PROSPECT OF UNLOCKING THE POTENTIAL AND SUSTAINABLE INTEGRATION OF THE UNDERUTILISED INDIGENOUS FRUIT TREE MILKWOOD (*MIMUSOPS ZEYHERI*) IN THE AGROECOSYSTEMS FOR COMBATING FOOD AND NUTRITIONAL INSECURITIES

Report to the
Water Research Commission by

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EXECUTIVE SUMMARY

Mimusops zeyheri is an indigenous wild fruit tree of ecological, nutritional, cultural, and economic importance in southern Africa, yet comprehensive scientific documentation of its traditional uses, population ecology, genetic and morphological variation, water use, and nutritional value remains limited. The species can contribute to household income generation, rural livelihoods and local value chain development through fruits, seeds and nutraceutical products. The tree presents opportunities for smallholder farmers and community enterprises through the development of value-added products such as jams and therapeutic products, while contributing to food security and sustainable economic participation in rural communities. This project, funded by the Water Research Commission (Project ID: C2023/2024-01331) and implemented by the University of Mpumalanga in collaboration with various organisations, provides an integrated multi- disciplinary assessment of *M. zeyheri* across two regions, Vhembe in Limpopo Province and Ehlanzeni in Mpumalanga Province, using ethnobotanical, ecological, morphological, sensory, water use and nutritional profiling approaches.

The project was motivated by the increasing need to identify climate-resilient underutilised indigenous trees that can contribute to the issues of food security, biodiversity conservation, sustainable livelihoods and economic development in rural areas. The project further sought to support the conservation and sustainable use of indigenous biodiversity while strengthening local value chains.

The project implemented a multidisciplinary approach across two regions, namely the Vhembe District in Limpopo Province and the Ehlanzeni District in Mpumalanga Province. The

project on integrated ethnobotanical surveys, ecological assessments, morphological characterization, sensory evaluation, chloroplast genetic analysis, sap flow measurements, and nutritional profiling of *M. zeyheri*. Data collection focused on size-class distribution, with statistical analyses applied to assess population structure and variation using ecological modelling and scoring indices. Morphological measurements were conducted on 40 trees in total from each agroecosystem (Vhembe District in Limpopo Province and the Ehlanzeni District in Mpumalanga Province), while sensory evaluation involved 100 adult participants using a 9-point hedonic scale. Genetic diversity was evaluated using the chloroplast markers matK and trnH-psbA. Water-use dynamics were assessed through sap flow measurements, and nutritional analyses were conducted using standard AOAC methods and ICP-OES elemental analysis.

Significant differences were found in stem circumference ($F=31.98$, $p=0.000031$), tree height ($p=0.017813$), and inter-tree distances ($p=0.01005$) between the two sites. Spatial analysis revealed a dispersed pattern in Vhembe (mean distance = 5.54m), likely driven by wind dispersal (anemochory), and a clustered pattern in Ehlanzeni (mean distance = 2.68m), suggesting animal-mediated dispersal (zoochory). Ecological health assessments revealed greater anthropogenic disturbance in Vhembe, where over half the population fell within the 50–100% disturbance index range, whereas Ehlanzeni showed minimal disturbance (0–5% index). Despite high regeneration potential, a dominance of smaller size classes suggests possible regeneration bottlenecks linked to harvesting pressures.

Mimusops zeyheri morphological characterisation was based on sampling 40 trees (20 per region), with fruit, nut, and leaf traits measured using standard morphometric procedures. Sensory evaluation was conducted with 100 adult participants (50 per site) using an eight-attribute, 9-point hedonic scale. Genetic diversity analysis employed chloroplast markers

matK and trnH-psbA, with a 75% sequencing success rate. Mpumalanga fruits were larger, with mean fruit lengths of 29.41±0.61mm and widths of 24.14±0.55mm, compared to Limpopo fruits measuring 27.41±0.47mm in length and 23.22±0.36mm in width. The results of the sensory evaluation show that Limpopo fruits received markedly higher ratings across all eight sensory attributes, with mean scores for taste, aroma, and mouthfeel ranging from 7.7 to 8.0. In contrast, Mpumalanga fruits scored between 5.0 and 5.4 on the 9-point hedonic scale. Genetic analyses using the matK and trnH-psbA chloroplast markers (with a 75% sequencing success rate) identified two major genetic lineages, distinguished by regional clustering, along with rare haplotypes such as LP3, MP9, and MP10, exhibiting longer branch lengths and unique nucleotide substitutions. Together, the morphological, sensory, and genetic evidence demonstrates strong regional structuring in *M. zeyheri* populations, shaped by both environmental gradients and historical lineage divergence. The greenhouse propagation experiments on *Mimusops zeyheri* provided important baseline information on the response of the species to different propagation approaches under controlled conditions. The low success rates observed during the trials indicate the need for additional research focusing on seed dormancy mechanisms, rooting hormones, environmental regulation, and propagation protocols suitable for indigenous fruit tree species.

The data on water use from sap flow measurements showed that the Limpopo site was utilizing approximately 14.73 litres per day of the entire period which mainly influenced by the microclimate of the area. During the first week of measurements the trees where taking up to 21.77 litres per day and then declined to 13.44 litres per day. These findings highlight the influence of diurnal and seasonal climate variability on transpiration in *M. zeyheri*. The study provides baseline information on the water-use characteristics of this underutilised indigenous fruit tree and offers a valuable reference for future research aimed at supporting its sustainable

management and potential integration into water-efficient agroforestry systems.

Lastly, the nutritional composition of *M. zeyheri* plant parts were determined using standard AOAC methods and elemental analysis by microwave-assisted digestion followed by ICP-OES. Data were analysed using one-way ANOVA and Pearson correlation in R. Nuts contained the highest dry matter (~94 %), crude fat (~54%), protein (~19 %), and energy value (~595 kcal/100 g^{-1}), together with the greatest concentrations of calcium (≈210 mg/100 g^{-1}), magnesium (≈143 mg 100 g^{-1}), zinc (≈95 mg/kg^{-1}), and potassium (≈495 mg/100 g^{-1}). Leaves were rich in protein (≈24 %) and fibre (≈22%), while pulp and fruit fibre provided high moisture, modest vitamin C (5–7 mg/100 g^{-1}), and β-carotene (~1.5–2.1 mg/100 g^{-1}). Correlation analysis revealed strong positive associations ($r > 0.95$, $p<0.01$) between dry matter, crude fat, protein, and divalent cations (Ca, Mg, Zn), indicating that nutrient concentration increases as moisture decreases. Provincial differences were minor, indicating ecological consistency. *M. zeyheri* offers a unique combination of energy-dense nuts, protein, mineral-rich leaves, and vitamin-containing pulp, making it a valuable, climate-resilient food source for improving dietary diversity and combating micronutrient deficiencies in rural South Africa. In line with the United Nations Sustainable Development Goals, the study contributes directly to SDG 1 (No Poverty) by identifying pathways for livelihood diversification and income generation through the sustainable use and value addition of *M. zeyheri*, SDG 2 (Zero Hunger) by demonstrating its potential to enhance dietary diversity and nutrition security through climate-resilient indigenous food resources. Besides, it contributes to SDG 13 (Climate Action) by highlighting the role of resilient indigenous tree species in supporting adaptation strategies amid increasing climate variability.

The project contributed significantly to postgraduate training and human capacity development. The project trained Ms Maropeng Matlala (Honours research project), Ms

Nonhlanhla Lubisi (Honours research project), Mr Mthobisi Dhladhla (MSc Agriculture) and Dr Christeldah Mkhonto (PhD in Biology). Dr Mkhonto successfully completed her Doctor of Philosophy (PhD) degree through research conducted within this project, while Mr Mthobisi is currently finalizing his MSc Agriculture degree. The project further capacitated a research assistant (Ms Kgaugelo Malesa) and the postdoctoral candidate (Dr Luambo Ramarumo) with skills development in plant ecology, genetics, nutritional analysis, sap flow measurements, and indigenous knowledge documentation, thereby strengthening research capacity in indigenous crop systems and sustainable agriculture.

Future research should incorporate high-resolution spatial mapping using GIS to identify priority conservation areas and harvesting hotspots, alongside biological assays and targeted compound isolation to validate the bioactivity, safety, and therapeutic relevance of key phytochemicals. In addition, the development of community-based, value-added products from *M. zeyheri* has strong potential to strengthen local value chains and rural livelihoods, while long-term ecological monitoring is essential to track population dynamics, regeneration success, and anthropogenic disturbance of *M. zeyheri* as a climate-resilient indigenous food and medicinal resource.

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ACRONYMS

AM16/32B	Campbell Scientific multiplexer (data logging device)
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ARC-TSC	Agricultural Research Council Tropical and Subtropical Crops
ARC-VIMP	Agricultural Research Council Vegetable, Industrial and Medicinal Plants
ATP	Adenosine Triphosphate
AWS	Automatic Weather Station
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database
BW	Body Weight
Ca	Calcium
CE	Clark–Evans (nearest neighbour index)
CHPM	Compensation Heat Pulse Method
CR1000	Campbell Scientific data logger
CTAB	Cetyltrimethylammonium Bromide
DBH	Diameter at Breast Height
DI	Daily Intake
EDI	Estimated Daily Intake
ETo	Reference Evapotranspiration
ETc	Actual Evapotranspiration
FAO	Food and Agriculture Organization
FFLP	Fruit Fibre Limpopo
FFMP	Fruit Fibre Mpumalanga

FNLP	Fruit Nuts Limpopo
FNMP	Fruit Nuts Mpumalanga
FPLP	Fruit Pulp Limpopo
FPMP	Fruit Pulp Mpumalanga
Fs	Sap Flow Rate
GA_3	Gibberellic Acid
GIS	Geographic Information System
HCl	Hydrochloric Acid
HFD	Heat Field Deformation
HSD	Honestly Significant Difference
H_2SO_4	Sulphuric Acid
HPV	Heat Pulse Velocity
IBM-SPSS	International Business Machines – Statistical Package for the Social Sciences
IBA	Indole-3-Butyric Acid
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IFAD	International Fund for Agricultural Development
IUCN	International Union for Conservation of Nature
K	Potassium
K80	Kimura 80 genetic distance model
Kc	Crop Coefficient
Kcb	Basal Crop Coefficient
LAI	Leaf Area Index
LI-COR	LI-COR Biosciences measurement equipment/system

LLP	Leaves Limpopo
LMP	Leaves Mpumalanga
LV	Limpopo (Vhembe site)
Mg	Magnesium
MJ	Megajoule
MPE	Mpumalanga (Ehlanzeni site)
Na	Sodium
NAA	Naphthalene Acetic Acid
NaOH	Sodium Hydroxide
NCBI	National Center for Biotechnology Information
NN	Nearest Neighbour
P	Phosphorus
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
RAPD	Randomly Amplified Polymorphic DNA
RAE	Retinol Activity Equivalents
RDI	Recommended Daily Intake
S	Sample titre
SANBI	South African National Biodiversity Institute
SCoT	Start Codon Targeted (marker system)
SD	Standard Deviation
SFD	Sap Flux Density
SNPs	Single Nucleotide Polymorphisms

SP	Sap Flow
SV	Sap Flow Velocity
TDP	Thermal Dissipation Probe
UN	United Nations
UNICEF	United Nations Children’s Fund
VPD	Vapour Pressure Deficit
W	Weight
WFP	World Food Programme
WHO	World Health Organization
WU	Water Uptake
WRC	Water Research Commission
WU	Water Uptake
WUE	Water Use Efficiency
Zn	Zinc

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CHAPTER ONE

BACKGROUND AND INTRODUCTION

1.1 Background

There is a growing demand for sustainable food systems that has renewed interest in indigenous and underutilised plant species that can contribute to household nutrition, environmental sustainability, and livelihood improvement in rural communities (Masipa, 2017). In different parts of southern Africa, indigenous fruit trees have historically played an important role in supplementing diets, supporting traditional health practices, and providing seasonal sources of food and income (Omotayo and Aremu, 2020). Despite their importance, many indigenous species remain poorly integrated into mainstream agricultural production systems and continue to receive limited scientific and commercial attention.

South Africa is characterised by diverse climatic regions and rich plant biodiversity, offering opportunities for the utilisation of indigenous fruit trees in climate-resilient agroecosystems. Indigenous species are often naturally adapted to local environmental conditions such as drought, poor soils, and climatic variability, making them valuable resources for sustainable agricultural development. In addition to supporting biodiversity conservation, these species may contribute to reducing dependence on conventional crops that are increasingly vulnerable to climate-related stresses.

Mimusops zeyheri Sond., commonly known as the Transvaal red milkwood, is one of the indigenous fruit tree species with potential for integration into sustainable agroecosystems. The species occurs naturally in several parts of southern Africa, including Limpopo and Mpumalanga in South Africa. The fruits are consumed fresh in many rural communities, while

different plant parts are associated with traditional medicinal uses and local livelihood practices. Previous studies have indicated that the species contains important nutritional components, including carbohydrates, dietary fibre, minerals, and bioactive compounds that may contribute to human nutrition and health (Omotayo and Aremu, 2020; Mkhonto et al., 2024).

Although *M. zeyheri* has ecological, nutritional, and socio-economic importance, there is still inadequate information regarding its propagation, domestication potential, water-use characteristics, and integration into agricultural systems. The lack of scientific information limits the development of sustainable cultivation strategies and restricts opportunities for conservation, value addition, and commercialisation. Furthermore, pressures such as habitat degradation, land-use change, overexploitation, and climate variability threaten the long-term sustainability of indigenous fruit tree populations.

The conservation and sustainable utilisation of indigenous fruit trees require approaches that combine scientific research with indigenous knowledge systems. Understanding local practices associated with collection, utilisation, and management of indigenous germplasm is important for supporting domestication and conservation initiatives. In addition, research on propagation methods, plant physiology, water-use efficiency, and nutritional composition is necessary to support the development of sustainable production systems for underutilised species.

Against this background, the current project was initiated to investigate the potential of *Mimusops zeyheri* for sustainable integration into agroecosystems as a strategy for contributing to food and nutrition security, biodiversity conservation, and climate-resilient

agricultural development in South Africa.

1.2 Introduction

Food and nutrition insecurity remains a persistent challenge across Africa, where approximately 20% of the population is undernourished due to inadequate dietary energy intake (Mayer and Anderson, 2020). This challenge is further intensified by climate change, with projected crop yield losses of up to 50% in parts of sub-Saharan Africa by 2050, threatening both livelihoods and food systems (Masipa, 2017). These pressures underscore the urgent need for resilient, diversified, and locally adapted food sources that can strengthen household nutrition and sustain rural economies. Africa possesses a rich diversity of indigenous tree species that are underutilised, particularly for their substantial nutritional, ecological, and livelihood value (van Noordwijk et al., 2014).

Trees play a critical role in supporting rural livelihoods, environmental conservation, and national economic development by providing food, medicine, income, and ecosystem services (Padulosi and Hoeschle-Zeledon, 2004; Omotayo and Aremu, 2020). When deliberately integrated into crop and livestock systems, trees form agroforestry systems that reconcile agricultural productivity with environmental sustainability (Gemechu and Jiru, 2021). Indigenous fruit trees, in particular, offer diverse nutrients and are increasingly recognised as strategic resources for enhancing dietary diversity, climate resilience, and food security. Among these species, *Mimusops zeyheri* Sond, (Transvaal red milkwood), is an indigenous fruit tree widely distributed in South Africa and neighbouring countries, yet it remains underutilised in formal agricultural systems (Omotayo and Aremu, 2020). Historically, its fruits have supplemented local diets during periods of food scarcity, particularly in rural and marginalised communities.

The species is valued for its fruits, leaves, and seeds, which provide nutritional, medicinal, and livelihood benefits (Ramarumo and Maroyi, 2020). Despite its importance, *M. zeyheri* receives limited research compared to conventional fruit crops, hindering its integration into diversified, and climate-resilient farming systems. Indigenous and wild fruit trees have long formed part of traditional food systems in southern Africa (Nkosi et al., 2020; Omotayo et al., 2020; Akinnifesi et al., 2005). Ancient indigenous communities relied on wild and semi-domesticated plant species as staple foods or dietary supplements, supported by extensive ethnobotanical knowledge. However, this indigenous knowledge is increasingly eroding due to urbanisation, cultural transformation, and modernisation, leading to the neglect of many nutritionally valuable species. Consequently, such species are now collectively referred to as underutilised or neglected crops, despite their potential to strengthen food basket security and rural livelihoods. Scientific evidence consistently indicates that utilisation and conservation of wild plant species are closely linked, as communities tend to conserve species they value and use.

In South Africa, several tribal communities practise localised conservation rooted in their reliance on indigenous fruit trees, including *M. zeyheri*. Nonetheless, there remains inadequate information on the collection, management, and preservation of indigenous germplasm for many underutilised species. Overharvesting, habitat loss, and climate-related disturbances further threaten their populations, highlighting the need for sustainable domestication strategies informed by local knowledge. Domestication and integration of *M. zeyheri* into agricultural cropping systems require a multidisciplinary approach that includes ethnobotanical documentation, propagation research, and nutritional characterisation.

Plant propagation is central to conservation and commercialisation efforts, enabling sustainable seedling production, genetic preservation, and large-scale cultivation. Both sexual and asexual propagation methods offer opportunities for improving seedling quality, shortening juvenile phases, and maintaining desirable traits. However, propagation success is influenced by biological, environmental, and socio-economic factors, including seed biology, nursery availability, farmer knowledge, and institutional support. In parallel, an improved understanding of the nutritional and phytochemical composition of *M. zeyheri* is essential to support its promotion as a functional food crop. Studies indicate that its fruit pulp, peel, seeds, and leaves contain carbohydrates, fibre, essential minerals, vitamins, unsaturated fatty acids, and bioactive compounds with antioxidant properties (Omotayo and Aremu, 2020; Mkhonto et al., 2024). Despite these promising attributes, comprehensive data on nutrient variability across ecological regions and plant parts remain limited, constraining their inclusion in nutrition-sensitive agriculture and food diversification initiatives.

This research project was therefore initiated to explore local indigenous knowledge associated with the collection of *M. zeyheri* germplasm, assess its propagation potential and water-use efficiency, and evaluate its nutritional composition, as a foundation for sustainable domestication and integration into cropping systems. The first phase focused on documenting the identification, distribution, and cultivation status of the species in Mpumalanga and Limpopo Provinces. In contrast, subsequent phases address propagation strategies, water-use efficiency, and nutritional and elemental profiling using established analytical methods. By strengthening scientific knowledge and local capacity, this study contributes to broader national and continental priorities related to food security, biodiversity conservation, climate resilience, and inclusive economic development, in alignment with

South Africa's National Development Plan, the African Union Agenda 2063, and the United Nations Sustainable Development Goals.

1.3 Problem statement

Despite the growing recognition of indigenous and underutilised fruit trees as important resources for food security, climate change adaptation and rural livelihoods, *Mimusops zeyheri* remains poorly researched and underutilised in southern Africa. Approximately 20% of the African population experiences undernourishment, reflecting persistent deficits in dietary energy and nutrient intake, while climate change is projected to reduce crop yields by up to 50% in parts of sub-Saharan Africa by 2050. These trends pose a serious threat to food security and the sustainability of rural livelihoods. Although the African region is rich in biodiversity with considerable potential to support resilient food systems, a large proportion of indigenous plant species, including wild fruit trees, remains largely untapped and excluded from mainstream diets and agricultural systems.

In South Africa, limited access to and acceptance of wild fruits as regular dietary components further constrain the utilisation of indigenous fruit tree species. Despite increasing global and regional interest in underutilised crops, research on wild fruit trees is scant and fragmented. Existing studies tend to focus on isolated attributes, with little integration of ethnobotanical knowledge, population ecology, genetic structure, morphological variability, nutritional value and phytochemical composition. This fragmented evidence base restricts the development of informed conservation strategies, limits domestication and agronomic improvement efforts, and hampers the effective inclusion of such species in climate-resilient and nutrition-sensitive food systems.

Underutilised crops help maintain biodiversity and offer valuable opportunities for climate change adaptation, food security and food sovereignty. However, their adoption within agricultural and development frameworks remains limited, partly due to gaps in knowledge related to their ecological distribution, abundance, nutritional composition and functional properties. In the case of *M. zeyheri*, increasing anthropogenic pressures, including land-use change, infrastructure development and overharvesting, threaten natural populations, yet empirical data on population dynamics, regeneration patterns and long-term viability across agro-ecological zones are lacking. Furthermore, although the species is widely used in traditional medicine and consumed as a wild food, its bioactive compounds and nutritional functionality have not been comprehensively validated using modern analytical approaches, necessitating this study.

Consequently, a critical knowledge gap persists at the intersection of indigenous knowledge systems, biodiversity conservation, nutritional science and climate adaptation. There is a clear need for a comprehensive and multidisciplinary understanding of underutilised indigenous fruit trees such as *M. zeyheri*, encompassing their distribution, abundance, ecological condition, genetic and morphological variation, and nutritional and phytochemical potential. Addressing this gap is essential for informing conservation and domestication strategies, supporting agronomic and value-addition initiatives, and facilitating the integration of *M. zeyheri* into sustainable and climate-resilient food systems. Without such an integrated evidence base, the potential of this species to contribute meaningfully to improved nutrition, livelihood resilience and climate change adaptation in South Africa will remain largely unrealised.

1.4 Significance of the research on *M. zeyheri*

Trees are critical resources for livelihoods, environmental conservation, and national economic development, particularly in rural contexts where communities depend directly on natural ecosystems for food, energy, and primary healthcare. In South Africa, the forestry and tree-based sector contributes to national economic activity and provides essential ecosystem services that support poverty reduction, food production, and household wellbeing. Indigenous fruit trees, in particular, represent an important yet underutilised resource within this system, despite their capacity to complement conventional agriculture and strengthen local food systems.

Evidence indicates that indigenous fruit trees such as *Mimusops zeyheri* provide diverse and nutritionally important components, including sucrose, glucose, fructose, and β-carotene, which can contribute meaningfully to daily dietary requirements (Wilson and Downs, 2012). In addition, the nuts of *M. zeyheri* have been identified as a potential source of calcium, making them valuable for both human and animal nutrition (Chivandi, 2013). These nutritional attributes directly support Sustainable Development Goal (SDG) 2 (Zero Hunger) by enhancing dietary diversity and addressing micronutrient deficiencies, while also contributing to SDG 3 (Good Health and Well-being) through improved nutrition outcomes.

Beyond its nutritional value, *M. zeyheri* also holds economic and environmental significance. The sustainable harvesting and sale of fruits, nuts, and planting material can provide income-generating opportunities for smallholder farmers and rural households, thereby supporting livelihood diversification and rural economies (Chivandi et al., 2008). These contributions align with SDG 1 (No Poverty) by strengthening income resilience in rural communities and promoting inclusive economic participation. However, despite this potential, the value of *M.*

zeyheri remains largely unrealised due to limited scientific documentation and weak integration into formal food and agricultural systems, 'birthing' this report.

The significance of the current study lies in its focus on documenting the distribution, abundance, and nutritional composition of the underutilised indigenous fruit tree *M. zeyheri,* thereby addressing key knowledge gaps that limit its broader utilisation. Unlocking the nutritional and economic value of this species is particularly important amid rising food insecurity and climate variability, where resilient indigenous species can play a crucial role in adaptation strategies. In this regard, the study also supports SDG 13 (Climate Action) by promoting climate-resilient food resources and adaptation pathways.

Furthermore, this study aligns with the National Environmental Management Act (1998), which promotes the conservation and sustainable use of South Africa's indigenous biodiversity. By generating rigorous, location-specific evidence on *M. zeyheri*, the study contributes to biodiversity conservation and sustainable land use, consistent with SDG 15 (Life on Land). The potential impact of enhancing the accessibility and scientific evaluation of knowledge related to this species is substantial, with implications for addressing malnutrition, food insecurity, and livelihood vulnerability not only in southern Africa but also in other regions with similar ecological and socio-economic conditions.

1.5 Aim and objectives

1.5.1 Aim

The study aimed to comprehensively examine *M. zeyheri* in two South African agro-ecosystems (Mpumalanga and Limpopo), focusing on its diversity, traditional knowledge, water-use efficiency and nutritional composition to promote its conservation, adoption, and

sustainable utilisation.

1.5.2 Specific objectives of the project on *M. zeyheri*

- To assess the distribution and abundance of *M. zeyheri* across two agro-ecologies.
- To identify and classify the genetic diversity of *M. zeyheri* and assess variations in production, fruit taste, and adaptability in two different agro-ecologies.
- To evaluate the effectiveness of different propagation methods for improving seedling establishment and growth of *M. zeyheri.*
- To determine water-use efficiency using sap flow for *M. zeyheri* fruit from two different agro-ecologies in South Africa.
- To determine the nutritional composition of *M. zeyheri* fruit from two different agro-ecologies in South Africa.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Underutilised indigenous fruit crops have gained increasing attention in South Africa for their potential to enhance food security and contribute to more sustainable agricultural systems. Although many of these fruit species have been consumed for generations and form part of traditional diets and health practices, they are largely neglected in formal research agendas and commercial agriculture. The existing literature consistently highlights their value as sources of essential nutrients and functional food components, particularly in resource-limited communities where access to diversified diets is often constrained. The topics discussed in this section provide substantial evidence of the role underutilised fruit crops play in traditional healthcare systems. The distribution and habitat of underutilised fruit crops are closely linked to specific ecological conditions and cultural landscapes.

Propagation and domestication remain key challenges for the broader adoption of underutilised wild fruit trees. Developing effective, affordable, and locally appropriate propagation techniques is therefore vital for improving the sustainability of these species. Within this broader framework, *Mimusops zeyheri* has emerged as an essential species due to its nutritional, medicinal, and livelihood potential. The available literature suggests that the species is well adapted to semi-arid environments. Yet, detailed information on its water-use efficiency, growth dynamics, and physiological responses to water availability is scarce. This literature review, therefore, provides a consolidated foundation for research on underutilised fruit crops, with a specific focus on *M. zeyheri*, to support future studies on its propagation, water-use efficiency, and integration into sustainable agricultural systems.

2.2 Importance of underutilised fruits in South Africa

Underutilised fruit crops are an integral part of food systems and contribute to them in various ways. Incorporating underutilised fruit trees into the food system means that people can access a broader spectrum of nutrients, vitamins, minerals, and antioxidants contributing to improved health and nutrition. Diversification of crops enhances food security by reducing the population's reliance on limited staple crops (Mango et al., 2018). Underutilised fruit species can serve as a safety net during famine or market volatility, helping ensure a more stable food supply and better nutrition. They can withstand drought and fare well in poor-quality soil (Kamlesh et al., 2019). Underutilised fruit crops can be consumed or sold fresh. They can also produce value-added processed products such as jams, juices, and dried fruits (Kumar et al., 2020). The products can generate income beyond the fresh fruit sales, benefitting the food processing industries. Vitamins, minerals, and antioxidants are often available at high levels in some underutilised fruit species. Underutilised fruit species frequently have unique properties and incorporating them into the diet can improve overall nutrition, especially in rural communities.

2.3 Medicinal potency of underutilised fruit crops

Growing health concerns have driven substantial demand for edible plants that offer health benefits beyond basic nutrition. Since early times, locals have used various fruits as folk medicine to treat several diseases, including cough, fever, asthma, diarrhoea, indigestion, and skin diseases. Besides the fruit, bark, leaves, stem, root, twigs, and sap have been used as ingredients for traditional medicine (Nazir et al., 2020). The medicinal properties of these plants are closely related to their phytochemicals, which can prevent, treat, and cure various diseases. Extracts from different parts of the plant, including fruit, have been employed for other benefits, such as anticancer, antifungal, antimicrobial, anti-inflammatory,

antiatherosclerotic, antidiabetic, and antiproliferative agents (Khoo et al., 2016). As the demand for food with promising health benefits increases, the search for wild edible fruit trees with medicinal and nutritional properties increases. Underutilised edible fruits are an essential natural resource that can ensure food, nutrition, and health security, particularly during times of need, such as natural disasters and pandemics. Besides, underutilised edible fruits are frequently consumed to treat and cure various ailments and are considered necessities rather than supplements by communities (Dasilaa and Singh, 2020). Progress in plant drug research has flourished, thanks to significant contributions from various disciplines, enabling the isolation, characterisation, and testing of bioactive molecules to produce plant-based pharmaceutical drugs. Several medicinal and health-related products have been obtained from plants; however, the abundance of plants has been neglected, and their potential remains unexplored for several reasons. Most underutilised plants are nutritionally rich and contain a variety of phytochemicals that need to be explored for their potential uses and to exploit their intrinsic and fundamental values, justifying this report.

Underutilised plants continue to languish as untapped resources due to limited access to their complete botanical data and other relevant information. However, current remarkable progress in analytical chemistry, clubbed with a meticulous screening of plant products, combinatorial synthesis, plant cell culture technology, and metabolic and genetic engineering, has enabled and assisted in interpreting and construing numerous constraints encountered in the research on underutilised plants such as sterility, long and low generation time, perennial nature, selection of high-yielding plant sources, peculiar morphological structures, intricate biodiversity, and complex biosynthetic pathways. Previous studies have shown that fruits are rich in bioactive phytochemicals that may provide desirable health benefits beyond basic nutrition, helping reduce the risk of chronic diseases (Dabas et al.,

2013; Dias et al., 2016). The most important groups of dietary phytochemicals in plants are phenolics, alkaloids, nitrogen-containing compounds, and terpenoids, as presented in Figure 2.1. Phenolics, such as phenolic acids and flavonoids, are known for their antioxidant properties and are found in various plant parts (Wu and Pike, 2001). These phytocompounds are characterised by the plant as a defence against predators. Among the phytocompounds produced by plants, anthocyanins contribute to the attractive colours of many fruits, ranging from red to blueberries, while carotenoids give fruits yellow to orange colours.

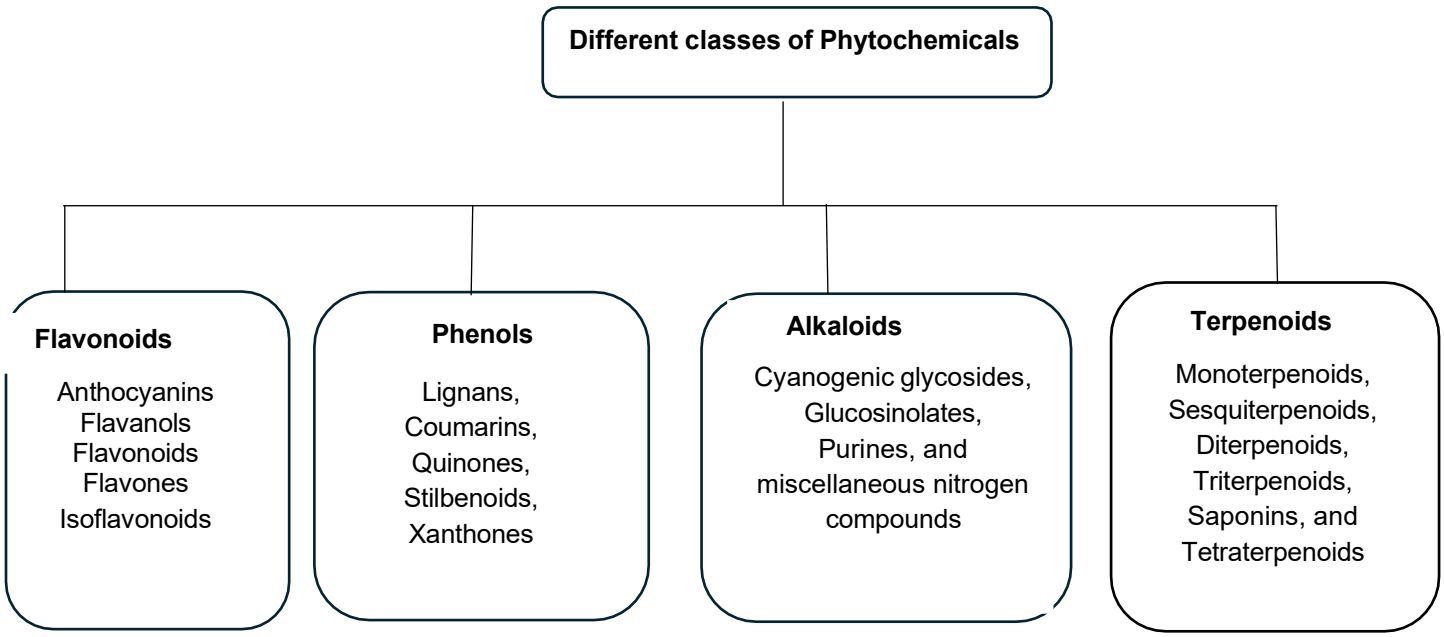


Figure 0-1:Classification of different phytocompounds that are found in plants

2.4 Distribution, habitat, and ethnobotanical survey of underutilised fruit crops

Genus *Mimusops* belongs to diverse genera of flowering plants (Hamdy et al., 2022). Hamdy et al. (2022) estimated the number of species in this genus at 47. Therefore, only four species among these 47 members of this genus are considered indigenous in Southern African countries (Dube et al., 2016). This includes *Mimusops caffra* E.Mey. ex A.DC., *Mimusops obovata* Nees ex Sond., *Mimusops zeyheri* Sond., and *Mimusops obtusifolia* Lam., (Hamdy

et al., 2022). In South Africa, *M. caffra* is distributed alongside the coastal belt in the KwaZulu-Natal and the Eastern Cape (van Staden, 2017a; Jami et al., 2018), with *M. obovata* widespread across the eastern escarpment of Limpopo and Mpumalanga Provinces through the Kingdom of Eswatini to KwaZulu-Natal and stretching towards the Eastern Cape Province (van Staden, 2018). The distribution of *M. obtusifolia* is considered uncertain in South Africa, though some scholars argue that its distribution extends across the northern provinces (Kamundi and van Staden, 2018). Moreover, the distributional range of *Mimusops zeyheri* is considered widespread in South Africa, stretching across the provinces of the north of Limpopo, Mpumalanga, Gauteng, North-West, and KwaZulu-Natal (van Staden, 2017b). This plant species adapted to different habitats, including the Tzaneen Sour Bushveld, Sekhukhune Mountain Bushveld, Limpopo Sweet Bushveld Soutpansberg Mountain Bushveld, Waterberg Mountain Bushveld, and Makhado Sweet Bushveld (van Staden, 2017b). Different tribal ethnic groups in the northern provinces of South Africa, called *M. zeyheri*, use distinct vernacular names, which differ from one group to another (van Staden, 2017b). For example, the Afrikaans vernacular name for *M. zeyheri* is Transvaalmelkhout, whereas its Venḓa name is Mumbubulu; in Ndebele, it is called Umbumbulu (van Staden, 2017b; Magwede et al., 2019). Therefore, these vernacular names reflect *M. zeyheri's* distributional range and diverse uses in South Africa. Ramarumo et al. (2020) stated that vernacular names for a particular species provide information about its uses and distributional range. Furthermore, it is mutual understanding that most indigenous locals do not give names to plants they do not utilise (Ramarumo and Maroyi, 2020).

