methodological framework	Nutritional, Phytochemical, and/ or biological signature
	knowledge on medicinal plants in Muda (Mozambique)		Pentecostal Churches "prophets" and lay villagers (7/67			
De Wet et al. (2013)	Medicinal plants used for the treatment of various skin disorders by a rural community in northern Maputaland, South Africa	Maputaland, **Republic of South Africa**	Local community members	87	Structured questionnaires	None
Doka (2009)	Ethnobotanical survey of Medicinal Plants in West Kordofan (Western Sudan)	West Kordofan, **Sudan**	Local practitioners	31	Semi-structured interviews	None
Karou et al. (2011)	Ethnobotanical study of medicinal plants used in the management of diabetes mellitus and hypertension in the Central Region of Togo	Central Region of **Togo**	THPs	55	Semi-structured questionnaires	None
Koné and Kamanzi Atindehou (2008)	Ethnobotanical inventory of medicinal plants used in traditional veterinary medicine in Northern Côte d'Ivoire (West Africa)	Northern **Côte d'Ivoire**	Local breeders and traditional practitioners	14	Structured questionnaires	None

Author	Study title	Location	Participant-type	Interviewee number	Methodological framework	Nutritional, Phytochemical, and/ or biological signature
Lawal et al. (2019)	Anti-inflammatory potential of South African medicinal plants used for the treatment of sexually transmitted infections	Venda region, Limpopo, **Republic South Africa**	N/A	N/A	In vitro testing	B, P
Lockett et al. (2000)	Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, Northeastern Nigeria	Rural Fulani, Northeastern **Nigeria**	Elderly focus group	Not specified (Four groups of 4-8 participants)	Semi-structured questionnaires; In vitro testing	N
Madzimure et al. (2013)	Efficacy of Strychnos spinosa (Lam.) and Solanum incanum L. aqueous fruit extracts against cattle ticks	Henderson Research Station (HRS), **Zimbabwe**	N/A	N/A	In vitro testing	B, P
Magassouba et al. (2007)	Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine	Low, Middle, Upper and Forest ecological zones of **Guinea**	Traditional practitioners	418	Structured questionnaires; In vitro testing	B, P
Maroyi (2011)	An ethnobotanical survey of medicinal plants used by the	Nhema communal	Traditional practitioners	9	Participatory Rapid Appraisal approach using	None

Author	**Study title**	**Location**	**Participant-type**	**Interviewee number**	**Methodological framework**	**Nutritional, Phytochemical, and/ or biological signature**
	people in Nhema communal area, Zimbabwe	area, **Zimbabwe**			open-ended interviews (unstructured)	
Mbhele et al. (2022)	Indigenous Knowledge on the uses and morphological variation among Strychnos spinosa Lam. at Oyemeni area, KwaZulu-Natal, South Africa	Oyemeni Area, KwaZulu-Natal, **Republic of South Africa**	Knowledgeable local community members	100	Structured questionnaires	None
Molander et al. (2014)	Hyaluronidase, phospholipase A2 and protease inhibitory activity of plants used in traditional treatment of snakebite-induced tissue necrosis in Mali, DR Congo and South Africa	**Democratic Republic of Congo**, **Mali** and **Republic of South Africa**	N/A	N/A	In vitro testing	B, P
Mhlongo and Van Wyk (2019)	Zulu medicinal ethnobotany: new records from the Amandawe area of KwaZulu-Natal, South Africa	Amandawe area of KwaZulu-Natal, **Republic of South Africa**	Local community members	37	Freelisting; rapid ethnobotanical appraisal	None

Author	Study title	Location	Participant-type	Interviewee number	Methodological framework	Nutritional, Phytochemical, and/ or biological signature
Novotna et al. (2020)	Medicinal plants used by 'root doctors', local traditional healers in Bié province, Angola	Bié province, **Angola**	Professional herbalists	10	Participatory observation; semi-structured interviews; transect walks	None
Ruffo (1991)	A Survey of medicinal plants in Tabora region, (Hilonga et al., 2018; Ruffo, 1991)	Tabora Region, **Tanzania**	Traditional health practitioners	27	Structured interviews	None
Tchacondo et al. (2011)	Herbal remedies and their adverse effects in Tem tribe traditional medicine in Togo	Tchaoudjo prefecture, **Togo**	Traditional health practitioners	54	Rapid ethnobotanical appraisal	None
Waterman et al. (2010)	Anthelmintic screening of Sub-Saharan African plants used in traditional medicine	**Madagascar** and **Central African Republic**	N/A	N/A	In vitro testing	B, P
Zwane et al. (2024)	Blood purification practices: Some ethnopharmacological insight from a rural community in KwaZulu-Natal, South Africa	Northern Maputaland, Kwazulu-Natal, **Republic of South Africa**	Knowledgeable local community members	55	Structured questionnaires	None
Derya et al. (2023)	Ethnobotanical survey of medicinal plants in	Sissala East municipality, **Ghana**	THPs and knowledgeable	50	Semi-structured questionnaires	None

Author	Study title	Location	Participant-type	Interviewee number	Methodological framework	Nutritional, Phytochemical, and/ or biological signature
	Sissala East municipality of the upper West region, Ghana		local community members			
Mohamadoua et al. (2024)	Ethnopharmacological Investigation of Plants Used in the Treatment of Breast Cancer in Northern Cameroon	Northern **Cameroon**	Traditional health practitioners	131	Semi-structured questionnaires	None
Magwede et al. (2019)	An inventory of Vhavenḓa useful plants	Venda, **Republic of South Africa**	Knowledgeable local community members	66	Semi-structured questionnaires	None
Mlilo and Sibanda (2021)	An ethnobotanical survey of the medicinal plants used in the treatment of cancer in some parts of Matabeleland, Zimbabwe	Matabeleland, **Zimbabwe**	Traditional health practitioners, Herbalists, apprentices, and family members of traditional health practitioners	25	Open-ended semi-structured inter- views	None
Chinsembu et al. (2019)	Ethnomedicinal plants used by traditional healers in the management of HIV/AIDS opportunistic	Lusaka, **Zambia**	Traditional health practitioners	40	Semi-structured interviews	None

Author	Study title	Location	Participant-type	Interviewee number	Methodological framework	Nutritional, Phytochemical, and/ or biological signature
	diseases in Lusaka, Zambia					
Aparicioa et al. (2021)	Ethnobotanical study of medicinal and edible plants used in Nhamacoa area, Manica Province, Mozambique	Nhamacoa area, Manica province, **Mozambique**	Randomly selected villagers	25	Free listing and semi-structured interviews; random selection	None
Shopoa et al. (2022)	Ethnobotanical study of medicinal plants traditionally used in Gokwe South District, Zimbabwe	Gokwe South District, **Zimbabwe**	knowledgeable local community members	46	Semi-structured questionnaire.	None
Yaoitcha et al. (2015)	Prioritization of useful medicinal tree species for conservation in Wari-Maro Forest Reserve in Benin: A multivariate analysis approach	Wari-Maro Forest Reserve, **Benin**	Traditionally most prominent authorities of local households	149	Semi-structured interviews	None
Hilonga et al. (2018)	Trade of wild-harvested medicinal plant species in local markets of Tanzania and its implications for conservation	Arusha, Dodoma, Mbeya, Morogoro and Mwanza regions, **Tanzania**	Local herbal vendors	40	Semi-structured interviews	None

Author	Study title	Location	Participant-type	Interviewee number	Methodological framework	Nutritional, Phytochemical, and/ or biological signature
Masumbu et al. (2023)	Ethnobotanical survey of medicinal plants claimed by traditional herbal practitioners to manage cancers in Malawi	Mzimba and Nkhata Bay district of northern **Malawi**	Traditional health practitioners	25	Semi-structured questionnaires and purposive sampling technique	None

** Nutraceutical profile – nutritional (N), phytochemical (P), and/ or biological (B) analysis of Strychnos spinosa*

Table 2.2: Ethnobotanical uses of *Strychnos spinosa* Lam. and modes of administration in Africa

Category of diseases	Ethnobotanical use	Plant part	Method of preparation	Administration	Reference
Gastrointestinal diseases	Stomach aches	Leaves, roots, bark, non-ripe fruit	Calcinated to powder (fruit), decoction (leaves boiled)	Orally	(Avakoudjo et al., 2019; Mbhele et al., 2022; Mohamadoua et al., 2024; Novotna et al., 2020; Yaoitcha et al., 2015)
	Diarrhoea (humans and livestock) Constipation	Roots, bark	Powder or decoction (roots)	Orally	(Avakoudjo et al., 2019; Maroyi, 2011; Tchacondo et al., 2011)
	Diseases of the digestive tract	Leaves, bark	Decoction	Not specified	(Mohamadoua et al., 2024)
	Vomiting	Roots	Powder	Orally	(Tchacondo et al., 2011)
	Abdominal pains	Roots	Not specified	Not specified	(Maroyi, 2011)
Infectious diseases	Malaria	Leaves, twigs	Decoction and infusion (leaves), grind twigs	Orally, topical (twigs ground, mixed with pomade)	(Asase et al., 2005; Avakoudjo et al., 2019; Mohamadoua et al., 2024; Yaoitcha et al., 2015)
	Venereal diseases (gonorrhoea, syphilis, lymphogranuloma inguinale)	Roots, fruit, root bark	Maceration (roots, fruit), decoction	Orally	(Bruschi et al., 2011; Chinsembu et al., 2019; Lawal et al., 2019; Magassouba et al., 2007; Ruffo, 1991; Shopoa et al., 2022; Tchacondo et al., 2011)
	Bacterial infections	Bark	Calcinated or infusion	Orally	(Avakoudjo et al., 2019)
	Tuberculosis	Not specified	Not specified	Not specified	(Mohamadoua et al., 2024)
	Sleeping sickness (anti-trypanosal activity)	Not specified	Not specified	Not specified	(Mohamadoua et al., 2024)
	Haemorrhoids	Root	Crushed	Enema	(Novotna et al., 2020)

Category of diseases	Ethnobotanical use	Plant part	Method of preparation	Administration	Reference
Pain and inflammation	Fever	Root, bark with ripe fruit	Maceration of roots, infusion of bark and roots	Orally	(Avakoudjo et al., 2019)
	Weariness	Leaves with roots	Infusion	Orally and/ or submerged	(Avakoudjo et al., 2019)
	Diabetes	Roots	Calcination	Orally	(Karou et al., 2011; Mohamadoua et al., 2024)
	Cataract	Bark, fruits, leaves or roots	Decoction	Topical (on eye)	(Shopoa et al., 2022)
	Hernia	Roots	Decoction	Orally	(Bruschi et al., 2011; Yaoitcha et al., 2015)
	Cures swelling of feet	Bark with ripe fruit	Decoction	Orally, topical	(Avakoudjo et al., 2019)
	Hypertension	Root, fruit	Infusion	Orally	(Avakoudjo et al., 2019; Doka, 2009; Mohamadoua et al., 2024)
	Ear disease	Leaf, bark and roots	Not specified	Not specified	(Yaoitcha et al., 2015)
	Prevents paralysis	Bark with ripe fruit	Decoction	Orally, topical	(Avakoudjo et al., 2019)
	Oedema	Bark, fruits, leaves or roots	Decoction	Orally	(Shopoa et al., 2022)
	Foot crevices	Fruit	Pulp	Not specified	(Avakoudjo et al., 2019)
	Cardiovascular	Not specified	Not specified	Not specified	(Mhlongo and Van Wyk, 2019)
	Food allergies	Bark, roots	Decoction (bark), powder (roots)	Orally	(Mbhele et al., 2022; Tchacondo et al., 2011)
	Unblocking respiratory deficiencies	Leaves with roots	Maceration	Orally	(Avakoudjo et al., 2019)

Category of diseases	Ethnobotanical use	Plant part	Method of preparation	Administration	Reference
Reproductive health	Facilitation of milk production (for mothers and cows)	Fruit	Ripe fruit calcinated and reduced to powder	Orally (sprinkled in porridge)	(Avakoudjo et al., 2019)
	Dysmenorrhea	Root, bark	Infusion of roots or bark, decoction of bark	Orally	(Avakoudjo et al., 2019; Hilonga et al., 2018; Mhlongo and Van Wyk, 2019)
	Prevention of miscarriage	Bark, fruits, leaves or roots	Decoction	Orally	(Shopoa et al., 2022)
	Labour induction	Roots	Maceration	Orally	(Bruschi et al., 2011)
	Prevention of sterility	Not specified	Not specified	Not specified	(Mhlongo and Van Wyk, 2019)
Trauma and emergencies	Snake bites	Roots, bark, fruit, radix/ cortex	Calcination, decoction (bark), un-ripe fruit licked	Orally, topical	(Bruschi et al., 2011; Derya et al., 2023; Lockett et al., 2000; Mbhele et al., 2022; Mohamadoua et al., 2024; Molander et al., 2014)
	Slows venom (anti-venom)	Roots	Infusion/ calcinated	Orally	(Avakoudjo et al., 2019)
Other uses	Cultural rituals (homestead protection, livestock increase, love charm, blood cleanser)	Fruit, roots, bark (mixed with *Sapium integerrimuma* (Hochst.) J. L´ Leonard)	Paste (pulp of non-ripe fruit), maceration (roots), crushed (bark)	Orally	(Avakoudjo et al., 2019; Mbhele et al., 2022; Zwane et al., 2024)
	Growth retardation	Leaves, bark, roots	Not specified	Not specified	(Yaoitcha et al., 2015)
	Cattle dermatitis	Leaves with leaves of *Crossopteryx*	Decoction	Orally and topical	(Koné and Kamanzi Atindehou, 2008)

Category of diseases	Ethnobotanical use	Plant part	Method of preparation	Administration	Reference
		febrifuga (G. Don) Benth			
	Fever in oxen	Bark	Calcinated or infusion	Orally	(Avakoudjo et al., 2019)
	Construction / craftwork	Wood	Wood dried	Not specified	(Magwede et al., 2019)
	Firewood	Wood	Wood dried	Not specified	(Avakoudjo et al., 2019; Mbhele et al., 2022)
	Solidification of babies	Leaves	Infusion and decoction	Orally and submerged	(Avakoudjo et al., 2019)
	Cattle ticks	Fruit	Ground (pulp and seeds of un-ripe fruit)	Topical	(Madzimure et al., 2013)
	Food/ drink (fermented maize meal/ porridge, jam, alcohol, juice)	Fruit	Pulp from ripe fruit	Orally	(Aparicioa et al., 2021; Asase et al., 2005; Avakoudjo et al., 2019; Magwede et al., 2019; Mbhele et al., 2022)
	Fodder	Leaves	Picked fresh	Orally	(Avakoudjo et al., 2019)
Skin related conditions	Chronic wounds/ sores	Fruit, fruit sap with leaves of *S. madagascariensis*, bark	Powder	Orally, topical	(De Wet et al., 2013; Masumbu et al., 2023; Mlilo and Sibanda, 2021)

2.3.2 Plant parts of *S. spinosa* in Africa

This study has revealed the use of six distinct plant parts of *S. spinosa* for the treatment of various health conditions as shown in Figure 2.2. The Figure shows that the roots of *S. spinosa* roots are commonly used to treat digestive disorders and fever, representing 25.7%, followed by the bark (22.9%) and fruits (22.9%). *S. spinosa* fruits are consumed raw or cooked in East African countries, such as Tanzania (Hilonga et al., 2018; Ruffo, 1991), where its bark is traditionally used for analgesic properties (Hilonga et al., 2018; Ruffo, 1991). In South Africa and Zimbabwe, the fruit is valued for its ripeness as a snack or food (Madzimure et al., 2013; Maroyi, 2011; Mlilo and Sibanda, 2021; Shopoa et al., 2022), while the wood is used for tools and firewood (De Wet et al., 2013; Lawal et al., 2019; Magwede et al., 2019; Mbhele et al., 2022; Mhlongo and Van Wyk, 2019; Molander et al., 2014; Zwane et al., 2024). Different parts of the plant are also used to treat respiratory and digestive issues.