As a result, it is arguable that those tribes use *M. zeyheri* for varied purposes, and vice versa. Scientific evidence suggests that indigenous people from different tribes differ in their knowledge about the utilisation of similar or other plants (Gouwakinnou et al., 2011; Smith et

al., 2021; Seile et al., 2022; Ndhlovu et al., 2023). For example, the study by Mabogo (1990) reported that the Vhavenda tribe of South Africa uses the root of *Opuntia ficus-indica* (L.) Mill. to remedy a toothache. In contrast, Mbambala et al. (2017) asserted that the same tribe uses the root of this similar species to treat mouth sores and wounds, while Maema et al. (2019) emphasised that the Bapedi tribe uses the same root to treat sexually transmitted diseases such as syphilis and gonorrhoea. Due to this, and since *M. zeyheri* is widespread across various tribal regions in the northern provinces of South Africa, there is no doubt that local people in those communities use it for multiple purposes. In South Africa, inadequate numbers of ethnobotanical studies have reported on the utilisation of *M. zeyheri* for both medicinal and fruit purposes (Constant and Tshisikhawe, 2018; Mogale et al., 2019; Semenya and Maroyi, 2019; Shai et al., 2020), whereas its contributions to local livelihoods, nutritional, and food security remain in-definitively studied (Omotayo et al., 2020). This justifies the utmost need to investigate further the ethnobotanical knowledge associated with various tribal groups' utilisation of *M. zeyheri* and its contributions to livelihoods, nutritional and food security, and its distribution across South African provinces, including Limpopo and Mpumalanga.

2.4.1 Taxonomic development

Mimusops taxonomy and nomenclature evolved with advances in botanical knowledge (Bustamante et al., 2019). Species within the genus were described, defined, and classed based on morphological, anatomical, and reproductive characteristics.

Table 0-1: Taxa included in this Sapotaceae phylogenetic research based on morphology and ndhF sequencing data

Taxon	Origin and voucher	*ndh*F GenBank acc. No.	Standard Reference

Mimusopinae			
Autranella congolensis (De Wild.) A. Chev.	Congo: *Bokdam 4401* (WAG)	AY230666	Pennington (1991)
Mimusops comorensis Engl.	Comoro Islands: *Pignal & Ginguette 1065* (P)	AY230700	Aubréville (1974)
Mimusops elengi L.	Thailand: *Chantaranothai 2305* (Khon Kaen Univ. Herb.)	AY230701	Pennington (1991)
Mimusops zeyheri Sond.	South Africa: *Dahlstrand 6386* (GB)	AY230702	Pennington (1991)

2.5 Propagation strategies for underutilised wild fruit trees

The propagation and conservation of indigenous trees have gained increasing attention in recent decades (Leakay et al., 2022). In Africa, indigenous fruit trees have been an important food source for centuries, but are now facing population declines due to deforestation, and characterization (Cemansky, 2015). Plant propagation has a long history, dating back centuries, as humans have cultivated plants for food and other uses (Kulak et al., 2022).

Over time, various methods of propagation were developed, including sexual (seed) and asexual (vegetative) techniques (Awotedu et al., 2021).

The 20th century saw significant advancements in vegetative propagation, such as the discovery of auxins for rooting cuttings and the adoption of steam characterizes for propagation media (Preece, 2003). Tree propagation methods have evolved. For example, in the 17th and 18th centuries in Sweden, common lime trees were primarily propagated by layering, while seed propagation became more prevalent later (Carvalho et al., 2008). In Africa, efforts to domesticate and commercialise wild fruit tree species have grown, with

research focusing on their nutritional and medicinal properties, genetic diversity, and integration into agro-forestry systems (Leakey et al., 2022). This shift towards indigenous species marks a departure from earlier forestry practices that characterize fast-growing exotic species for timber production (Leaky et al., 2022). The future of indigenous trees lies in their cultivation, with farmers playing a crucial role in their preservation and sustainable use (Cemansky, 2015; Leakey et al., 2022). Propagation challenges with seed production and vegetative propagation are discussed below.

2.5.1 Challenges with seed propagation

Seed propagation is a critical method in plant reproduction, involving the use of seeds to produce genetically diverse offspring through sexual reproduction (Curtis, 2007). This process contrasts with asexual propagation, where offspring are clones of the parent plant (Curtis, 2007). In clonal plants, seed propagation plays a vital role in maintaining genetic diversity and enhancing resilience against pathogens (Sakai and Takada, 2017). For instance, in the Mediterranean region, *Cynara cardunculus* is often propagated by seeds, which can introduce new genotypes and improve agronomic outcomes (Calabrese et al., 2019). The balance between vegetative and seed propagation is influenced by environmental factors and the need for disease resistance, underscoring the importance of seed propagation across agricultural practices and natural ecosystems (Sakai and Takada, 2017; Calabrese et al., 2019). Seed propagation is effective for many species, though some require treatments such as thiourea or dung to enhance germination (Krishnan and Rao, 2019; Tripathi et al., 2022).

Research on seed propagation of wild fruits highlights various techniques and species-specific requirements. Kumar and Parmar (2000) found that species such as Carissa and

Cordia propagate readily from seed, whereas Ficus and Rubus require specific germination treatments. Tripathi et al. (2022) reported that species such as karonda and wood apple achieved over 60% germination without dormancy, with successful propagation through cuttings and air-layering. Hossain (2011) demonstrated that soaking *Flacourtia jangomas* seeds in cold water for 48 hours significantly improved germination and biomass production, while Indole-3-Butaric Acid (IBA) treatment enhanced rooting in cuttings. Dirres (2016) identified 23 wild edible species in Ethiopia, of which 12 showed reasonable germination rates; however, only 11 survived in the field, underscoring the need for further research on their propagation and economic potential. Overall, these studies emphasise the importance of species-specific propagation strategies for the successful cultivation of wild fruits. Seed dormancy is a complex trait that delays germination until favourable conditions arise and is influenced by genetic and environmental factors (Graeber et al., 2012; Finch-Savage and Leubner-Metzger, 2006). Dormancy mechanisms include embryo and coat-imposed dormancy, regulated by hormones such as abscisic acid and gibberellins (Graeber et al., 2012; Qaderi, 2023). Environmental factors, such as temperature and moisture, affect dormancy levels in natural seed banks, with seeds continuously changing their dormancy status over time (Batlla and Benech-Arnold, 2010). Primary dormancy is innate, while secondary dormancy is induced after seed dispersal (Qaderi, 2023). Seed banks can be transient or persistent, with some seeds requiring after-ripening for germination (Qaderi, 2023).

2.5.2 Vegetative propagation of *Mimusops zeyheri*: Cloning techniques

Vegetative propagation is a crucial method for the reproduction of wild fruit trees, allowing for the maintenance of desirable traits and rapid multiplication (Awotedu et al., 2021). Techniques such as stem cuttings, air layering, and grafting have been shown to propagate various

species, including wild jamun (*Syzygium cumin*), Alangeum (A*langium salvifolium)*, carombola (*Averhoa carambola*), Canito (C*hrysophyllum cainito*), kokum (Garcinia indica), yellow mangosteen (*Garcinia xanthochymus*), and Malabar tamarind (*Garcinia gummigutta*), effectively (Triphati et al., 2022). In the sub-Himalayan region, certain wild fruits can be propagated both sexually and asexually, with species such as *Carissa* and *Cordia* readily propagated from seed. In contrast, others require specific treatments for germination (Kumar and Parmar, 2000). Additionally, soilless cultivation systems have emerged as innovative methods to enhance propagation quality and quantity, particularly through techniques such as micropropagation (Schuch and Peil, 2012). Historically, the shift from sexual to vegetative propagation in fruit trees such as olives and grapes has facilitated their domestication, though it has also reduced genetic diversity (Salgotra and Chauhan, 2023).

2.6 Background on *M. zeyheri* and water use efficiency

Mimusops zeyheri Sond., commonly known as the Transvaal red milkwood, is a member of the Sapotaceae family and is referred to by various local names, including Moepel (Afrikaans), Mmupudu (Sepedi), Umpushane (isiZulu), Mbubulu (Venda), and Mgamba kapu (siSwati) (Teffo, 2024). This evergreen tree can reach up to 20 m in height and is characterised by its brown bark, milky latex, and a rounded, spreading crown (El-Hadidy et al., 2022). *Mimusops zeyheri* produces substantial quantities of fruit that are laterally borne and rich in vitamin C (Mashela et al., 2013). Historically, it played a significant role in rural communities as a source of food and traditional medicine; however, in recent years, its use has declined, leaving it vastly underutilised despite its ecological and nutritional value (Lubisi et al., 2023; Matlala et al., 2024). Understanding the water-use efficiency of *M. zeyheri* is crucial for evaluating its potential for sustainable cultivation and conservation, particularly in the context of water scarcity and climate change.

Hatfield and Dold (2019) define water use efficiency (WUE) as the amount of carbon assimilated into biomass per unit of water used by a crop, essentially describing the relationship between water consumption and plant productivity. The WUE directly influences crop health and post-harvest characteristics, and ultimately the market value (Xing et al., 2025). For agriculture to achieve long-term sustainability, improving water use efficiency (WUE) is essential, particularly in regions facing water scarcity. WUE reflects the amount of biomass or yield produced per unit of water consumed by a crop (Alharbi et al., 2024). In the context of climate change and increasing competition for limited freshwater resources, enhancing WUE is a critical strategy for ensuring global and regional food security (Rockström et al., 2010; Blum, 2009). A range of approaches, including innovative technologies, crop-specific agronomic practices, and the adoption of sustainable irrigation systems, have been shown to improve WUE in both staple and horticultural crops significantly (Singh, 2010; Zwart and Bastiaanssen, 2004). Some species are genetically predisposed to achieve high yields with minimal water, which accounts for differences in WUE even within a species (Kilemo, 2022). This variation is also linked to differences in photosynthetic pathways between C_3 and C_4 plants (Kilemo, 2020).

In addition to genetic and physiological factors, WUE is influenced by climatic conditions, including temperature, humidity, and light intensity (Lin et al., 2023). Moreover, anthropogenic interventions, such as irrigation, fertiliser application, and tillage, can enhance crop yield by altering carbon and water cycles, thereby improving WUE (Du et al., 2019). Plant physiological traits, including leaf anatomy, stomatal size and density, stomatal control, residual water losses, nocturnal conductance, and leaf hydraulic conductance, significantly influence water use efficiency by affecting the balance between carbon gain and water loss (Petrík et al., 2023). Despite the growing body of research on WUE, indigenous and underutilised crops such

as *Mimusops zeyheri* remain neglected, mainly in agricultural research. This species, an African fruit tree with considerable nutritional and ecological potential, debatably, has not been systematically studied for its water-use dynamics or its role in sustainable production systems.

2.7 *M. zeyheri* growth, health, and nutritional composition

The *Mimusops* genus in the Sapotaceae family is fascinating because it comprises a wide range of fruit-bearing species used by rural populations in warm and subtropical Africa. Species such as *Mimusops djave*, *Mimusops elengi*, and *Mimusops kummel* produce edible fruits. These fruits vary in terms of sweetness, acidity, and nutritional value. Documented studies across the species reveal consistent trends, including a high carbohydrate content, high vitamin C levels, and a diverse range of mineral compositions (Chivandi et al., 2011b). Many Mimusops plants also contain a significant amount of crude fibre, making them healthier, especially in areas where people typically do not consume much fibre (Dreher, 2018). Many *Mimusops* species possess antioxidant properties and contain a significant amount of phenolics in their phytochemical composition. For example, the seeds and leaves of *M. elengi* are rich in flavonoids and tannins, and the fruits are very effective at characterize free radicals (Zahid et al., 2016). Many seeds in this genus are high in lipids and unsaturated fatty acids, such as oleic acid and linoleic acid, playing a significant role (Cheng et al., 2022; Dutta and Deka, 2014; Kajal Dutta and Deka, 2014). People are aware that these oils are beneficial and can be utilised in various applications, including medicine, food, and industry. Several types of bioactive chemicals are found in leaves, which is why they are often used in traditional treatments.

Despite these general consistencies, reported nutrient concentrations vary widely across the *Mimusops* genus. Vitamin C levels, for instance, range from modest to very high depending on species and environmental conditions (Jitendra et al., 2015). The mineral composition also

differs markedly, influenced by soil type, altitude, and moisture availability. In some species, calcium and magnesium dominate the mineral spectrum, while in others, potassium and iron are more prominent (Kadam et al., 2012). These differences highlight the ecological sensitivity of the genus and underscore the need for multi-site studies when characterizes any specific *Mimusops* species. *Mimusops zeyheri*, the Transvaal red milkwood, fits within these broader nutritional patterns but is distinguished by a relatively small body of scientific work compared with other *Mimusops* species. Available studies show that its fruit pulp contains substantial carbohydrates and dietary fibre, aligning with the general profile of the genus. Vitamin C is consistently highlighted, although reported levels vary across studies, with some documenting moderate levels while others report concentrations comparable to or higher than those found in *Chrysophyllum albidum* (Onyekwelu et al., 2015*)*. Mineral concentrations also reflect variability. Potassium is often the dominant mineral, followed by calcium, magnesium, and iron, yet the exact proportions differ between study sites, suggesting the influence of ecological conditions. Investigations into southern African indigenous fruit species indicate considerable phytochemical potential; for example, in a study of eight underutilised fruits, the seed of *Dovyalis caffra* (Kei apple) contained total phenols of ~5,084.5mg/kg and hydroxycinnamic acids of ~3,403.8mg/kg. In contrast, flavonoids in the seed of *Colpoon compressum* reached ~1,127.2mg/kg (Pfukwa et al., 2022). Likewise, solvent extractions of *Ximenia caffra* revealed a high polyphenolic content in the pulp and skin (Oosthuizen et al., 2018). These data underscore a strong precedent for indigenous fruit materials to harbour high concentrations of bioactive phenolics and antioxidants. However, in the particular case of *Mimusops zeyheri*, the literature fails to provide equivalent quantitative metrics for peel, pulp, seed, or leaf fractions. Thus, the absence of characterize extraction data and analytical values for *M. zeyheri* represents a significant lacuna in the literature, impeding reliable comparison with other

native fruit taxa.

Furthermore, although seed composition data from other Sapotaceae members indicate total lipid contents of ~28-34% and unsaturated fatty acid proportions of ~60-67% (Agustina et al., 2022; Bouknana et al., 2024). There is insufficient regionally stratified data for *M. zeyheri* to determine whether similar or divergent nutrient profiles apply. Similarly, while leaves of related species such as *Mimusops elengi* have been quantified (e.g., ~18- 26 mg QE/g flavonoids; ~3-5% condensed tannins (Mannan et al., 2017), no comparable quantitative leaf chemistry has been reported for *M. zeyheri*. This gap hampers the capacity to contextualise *M. zeyheri* within broader functional-food-oriented value chains or to ascertain its regional variation. The current state of knowledge places *M. zeyheri* is a promising species, but one for which fundamental phytochemical and nutritional characterisation is under-addressed. Such deficits should be regarded not merely as incidental absences but as key obstacles to advancing indigenous fruit research, commercialisation, and rural value-chain development.

2.8 Phytochemical constituents and antioxidant capacity of *Mimusops zeyheri*

Although *Mimusops zeyheri* is recognised for its nutritional and ethnobotanical value, the phytochemical literature on the species is notably underdeveloped. Existing work indicates the presence of broad classes of secondary metabolites, principally phenolics, flavonoids, and tannins, across the fruit and other plant tissues (Chivandi et al., 2011b; Rammone et al., 2020). These compounds are widely associated with antioxidant capacity and physiological functions relevant to plant defence and human health. However, the evidence base for *M. zeyheri* is minimal. The study by Teffo et al. (2025) characterizes the phytochemistry of *M. zeyheri*. Still, the analysis is confined to the fruits and provides only high-level indications of phenolic abundance and antioxidant potential. As such, Teffo et al.'s (2025) contribution, while

valuable for signalling phytochemical activity, does not constitute comprehensive profiling; the peel, seeds, leaves, bark, and other tissues remain chemically undocumented. This partial coverage leaves significant uncertainty regarding the full spectrum of bioactive constituents present in the species. A more pressing concern arises from overlooking environmental and methodological factors known to influence phytochemical expression. Research on native fruits such as *Ximenia caffra*, *Dovyalis caffra*, and *Sclerocarya birrea* indicates that phenolic content, antioxidant capacity, and related metabolites exhibit significant variability across agroecological zones, soil types, rainfall patterns, and harvest times. Without similar multi- site, multi-season studies for *M. zeyheri*, it remains challenging to determine whether the limited findings so far genuinely reflect broader patterns or are merely due to local environmental differences. Furthermore, inconsistencies in extraction solvents, analytical techniques, and reporting units across the limited body of existing research make cross-study comparisons more challenging. Overall, the lack of quantitative data, the narrow focus on fruit tissues, and the absence of geographically diverse evidence highlight a significant knowledge gap. Addressing this gap is crucial for understanding the biochemical ecology of *M. zeyheri* and evaluating its potential role in functional foods, nutraceuticals, and the broader use of underexplored indigenous fruit resources.

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CHAPTER THREE

ASSESS THE DISTRIBUTION AND ABUNDANCE OF *M. ZEYHERI* ACROSS TWO AGRO-ECOLOGIES

Abstract

Mimusops zeyheri Sond., a valuable indigenous tree species with notable ecological and ethnobotanical importance, occurs across diverse agro-ecological regions in South Africa. This study investigated the population dynamics, spatial distribution, morphological variation, and environmental conditions of *M. zeyheri* in two distinct regions: Ehlanzeni District (Mpumalanga) and Vhembe District (Limpopo). Data collection focused on size-class distribution, with statistical analyses applied to assess population structure and variation. Significant differences were found in stem circumference (F=31.98, p= 0.000031), tree height (p=0.017813), and inter-tree distances (p=0.01005) between the two sites. Spatial analysis revealed a dispersed pattern in Vhembe (mean distance = 5.54 m), likely driven by wind dispersal (anemochory), and a clustered pattern in Ehlanzeni (mean distance = 2.68m), suggesting animal-mediated dispersal (zoochory). Ecological health assessments indicated greater anthropogenic disturbance in Vhembe, where over half the population fell into the 50–100% disturbance index range, while Ehlanzeni showed minimal disturbance (0–5% index). Despite high regeneration potential, a dominance of smaller size classes suggests possible regeneration bottlenecks linked to harvesting pressures. These findings underscore *M. zeyheri* as a potential agroforestry species that contributes to climate resilience and food security.

3.1 Introduction

Indigenous wild fruit trees play a crucial role in the livelihoods of many rural communities in South Africa, particularly in Limpopo and Mpumalanga (Semenya and Mokgoebo, 2020; Shai et al., 2020). Wild fruit trees ensure food security by providing edible fruits and health care through ethnomedicine derived from different plant parts and contribute to local economies through harvest and trade (Borelli et al., 2020). Despite their crucial contributions to rural livelihoods, wild fruit trees remain neglected and are declining due to anthropogenic disturbance. According to Wessels (2021), 81-97% of African wild fruit tree species may experience a decline in suitable habitats, with 24-42% expected to go extinct by 2085. The population ecology and distribution patterns of wild fruit trees are crucial for understanding ecosystem dynamics and sustainable resource management. The distribution of wild fruit trees is region-specific and primarily shaped by biophysical factors, including topography, soil properties, climate, and land use patterns (Liu et al., 2021). In South Africa, the distribution of wild fruit species is influenced by diverse vegetation and landscape dynamics across regions, with varying cultivation suitability (Sardeshpande and Shackleton, 2020). The Northern and Western Cape Provinces are less suitable for wild fruit growth due to the predominance of shrubs and barren land (Salami et al., 2022). In contrast, provinces such as Limpopo, Mpumalanga, KwaZulu-Natal, and the Free State are reported to be more favourable for cultivating wild fruit trees (Genis, 2020; Mothupi and Shackleton, 2025; Salami et al., 2022).

Several wild fruit species, including milk plum and 29 others prioritized for domestication and commercialisation, are found in coastal provinces such as KwaZulu-Natal (Nkosi et al., 2020), with *Strychnos spinosa* ranking the highest. Limpopo and Mpumalanga Provinces are recognised for their remarkable endemic plant richness, attributed to their varying climatic conditions and characteristic summer rainfall montane

niches. However, several studies have shown that the conservation knowledge and utilisation patterns of wild fruit trees across the provinces have received insufficient scientific attention, indicating a substantial research and documentation gap (Lubisi et al., 2024; Shai et al., 2020).

Mimusops zeyheri Sond. (Sapotaceae), recognised as a keystone species, occurs widely in rural communities across Limpopo Province, particularly in Vhembe District and Mpumalanga Province, South Africa. The ecological distribution of *M. zeyheri* is influenced by soil composition, with reports documenting varying growth responses across clay, loamy, and sandy soils; clay soils show remarkable leaf growth patterns (Omotayo et al., 2020). The tree has several reported ecological benefits, including soil stabilisation and providing habitat for birds and endophytes (Dube et al., 2016). Although recognised for its ecological and traditional role, there is a notable gap in the comprehensive literature documenting *M. zeyheri's* wild tree distribution patterns, size structure variations, and comparative studies of environmental factors influencing local distribution across different agro-ecologies. While several ethnobotanical studies have documented the traditional and spiritual uses, as well as the regional perceptions of *M. zeyheri* among local rural communities, detailed ecological studies aimed at quantifying tree population densities, size structures, ecological health, and spatial distribution patterns related to environmental variables remain scarce.

Perceptions of the utilisation, population, and distribution patterns of the species indicate that it is underutilised and, therefore, faces a threat of genetic erosion. According to rural communities in Vhembe District of Limpopo, *M. zeyheri* faces mounting pressures stemming from habitat fragmentation, agricultural expansion, and unsustainable harvesting practices (Atanasso et al., 2021; Lubisi et al., 2023).

Sustainable utilisation and conservation of wild fruit trees require documenting their population ecology and distribution patterns. Also, for their sustainable utilisation and conservation, they require the documentation of their population ecology and distribution patterns. Several reports indicate that most wild fruit trees are abundant somewhere in their range, with larger ranges correlating with a higher abundance (Murphy et al., 2006; Stevenson, 2001; Vizentin-Bugoni et al., 2021). This underscores the importance of understanding tree distribution and population ecology as a crucial part of terrestrial ecological management efforts, as such data provides insights into the species' biological requirements and potential responses to changes in the ecosystem. The study aimed to document the distribution and population structure of *M. zeyheri* in Vhembe and Ehlanzeni Districts of South Africa. The study aimed to contribute to the development of sustainable strategies that integrate traditional knowledge with ecological management approaches to ensure the continued contribution of the plant species to both biodiversity and overall human well- being.

3.2. Materials and methods

3.2.1 Study site

The study was conducted in two regions of South Africa: Vhembe District (22.7696°S, 29.9741°E) of Limpopo Province and Ehlanzeni District (29°48′46″S, 30°38′11″ E) of Mpumalanga Province. The two regions were selected based on their contrasting climatic, edaphic, and land-use characteristics that influence the distribution and abundance of wild fruit trees, including *Mimusops zeyheri*. Vhembe District is the northernmost part of Limpopo Province and covers approximately 25,597 km², or nearly 20% of the province's total land area. Land use patterns in Vhembe District are diverse, ranging from livestock rearing and agriculture (mainly maize and sorghum cultivation) to commercial plantations. Vhembe District experiences a humid climate, with temperatures ranging from 18 °C in winter to 28 °C in

summer, and annual rainfall of 755-798mm. The district receives summer rainfall (October-March), with peak precipitation observed mainly in December and February. The district is characterised by an altitudinal gradient ranging from 200 meters above sea level in the eastern lowlands to 1,700 meters above sea level in the highlands. The district is further characterised by the Soutpansberg mountain range, with its highest peak at 1747m, which influences the local climate and the region's temperate and tropical mosaic biota assemblage, which is not found elsewhere in the country.

The district is home to various hydrological features, including Limpopo River, which serves as an international border between Zimbabwe and Botswana, and Ndzhelele River, which supports aquatic and riparian vegetation species such as *Vachellia robusta* subs. robusta (fever tree) and *Diospyros mespiliformis* Hochst. Ex A.DC. (jackal-berry). The district's lowland areas are dominated by *Colophospermum mopane* (J. Kirk ex Benth.) J. Kirk ex J. Léonard vegetation woodland mixed with *Terminalia serica* Burch. Ex DC. and acacia species adapted to clay soil and humid conditions. Soutpansberg is home to diverse *Afromontane* species, including *Zanthoxylum capense* (small knob wood) and *Schefflera umbellifera* (Sond.). Baill. (false cabbage tree), *Xymalos monospora* (lemon wood), *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb. (yellowwood) and *Curtisia dentata (Burm.f.) C.A.Sm.* (assegai tree) (Constant and Tshisikhawe, 2018; Hahn, 2017). The transitional zone is dominated by mixed woodland species such as *Combretum molle* R.Br. ex G.Don (velvet bushwillow), *Grewia hexamita* Burret (giant raisin), *Euphorbia cupularis Boiss.* (Lebombo candelabra tree) and Ficus species. The higher plateaus are characterised by grassland species, such as *Panicum maximum* Jacq (guinea grass) and *Themeda triandra* Forssk. (red grass), as well as scattered eragrostic species that resemble a parkland savanna structure. Ehlanzeni District is one of the most ecologically significant regions,

covering a total land area of 28,795 km². The district has a diverse topography and climate, with forest and woodland covering 39.11% of the area, followed by thicket, bush, and grassland. It receives an annual rainfall of 750–860mm, with both flat lowlands and dissected mountainous terrain. These conditions support forestry, agriculture, and tourism, which are key economic drivers. The region has remarkably diverse vegetation and is home to over 1500 plant species, including endangered and endemic taxa.

The region is dominated by C4 grasses, as well as herbaceous and perennial plant species, such as *Aloe spicata L.f.*, *Tulbaghia violacaea* Harv., *Bidens pipelosa* L., Wild fruit trees such as *Sclerocarya birrea* (A. Rich) Hoschst (Marula), *Mangifera indica* L. (Mango), and *Acacia mearnsii* De Wild. (Black wattle) The region is known for its mountainous peaks, which serve as tourist attractions, including Mariepskop Mountains at 1,944m, Moholoholo at 1,794m, and Hebronberg at 1,767m. The region is home to several water bodies, including Olifants River, which borders Mpumalanga and Limpopo, and the Crocodile River, significant for irrigation in citrus farming, aquatic life, and domestic use.

3.2.2 Sampling

The study employed a systematic random sampling method, as described by Amini et al. (2015). A stratified sampling approach was used to ensure representative coverage, and 41 trees were selected at each study site to eliminate bias. The tree circumference was measured using a diameter tape at the ground level. Measurements included trees of all sizes, including saplings, nutlings, and mature tree classes. The tree height was measured using the tangent method, with assistance from an experienced ecological field guide, as described by Larjavaara and Muller-Landau (2013), with a slight modification.

The technique required the data collector to stand far enough from the tree so the angle from the ground to the top of the tree remains at or slightly below 45 °C. The ecological

disturbance of the trees was measured using an index scoring system, as described in Table

Table 3-1: Ecological disturbance modelling index scoring system

Assessment class	Description	Percentage index score
A	Still pristine or close to pristine	0 < 5%
B	Least damage	5 > < 25%
C	Mostly damaged	25 > < 50%
D	Moderately damaged	50 > < 75%
E	Severely damaged	75>< 100%

3.2.3 Data analysis

Field data was coded and entered into a Microsoft Excel spreadsheet. The coded data were analysed using a single-factor ANOVA ecological scoring model and a paired T-test for sample means. Furthermore, some data was analysed using graphs and percentages.

3.3. Results and discussion

3.3.1 Tree size class distribution

Figure 3.1 shows the size classes of *M. zeyheri* based on circumference measurements taken at the ground level. The results show a predominance of trees in the smaller circumference class (0 - 15cm) at both regions, suggesting that the population of *M. zeyheri* is under threat in both provinces.

Although not reported under the Red List of South African Plants, according to the International Union for Conservation of Nature (IUCN), the populations of any plant species are considered stable only if mature trees are more represented (IUCN, 2025), which is contrary to what the results of the study found in the populations of *M. zeyheri* in Limpopo and Mpumalanga. Ramarumo and Maroyi (2020) concurred that mature plants indicate a

healthy population structure and future reproductive potential, helping avoid extinction risk. In contrast, Tshisikhawe et al. (2012) maintained that a healthy population structure resembles a mix of nutlings and mature trees. Therefore, it is arguable that although Tshisikhawe et al. (2012) and Ramarumo and Maroyi (2020) both focused on the sustainable conservation of threatened plant species, the IUCN is the only international scientific body that determines the conservation status of species (Bamigboye and Tshisikhawe, 2020). However, notably, the current study focused solely on the distribution and population structure of the understudied fruit plant, *M. zeyheri,* in Vhembe and Ehlanzeni Districts of South Africa and did not apply Version 3.1 of the IUCN's Red List Categories and Criteria for data collection.

Factors affecting tree distribution, growth, and survival include fire, soil type, terrain slope, heterospecific tree density, and human-induced disturbances (Atanasso et al., 2021; Lubisi et al., 2023b). Size class distribution is a significant ecological indicator of population structure and tree sustainability. The prevalence of smaller individuals suggests active tree regeneration. In comparison, the absence of larger trees indicates a high tree mortality rate, attributed to increased harvesting pressures for food, timber, or habitat expansion. Trees in Ehlanzeni were mainly found within homesteads and in their vicinity, exposing them to human influence, as communities have access to the trees for fruits, timber, and other plant parts used for medicinal purposes.

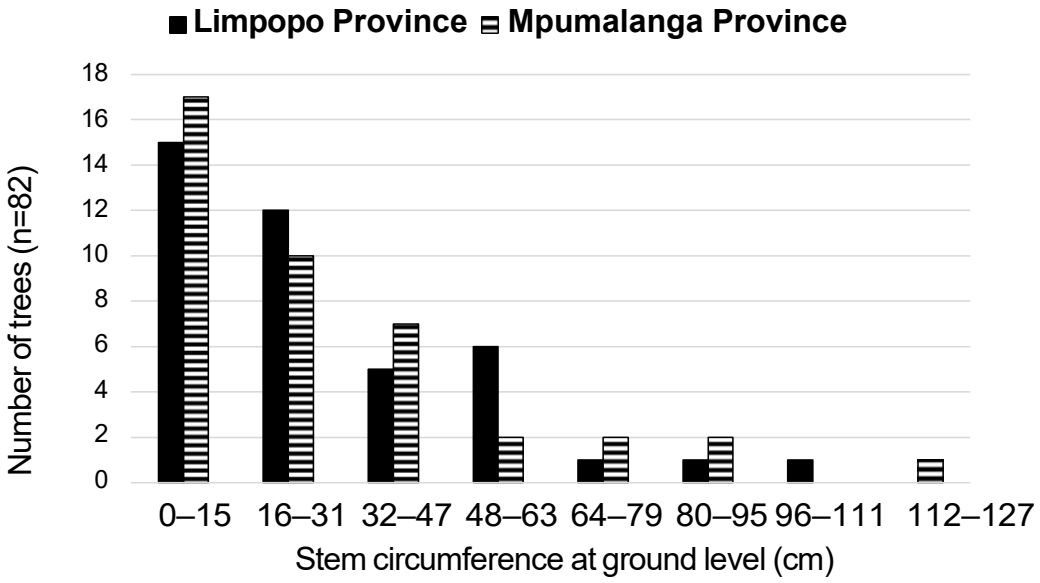


Figure 3-1: Circumference size-class of *Mimusops zeyheri* in Limpopo and Mpumalanga Provinces of South Africa, n=82

3.3.2 Tree height class distribution

Wild fruit trees exhibit significant variations in height distribution across different regions and altitudes. Figure 3.2 shows the distribution of *M. zeyheri* tree height categories across the two areas. Table 3.2 shows a significant difference in tree height across the two regions. Trees in Ehlanzeni exhibited a higher mean height (5.65m) than those in Vhembe (2.71m) with a p-0.017813 and a F-5,853646 (Table 3.2). *M. zeyheri* reaches up to 25m in height at full maturity (Lemmens, 2005b), which is higher than the findings of this study. The variations in tree height may be attributed to differences in climatic conditions between the two study regions. Ehlanzeni of Mpumalanga Province receives high rainfall. It has more humid conditions that support larger trees (Greyling, 2018); conversely, Vhembe of Limpopo Province is drier, receiving lower rainfall, potentially limiting the growth of *M. zeyheri* (Mavhungu, 2022*)*. Similar results were reported by Merwe et al. (2023), where a strong correlation was noticed between tree height and maximum temperature with spring rainfall in *Pinus patula* plantations in Mpumalanga.

Mean annual and maximum seasonal temperatures are emphasised as significant factors influencing dominant tree height in African forests (Merwe et al., 2023).

A study by Mandigora and Drew (2022) suggests that climate change may slow the growth of dominant trees, necessitating the development of adapted wild-tree management strategies. Climatic conditions often influence soil fertility and soil moisture availability, critical determinants of tree growth and productivity. The differences in the two agro-ecologies may also suggest that edaphic conditions, such as soil nutrients, water retention, and organic matter, influence tree growth rates. Sandy soils typical in Limpopo generally lead to reduced growth, leaf development, and plant density compared to loamy soils common in Mpumalanga that support greater tree vigour (Kreuzwieser and Gessler, 2010; Lévesque et al., 2016; Ravhuhali et al., 2022). Some trees, such as *Moringa oleifera,* can adapt well to varying soil conditions without compromising growth or height (Bopape-Mabapa et al., 2021). These findings underscore the complex interactions between climate variables and tree growth in the South African ecosystem, emphasising the need for soil physicochemical analysis across the two agroecologies to validate the observed patterns.

Table 3-2: Statistical comparison for height between *Mimusops zeyheri* in Limpopo and Mpumalanga Provinces

Anova: Single Factor – Statistical Summary						
Groups	*Count*	*Sum*	*Average*	*Variance*	*F*	*P-value*
Limpopo	41	111.4	2.717073	2.935951	5.853646	0.017813
Mpumalanga	41	230.9	5.631707	56.56522		

Figure 3.2 emphasises the marked divergence in height structure between provinces, with Mpumalanga showing a higher proportion of taller individuals (>6m) than Limpopo. In Limpopo, most trees fall into shorter height categories, suggesting either younger stands or suppressed growth likely driven by disturbance or harvesting. The scarcity of tall trees in Limpopo suggests limited progression into mature classes, consistent with ongoing cutting and land-use pressure, as shown in subsequent figures depicting disturbances.

Studies have reported a positive correlation among tree height, diameter, and canopy cover for win fruit trees (Ekasari and Kurnia, 2023; Gopal et al., 2020; Li et al., 2021; Woldegebriel, 2025). Tree height is influenced by several factors, including altitude, which affects the morphological and biological characteristics of wild fruit tree species (Oršanić et al., 2009). According to Schäfer et al. (2000), taller trees exhibit lower stomatal conductance, with a 60% decrease reported in *Fagus sylvatica* trees 30m high.

Woodruff et al. (2007) reported that taller trees maintain high stomatal conductance at more negative leaf water potentials, making photosynthesis possible during periods of water stress. Tree height is closely linked to tree productivity (Wang et al., 2019). Fu et al. (2018) report that the height has been proposed as an alternative productivity indicator, mainly useful in natural and mixed-species wild areas where age-based indices are less applicable. Tree height has significant implications for biomass estimations, competitive fitness, and ecological modelling (Hunter et al., 2013; Sullivan et al., 2018). The results of the study indicate a lack of larger individuals in both agroecosystems, suggesting a low recruitment from smaller to larger size classes. Therefore, this suggests an unhealthy, structured population that does not exhibit a steady transition across different size classes.

This trend may be attributed to harvesting pressure and the risks of overexploitation. *M. zeyheri* is valued for its medicinal, nutritional, and timber properties, making it susceptible to overexploitation. Harvesters often select mature trees with larger branches for timber and firewood, explaining the scarcity of larger trees observed in the study (Young et al., 2017). These findings underscore the importance and necessity of conservation efforts, including protection against overexploitation, land expansion, and the preservation of wild cover at the landscape level.

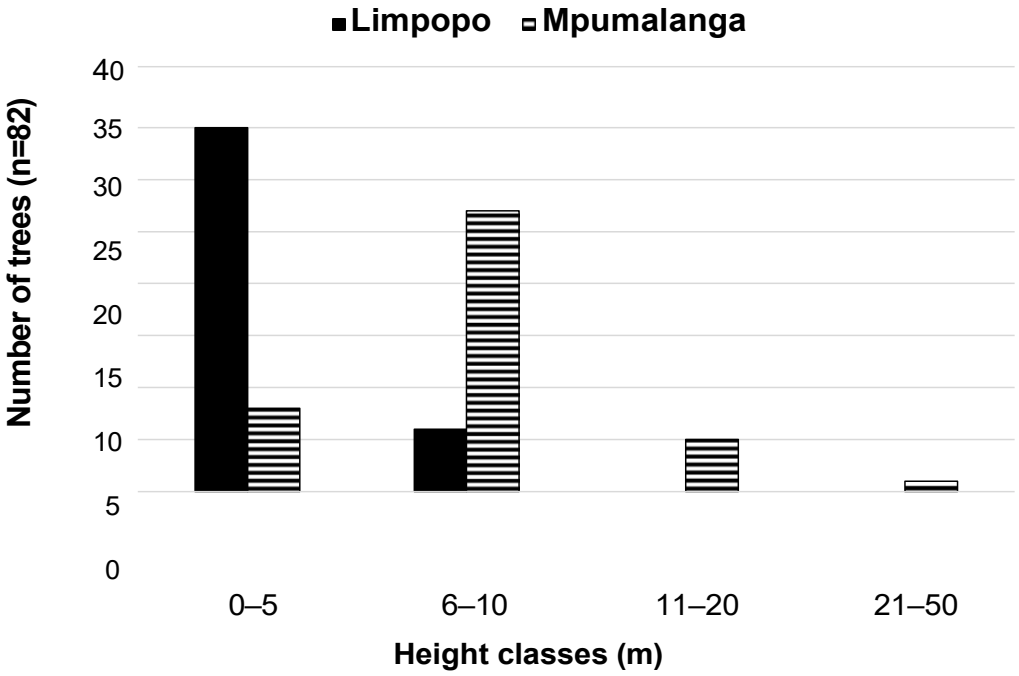


Figure 3-2: Height size-class comparison of *Mimusops zeyheri* found in Limpopo and Mpumalanga Provinces

3.3.3 Tree distance and spatial distribution patterns

The results in Table 3.3 show a significant difference in the distribution distance of *M. zeyheri* trees between the two agro-ecologies. The mean distribution distance is notably greater in Vhembe (5.54m) than in Mpumalanga (2.68m), suggesting a more dispersed tree population structure in Vhembe. Tree-to-tree distance is influenced by various factors in the ecosystem,

including intra-competition among trees. Nut dispersal mechanisms, including anemochory and zoochory, are everyday factors affecting the distribution of wild fruit trees (Carvalho et al., 2025). Anemochory is a common phenomenon in open and rocky areas (Pereira et al., 2022), and according to the study's results, it is the dominant dispersal mode in Vhembe. A higher degree of variance (29.89) in the area suggests greater randomness in nut dispersal patterns, attributable to a significant extent to wind dispersal.

Given the small, lightweight size of the *M. zeyheri* nut, wind dispersal over long distances is possible, explaining the larger tree-spacing patterns in the region. The low variance (10.28) in the distance between trees in Ehlanzeni (Table 3.3) indicates a dominance of zoochory. These results are further emphasised by the data shown in Table 3.4, reflecting a clustered distribution pattern (also visible in Figure 3.3) commonly associated with nut dispersal by frugivorous animals, such as birds, bats, and monkeys, which are known for depositing nuts in a specific location rather than scattering them randomly (Corlett, 2021; Muller- Landau and Hardesty, 2005). Both dispersal mechanisms and patterns are critical in maintaining ecosystem connectivity and diversity.

Several studies on tree size and intra-tree distance have revealed intricate relationships between tree dimensions, such as height, canopy cover, species richness, and tree spatial distribution (Chen and Niu, 2020; Temesgen et al., 2014; Zhang et al., 2004). According to He and Yan (2018), larger trees exhibit a more conservative strategy, while smaller-sized trees portray acquisitive traits, revealing an intraspecific economic spectrum. This is evident in the study, as *M. zeyheri* trees in Vhembe exhibit a conservative strategy. In contrast, *M. zeyheri* trees in Ehlanzeni exhibit a more acquisition-oriented pattern, as evidenced by their clustering.

According to Metsaranta (2020), the spatial distribution of trees is time-dependent, as trees may shift from a random pattern to a more regular pattern in response to mean tree spacing and crown width. Additionally, Gobbetti and Marton (2004) report that the projected mean distance between trees is proportional to their average diameter and basal area, independent of their diameter distribution.

This corresponds with reported results in acacia species of the savannah grassland, where regular tree dispersion patterns and a positive relationship between tree size and nearest neighbour distance were observed (Pillay and Ward, 2012). It is essential to consider multiple factors when studying intra-spacing and tree-size patterns.

Table 3-3: Statistical comparison between *Mimusops zeyheri* distributed distances in Limpopo and Mpumalanga Provinces of South Africa

t-Test: Paired Two Sample for Means	Limpopo	Mpumalanga
Mean	5.541463415	2.680487805
Variance	29.8914878	10.28373476
Observations	41	41
Pearson Correlation	-0.164680459	
Hypothesised Mean Difference	0	
Df	40	
t Stat	2.702490679	
P(T<=t) one-tail	*0.005025019*	
t Critical one-tail	1.683851013	
P(T<=t) two-tail	*0.010050039*	
t Critical two-tail	2.02107539	

Table 3-4: Clark–Evans nearest-neighbour statistics for *Mimusops zeyheri* populations in Limpopo and Mpumalanga

Region	U (Unique tree positions)	Area (m²)	Density (trees/m²)	Observed NN distance $\bar{r}_{obs}$(m)	Expected NN distance $\bar{r}_{exp}$(m)	R(CE Index)
Limpopo	18	2 102.62	0.00856	4.67	5.40	0.86
Mpumalanga	39	1 009 470	3.86×10^{-5}	2.65	80.44	0.03

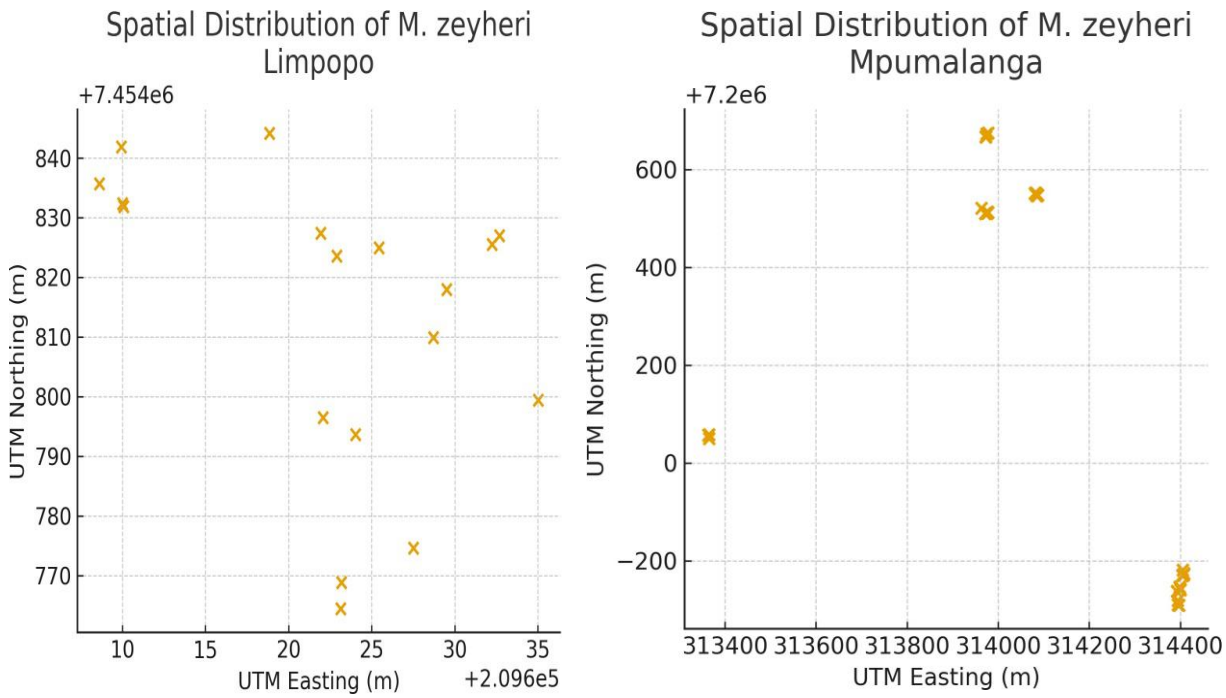


Figure 3-3: Limpopo and Mpumalanga tree spatial scatter plots

3.3.4 Tree health assessment

The damage assessment across the two provinces reveals a stark spatial divergence in the health of *Mimusops zeyheri*. In Mpumalanga, most trees were classified as pristine (< 5% damage), with 18 individuals recorded in this category, while smaller numbers fell into the least damaged (5<>25%; 9 trees), moderately damaged (25<>50%; 8 trees) and mostly damaged (50<>75%; 4 trees) classes. Only two individuals fell into the severely damaged category (75<>100%), suggesting that, at least in this province, widespread physiological decline or structural degradation has not yet occurred. In contrast, Limpopo shows fewer pristine individuals (12 trees < 5%) and comparatively higher proportions of damaged trees, especially in the mostly damaged class (50<>75%; 10 trees), followed by moderately damaged (25<>50%; 9 trees) and severely damaged (75<>100%; 3 trees) categories. The greater representation of individuals in the higher disturbance classes in Limpopo signals a more stressed population, potentially reflecting cumulative impacts of human disturbance, environmental stress, or both.

These observed patterns must be interpreted against the socio-economic context of rural livelihoods in southern Africa, where *M. zeyheri* serves as a multipurpose resource. As highlighted by recent work and the subsequent chapter, this species provides not only edible fruit but also wood for fuel or construction and bark, leaves, or roots used in traditional medicine (Rammone et al., 2020; Teffo et al., 2025a). The high demand for firewood and medicinal bark in many rural communities frequently leads to unsustainable harvesting practices, such as repeated branch cutting, stem debarking, or the selective removal of large limbs, as seen in Figure 3.4. Studies of tree populations subjected to such pressure show that these actions can severely weaken individuals, reduce canopy cover, damage vascular tissue, increase susceptibility to pests or pathogens, leading to mortality or failure to

reproduce (Ohse et al., 2023; Umebayashi et al., 2019). Considering this background, the elevated damage levels in the Limpopo sample are unlikely to be random or due solely to environmental factors, but rather reflect anthropogenic pressure from fuelwood collection, medicinal harvesting, and other resource uses.

The ecological implications of continued over-harvesting and structural damage in *M. zeyheri* populations are serious, as shown in Figure 3.4. As an evergreen fruit-bearing tree native to southern African savannas and woodlands, *M. zeyheri* contributes to biodiversity by providing food and habitat for fauna, supporting soil stability, offering shade, and regulating microclimate (Mkhonto et al., 2024). Loss or degradation of mature individuals, especially those with damaged canopies or compromised bark, can reduce fruit yield, impair natural nut dispersal (which often depends on frugivores), thus weakening recruitment and long-term persistence (Manojkumar et al., 2025).

Moreover, the removal of woody biomass for firewood, if uncompensated by planting or regeneration, leads to a gradual reduction in population density and overall woodland structure (Lindenmayer and Laurance, 2017). This dynamic has been documented in comparable contexts across southern Africa, where unregulated harvesting of poles, bark, and firewood results in habitat modification, reduced canopy cover, and increased tree mortality (Leaver and Cherry, 2020; Ryan et al., 2016). Over time, such degradation may shift *M. zeyheri* from a stable, widespread component of woodland ecosystems to a sparse, stressed remnant with negative consequences for biodiversity, ecosystem function, and local livelihoods.

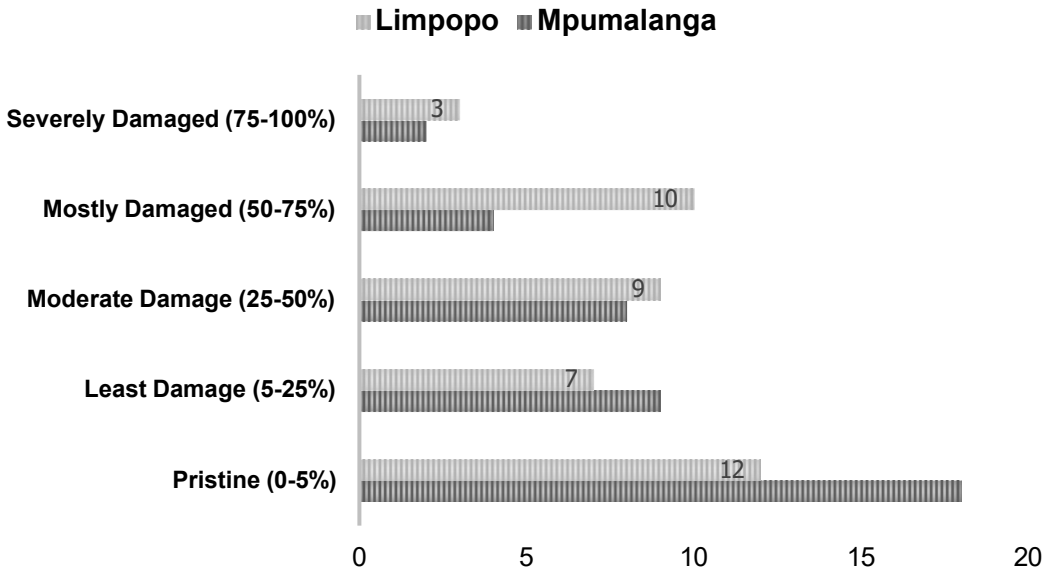


Figure 3-4: Disturbance scores of *Mimusops zeyheri* populations in Limpopo and Mpumalanga Provinces, South Africa

3.3.5 Observed damages and disturbances

The removal of *Mimusops zeyheri* for residential development, noted in Figure 3.6, significantly contributes to population decline by eliminating mature specimens and their reproductive capacity, in contrast to the partial or transient damage inflicted by the harvesting of fuelwood or medicinal plants (Shackleton and Scholes, 2011). Deforestation for the establishment of new towns destabilises the soil, reduces carbon storage, and obliterates minor ecosystems that sustain diverse fauna. This has enduring consequences for biodiversity and ecological functionality (Midgley et al., 2019). Studies on indigenous fruit trees such as *Sclerocarya birrea* and Adansonia digitata indicate that removing mature trees reduces fruit availability, alters nut dispersal, and hinders recruitment, particularly in slow-growing species dependent on substantial, long-lived adults.



Figure 3-5: *Mimusops zeyheri* stems from a cut-off tree (A), damaged bark (B), and regeneration from a cut branch (C)



Figure 3-6: Cleared landscape at one of the study sites in Limpopo, showing cut or burnt woody vegetation, including former populations of *M. zeyheri*

Given the dual ecological importance and high socioeconomic value of *M. zeyheri*, the results underscore the need for urgent, integrated conservation and management strategies. First, sustainable harvesting guidelines must be developed and promoted. For example, restricting or rotating bark and branch removal, limiting firewood extraction to dead or fallen wood, and avoiding indiscriminate debarking that kills individual trees are practices that have been shown to reduce long-term damage when properly managed (Ryan et al., 2016). Second, community awareness and participatory management programmes should be instituted, including educating harvesters about the long-term consequences of overexploitation, involving local leaders or traditional healers in monitoring efforts, and promoting alternative energy sources or the planting of fast- growing fuelwood species. Lastly, active propagation and domestication of *M. zeyheri* should be encouraged by establishing woodlots or agro-forestry plots to reduce reliance on wild populations while providing a sustainable supply of fruit, medicine, and wood.

3.4 Concluding remarks

The study provides valuable insights into the ecology and population dynamics of *M. zeyheri* across the two study agro-ecologies in South Africa. The findings reveal varying levels of plant health, morphology, and spatial distribution patterns, highlighting the intricate connection between anthropogenic and ecological factors that affect wild fruit trees. The predominance of smaller trees across both regions raises concern, as it reflects bottlenecks that may compromise the long-term sustainability of the species in both areas. The study's findings, therefore, indicate that the health of the *M. zeyheri* population is under threat. There is an urgent need to protect species' populations. Local municipalities and traditional leaders need to prioritise collaborative efforts to establish protected areas within their respective territories.

The results indicate the tree's potential regeneration rate, making it an ideal candidate for domestication. There is therefore a need to develop species-specific propagation trials of *M. zeyheri* under greenhouse conditions using different methods, including both nut and vegetative strategies. *M. zeyheri* should be recognised as a valuable species and, therefore, integrated into agricultural cultivation policies for potential contribution to sustainable ecological restoration and food security. The recommended policies should also seek to integrate awareness-raising programmes that educate and train communities on the importance and sustainability of plant species. There is a need for more research focused on assessing species' economic diversity, evaluating their susceptibility to climate change, and conducting in-depth analyses of their reproductive biology to inform the development and application of tailored conservation interventions. This will ensure the safeguarding of critical species, and the indigenous knowledge associated with them, while simultaneously supporting local communities' economies and cultural traditions.

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CHAPTER FOUR

ASSESSMENT OF PROPAGATION TECHNIQUES FOR ESTABLISHMENT AND GROWTH IMPROVEMENT OF *M. ZEYHERI*

Abstract

Plant propagation serves as a fundamental component of plant conservation, domestication, preservation of valuable indigenous plant genetic resources and commercialisation. For indigenous tree species such Mimusops zeyheri Sond., propagation remains an important strategy for overcoming challenges associated with low natural regeneration, habitat degradation, and limited seedling availability. This report documents the investigation on propagation techniques for establishment and growth of *M. zeyheri*. The report further outlines the major challenges encountered during the implementation of the propagation trials and evaluates factors limiting successful vegetative propagation of the species. The research identified several factors that negatively influenced the achievement of the propagation objectives. These challenges included limited availability of planting material and seedlings in local nurseries, inadequate baseline information on seed biology and germination requirements, environmental variability affecting greenhouse performance, and low rooting success in vegetative propagation trials. The report further highlights the importance of developing sustainable propagation and conservation strategies to support future domestication efforts. Recommended interventions include the establishment of community-based nurseries, strengthening conservation initiatives, improving access to propagation material, and addressing market-related limitations associated with underutilised indigenous species.

4.1. Introduction

Indigenous fruit trees contribute significantly to rural livelihoods in Africa, providing food, income, and medicine (Mng'omba et al., 2008). Indigenous fruit trees in South Africa are facing decline despite their potential for poverty alleviation, food security, and income generation (Nkosi et al., 2020; Akinnifesi et al., 2005). Many indigenous fruit trees have lost their natural habitat to deforestation, which has resulted in exponential human population growth (Cemansky, 2015). Inadequate research on cultivation also poses significant challenges to their sustainability (Omotayo and Aremu, 2020). Wild fruit trees are rich in nutrients and vitamins and play a crucial role during times of food scarcity (Akinnifesi et al., 2005).

Mimusops zeyheri, an indigenous fruit tree in South Africa, recognized as an underutilised wild crop with significant medicinal, nutritional, and livelihood benefits (Omotayo et al., 2020). Despite its potential to combat food insecurity and poverty, *M. zeyheri* faces threats, including habitat destruction and overharvesting (Matlala et al., 2024). Local communities perceive a decline in their population, with fire identified as a significant factor affecting its distribution (Lubisi et al., 2023). The tree offers diverse uses, mainly its fruits and leaves, with decoction being a standard preparation method (Matlala et al., 2024). Rich in vitamins, proteins, and fatty acids, *M. zeyheri* has potential as a functional food source (Omotayo et al., 2020). However, its successful utilisation requires multidisciplinary research to address gaps in standardized nutrient profiles, genetic diversity studies, and conservation efforts (Mkhonto et al., 2024). Hence, there is a need for sustainable cultivation of *M. zeyheri* to significantly improve food security and economic stability in local communities where smallholder farmers sell fruit for commercial gain (Matlala et al., 2024).

One way to ensure the sustainable cultivation of *M. zeyheri* and other indigenous wild fruit trees is through exploring different propagation methods. Plant propagation plays a crucial role in the conservation and commercialisation of plants. It helps preserve biodiversity, supports sustainable livelihoods, and contributes to economic development (Fajinmi et al., 2023). Efforts to conserve and domesticate indigenous fruit trees involve germplasm selection, propagation, and evaluation (Akinnifesi et al., 2005). Various propagation methods exist, with vegetative propagation showing promise in shortening the juvenile phase of some species (Akinnifesi et al., 2005). Seed germination studies have identified effective pre- treatments for several indigenous fruit tree species, with many achieving high germination rates through simple cleaning and soaking (Makuvara et al., 2022).

Propagation is crucial for plant cultivation, encompassing both sexual and asexual methods. Sexual propagation involves seeds and genetic recombination, while asexual propagation, such as tissue culture and cuttings, produces genetically identical offspring (Singh, 2021). In vitro propagation, mainly through tissue culture, allows for the rapid multiplication of disease-free plants, which is essential for maintaining desirable traits (Ahmadian et al., 2013). Clonal propagation, especially of medicinal and aromatic plants, is vital for conservation and economical cultivation, as it ensures true-to-type plants with optimal active ingredient levels (Waman et al., 2019). Additionally, advancements in propagation techniques are critical for fruit trees, enhancing seedling quality and disease resistance (Petri et al., 2019). Overall, effective propagation methods are fundamental for sustainable agriculture and biodiversity conservation.

Factors affecting indigenous fruit tree propagation success include the choice of appropriate techniques, defoliation practices, and farmer's attitudes. Partial defoliation of rooted marcots has

shown higher survival rates for species like *Irvingia gabonensis* and *Dacryodes edulis* (Anegbeh et al., 2006).

Farmers' attitudes towards indigenous fruit tree cultivation are influenced by socio- economic factors such as gender, education, and farm size (Agea et al., 2010). Other challenges include limited information on food values and scarcity of seedlings in local nurseries. Environmental factors, knowledge gaps in seed biology, invasive species, and anthropogenic activities also affect indigenous fruit tress regeneration (Gemechu and Jiru, 2021). Strategies such as establishing community nurseries, addressing market issues, and implementing conservation measures are recommended to improve propagation success (Agea et al., 2010; Gemechu and Jiru, 2021). This report documents various propagation strategies for underutilised wild trees, highlighting their effectiveness and associated challenges. The report further outlines the major challenges encountered during the implementation of the propagation trials and evaluates factors limiting successful vegetative propagation of the species.

4.2 Materials and methods

4.2.1 Challenges in propagation of *Mimusops zeyheri*

This section documents the challenges encountered during the propagation of Mimusops zeyheri using both seeds and vegetative propagation materials. The study highlights limitations associated with seed germination, rooting success, environmental conditions, and the availability of suitable propagation materials under greenhouse conditions. It further outlines the factors that affected propagation efficiency and discusses the implications of these challenges for the sustainable cultivation and domestication of the species.

4.2.1.1 Seed dormancy limitations

Mimusops zeyheri seeds were collected from natural populations in Limpopo and Mpumalanga Provinces, while additional seed material was sourced from the South African National

Biodiversity Institute (SANBI).

The seeds possess a hard seed coat (testa) and exhibit physiological dormancy as shown in Figure 4.1, which contributes to delayed, irregular, and often poor germination. Germination success is influenced by several factors, including environmental conditions, seed coat impermeability, and hormonal regulation within the seed (Waskow et al., 2021). In this study, various pre-treatment methods, such as mechanical scarification and hot water treatment, were employed to improve germination, however, these approaches were not successful.



Figure 4-1: *Mimusops zeyheri* seeds with hard testa on, unshelled

(Image captured by Dr C. Mkhonto)

Other challenges faced in this study included limited information on local nurseries producing seedlings of *Mimusops zeyheri*. Strategies such as establishing community nurseries, addressing market issues, and implementing conservation measures are recommended to improve propagation success of species such as *Mimusops zeyheri* (Agea et al., 2010; Gemechu and Jiru, 2021). Various propagation methods exist, with vegetative propagation

showing promise in shortening the juvenile phase of some species (Akinnifesi et al., 2005).

4.2.1.2 Vegetative propagation challenges

Vegetative propagation of *Mimusops zeyheri* has proven to be challenging under greenhouse conditions (Figure 4.2A and Figure 4.2B). One of the major constraints observed during the study was poor air circulation, which resulted in rapid wilting of the cuttings shortly after planting. Maintaining suitable environmental conditions, including adequate moisture, temperature, and growing media, was therefore essential for the survival of the propagated material. The species is known to be a slow-growing tree and appears to have specific requirements for successful establishment and growth. As a result, careful irrigation management and the evaluation of different growing media were necessary to support the propagation trials. Despite these efforts, rooting success remained low, particularly when stem cuttings collected from both young and mature trees were used. Poor root development may be associated with physiological factors within the plant, including the presence of inhibitory compounds or endogenous hormone levels that limit root initiation (Durege et al., 2019). To improve rooting success, several rooting hormones, including Dynaroot powder, indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA), were applied. These treatments were intended to stimulate root formation; however, vegetative propagation of *M. zeyheri* remained difficult, highlighting the need for further research to optimise propagation protocols for this species.



Figure 4-2: Propagation trial of *Mimusops zeyheri* with sandy soil mixed with compost as growing media (A) propagated from old and young trees (B).
(Image captured by Dr C. Mkhonto)

4.2.1.3 Challenges associated with seed germination

Seed propagation of *Mimusops zeyheri* remains challenging due to low and uneven germination rates. The species possesses a hard seed coat (testa), which can restrict water uptake and delay germination (Figure 4.3A and Figure 4.3B). Previous studies have suggested that treatments such as gibberellic acid (GA_3) and smoke water may improve germination by helping to overcome seed dormancy (Elsadek and Yousef, 2019). In addition, optimising stratification periods has been identified as a potential approach to enhance germination success.

At the University of Mpumalanga research farm, several measures were implemented to improve germination. The seeds were manually de-shelled using a secateur to remove the hard testa (Figure 4.3A and Figure 4.3B). Thereafter, the seeds were soaked in hot water for 30 minutes before planting. Despite these pre-treatments, germination remained slow, and seedling establishment required an extended period under controlled glasshouse conditions.



Figure 4-3: *Mimusops zeyheri* seeds used during the propagation trials with hard shell (A) and without shell (B). (Image captured by Dr C. Mkhonto)

4.2.1.4 Challenges associated with rooting hormone, fertiliser and media applications

Several propagation trials were conducted at the University of Mpumalanga research farm to evaluate the effects of rooting hormones, fertiliser applications, and different growing media on the propagation of *Mimusops zeyheri*. Despite the use of dynaroot rooting hormones at different concentrations, root development in stem cuttings remained poor. Both young and mature cuttings showed limited rooting success, indicating that the species is difficult to propagate through vegetative methods.

Different growing media, including sand mixed with compost, hygromix, and water, were tested to improve plant establishment. However, the cuttings frequently wilted, while many of the seeds rotted before successful establishment. Fertiliser applications using Osmocote Pro Slow-Release Fertiliser at different rates also did not result in significant improvements in growth or survival. Additional modifications, such as soaking cuttings in water before planting, extending hormone exposure periods, covering cuttings to retain moisture, and using regenerating branches from older trees, were introduced to improve propagation success. However, these interventions produced limited improvement. The results suggest that *M. zeyheri* has specific physiological requirements for rooting and establishment that are not yet fully understood. Further research is therefore required to optimise rooting conditions, identify suitable propagation media, and develop effective propagation protocols for this species.

4.2.1.5 Challenges associated with propagation: A case of ARC-VIMP

Propagation trials for *M. zeyheri* trees were conducted at the Agricultural Research Council–Vegetable, Industrial and Medicinal Plants (ARC-VIMP) using both seeds and stem cuttings. Although these methods offer opportunities for producing planting material, several challenges were encountered during the study. Seed propagation was affected by slow and uneven germination. Even after seed cleaning and soaking treatments, germination rates remained variable and depended on factors such as seed maturity and environmental conditions. This made it difficult to obtain uniform seedlings within a short period. Vegetative propagation using semi-hardwood stem cuttings also presented challenges. While some rooting was achieved, successful root development required high humidity, rooting hormones, and careful environmental management. Rooting was generally slow and inconsistent, limiting the number of cuttings that established successfully. In addition, maintaining suitable moisture and humidity levels in the propagation environment required continuous monitoring.

These findings indicate that both seed and stem-cutting propagation of *M. zeyheri* can be challenging and require specific environmental conditions to improve success rates. Further research is needed to optimise propagation protocols and enhance the production of quality planting material for conservation and cultivation purposes.



Figure 4-4: Sowing of *Mimusops zeyheri* seeds in seedling trays under glasshouse conditions and vegetative propagation using semi-hardwood stem cuttings (Imaged captured by the ARC-VIMP team)

4.3 Closing remarks and recommendations

The propagation trials conducted under different experimental conditions at the University of Mpumalanga and the Agricultural Research Council-Vegetable Industrial Medicinal Plants demonstrated that the propagation of *Mimusops zeyheri* remains a complex and challenging process. Both seed and vegetative propagation methods showed limited success. Seed propagation was constrained by slow and uneven germination, low seed viability, and seed dormancy, while vegetative propagation was affected by poor rooting, wilting of cuttings, and inconsistent establishment under greenhouse conditions. The use of different growing media, rooting hormones and fertiliser applications provided valuable insights; however, these interventions did not consistently improve propagation success.

The study confirmed the importance of *M. zeyheri* as an indigenous fruit tree with potential contributions to food security, nutrition, biodiversity conservation, and rural livelihoods. However, the slow growth habit of the species, together with challenges in propagation, limits its large-scale cultivation and commercialisation. The findings also highlighted the need for a better understanding of the physiological, biochemical, and genetic factors that influence germination, rooting, and plant establishment.

Future research should focus on improving seed germination through the evaluation of dormancy-breaking techniques, including chemical and mechanical scarification, and hormonal treatments. There is also a need to investigate suitable seed storage conditions to maintain seed viability over longer periods. Vegetative propagation protocols should be further refined through the assessment of different cutting types, rooting hormone concentrations, misting systems, and the use of microbial inoculants or biostimulants that may enhance root development.

Successful development of propagation protocols will require continued collaboration among research institutions, government departments, conservation agencies, and local communities. Such partnerships will contribute to the sustainable conservation, cultivation, and commercial utilisation of *Mimusops zeyheri*, thereby supporting biodiversity conservation, food and nutrition security, and rural economic development.

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CHAPTER FIVE

GENETIC DIVERSITY OF *M. ZEYHERI* AND ASSESS VARIATIONS IN PRODUCTION, FRUIT TASTE, AND ADAPTABILITY IN TWO DIFFERENT AGRO-ECOLOGIES

Abstract

Understanding variation within *Mimusops zeyheri* is essential for guiding its conservation, domestication, and potential development as a high-value indigenous fruit tree. This chapter examined the morphological, sensory, and genetic diversity of *Mimusops zeyheri* across two populations sampled in Vhembe (Limpopo) and Ehlanzeni (Mpumalanga), using 40 trees in total (20 per region). Morphological assessment showed clear population-level differences in reproductive traits: Mpumalanga fruits were larger, with mean fruit lengths of 29.41±0.61mm and widths of 24.4±0.55mm, compared to Limpopo fruits measuring 27.41±0.47mm in length and 23.22±0.36 mm in width. Nut size followed the same pattern, averaging 2.11± 0.36cm in Mpumalanga and 1.81±0.31cm in Limpopo. By contrast, leaf morphology remained stable across regions, with overlapping mean leaf lengths (7.23–7.52cm), widths (3.93–4.03cm), and leaf areas (21.69–22.80cm²). Sensory evaluation, conducted with 100 adult participants (50 per site), revealed a strong regional contrast: Limpopo fruits received markedly higher ratings across all eight sensory attributes, with mean scores for taste, aroma, and mouthfeel ranging from 7.7 to 8.0, while Mpumalanga fruits scored between 5.0 and 5.4 on the 9-point hedonic scale. Genetic analyses using the *matK* and *trnH-psbA* chloroplast markers (75% sequencing success) identified two major genetic lineages, distinguished by regional clustering, along with rare haplotypes such as LP3, MP9, and MP10, which showed longer branch lengths and

unique nucleotide substitutions. Together, the morphological, sensory, and genetic evidence demonstrates strong regional structuring in *M. zeyheri* populations, shaped by both environmental gradients and historical lineage divergence. These findings highlight the importance of conserving multiple genetic groups and provide a basis for selecting superior fruit quality and adaptive traits for future domestication initiatives.

5.1. Introduction

Variation within a species underlies its capacity to adapt, persist, and evolve in changing environments. In fruit-bearing trees, the interaction between genetic make-up, morphological form, and sensory attributes determines both ecological fitness and human value (Rival, 2021). For many indigenous African fruit species, this intricate web of variation remains largely unexplored. *Mimusops zeyheri* Sond. is a fitting example. Valued by rural communities for its sweet, aromatic fruits and durable timber, it is cultivated informally despite its considerable ecological and nutritional potential (Omotayo et al., 2020). What remains less understood, however, is how the species varies genetically and phenotypically across regions and how such variation relates to taste and consumer preference.