In Nigeria and Ghana, the leaves and roots of the plant are commonly used in herbal preparations to treat ailments, such as fevers and headaches, with some communities believing it has protective properties (Asase et al., 2005; Lockett et al., 2000). On the contrary, in the Democratic Republic of Congo and Central African Republic, *S. spinosa* fruit is appreciated more for its nutritional value and, sometimes, fermented to enhance the flavour and nutritional profile of the fruit (Mohamadoua et al., 2024; Molander et al., 2014; Novotna et al., 2020; Waterman et al., 2010). The leaves and roots of the plant are also used in traditional medicine, particularly in treatments related to women's health (Mohamadoua et al., 2024; Molander et al., 2014; Novotna et al., 2020; Waterman et al., 2010).

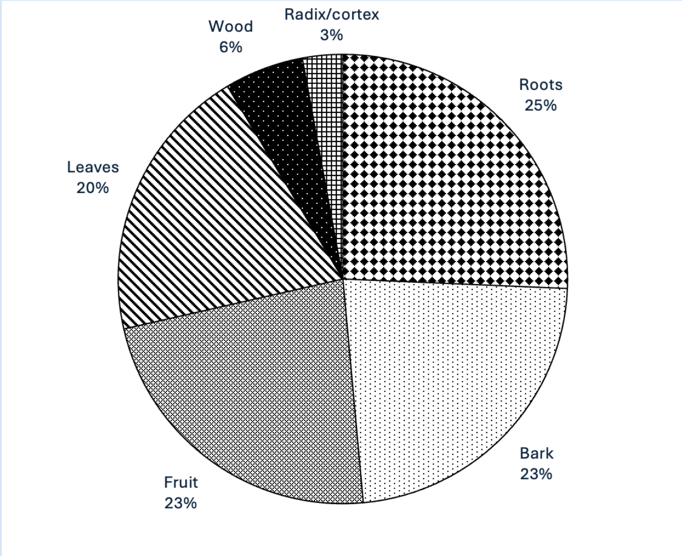

Figure 2.2: Different *S. spinosa* plant parts used to manage various ailments and conditions in Africa

2.3.3 Methods of preparation and administration

Different techniques for preparation, such as decoctions, crushed plant parts, infusions, macerations, or calcinations, have been documented (Figure 2.3A). Decoctions (30%), crushed plant parts (22%), and infusions (17%), involve soaking a part of the plant in hot water for a period ranging from overnight to several hours, macerations (9%), involve softening plant parts by soaking them in a liquid, while calcinations (9%), involve heating plant material to high temperatures in the absence of air. Some studies (13%) did not specify methods of preparation (Figure 2.3A), while others revealed that the parts of *S. spinosa* are often crushed, ground, or prepared in powder form, highlighting the critical role of dried plant materials in traditional medicine. This method provides benefits, such as longer shelf life and flexibility in usage, which can be especially valuable in resource-limited settings (Adeniyi et al., 2018; Schultz et al., 2020). Interestingly, the results are similar to other studies (Adeniyi et al., 2018; Dabré et al., 2023; Dlova and Ollengo, 2018; Kshirsagar et al., 2019) where decoction was identified as the predominant preparation method. The ease of making decoctions is a key reason why they are the main method used in traditional healing practices. Several indigenous cultures worldwide, use decoctions to harness the healing properties of different plants. Additionally, decoctions eliminate contaminants and are deemed a safer option than other preparation methods (Dlova and Ollengo, 2018).

The distribution of administration methods for *S. spinosa*, as shown in Figure 2.3B, reveals a strong preference for oral administration, accounting for 59% of studies, followed by topical application (16%). The least documented means of administration was enemas (2%). Some studies (23%) did not specify any administration methods. The predominance of oral administration aligns with the findings of Yadav et al. (2014), which revealed that *Strychnos potatorum* L.f, exhibits significant pharmacological efficacy, when administered orally. This predominance of oral administration suggests that *S. spinosa* may contain bioactive compounds that are effective when ingested. The high incidence of oral usage may stem from the presence of similar bioactive substances readily absorbed through the gastrointestinal tract, potentially aiding in the treatment of different internal disorders (Ndarubu et al., 2020; Ngadze et al., 2016; Tittikpina et al., 2020). Topical application highlights its potential for addressing skin conditions and external ailments. Albahri et al. (2023) found that topical applications of plant extracts demonstrate notable wound-healing properties attributed to their antibacterial and anti-inflammatory effects. Application of *S. spinosa* in topical forms indicates it may possess similar characteristics, making it valuable for treating skin-related conditions.

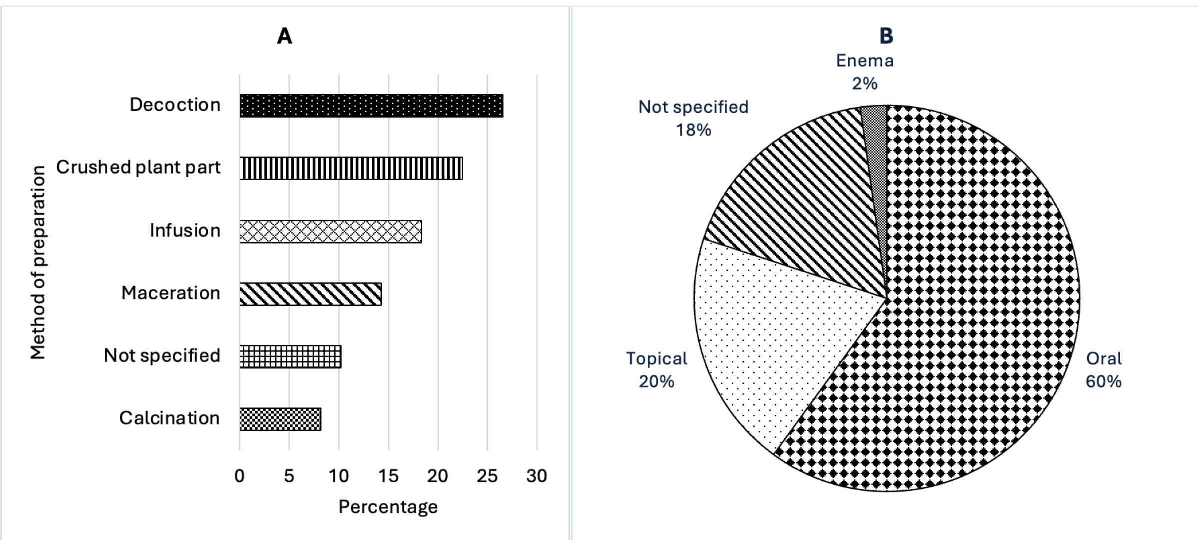

Figure 2.3: Methods of preparation (A) and administration (B) of *S. spinosa* for the treatment of different ailments and conditions in Africa

2.3.4 Socio-ecological impact and implications for conservation

The limited research on the socio-ecological impacts of harvesting and using *S. spinosa* in African communities, highlighting potential biodiversity and ecosystem changes, requires in-depth analysis (Asase et al., 2005; Hilonga et al., 2018; Yaoitcha et al., 2015). Future research should focus on assessing the socio-ecological impacts of the use of *S. spinosa*, taking into consideration factors, such as resource sustainability, environmental conservation and community well-being to inform sustainable management practices. Considering the nutritional and economic potential of *S. spinosa*, it is evident that *S. spinosa* offers opportunities to tackle food and nutrition insecurity as well as promote economic prosperity among marginalised rural populations (Omotayo and Aremu, 2021). With the high content of essential nutrients of the plant, such as vitamin C, iron, and zinc, *S. spinosa* stands out as a valuable resource for enhancing food security and improving health outcomes in developing nations. To fully harness the benefits of *S. spinosa*, further research is recommended on domestication processes, value chain integration and economic viability (Omotayo and Aremu, 2021). Collaboration among stakeholders is crucial to explore the full potential of this plant, as a sustainable solution to food shortages and malnutrition experienced by rural communities (Nontu et al., 2024).

This study has revealed significant implications for biodiversity conservation and sustainable resource management. The study emphasises the importance of degraded areas as a source of medicinal plants for indigenous communities, indicating the need to prioritise protecting and restoring these ecosystems (Asase et al., 2005; Maroyi, 2011). Furthermore, this study highlights the need to integrate indigenous knowledge into conservation strategies, as traditional practices and resource utilisation patterns can provide valuable insights for sustainable management (Asase et al., 2005; Hilonga et al., 2018; Yaoitcha et al., 2015). There is, therefore, the need to develop collaborative approaches that respect and engage with local communities, ensuring equitable sharing of benefits and protection of traditional ecological knowledge (Robinson et al., 2021).

2.4. Research gap and limitations of the study

Potential limitations in available literature, methodological constraints, or cultural biases, are some of the shortcomings of the study. Thus, future research should address these limitations by expanding the scope, incorporate more diverse sources of IK, and employ innovative methodologies that prioritise community engagement and reciprocity (Gómez-Baggethun, 2021; Magni, 2017). Further research is needed to explore biological activity, toxicological properties, and potential applications of *S. spinosa*, based on novel use reports and identified knowledge gaps. Collaborative efforts between researchers, local communities, and conservation organisations can contribute to a more comprehensive understanding of this plant species and its role in indigenous knowledge systems, biodiversity conservation and sustainable resource management (McClatchey et al., 2009).

Several studies have documented the traditional uses of *S. spinosa* within indigenous communities in only two provinces of South Africa (De Wet et al., 2013; Magwede et al., 2019; Mbhele et al., 2022; Mhlongo and Van Wyk, 2019; Zwane et al., 2024). These studies have highlighted the diverse uses of the plant, including medicinal, cultural, and socio-economic purposes (De Wet et al., 2013; Magwede et al., 2019; Mbhele et al., 2022; Mhlongo and Van Wyk, 2019; Zwane et al., 2024). There is need for a comprehensive and cohesive compilation of various traditional uses across different communities. There is also the need for further research to systematically document and compare traditional uses of *S. spinosa* within various indigenous communities to provide a clearer understanding of its significance and cultural value (Omotayo and Aremu, 2021; Tittikpina et al., 2020).

More studies are needed to authenticate the ethnobotanical uses of *S. spinosa* by assessing its nutritional and bioactive compounds, including its phytochemical composition in Africa (Bero et al., 2009; Bizimana et al., 2006; Lawal et al., 2019; Madzimure et al., 2013; Magassouba et al., 2007; Molander et al., 2014; Waterman et al., 2010). However, existing literature often needs a community-specific approach to authenticating the phytochemicals within different indigenous communities. Further research should adopt a community-based participatory approach to identify and authenticate phytochemicals in *S. spinosa* samples, acknowledging variations that may exist across different communities and potential implications for traditional medicinal practices (Ferreira and Gendron, 2011).

This study emphasises the critical importance of IK in the conservation and sustainable management of *S. spinosa*, a plant species of significant cultural and ecological value in Africa (Omotayo and Aremu, 2021). The findings indicate that IK contains invaluable insights into the traditional uses, socio-ecological impacts, and potential phytochemical applications of *S. spinosa*, highlighting its multifaceted roles in local communities (Mbhele et al., 2022).

The comprehensive exploration of current literature underscores the urgent need to document and preserve this knowledge, particularly as it faces threats from modernity and globalisation (Gómez-Baggethun, 2021; Magni, 2017). As communities navigate environmental challenges and shifting socio-economic landscapes, it becomes paramount to empower local populations by recognising and integrating their traditional ecological knowledge. In addition, fostering collaboration between indigenous communities and scientific research, can lead to more effective conservation strategies that protect biodiversity and promote cultural heritage (Nontu et al., 2024; Robinson et al., 2021). This study serves as a call to action for policymakers, researchers, and environmental practitioners to value and incorporate indigenous perspectives into sustainable resource management frameworks (Magni, 2017; Tittikpina et al., 2020). Ultimately, efforts to understand and preserve *S. spinosa* through the lens of IK, can enhance environmental resilience and support the rights and well-being of local communities.

While numerous studies have documented the ethnobotanical uses of *S. spinosa*, there is limited research examining harvesting practices and market dynamics associated with the species, particularly within localised contexts such as Bushbuckridge Local Municipality. Existing literature largely focuses on nutritional, medicinal and phytochemical properties, with comparatively less attention given to how the species is sourced, traded and accessed within informal medicinal plant markets (Avakoudjo et al., 2019; Omotayo and Aremu, 2021). Furthermore, recent reviews have highlighted the need for greater attention to sustainable harvesting practices, reinforcing the limited focus of current research in this area.

2.5. Concluding remarks

This study highlights a significant gap in understanding the nutritional and bioactive compounds of *S. spinosa*, a plant valued for its traditional medicine and diets. Previous studies have mainly focused on qualitative methods, lacking quantitative assessments of phytochemical composition and therapeutic applications. The roots of the plant are used for gastrointestinal conditions and to treat fever, among other health conditions, while its bark and fruits are known for their analgesic properties. Cultural practices enrich application, reflecting protective qualities and integration into herbal preparations. The bioactivity of *S. spinosa* in African communities is crucial for pharmacological benefits and preserving traditional practices. Thus, nutraceutical and pharmacological analysis is essential to enhance understanding of nutritional content, pharmacological benefits and potential therapeutic effects related to *S. spinosa*. Furthermore, encouraging collaboration among ethnobotanists, pharmacologists, and local communities can support the sustainable use of *S. spinosa*, ensuring its preservation while facilitating its potential integration into contemporary medicinal applications. By bridging the divide between traditional knowledge and scientific inquiry, the contributions of *S. spinosa* can be maximized, thus supporting both health outcomes and cultural heritage.

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Chapter 3: Exploring Indigenous Knowledge and uses of *Strychnos spinosa* Lam. in Bushbuckridge, Mpumalanga Province: Community insights and sustainability Challenges

3.1. Introduction

Ethnobotanical knowledge plays a vital role in understanding the relationship between indigenous communities and plant resources, contributing to both cultural preservation and biodiversity conservation (Zidny et al., 2020). There have been calls to integrate Indigenous Knowledge (IK) into formal education systems (da Silva et al., 2023; Dlutu, 2025; Riley et al., 2024). This integration can enhance cultural relevance and sustainability in education, fostering a more holistic understanding of ecological practices. Environmental education, in particular, offers a platform to bridge traditional and scientific knowledge systems, promote biodiversity conservation and cultural preservation (Maila and Loubser, 2003).

Within this context, plant species used in traditional systems provide important case studies for understanding the application of IK in everyday practices. One such significant plant is *Strychnos spinosa* Lam., commonly known as the 'Monkey Orange' in English, 'umKwakwa' in siSwati, and 'Morapa' in Sepulana (Rodrigues et al., 2018), a tree native to sub-Saharan Africa. Traditionally valued for its edible fruit, medicinal properties, and cultural significance, *S. spinosa* is widely used across various African regions (Aremu and Moyo, 2022; Harvey-Brown, 2024; Mbhele et al., 2022a, b; Omotayo and Aremu, 2021). Nevertheless, despite its ethnobotanical importance, comprehensive documentation of its indigenous uses, particularly in South Africa, remains limited (Aremu and Moyo, 2022; Mbhele et al., 2022a; Omotayo and Aremu, 2021; Thothela et al., 2025; Tittikpina et al., 2020).

In South Africa, *S. spinosa* is predominantly found in the Limpopo, KwaZulu-Natal, Eastern Cape and Mpumalanga provinces, thriving in savannas, woodlands and transitional forested regions (Mbhele et al., 2022b). This species is well adapted to semi-arid environments and plays a crucial role in human and ecological systems. Local communities have historically depended on this plant for food, medicine and various traditional practices. However, shifting socio-economic dynamics, changes in land use, and environmental pressures, pose challenges to its sustainable utilisation (Omotayo and Aremu, 2021). The Bushbuckridge region in Mpumalanga Province, South Africa, known for its rich biodiversity and strong cultural heritage

(BusinessTrust, 2007), presents a unique opportunity for studying the ethnobotanical applications of *S. spinosa*. According to Mudau et al. (2024), Bushbuckridge is an understudied geographical area as very limited research exists about the area. The area faces challenges, such as habitat loss and socio-economic challenges, making it essential to document traditional plant knowledge for conservation initiatives (Constant and Tshisikhawe, 2018).