In recent years, researchers have become increasingly interested in underutilised fruit trees such as *M. zeyheri*, as they seek hardy, nutrient-rich fruits suitable for semi-arid and variable climates (Chivhandi et al., 2011; Omotayo et al., 2020). These species are genetic reservoirs that can help guide future breeding and conservation measures. However, the promise and potential of M. zeyheri cannot be realised without a thorough analysis of its internal diversity. Multiple traditional research studies have documented mainly ethnobotanical uses and nutritional attributes of the species in isolation (Lubisi et al., 2023; Matlala et al., 2024; Omotayo et al., 2020). While important, such compartmentalised studies rarely show the complete biological

narrative connecting genetics, environment, and perception. Assessing genetic diversity within *M. zeyheri* populations provides an essential foundation for their conservation and improvement. Although the species is increasingly recognised for its nutritional and ecological value, molecular-level studies are scarce. Limited studies have applied DNA-based markers to explore variation among its genotypes, and even fewer have linked that variation to fruit quality or adaptation.

Without this knowledge, breeding and domestication efforts remain largely empirical. In any plant improvement programme, progress depends on the extent of genetic variation and how it can be utilised. The wider the genetic base, the greater the chance of combining traits that enhance yield, resilience, or fruit quality (Sattar et al., 2021). Within *M. zeyheri*, this diversity underpins more than adaptation; it is the key to nutritional and phytochemical potential. Variability in genes controlling the synthesis of sugars, minerals, and secondary metabolites likely explains differences in fruit sweetness, colour, and antioxidant activity across populations (Carbone et al., 2009; Gonzali and Perata, 2021). Understanding these genetic foundations allows researchers to identify genotypes that are both agronomically promising and nutritionally superior. Populations with distinctive genetic traits may also exhibit biochemical advantages, including potentially higher concentrations of phenolics, vitamins, or essential minerals, contributing to the species' dietary and medicinal value (Carreno-Quintero et al., 2013; Pott et al., 2021).

To investigate these relationships, this study combined molecular, morphological, and sensory analysis. *Mimusops zeyheri* Leaf, fruit, and nut characteristics were recorded to document visible variation between and within regions. DNA was extracted from leaf tissue to evaluate genetic diversity and population structure, providing a molecular perspective on differentiation that morphology alone cannot explain. At the same time, sensory evaluation captured how people

experience the fruits, their sweetness, aroma, and texture, offering a human dimension that links genotype to perception. Such an integrated approach helps clarify how *M. zeyheri* has adapted to distinct environments and points towards its potential for selection, propagation, and eventual domestication as a high-value indigenous fruit tree.

5.2 Materials and methods

5.2.1 Morphological sample collection and analytical procedure

Morphological and physiological data were obtained from *Mimusops zeyheri* trees growing in the same sites where leaf samples for DNA analysis were collected, namely Vhembe District of Limpopo and Ehlanzeni District of Mpumalanga. In each region, twenty trees were selected to correspond with those sampled for molecular work. From every individual, representative samples of fruits, leaves, and bark were collected. Mature leaves and fully ripened fruits were selected to ensure comparability across regions and minimise developmental bias. Bark samples were carefully removed from the main stem at breast height using a clean knife to prevent damage to the trees. After collection, the fruits were placed in labelled paper bags, transported to the laboratory, and processed within seventy-two hours to minimise shrinkage and moisture loss. In the laboratory, fruits were washed under running distilled water to remove dirt and debris, air-dried on absorbent paper, and then manually dissected with a sterile stainless-steel scalpel to separate the nuts from the pulp. Each fruit and its corresponding nut were assigned unique identification codes to prevent mix-ups. Morphological measurements were performed using a digital Vernier calliper with an accuracy of ±0.01mm. Fruit length was measured as the distance from the apex to the base of the fruit, while fruit width was taken as the maximum equatorial diameter after rotating the fruit to locate its widest point.

Nuts were cleaned and air-dried for twenty-four hours before measuring their maximum longitudinal size, expressed in centimetres. All measurements were replicated thrice by the

same collector to reduce observer bias, and the mean of the three measurements was recorded as the final value.

5.2.2 Data analysis

All quantitative data were first entered and cleaned in Microsoft Excel before being analysed using R (Version 4.3.2) and IBM SPSS Statistics (Version 29). Descriptive statistics, including means, standard deviations, and coefficients of variation, were calculated for each trait to summarise morphological variability within and between regions. Differences among populations (Limpopo vs Mpumalanga) were tested using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's HSD test was used for pairwise comparisons.

5.2.3 Sensory evaluation

5.2.3.1 Fruit collection and sensory procedure

Fruits used for sensory evaluation were collected directly from the same *Mimusops zeyheri* trees sampled for morphological and genetic analyses in Vhembe District (Limpopo) and Ehlanzeni District (Mpumalanga), followed by the steps shown in Figure 5.1. Only fully ripe, undamaged fruits with uniform colour and firmness were selected to maintain consistency across sites. After harvesting, fruits were rinsed with distilled water, air-dried, and stored in insulated coolers to preserve freshness during transport. Sensory sessions were conducted separately in each region, and participants were intentionally presented with fruits sourced from their own locality to avoid unfamiliarity effects. Before serving, fruits were brought to room temperature and cut into uniform bite-sized portions using sterilised utensils. Each portion was placed in identical, coded, non-transparent containers to minimise visual bias, and all samples were served in a randomised sequence.

The sensory assessment was conducted separately in Limpopo and Mpumalanga, with 50 willing participants in each region. Participants were adults aged 18 years and above who volunteered after being informed of the purpose and nature of the study. Sensory evaluations were conducted in small groups under calm, well-lit conditions to avoid external distractions. Respondents were instructed not to discuss their impressions with others while scoring to maintain independent evaluations. Between samples, participants rinsed their mouths with clean water to reduce carry-over effects. Each participant rated the eight sensory attributes (appearance, aroma, taste, sweetness, texture, mouthfeel, aftertaste, and overall acceptability) using a standardised 9-point hedonic scale, where 1= “Dislike” and 9 = “Very Liked.”(Appendix 1) Completed score sheets were checked for completeness before collection.

5.2.3.2 Data analysis

Data from the sensory evaluation were entered into a cleaned spreadsheet and analysed using descriptive and inferential statistical procedures. Mean, standard deviation, minimum, and maximum values were calculated for each sensory attribute at each site. To assess differences between the two regions, Welch’s t-test was applied because it accounts for unequal variances and sample sizes. Multivariate analyses were then conducted to explore relationships among sensory attributes. A radar chart was used to visualise attribute profiles across sites, and Principal Component Analysis (PCA) was performed to identify the characteristics that contributed most to variation in sensory perception. All analyses were carried out using R (Version 4.3.2), and graphical outputs were generated using the ggplot2, FactoMineR, and factoextra packages.

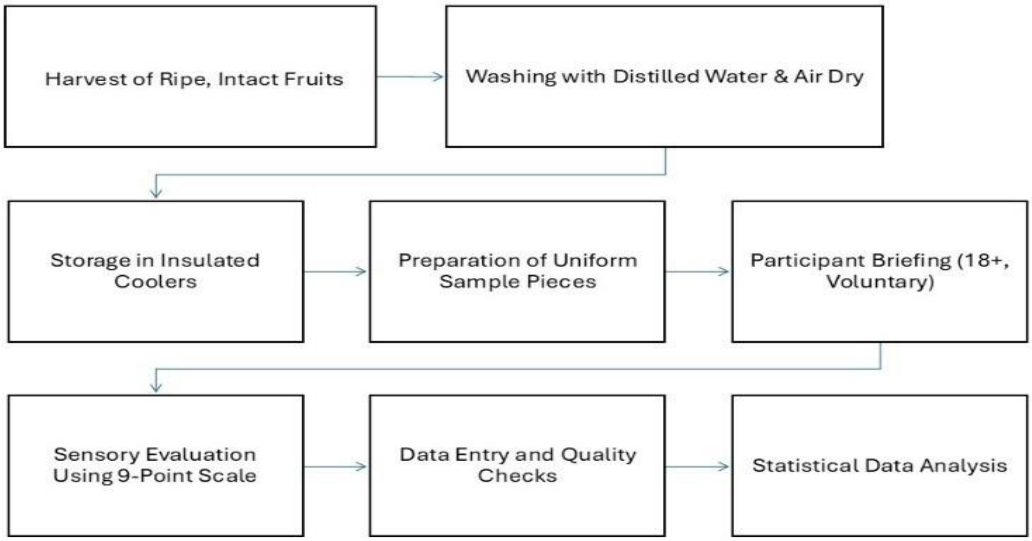


Figure 5-1: Flow diagram of the sensory evaluation process

5.2.4 Genetic analysis

5.2.4.1 Leaf sample collection

Leaf samples of *Mimusops zeyheri* were collected from two distinct populations representing contrasting agro-ecological zones: Vhembe District in Limpopo Province and Ehlanzeni District in Mpumalanga Province. At each site, 20 individual trees were randomly selected, and one mature, healthy leaf was sampled from each, yielding 40 samples. To avoid sampling from genetically identical ramets, trees were spaced at least 30 m apart. Each leaf sample was immediately placed in a separate brown paper bag, labelled with its collection site and number (M1–M20 for Mpumalanga and LP1–LP20 for Limpopo), as shown in Figure 5.2. The bags were kept in a portable cooler during transportation from the field to the laboratory to minimise degradation of genetic material. Upon arrival, samples were air-dried and stored at 4 °C until DNA extraction and subsequent molecular analyses.

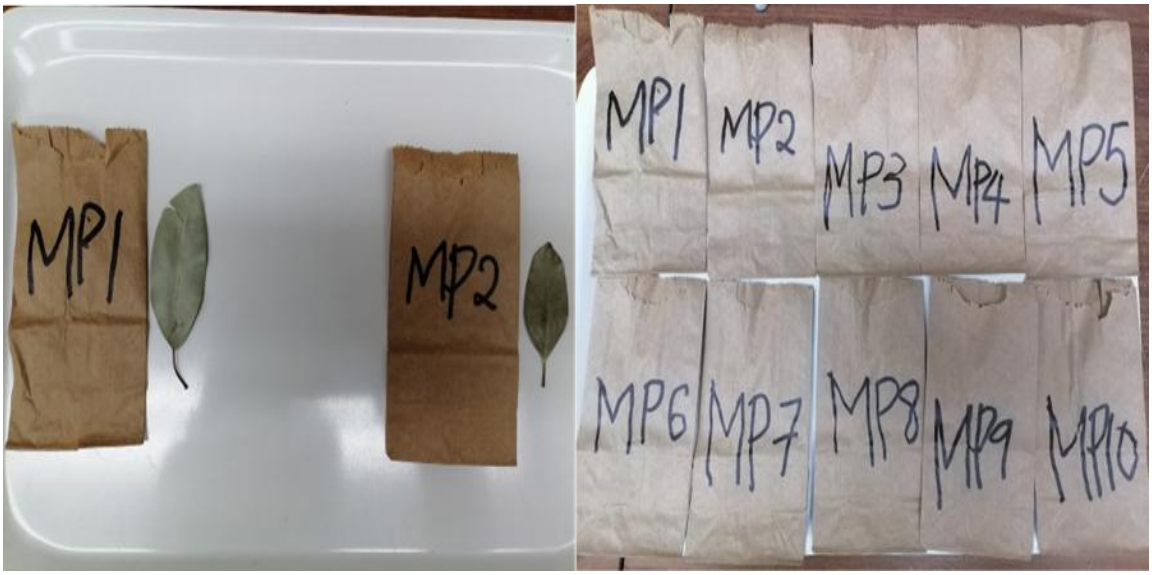


Figure 5-2: Collected leaf samples from Mpumalanga.

(Image captured by Dr C. Mkhonto)

5.2.4.2 DNA extraction, sequencing, and analysis

DNA analysis was performed at the University of Johannesburg African Centre for DNA Barcoding, Department of Botany and Plant Biotechnology. DNA extraction is an internationally recognised technique for extracting DNA from plant samples. It is a process of purification of DNA from a sample using a combination of chemical and physical methods. The DNA extraction method was performed using the CTAB (cetyltrimethylammonium bromide) method described by Doyle and Doyle (1987). The extracted DNA was processed using Polymerase Chain Reaction (PCR). This internationally recognised technique amplifies DNA fragments via temperature cycling, generating thousands to millions of copies of a specific DNA region. The core barcoding region *matK* and an additional gene *trnH-psbA* were selected for this test. Negative and positive controls were used to ensure the procedure's correctness (i.e., no contamination was detected).

All PCR reactions were performed using Amplicon TAQ DNA Polymerase Master Mix Red (www.amplicon.com) with the addition of 3.2% bovine serum albumin. The PCR instrument used is a Gene Amp® PCR System 9800 Fast Thermal Cycler (Applied Biosystems). PCR products were visualised on 1% agarose gels, and visible products were selected for DNA sequencing. The amplified fragments were then bidirectionally sequenced using an internationally recognised Sanger cycle-sequencing method, which provides the actual DNA sequence of the amplified regions. The process is based on the selective incorporation of chain- terminating dideoxy-nucleotides by DNA polymerase during DNA replication. Cycle sequencing was performed using the BigDye® Terminator V3.1 (Thermo Fisher Scientific) kit. The DNA code was visualised using a 3500 Genetic Analyser (Thermo Fisher Scientific). Negative and positive controls were used to ensure the procedure's correctness. Complementary strands were assembled, edited, and aligned using Geneious v.8.1.9 (Biomatters). The sequence data for the targeted regions was analysed. The Basic Local Alignment Search Tool (BLAST) algorithm was used to compare query sequences against the GenBank (NCBI) and Barcode of Life Database (BOLD), both of which are internationally recognised sequence databases. A BLAST search compares a query sequence with the library of sequences available in GenBank and identifies sequences that resemble the query sequence above a certain threshold.

5.3 Results and discussion

5.3.1 Bark morphology

Bark morphology in *Mimusops zeyheri* exhibits significant ecological variation across its range, with distinct differences between populations from Mpumalanga and Limpopo (Figure 5.3). Individuals from Mpumalanga usually develop dark brown to blackish bark that is deeply fissured and coarse, often supporting lichens, an indication of the humid, shaded, and stable microclimates characteristic of forest margins and moist valleys. This phenotype aligns with broader patterns in the Sapotaceae, where greater bark fissuring, thicker periderm layers, and darker pigmentation

are associated with higher moisture availability, enhanced cambial activity, and sustained secondary growth (Pumijumnong et al., 2023; Rahman et al., 2019). In contrast, Limpopo populations have lighter grey-brown, thinner, and smoother bark, typical of trees growing on rocky outcrops and in semi-arid savanna. These environments experience intense sunlight, shallow soils, and limited moisture, which favour reflective bark surfaces, limited cork development, and minimal fissuring to reduce water loss. These xeromorphic traits align with observations in other Sapotaceae genera, where species in drier habitats often develop thinner, less fissured bark as adaptive responses to heat and moisture stress (Pausas, 2015; Peguero-Pina et al., 2020). Moganedi et al. (2011) also highlight the importance of environmental factors like soil type, rainfall, and altitude in understanding morphological differences, emphasising the influence of ecological gradients on regional variation. In general, the bark morphology of M. zeyheri exhibits considerable phenotypic plasticity, driven by environmental diversity throughout its distribution, aligning with patterns observed in the Sapotaceae family.

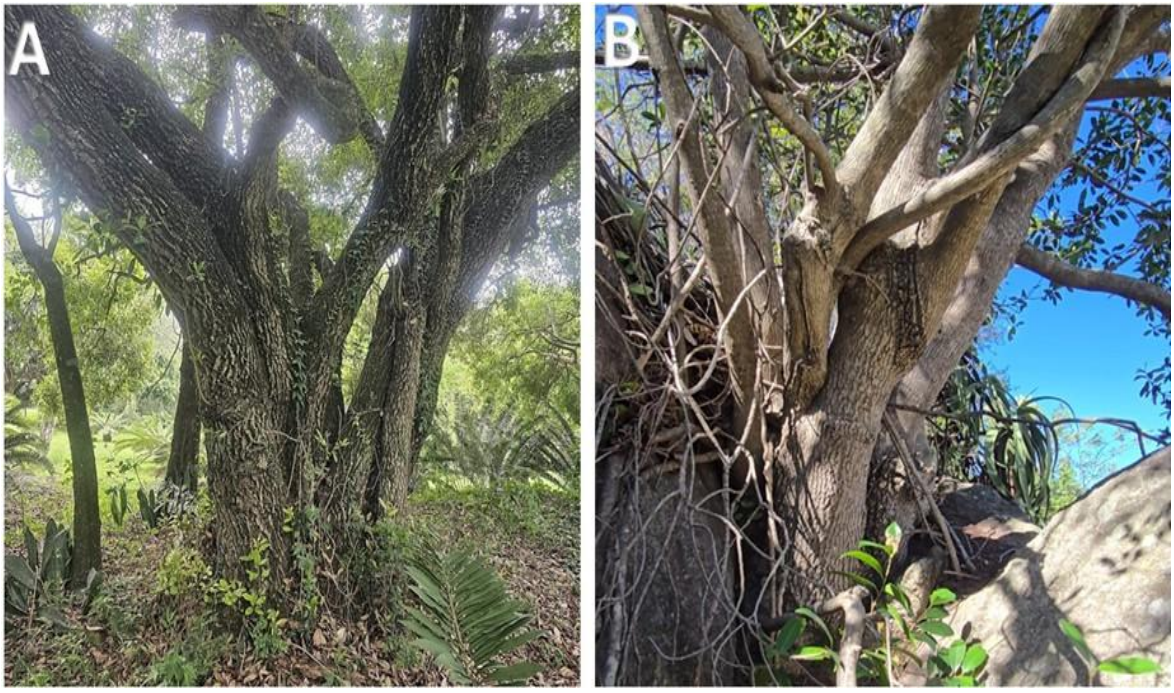


Figure 5-3: Comparative bark morphology of *M. zeyheri* populations from two regions in South Africa, Mpumalanga, Ehlanzeni District (A), and Limpopo, Vhembe District (B). (Image captured by Dr C. Mkhonto)

5.3.2 Nut and fruit morphology

Nut and fruit size results of M. zeyheri from Limpopo and Mpumalanga are shown in Table 5.1. The results revealed a clear and consistent pattern: across all reproductive traits measured, nut size, fruit length, and fruit width, the Mpumalanga population exhibits noticeably larger values than the Limpopo population. Mpumalanga individuals produced larger nuts (2.11±0.36cm) and larger fruits, both in length (29.41±0.61mm) and width (24.14±0.55mm), whereas Limpopo individuals had smaller nuts (1.81±0.31cm) and smaller fruit dimensions overall. These differences represent a coordinated upwards shift in reproductive morphology in the Mpumalanga population rather than isolated variation in individual traits. Joint analysis of nut and fruit traits revealed a strong morphological distinction between the two regions, supporting the view that these populations differ in their overall reproductive phenotype.

Although larger fruits and larger nuts align when the species is considered as a whole, within-site correlations are weak (r= –0.328 between nut size and fruit length in Limpopo and r=0.117 in Mpumalanga), indicating that individual trees do not consistently produce larger nuts when they produce larger fruits.

Instead, the association between fruit and nut size appears to be structured at the population level. This type of population-level divergence in reproductive traits, with relatively loose tree- level correlations, has also been reported for other African fruit trees in the Sapotaceae and related families, including *Vitellaria paradoxa*, *Sclerocarya birrea,* and *Chrysophyllum albidum* (Djekota et al., 2014; Dlamini, 2011; Sanou et al., 2005), where fruit and nut characteristics cluster by region or stand rather than tightly scaling within individual trees.

Beyond the descriptive statistics, the size differences observed in *M. zeyheri* are consistent with broader patterns in indigenous African fruit trees, where intraspecific variation in nut morphology is shaped by reproductive strategy, maternal resource allocation, and the ecological roles nuts play in regeneration (Negash, 2021; Schupp et al., 2019). Larger nuts, such as those characteristics of the Mpumalanga population, typically contain greater stored reserves, which can support stronger early nutling growth and improve survival under competitive or shaded conditions. This trend has been documented in *Vitellaria paradoxa* and *Sclerocarya birrea*, where heavier nuts tend to produce nutlings with greater initial vigour and resilience (Sanou et al., 2005). Nut size differences in *M. zeyheri* may also influence germination patterns. Research on other Sapotaceae, including *Chrysophyllum albidum*, shows that large nut morphotypes often germinate more slowly but sustain emergence over a more extended period.

In contrast, smaller nuts may germinate more rapidly, reducing the window of vulnerability to granivores and fungal pathogens (Dadegnon et al., 2015). This suggests that the larger nuts in Mpumalanga may favour robust but possibly slower-establishing nutlings. In comparison, the smaller nuts in Limpopo may support quicker germination and a faster response to transient recruitment opportunities.

At the same time, the smaller nuts typical of the Limpopo population should not be viewed as ecologically inferior. Numerous studies highlight the advantages of small nuts: they are generally produced in higher numbers, disperse more widely, and are more easily transported by birds and small mammals (Morales and Morán López, 2022; Nakashima and Do Linh San, 2022). For fleshy-fruited trees that depend heavily on animal-mediated dispersal, such as *M. zeyheri*, smaller nuts may enhance colonisation potential by reaching a broader range of microsites, including gaps and disturbed patches. The coexistence of these contrasting nut-size strategies within a species is well documented in savanna trees, where recruitment success is shaped not by a single “optimal” nut size but by trade-offs between dispersal, establishment, and maternal investment (Wright et al., 2024). In this context, the variation observed in *M. zeyheri* is consistent with the wider botanical literature, suggesting that both nut types contribute to population persistence through different but complementary roles within the regeneration niche.

The documented differences in fruit size reinforce this interpretation. Larger fruits in Mpumalanga provide greater pulp rewards, which are likely to be attractive to frugivores capable of longer-distance dispersal, while the associated larger nuts enhance early nutling performance (Just et al., 2024).

In contrast, Limpopo's smaller fruits and nuts may represent a strategy that stresses fecundity and dispersal breadth: smaller propagules, produced in larger numbers, can be handled by a wider array of dispersers and spread across more heterogeneous environments. These dynamics mirror classical nut-dispersal theory, which emphasises the balance between nut size, dispersal potential, and nutling performance as a central component of plant reproductive ecology (Castro et al., 2024; Fricke et al., 2019; Ramlal et al., 2025).



Figure 5-4: Comparison of *M. zeyheri* nut morphology from two regions in South Africa, Mpumalanga, Ehlanzeni District (D), and Limpopo, Vhembe District (C). (Image captured by Dr C. Mkhonto)

Table 5-1: Nut size and fruit morphological traits of *M. zeyheri* from Mpumalanga and Limpopo, including correlations between nut size and fruit dimensions

Province	Nut Size (cm)	Fruit Length (mm)	r (Nut–Fruit Length)	Fruit Width (mm)	r (Nut–Fruit Width
Mpumalanga	2.11±0.36[a]	29.41±0.61[a]	0.117	24.14±0.55[a]	–0.078
Limpopo	1.81±0.31[b]	27.41±0.47[b]	–0.328	23.22±0.36[b]	–0.063

Values represent mean ± standard deviation. Different superscript letters indicate statistically significant differences between provinces ($p<0.05$)

5.3.3 Leaf morphology

The leaf morphometric results in Figure 5.5 revealed minimal differences in leaf size between Limpopo and Mpumalanga populations of *M. zeyheri*. Mean leaf length was 7.23cm in Limpopo and 7.52cm in Mpumalanga, while mean leaf width measured 3.93cm and 4.03cm, respectively. Calculated leaf area also showed substantial similarity, with averages of 21.69 cm² (Limpopo) and 22.80 cm² (Mpumalanga). The narrow range of differences, typically less than one centimetre, indicates substantial overlap in leaf morphology across the two regions. This pattern of morphological stability is consistent with findings from broader leaf-trait ecology literature, where evergreen tropical species often exhibit conserved leaf dimensions across sites with comparable environmental conditions (Moles and Leishman, 2008; Poorter et al., 2009). *M. zeyheri*, a sclerophyllous species in the Sapotaceae, is characterised by thick, leathery leaves that maintain structural consistency across environmental gradients (Chivandi et al., 2012b; Mkhonto et al., 2024b). The slight tendency towards larger leaves in Mpumalanga may relate to marginally higher local moisture availability, as leaf size is known to respond to microclimatic conditions such as rainfall and temperature (Wang et al., 2022; Wright et al., 2017). However, the differences observed here are small and fall well within expected intraspecific variation for African woody species.

Notably, the considerable overlap in leaf area distributions suggests that both populations occupy a shared morphospace, reinforcing the ecological interpretation that leaf size in *M. zeyheri* is a highly stable functional trait. This aligns with global analyses showing that key morphological traits often exhibit limited divergence across populations unless subjected to strong selective pressures or highly contrasting climates (Díaz et al., 2016). Thus, the results indicate that *M. zeyheri* maintains consistent leaf morphology across its

range in Limpopo and Mpumalanga, with only subtle, non-adaptive variation.

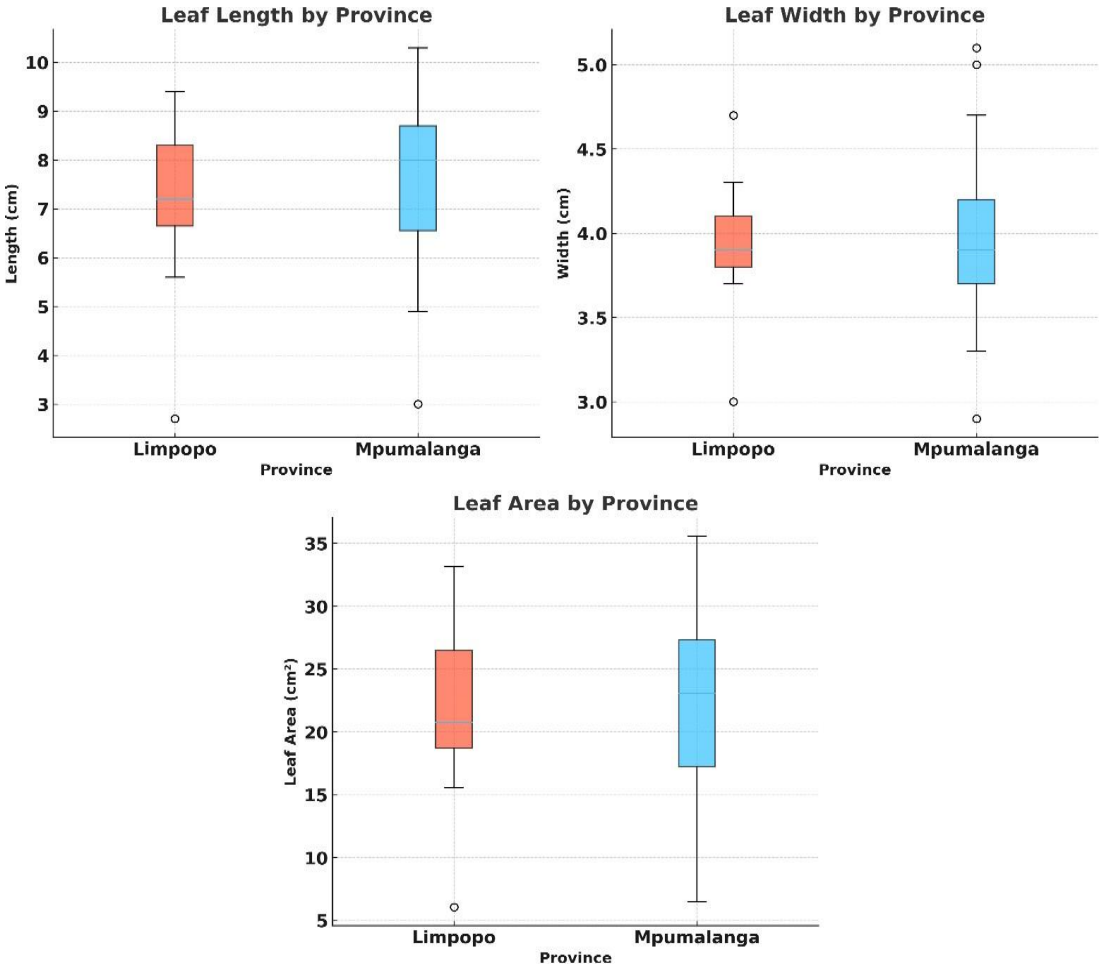


Figure 5-5: Variation in leaf length, leaf width, and leaf area of *M. zeyheri* from Limpopo and Mpumalanga

The Mpumalanga population of *M. zeyheri* displayed abundant reproductive activity during the sampling period, with numerous open flowers and developing buds observed along the axillary nodes (Figure 5.6A). Flowers exhibited the typical multi-segmented, star-shaped corolla characteristic of the species, with creamy-white tepals radiating symmetrically from the centre. Compared to Limpopo samples, Mpumalanga individuals carried significantly more flowering clusters per branch, suggesting favourable phenological conditions during the sampling interval.

Vegetatively, the leaves were elliptic, glossy, and medium to dark green with entire margins (Figure 5.6B). No gall formation was observed in Mpumalanga trees, in contrast to several Limpopo individuals that exhibited widespread galling. Leaf size remained relatively constant across populations, with only slight variation, consistent with the quantitative measurements recorded in this study.

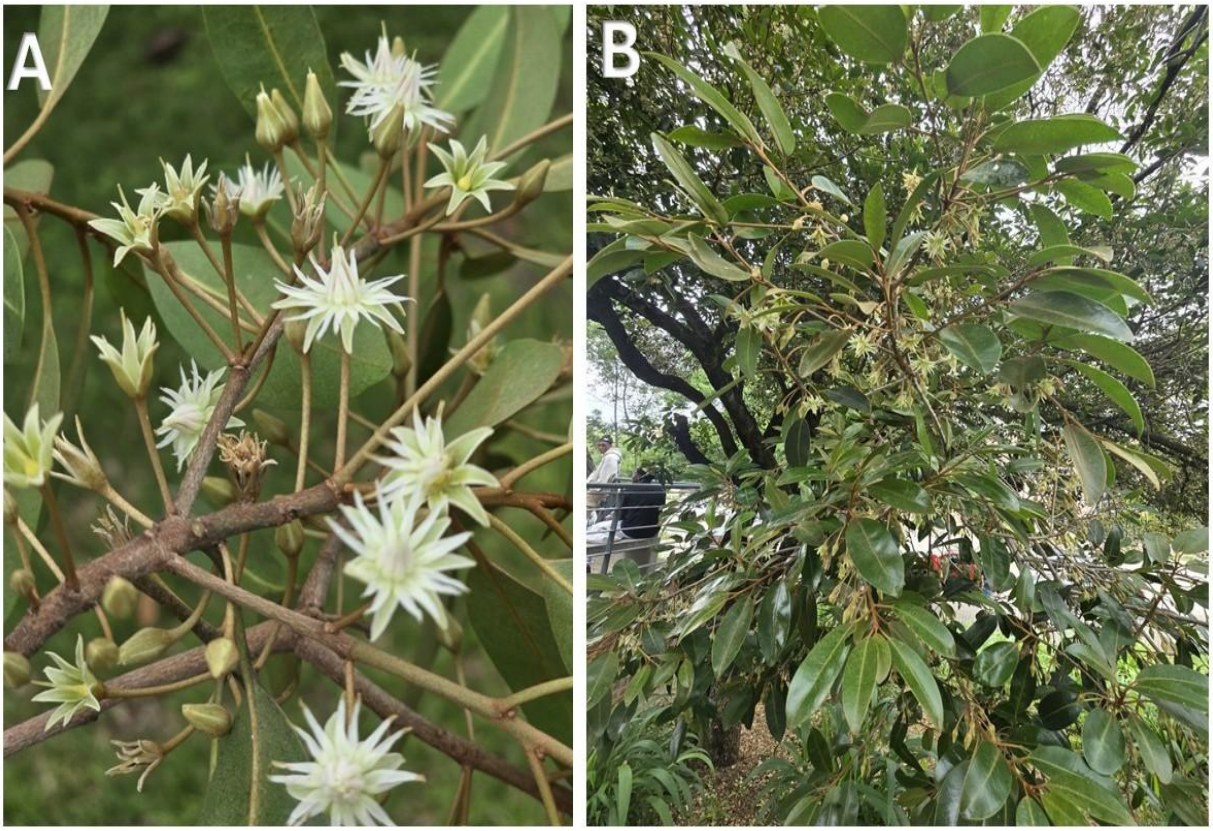


Figure 5-6: Early reproductive (A) and vegetative (B) morphology of *M. zeyheri* from the Mpumalanga populations. (Image captured by Dr C. Mkhonto)

The Limpopo population of *Mimusops zeyheri* exhibited early reproductive development during the sampling period, with several branches showing compact clusters of immature axillary flower buds (Figure 5.7A). These buds were small, oval, and tightly grouped, indicating that the trees were in the initial stages of floral initiation before anthesis.

Vegetatively, leaves were elliptic, glossy, and medium green; however, a notable feature of the Limpopo samples was the presence of pronounced gall formation across the leaf lamina (Figure 5.7.B). These galls, likely resulting from insect-induced hypertrophy, varied in density among individuals but did not visibly distort overall leaf shape or venation patterns. Together, these observations reflect a Limpopo population characterised by early-stage reproductive activity and moderate biotic stress, while maintaining the species' general vegetative morphology.



Figure 5-7: Early reproductive (A) and vegetative (B) morphology of *Mimusops zeyheri* from the Limpopo populations. (Image captured by Dr C. Mkhonto)

5.3.4 Sensory evaluation

5.3.4.1 Participants' sensory responses to fruit attributes

The descriptive statistics in Table 5.3 show a distinct and uniform pattern in consumer evaluations of *M. zeyheri* fruits across the two study sites. Fruits sourced from Limpopo exhibited superior mean scores across all eight sensory attributes in comparison to those from MPE (Mpumalanga–Ehlanzeni). Various attributes, notably taste, aroma, mouthfeel, and overall acceptability, exhibit significant differences, with Limpopo, Vhembe (LV) fruits consistently scoring between 7.7 and 8.0, while MPE fruits range from 5.0 to 5.4. The 9-point scale (1 = very poor; 9 = excellent) highlights the extent of this difference; LV fruits are positioned between "very good" and "excellent," whereas MPE fruits are nearer to "fair" or "average." This indicates a significant difference in sensory appeal between the two geographical zones. These differences reflect more than just inherent fruit quality; they echo how communities interact with wild fruits in their daily lives. Vhembe is a region where wild edible fruits are still widely collected, traded informally, and incorporated into the diets of rural households. Greater cultural familiarity often translates into more positive sensory assessments, as individuals develop an appreciation for flavour nuances, textural expectations, and ripeness indicators over time.

The radar chart (Figure 5.8) and PCA (Figure 5.9) offer a coherent understanding of how respondents from both sites perceived the sensory qualities of *Mimusops zeyheri*. The PCA supports and extends these radar observations by showing how these same attributes interact to shape respondents' overall perception of the fruit. Attributes that appeared more pronounced in the radar chart, particularly aroma, taste, and mouthfeel, also carried the strongest loadings in the PCA, demonstrating that they were the primary drivers in distinguishing how the fruit was interpreted. Overall, respondents from both study sites rated

fruit favourably across several attributes, indicating that the species possesses generally appealing sensory characteristics. Attributes such as appearance, aroma, taste, and overall acceptability received moderate-to-high scores in both Vhembe and Ehlanzeni, reflecting a broad appreciation of the fruit's quality across regions. Although Vhembe consistently recorded slightly higher mean values, the overall shape of the profiles suggests that both respondent groups recognised *M. zeyheri* as a palatable fruit. Across both sites, aroma emerged as a key sensory driver of acceptance. The literature consistently shows that aroma plays a decisive role in shaping consumer expectations because it results from the combined effects of numerous interacting volatile organic compounds (Kilinçoğlu et al., 2025). In this study, aroma scores differed only slightly between the two sites, yet the attribute remained influential for both groups. Similar findings were reported in sensory studies on underutilised fruits such as *Hippophae salicifolia*, where aroma variations contributed to subtle differences in overall liking across products processed into juice, jam, or chutney (Sherpa, 2021). These patterns emphasise that even modest differences in aroma intensity can shape how consumers perceive freshness and flavour richness.

Appearance played an essential role in shaping respondents' views of the fruit at both sites. Colour and surface characteristics are widely recognised as early indicators of ripeness and quality compounds (Kilinçoğlu et al., 2025), as reflected in the similar, generally positive scores for appearance in both Vhembe and Ehlanzeni. Comparable findings have been reported in other wild fruit studies, such as *Elaeagnus umbellata*, where appearance remained a stable attribute across different localities (Ali et al., 2026; Hussain et al., 2011). Texture and mouthfeel also contributed meaningfully to overall acceptability. Attributes such as firmness, juiciness, and fibrousness are known to influence preference in both fresh and processed fruits (Ilhan, 2024). In this study, both sites rated these attributes relatively well,

with Vhembe showing slightly higher values. This indicates that the samples retained the tactile qualities typically associated with ripe *M. zeyheri*.

Notably, the way the fruit was prepared for evaluation may have influenced how aroma and texture were perceived. Handling steps, such as cutting or short exposure to air, can affect the release of volatile compounds and surface moisture. Similar effects have been documented in sensory work on *Hippophae salicifolia*, where different processed forms showed varied sensory outcomes (Sherpa, 2021). These preparation-related factors may explain some of the smaller differences observed between the two sites, while both still recognise *M. zeyheri* as a fruit with appealing sensory traits. Furthermore, maturity and harvest timing are crucial factors that greatly affect the perceived flavour, aroma, and texture. A study by Ilhan (2024) highlights that soluble solids and flavour compounds undergo substantial changes as the fruit matures, which influence individuals' senses. The observed patterns suggest that sensory scores result from a complex interaction among biological, environmental, and experiential factors, rather than being exclusively dictated by chemical composition.

Table 5-2: Summary statistics for *M. zeyheri* fruit sensory evaluation from Mpumalanga and Limpopo

Attribute	LV Mean	MPE Mean	Diff	LV std	MPE std	LV Min	MPE Min	V Max	MPE Max
Appearance	7.92	5.30	2.62	0.78	1.23	6	3	9	8
Ripeness	7.94	5.24	2.70	0.84	1.10	6	3	9	7
Aroma	7.90	5.38	2.52	0.71	1.18	6	3	9	8
Taste	7.94	5.28	2.66	0.71	1.14	7	3	9	7
Texture	7.94	5.30	2.64	0.74	1.15	7	4	9	8
Mouthfeel	8.04	5.20	2.84	0.78	1.16	7	3	9	8
Nuttiness	5.74	3.96	1.78	0.83	0.83	5	3	7	5
Overall Acceptability	7.76	5.18	2.58	0.62	0.90	7	4	9	7

A summary is given of the descriptive statistics for all sensory attributes of *Mimusops zeyheri* fruits evaluated across the two study sites: LV (Limpopo, Vhembe) and MPE (Mpumalanga, Ehlanzeni). The table reports the mean, standard deviation, minimum, and maximum values for each attribute, together with the mean differences between sites. Sensory ratings were collected on a 9-point hedonic scale, with 1 indicating "very poor" and 9 indicating "excellent." Comparisons between LV and MPE were performed using Welch's independent samples t-test, and the results showed statistically significant differences ($p<0.05$) for all attributes.

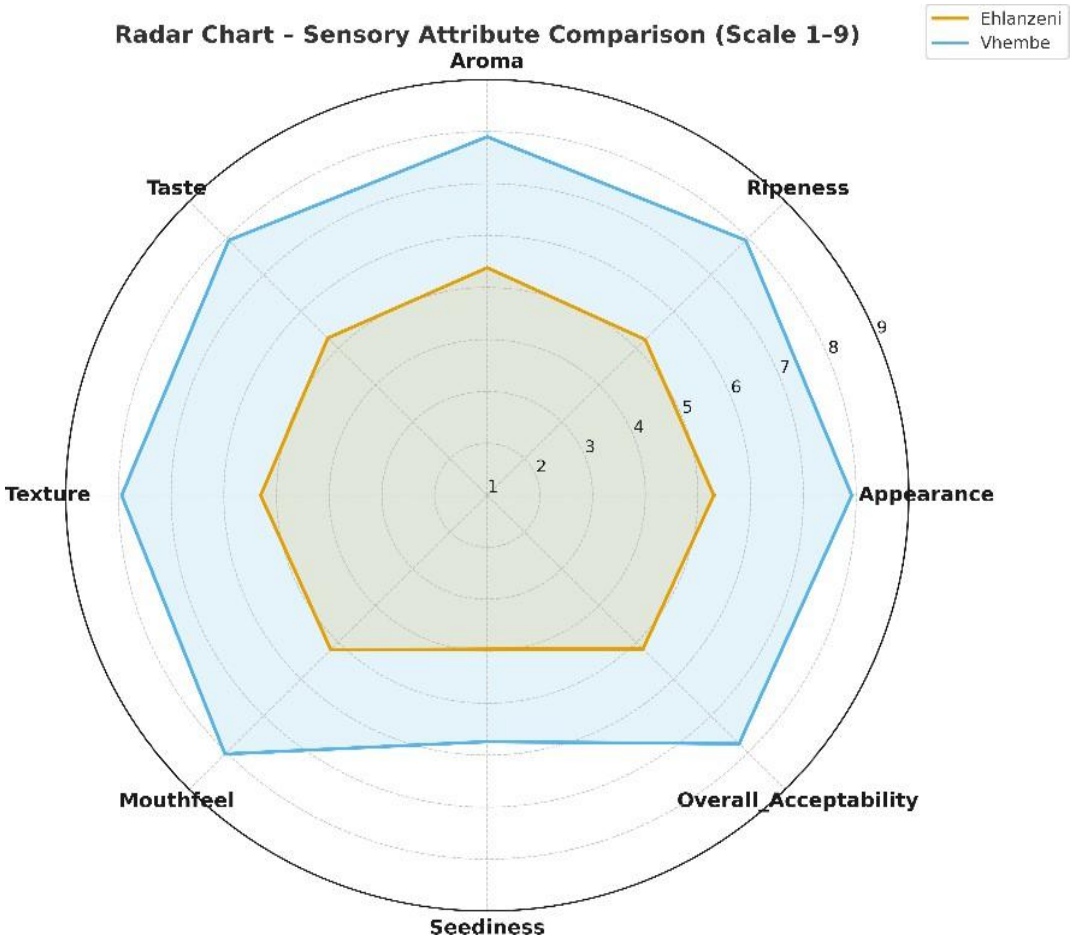
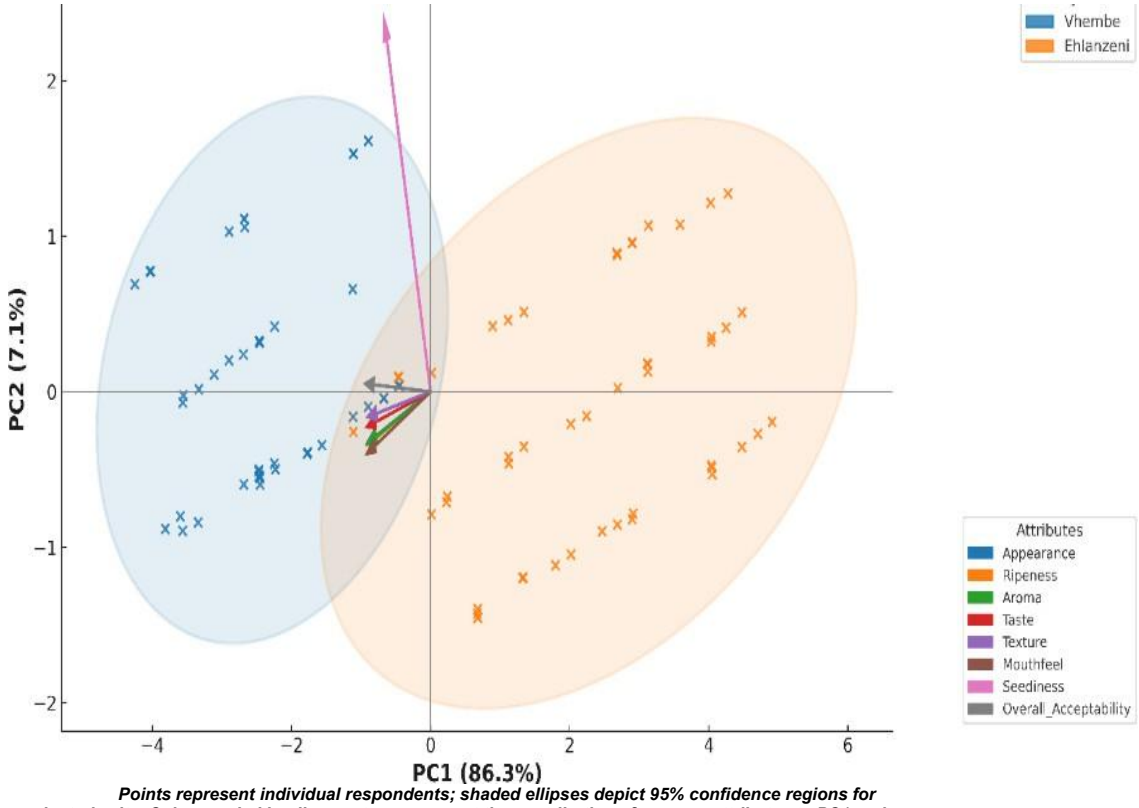


Figure 5-8: Radar plot showing the mean sensory evaluation scores of *Mimusops zeyheri* fruits across the two study sites, LV (Limpopo, Vhembe) and MPE (Mpumalanga, Ehlanzeni)



Points represent individual respondents; shaded ellipses depict 95% confidence regions for each study site. Colour-coded loading vectors represent the contribution of sensory attributes to PC1 and PC2, with corresponding attribute colours shown in the legend

Figure 5-9: Principal Component Analysis (PCA) biplot illustrating respondents' overall sensory perception patterns for *Mimusops zeyheri* fruits from LV (Limpopo–Vhembe) and MPE (Mpumalanga, Ehlanzeni).

5.3.5 DNA analysis

DNA barcoding was employed to assess genetic diversity and relatedness among *Mimusops zeyheri* populations collected in Limpopo and Mpumalanga using two chloroplast markers, *matK* and *trnH-psbA*. These markers provide complementary information on sequence divergence and phylogenetic clustering, allowing the identification of genetic variation within and between populations. Table 5.4: Voucher table shows PCR and sequencing results. Only 20 of the 40 samples received were processed, resulting in a 75% success rate for the samples listed above. Unsuccessful reactions were repeated twice before being concluded.

Table 5-3: PCR and voucher sequencing results

		PCR		Cycle Sequencing	
No	ID	matK	trnH-psbA	matK	trnH-psbA
1	LP1				
2	LP2				
3	LP3				
4	LP4				
5	LP5				
6	LP7				
7	LP8				
8	LP9				
9	LP10				
10	LP11				
11	MP1				
12	MP2				
13	MP3				
14	MP4				
15	MP5				
16	MP6				
17	MP7				
18	MP8				
19	MP9				
20	MP10				

Successful

Unsuccessful

5.3.5.1 Sequence alignment and marker efficiency

Figure 5.10 presents the DNA barcode profiles of *Mimusops zeyheri* individuals from Limpopo (LP) and Mpumalanga (MP), generated using the chloroplast *trnH-psbA* intergenic spacer. Each horizontal bar corresponds to a single sample, with colours representing nucleotide states at aligned positions across the region. The distinct vertical colour shifts indicate polymorphic sites, while uniform colour stretches denote conserved regions. Samples LP1–LP9 and MP3–MP10 exhibit apparent sequence variation, with several accessions showing unique colour blocks, indicating nucleotide substitutions, insertions, or deletions relative to the consensus sequence. The barcode patterns exhibit significant sequence variation across the trnH-psbA region, underscoring why this marker is effective at distinguishing between closely related populations. The mix of colored segments and the lack of uniformity between individuals confirm that trnH-psbA accumulates mutations more quickly than coding regions like matK, a phenomenon extensively documented in flowering plants (Pang et al., 2012; Fazekas et al., 2008).

This higher variability proves helpful when studying populations, since it reveals subtle genetic differences tied to where populations live and how they have adapted to local conditions. The observed apparent visual differences between LP and MP samples indicate region-specific variations, which align with the moderate genetic separation observed in the cluster and tree-based analyses.

The concentration of variable sites in LP3, MP5, and MP8, as indicated by more frequent colour changes, suggests the presence of private alleles or micro-haplotypes associated with specific locations. Similar patterns are reported in studies of *Chrysophyllum albidum* and *Manilkara zapota*, where geographically isolated populations carried distinct chloroplast variants linked to differences in climate and soil (Ogundipe et al., 2020; Vanijajiva, 2020). The environmental contrast between Limpopo's dry savanna and Mpumalanga's wetter highveld could be driving similar selective pressures that shape chloroplast diversity in *M. zeyheri*, especially since chloroplast genes often play roles in stress tolerance, photosynthetic performance, and metabolic control (Zhang et al., 2020). It is worth noting that the level of barcode variation fits with known difficulties in getting clean DNA from Sapotaceae species (Gonzalez et al., 2009). Their tissues contain latex, polysaccharides, and phenolic compounds that interfere with DNA extraction, hampering PCR and increasing the chances of sequence errors or incomplete reads (Gonzalez et al., 2009; Vivas et al., 2014). Even with these challenges, the consistent polymorphic patterns across samples suggest that the sequences represent genuine genetic differences rather than technical issues. Successfully capturing meaningful variation demonstrates that trnH-psbA holds up well even in tree species loaded with problematic secondary compounds (Vivas et al., 2014).

The barcode also has ecological and biochemical relevance. Chloroplast variation can affect pathways involved in photosynthesis, carbon fixation, and the production of primary metabolites, which indirectly influence fruit nutrition (Pott et al., 2019). Studies on *Vitellaria paradoxa* and *Pouteria campechiana* revealed that populations with different chloroplast haplotypes also exhibited differences in fruit phenolics, carotenoids, and antioxidant levels (Allal et al., 2011; Jiménez-Parra et al., 2025). In *M. zeyheri*, the connection between barcode differences and regional sensory variations suggests chloroplast genetic structure might contribute to the variation in fruit sweetness, aroma, and pulp colour documented in your morphological and sensory assessments.

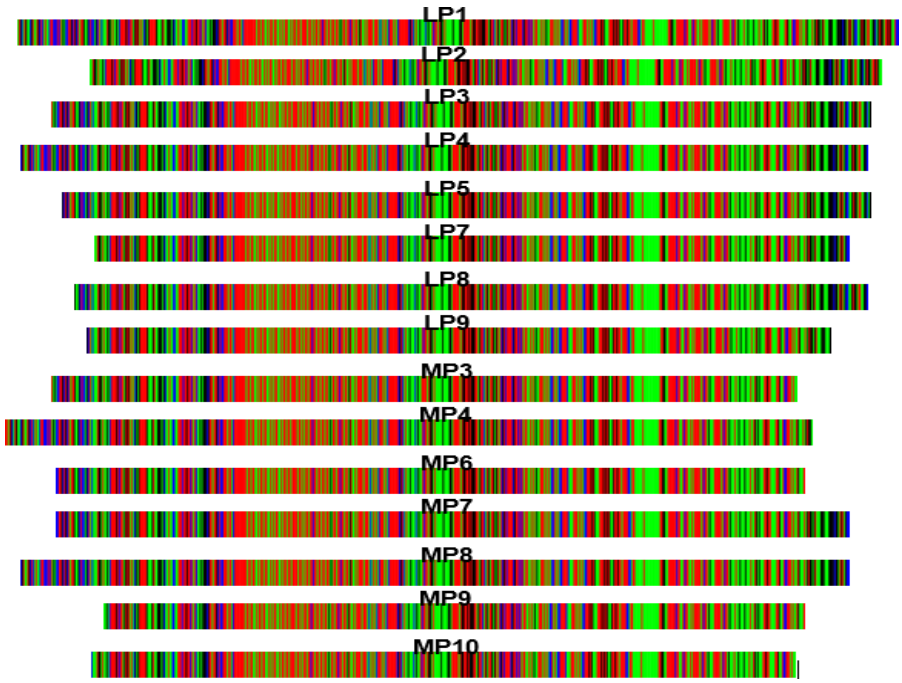


Figure 5-10: DNA barcode representation of *M. zeyheri* samples from Limpopo (LP) and Mpumalanga (MP) based on the trnH-psbA marker

5.3.5.2 Genetic distance and clustering

The heatmaps and clustering thresholds in Figure 5.11 provide a coherent picture of how *Mimusops zeyheri* individuals group genetically across the two regions. The patterns in Table 5.5 reinforce what the heatmaps visualise: the species contains two broad genetic lineages when assessed with the trnH-psbA marker, but a finer, four-cluster structure when using matK at a much stricter distance threshold. In both cases, individuals sort into well-defined blocks of similarity that closely correspond to the warm and cool-colour gradients in the heatmaps. The presence of two singletons in the matK results (LP3 and MP5) suggests that specific individuals carry rare haplotypes that lie outside the main genetic groups, a pattern frequently seen in long-lived trees where small, isolated maternal lineages persist over generations (Bradshaw, 2019).

The structure of the matK clusters, detected at an optimised threshold of 0.0005, suggests a shallow, subtle lineage split. The first two clusters each contain a mix of Limpopo and Mpumalanga samples, indicating that shared ancestry or historical gene flow still links the two regions. The singleton cluster containing LP3 stands out, as such rare, outlying haplotypes have been documented in other Sapotaceae species, such as *Chrysophyllum albidum*, where isolated individuals harbour private mutations not yet diluted by cross- regional pollen flow (Alowanou-Kélé et al., 2025). In contrast, the trnH-psbA marker, operating at a much broader threshold (0.256), partitions the dataset into two major clusters that align neatly with the contrasting blocks in the heatmap. This shift from four to two clusters when switching markers highlights how different genomic regions capture distinct layers of evolutionary history, one reflecting fine-grained, recent divergence, while the other captures deeper population structure (Shum and Palumbi, 2021; Wang et al., 2019).

The internal cohesion of these clusters raises essential questions about the processes sustaining such patterns. In several Sapotaceae species, such as Pouteria torta and Synsepalum dulcificum, restricted nut dispersal has resulted in similarly compact genetic blocks, where offspring rarely move far beyond the maternal tree (Chibuzor et al., 2017; Hardy et al., 2006; Tchokponhoué et al., 2023). If M. zeyheri relies on frugivores with short foraging ranges, this could explain why lineages remain tightly knitted within sites. Over time, this restricted movement affects more than just genetics: it limits the spread of advantageous traits, constrains mating opportunities, and encourages the persistence of localised trait complexes linked to growth rhythm, fruit chemistry, and nutling vigour (Chibuzor et al., 2017). Such structuring also affects the tree's phytochemistry and overall performance. Studies on species like Chrysophyllum viridifolium have shown that populations with narrow genetic bases tend to exhibit more uniform secondary metabolite profiles, often influencing their resistance to pests or environmental stress (Ismail, 2025). If each genetic cluster of M. zeyheri carries its own phytochemical "signature," this may translate into regional differences in fruit qualities such as sweetness, aroma, or antioxidant capacity patterns that were also hinted at in the sensory evaluations. This is consistent with the report by Moore et al. (2014), who further emphasise that high intraspecific variation provides plants with the capacity to evolve rapidly, suggesting that genetic diversity is crucial for adaptive responses to environmental challenges. The heatmap, therefore, offers more than an abstract genetic picture pointing to underlying biochemical and ecological differentiation with real consequences for how these trees cope with their environments.

Finally, the separation observed between clusters suggests that conservation and utilisation strategies should treat the two groups as distinct genetic resources. Lessons from *Manilkara multifida* and *Pouteria reticulata* show that mixing genetically divergent lineages can, under some circumstances, reduce nutling survival or disrupt locally adapted traits (Schroeder et al., 2014; Waqar et al., 2021). Preserving both lineages of *M. zeyheri* is therefore crucial, not only to safeguard genetic diversity but also to maintain the full spectrum of nutritional and physiological traits the species offers. The heatmaps, when viewed in this light, underscore the importance of region-specific management and highlight promising avenues for linking genetic structure with phytochemical potential, fruit quality variation, and long-term population stability.

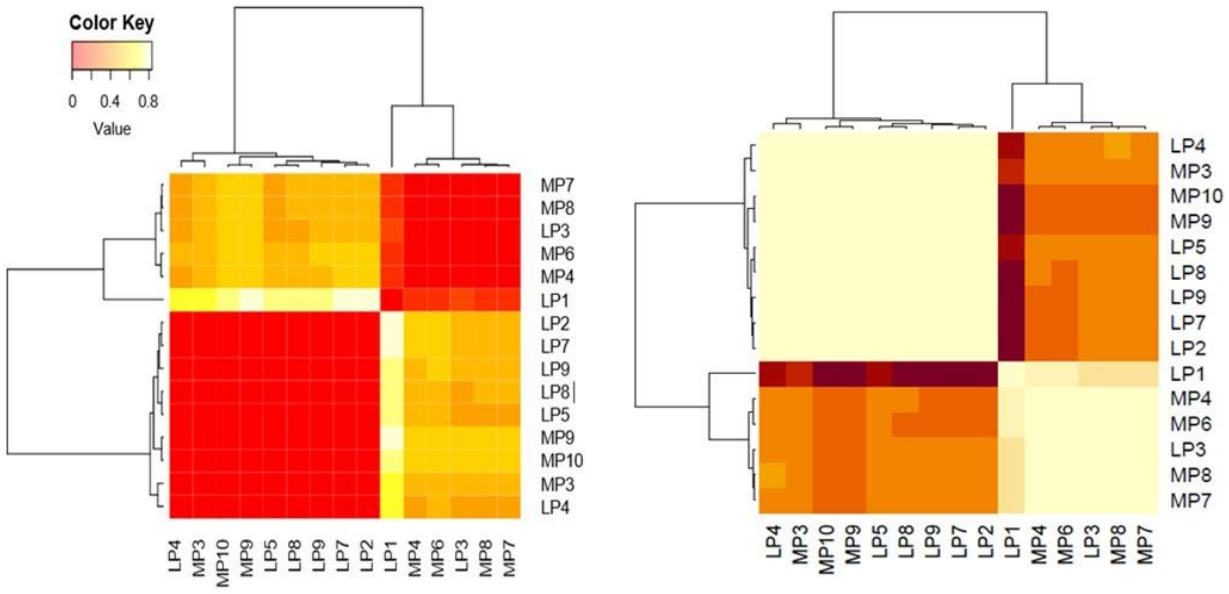


Figure 5-11: Heatmap showing pairwise genetic distances among *Mimusops zeyheri* samples from Limpopo and Mpumalanga based on combined chloroplast sequences

Table 5-4: Cluster composition and optimised genetic distance (K80) thresholds for *M. zeyheri* based on matK and trnH-psbA markers

Marker	Optimised threshold (K80)	No. clusters	Cluster ID → composition (sample codes)
matK	0.0005	4	C1: LP1, MP1, MP4, MP6, MP7, MP8 C2: LP2, LP4, LP5, LP7, MP3, MP9 C3 (singleton): LP3 C4 (singleton): MP5
trnH-psbA	0.256	2	C1: LP1, LP3, MP4, MP6, MP7, MP8 C2: LP2, LP4, LP5, LP7, LP8, LP9, MP1, MP3, MP9, MP10

5.3.5.3 Cluster optimisation and grouping

The silhouette analysis reveals a two-cluster model corresponding to the geographic separation of sampling regions (Figure 5.12), which mirrors patterns observed in other Sapotaceae across environmentally variable landscapes. The pronounced peak at k=2 indicates *M. zeyheri* displays similar regional structuring, probably shaped more by ecological gradients than by recent habitat fragmentation. The density distribution supports this interpretation: within-population distances cluster tightly, reflecting relatively uniform haplotypes within each region, while between-population distances spread more broadly, signalling accumulated divergence (Elhaik, 2012; Lawson et al., 2012). This pattern points to restricted gene flow between Limpopo and Mpumalanga populations, likely resulting from limited pollen movement across topographic barriers, a common mechanism driving chloroplast differentiation in wild fruit trees. *Sideroxylon obtusifolium* offers a parallel case, displaying marked chloroplast divergence across dune systems where wind patterns limit pollen dispersal (Gomes et al., 2021). The terrain separating the Soutpansberg foothills from the Mpumalanga escarpment may create comparable barriers for *M. zeyheri*.

The clear barcode gap indicates that mutation rates in these plastid regions are high enough to distinguish populations over relatively short evolutionary periods. In long-lived trees, such gaps often reflect historical environmental pressures rather than distance alone (Mayol et al., 2015; Pham et al., 2017). *Chrysophyllum cainito*, for example, shows chloroplast divergence linked to moisture availability, with drought-zone trees carrying distinct haplotypes (De Faria et al., 2017; Petersen, 2012). The observed genetic structure also corresponds to regional differences in fruit traits. Studies on *Pouteria caimito* and *Synsepalum dulcificum* show that chloroplast haplotypes can align with differences in carotenoids, phenolics, and flavour profiles (Achigan-Dako et al., 2015; Yang et al., 2019). A similar link may explain the sweetness and sensory differences documented between Limpopo and Mpumalanga fruits. The presence of two well-defined genetic clusters provides a practical framework for both domestication and conservation planning. Retaining representation from each lineage ensures that breeding programmes can access the full complement of genetic diversity, including traits linked to fruit quality, resilience, and potential commercial value. This structure supports a dual-lineage strategy for germplasm collection, orchard development, and restoration initiatives, thereby strengthening the long-term adaptability and utilisation potential of *M. zeyheri* across varied agricultural and ecological settings.

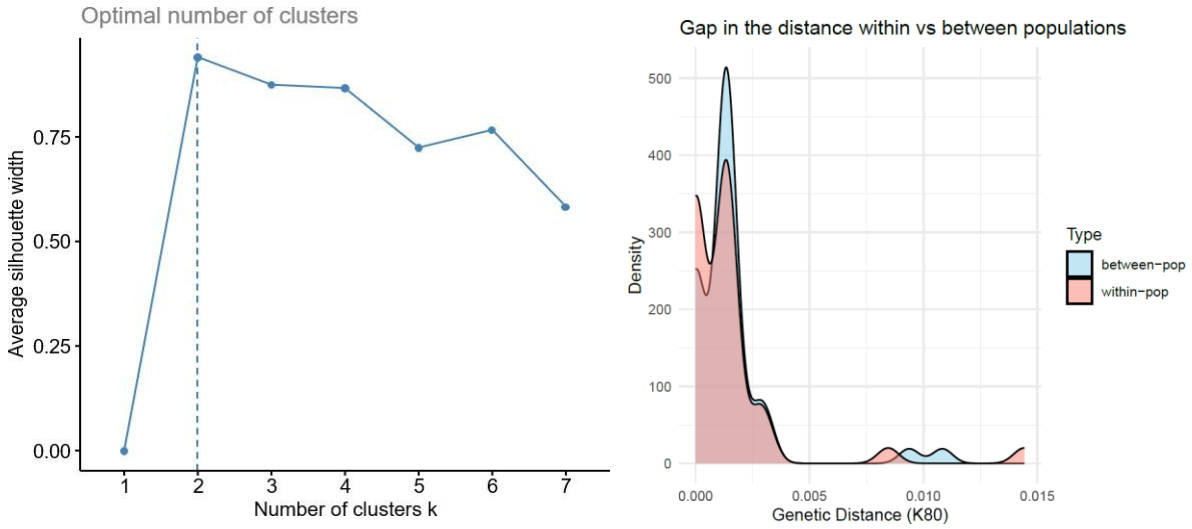


Figure 5-12: Optimal number of genetic clusters and distribution of genetic distances within and between populations of *Mimusops zeyheri*

5.3.5.6 Phylogenetic tree and population grouping

The phylogenetic network in Figure 5.13 provides a distinct layer of insight into the genetic structure of *M. zeyheri*, revealing evolutionary patterns that are not immediately apparent from the heatmaps or clustering thresholds. Two principal lineages are visible, marked by the contrast between the red and grey groupings, and the branch lengths indicate varying degrees of divergence among individual samples. Notably, LP3, MP9, and MP10 occupy longer, more isolated branches, a pattern consistent with the presence of rare or uniquely derived haplotypes. Similar observations have been documented in other Sapotaceae species, such as *Manilkara zapota* (Liu et al., 2019) and *Chrysophyllum africanum* (Swenson et al., 2008), where edge-positioned haplotypes often represent remnants of older maternal lineages or the outcomes of historical isolation events. These isolated branches can sometimes signal past demographic contractions, restricted nut dispersal, or lineage-specific mutations that have persisted in small local populations (Lin et al., 2025; McDonough et al., 2015).

At the same time, clustering several Limpopo and Mpumalanga individuals into short branch segments supports the idea of shared ancestry, mirroring the mixed-composition clusters derived from the matK marker. This grouping pattern suggests that, despite geographical separation, the two regions have not been completely genetically isolated. Cross-regional similarities in fruiting phenology or pollination biology could facilitate occasional gene flow, helping maintain some degree of connectivity between populations. A comparable pattern has been seen in *Micropholis,* where populations from different ecological zones still cluster tightly in phylogenetic reconstructions due to shared pollinators and overlapping flowering periods (Sánchez et al., 2022). For *M. zeyheri*, this shared ancestry hints at a historical continuum rather than sharply divided evolutionary trajectories. However, what sets the phylogenetic network apart from the previous analyses is the depth of the evolutionary splits it reveals.

The separation between major branches suggests that some lineages may have persisted independently for long periods, potentially predating current environmental conditions in Limpopo and Mpumalanga. Such patterns in perennial trees often reflect ancient demographic events rather than recent ecological pressures (Sánchez et al., 2022). This deeper structure in *M. zeyheri,* therefore, signals that the genetic landscape is shaped by long-term lineage stability, maternal inheritance patterns, and historical refugia more than by contemporary environmental differences.

The implications of these findings extend beyond phylogenetic interest. Rare or peripheral haplotypes such as those represented by LP3, MP9, and MP10 may carry biochemical or physiological traits not found in the dominant lineages. Research on *Vitellaria paradoxa* and *Synsepalum dulcificum* has shown that peripheral haplotypes often exhibit unique profiles of secondary metabolites and altered mineral composition (Achigan-Dako et al., 2015).

If similar patterns hold for *M. zeyheri*, overlooking these divergent lineages could lead to the loss of phytochemically valuable or ecologically resilient genetic resources. In breeding or domestication programmes, incorporating such rare lineages is crucial, as they may contribute traits linked to drought tolerance, fruit sweetness, nut size, or resistance to fungal pathogens. Conservation efforts must therefore account for the full breadth of genetic variation, ensuring that unique haplotypes are not inadvertently excluded through practices that favour only the common or high-yielding genotypes.

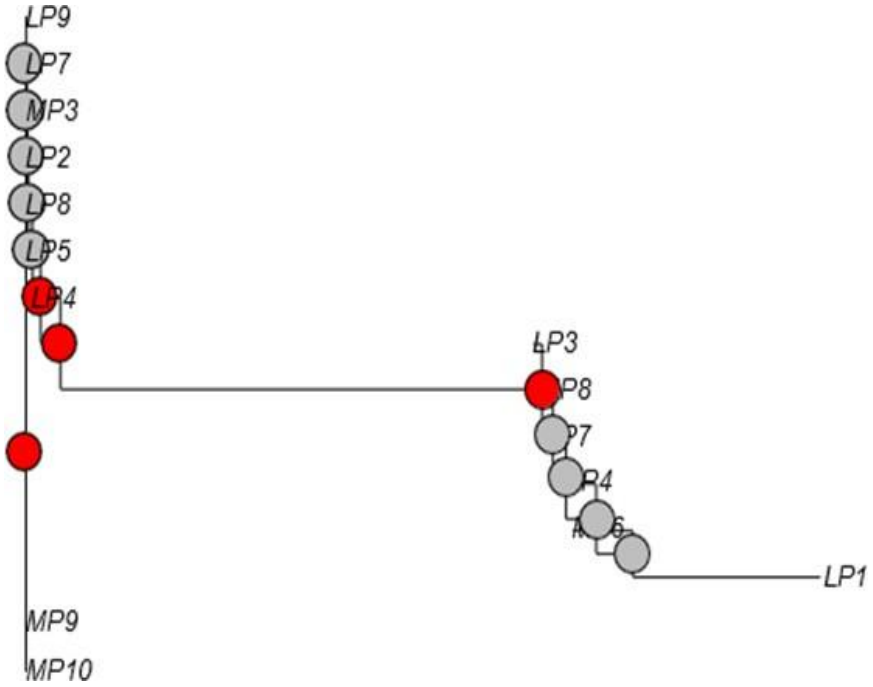


Figure 5-13: Neighbour-joining phylogenetic tree showing genetic relationships among *Mimusops zeyheri* samples from Limpopo and Mpumalanga

5.4 Concluding remarks

This study shows that *Mimusops zeyheri* exhibits meaningful diversity across the Limpopo and Mpumalanga populations, expressed in both morphological and sensory traits, and supported by distinct chloroplast lineages. Fruit and nut measurements revealed clear population-level differences: Mpumalanga trees produced larger reproductive structures,

while leaf morphology remained largely stable across sites. Sensory evaluations told a different story: despite their smaller size, fruits from Limpopo consistently received higher ratings for taste, aroma, and overall acceptability, suggesting that biochemical qualities rather than fruit dimensions shape consumer preference.

The genetic analyses confirmed two broad lineages with additional rare haplotypes, indicating long-term divergence, limited maternal gene flow, and the persistence of localised genetic variants. Several gaps tamper with the interpretation of these findings. Morphology was limited to external traits, leaving the chemical basis of sensory differences unexplored. Sensory scoring, though useful, was unsupported by instrumental assessments of sugars, volatile compounds, or secondary metabolites. The molecular analysis relied solely on chloroplast markers, capturing only maternal inheritance and do not fully resolve population structure or adaptive variation.