According to the International Union for Conservation of Nature (IUCN), *S. spinosa* is currently listed as “Least Concern” (Harvey-Brown, 2024). While this suggests the species is not under immediate global threat, local pressures, such as overharvesting, habitat fragmentation and climate change could contribute to its decline. In South Africa, where *S. spinosa* is essential in rural livelihoods, lack of formal conservation measures specific to this species raises concerns about its long-term sustainability (Avakoudjo et al., 2019). Thus, integrating IK with modern conservation strategies is crucial for ensuring the continued availability of *S. spinosa*.

Central to this integration, is the transmission of IK, which is typically passed down through oral traditions, intergenerational storytelling, observation, and participation in cultural and ecological practices (Kumar et al., 2021; Zidny et al., 2020). Elders often serve as custodians of IK, mentoring younger generations through practical engagement in harvesting, preparation and usage of indigenous plants (Constant and Tshisikhawe, 2018; Kumar et al., 2021). However, the increasing influence of formal education system, urban migration, and changing lifestyles, has led to a decline in youth participation and interest in traditional practices (Dahlberg and Trygger, 2009; Georgiadis, 2022; Kumar et al., 2021). This disruption in knowledge transmission affects the continuity of sustainable practices, leading to potential misuse or overharvesting of species, such as *S. spinosa (Constant and Tshisikhawe, 2018; Stepp, 2018)*. Furthermore, without proper transmission mechanisms, critical knowledge regarding the ecological roles, seasonal harvesting norms, and cultural significance of such plants may be lost, potentially compromising both conservation and community health outcomes (Kumar et al., 2021; Stepp, 2018).

S. spinosa is a deciduous shrub or small tree belonging to the family Loganiaceae (Mbhele et al., 2022a; Rodrigues et al., 2018). It typically reaches heights of 3 to 12 metres and is characterised by a rounded canopy and a short, often fluted trunk that bears rigid, spiny branches (Mbhele et al., 2022a, b). The bark varies from grey to pale brown and becomes deeply fissured with age (Mbhele et al., 2022a).

Leaves are simple, opposite, and broadly ovate to elliptic, measuring approximately 3–10 cm in length and 2–8 cm in width. They have a glossy green upper surface and smooth margins (Mbhele et al., 2022a, b). Flowering occurs during the dry season, producing small, greenish-white, fragrant flowers that are densely grouped at the tips of branches (Rodrigues et al., 2018). The fruits are large, spherical berries with a hard yellowish shell when ripe, enclosing a sweet-sour edible pulp and numerous flattened seeds (Mbhele et al., 2022a, b; Rodrigues et al., 2018). *Strychnos spinosa* thrives in diverse habitats, including savannas, riverine woodlands, coastal thickets and well-drained sandy soils (Harvey-Brown, 2024; Omotayo and Aremu, 2021). This species is highly drought-tolerant and ecologically important as a food source for wildlife, including baboons, antelopes and elephants (Harvey-Brown, 2024; Omotayo and Aremu, 2021).

The impact of formal education on traditional ecological knowledge (TEK), particularly regarding plant use, is a debated topic in ethnobotanical studies(Battiste, 2002; Ruiz-Mallén et al., 2013; Shephard et al., 2023). Some research suggests that increased educational attainment may weaken TEK by reducing dependence on traditional knowledge systems, primarily when modern curricula do not encompass indigenous viewpoints (Dlutu, 2025; Mbelebele et al., 2024). In contrast, the study by Cruz et al. (2013) proposes that education can raise awareness and reinforce IK if effectively integrated into community-focused or formal learning environments.

The aim of this study was to document indigenous uses of *S. spinosa* among different communities within Bushbuckridge Local Municipality, Mpumalanga Province, with a focus on its medicinal, nutritional and cultural applications. It also sought to examine how socio-demographic factors, particularly level of education, influence the depth and diversity of knowledge related to plant use in the study area. In doing so, the study contributes to the ongoing discussion on the relationship between education, cultural knowledge transmission and the sustainable use of plant resources.

3.2. Material and methods

3.2.1. Study area

A purposive sampling method was used to select six study areas based on the recommendations of local elders and authorities, as well as the inclusion criteria, which were the presence of indigenous knowledge holders and the plant under investigation, for ethnobotanical data collection. The study was conducted across specific settlements in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa, namely Buyisonto, MP Stream, Matsavana, Ximhungwe, Khomanani and Dumphries (Figure 3.1). Bushbuckridge is the main town in the Bushbuckridge Local Municipality, Ehlanzeni District. It is the largest municipality within the district, with 135 settlements and 34 wards, covering over a third of its geographical area. The town itself spans over 10.248km², with a population of 750.821 inhabitants. Over 50% of the population consists of females, and approximately two-thirds of this population aged between 15 and 64 years (Bushbuckridge Local Municipality., 2020).
Geographically, the town is between 240 50' 30" Latitude and 320 04' 46" Longitude (Shai et al., 2020). According to Mograbi et al. (2018), the climate has hot, wet summers (500 to 900 mm annual rainfall; October-March: average minimum–average maximum: 20–31°C) and warm, dry winters (April-September: 8–26°C). The area is characterised by diverse ecological systems and a rich cultural heritage, which supports the utilisation of various indigenous plants, including *S. spinosa* (BusinessTrust, 2007; Shai et al., 2020).

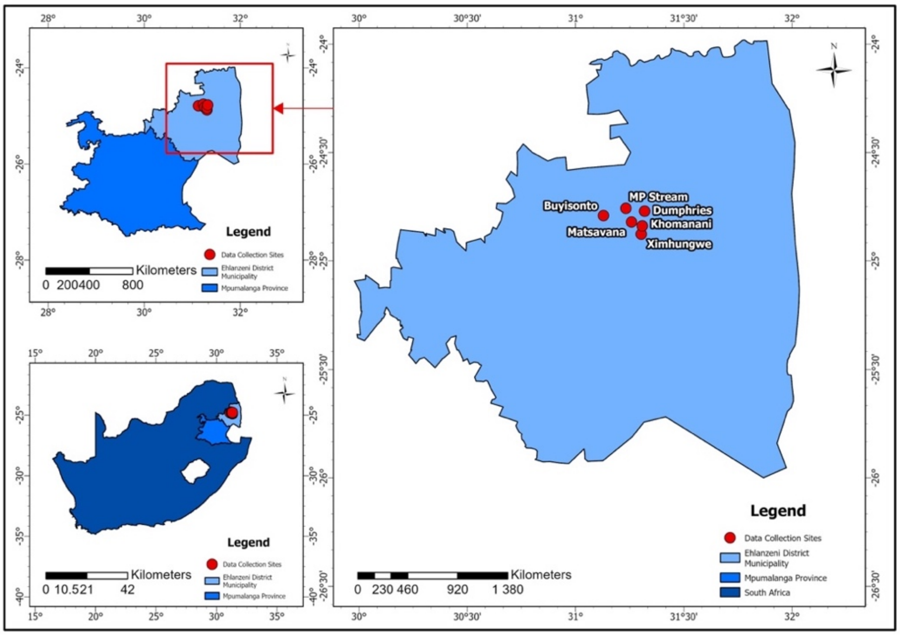

Figure 3.1: Spatial distribution of the six selected study areas in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa.

3.2.2. Data collection

Data was collected in 2024 and 2025, over a period of five to seven months (October - April 2025); within the six study areas of Bushbuckridge Local Municipality, Mpumalanga Province, South Africa (Figure 3.1). Semi-structured interviews, consisting of open-ended questions, were used to solicit detailed accounts of the uses, harvesting practices and cultural significance of *S. spinosa* (Appendix D). A total of 108 participants aged 18 years and above were purposively selected to participate in the study based on their willingness, knowledge and experience with *S. spino*sa. Participants included in the study were community members, herbalists and indigenous knowledge holders residing in the study area. Demographic characteristics, such as sex, level of education and occupation were considered to ensure diverse representation. The interviews were conducted in Xitsonga, SiSwati or Sepedi, and later translated into English.

According to a report by BusinessTrust (2007), Xitsonga is the predominant language in the region and is spoken by 58% of the population in Bushbuckridge Local Municipality. In comparison, 27% of the population speak Sepedi. Focus group discussions were also held with community members to encourage collective dialogue on sustainable practices and concerns regarding the plant. Lastly, key participant observations were conducted during community gatherings and harvesting activities. This helped contextualise the qualitative data and understand the plant’s practical uses and preparation methods.

3.2.3. Data analysis

Descriptive statistics, such as mean, frequency, and percentage, were used to identify and characterise the socio-demographic characteristics of participants. The data was compiled and converted into statistical measures, using established ethnobotanical indices. Two quantitative parameters - Use Value (UV) and Relative Frequency of Citation (RFC) - were used to analyse the data.

Use Value (UV)

The Use Value (UV), proposed by Phillips and Gentry (1993), measures the average number of uses participants know for a species and is calculated as follows:

$$UV_s = \frac{\sum UV_{is}}{n}$$

where: UV_{is} = number of uses informant $_i$ knows for species $_s$.

In a study by (Tardío and Pardo de Santayana, 2008), this index considers the spread of the use (number of participants) for each species along with its adaptability, that is, the diversity of its applications.

Relative Frequency of Citation (RFC)

The Relative Frequency of Citation (RFC) measures plants that were the most frequently mentioned as useful. It is calculated as follows:

$$RFCs = \frac{FCs}{N} = \frac{\sum_{i=i1}^{iN} URi}{N}$$

where: FCs = number of informants who mention the use of the species, also known as frequency of citation; *N* = number of informants participating in the survey. This index does not consider the variable u (use-category). The index theoretically varies from 0, when nobody refers to the plant as useful, to 1 in the unlikely case that all the informants would mention the use of the species(Tardío and Pardo de Santayana, 2008).

Pearson’s chi-square test

All data collected in the ethnobotanical survey was transcribed and subjected to descriptive analysis, using Excel and in-depth analysis processed by System Professional for Social Sciences (SPSS) version 19 software. Pearson’s chi-square test was used to determine whether demographic variables (sex and age) were significantly associated with knowledge categories (culinary, medicinal, combined-use and ornamental). Statistical significance was set at $p < 0.05$.

One-way ANOVA analysis

A one-way Analysis of Variance (ANOVA) was conducted to evaluate the impact of level of education on the diversity of knowledge concerning uses of *S. spinosa*. This method is commonly used in ethnobotany to determine whether the average values of a continuous variable (e.g., Use Value) differ significantly across multiple categorical groups (e.g., various education levels). The Use Value (UV) for each respondent was calculated based on the count of unique uses (culinary, medicinal, ornamental) recognised for *S. spinosa*. Respondents were classified into four education categories: No formal education, Primary School, High School (combined with Middle School), and University (combined with college). Only respondents who provided clear, codable descriptions of their use cases were included in the analysis.

3.3. Results

3.3.1. Demographic overview of participants

A total of 108 participants were interviewed across the six settlements found in Bushbuckridge (Table 3.1), comprising 59 females (55%) and 49 males (45%). The age distribution shows diverse representation, with most participants (81%) falling above the 41-year age bracket. Only 9% of participants were youth under 30 years, who showed limited indigenous knowledge regarding *S. spinosa*. Levels of education of participants varied: 2% no formal education, 3% had primary education, 48% had secondary education, and 47% had tertiary qualifications. Notably, 52% were unemployed.

Table 3.1: Demographic characteristics of participants (n) with knowledge and uses of *S. spinosa* in Bushbuckridge, Mpumalanga Province, South Africa (n=108)

Variable	Frequency	Percentage (%)
Sex		
Male	49	45
Female	59	55
Age (years)		
18 – 24	6	5
25 – 30	4	4
31 – 40	11	10
41 – 50	27	25
51 – 60	32	30
Over 60	28	26
Level of education		
No formal education	2	2
Primary school	3	3
High school	52	48
Tertiary	51	47
Occupation		
Unemployed	56	52
Employed	25	23

Variable	Frequency	Percentage (%)
Self-employed	13	12
Scholar	6	6
Retired	5	5
Undisclosed	3	2
Settlement distribution		
Buyisonto	30	27
Matsavana	20	19
Mp Stream	20	19
Ximhungwe	15	14
Dumphries	13	12
Khomanani	10	9

3.3.2. Patterns of utilisation of knowledge

S. spinosa was primarily used for culinary purposes (38%), followed by 31% for both medicinal and culinary reasons. Twenty-six percent of participants used the plant only for medicinal purposes, 2% used it for ornamental purposes, while 1% used the plants for three separate purposes as follows: ornamental-culinary; ornamental-medicinal-culinary; and ornamental-medicinal (Figure 3.2). Ornamental uses, which were rarely cited, were disproportionately mentioned by male participants, thus potentially reflecting traditional gender roles in crafting and cultural ceremonies.

3.3.3. Influence of sex and age on knowledge patterns

To explore how demographic factors relate to knowledge of *S. spinosa*, chi-square tests of independence was used to analyse observed frequencies of knowledge categories classified according to sex and age (see Table 3.2). The results from these tests revealed a significant association between sex and knowledge category (χ^2= 82.06, df= 3; $p < 0.0001$). The distribution of responses showed that females primarily reported uses related to culinary (41) and medicinal (18), while none mentioned ornamental or combined uses. In contrast, male participants indicated both culinary and medicinal uses (33), with some reporting ornamental applications (6).

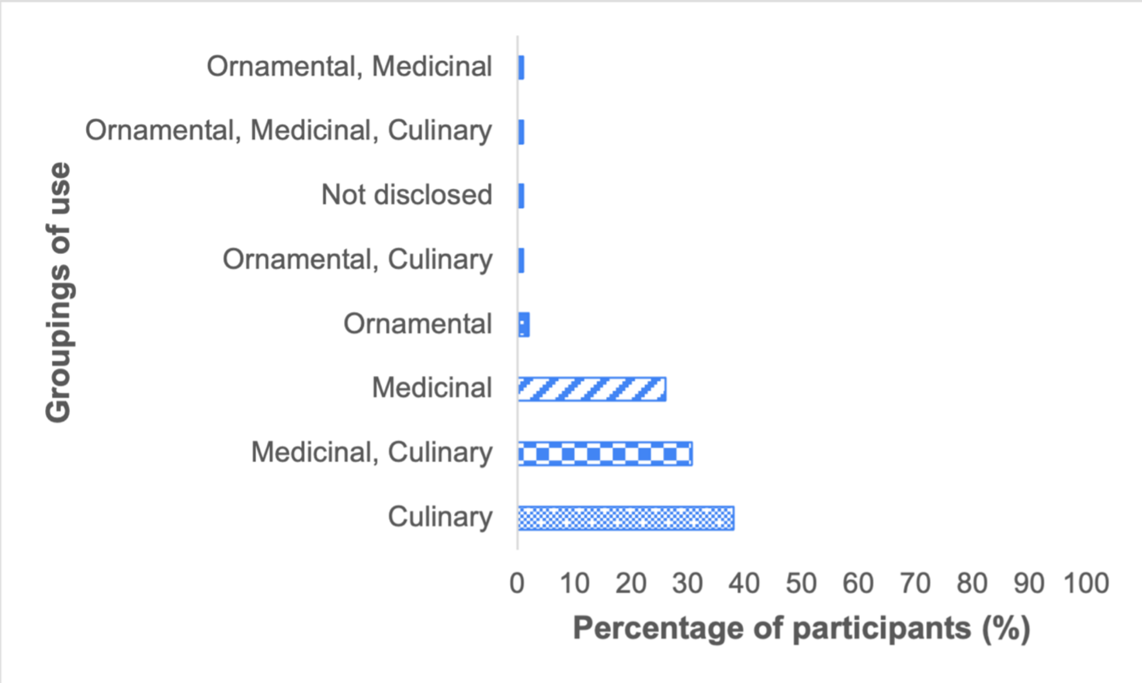

Figure 3.2: Categorisation of use of *S. spinosa* among participants, showing percentages for culinary, medicinal, combined and ornamental purposes based on ethnobotanical survey data in Bushbuckridge, Mpumalanga Province, South Africa

3.3.4. Chi-square

A second chi-square test was used to assess differences between participants under 41 years and those aged 41 and older, revealing a highly significant association (χ^2 = 30.02, df = 3; $p < 0.0001$). Younger participants (<41) exclusively reported combined-use knowledge (10), while the older group (41+) showed a diverse distribution across culinary (41), medicinal (32), combined (19) and ornamental (6) uses (Table 3.2).

Table 3.2: Statistical associations between demographic variables (sex and age) and categories of ethnobotanical knowledge regarding S. spinosa, including Chi-Square values (χ^2), degrees of freedom (df) and p-values (p)

Variable	Combined Uses	Culinary	Medicinal	Ornamental	χ^2	df	p-value
Sex							
Female	0	41	18	0	82.06	3	1.11×10^{-17}
Male	33	0	10	6			
Age group							
*≥41	19	41	32	6	30.02	3	1.37×10^{-6}
*<41	10	0	0	0			

*Age categories were collapsed for inferential analysis (refer to Table 3.1 for original age ranges).

Table 3.3: Different uses of *S. spinosa*, plant parts utilised, methods of preparation, frequency of citations by the 108 participants (N) in Bushbuckridge, Mpumalanga Province and ethnobotanical indices (RFC and UV)

Grouping of use	Specific use	Reference	Plant part	Method of preparation	No. of participants who mentioned use (FC) (%)	RFC = FC/N	UV
Culinary	Edible fruit	(Aremu and Moyo, 2022; Asase et al., 2005; Avakoudjo et al., 2019; Mbhele et al., 2022)	Fruit	Natural form	66 (61)	0.6	2.4
	Jam	(Mbhele et al., 2022; van Rayne, 2021)	Fruit	Macerated			
	Alcoholic beverage	(Mbhele et al., 2022)	Fruit	Aqueous extract			
	Additive in traditional dishes	(Mbhele et al., 2022)	Fruit, stem, root, seed, leaves	Dry extract			

Grouping of use	Specific use	Reference	Plant part	Method of preparation	No. of participants who mentioned use (FC) (%)	RFC = FC/N	UV
Medicinal	Stomach aches	(Aremu and Moyo, 2022; Avakoudjo et al., 2019; Mbhele et al., 2022)	Stem, seed, roots, fruit, leaves	Macerated,	58 (54)	0.5	3.2
	Constipation	(Avakoudjo et al., 2019)	Seed, fruit, stem	Macerated, dry extract			
	Indigestion	(Omotayo and Aremu, 2021)	Seed, fruit, root	Macerated			
	Piles	(Novotna et al., 2020)	Root	Macerated, dry extract			
	Bile issues	-	Seed	Macerated, aqueous extract			
	Erectile dysfunction	(Mhlongo and Van Wyk, 2019;	Fruit, seed	Macerated			

Grouping of use	Specific use	Reference	Plant part	Method of preparation	No. of participants who mentioned use (FC) (%)	RFC = FC/N	UV
		Tittikpina et al., 2020)					
Ornamental	Cultural significance	(Avakoudjo et al., 2019)	Whole plant, root, latex, fruit	Natural form, poultice	11 (10)	0.1	0.2
	Wood carvings	(Magwede et al., 2019)	Stem, fruit	Natural form			

3.3.5. Plant parts used and preparation methods

Table 3.3 shows seven distinctive *S. spinosa* plant parts used for different purposes in Bushbuckridge. The fruit was the most commonly used part of the plant, closely followed by the seeds. The stem, roots, and leaves were also frequently used, though to a lesser extent. Latex and flowers were the least commonly used plant parts. The most common applications include using the plant's fruit to make jam, various plant parts for treating conditions like piles and indigestion, and the whole plant in cultural ceremonies.

Maceration was the most commonly used preparation method. Aqueous and dry extracts were also common, although they were secondary to maceration. Various plant parts of *S. spinosa*, in their natural form, were occasionally used but less frequently than methods, such as maceration or aqueous extraction. The least commonly mentioned preparation method was the poultice.

3.3.6. Ethnobotanical indices

Medicinal use recorded the highest UV of 3.2, followed by culinary use (UV of 2.4) and ornamental use (UV of 0.2). The average UV across all use categories was 1.9, and the RFC was 0.4, indicating the cultural and practical significance of *S. spinosa* (Table 3.3).

A one-way ANOVA was performed to assess the Use Value (UV) of *S. spinosa* among different levels of education in Bushbuckridge, Mpumalanga Province, South Africa (Table 3.4). The analysis revealed a statistically significant variation in UV scores across groups ($F(3, 103) = 2.83$, $p = 0.042$), with high school-educated participants showing the most diverse uses (Mean UV = 1.44). Participants with university education had a mean UV of 1.22, and those with minimal formal education recorded the lowest UV.

Table 3.3: Summary of the average Use Value (UV) scores of knowledge of *S. spinosa* among participants in Bushbuckridge, Mpumalanga Province, South Africa

Level of education	N*	Mean UV	Std Dev	Min UV	Max UV
High School	52	1.44	0.57	0	3
University	50	1.22	0.46	0	2
No formal education	2	1	0	1	1
Primary School	3	1	0	1	1

N: Number of education group participants with a valid, usable UV score*

3.3.7. Sourcing and harvesting practices

Approximately 93% of participants sourced *S. spinosa* from local bush environments, with 6% cultivating *S. spinosa* while 1% purchased the plant (Table 3.5). The average quantity harvested per participant was approximately 5kg per month. However, variations were observed depending on seasonal availability. Harvesting tools predominantly included were machetes and traditional digging sticks.

3.3.8. Market dynamics and economic considerations

A total of 107 participants, representing 99% of the participants, sold *S. spinosa* products locally, with an average market price of R30 per cup (Table 3.5). The average pricing was consistent across the demographic groups, indicating that pricing is likely determined by local market norms rooted in the shared cultural valuation of *S. spinosa* rather than individual characteristics such as age, gender or education. Field observations and participant interviews revealed that harvesting of *S. spinosa* is primarily for local consumption and small-scale trade, rather than for industrial or pharmaceutical supply chains. The fruit is commonly sold in informal community markets, along roadsides, and within households, with minimal involvement from formal commercial sectors. Although harvesting for personal use dominates, overall local demand during fruiting seasons places noticeable pressure on wild populations, particularly where fruit trees are close to human settlements.

3.3.9. Environmental awareness and concerns

As shown in Table 3.5, 41% of participants considered *S. spinosa* rare in their environment, 31% viewed it as abundant, while 28% regarded it as common. Concurrently, 71% expressed concerns about its dwindling availability over the past decade. Over 46% of participants reported insufficient conservation measures. Participants indicated significant awareness regarding the long-term impacts of human activities on the environment. Over 80% of participants acknowledged that urbanisation, agriculture, and climate change, challenged the sustainable availability of *S. spinosa* and other indigenous plant species.

Table 3.4: Summary of practices and perceptions of the 108 participants on sourcing, harvesting, marketing and conservation of *S. spinosa* in Bushbuckridge, Mpumalanga Province, South Africa

Category	Findings
Sourcing of plant material	101 sourced from local bush; 7 cultivated; 1 purchased
Quantity of harvest	Average 5kg per participant per month (seasonal variations noted)
Harvesting tools	Predominately machetes and traditional digging sticks
Market participation	107 sold portions of harvest plant material
Market price	Average of R30 per cup
Perceived plant abundance	44 rare; 34 abundant; 30 common
Concerns over decline in availability	77 noted a decline over the last decade
Conservation awareness	50 reported insufficient conservation measures
Environmental threats identified	87 linked decline to urbanisation, agriculture and climate change

3.4. Discussion

The findings confirm the report by BusinessTrust (2007) that females represent 53% of the total population in Bushbuckridge, Mpumalanga Province, South Africa and account for 56% of heads of households. The findings further confirm that IK of *S. spinosa* remains prevalent among older generations but is declining among the youth. This difference suggests that younger participants may not possess older individuals' in-depth or specialised knowledge. The findings point to the concentration of detailed ethnobotanical knowledge among older adults and correspond with established research on the decline of IK due to shifting socio-cultural dynamics, urbanisation and reduced engagement in traditional practices (Dahlberg and Trygger, 2009; Hunter et al., 2014; Kumar et al., 2021; Stepp, 2018). This further aligns with studies by Nxusani et al. (2023) and Dovie et al. (2008) which revealed that younger generations often possess less detailed knowledge of indigenous plants compared to older individuals, highlighting a generational shift in knowledge acquisition. The high unemployment confirms the views highlighted in the study by MacGinty (2024), whereby graduate unemployment continues to be challenging, especially in rural areas where opportunities are scarce.

The deviation from expected figures - where males were anticipated to report 15 combined-use cases but reported 33 - indicates that sex plays a significant role in the type of ethnobotanical knowledge possessed. This reinforces the view that societal gender roles affect plant knowledge, aligning with findings by Constant and Tshisikhawe (2018) regarding knowledge hierarchies in traditional societies and further supported by research emphasising male participation in ritualistic and artisanal plant uses (Howard, 2003; Thomas et al., 2009).

The skewed distribution of *S. spinosa* uses supports arguments made by Stepp (2018) that ecological knowledge often reflects both practical use and symbolic meaning, especially within specific societal roles. This further aligns with the literature on the plant's cultural and ceremonial roles in African traditions (Bushwise, 2022; Hunter et al., 2014; Zenebe et al., 2012). Historical IKS in Bushbuckridge reflect long-standing community-based conservation ethics (Shackleton et al., 2000). Traditional practices, such as rotational harvesting, seasonal restrictions, and taboos against collecting unripe fruits, have functioned as informal yet effective conservation mechanisms that regulate plant use and regeneration (Constant and Tshisikhawe, 2018). Elders emphasised respect-based stewardship, whereby certain families or clans acted as custodians of fruit trees, ensuring equitable access and intergenerational resource protection. Similar patterns of customary governance and local management have been documented in other rural South African contexts (Constant and Tshisikhawe, 2018; Dovie et al., 2008; Shackleton et al., 2000). Collectively, these practices align closely with the principles of community-based natural resource management (CBNRM) and demonstrate the continued relevance of Indigenous ecological ethics in modern conservation planning.

According to Matlala et al. (2024), Zenderland et al. (2019) and Kalita et al. (2024), the value of a plant species can differ according to its cultural, economic and ecological importance to a community. Use Value scores typically range from 0 to higher values, generally between 1 and 5, though scores can exceed this range. A higher Use Value signals a considerable degree of usefulness, reflecting that the plant fulfils various roles in the community, including culinary, medicine and cultural rituals. Although some overlap occurred among participants citing multiple use categories in Bushbuckridge, the mean RFC provided a representative measure of the plant's general recognition within the community.

Various studies have also revealed that a high RFC value suggests that local communities successfully transfer traditional knowledge and that the plant species may be readily available in the region, highlighting its regular and extensive use (Derya et al., 2023; Hilonga et al., 2018; Masumbu et al., 2023; Matlala et al., 2024). The diverse application of *S. spinosa,* highlighted through the high UV and RFC scores, demonstrates the plant's importance in Bushbuckridge for nutrition, health and culture (Aremu and Moyo, 2022; Mbhele et al., 2022a; Tittikpina et al., 2020).

Several participants confirmed using various parts of the plant together, demonstrating a holistic treatment approach (Dahlberg and Trygger, 2009). The combination of fruit and seed was widespread, highlighting their therapeutic significance (Aremu and Moyo, 2022). Furthermore, some preparations included three or more plant parts, indicating complex traditional knowledge within herbal medicine (Dahlberg and Trygger, 2009).

The variety of preparation methods suggests a profound indigenous understanding of plant effectiveness and usage. The choice of macerations and aqueous extracts corresponds with traditional medicine practices, prioritising extract-driven remedies (Mbhele et al., 2022a; Omotayo and Aremu, 2021). Additionally, incorporating seeds and fruits, highlights their significance in addressing numerous health issues.

The impact of education on IK is complex. Traditional plant knowledge may not necessarily increase with more education and might peak at certain levels of formal education. Several studies suggest a negative correlation between formal education and TEK. For instance, research on the Waorani communities in Ecuador and the Baiga in India, revealed that increased schooling correlated with decreased medicinal plant knowledge (Weckmüller et al., 2019). However, this trend is not universal. In Western Nigeria and parts of Brazil, studies revealed weak or no correlations (Bruyere et al., 2016; Trimarco, 2016), while others, like a study from Indonesia, suggests that integrating TEK into the curriculum could help preserve it (Rinto et al., 2023). This study, which shows a statistically significant difference in UV according to level of education, contributes to this broader conversation, suggesting that while formal education may reduce reliance on traditional knowledge, the relationship is complex and context dependent.

The findings highlight a heavy reliance on wild harvesting, using traditional tools, similar to studies by Omotayo and Aremu (2021) and Ngadze et al. (2016). The findings also align with a study by Constant and Tshisikhawe (2018), in which many participants from the Vhavenda community indicated that they collected medicinal plants from surrounding bushlands. Traditional tools were commonly used during the harvesting process, and seasonal changes influenced quantities gathered, as seen in various regions where *S. spinosa* is predominantly harvested from surrounding bushlands in KwaZulu-Natal Province (Mbhele et al., 2022a). Studies on African rural plant markets show that pricing of harvested plant material is often standardised within community frameworks to maintain harmony and equity (Avakoudjo et al., 2019; Hilonga et al., 2018).

There is a perceived significant decline in the plant's abundance, which cannot be attributed solely to harvesting pressure, as both respondents and existing literature indicate multiple factors, including habitat loss, climate change, or shifts in land use contribute to the decline (Avakoudjo et al., 2019; Georgiadis, 2022; Stepp, 2018) These factors collectively threaten the regeneration of *Strychnos* species and the ecological balance of the savanna landscapes where the species occurs (Avakoudjo et al., 2019). A considerable proportion of respondents acknowledged this trend, which suggests that the species may face ecological stress. This finding aligns with research highlighting global biodiversity decline driven by climate change and unsustainable land practices (Georgiadis, 2022; Stepp, 2018). Participants' awareness of environmental threats, such as climate change and urbanisation, indicates a readiness to engage in conservation, if supported.

Studies have highlighted that traditional ecological knowledge can inform sustainable natural resource management and emphasise the role of indigenous practices in biodiversity conservation, as well as challenges posed by modern development (Abdullah and Khan, 2023; Constant and Tshisikhawe, 2018; Jessen et al., 2022; Kumar et al., 2021; Sinthumule, 2023). A study by Georgiadis (2022) also underscores the importance of integrating ethnobotanical knowledge into conservation strategies. The heavy reliance on wild harvesting and the perception of resource decline, emphasise the need for sustainable harvesting strategies and community-driven conservation. Experimental work by Ngadze (2018) demonstrated that *S. spinosa* can be propagated successfully under nursery conditions using seed pre-treatments and proper management, while Mbhele et al. (2022b) highlighted substantial morphological and genetic variation that could underpin breeding and domestication efforts. Expanding such research and investment in cultivation could help reduce harvesting pressure on wild populations.

While *S. spinosa* is ecologically and culturally significant, there are no government programmes explicitly dedicated to its conservation (SANBI, 2023). The species benefits indirectly from broader national frameworks, such as the National Biodiversity Strategy and Action Plan (NBSAP) and the National Protected Areas Expansion Strategy (NPAES), which aim to safeguard savanna and woodland ecosystems where *S. spinosa* naturally occurs (SANBI, 2023). Conservation at the species level currently depends on community-based initiatives and traditional ecological knowledge practices, underscoring the need for policy integration and targeted resource management support (Mbelebele et al., 2024)

The findings of this study have significant implications for policy development and practical implementation in biodiversity conservation and education. The reliance of local communities on *S. spinosa* for food, medicine, and cultural purposes underscores the need for community-based natural resource management approaches that actively involve IK holders in decision-making processes. Incorporating IK into local conservation strategies and municipal by-laws could enhance compliance, promote sustainable harvesting, and strengthen stewardship at the community level.