Finally, the study was confined to two provinces, Limpopo and Mpumalanga, yet *M. zeyheri* occurs across a wider environmental gradient that may hold additional diversity. Future work should extend genetic analyses to nuclear markers or genome-wide SNPs, incorporate detailed phytochemical profiling, and broaden geographic sampling to establish a fuller understanding of variation within the species. For conservation and utilisation, the two primary genetic lineages, together with the rare haplotypes identified, should be treated as distinct resources.

Domestication efforts may benefit from combining the favourable sensory traits prevalent in Limpopo with the larger fruit size observed in Mpumalanga, supporting the development of high-quality, climate-resilient cultivars.

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CHAPTER SIX

UNDERSTANDING SAP FLOW DYNAMICS OF *MIMUSOPS ZEYHERI* SOND: INSIGHTS FROM VHEMBE AND EHLANZENI DISTRICTS

Abstract

Mimusops zeyheri Sond. is an underutilised indigenous fruit tree species with limited information available on its water use characteristics and transpiration dynamics. This study aimed to investigate the sap flow dynamics and water use patterns of *Mimusops zeyheri* under natural field conditions in the Vhembe District of Limpopo Province and the Ehlanzeni District of Mpumalanga Province, South Africa. The aim of the study was to evaluate sap flow measurements using the Thermal Dissipation Probe (TDP) sensors in the Vhembe district and Ehlanzeni district. Sap flow measurements were performed on mature *Mimusops zeyheri* trees using stem diameter, bark thickness, phloem and xylem dimensions were measured to facilitate sap flow calculations and determine sapwood area. Meteorological variables, including temperature, rainfall, relative humidity, solar radiation, wind speed, and reference evapotranspiration (ETo), were obtained from automatic weather stations located near the study sites. The results demonstrated that sap flow and transpiration in *Mimusops zeyheri* were strongly influenced by prevailing environmental conditions. Trees at the Limpopo site recorded an average water use of approximately 14.73 L day^{-1}, with peak water use reaching 21.77 L day^{-1} during warm and dry conditions. At the Mpumalanga site, average water use ranged from 2.8 to 3.89 L day^{-1} during periods of high atmospheric demand but declined to between 1.34 and 1.60 L day^{-1} during periods of heavy rainfall and overcast conditions. The generated baseline information contributes to a better understanding of the ecophysiology of *Mimusops zeyheri* and highlights its potential for integration into climate-resilient agroforestry systems.

6.1. Introduction

Effective management of water in agriculture is a key area where sap flow measurements are particularly valuable, especially within production systems. In regions such as Limpopo and Mpumalanga, where water availability is constrained by variable rainfall and increasing climate pressure, irrigation plays a critical role in sustaining crop productivity and improving agricultural resilience (Serote et al., 2023; Department of Water and Sanitation, 2025). This is especially true in dryland environments, where rainfall alone is often not sufficient to meet the water requirements of crops, making careful monitoring and management of water resources essential for optimal production (Koch and Missimer, 2016). Measuring how plants use water is important for managing rain-fed ecosystems, and sap flow techniques provide an effective way to do this (Sharma et al., 2011). These methods offer a relatively affordable yet reliable approach for estimating the amount of water used by individual trees (Granier and Breda, 1996). The factors that influence sap flow are well understood, with environmental conditions such as vapour pressure deficit (VPD) playing a major role in driving tree transpiration (Tharaga et al., 2023). By continuously recording sap flow, it is possible to support real-time and indirect management of precision irrigation in agriculture (Paço et al., 2004; Santos et al., 2012; Yamane et al., 2011). As a result, sap flow techniques have been widely applied across different fields, including research in horticulture (Zuñiga et al., 2014), forestry, and ecology (Mapeto et al., 2018), to assess plant water use and transpiration rates (Green, 1998; Smith and Allen, 1996).

The previous report by Goodwin et al. (2012) showed that weather data are commonly used to estimate grass-reference evapotranspiration (ETo) using the crop coefficient (Kc) based on the FAO-56 approach. These estimates are then applied to determine the actual evapotranspiration (ETc) of trees.

The basal crop coefficient (Kcb), which reflects plant transpiration relative to a reference surface, is derived from tree water use and understory conditions, and it varies across different ecosystems (Goodwin et al., 2012). The basal crop coefficient (Kcb) generally follows a seasonal trend, increasing during the early growth stages as leaf area expands, reaching a stable level during mid-season, and gradually decreasing toward the end of the growing period (McClymont et al., 2009). Despite its importance, there is no information available on the water requirements of milkwood trees, and this is the first study of its kind.

Sap flow measurements are used to determine water movement within individual trees and can be scaled up to estimate transpiration at stand level (Forster, 2017). This method is particularly useful in complex and heterogeneous landscapes where techniques such as eddy covariance are difficult to apply (Regalado et al., 2009). Sap flow specifically measures transpiration as part of the evapotranspiration process by estimating the rate of sap movement through the xylem (Green et al., 2003; Juhasz et al., 2008). However, challenges such as sapwood structure and depth, tree age, and species diversity can affect measurement accuracy and make stand-level extrapolation difficult (Sam, 2016). Several sap flow methods have been developed and improved over time, including heat pulse velocity (HPV), thermal dissipation probes (TDP), trunk segment heat balance, stem steady-state heat balance, and heat field deformation (Figure 6.1) (Steppe et al., 2010).

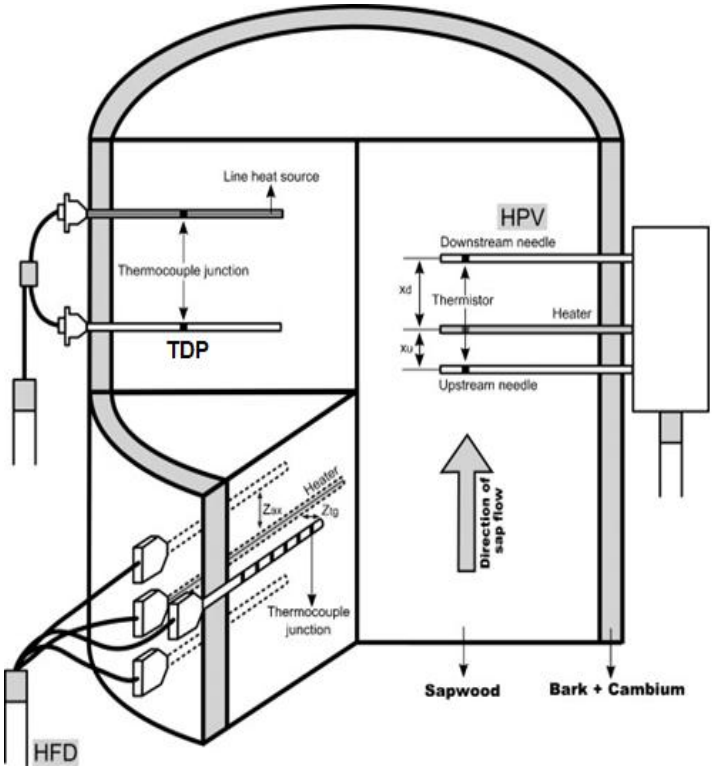


Figure 6-1: Schematic diagram of the compensation heat pulse velocity (HPV)(CHPM) sensor, the thermal dissipation probe (TDP) and the heat field deformation (HFD) sensor, installed radially into a stem segment (Steppe et al., 2010).

The thermal dissipation probe (TDP) method, developed by Granier in 1984, is a simple and cost-effective technique for measuring sap flow in trees (Granier, 1985). It uses two temperature probes (thermocouples) inserted into the sapwood, with the upper probe containing a small heater, while the lower probe remains unheated. The temperature difference between the two probes is used to estimate sap velocity, which is then converted into volumetric flow using the sapwood area of the tree. Accurate measurement of sapwood area and species-specific calibration are important for reliable results, although many studies report that TDP is often used without proper calibration (Steppe et al., 2010; Sam, 2016). Calibration methods such as weighing lysimeters and potometers have been applied to improve accuracy (Pasqualotto et al., 2019).

The TDP method has been successfully used in crops such as olive, grapevine, apple (Zanoteli et al., 2022), sweet cherry (Tharaga et al., 2023), pomegranate (Mahdi et al., 2022), Kiwifruit (Wu et al., 2022), and peach. Sap flow rates are strongly influenced by soil water availability and environmental factors such as temperature and humidity, which affect transpiration and stomatal behaviour (Scalisi et al., 2019).

The studies conducted on sap flow techniques of indigenous tree species in South Africa are far fewer than studies on commercial forestry or deciduous fruit trees. This supports the statement that research on underutilized indigenous trees remains limited. For example, Gush and Dye (2009), compared the water-use efficiency of selected indigenous and exotic tree species using sap flow and biomass measurements. The second study quantified sap flow in two indigenous tree species, *Searsia pendulina* and Searsia lancea, established on gold mining sites in central South Africa (Dye et al., 2017). Although these studies demonstrate the successful application of sap flow techniques in indigenous species such as *Searsia lancea* and *Searsia pendulina*, detailed investigations on underutilized indigenous fruit trees such as *Mimusops zeyheri* remain scarce. Most South African sap flow research has focused on forestry species, rehabilitation systems, and commercial orchards rather than indigenous multipurpose trees. This deliverable report documented the investigation of sap flow dynamics of *Mimusops zeyheri* in the Vhembe and Ehlanzeni districts in order to understand its water use patterns under different environmental conditions.

6.2 Methodology

6.2.1 Study site description

The study was conducted in two locations within the Lowveld and Vhembe regions of South Africa to evaluate the sap flow dynamics of *Mimusops zeyheri*. The first site was located at the University of Mpumalanga Campus in Mbombela (formerly Nelspruit), Mpumalanga Province, situated at approximately 25°26′S, 30°58′E. The second site was located near Makhado in the Vhembe District, Limpopo Province, at WH Smith Farm (33 Witvlag Road), with approximate coordinates of 23°02′S, 29°54′E. Figures 6.2A and B present maps showing the geographical location of the two study sites, namely the University of Mpumalanga Campus in Mbombela, Mpumalanga Province, and WH Smith Farm near Makhado in the Vhembe District, Limpopo Province, where sap flow measurements of *Mimusops zeyheri* were conducted. These two sites were selected to represent different environmental conditions within the natural distribution range of the species. The selection is mainly based on the availability of the *Mimusops zeyheri* trees which grows naturally in different areas in the low-veld and mainly thrive under tropical climatic conditions.

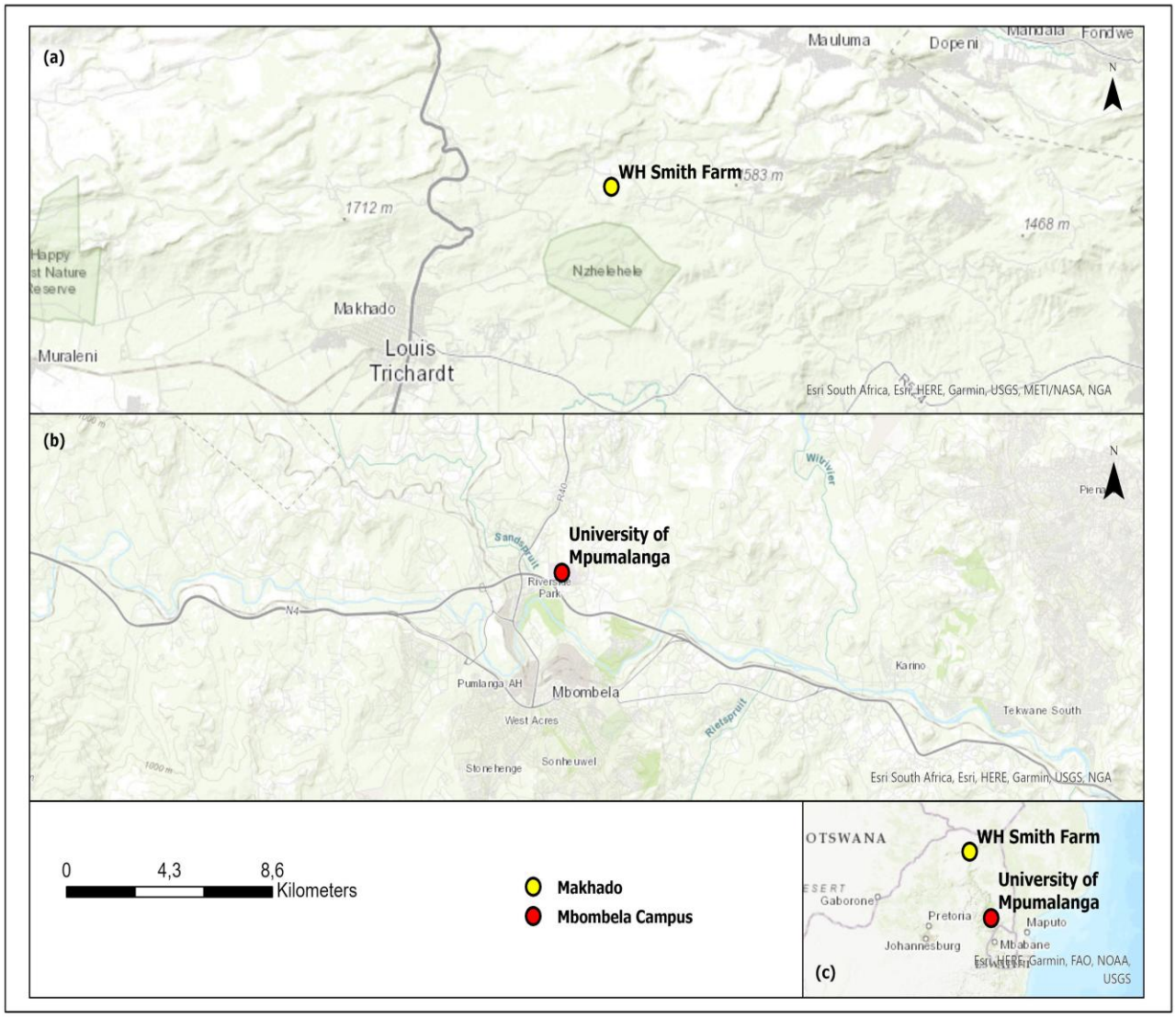


Figure 6-2: Maps of Mpumalanga and Limpopo Provinces, highlighting Mbombela municipality in Ehlanzeni and Vhembe district, respectively.

The study focused on naturally growing *Mimusops zeyheri* trees found within protected and accessible areas at both locations (Figure 6.2A and B). Two mature *Mimusops zeyheri* trees were selected for sap flow monitoring, with sensors installed on stems located within safe and secure zones to ensure continuous data collection and equipment protection. At the University of Mpumalanga site, the selected tree had two main elongated stems, both of which were fitted with TDP 30 and TDP 50 Thermal Dissipation Probe (TDP) sap flow sensors to monitor water movement within the xylem.

Stem diameter measurements were taken to support sensor placement and the calculation of sapwood area required for sap flow estimation. The sapwood was determined using the tree core and the vernier caliper for measuring the stem diameter. Bark thickness, phloem thickness, and xylem thickness were measured from the extracted tree core. The hardwood thickness was subsequently measured from the same core sample. The Mbombela site is located approximately 5 km from the Agricultural Research Council Tropical and Subtropical Crops (ARC-TSC) research station, which operates a weather station within the vicinity. Meteorological data from this station, including temperature, rainfall, relative humidity, solar radiation, and vapour pressure deficit (VPD), were used to assess weather conditions during the measurement period and to determine correlations between environmental variables and sap flow dynamics. Because the trees were isolated and surrounded by mixed vegetation, measurements such as leaf area index (LAI) could not be accurately determined using instruments such as the LI-COR LAI-2000 or LI-COR quantum sensors. The presence of neighbouring trees interfered with canopy-based measurements, making standard stand-level assessments impractical.

6.2.2 Meteorological data

Meteorological variables were measured from an automatic weather station (AWS) installed in the open space near the experimental site. The AWS comprised of the CS215-L-Digital air temperature (°C) and relative humidity (%) sensor (Campbell Scientific Inc., Logan, Utah, USA), the Rain-O-Matic Professional Rain gauge (PRONAMIC, Rindum gaards, Denmark) for rainfall (mm), SP Lite2 Pyranometer (Kipp and Zonen, Delft, Netherlands) for solar radiation (Rs) (MJ m-2), DS-2 Sonic Anemometer (Pacific Data Systems Australia Pty Ltd, Brisbane, Queensland, Australia) for horizontal wind speed (m s^{-1}) and direction (Compass).

The data logger used for programming and data storage was the DSG S300E (Dacom Farm Intelligence, Emmen, Netherlands). The Penman-Monteith equation was incorporated into the program to give an output value of grass reference evaporation (ETo, mm/day).

6.2.3 Stem diameter measurement

Tree-stem diameter and sapwood depth are required for better selection of the possible sap flow methods. Stem diameter was measured using a vernier calliper. The conversion was done based on stem diameters of each branch on the tree, as there was no stand level like in orchards. The data was measured on stem diameters of branches from 6 trees in the Limpopo study site and 3 trees at the Mpumalanga study site. The total number of branches measured in Limpopo site was 22 branches ranging from 76-184 cm, measured using the vernier calliper. While the number of branches measured for the Mpumalanga side were 14 branches which include water shoots from the milkwood trees, ranging from 86 to 192 cm. This data was used to convert the sap flow from grams/hour to mm/hour. This information was used to determine tree diameter representativeness for the various diameter classes (Table 6.1). This process was followed by selecting trees suitable for installing probes to measure sap flow for characterising water relations and tree water use during the fruit growing season.

Table 6-1: Variation in stem diameter and the TDP probe specifications used for measurements at University of Mpumalanga Site.

Probes TDP*	Probe length (mm)	Probe spacing (mm)	Probe Diameter (mm)	Tree-stem diameter (mm)	Bark thickness (mm)
TDP 30	30	40	1.20	76	3.5
TDP 50	50	40	1.65	184	4.8

Table 6-2: Variation in stem diameter and the TDP probe specifications used for measurements at WH Smith farm, Makhado Area Witvlag.

Probes TDP*	Probe length (mm)	Probe spacing (mm)	Probe Diameter (mm)	Tree-stem diameter (mm)	Bark thickness (mm)
TDP 30	30	40	1.20	121	4.4
TDP 50	50	40	1.65	192	5.6

6.2.4 Transpiration measurement

Sap flow was measured using Granier thermal dissipation probes (TDP) (Dynamax, Texas, USA) (Granier, 1985; Granier et al., 1996b). The TDP probe with two-temperature thermocouple probes is usually inserted at two levels of the tree stem, the upper thermocouple containing the heater and the thermocouple. The lower needle functions as the reference temperature sensing probe. In this study, two lengths of TDP were used to measure the tree xylem sap flow: 30 and 50mm in length and 1.2 and 1.65 mm in diameter, respectively, with both TDPs having a 40-mm spacing between the T2 (upper-heated probe) and T1 (upstream below heated probe). The TDP probes were installed in each tree on two branches as the trees are trained in spanish bush, and there is no straight-up stem from the ground. To avoid installing sensors they were installed at least 1m above the rootstock zone on six representative trees in the study site (Figures 6.3). Aluminium foil was wrapped around the probes to reduce the thermal noise caused by solar radiation. The TDP probes were connected to a Campbell CR1000 datalogger with a Campbell multiplexer (AM16/32B). The data logger power supply effectively uses a 12-V car battery (36 Ah). Due to the power supply and site visits, sap flow data were downloaded 2-3 times a month.



Figure 6-3: Dynamax TDP sap flow sensor inserted on a representative stem; and TDP sap flow at two heights

6.2.5 Sap flow calculations

A study conducted by Lu et al. (2004) described the Granier thermal dissipation probe (TDP) method for measuring sap flow in trees in detail. The Granier method also requires the sapwood's physical dimensions to convert velocity to sap flow rate. The data were used to calculate sap flow, according to the equation derived empirically (Granier, 1985) with the sap flow rate calculated using:

$$k = \frac{(dTM - dT)}{dT} \qquad \text{Equation 1}$$

where *k* is the non-dimensional sap flow index, *dTM* is the temperature difference when there is no sap flow and *dT* is the difference in temperature between the upper and lower needles. When *dT* = 0, *k* is undefined and, if *dT* = *dTM*, *k* = 0 (corresponding to zero flow). Clearly, when dT = 0, K equals infinity and, if dT = dTM, K =0 (zero flow).

Granier (1985) found empirically that the average sap flow velocity (*SV*, in cm s^{-1}) calculated from *k* using:

$$SV = 0.0119 \times k^{1.231} \text{ Cm/s} \qquad \text{Equation 2}$$

Based on an experimental regression, the method relates sap flux density (*SFD*) (g m^{-2} s^{-1}) to a temperature difference ΔT (K), measured between a continuously heated probe and an unheated probe (T1 of Figure 6.3) located in the lower part of the tree. The *SFD* is calculated using:

$$SFD = 119 \times k^{1.231} \qquad \text{Equation 3}$$

where *k* expresses the temperature difference (Equation 1), the *SFD* must be extrapolated to the sapwood's whole cross-sectional area (active water-conducting xylem). Thus, the total sap flow of the tree (*SP*) can be calculated as the product of the sapwood cross-section area and the heated probe level (*SA*, m^2 m^{-2}). Converting sap velocity to sap flow rate (Nadezhdina, 1999; Steppe et al., 2010):

To convert the velocity to sap flow rate, one uses:

$$Fs = As * V * 3600 \text{ (s/h) cm3/h} \qquad \text{Equation 4}$$

where Fs (cm^3/h) is the sap flow (cm^3/h), and *As* is the cross-sectional area of sap conducting wood (cm^2). Typical midday values for V are from 10 to 80 cm/h, as reported by Granier (1,3). Microsoft Excell was used to embed the above expression; it calculates the temperature difference and converts the latter into sap velocity.

6.2.6 Correction factor

Correction of the probe temperature difference measurements for xylem and sapwood areas are required (Clearwater et al., 2013). The potential for error in calculating velocity and mass flow depends on the sapwood area's calculation and probe averaging sap velocity.

Previous users of the TDP method have assumed that the probe measures the mean sap velocity along its length (Clearwater et al., 1999; Lu et al., 1995). However, if sap velocity varies along the probe's length, the heat dissipation and probe surface temperature will also vary (Lu et al., 2004). In the case where the probe is inserted into non-conducting xylem while the remainder is in sapwood with relatively uniform sap velocity, then it can be assumed that the measured ΔT is a weighted mean of ΔT in the sapwood (ΔTsw) and ΔT in the active xylem (ΔTm):

$$\Delta T = a\Delta T_{sw} + b\Delta T_m \qquad \text{Equation 6}$$

where a and b are the proportions of the probe in the sapwood and active xylem, respectively, while b is expressed as $b = 1 - a$. This approach assumes that inactive xylem's thermal properties are the same as that in the sapwood when SV = 0. If the depth of sapwood is known, then Equation 2 can be rearranged to find the sapwood's actual temperature and sap velocity. The detailed information about the correction method used in this study was based on Clearwater et al. (1999).

6.2.7 Water use calculation

The hourly recorded sap flow values were integrated into daily cumulative sap flow (l d^{-1} $tree^{-1}$) and considered daily water uptake (WU) and from the daily cumulated sap flow (l day^{-1}).

6.3 Results and discussion

The results indicated that weather conditions played a significant role in influencing daily sap flow patterns. The two experimental sites, namely the University of Mpumalanga and WH Smith Farm (33 Witvlag Road), experienced varying environmental conditions, including extremely hot days, rainfall events, calm periods, and windy conditions.

These fluctuations in climatic factors contributed to differences in tree physiological responses, resulting in variations in sap flow behaviour between the two sites.

Figure 6.4 illustrates the pattern of water use observed during the measurement period in Limpopo Province at the WH Smith Farm (33 Witvlag Road). The data recorded higher sap flow during the first and second weeks of December 2025, reflecting increased water demand by the trees under warmer conditions. This was followed by a gradual decline in sap flow, which corresponded with the occurrence of consecutive heavy rainfall events in the Limpopo area. The substantial rainfall in December 2025 likely reduced plant water stress and lowered the need for active water uptake from the soil.

Following the cessation of rainfall, sap flow rates increased again, mainly due to rising temperatures and higher solar radiation, which promoted greater transpiration demand. However, this increase was short-lived, as additional heavy rainfall occurred during the last week of December 2025. The renewed rainfall once again reduced transpiration rates and water uptake, as the trees were no longer under moisture stress and remained sufficient.

The trees in Limpopo were utilizing approximately 14.73 litres per day of the entire period which mainly influenced by the microclimate of the area. During the first week of measurements the trees where taking up to 21.77 litres per day and then declined to 13.44 litres per day.

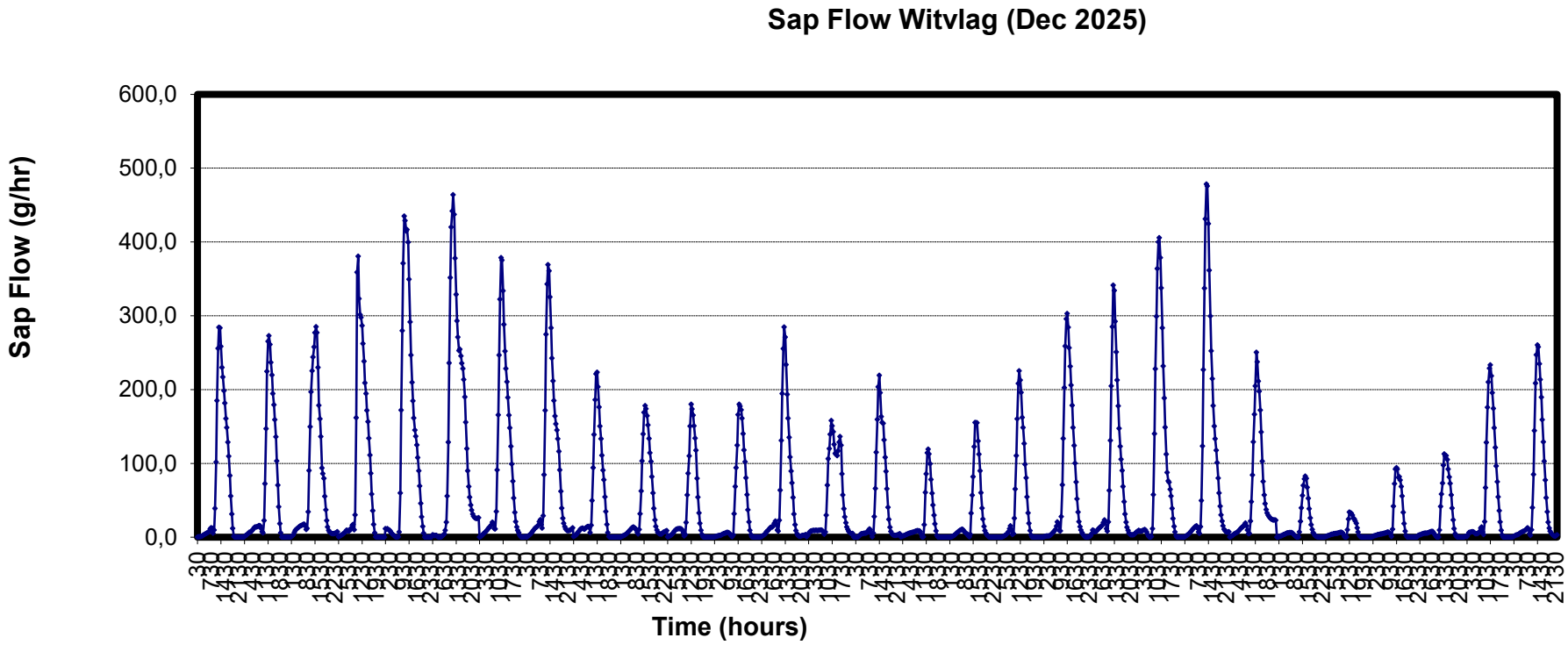


Figure 6-4: Sap flow trends of *Mimusops zeyheri* trees measured using TDP 50 and TDP 30 at Witvlag area of Limpopo Province in South Africa.

The amount of water utilised by *Mimusops zeyheri* varied considerably depending on prevailing climatic conditions as well as the structural characteristics of the trees. The growth of such patterns influences sap flow distribution and may affect the overall movement of water within the xylem, making individual stem measurements important for accurate estimation of whole-tree water use. At the University of Mpumalanga site in Mbombela, the monitored trees recorded an average water use of approximately 2.8 litres per day during the first week of measurements. During the second week of December 2025, water use increased further to approximately 3.89 litres per day, which was likely associated with higher temperatures, and greater atmospheric demand for transpiration. Under such conditions, the trees experienced higher evaporative demand, resulting in increased sap flow and water uptake.

In contrast, substantially lower water use was observed during periods of heavy rainfall affecting both Limpopo and Mpumalanga Provinces. The prolonged cloudy and overcast conditions reduced incoming solar radiation and lowered transpiration demand, leading to reduced sap flow rates. During these periods, water consumption declined to between 1.34 and 1.60 litres per day. The reduced water uptake suggests that the trees were not under moisture stress, as soil water availability remained sufficient and environmental conditions did not favour high transpiration rates.

The observed variation in water use by *Mimusops zeyheri* confirms the strong influence of climatic conditions and tree structure on sap flow dynamics, which is consistent with findings reported in previous studies (Scalisi et al., 2019). The higher water use recorded during the first and second weeks of December at the University of Mpumalanga site (2.8 to 3.89 L day^{-1}) was associated with increased temperatures and greater solar radiation, which likely increased transpiration demand.

Similar observations were reported by Scalisi et al. (2019), who found that sap flow rates are closely linked to environmental drivers such as temperature, humidity, and vapour pressure deficit (VPD), all of which regulate stomatal conductance and transpiration. In this study, water use declined to between 1.34 and 1.60 L day^{-1} during these conditions, indicating that *Mimusops zeyheri* responded physiologically to favourable soil moisture conditions and reduced atmospheric demand. In regions such as Mbombela and the tropical areas of Limpopo around Venda, where rainfall is highly variable, sap flow data can support the development of predictive models for irrigation scheduling. Goodwin et al. (2012) highlighted the importance of integrating transpiration measurements with environmental variables to improve estimates of crop water requirements. Similarly, Tharaga et al. (2023) showed that sap flow data can be used to establish regression models linking transpiration with climatic variables, thereby improving precision irrigation strategies. The present study extends this application to an underutilized indigenous tree species, demonstrating the potential of *Mimusops zeyheri* for future water-use studies in agroforestry systems.

However, the limitations encountered in this study are also consistent with challenges reported in the literature. Sam (2016) noted that scaling sap flow measurements from individual trees to stand level is particularly difficult in heterogeneous systems where tree age, canopy structure, and spacing vary significantly. This was evident in the present study, where naturally growing *Mimusops zeyheri* trees were scattered among other vegetation and did not form a homogeneous canopy. It is very difficult to explain the sap flow data converted to equivalent depth units (i.e. mm/d). The multi-stem growth habit of *Mimusops zeyheri*, where double to triple stems arise from a single rootstock, further complicated whole-tree water use estimation.

According to Forster (2017), stem architecture and sapwood variability are major sources of uncertainty in sap flow studies because water movement differs across stems and branches. This structural complexity required individual stem measurements using TDP 30 and TDP 50 sensors but still limited the precision of scaling to whole-tree transpiration.

In addition, technical limitations affected data continuity during the monitoring period. The flat trend observed in Figure 6.5 from 29 December 2025 to 18 January 2026 resulted from battery malfunction in the sap flow system, which interrupted continuous recording. Such equipment-related challenges are common in long-term field monitoring studies and may affect interpretation if not properly accounted for (Pasqualotto et al., 2019). Despite these constraints, the study provides important baseline information on the sap flow behaviour of *Mimusops zeyheri* under natural field conditions and contributes valuable knowledge for future research on indigenous and underutilized tree species in South Africa.

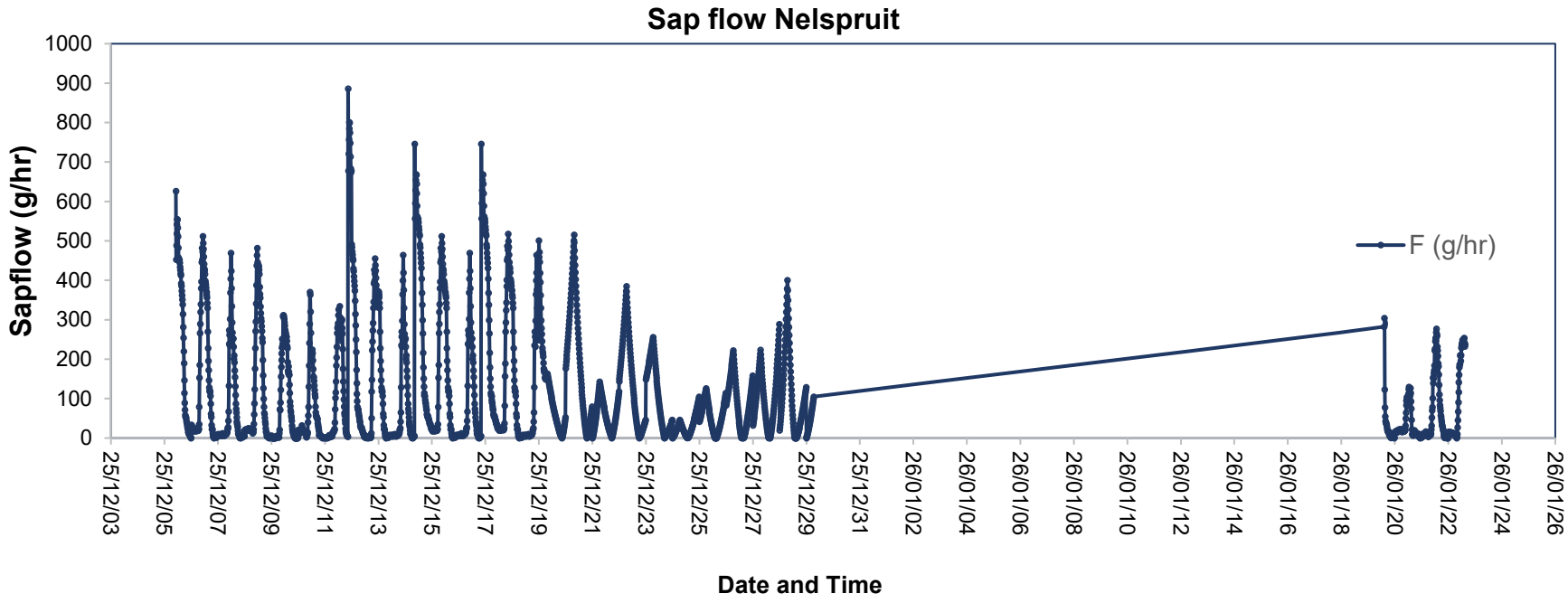


Figure 6-5: Sap flow Dynamics of *Mimusops zeyheri* trees at the University of Mpumalanga, Mpumalanga Province measured from TDP 30 and TDP50.