Furthermore, the study highlights the potential value of integrating TEK into formal and non-formal environmental education curricula, as recommended by Dlutu (2025) and da Silva et al. (2023). Embedding IK in education systems can foster intergenerational knowledge transmission, enhance learners' sense of environmental responsibility, and ensure that conservation initiatives remain culturally relevant and socially inclusive. As argued by Mbelebele et al. (2024), harmonising traditional knowledge with modern sustainability frameworks can bridge gaps between scientific and local worldviews, ultimately contributing to both biodiversity conservation and socio-ecological resilience.

Feasible conservation strategies for *S. spinosa* include community-led cultivation, agroforestry integration, and ex situ propagation of seedlings. Reviews and development frameworks on indigenous fruit trees show that domestication and farmer-managed agroforestry systems can sustain supply and livelihoods while conserving genetic resources (Leakey and Akinnifesi, 2017). According to Omotayo and Aremu (2021), the propagation of *S. spinosa* under managed conditions is feasible, and the process from seed to fruiting can take approximately three to five years, which supports nursery-based seedling production for home gardens and local reforestation programmes (Omotayo and Aremu, 2021). Community and school-garden models, alongside ex situ propagation, have already been used in South Africa to mainstream useful native plants at local sale, demonstrating pathways relevant to *S. spinosa* roll-out (Ulian et al., 2015) Conservation planning should also align with the Nagoya Protocol and South Africa's biocultural heritage framework, ensuring equitable benefit-sharing and protection of community-held knowledge (Diversity, 2011).

The Department of Forestry, Fisheries and the Environment (DFFE) and the South African National Biodiversity Institute (SANBI) contribute indirectly to the conservation of *S. spinosa* through ecosystem-level management, woodland rehabilitation, and biodiversity stewardship programmes (Department of Forestry, 2016; Department of Forestry Fisheries and the Environment., 2023; SANBI, 2023). Current national frameworks such as the National Biodiversity Strategy and Action Plan (2015 – 2025) and SANBI's stewardship and restoration initiatives focus on protecting savanna and woodland ecosystems rather than individual non-threatened species. According to SANBI (2024), *S. spinosa* is categorised as Least Concern, and no specific management plan exists. Greater government support is therefore required to mainstream this species into local environmental management and sustainable-use initiatives that combine ecosystem-based conservation with community-led stewardship.

3.5. Limitations and future research

While this study provides valuable insights into the ethnobotanical knowledge and use of *S. spinosa* in Bushbuckridge, several limitations should be acknowledged. The reliance on purposive sampling may limit the generalizability of the findings to broader populations, as participants were selected based on their prior knowledge of the species. In addition, the self-reported nature of ethnobotanical data introduces potential response bias, as participants may over- or understate specific uses or practices. The study did not include ecological population assessments of *S. spinosa*, which would be essential for quantifying species abundance, regeneration rates, and the ecological impacts of harvesting.

Future research should incorporate longitudinal ecological monitoring to track population trends of *S. spinosa* under changing environmental and socio-ecological conditions. Comparative studies across multiple provinces or bioregions are recommended to explore regional variations in traditional use, conservation awareness, and management practices. Such interdisciplinary approaches would deepen understanding of how IKS can support both sustainable utilisation and biodiversity conservation across southern Africa.

3.6. Concluding remarks

This chapter highlights the deep connection of the Bushbuckridge community with *S. spinosa*. Participants rely on this plant for its nutritional, medicinal and economic benefits. However, the findings reveal a sharp decline in availability due to habitat loss, overharvesting and climate change. Participants in the study confirmed the limited conservation efforts, further exacerbating sustainability challenges.

There is a need for a multifaceted approach to address the long-term conservation of *S. spinosa*. The community should be empowered to spearhead conservation initiatives that encourage sustainable harvesting and cultivation practices, such as establishing community nurseries to support propagation and replanting, enforcing local harvesting quotas, and running awareness campaigns on sustainable use. Additionally, integrating the species into agroforestry and household garden systems and fostering collaboration among municipalities, SANBI, and traditional councils will help embed IK into formal conservation frameworks. Such coordinated efforts would improve ecological sustainability while strengthening community empowerment.

Moreover, education and awareness campaigns can play a vital role in passing down knowledge about this plant from one generation to the next, fostering greater community involvement in conservation. Lastly, more research is needed to understand the ecological impacts of using *S. spinosa* and to develop effective propagation strategies. By blending traditional knowledge with contemporary conservation practices, communities can ensure that *S. spinosa* is protected for future generations, while supporting local people's livelihoods.

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Chapter 4: Molecular authentication of medicinal samples in Bushbuckridge, Mpumalanga Province, South Africa: A DNA barcoding approach to identify *Strychnos* species from processed or poorly conserved market samples

4.1. Introduction

Traditional medicine is an essential component of primary healthcare for many communities in South Africa, particularly in rural and peri-urban areas where access to conventional medical services may be limited or unaffordable (Mander et al., 2007; Thipanyane et al., 2022; Zuma et al., 2016). Over 70% of South Africans are estimated to rely on traditional remedies, with the traditional medicine trade forming a crucial part of both cultural identity and the informal economy (Mashigo et al., 2024; Mothibe and Sibanda, 2020; World Health Organization., 2013; Zuma et al., 2016). However, the authenticity and safety of herbal medicines are increasingly challenged by overharvesting, misidentification, and substitution with morphologically similar or toxic species (Fitzgerald, 2023; Jităreanu et al., 2023; Ozioma and Chinwe, 2019; Street et al., 2008; World Health Organization., 2007; Xaba et al., 2022).

One region where traditional plant use is prevalent, is Bushbuckridge, located in Mpumalanga Province (Chauke et al., 2025; Potgieter, 2015). This area is a repository of plant biodiversity and a significant source of medicinal material that feeds into local and national muthi markets (Chauke et al., 2025; Manzini, 2005). Despite its ecological and ethnobotanical importance, Bushbuckridge remains uncharacterised mainly at the molecular level (Chauke et al., 2025; Tamburini et al., 2022). Most previous efforts, such as those conducted at the Faraday Medicinal Market in Johannesburg by Mankga et al. (2013), have focused on urban trade hubs, leaving a knowledge gap in rural collection and usage contexts (Rattray et al., 2024; Williamson et al., 2016).

Various studies revealed that harvesting of medicinal plants was traditionally carried out by trained traditional health practitioners (THPs) who adhered to indigenous conservation practices (David, 2008; WHO-AFRO, 2010; Zuma et al., 2016). However, over the past two decades, studies indicate a shift in this dynamic. The collection and trade of medicinal plants have expanded beyond traditional healers, becoming integral to South Africa’s informal economy (Kurui et al., 2016; Ouma, 2022). This expansion has resulted in increased participation from individuals seeking income through the commercialisation of natural resources (Mander et al., 2007; Mofokeng et al., 2022).

This growing demand and reliance on wild-sourced material have placed increasing pressure on native plant populations (Mofokeng et al., 2022). Overharvesting has been identified as a significant threat to biodiversity, elevating the extinction risk for numerous species and contributing to the depletion of widely used medicinal plants (Chen et al., 2019; Nenungwi et al., 2025). Loundou (2008) and Williams et al. (2013) highlighted how species, such as *Ocotea bullata*, *Warburgia salutaris*, and *Bowiea volubilis*, once considered abundant, are now listed as threatened due to unsustainable harvesting practices. Additionally, other species, including *Cassine transvaalensis* and *Erythrophleum lasianthum* are becoming increasingly vulnerable due to overexploitation (Grace et al., 2003; Williams et al., 2013). These developments underscore the urgent need for enhanced regulatory frameworks and sustainable resource management strategies to conserve South Africa's medicinal plant diversity (Department of Forestry Fisheries and the Environment., 2023).

The genus *Strychnos* is a pantropical group within the Loganiaceae family (Gentianales), consisting of about 200 species, 75 of which are found in tropical Africa and have relatively uniform generic traits (Setubal et al., 2021). The species is commonly utilised in traditional Southern African medicine for various ailments, including digestive issues, respiratory problems and fevers (Mbhele et al., 2022; Thothela et al., 2025; Tittikpina et al., 2020).

Despite this, some *Strychnos* species, notably *S. nux-vomica*, contain potentially harmful alkaloids like strychnine, highlighting the importance of accurate taxonomic identification (Guo et al., 2018; Maji and Banerji, 2017; Olivier et al., 2022; Tiwana et al., 2024). Relying solely on morphological features is often inadequate, given that many market samples are sold in dried, powdered or seed forms that lack diagnostic morphological features (Haider, 2011; Kool et al., 2012). This limitation highlights the importance of developing effective techniques to support and enhance traditional plant identification, which is crucial for preserving biodiversity, traditional knowledge related to these plants (Mamathaba et al., 2022; Mankga et al., 2013), and ensuring human health (Amin et al., 2020).

Molecular studies on the genus *Strychnos* remain limited, despite the ethnobotanical and pharmacological relevance of the genus. Most published work has focused on phylogenetics using fresh or herbarium specimens, including global ITS-based phylogenetic reconstructions (Setubal et al., 2021) and southern African species-level analyses (Adebowale, 2014; Adebowale et al., 2016a; Adebowale et al., 2016b), which examined species boundaries and clade structure across approximately 48 African taxa. However, these studies did not address commercial herbal products nor the authenticity of traded material.

To date, no research has applied DNA barcoding to verify *Strychnos* products sold in rural medicinal markets in South Africa. This represents a critical gap, as visually degraded samples and processed powders are common in traditional trade and are highly prone to substitution or misidentification (Adebowale et al., 2016a; Adebowale et al., 2016b; CBOL Plant Working Group., 2009; Setubal et al., 2021). Therefore, molecular verification of market material is urgently needed to complement taxonomic studies, support safe use of traditional medicine, and monitor wild-harvested species under increasing commercial pressure.

DNA barcoding is considered a reliable approach for verifying the identity of species, regardless of the physical condition of the sample (Harris et al., 2024; Hebert et al., 2003; Mankga et al., 2013). This method entails sequencing short, standardised genetic markers, like the officially recommended two-locus barcode complementary combination for land plants plastid genes, ribulose bisphosphate carboxylase large chain (*rbcLa*) and Maturase K (*matK*), which can theoretically differentiate even closely related species (CBOL Plant Working Group., 2009; Cheng et al., 2023; Farha et al., 2022; Hebert et al., 2003; Lahaye et al., 2008). *rbcLa* provides a highly universal, conserved region that amplifies reliably across angiosperms, including degraded or processed herbal material, while *matK* offers greater nucleotide variation suitable for species-level discrimination (Hollingsworth et al., 2011; Mankga et al., 2013; van der Bank et al., 2016; Yu et al., 2021).

The use of the *rbcLa*- *matK* mutlilocus plastid system has been widely validated in medicinal plant authentication and in resolving species boundaries in complex genera such as *Strychnos* (Adebowale, 2014; Adebowale et al., 2016b). Alternative markers, such as the second internal transcribed spacer region (ITS2), although highly variable, often fail to amplify from degraded samples and are prone to fungal coamplification and paralogy issues (Chen et al., 2010). Intergenic spacers such as trnH-psbA exhibit alignment difficulties and high length polymorphism (Kress et al., 2009).

Although previous phylogenetic studies have clarified species boundaries in southern African *Strychnos* (Adebowale, 2014; Adebowale et al., 2016b; Setubal et al., 2021), none have investigated traded herbal material. *Strychnos* species vary markedly in phytochemistry, with some taxa producing toxic strychnine-type alkaloids (*S. nux-vomica, S. icaja*). In contrast, other species, such as *S. spinosa,* are culturally important edible species (Leeuwenberg, 1969). Misidentification has implications for safety, pharmacovigilance, and conservation. Therefore, molecular authentication of market samples is essential for validating species use patterns, monitoring adulteration risks, and identifying conservation-relevant harvest pressure in rural trade systems.

DNA barcoding and phylogenetic analysis were used in this study to authenticate poorly preserved and processed *Strychnos* samples collected at herbal markets in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa. This work provides the first molecular documentation of *Strychnos* trade in rural Mpumalanga, contributing new data for biodiversity monitoring, pharmacovigilance and the implications for IKS.

4.2. Materials and methods

4.2.1. Sample collection

Six market samples were purchased in herbal markets in Bushbuckridge, Mpumalanga Province, South Africa (Figures 4.1 and 4.2). The common name of the market samples was used to confirm the purchase of the species under investigation. The samples included various fruit plant parts, such as whole fruits and seeds. The market samples were sometimes poorly stored and/or in processed states (Figure 4.3), such as powdered material, making it impossible to assign scientific names to the samples at time of purchase.

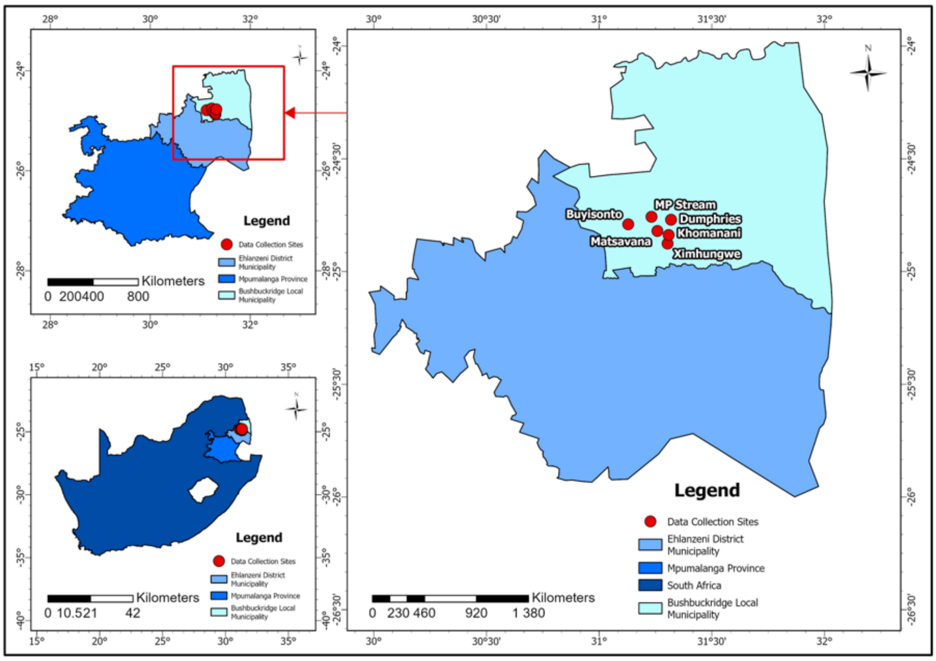

Figure 4.1: Spatial distribution of the six areas where market samples were sourced in Bushbuckridge, Mpumalanga Province, South Africa. Map was produced using GIS software and administrative boundary data from GADM (version 4.1)

Figure 4.3: *Strychnos* herbal market samples collected in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa. Samples 1-2: Seeds; Samples 3-4, 6: Fruit; Sample 5: Processed powder

Figure 4.2: Storage state of some of the *Strychnos* samples found in herbal markets in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa

4.2.2. DNA extraction and PCR amplification

All the market samples collected for the study were sent to the African Centre for DNA Barcoding (ACDB) in Johannesburg, South Africa, where total DNA was extracted, and the two core DNA barcodes (*mat*K and *rbc*La) amplified and sequenced according to ACDB protocols. DNA extraction was performed using the 2× CTAB method (Doyle and Doyle, 1987). Polyvinyl pyrrolidone (2% PVP) was added to reduce the effect of high polysaccharide concentrations in the samples. After precipitating the DNA with 100% ethanol, it was stored at -20 °C for a minimum of two weeks (Fay et al., 1998). DNA extracts were purified using QIAquik silica columns (Qiagen Inc., Hilden, Germany) according to the manufacturer's protocol.