Climatic variables during the measurement period played an important role in influencing sap flow rates. A correlation was observed between the hourly weather variables and sap flow measured in grams per hour (Figure 6.6). The transpiration rate derived from sap flow followed a pattern similar to that of reference evapotranspiration (ETo), although ETo values were consistently higher than the measured transpiration rates. The diurnal pattern further showed a positive relationship between transpiration rate and evapotranspiration, indicating that both responded similarly to changes in environmental conditions, even though one variable does not directly determine the other. The transpiration rate and reference evapotranspiration (ETo) followed a clear diurnal pattern, with both increasing during the warmer parts of the day and declining during the cooler evening and night periods. This trend reflects the strong influence of climatic factors such as solar radiation, air temperature, humidity, and wind speed on plant water use.

According to Food and Agriculture Organization of the United Nations guidelines described by Allen et al. (1998), reference evapotranspiration (ETo) represents the evaporative demand of the atmosphere from a well-watered reference surface and is primarily driven by weather conditions such as radiation, temperature, wind speed, and vapour pressure deficit. Relative humidity showed an inverse relationship with transpiration. The higher humidity levels were associated with lower transpiration rates, while lower humidity under drier atmospheric conditions resulted in increased transpiration.

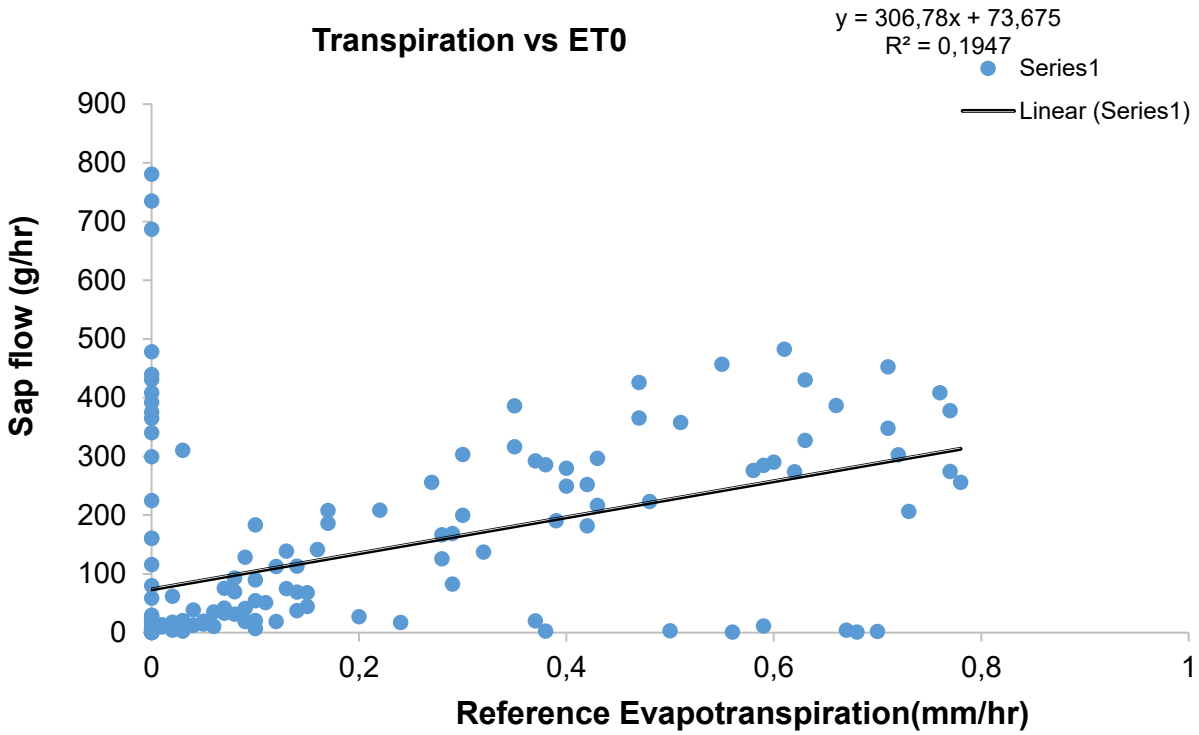


Figure 6-6: The correlation analysis between sap flow and reference evapotranspiration rates.

Air temperature is another important factor influencing transpiration. Ground temperature does not always increase directly with solar radiation, as it is also affected by wind conditions, humidity, and cloud cover (Figure 6.7). Under extremely high temperatures, trees may partially close their stomata to reduce excessive water loss and prevent water stress. In addition, when high temperatures are accompanied by windy conditions, evapotranspiration losses are generally greater than during calm days, as wind enhances the movement of water vapour away from the leaf surface and increases atmospheric demand.

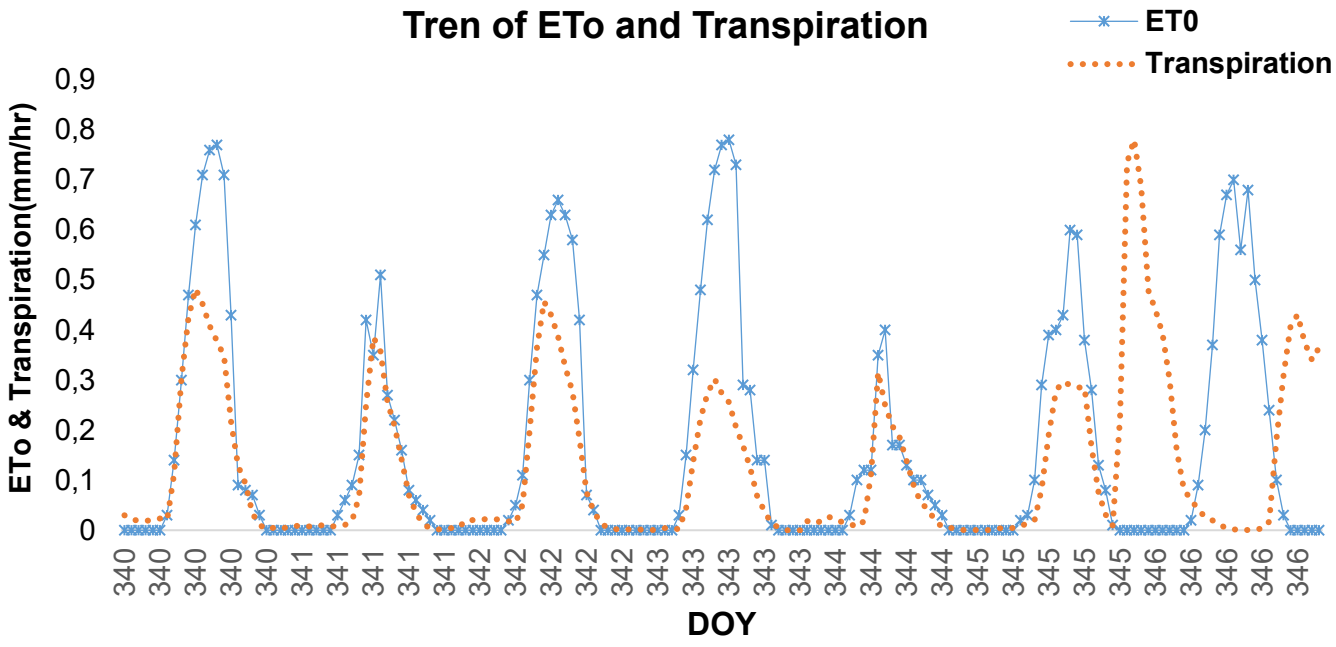


Figure 6-7: Diurnal trend of evapotranspiration and transpiration rates in mm.

The relationship between relative humidity and transpiration is an inverse relationship (Figure 6.8). When the humidity is high, the transpiration rates become lower. The data suggest that when the humidity is low, and the area is dry the rate of transpiration becomes higher. This also implies that the rate of relative humidity influences the vapour pressure deficit in the microclimate of the area where the tree is growing.

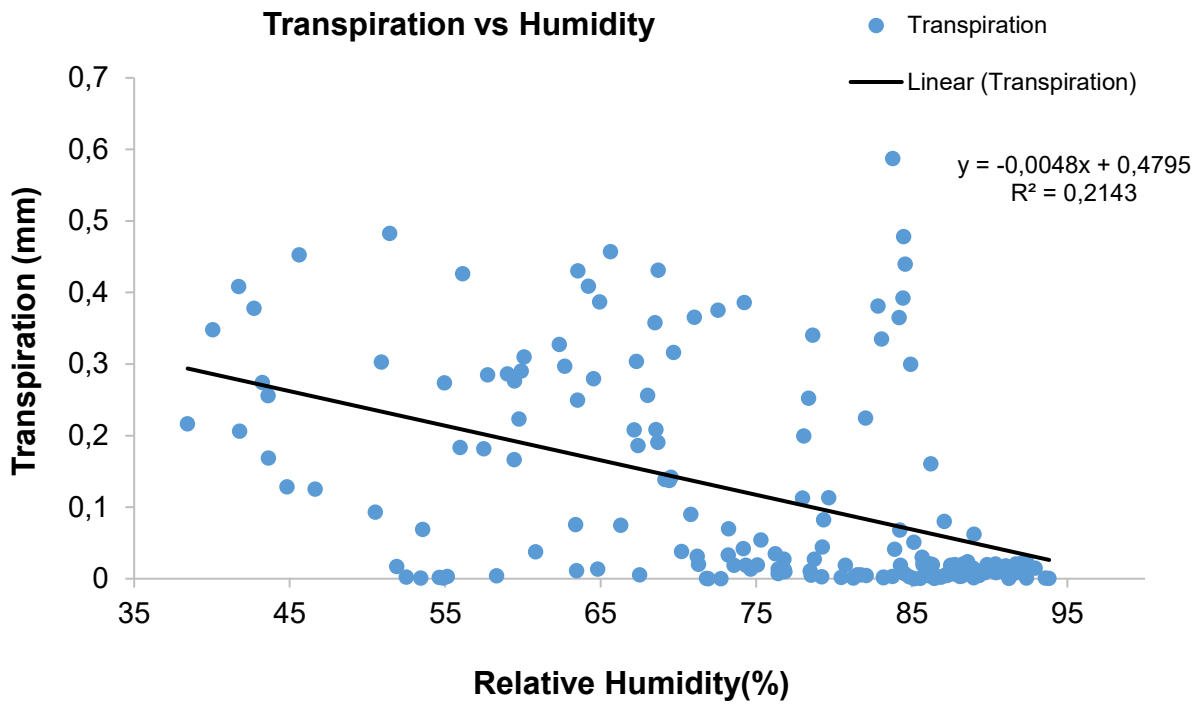


Figure 6-8: The correlation between transpiration rates and relative humidity.

Figure 6.9 illustrates the relationship between net radiation (W m^{-2}) and transpiration (mm) in *Mimusops zeyheri*. The graph shows a positive relationship between the two variables, indicating that transpiration generally increased as net radiation increased. This trend is represented by the upward slope of the regression line (y = 0.0002x + 0.0747), suggesting that higher solar energy availability promoted greater water loss through transpiration.

Net radiation provides the energy required for evaporation and transpiration processes. During periods of high radiation, stomata remain more active, and the atmospheric demand for water increases, resulting in higher sap flow and transpiration rates. This explains why most of the higher transpiration values were observed at radiation levels above 400 W m^{-2}.

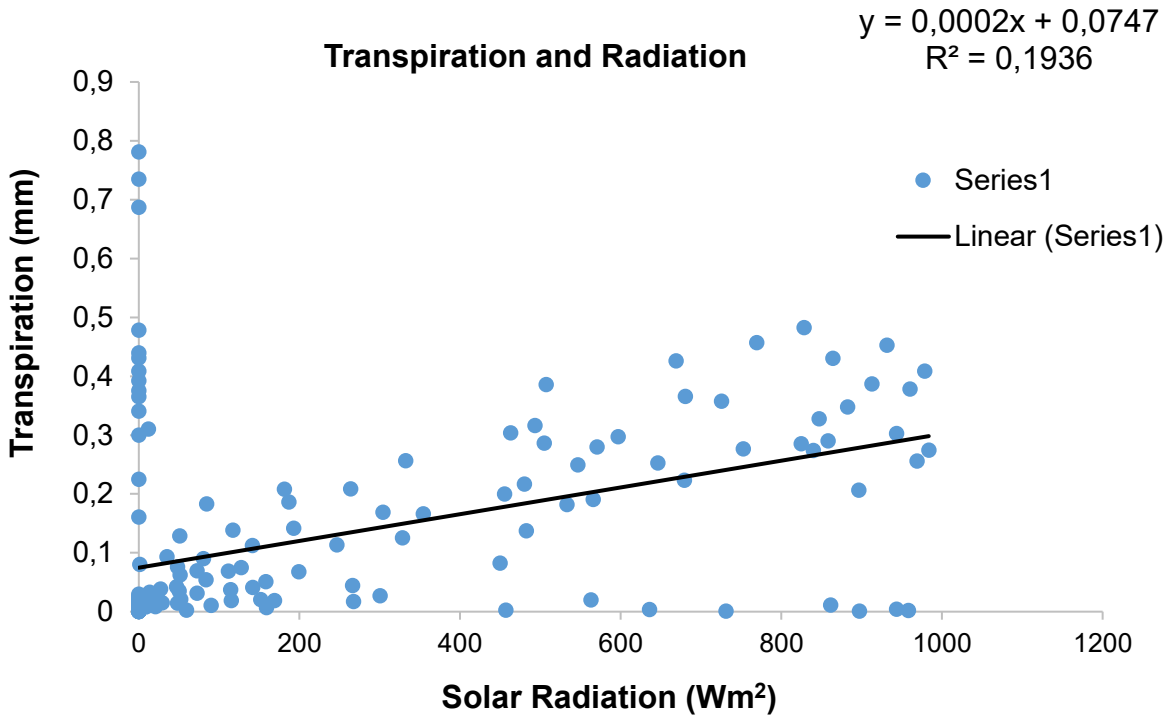


Figure 6-9: The correlation between radiation and transpiration rates

Figure 6.10 presents the relationship between air temperature (°C) and transpiration (mm) in *Mimusops zeyheri*. The graph shows a positive relationship between temperature and transpiration, indicating that transpiration generally increased as air temperature increased. This is illustrated by the upward trend of the regression line (y = 0.0156x − 0.2399), which suggests that warmer conditions promoted greater water loss from the tree through transpiration. As temperature rises, the atmosphere becomes capable of holding more water vapour, which increases the evaporative demand around the leaves. This creates a stronger gradient for water movement from the leaf surface to the surrounding air, resulting in increased transpiration. Higher temperatures also increase stomatal activity under favourable moisture conditions, allowing more water to move through the xylem and be released into the atmosphere. Figure 6.10 presents the relationship between air temperature (°C) and transpiration (mm) in *Mimusops zeyheri*.

The graph shows a positive relationship between temperature and transpiration, indicating that transpiration generally increased as air temperature increased. This is illustrated by the upward trend of the regression line (y = 0.0156x − 0.2399), which suggests that warmer conditions promoted greater water loss from the tree through transpiration. As temperature rises, the atmosphere becomes capable of holding more water vapour, which increases the evaporative demand around the leaves. This creates a stronger gradient for water movement from the leaf surface to the surrounding air, resulting in increased transpiration. Higher temperatures also increase stomatal activity under favourable moisture conditions, allowing more water to move through the xylem and be released into the atmosphere.

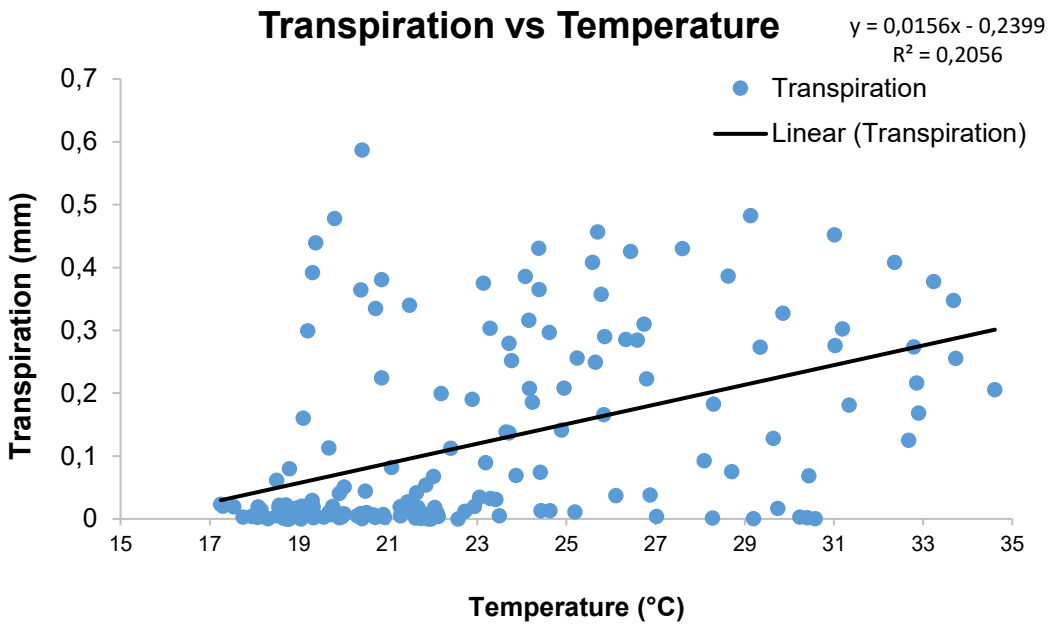


Figure 6-10: The correlation between transpiration rates and air temperature

The daily reference evapotranspiration (ETo) and transpiration rates for the Mbombela site were analysed using the available weather data, and all correlation analyses were based on this location. These daily values are presented in Table 6.3. The evaporation rate shown in Table 6.3 was calculated as the difference between reference evapotranspiration and sap flow-derived transpiration, with all values expressed in mm day^{-1}.

This provided an estimate of the water lost through surface evaporation in addition to transpiration from the tree. During December 2025, several days with temperatures ranging between 28 and 30 °C were associated with higher evapotranspiration and evaporation rates, particularly on the 9th, 12th, and 13th of December. On 6 December, the maximum temperature recorded in Mbombela was 33.68 °C, while 34.61 °C was recorded on 9 December and 33.70 °C on 13 December. These periods of elevated temperature corresponded with increased transpiration rates in *Mimusops zeyheri*, indicating the strong influence of temperature on water use by the trees.

Higher temperatures increase the evaporative demand of the atmosphere and raise vapour pressure deficit (VPD), which promotes greater water movement from the leaf surface to the surrounding air. As a result, transpiration rates tend to increase when sufficient soil moisture is available. Allen et al. (1998) explained that temperature is one of the major climatic factors influencing evapotranspiration because it directly affects both soil evaporation and plant transpiration. Similarly, Green et al. (2003) reported that sap flow rates are closely related to temperature and radiation, particularly during periods of high atmospheric demand. These findings support the observed increase in transpiration rates of *Mimusops zeyheri* during warmer days in Mbombela.

Table 6-3: The daily totals of evapotranspiration from the weather station and the sap flow derived transpiration rate.

Date	ETO(mm/day)	Transpiration(mm)	Evaporation(mm)
2025/12/06	5.2	3.56	1.64
2025/12/07	2.46	1.89	0.57
2025/12/08	4.53	2.96	1.57
2025/12/09	5.46	2.14	3.32
2025/12/10	1.94	1.58	0.36
2025/12/11	3.73	2.98	0.75
2025/12/12	5.13	4.08	1.05
2025/12/13	5.33	2.77	2.56

6.4 Conclusion

The study demonstrated that sap flow measurements can provide valuable insights into the water use patterns of Mimusops zeyheri under natural field conditions. The results showed that climatic factors such as temperature, solar radiation, rainfall, relative humidity, and vapour pressure deficit strongly influenced transpiration rates and daily water use. Higher sap flow rates were observed during warm and dry periods, while reduced transpiration occurred during periods of heavy rainfall and prolonged overcast conditions.

Although the monitoring period was short, the findings establish a foundation for future long-term studies on seasonal water use, irrigation planning, and the ecological adaptation of Mimusops zeyheri. This baseline information is particularly valuable for improving understanding of indigenous tree water use and supporting the integration of underutilized species into sustainable agroforestry and climate-resilient land management systems.

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CHAPTER SEVEN

NUTRITIONAL VALUE OF *MIMUSOPS ZEYHERI* SEEDS FROM TWO AGROECOLOGIES

Abstract

Indigenous wild fruit trees are receiving growing attention for their ecological roles and nutrient-rich qualities, which make them essential in efforts to reduce hidden hunger and build climate-resilient food systems. One notable species is *Mimusops zeyheri*, a drought-tolerant wild fruit native to southern Africa. Despite its ecological significance and reported contribution to rural livelihoods, its nutritional value and broader dietary impact remain only loosely documented. Ripe fruits and leaves were collected from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni) Provinces. Proximate composition was determined using standard AOAC methods, and microwave-assisted digestion followed by ICP-OES was used for elemental analysis. Data were analysed using one-way ANOVA and Pearson correlation in R. Nuts contained the highest dry matter (~94%), crude fat (~54%), protein (~19%), and energy value (~595 kcal/100 g^{-1}), together with the most significant concentrations of calcium (≈210 mg/100 g^{-1}), magnesium (≈143 mg 100 g^{-1}), zinc (≈95 mg/kg^{-1}), and potassium (≈495 mg/100 g^{-1}). Leaves were rich in protein (≈24 %) and fibre (≈22 %), while pulp and fruit fibre provided high moisture, modest vitamin C (5–7 mg/100 g^{-1}), and β-carotene (~1.5–2.1 mg/100 g^{-1}). Correlation analysis revealed strong positive associations ($r > 0.95$, $p < 0.01$) among dry matter, crude fat, protein, and divalent cations (Ca, Mg, Zn), reflecting higher nutrient concentrations as moisture decreases. Provincial differences were minor, indicating ecological consistency. *M. zeyheri* offers a unique combination of energy-dense nuts, protein, mineral-rich leaves, and vitamin-containing

pulp, making it a valuable, climate-resilient food source for improving dietary diversity and combating micronutrient deficiencies in rural South Africa.

7.1 Introduction

Global food systems face increasingly significant pressures from population growth, climate change, and environmental degradation, threatening the capacity of conventional agriculture to meet future nutritional needs sustainably. More than 700 million people experience chronic hunger, while billions suffer from "*hidden hunger*" due to deficiencies in micronutrients such as iron, zinc, and vitamin A (Redón Lago, 2021; Weffort and Lamounier, 2024). Projections indicate that by 2050, the world population will exceed 9.7 billion, necessitating a minimum 50 percent increase in food production compared to 2012 (Alexandratos and Bruinsma, 2012). However, further intensification of major cereals is constrained by a lack of arable land, limited water resources, and climate- related stresses, heatwaves, erratic rainfall, and emerging pests, which already reduce yields of maize and wheat.

In sub-Saharan Africa, inadequate intake of proteins, energy, and minerals has contributed to a double burden of malnutrition, characterised by the coexistence of undernutrition alongside overweight, obesity, and other non-communicable diseases (Ritchie and Roser, 2017). The prevalence of stunting induced by malnutrition in sub- Saharan countries ranges from 7.9 to 57.7%, with a mean of 30%. The prevalence of undernourishment is projected to increase to 25.7% by 2030 (Akombi et al., 2017; Atukunda et al., 2021). Major risk factors include male gender, low birth weight, home delivery, limited maternal education, household poverty, and rural residence (Seretew et al., 2024). Furthermore, malnutrition is closely associated with impaired brain development, which can restrict cognitive capacity, reduce educational attainment, compromise immune function, and diminish economic productivity (Fernandes and Le, 2021).

Although many sub-Saharan African nations, including South Africa, have introduced national nutrition programmes aligned with the Sustainable Development Goals, progress remains slow, especially towards SDG 1-eradicating extreme poverty and SDG 2-eliminating hunger and malnutrition by 2030 (Atukunda et al., 2021).

Several studies reported the potential of indigenous and under-utilised plant species to diversify food systems, enhance food security, and strengthen resilience to climate variability (Mayes et al., 2012; Ndlovu et al., 2024; Zuza et al., 2024). Research conducted across Africa and other biodiversity-rich regions demonstrates that numerous wild and semi-domesticated plants are rich in proteins, essential fatty acids, vitamins, and trace minerals, and contain bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial activities (Aworh, 2018; Ndlovu et al., 2024).

According to Lubisi et al. (2023a), indigenous fruit trees contribute directly to better health and climate action by supporting nutrition, livelihoods, and ecosystem stability, particularly in sub-Saharan Africa. Although sub-Saharan Africa is rich in biodiversity and edible wild fruit species, it continues to experience some of the highest levels of food insecurity (Akinnifesi et al., 2007). This is partly due to the limited cultivation of edible plant species and a low level of commercial trade (Akinnifesi et al., 2007). Indigenous fruit trees such as *Adansonia digitata*, *Sclerocarya birrea*, and *Annona senegalensis* contribute significantly to rural diets and household economies yet remain marginal in agricultural policy and research (Omotayo and Aremu, 2020). These species frequently thrive on marginal soils with little management, providing natural buffers against drought and other climate shocks. In South Africa, communities in Limpopo and Mpumalanga increasingly rely on wild fruit trees such as *M. zeyheri,* as erratic rainfall and recurrent drought threaten maize, the national staple.

Expanding scientific knowledge of *M. zeyheri's* nutritional and elemental composition is therefore essential for climate-smart agriculture, agroforestry, and community-based resource management. *Mimusops zeyheri* is drought-tolerant and adapted to sandy, nutrient-poor soils, making it ecologically valuable in semi-arid landscapes (Omotayo et al., 2020). *M. zeyheri* also contributes to climate-change mitigation through long-lived biomass and deep-root systems that enhance carbon sequestration, stabilise soils, reduce erosion, and improve microclimates, ecosystem services essential to sustainable agriculture (Mkhonto et al., 2024b). Minimal input requirements and strong drought tolerance make this species well-suited to climate-smart agroforestry initiatives (Mkhonto et al., 2024b), consistent with South Africa's National Development Plan and the African Union's Agenda 2063.

Ethnobotanical surveys record that almost every part of the plant is traditionally utilised. The yellow-orange fruits are eaten fresh or fermented into beverages; leaves and bark are used in remedies for diarrhoea, respiratory ailments, and skin infections; nuts yield oil applied in cooking and cosmetics; and the wood is valued for its durability (Lubisi et al., 2023; Mkhonto et al., 2024; Omotayo et al., 2020). Such diverse uses point to a rich nutrient and phytochemical profile, yet systematic laboratory analyses remain limited.

Extensive data on the proximate and mineral composition of *M. zeyheri* are needed to evaluate its potential contribution to dietary requirements and public health initiatives. Identifying nutrient-dense indigenous foods is particularly relevant in rural South Africa, where micronutrient deficiencies remain widespread. Hence, this study aimed to document the nutritional (proximate and elemental) composition of *M. zeyheri* from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni).

7.2 Materials and methods

7.2.1 Sample collection and preparation

Fresh leaves and ripe fruits of *M. zeyheri* were collected in August 2025 during their ripening season from two provinces in South Africa. The collection sites were in Vhembe District of Limpopo Province (23.9000° S, 29.4500° E) and Ehlanzeni District of Mpumalanga Province (25.4652° S, 30.9785° E). These two distinct agro-ecologies were chosen to provide a comprehensive representation of the plant's natural habitat across different regions. Voucher specimens from the leaves were prepared through drying and pressing (C003) and were deposited at the South African National Biodiversity Institute (SANBI) herbarium in Pretoria for authentication. The plant leaves and ripe fruits were separated from the other plant parts and washed thoroughly with tap water, then again with distilled water to remove any remaining debris that could cause contamination. The leaves were oven-dried for 48 hours at 40 °C. The fruits were subjected to fibre and pulp separation and freeze drying at -80 degrees using the FD-12 Series freeze dryer (Lyophilizer). The dried plant parts were pulverised using a Waring TNNK160K commercial blender at low speed. The powdered samples were stored in airtight containers to preserve the plant biomolecules and kept at -80 degrees in a freezer.

7.2.2 Proximate analysis

7.2.2.1 Moisture

Five (5) g of each *Mimusops zeyheri* plant sample (leaves, fruit peel, fruit pulp, and nuts) was weighed in triplicate into pre-weighed crucibles for all proximate determinations. For moisture content, the crucibles were placed in a hot-air oven at 80°C and dried to

constant weight. After cooling in a desiccator to room temperature, the crucibles were re-weighed, as described by Nielsen (2009). Moisture was calculated as (1):

$$\%\ Moisture\ content = \frac{W2-W3}{W2-W1} \times 100 \quad (1)$$

where W1W_1W1 is the initial weight of the sample, and W2W_2W2 is the weight after drying. Dry matter was expressed by difference as (2)

$$\%Dry\ matter = 100 - Moisture \quad (2)$$

7.2.2.2 Ash content

5 g of the oven-dried sample was incinerated in a muffle furnace at 550 °C for six hours until a light-grey ash was obtained. The crucibles were cooled in a desiccator and weighed (W3). Ash percentage was determined using (3):

$$\%\ Ash = \frac{W3-W1}{W2-W1} \times 100 \quad (3)$$

7.2.2.3 Crude fat

To remove the lipid fraction, 5 g of the finely ground sample was precisely weighed into a cellulose extraction thimble and Soxhlet-extracted with petroleum ether (boiling range: 45–60 °C) for 5 hours. Following the extraction cycle, the solvent was removed, and the fat residue was dried to a consistent weight in an oven set at 105 °C. It was then allowed to cool in a desiccator and weighed again. Crude fat percentage was determined as (4):

$$\%\ Fat = \frac{W1-W2}{W1} \times 100 \quad (4)$$

7.2.2.4 Crude fibre

Two (2) g of the previously defatted sample (W_s) was weighed into a 250 mL conical flask and extracted according to the AOAC procedure with modifications described by Jacob

et al. (2016). Two hundred millilitres of 1.25% sulphuric acid were added, and the mixture was gently boiled for 30 minutes. The hot suspension was filtered through a Buchner funnel lined with poplin cloth and rinsed repeatedly with hot distilled water until a neutral pH was confirmed with litmus paper. The residue was returned to the conical flask, treated with 200 mL of 1.25% sodium hydroxide, boiled for 30 minutes again, filtered through poplin cloth, and washed with hot water until no trace of alkali remained. The neutralised residue was transferred to a pre-weighed porcelain crucible and dried in a hot-air oven at 105 °C to constant weight to obtain Wcd. The crucible was then placed in a muffle furnace at 550 °C for 6 hours, cooled in a desiccator, and reweighed to obtain the ash weight (Wca). The percentage crude fibre was calculated as (5):

$$\% \ Fibre = \frac{W_{cd} - W_{ca}}{W_S} \times 100 \quad (5)$$

where W_{cd} is the weight of the dried residue, W_{ca} is the weight of the ash, and W_S is the initial weight of the defatted sample.

7.2.2.5 Protein

To determine crude protein nitrogen content, the Kjeldahl method described by Goyal et al. (2022) was used with slight modifications. 1g of finely ground sample was weighed into a digestion flask, and two selenium tablets were added as a catalyst. Twelve millilitres of concentrated sulphuric acid (H_2SO_4) were introduced and the mixture heated gently until the digest became clear, indicating complete conversion of organic nitrogen to ammonium sulphate. The clear digest was cooled, transferred to a 50 mL volumetric flask, and diluted to the mark with distilled water. An aliquot of 10 mL of the digest was mixed with 10 mL of 40 % sodium hydroxide (NaOH) in a Kjeldahl distillation unit. A receiving flask containing 5 mL of 2 % boric acid solution and three drops of mixed indicator was

placed under the condenser outlet. During distillation, ammonium ions were converted to ammonia gas, which was absorbed into the boric acid receiver to form ammonium borate. The captured ammonia was titrated with 0.01 M hydrochloric acid (HCl) until the indicator colour changed from green to pink, signifying the endpoint.

$$\% N = \frac{(S-B)\times Nacid\times 0.014\times D}{W\times V}\times 100 \quad (6)$$

$$\%Crude\ protein = 6.25^{*} \times \%N(*\ Correction\ factor) \quad (7)$$

where S is the sample titre (mL), B the blank titre (mL), $N_{(}acid_{)}$ the normality of HCl, 0.014 the milliequivalent weight of nitrogen (g), D the total dilution after digestion (mL), V the aliquot volume distilled (mL), and W the sample weight (g).

7.2.2.6 Carbohydrate

Total carbohydrate was obtained by difference to ensure the proximate fractions summed to 100% using a formula described by Sibiya et al. (2021).

$$\%Carbohydrate = 100 - (\%Ash + \%Protein + \%\ Fat + \%\ Fibre) \quad (8)$$

7.2.2.7 Energy value

The energy value (metabolisable energy) was estimated with Atwater factors of 4 kcal g^{-1} for protein and carbohydrate and 9 kcal g^{-1} for fat:

$$Energy\ (kcal\ 100\ g^{-1}) = 4 \times (Protein\ +\ Carbohydrates) + 9 \times CrudeFat \quad (9)$$

7.2.3 Elemental analysis

Elemental analysis of *Mimusops zeyheri* plant parts was carried out using a microwave-assisted acid digestion followed by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES), following a procedure described by Al-Juhaimi et al. (2023). After the prepared dry powders of each sample (FFLP, FFMP, FPLP, FPMP, FNLP, FNMP)

were weighed, approximately 0.20 g was placed in TFM digestion vessels with 5 mL of trace-metal grade nitric acid and 2 mL of hydrogen peroxide. The sealed vessels were heated in a microwave digestion system to 210 °C under pressure to ensure complete oxidation of organic matter. Once cooled, digests were transferred to acid-washed volumetric flasks and diluted to a fixed volume with ultrapure water. Method blanks, matrix spikes, and a certified reference plant material were included in every batch to check contamination, recovery, and precision.

The concentrations of macro- and micro-elements (Ca, Cu, Fe, K, Mg, Na, P, and Zn) were quantified on an ICP-OES instrument equipped with a concentric nebuliser and cyclonic spray chamber. Calibration curves were prepared from multi-element standards spanning the expected concentration ranges, and verification standards were analysed at regular intervals. Wavelengths free of spectral interferences were selected for each element, and instrumental drift was corrected using internal quality controls. Elemental concentrations were calculated from the instrument response, converted to mg kg^{-1} dry weight, and then normalised to mg 100 g^{-1} fresh weight using independently determined moisture contents. All analyses were performed in triplicate.