PCR amplification was performed for both genes, using ReadyMix Mastermix (Advanced Biotechnologies, Epson, Surrey, UK). Bovine serum albumin (BSA) (3.2%) was added to all reactions to stabilise enzymes, minimise secondary structure issues, and enhance annealing (van Pelt-Verkuil et al., 2008). PCR amplification was performed with the GeneAmp PCR System 9700 machines. The PCR programmes used were as follows: (a) for *rbc*La, pre-melt at 94 °C for 60 seconds, denaturation at 94 °C for 60 seconds, annealing at 48 °C for 60 seconds, extension at 72 °C for 60 seconds (for 28 cycles), followed by a final extension at 72 °C for 7 minutes; and (b) for *mat*K, the protocol involved pre-melt at 94 °C for 3 minutes, denaturation at 94 °C for 60 seconds, annealing at 52 °C for 60 seconds, extension at 72 °C for 2 minutes (for 30 cycles) and a final extension at 72 °C for 7 minutes.

Table 4.1: PCR primers utilised for amplification and sequencing of the rbcLa and matK loci in Strychnos market samples from Bushbuckridge Local Municipality, Mpumalanga Province, South Africa.

Locus	Primer	Primer sequence (5'-3')	Reference
matK	*matK-472F*	CCCRTYCATCTGGAAATCTTGGTTC	(Yu et al., 2011
matK	*matK-1248R*	GCTRTRATAATGAGAAAGATTTCTGC	(Yu et al., 2011
rbcLa	*rbcLa-F*	ATGTCACCACAAACAGAGACTAAAGC	(Levin et al., 2003)
rbcLa	*rbcLa-R*	GTAAAATCAAGTCCACCRCG	(Levin et al., 2003)

4.2.3. Sequencing and data analysis

Cycle sequencing reactions were performed in a GeneAmp PCR System 9700 thermal cycler using the ABI PRISM® BigDye® Terminator v3.1 (Applied Biosystems, Inc., California, USA). Cycle sequencing products were precipitated in ethanol and sodium acetate to remove excess dye terminators. They were then suspended in 10 µl HiDi formamide (ABI) before sequencing on an ABI 3130 xl Genetic Analyzer (ABI). Complementary strands were assembled and edited using Sequencher v3.1 (Gene Codes, Ann Arbor, Michigan, USA) (Daru et al., 2017).

All generated sequences were manually cleaned and aligned in PAUP* v4.0b10 (Mankga et al., 2013). The DNA sequences obtained from the degraded and poorly conserved market samples were BLASTed against the NCBI GenBank database of medicinal plants accessible through the BOLD system (Nakazato and Jinbo, 2022). Only sequences with ≥99% identity and complete query coverage were considered trustworthy identifications (Mankga et al., 2013; Wu et al., 2021).

4.2.4. Phylogenetic reconstruction

To validate species identities beyond BLAST similarity, a phylogenetic framework was constructed using the combined *rbcLa* + *matK* dataset. All sequences generated in this study were aligned with reference *Strychnos* accessions obtained from GenBank (Appendix B). Alignments were performed in Geneious v11.1.5 and checked manually to ensure positional homology (Adebowale, 2014; Adebowale et al., 2016b; Setubal et al., 2021).

Bayesian Inference (BI) analyses were performed in MrBayes v3.2.7 (Ronquist et al., 2012) implemented in Geneious v11.1.5. The best evolution model, GTR+I+G, was selected based on the Bayesian Information Criterion (BIC) in jModelTest 2.1.10 (Darriba et al., 2012). Analyses were performed with 5 million generations, sampling every 200 generations. Convergence was assessed by examining the average standard deviation of split frequencies (<0.01) and by confirming that all parameters reached effective sample sizes (ESS > 200) in Tracer v1.7.2 (Rambaut et al., 2018). The initial 25% of trees were discarded as burn-in before constructing the majority-rule consensus tree. Posterior probability (PP) values ≥0.90 were considered strong support.

Maximum Parsimony (MP) analyses were conducted in PAUP* v4.0a, using a heuristic search with Tree Bisection–Reconnection (TBR) branch swapping, 1,000 random addition sequence replicates, and retention of multiple equally parsimonious trees.

Bootstrap support was calculated using 1,000 bootstrap replicates, with values ≥70% interpreted as supported nodes (Daru et al., 2013; Mankga et al., 2013). Two Gentianales species, *Gelsemium elegans* and *Gardneria multiflora*, were included as outgroups following previous *Strychnos* phylogenetic studies ((Adebowale et al., 2016b; Setubal et al., 2021).

4.3. Results

4.3.1. BLAST identification

Results from the BLAST analysis of medicinal market samples from Bushbuckridge for the *rbcLa* and *matK* markers confirmed high sequence identities for known *Strychnos* species (Table 4.1). The *rbcLa* sequences exhibited a 99.8% identity with *S. madagascariensis*, 99.7% with *S. innocua* and 100% with an unresolved *Strychnos sp.* CP180. Sequences from *matK* confirmed identifications of *S. xanthoclada* (99.6%), *S. madagascariensis* (99.7%) and *S. innocua* (99.8%).

Table 4. 2: Summary of results of DNA barcoding for Strychnos market samples from Bushbuckridge using rbcLa and matK markers

Sample type	Marker	Top GenBank match (Accession No.)	Species identified	Identity percent age	Match confidence
Seed	*rbcLa*	MN216454.1	*Strychnos sp. CP180*	100%	High
Fruit	*rbcLa*	EU213654.1	*Strychnos madagascariensis*	99.8%	High
Fruit	*rbcLa*	KU681041.1	*Strychnos innocua*	99.7%	High
Fruit	*matK*	JX517383.1	*Strychnos xanthoclada*	99.6%	High
Seed	*matK*	MW504166.1	*Strychnos innocua*	99.8%	High
Powder	*matK*	EU214297.1	*Strychnos madagascariensis*	99.7%	High

Note: Only BLAST matches with ≧99% identity and full query coverage were considered valid for authentication.

Table 4. 3: Comparison of marker performance for identification of Strychnos species in market samples

Marker	Amplification success	Reliable matches (≧99%)	Unique species identified	Observed ambiguities	Comments
rbcLa	100% (3/3)	3	*Strychnos sp*. CP180, *Strychnos madagascariensis*, *Strychnos innocua*	None	High amplification success and reliable alignment; provides genus-level information but has limitations in distinguishing closely related species.
matK	100% (3/3)	3	*Strychnos madagascariensis, Strychnos innocua, Strychnos xanthoclada*	None	Increased nucleotide variability allowed for a more detailed resolution among closely related species within the same genus.
Combined	100% (6/6)	6	4 total species	None	The dual-marker strategy improved both resolution and confidence in species authentication.

4.3.2. Phylogenetic validation

The BI phylogeny generated from the combined *rbcLa* + *matK* dataset produced a well-resolved topology that clearly distinguished major *Strychnos* lineages (Figure 4.4) and confirmed the placement of all Bushbuckridge samples. All six market samples (Sample001 - Sample006) clustered closely within a single *Strychnos madagascariensis* clade with Posterior probability (PP) value of 0.83, alongside verified GenBank voucher sequences RL1433 and RL1460 (Appendix E). None of the samples grouped with other southern African species, such as *S. spinosa*, *S. decussata*, *S. innocua*, *S. xanthoclada* or *S. pungens*, suggesting that the traded material represents a single taxon with no signs of adulteration or species substitution. Outgroup taxa (*Gelsemium elegans* and *Gardenia multiflora*) were correctly positioned outside the *Strychnos* clade, as expected, confirming proper rooting of the phylogeny.

As seen in Figure 4.5, the Maximum Parsimony (MP) bootstrap consensus tree produced a topology that complements the BI analysis. All Bushbuckridge samples again formed a coherent monophyletic group with *S. madagascariensis*, supported by strong bootstrap values (≥91%). No sample showed affinity to other *Strychnos* species in the reference dataset, and the MP tree similarly identified major species clusters such as *S. panamensis*, *S. toxifera*, *S. usambarensis*, *S. brasiliensis*, and *S. decussata* as distinct, well-supported lineages.

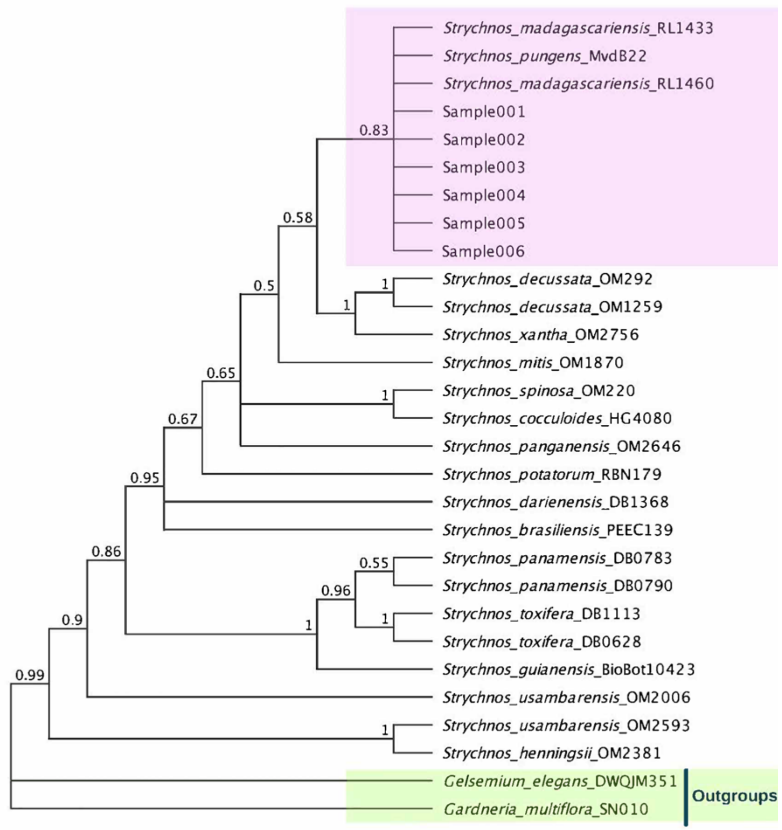

Figure 4.4: Bayesian Inference (BI) phylogeny of *Strychnos* species inferred from combined *rbcLa* + *matK* dataset.

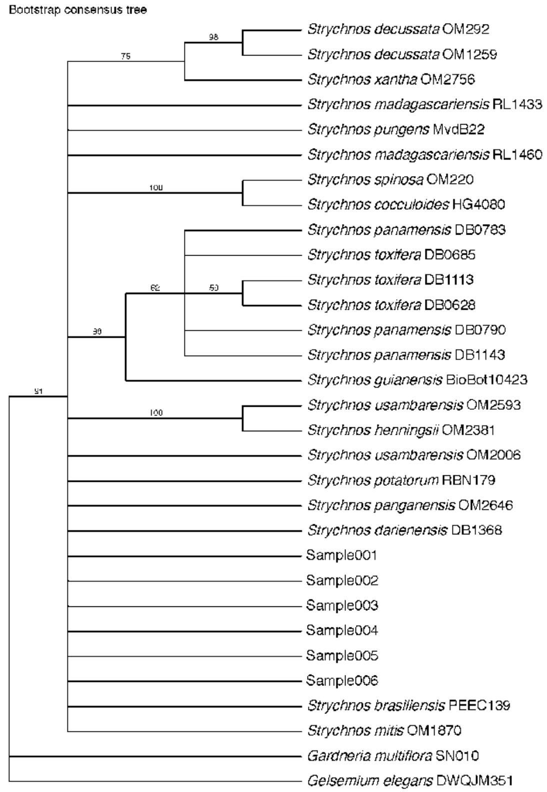

Figure 4.5: Maximum Parsimony (MP) bootstrap consensus tree of *Strychnos* species based on combined *rbcLa* + *matK* sequence data.

4.4. Discussion

The findings of this study demonstrate that DNA barcoding, using the core plant markers *rbcLa* and *matK* is highly effective for authenticating *Strychnos* material traded in Bushbuckridge Local Municipality herbal markets. Both markers amplified successfully across all six samples, confirming their reliability even for degraded or processed commercial material, a trend consistent with global barcoding initiatives (CBOL Plant Working Group., 2009; Kress and Erickson, 2007; Mankga et al., 2013) (Table 4.2), The results also provide the first molecular evidence for the identity of *Strychnos* species sold in rural Mpumalanga markets, addressing a gap identified in previous South African studies, which focused predominantly on urban trade hubs such as Johannesburg's Faraday Market .

4.4.1. Marker-specific performance

The *rbcLa* gene consistently yielded identifications with ≥99% identity and full query coverage. Nevertheless, its resolution was constrained, identifying only *Strychnos madagascariensis* and an undetermined *Strychnos sp.* (GenBank CP180). This observation is consistent with the well-documented trend of lower sequence variability at the *rbcLa* locus, often limiting its ability to differentiate between genera or closely related species clusters (CBOL Plant Working Group., 2009; Chen et al., 2010).

Compared to *rbcLa*, *matK* showed greater taxonomic resolution, effectively distinguishing *S. madagascariensis*, *S. innocua* and *S. xanthoclada* with high confidence. The enhanced performance of *matK* aligns with previous findings that highlight its relatively higher nucleotide substitution rate and greater informativeness at the species level (Lahaye et al., 2008; Roy et al., 2010). This marker's utility becomes particularly valuable when working with morphologically indistinct or degraded market samples.

Together, the marker-specific patterns observed closely mirror those reported for African medicinal flora and within *Strychnos* phylogenetic research (Adebowale et al., 2016; Setubal et al., 2021)

4.4.2. Combined marker authentication

The combined use of both markers enhanced resolution of species, yielding four unique *Strychnos* species: *S. madagascariensis*; *S. innocua*; *S. xanthoclada*; and *Strychnos sp.* (GenBank CP180). The latter represents a distinct barcode lineage and may reflect incomplete reference representation, an issue widely acknowledged in DNA barcoding research, especially in underrepresented African genera (Copilaş-Ciocianu et al., 2022; Gostel and Kress, 2022; Yu et al., 2021).

Preliminary BLAST outputs identified *S. pungens* with high similarity; however, it was not included in the results due to slightly lower identity percentages or partial query coverage. Additionally, BLAST hits to *S. nux-vomica*, a highly concerning toxic species, were also rejected due to matches originating from whole chloroplast genomes rather than barcode regions, echoing warnings by(Cheng et al., 2023) and (Farha et al., 2022) that full plastome matches may inflate identity values and mislead species authentication.

The results highlight the significance of strict filtering, using barcode-region alignment, identity thresholds and query coverage in analysing BLAST matches. While hits to full genomes may show high identity, they are generally less reliable for authentication than barcode-specific match accessions (Cheng et al., 2023; Farha et al., 2022). The identifications also validate that multi-locus barcoding strategies significantly enhance species authentication, particularly within complex genera such as *Strychnos,* which contains both medicinal and toxic members (Fazekas et al., 2008; Mankga et al., 2013).

4.4.3. Phylogenetic validation of identifications

The phylogenetic placement of all six Bushbuckridge Local Municipality market samples forming a robust monophyletic cluster within *Strychnos madagascariensis* (PP = 0.83) and grouping closely with verified GenBank accessions (RL1433, RL1460), aligns fully with previously published *Strychnos* phylogenies (Adebowale, 2014; Setubal et al., 2021) and shows that the combined markers can reliably define species boundaries within the genus. The use of suitable outgroups (*Gelsemium elegans*, *Gardenia multiflora*) ensured correct rooting, further strengthening the robustness of the resulting topology. The agreement between BLAST and phylogenetic validation enhances the credibility of species identification and reflects best practices recommended by barcoding experts for authenticating commercial herbal products (Yu et al., 2021)

4.4.4. Biogeographical factors and uncertainty

Identification of *S. madagascariensis* is strongly aligned with previous ethnobotanical studies conducted in Bushbuckridge, wherein, the species is recognised for its edible fruit and traditional applications (Chauke et al., 2025; Maroyi, 2021; Shai et al., 2020 ; van Rayne et al., 2020). However, the confirmed occurrence of *S. innocua* and *S. xanthoclada* within this region remains poorly documented in the published floristic or ethnobotanical literature (Hyde et al., 2025; Leeuwenberg, 1969; Orwa et al., 2009; Shai et al., 2020). Such uncertainty underscores the importance of DNA barcoding for revealing neglected or underreported taxa in ethnomedicinal trade networks (Campanaro et al., 2019). However, the ecological presence of *S. innocua* and *S. xanthoclada* in Bushbuckridge needs to be confirmed through herbarium records or organised field surveys (Cowell et al., 2023 ; Greve et al., 2016; Molano-Flores et al., 2023).

Interestingly, *S. spinosa*, commonly referred to as the 'monkey orange', is well-known in Bushbuckridge for its edible fruits, medicinal bark and economic significance (Le Roux, 2005; Mbhele et al., 2022a; Omotayo and Aremu, 2021). However, it was not found among the validated samples. Despite its recognised ethnobotanical importance and ecological prevalence in the area, no barcode matches met the ≥99% identity requirement with full query coverage for *S. spinosa*. This may indicate that *S. spinosa* was absent from the sampled materials, or that the genetic similarities between species, along with a possible gap in local barcode representation, limited the ability of markers to distinguish between the species (Mampang et al., 2023; Mbhele et al., 2022b; Mbhele et al., 2023). Additionally, it could point to market substitutions or sourcing from outside the region (Fitzgerald, 2023; Mbhele et al., 2023).

According to Gwiazdowski (2024), a regional DNA barcode reference library containing vouchered specimens improves identification accuracy by offering a more specific and dependable database for comparison. This localised strategy aids in clarifying ambiguities that could emerge from utilising wider, less detailed databases, especially for species found within a particular geographic region (Copilaş-Ciocianu et al., 2022; Gostel and Kress, 2022).

4.4.5. Conservation and safety implications

Importantly, no evidence of species substitution or adulteration was observed. This is significant given that prior studies in other regions, such as the Faraday market in Johannesburg, revealed discrepancies between vernacular names and actual identity of species (Mankga et al., 2013). The accuracy noted in this study may reflect localised knowledge fidelity within Bushbuckridge or a limited sample size and warrants further regional comparison (Gitima et al., 2025; Potgieter, 2015).

Using core barcode markers (*rbcLa* + *matK*) provides a strong and scalable approach to verify the authenticity of medicinal plants, regardless of whether the samples are powdered or poorly preserved (Letsiou et al., 2024; Mampang et al., 2023; Mankga et al., 2013; Pretorius, 2012). This strategy enhances pharmacovigilance, conservation efforts, and public health safety by ensuring taxonomic accuracy in the traditional medicine market (Hebert et al., 2003; Mankga et al., 2013; Yu et al., 2021).

Incorporating molecular authentication into the quality control of traditional medicine must respect Indigenous Knowledge Systems (IKS), safeguard community intellectual property, and ensure fair benefit-sharing (Department of Science and Technology., 2004; Seleke et al., 2025), ensuring alignment with the Nagoya Protocol and the Protection, Promotion, Development and Management of Indigenous Knowledge Act, 2019 (Constitution of the Republic of South Africa., 2019). Achieving this necessitates a collaborative strategy that engages communities throughout the process and establishes clear guidelines for access, benefit-sharing, and intellectual property protection (Department of Science and Technology., 2004; Parliamentary Monitoring Group, 2016).

4.5. Concluding remarks

This chapter provides the first DNA barcoding assessment of *Strychnos* species traded in Bushbuckridge Local Municipality, Mpumalanga Province. The combined use of *rbcLa* and *matK*, validated by both BLAST analysis and Bayesian phylogenetic analysis, confirmed that all market samples belonged to *S. madagascariensis,* with no evidence of adulteration or misidentification. These results demonstrate the reliability of plastid barcodes for species authentication in traditional medicine markets and highlight their value for biodiversity conservation, public health, and regulatory oversight. Integrating DNA barcoding into local regulatory frameworks is thus recommended for medicinal plant markets. This would improve current conservation initiatives and promote a safer, more transparent trade in ethnomedicinal products, thereby meeting the objectives of South Africa's environmental and biodiversity policy frameworks, which emphasise the sustainable use of biological resources, protection of IKS and equitable benefit-sharing from the use of biodiversity.

This chapter establishes a molecular foundation upon which future regional barcode libraries, expanded sampling, and integrative taxonomic approaches can build to improve the management of South Africa's ethnomedicinal plant resources.

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Chapter 5: General discussion, synthesis and conclusion

5.1. Introduction

Sub-Saharan Africa, recognised as a global biodiversity hotspot, continues to experience increasing pressures driven by climate variability, land-use change, and a growing reliance on natural resources to sustain household livelihoods (Chazdon et al., 2009; Gebru et al., 2019). These pressures are particularly evident in rural communities, where wild plant species contribute significantly to food security, primary healthcare and cultural continuity. In such contexts, IKS remain central to the interpretation, use and management of plant resources, offering context-specific strategies that support both human well-being and ecological resilience (Gautam, 2024; Vira and Kontoleon, 2010).

The value of these knowledge systems becomes more pronounced as species face ecological threats, fluctuating availability and limited formal conservation interventions. As global conservation frameworks increasingly recognise the importance of community-managed landscapes and Other Effective Area-Based Conservation Measures (OECMs), attention is shifting toward understanding how local knowledge and practices influence the sustainability of key indigenous species (Hoffmann, 2021). Within this shift, wild fruit trees such as *S. spinosa* occupy an important socio-ecological niche, providing nutritional, medicinal and economic benefits across the region while remaining vulnerable to habitat modification and changing harvesting pressures (Omotayo and Aremu, 2021).

At the same time, ensuring the credibility and safe use of plant-based products requires approaches that bridge cultural knowledge with scientific verification tools. Molecular techniques such as DNA barcoding provide opportunities to identify processed materials in local markets, thereby strengthening trust, enhancing product integrity and supporting communities whose livelihoods depend on natural-resource trade.

Against this backdrop, the present chapter integrates findings from the systematic review, ethnobotanical assessment and molecular authentication components of the study to provide a cohesive interpretation of *S. spinosa* within its broader ecological, cultural and socio-economic context. The synthesis highlights the ways in which IK, harvesting practices, market dynamics and DNA-based evidence intersect to shape the sustainable use of the species. This discussion then forms the basis for the recommendations presented at the end of the chapter, aimed at supporting conservation planning, knowledge preservation and the long-term utilisation of *S. spinosa* in Bushbuckridge and similar settings.

5.2. Synthesis of empirical findings

This study focused on traditional uses, market trade, and taxonomic authenticity of *S. spinosa* in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa. Chapter 2 demonstrated the extensive nutritional, medicinal and cultural value of *S. spinosa* across sub-Saharan Africa, while highlighting critical knowledge gaps on its phytochemical and pharmacological properties. The ethnobotanical survey confirmed *S. spinosa* as a culturally significant and ecologically valuable species within Bushbuckridge Local Municipality. High Use Value (1.9) and Relative Frequency of Citation (0.4) reflect widespread reported reliance on *S. spinosa* among participants, with 93% harvesting from the wild and 99% participating in trade. However, this reliance reflects local identification systems rather than confirmed taxonomic identity, as molecular analysis did not confirm the presence of *S. spinosa* in the analysed market samples. This suggests potential species misidentification or substitution within trade networks.

Participants reported declining population availability due to agricultural expansion, climate pressures, and land-use change, alongside limited formal conservation initiatives. The DNA barcoding component confirmed *Strychnos* identity in all six market samples analysed using *rbcLa* and *matK* markers. All six samples aligned with *Strychnos* species at over 99% sequence identity, with phylogenetic placement supporting the BLAST results. Notably, there was no evidence of adulteration or substitution, even in degraded or processed samples. This molecular verification provides the first scientific evidence that local vendors and knowledge holders maintain the taxonomic integrity of materials they sell. This is an important finding for consumer trust, medicinal safety and the commercial viability of *Strychnos*-based products.

A key finding of this study is the discrepancy between ethnobotanical reports and molecular identification results. While participants consistently identified and reported the use of *S. spinosa*, DNA barcoding did not confirm its presence in the analysed market samples. Instead, samples were identified as closely related *Strychnos* species. This discrepancy does not necessarily indicate incorrect knowledge, but rather reflects differences between local and scientific systems of plant classification. Indigenous Knowledge systems often rely on morphological, functional and cultural characteristics, which may group closely related species under a single vernacular name. In contrast, DNA barcoding distinguishes species at a genetic level, revealing distinctions that may not be apparent through traditional identification methods.

In addition, the findings may reflect substitution or mixing of species within informal medicinal plant markets, where plant materials are often processed and lack distinguishing morphological features. This highlights the importance of molecular authentication in validating species identity, particularly in trade contexts.

These results demonstrate the value of integrating ethnobotanical knowledge with molecular approaches, as this provides a more comprehensive understanding of plant use while also identifying potential inconsistencies that may have implications for safety, reliability and conservation.

The study presents a coherent and multi-layered understanding of the cultural significance, utilisation patterns, and taxonomic identity of plant materials identified as *S. spinosa* in African contexts, specifically in Bushbuckridge Local Municipality. Building on this foundation, the ethnobotanical inquiry in Bushbuckridge Local Municipality demonstrated *S. spinosa* remains embedded in local livelihoods, healthcare practices, and cultural identity. High ethnobotanical indices confirmed strong knowledge retention and utilisation, while participants' narratives highlighted dependence on wild population and growing concern about resource decline driven by land-use change, climate variability and inadequate formal conservation interventions. These findings emphasise the central role of IK in sustaining plant-based practices, while also revealing ecological and socio-economic pressures that may threaten species persistence and knowledge continuity.

The molecular authentication complemented and extended the ethnobotanical insights from the study by interrogating the integrity of traded plant material. DNA barcoding successfully confirmed *Strychnos'* identity in all six samples, yet did not detect *S. spinosa*. This outcome raises critical questions regarding market substitution, ecological scarcity, and the completeness of reference databases. These molecular results reinforce ethnobotanical concerns around resource pressure and trade uncertainty, while demonstrating the importance of scientific verification in contexts where morphological identification is constrained by processed or adulteration. Together, these illustrate the value of integrating IK with molecular diagnostics to address taxonomic identity, safety, and sustainable use. The synthesis shows that dependence on *S. spinosa* for cultural and economic reasons stays high; however, ecological risks and trade gaps require coordinated efforts in conservation, research, and policymaking. The integrated evidence base strengthens the rationale for transdisciplinary approaches that validate IK, safeguard biodiversity, and enhance pharmacovigilance in traditional medicine and food value chains.

The incorporation of DNA barcoding simultaneously responded to increasing global emphasis on authenticity, traceability, and quality assurance in traditional medicine value chains. Given that the trade often involves dried, powdered, or morphologically altered plant material, molecular tools offer a reliable means of verifying species identity where traditional morphological identification becomes challenging. The molecular approach used in this study aligns with emerging pharmacovigilance frameworks and biodiversity governance instruments that promote safe, ethical, and scientifically validated use of natural resources.

The integration of IK with molecular identification enabled a holistic assessment of *S. spinosa* that moves beyond descriptive ethnobotany toward applied conservation science. The alignment of IK and molecular authentication reflects South Africa's commitments under national biodiversity policies and the Nagoya Protocol, emphasising equitable knowledge recognition and benefit-sharing. By linking cultural relevance, commercial demand, and species identification, the research orientation ensured that the study remained responsive to both community realities and scientific imperatives, thereby contributing to policy-relevant conservation discourse.

The integrated orientation highlighted reinforces the subsequent discussion and strengthens the relevance of the study's findings for conservation policy, community empowerment, and future research on Indigenous medicinal plants.

The current findings highlighted both the resilience and vulnerability of traditional medicine systems. Indigenous knowledge holders retain a rich body of insights into the uses, harvesting practices and cultural value of *S. spinosa*. Market dynamics concurrently expose the system to risks of adulteration and misidentification of species. DNA barcoding proved to be a reliable tool for verifying identity of species, especially in processed or morphologically ambiguous samples.

Absence of *S. spinosa* in the samples analysed indicates several possibilities, including ecological scarcity due to overharvesting or habitat loss, market substitution driven by availability, or incomplete representation in barcode reference libraries. Integration of IKS with molecular diagnostics becomes a vital way forward. This integration should be supported by regulatory frameworks that involve local communities in decision-making and benefit-sharing, and adhere to international agreements, such as the Nagoya Protocol and South Africa’s White Paper on the Conservation and Sustainable Use of Biodiversity.

5.3. Limitations of the study

This study was subject to seeral limitations. The study was limited to selected study area within Bushbuckridge Local Municipality, Mpumalanga Province. Thus, these results might not be applicable to other regions or communities with different cultural or ecological contexts. Analysis of market samples was also limited to a small number of vendors and herbal outlets, which limits the generalizability of the findings since the diversity of *Strychnos* species in trade across the region may not be fully represented.

The use of purposive and snowball sampling in both ethnobotanical and molecular components may have introduced bias, and the small number of authenticated market samples (n = 6) reduced the statistical strength of molecular results. Although these approaches may limit generalizability, the goal of this study was not statistical representativeness, but rather an in-depth understanding and accurate species authentication within authentic local knowledge and trade contexts. Although DNA barcoding with *rbcLa* and *matK* produced reliable results, the study did not include other *loci*, such as *ITS2* or *trnH-psbA*, which could have offered better species discrimination. Interestingly, the focal species, *S. spinosa*, was not found among the validated samples, possibly due to limited reference sequences in GenBank, low barcode divergence, or actual absence from the market samples.

Some species identified through DNA barcoding (e.g., *S. innocua* and *S. xanthoclada*) were scarcely documented in Bushbuckridge's floristic and ethnobotanical literature, indicating the need for further herbarium verification or ecological surveys. Constraints, including confinement of study site, limited access to the laboratory, funding, and time, affected the number of samples processed and the scope of comparative barcoding.

Despite these limitations, the study provides valuable insights into the relationship between IK and species authentication.

5.4. Recommendations

The following recommendations, which support evidence-based conservation, ethical trade practices, and the integration of IK with scientific tools to ensure the long-term sustainability and cultural continuity of *S. spinosa* and other culturally important species in South Africa, are made based on the findings of this study:

- According to results, IK of *S. spinosa* is strongly concentrated among older participants in Bushbuckridge Local Municipality. In comparison, younger participants demonstrated limited awareness and involvement; 81% of participants were older than 41 years, and only 9% were youth under 30 years (Table 3.1). Targeted youth engagement programmes are thus needed. Policies that make traditional plant-based livelihoods, training and conservation activities more accessible and appealing to younger generations would help safeguard long-term knowledge transmission and reduce the risk of cultural erosion.
- From the current findings, *S. spinosa* holds high medicinal, nutritional and cultural value across sub-Saharan Africa and Bushbuckridge Local Municipality, yet over 70% of published studies lack phytochemical and biochemical analysis, and local knowledge remains largely undocumented. The insufficient documentation places IK at risk of loss, misinterpretation and generational erosion. Therefore, the establishment of community-led IK documentation programmes, involving elders, traditional healers and youth, is recommended. Such initiatives should create repositories of traditional uses, harvest protocols and cultural practices for *S. spinosa* and related species.

- The study highlighted that 93% of participants rely on wild harvesting, and many reported declining availabilities, particularly due to urbanisation, agriculture and climate change (Table 3.5). This continued exclusive reliance on wild populations poses a long-term threat to resource availability. Training workshops should thus be implemented on sustainable harvesting, seasonal timing, rotational gathering and habitat stewardship, derived through municipal environment units and local NGOs.
- Trials for cultivating *S. spinosa* have been limited or unsuccessful, and communities currently have no formal options beyond wild harvesting (Chapters 1 and 3). Without domestication efforts, pressure on wild populations will continue to increase. Therefore, a collaboration with agricultural extension officers and research institutions is recommended to establish community nurseries, distribute seedings, and develop small-scale orchard trails for *S. spinosa*.
- Market engagement is exceptionally high with 99% of participants participating in trade (Chapter 3, Table 3.5), yet market sample storage practices and product quality are questionable (Chapter 4, Figure 4.3). Poor quality control undermines consumer trust and limits economic potential. Municipalities should introduce basic market standards, including safe storage, drying practices, packaging guidelines and vendor training.
- Further ethnobotanical surveys should be undertaken to document the broader IK associated with *S. spinosa* and other culturally important species in Mpumalanga. Expanding research to additional municipalities within the province, particularly those where formal ethnobotanical records remain limited, would help fill critical knowledge gaps and strengthen regional IK repositories. Such documentation initiatives, guided by participatory methods and community engagement principles, would support the preservation, promotion and long-term management of IK as mandated in the Indigenous Knowledge Systems (IKS) Act No. 6 of 2019. Additionally, future surveys should include questions asking community members whether they can differentiate among various *Strychnos* species. This would help determine whether the use of a common name indicates accurate species identification or just general knowledge, thereby enhancing the interpretation of ethnobotanical data when combined with molecular verification.

- DNA barcoding confirmed high species identity (≥99%) for all six Bushbuckridge Local Municipality market samples (Chapter 4). However, none of the analysed samples matched *S. spinosa,* instead corresponded to closely related *Strychnos* species. This suggests potential misidentification or use of shared vernacular names that do not distinguish between closely related species in local markets. These findings highlight the importance of incorporating periodic DNA authentication programmes, in partnership with ACDB, SANBI and the University of Mpumalanga, to validate species identity in medicinal plant markets and strengthen consumer trust, traceability and product credibility.
- The study revealed inadequate formal conservation efforts, yet communities already practice informal stewardship, such as selective harvesting and cultural restrictions (Chapter 3). Bushbuckridge Local Municipality's informal practices meet the criteria for Other Effective Area-Based Conservation Measures (OECMs) but lack formal recognition. The recognition of community-managed *S. spinosa* landscapes as OECMs, should thus be supported ensuring benefit-sharing, capacity strengthening, and alignment with the National Biodiversity Strategy and Action Plan.
- This study indicated high nutritional value, and diverse phytochemicals were highlighted, but communities lack structured pathways to commercialise value-added products. This underutilization limits income generation and local economic growth. The promotion of small enterprises such as juice extraction, dried fruit products, seed oil processing, and inclusion in local nutrition schemes is recommended.
- The study highlights gaps in research-community feedback loops and limited community involvement in decision-making (Chapters 3 and 4). This lack of communication weakens conservation planning and sustainable use efforts. Therefore, there should be the development of co-management frameworks, research dissemination workshops, and participatory monitoring programmes involving IK holders, traditional health practitioners, and municipal authorities.

Future research should build upon this study by expanding sampling frameworks and analytical approaches. Broader market surveys across multiple regions and informal trade networks are needed to assess species substitution patterns more comprehensively. Incorporating probability-based or mixed sampling designs will improve representativeness and minimise potential recruitment bias associated with purposive and snowball sampling approaches.

Ecological assessments are required to establish the current distribution and population trends of *S. spinosa* and related *Strychnos* species, particularly in the context of land-use change, climate variability, and harvesting pressure. Parallel development of regional DNA barcode reference library will strengthen molecular authentication and contribute to improved monitoring, traceability, and pharmacovigilance for medicinal plant species.

Further interdisciplinary studies integrating molecular, phytochemical, nutritional, and pharmacological analyses are encouraged to validate therapeutic claims and explore value-added opportunities. In addition, future work should adopt community-engaged research models that support Indigenous knowledge holders, promote sustainable harvesting practices, and contribute to capacity-building and benefit-sharing frameworks. Such approaches will ensure that scientific advances reinforce cultural heritage, ecological stewardship, and equitable access to biodiversity resources.

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Appendix A: Certificate - Approval of research proposal

UNIVERSITY OF MPUMALANGA

FACULTY OF AGRICULTURE AND NATURAL SCIENCES

Postgraduate Studies Committee

Certificate of Approval – Research Proposal

Date of this Approval:	**13 June 2024**

Student Details

1	Student Name:	S Thothela
2	Student Number:	230351360
3	School	School of Biology and Environmental Sciences
4	Degree Registered for:	MSc
5	Date of First Registration:	February 2024
6	Supervisor(s):	Dr TP Ndhlovu Ms E Kola Dr MTB Dalu

The research proposal entitled '**Indigenous knowledge on the uses and factors that determine the use of *Strychnos spinosa* Lam. found in Bushbuckridge, Mpumalanga, South Africa'** has been evaluated and approved by the Postgraduate Studies Committee of the Faculty of Agriculture and Natural Sciences.

Chairperson: Prof. Daniel Parker

UNIVERSITY OF MPUMALANGA
13 -06- 2024
FACULTY OF AGRICULTURE AND NATURAL SCIENCES

Signature:

Date & Official Stamp:

Appendix B: Ethical approval letter

UNIVERSITY OF MPUMALANGA

Creating Opportunities

MEK Ngcobo

School of Agricultural Sciences

Mbombela Campus.

Dear Sanele Nomfundo Sindisiwe Thothela (230351360)

Protocol Reference Number: UMP/Thothela230351360/SBES/MSC/2024/01

Project Indigenous Knowledge on the Uses and Factors that Determine the Use of *Strychnos spinosa* Lam. found in Bushbuckridge, Mpumalanga, South Africa.

Approval Notification: In response to your application received on **15/07/2024**, The Research Ethics Committee: Faculty Research Ethics Committee has considered the above mentioned application and the protocol has been granted **FULL APPROVAL**.

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. In case you have further queries, please quote the above reference number.

PLEASE NOTE: Research data should be stored securely in the School/ division for a period of 5 years.

The Ethical Clearance certificate is only valid for a period of 3 years from date of issue. Thereafter, Recertification must be applied for on an annual basis.

Wishing you the best with your study.

Yours faithfully,

....

MEK Ngcobo (Chair)

Cc: Faculty Research and Innovation Committee Chair: ....

DECLARATION OF INVESTGATOR(S)

I/We fully understand the conditions under which I am/we are authorised to carry out the abovementioned research and guarantee to ensure compliance with these conditions. I agree to completion of a yearly progress report.

....

Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER ON ALL ENQUIRIES

Appendix C: Permission letter from the Municipality

R533 Graskop Road.
Opp Mapulaneng DLTC
Bushbuckridge
Co-ordinates: 31°3'59.796"E 24°50'24
Tel: 013 004 0291/92/95

BUSHBUCKRIDGE
LOCAL MUNICIPALITY

Private bag x 9308
Bushbuckridge
1280
Email:info@bushbuckridge.gov.za
Website: www.bushbuckridge.gov.za

Enquiries: Mnisi M.M (Skills Development Facilitator)
Ref No: 4/4/2
Email: mnisim@bushbuckridge.gov.za

15 July 2024

Ms Thothela SNS
Farm71, Portion1, Southern Acres Estate
White River
1240

Cell No: 0793043404
Email: sanele.thothela@gmail.com

Attention: Ms Thothela

RE: PERMISSION TO CONDUCT RESEARCH STUDY

A permission is granted to conduct research study in our Municipality under the topic:" **Indigenous Knowledge on the uses and Factors that Determine the use of Strychnos Spinosa Lam. Found in Bushbuckridge, Mpumalanga, South Africa".**

While conducting your study, you need to take into consideration that all your actions will be subjected to the policies and procedures approved by the Municipal Council.

The Municipality wish you best of luck in your research endeavor. The Skills Development Facilitator, Ms. Mnisi MM contact 0605047520 will be the person to liaise with when need arises.

Kind regards

Mr J Ngobeni
Municipal Manager

Together speeding service delivery

Appendix D: Semi-structured interview questionnaire

ETHNOBOTANICAL QUESTIONNAIRE

Documentation of Ethnobotanical Knowledge about *Strychnos spinosa* Lam.

Socio-Demographic Data

Location		
Gender	1. Male 2. Female 3. Other	
Age	1. 18-24 2. 25-30 3. 31-40 4. 41-50 5. 51-60 6. >60	
Occupation	1. Employed 2. Self-employed 3. Unemployed 4. Scholar 5. Retired 6. Other	
Education level	1. No formal education 2. Primary school 3. High school 4. Tertiary	
Household size	1. 1 2. 2 3. 3 4. 4 5. 5 or more	
How many females in household	1. 1 2. 2 3. 3 4. 4 5. 5 or more	

How many children (aged 0-14) in household	**1. 0** **2. 1** **3. 2** **4. 3** **5. 4** **6. 5 or more**	
How many elderly people (aged 65 and above) in household	**1. 0** **2. 1** **3. 2** **4. 3** **5. 4** **6. 5 or more**	
Employed household members	**1. 0** **2. 1** **3. 2** **4. 3** **5. 4** **6. 5 or more**	
How often do you participate in community activities?	**1. Daily** **2. Weekly** **3. Monthly** **4. Yearly** **5. Rarely/Never**	
Are you aware of any community support networks?	**1. Yes** **2. No**	
I rely on the knowledge and skills of community members to solve problems?	**1. Strongly agree** **2. Agree** **3. Neutral** **4. Disagree** **5. Strongly disagree**	
Do I contribute to community development?	**1. Strongly agree** **2. Agree** **3. Neutral** **4. Disagree** **5. Strongly disagree**	

Questions to Ask Informant

Question 1:	Have you ever used *Strychnos spinosa* Lam. for medicinal or traditional purposes? If yes, please describe the purpose/ use.	
1. Yes **2. No**		**1. Ornamental** **2. Medicinal** **3. Culinary** **4. Industrial** **5. Other**
Question 2:	How did you learn about the uses of *Strychnos spinosa* Lam.?	
Question 3:	Which part of the plant have you used?	
1. Root **2. Stem** **3. Leaf** **4. Fruit** **5. Flower** **6. Seed** **7. Root bark** **8. Stem bark** **9. Latex** **10. Other (Please state)**		
Question 4:	How is it used?	
1. Dried **2. Fresh**		
Question 5:	How do you prepare it for use?	
1. Natural form **2. Infusion** **3. Crushed** **4. Poultice** **5. Powder** **6. Juice** **7. Other (Please state)**		
Question 6:	Why do you prepare it that way?	
1. Cheaper **2. More abundant**		

3. **Other (Please state)**	
Question 7:	How is the preparation administered?
1. **Internal (oral, nasal, other body parts)** 2. **External (dermal)** 3. **Other (Please state)**	
Question 8:	How and where do you source plant material?
1. **Collected from the wild** 2. **Cultivated** 3. **Purchased** 4. **Other (Please state)**	
Question 9:	Quantity harvested per day/ month/year
Question 10:	Is it sold?
1. **Yes** 2. **No**	
Question 11:	To whom is it sold? Price/kg?
Question 12:	Is the plant perceived to be abundant/common/rare?
1. **Abundant** 2. **Common** 3. **Rare**	
Question 13:	Changes in abundance of the plant for the last 10 years (more abundant/same/rare)
1. **More abundant** 2. **Same** 3. **Rare**	
Question 14:	Are there methods used to regenerate or otherwise actively manage the plant?

1. **Yes** 2. **No** 3. **Unknown**			
Question 15:	What are these methods?		
Question 16:	What kinds of tools are being used for harvesting?		
1. **Bare hand** 2. **Bare hand with utensil (e.g.: knife)** 3. **Machine** 4. **Other**			
Question 17:	How has your access to the plant species been impacted by human activity in your area?		
1. **Positively impacted** 2. **Neutral** 3. **Negatively impacted**			
Question 18:	What changes have you noticed in the plant species population in the past year?		
1. **Increase** 2. **No change** 3. **Decrease**			
Question 19:	How concerned are you about the long-term impact of human activities on the environment?		
1. **Very concerned.** 2. **Moderately concerned.** 3. **Not concerned**			

Appendix E: GenBank sequences

Barcode gene: *rbcLa*

Accession number	Species	Voucher
JF265609	*Strychnos_decussata*	OM292
EU213520	*Strychnos_decussata*	OM900
EU213521	*Strychnos_decussata*	RL1560
JX573002	*Strychnos_decussata*	OM1259
EU213522	*Strychnos_decussata*	RL1561
JX573009	*Strychnos_xantha*	OM2756
EU213523	*Strychnos_madagascariensis*	RL1433
JF265612	*Strychnos_pungens*	MvdB22
EU213524	*Strychnos_madagascariensis*	RL1460
EU213526	*Strychnos_spinosa*	OM220
KJ594512	*Strychnos_panamensis*	DB0783
JX573008	*Strychnos_usambarensis*	OM2593
JQ592408	*Strychnos_guianensis*	BioBot10423
KJ594515	*Strychnos_toxifera*	DB0685
KJ594517	*Strychnos_toxifera*	DB1113
JF265614	*Strychnos_usambarensis*	OM2006
KJ594516	*Strychnos_toxifera*	DB0628
KJ594511	*Strychnos_panamensis*	DB0790
KJ594509	*Strychnos_panamensis*	DB1143
JF265611	*Strychnos_potatorum*	RBN179
JX573005	*Strychnos_panganensis*	OM2646
KJ594508	*Strychnos_darienensis*	DB1368
MH657329	*Gardneria_multiflora*	SN010
PQ332324	*Gelsemium_elegans*	DWQJM351
MG718519	*Strychnos brasiliensis*	PEEC139
JQ025096	*Strychnos henningsii*	OM2381
JX573001	*Strychnos cocculoides*	HG4080

Barcode gene: *matK*

Accession number	Species	Voucher
JF270951	*Strychnos_decussata*	OM292
EU214292	*Strychnos_decussata*	OM900
EU214293	*Strychnos_decussata*	RL1560
JX517983	*Strychnos_decussata*	OM1259
EU214294	*Strychnos_decussata*	RL1561
JX517510	*Strychnos_xantha*	OM2756
EU214295	*Strychnos_madagascariensis*	RL1433
JF270954	*Strychnos_pungens*	MvdB22
EU214296	*Strychnos_madagascariensis*	RL1460
EU214298	*Strychnos_spinosa*	OM220
KJ594053	*Strychnos_panamensis*	DB0783
JX517734	*Strychnos_usambarensis*	OM2593
JQ588159	*Strychnos_guianensis*	BioBot10423
KJ594055	*Strychnos_toxifera*	DB0685
KJ594057	*Strychnos_toxifera*	DB1113
JF270956	*Strychnos_usambarensis*	OM2006
KJ594056	*Strychnos_toxifera*	DB0628
KJ594052	*Strychnos_panamensis*	DB0790
KJ594051	*Strychnos_panamensis*	DB1143
JF270953	*Strychnos_potatorum*	RBN179
JX517363	*Strychnos_panganensis*	OM2646
KJ594050	*Strychnos_darienensis*	DB1368
MH658905	*Gardneria_multiflora*	SN010
PQ331304	*Gelsemium_elegans*	DWQJM351
MG719023	*Strychnos brasiliensis*	PEEC139
JQ025002	*Strychnos henningsii*	OM2381
JX517336	*Strychnos cocculoides*	HG4080
JX518090	*Strychnos mitis*	OM1870

Appendix F: Language editing certificate

Faculty of Humanities **Department of English**

31 October 2025

TO WHOM IT MAY CONCERN

CERTIFICATE OF LANGUAGE EDITING

I, Paul Nepapleh Nkamta, confirm and certify that I have read through and edited the dissertation titled: "**Indigenous Knowledge on the uses and factors that determine the use of *Strychnos spinosa* Lam. found in Bushbuckridge, Mpumalanga, South Africa"** by **Sanele Nomfundo Sandisiwe Thothela**, student number: **230351360**.

The views and research procedures detailed and expressed in the dissertation remain those of the researcher/s.

Yours sincerely

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Appendix G: Turnitin report

Indigenous Knowledge on the uses and factors that determine the use of Strychnos spinosa Lam. found in Bushbuckridge, Mpumalanga, South Africa

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