7.2.3.1 Estimated daily intake

The estimated daily intake (EDI) of each element from wild fruit consumption was derived using its concentration in mg kg^{-1} (Kavcar et al., 2009), a standard fruit intake of 0.4 kg day^{-1} (Mehri et al., 2024), and an average adult body weight of 70 kg (Zergui et al., 2024), as shown in Table 7.1. The calculation followed the equation:

$$EDI = \frac{C \times DI}{BW} \quad (10)$$

where *C* is the element concentration, *DI* is the daily fruit intake, and *BW* is body weight

Table 7-1: Recommended Daily Intakes (RDI) for key nutritional elements in food

Element	Adult recommended intake	Source
Calcium (Ca)	1,000 mg day^{-1}	(Balk et al., 2017)
Copper (Cu)	0.9 mg day^{-1}	(Taylor et al., 2020)
Iron (Fe)	8 mg day^{-1} (men) 18 mg day^{-1} (women of child-bearing age)	(Wang et al., 2012)
Potassium (K)	4,700 mg day^{-1}	(EFSA Panel on Dietetic Products et al., 2016)
Magnesium (Mg)	310–420 mg day^{-1}	(Zhang et al., 2016)
Sodium (Na)	<2,000 mg day^{-1} (upper limit)	(EFSA Panel on Nutrition et al., 2019)
Phosphorus (P)	700 mg day^{-1}	(Fulgoni and Fulgoni III, 2021)
Zinc (Zn)	8 mg day^{-1} (women); 11 mg day^{-1} (men)	(Rondanelli et al., 2021)
Vitamin C	75 mg day^{-1} (women); 90 mg day^{-1} (men)	(Wang et al., 2021)
β-Carotene	900 µg retinol activity equivalents (RAE) day^{-1} (men); 700 µg RAE day^{-1} (women)	(do Nascimento et al., 2019)

7.2.4 Data analysis

Data were expressed as the mean of three independent replicates ± standard deviation (SD). Statistical analyses were performed in R version 4.3.1. One-way ANOVA followed by Tukey's HSD test ($p \leq 0.05$) was used to determine significant differences among plant parts and provinces. Pearson correlation coefficients between proximate composition (moisture, dry matter, ash, crude fat, crude fibre, protein, carbohydrate, energy value) and

elemental composition (Ca, Cu, Fe, K, Mg, Na, P, Zn) were calculated using the cor() function in R. Correlation coefficients (r) range from –1 to +1, where values ≥ +0.8 indicate a strong positive relationship and values ≤ –0.8 indicate a strong negative relationship.

7.3 Results and discussion

7.3.1 Proximate analysis

Table 7.2 shows the proximate composition (moisture, dry matter, ash, protein, fibre, carbohydrate, and energy value) of *M. zeyheri* fruit fibre, fruit pulp, nuts, and leaves collected from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni).

7.3.1.1 Moisture

Moisture analysis shows a distinct and highly stable gradient across the different *Mimusops zeyheri* plant parts, with the edible pulp exhibiting the highest water content of about 92% in Limpopo Province and 91% in Mpumalanga Province, followed by the fruit fibre at roughly 89%, leaves at approximately 65%, and nuts remaining extremely dry at close to 6%. These contrasts are statistically significant ($p<0.05$), emphasising that intrinsic plant tissue and physiology, rather than geographic or regional factors, are the primary determinants of moisture variation in *Mimusops zeyheri* plant parts. Such patterns are consistent with findings reported in other Sapotaceae species; for instance, *Sclerocarya birrea* pulp typically contains 80-85% moisture, while its nuts average 4–6% (Halidou et al., 2022; Magaia et al., 2013), and *Chrysophyllum albidum* pulp shows 83-88% water with nuts below 7% (Adunni Abiodun and Oladapo, 2011). Similar nut dryness is also documented for *Vitellaria paradoxa* (Obeng-Akrofi et al., 2025) and *Manilkara zapota* (Kumari et al., 2016), where nut moisture stabilises around 5-7%. This consistently low nut moisture represents an important adaptive trait, limiting microbial activity and

preserving high-fat reserves for extended periods, thereby supporting both nut viability and long-term food security, particularly in rural areas with limited access to conventional preservation technologies and thereby safeguards a reliable long-term food supply. From a utilisation perspective, these findings highlight two contrasting requirements: the juicy pulp, attractive to dispersers and rich in simple sugars, requires careful post-harvest handling or drying to prevent spoilage, whereas the dry nuts can be stored or transported over long distances without elaborate preservation, making them ideal for year-round oil extraction or direct consumption in rural households.

7.3.1.2 Dry matter

Dry matter varies inversely with moisture, reflecting the classic negative correlation observed in fruit tissues. Nuts have the highest levels, at 94.0% in Limpopo and 93.5% in Mpumalanga, followed by leaves at around 34-35%. The fruit fibre averaged at about 11%, and the pulp contains only 7-8%. These values resemble those reported in high-oil-nut plants such as *Irvingia gabonensis*, which often surpass 90% dry matter (Zoué et al., 2013), and marula nuts, typically 90-94% (Mthiyane and Mhlanga, 2017). The extremely high *M. zeyheri* nut dry matter underscores its role as a dense nutrient reservoir. Since nuts contain very little water, their nutrients, especially fats and proteins, are naturally more concentrated, which explains their high energy content. This low moisture level also makes nuts easier to store and transport, as they require minimal drying and are less prone to spoilage, making them ideal for food processors and consumers. In contrast, the pulp has a much higher moisture content, giving it a softer texture and making it more appealing to fruit-eating animals. Comparable part-driven differences occur in shea fruit, where nuts average 91% dry matter and the mesocarp seldom exceeds 15% (Honfo et al., 2014).

The implications for product development are clear: nuts are ideal for nutrient-rich flours and oils, whereas pulp-based products must account for large water loss during drying.

7.3.1.3 Ash

Ash content, which represents the total mineral fraction of plant tissue, varies markedly among the different parts of *Mimusops zeyheri*, and the sequence of these differences follows a clear physiological trend. In the current study, the leaves contained the highest levels, 11.85% in Limpopo and 12.13% in Mpumalanga, figures that exceed many cultivated vegetables and align with values reported for *Adansonia digitata* with 8-12% (Eke et al., 2013) and moringa oleifera leaves with 10-11% (Owusu et al., 2008). By contrast, the fruit fibre averaged about 4%, while the pulp and nuts ranged only between 2.6% and 3%. These results reflect the functional requirements of each tissue: leaves must store substantial amounts of calcium, magnesium, potassium, and trace elements to sustain photosynthesis and other metabolic activities (Kaur et al., 2023). Noted in this study, earlier work on *M. zeyheri* foliage reported calcium levels above 2 g 100 g^{-1} and potassium levels above 1.5 g 100 g^{-1} dry weight (Omotayo et al., 2020), supporting the high mineral values observed here. From a dietary perspective, these concentrations position the leaves as a valuable source of essential minerals that can complement cereal-based diets typically low in micronutrients, and the promotion of dried leaf powders could therefore help address mineral deficiencies and strengthen food security strategies in rural communities. The broader concept of ash content explains why such differences occur. Ash represents the inorganic residue left after complete combustion of plant material (Kuswa et al., 2024) and reflects the mineral composition of the tissue, which

depends on both species and plant part (Babayemi et al., 2010; Watanabe et al., 2007). As evident from this study, the marked contrast between *M. zeyheri* leaves, fibre, pulp, and nuts is consistent with patterns reported for other species. For example, Zeng et al. (2014) documented distinct ash levels across organs of *Pinus massoniana*, while Bakker and Elbersen (2005) observed that wetland species often exhibit higher ash content than C4 grasses, demonstrating that water uptake and environmental conditions influence mineral accumulation. These comparisons underscore that the high leaf ash content observed here is consistent with established physiological and ecological principles.

7.3.1.4 Crude fat

Differences in oil content and composition among plant species and their various organs arise from a range of ecological and physiological influences. Factors such as local climate, soil characteristics, pest pressure, and the fruit's developmental stage all affect lipid production and storage, accounting for the substantial variation observed both within and across species (Bacelar et al., 2024). This is consistent with the current findings of this study, as *Mimusops zeyheri* plant parts follow a comparable high-oil pattern. Fat accumulation was concentrated in the nuts, with fat levels of 54.0% in Limpopo and 54.1% in Mpumalanga, values that match or exceed those reported for other oil-rich wild fruits such as *Irvingia gabonensis* 48–67% (Mateus-Reguengo et al., 2020) and Allanblackia species with nearly 50% (Adubofuor et al., 2013; Loumouamou et al., 2014). In comparison, the *M. zeyheri* leaves contained about 6 % fat, while the fruit fibre (~1.8%) and pulp (<1%).

Chivandi et al. (2011b) reported that *M. zeyheri* nuts show that the oil is dominated by oleic (~43%) and linoleic (~35%) acids, a profile that provides oxidative stability and

cardiovascular protection similar to or greater than that of popular edible oils such as sunflower and soybean. Tocopherols and phytosterols, mainly reported in Sapotaceae species (Chakradhari et al., 2019), add antioxidant and cholesterol-lowering properties, reinforcing *M. zeyheri* nut oil's health value and highlighting its potential as a premium, heart-healthy alternative.

7.3.1.5 Crude fibre

Wild fruit trees contain significant amounts of crude fibre, making them valuable sources of nutrition for indigenous communities. Leaves contain the highest levels at 21.7–21.8%, nuts follow at about 9.5%, the fruit fibre averages 6%, and the pulp contains only 4–5%. Leaf values mirror those of baobab leaves (18-24%) and other traditional vegetables like *Corchorus olitorius* with a 20-22% range (Sha'a et al., 2019). High dietary fibre supports gut health, lowers cholesterol, and moderates blood glucose, justifying the traditional use of dried leaves as a relish (Sharma and Singh, 2025; Suresh et al., 2024). *M. zeyheri* nut fibre content is higher than that of *Sclerocarya birrea* nuts reported at 4-7% (Chauke et al., 2025) and comparable to *Irvingia gabonensis* reported between 8-10% (Adeyeye, 2013). Defatted nut meal could therefore serve as a functional fibre supplement in composite flours or high-fibre bakery products. In contrast, fruit fibre, pulp, and nuts facilitate consumption by frugivores and humans without additional processing.

7.3.1.6 Protein

Protein is an important part of a healthy diet, as it helps build and repair muscles and bones, and produce hormones and enzymes. Protein levels in *Mimusops zeyheri* vary by plant part, reflecting the distinct roles of each tissue. On a dry-weight basis, the leaves contain about 24.4% protein, surpassing many cultivated vegetables, including spinach

(~22%) and amaranth (~21%) (Pathan et al., 2019). Nuts follow closely at 19.1-19.7%, exceeding the 12–17% typically reported for *Chrysophyllum albidum* (Pathan et al., 2019) and well above the 10–12% found in *Vitellaria paradoxa* (Abdul-Mumeen et al., 2024). By contrast, the fruit fibre pericarp and the sweet pulp contribute only trace amounts, roughly 1% and 0.3–0.4%, respectively. Amino-acid analysis shows that the nuts provide essential amino acids, such as lysine and leucine, at levels comparable to those in soybean meal (Chivandi et al., 2011b), making them an excellent supplement to cereal-based diets where animal protein is scarce or costly. The protein-rich leaves can also be dried and milled into powders for soups and sauces, offering a low-cost way to improve household nutrition.

Proteins are fundamental to human health; they form the structural framework of cells, enable tissue growth and repair, and are required for enzymes, hormones, and other vital biomolecules (Chandana et al., 2024). They also support immune defence and blood-clotting processes, serving both regulatory and structural functions. Although most fruits contain only minor amounts of nitrogenous compounds (typically 0.1-1.5%), a few, including berries, cherimoya, and avocado, are recognised for their relatively higher protein content (Prosekov et al., 2018; Sánchez-Moreno et al., 2006). Similar to these exceptions, *M. zeyheri* stands out among wild fruits for its unusually high protein levels. As noted by Cao et al. (2020), adequate dietary protein is essential for maintaining healthy skin and other organs, and deficiencies can quickly impair these functions.

7.3.1.7 Carbohydrates

Carbohydrates play a central role in human nutrition, and their presence, even at the modest levels recorded in *Mimusops zeyheri,* has important implications for rural diets

and livelihoods. In this study, nuts showed the highest calculated carbohydrate content at 7.76-7.84%, while pulp and fruit fibre contained only 0.05-0.82% and leaves around 0.5%. Previous direct carbohydrate analyses have reported combined sucrose, glucose, and fructose levels of 12-18% on a fresh-weight basis of *Strychnos spinosa*, higher than those observed in *M. zeyheri* (Omotayo and Aremu, 2021). Such naturally occurring sugars explain the fruit's pleasant sweetness and mean that, despite the low figures in the proximate table, the pulp can deliver a rapid, readily absorbable source of energy. This quality is particularly valuable in rural settings where quick, high-glycaemic energy can supplement otherwise monotonous diets or sustain labour-intensive agricultural work. From a dietary perspective, carbohydrates remain the primary fuel for human metabolism, supplying about 45-65% of daily energy needs according to the World Health Organisation (Ahsan, 2021) and the Food and Agriculture Organisation (FAO, IFAD, UNICEF, WFP, and WHO, 2024; Mann et al., 2007). Carbohydrates provide glucose for the brain and red blood cells, spare protein from being used as energy, and are essential for glycogen storage that supports physical activity. In rural households, staple carbohydrate-rich foods such as maize meal (sadza or pap), sorghum, millet, cassava, sweet potato, and yam are not merely sources of calories but also cultural anchors for meals, festivals, and social gatherings (Ekpa et al., 2019). These staples are often accompanied by small amounts of legumes or leafy vegetables to improve protein quality, but the carbohydrate base remains the dietary pillar.

Against this backdrop, the sweet pulp of *M. zeyheri* offers a seasonal, locally available complement to these staples. It can diversify carbohydrate sources, provide natural sugars for children and field workers, and reduce dependence on purchased refined

sugar. The nuts, though far lower in carbohydrate, add a slow-release energy component when incorporated into composite flours or porridge blends, improving both nutrient density and satiety. By drawing on traditions in which wild fruits such as *S. birrea*, *A. digitata*, and *Strychnos spp.* are gathered and shared, *M. zeyheri* can fit naturally into rural food systems. Its combination of sweet pulp and energy-dense nuts mirrors the multipurpose roles of other wild fruits, strengthening household food security and providing an emergency food reserve during periods of staple-crop shortages.

7.3.1.8 Energy value

The energy content of *M*. *zeyheri* clearly reflects the distribution of fat and dry matter and differs significantly across plant parts ($p<0.05$). Nuts exhibit the greatest caloric load-596.52 and 594.75 kcal/100 g^{-1}, surpassing common oilnuts such as groundnut with ~567 kcal/100 g^{-1} (Mitu, 2020). Leaves provide a moderate 155–157 kcal/100 g^{-1}, while the fruit fibre contributes only 21-23 kcal/100 g^{-1}, and the pulp the lowest at 10.7-12.8 kcal/100 g^{-1}. When the accurate soluble sugar content of the pulp is considered, its energy value likely rises to around 80-140 kcal/100 g^{-1}, similar to other sweet fruits like mango or sapodilla (Akelom et al., 2022). The plant part values underscore how strongly tissue composition, especially fat concentration, determines total energy yield.

From a dietary standpoint, the high-energy nuts offer an essential supplement in food-insecure rural areas. A small quantity can substantially increase the caloric density of porridges or baked goods, helping households meet energy requirements where staple cereals or cooking oils are limited. By contrast, the low-energy pulp and fibre, despite their natural sugars, act more like classic fruits, adding bulk and hydration without contributing excessive calories. This characteristic aligns with evidence that diets rich in

low-energy-dense foods, such as fruits, help control total energy intake, because they provide satiety and meal weight with relatively few calories (Rolls, 2017). Such foods can therefore support weight management and reduce the risk of overconsumption, while still supplying vitamins and phytochemicals.

Nutrition studies further show that high-energy foods, particularly those high in refined grains or added sugars, are associated with obesity, whereas fruit consumption is generally not linked to weight gain (Kristoffersen et al., 2025; Tetens and Alinia, 2009). In this context, *M. zeyheri* presents a valuable dual option; its nuts supply concentrated energy when dietary calories are scarce, and its pulp provides a refreshing, low-energy fruit component that can be eaten freely without increasing the risk of excess energy intake. For rural livelihoods, this duality is advantageous; families can harvest and dry the nuts as a calorie reserve, while the fresh pulp provides a hydrating snack that supports dietary diversity and weight control, reflecting both nutritional and cultural benefits within traditional food systems.

Table 7-2: Proximate nutritional composition of *M. zeyheri* fruit fibre, pulp, nuts, and leaves from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni)

	Moisture	Dry matter	Ash	Crude Fat	Crude fibre	Protein	Carbohydrates	Energy Value
FFLP	88.87±0.19^{b}	11.13±0.19^{e}	4.1±0.14^{c}	1.76±0.03^{d}	6.16±0.22^{d}	0.88±0.06^{f}	0.05±0^{c}	21.4±0.1^{e}
FFMP	88.6±0.2^{b}	11.4±0.2^{e}	4.12±0.1^{c}	1.87±0.04^{d}	6±0.18^{d}	1.02±0.01^{f}	0.5±0^{c}	22.97±0.35^{e}
FPLP	92.19±0.37^{a}	7.81±0.37^{f}	2.61±0.08^{d}	0.82±0.01^{e}	4.42±0.18^{e}	0.34±0.01^{g}	0.5±0^{c}	10.73±0.12^{f}
FPMP	91.3±1.37^{a}	8.7±1.37^{f}	2.68±0.1^{d}	0.85±0.04^{e}	4.56±0.26^{e}	0.45±0.01^{g}	0.82±0.45^{c}	12.77±2.11^{f}
FNLP	5.98±0.03^{e}	94.02±0.03^{a}	2.90±0.05^{d}	54.02±0.43^{a}	9.52±0.26^{c}	19.74±0.63^{c}	7.84±0.35^{b}	596.52±2.49^{a}
FNMP	6.52±0.07^{e}	93.48±0.07^{a}	2.98±0.01^{d}	54.13±0.2^{a}	9.48±0.17^{c}	19.13±0.54^{c}	7.76±0.34^{b}	594.75±1.33^{a}
LLP	65.09±0.46^{c}	34.91±0.46^{c}	11.85±0.11^{a}	6.21±0.08^{c}	21.7±0.59^{a}	24.43±0.25^{a}	0.5±0^{c}	155.65±0.83^{c}
LMP	65.24±0.54^{c}	34.76±0.54^{c}	12.13±0.08^{a}	6.3±0.08^{c}	21.79±0.51^{a}	24.48±0.51^{a}	0.5±0^{c}	156.68±2.43^{c}

All analyses are the means of triplicate measurements ± standard deviation. Significant differences among the samples were assessed using the Tukey test. Means that share a superscript letter within a column are not significantly different at p ≤ 0.05. Key: FFLP (Fruit fibre Limpopo), FFMP (Fruit fibre Mpumalanga), FPLP (Fruit pulp Limpopo), FPMP (Fruit pulp Mpumalanga), FNLP (Fruit nuts Limpopo), FNMP (Fruit nuts Mpumalanga, LLP (Leaves Limpopo), LMP (Leaves Mpumalanga).

7.3.2 Elemental composition

Table 7.3 presents the elemental composition of *M. zeyheri* fruit fibre, pulp, nuts, and leaves collected from the selected study area. Elements analysed include calcium, copper, iron, potassium, magnesium, sodium, phosphorus, zinc, β-carotene, and vitamin C. The results show apparent variation by plant part, with nuts and leaves generally richer in minerals than the pulp and fibre. At the same time, provincial differences are minor, indicating that nutrient levels are primarily determined by plant tissue rather than location.

7.3.2.1 Calcium

Calcium was the most abundant micromineral in *M. zeyheri* nuts, with the nut sample from Mpumalanga (FNMP) containing 214 mg 1 g^{-1} and the nut sample from Limpopo Province containing 209 mg 100 g^{-1}. These values were markedly higher than those of the fresh fruit fibre from Limpopo (FFLP, 145 mg 100 g^{-1}) and Mpumalanga (FFMP, 152 mg 100 g^{-1}), as well as the pulp from Limpopo (FPLP, 127 mg 100 g^{-1}) and Mpumalanga (FPMP, 136 mg 100 g^{-1}). Nuts from Mpumalanga, therefore, carried a slightly greater calcium load compared to those found in Limpopo Province, although both provinces' nuts exhibited far richer calcium than the corresponding fruit tissues. This strong concentration in the nuts reflects the well-known nut-mineral partitioning observed in the Sapotaceae family, in which developing radicle (nut embryo) cells accumulate structural and signalling cations for germination and early nutling growth (Alamgir, 2017). Within the Mimusops genus, comparable levels have been documented. Nuts of *Mimusops caffra,* as reported by Mngadi et al. (2017a), contain about 2.3% calcium on a dry-matter basis (≈ 2,330 mg 100 g^{-1}), which exceeds even the richest fractions of *M. zeyheri*, while *Mimusops elengi* fruit pulp contains around 212 mg 100 g^{-1} (Srivastava et al., 2024).

Adequate dietary calcium is fundamental for skeletal development, bone mineralisation, neuromuscular excitability, blood clotting, and cell signalling. Chronic insufficiency causes rickets in children and contributes to osteopenia and osteoporosis in adults, while marginal intakes may impair nerve conduction and muscle contraction (Shlisky et al., 2022). The high calcium density of *M. zeyheri* nuts from both provinces, therefore, provides a valuable dietary source in communities where dairy products are scarce and cereal-based diets predominate. Calcium also interacts with plant phenolics, destabilising bacterial cell walls. It can enhance host immune responses (Negi et al., 2023), effects that are consistent with the long-standing ethnomedicinal use of *Mimusops* nuts and bark for treating skin infections and wounds (Mkhonto et al., 2024b).

7.3.2.2 Copper

Copper concentrations were strikingly uniform across all plant parts and both provinces, averaging about 94–95 mg/kg^{-1} dry weight. For example, the fresh fruit flesh from Limpopo (FFLP) contained 94 mg/kg^{-1}, the mid-portion flesh from Mpumalanga (FFMP) 94 mg/kg^{-1}, and the nuts from both Limpopo (FNLP) and Mpumalanga (FNMP) each 95 mg/kg^{-1}. This consistency indicates efficient systemic uptake and distribution throughout the plant regardless of geographic origin. Based on proximate analyses of *M. elengi* fruit pulp, copper concentrations ranged from about 82 to 105 mg kg^{-1} dry weight (Lim, 2012), depending on maturity stage and soil conditions. Similarly, *Mimusops caffra* nut studies report copper values of roughly 88-110 mg kg^{-1} dry weight (Mngadi et al., 2017a). These ranges place both *M. elengi* and *M. caffra* squarely in the upper tier of copper content when compared with most African wild fruits, which typically present copper

concentrations below 60 mg kg^{-1}. The overlap of these reported ranges with the 94-95 mg kg^{-1} recorded for *M. zeyheri* underscores the consistent copper richness across the genus. It confirms that *M. zeyheri* is fully comparable to, and in some cases matches the highest values found in, its close relatives.

Copper is an essential trace element that functions as a cofactor for many enzymes, among them cytochrome c oxidase, which drives cellular respiration, and superoxide dismutase, which protects tissues from oxidative stress (Arredondo and Núñez, 2005). It also supports iron transport, collagen cross-linking, and the synthesis of key neurotransmitters. Although dietary deficiency is uncommon, inadequate intake has been documented in malnourished infants, in patients receiving parenteral nutrition without copper supplementation, and in individuals with chronic malabsorption (Cordano, 1998). Such a deficiency can lead to anaemia, neutropenia, connective-tissue disorders, and impaired immune responses. The consistently high copper concentrations measured in *Mimusops zeyheri* fruit, therefore, represent a meaningful contribution to daily trace-element requirements, especially in communities where mineral intake may be limited. Copper is also well recognised for its antimicrobial action: ionic copper can generate reactive oxygen species, disrupt microbial membranes, and damage nucleic acids, producing both bactericidal and fungicidal effects (Salah et al., 2021). The detection of copper in fruits collected from both Limpopo and Mpumalanga Provinces provides scientific support for the traditional use of *M. zeyheri* preparations in the management of skin infections and the enhancement of wound healing.

7.3.2.3 Iron

Iron concentrations ranged narrowly between 93 and 100 mg kg^{-1} across all tissues, with the Limpopo nut lower-portion (FNLP) recording the highest value at 100 mg/kg^{-1} and the Mpumalanga nut mid-portion (FNMP) close behind at 94 mg/kg^{-1}. Flesh and pulp samples from both provinces (FFLP 94 mg/kg^{-1}; FFMP 95 mg/kg^{-1}; FPLP 93 mg/kg^{-1}; FPMP 93 mg kg^{-1}) showed no meaningful provincial differences. This uniform distribution means that whether people consume pulp, flesh, leaves, or nuts from either province, they receive a comparable amount of iron. Comparative studies show that *M. zeyheri* is richer in iron than many other members of its genus: *M. elengi* pulp contains roughly 0.6 mg 100 g^{-1} fresh weight (Kadam et al., 2012b), while *M. caffra* nuts (Mngadi et al., 2017a), though noted for their mineral density, do not exceed the levels reported here when adjusted to equivalent moisture bases. Iron is indispensable for haemoglobin synthesis, oxygen transport, and the functioning of numerous enzymes that utilise oxidative processes. Iron deficiency remains the most common micronutrient disorder worldwide, leading to iron-deficiency anaemia characterised by fatigue, impaired cognition, compromised immunity, and increased maternal and infant morbidity (Obeagu et al., 2025). The consistently high-iron content of *M. zeyheri* from both Limpopo and Mpumalanga, therefore, represents a significant nutritional advantage for populations in sub-Saharan Africa, where anaemia prevalence remains high.

7.3.2.4 Potassium

Potassium showed the greatest variability among elements, with clear provincial contrasts. The Limpopo pulp (FPLP) contained 210 mg/100 g^{-1}, while the Mpumalanga pulp (FPMP) reached 381 mg/100 g^{-1}. Fresh fruit fibre followed a similar pattern, with 318

mg/100 g^{-1} in Limpopo (FFLP) and 355 mg/100 g^{-1} in Mpumalanga (FFMP). The nuts were unequivocally the richest source, especially the Limpopo lower-portion nut (FNLP) at 495 mg/100 g^{-1}, though the Mpumalanga nut (FNMP) was also high at 421 mg/100 g^{-1}. These results indicate that Mpumalanga fruit tissues generally contained more potassium than those from Limpopo, whereas Limpopo nuts retained a slight advantage. Within the genus, *Mimusops kummel* fruit has been reported to contain approximately 760 mg/100 g^{-1} potassium (Mngadi et al., 2017a), which is even higher than the *M. zeyheri* nuts, highlighting the potassium-dense character of the genus. Potassium is vital for maintaining fluid balance, generating membrane potentials, and enabling normal neuromuscular and cardiac function. Inadequate potassium intake predisposes to hypokalemia, a condition that manifests as muscular weakness, arrhythmias, and glucose intolerance, and is implicated in the pathogenesis of hypertension (Yadav et al., 2024). The combination of high potassium and relatively low sodium in *M. zeyheri* nuts gives them a favourable sodium-to-potassium ratio, making them particularly suitable for managing blood pressure and reducing the risk of cardiovascular diseases.

7.3.2.5 Magnesium

Magnesium followed a pattern similar to that of calcium and potassium, with the highest concentrations in the nut and only slight differences across agroecologies. The Limpopo nut lower-portion (FNLP) recorded 142 mg/100g^{-1} and the Mpumalanga nut mid-portion (FNMP) 145 mg 100 g^{-1}, both higher than the fresh fruit flesh (FFLP 118 mg/100 g^{-1}; FFMP 122 mg/100 g^{-1}) and pulp (FPLP 117 mg/100 g^{-1}; FPMP 121 mg 100 g^{-1}). Other *Mimusops* species confirm this nut-rich pattern; *M. caffra* nuts contain magnesium concentrations in the same range (Mngadi et al., 2017a).

M. zeyheri leaves were analysed by independent studies contain about 0.06 % magnesium on a dry-matter basis. Magnesium is a cofactor for more than 300 enzymatic reactions, including those involved in energy metabolism, DNA and RNA synthesis, and regulation of ion channels (Mathew and Panonnummal, 2021). The magnesium-rich nuts of *M. zeyheri*, found in both Limpopo and Mpumalanga, can therefore play a crucial role in enhancing dietary magnesium intake in regions where cereals and refined grains predominate.

7.3.2.6 Sodium

Sodium concentrations were higher in the pulp and flesh and showed apparent provincial variation. The Limpopo lower-portion pulp (FPLP) reached 173 mg/100 g^{-1}, whereas the Mpumalanga mid-portion pulp (FPMP) was lower at 126 mg/100 g^{-1}. Fresh fruit flesh followed the same trend, with Limpopo (FFLP) at 168 mg/100 g^{-1} compared to Mpumalanga (FFMP) at 118 mg/100 g^{-1}. Nuts were lower in both provinces, particularly the Mpumalanga nuts (FNMP) at 85 mg/100 g^{-1}, while the Limpopo nuts (FNLP) contained 154 mg/100 g^{-1}. Compared with other *Mimusops* fruits such as *M. elengi*, which typically contain less than 10 mg/100 g^{-1} (Sayed et al., 2023), the pulp of *M. zeyheri* is relatively sodium-rich, although still well below the levels associated with adverse health effects when consumed as part of a balanced diet. The combination of low sodium and high potassium in *M. zeyheri* nuts provides a favourable electrolyte balance that supports blood pressure regulation and cardiovascular health.

7.3.2.7 Phosphorous

Phosphorus concentrations were most significant in the nut fractions, with the Limpopo lower-portion nut (FNLP) recording 128 mg/100 g^{-1} and the Mpumalanga mid-portion nut (FNMP) 111 mg/100 g^{-1}. Fresh fruit flesh and pulp contained far less: Limpopo flesh

(FFLP) 101 mg/100 g^{-1} and Mpumalanga flesh (FFMP) only 17 mg/100 g^{-1}, while the pulps contained 96 mg/100 g^{-1} (FPLP) and 110 mg/100 g^{-1} (FPMP). Nuts of *Ximenia caffra* contain even higher phosphorus levels, at around 345 mg/100 g^{-1} dry matter (Chivandi et al., 2012b), demonstrating that the genus consistently produces phosphorus-rich nuts. Phosphorus is a critical component of nucleic acids, phospholipids, and high-energy phosphate compounds such as ATP (Singh, 2019). Dietary deficiency, although rare, leads to hypophosphatemia, which manifests as bone pain, osteomalacia, muscle weakness, and, in severe cases, hemolysis and neurological dysfunction (Singh, 2019).

7.3.2.8 Zinc

Zinc concentrations were stable at about 94-95 mg/kg^{-1} across all tissues and both provinces, with no meaningful geographic differences between the Limpopo (FFLP, FPLP, FNLP) and Mpumalanga (FFMP, FPMP, FNMP) samples. Zinc is indispensable for more than 300 enzymes involved in DNA synthesis, immune response, and reproductive health (Bonaventura et al., 2015). Zinc deficiency remains a widespread issue, leading to impaired immunity, delayed wound healing, growth retardation in children, and loss of taste and smell (Hussain et al., 2022). The uniform zinc levels across *M. zeyheri* plant parts from both provinces mean that both the readily consumed pulp and the nuts can help close dietary zinc gaps.

7.3.2.9 β-Carotene

β-carotene was found only in the fleshy portions of the fruit. The highest level was observed in the mid-portion of fresh fruit flesh from Mpumalanga (FFMP), where it reached 2.16 mg/100 g^{-1}. Limpopo samples contained slightly less, with the fresh fruit flesh (FFLP) recording 1.67 mg/100 g^{-1} and the Limpopo pulp (FPLP) 1.50 mg/100 g^{-1}, while the Mpumalanga pulp (FPMP) showed a similar value of 1.50 mg/100 g^{-1}.

Such a distribution reflects the well-known tendency of carotenoids to accumulate in the colored mesocarp, a pattern also reported in other members of the Sapotaceae. For instance, studies of *Mimusops elengi* describe a bright orange pulp that is likewise rich in provitamin A carotenoids (Gami and Parabia, 2010). β-Carotene functions as both a potent antioxidant and the primary dietary precursor of vitamin A, a nutrient essential for vision, the maintenance of epithelial tissues, and normal immune responses.

No β-carotene was detected in the nut tissues, which is consistent with their anatomical and metabolic characteristics. Carotenoids are synthesised and stored in chromoplasts, which are abundant in the pigmented mesocarp but are largely absent from nuts. The nuts consist mainly of storage lipids and proteins. They are enclosed by dense, non-photosynthetic tissues that contain very few plastids, conditions that do not support carotenoid biosynthesis or long-term stability (Smolikova and Medvedev, 2015). The thick nut coat and the minimal exposure of the nut to light further limit the formation and retention of these pigments (Kapoor et al., 2022). These structural and biochemical characteristics explain why *M. zeyheri* nuts lack detectable β-carotene even though the surrounding fruit flesh is relatively rich in this provitamin compound.

7.3.2.10 Vitamin C

Vitamin C is present in *Mimusops zeyheri* mainly in the fruit pulp and flesh, with concentrations of about 5.4 mg/100 g^{-1} in the Limpopo lower-portion flesh (FFLP), 6.4 mg/100 g^{-1} in the Mpumalanga mid-portion flesh (FFMP), 6.9 mg/100 g^{-1} in the Limpopo lower-portion pulp (FPLP), and 6.6 mg/100 g^{-1} in the Mpumalanga mid-portion pulp (FPMP). Although these levels are moderate compared with citrus fruits, they are

nutritionally essential, particularly for infants and children whose tissues are expanding rapidly. Vitamin C is indispensable for collagen formation because it acts as a cofactor for prolyl and lysyl hydroxylases, enzymes that stabilise the collagen triple helix (Boo, 2022). Collagen provides the structural framework for skin, cartilage, bone, and blood vessels. During periods of rapid skeletal growth, adequate vitamin C is essential for proper cartilage formation in growth plates, normal dentine development, and the structural integrity of blood vessels (Abdullah et al., 2018). When intake is inadequate, children are at risk of defective collagen synthesis, which manifests as bleeding gums, fragile capillaries, painful joints, and delayed wound healing, and in severe cases leads to scurvy and impaired skeletal development (Das et al., 2024; Gandhi et al., 2023).

Vitamin C also strengthens the immune system, an essential function while a child's defences are still maturing. It accumulates in white blood cells and enhances neutrophil migration, phagocytosis, and the oxidative burst that destroys invading microbes, thereby reducing the severity and duration of common infections such as colds and respiratory illnesses (Carr and Maggini, 2017; Selvamary et al., 2020). Vitamin C improves the absorption of non-heme iron by reducing ferric to ferrous iron in the intestine, a process that is critical in childhood when iron requirements are high for expanding blood volume and brain development (Wintergerst et al., 2006). Additionally, better Vitamin C status supports normal cognitive performance, attention, and psychomotor development, providing indirect but significant benefits for neurological maturation.

Table 7-3: Elemental composition of *M. zeyheri* fruit fibre, Pulp, nuts, and leaves from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni)

	Ca	Cu	Fe	K	Mg	Na	P	Zn	B-carotene	Vitamin C
FFLP	145.1±2.1 ^{b}	94.5±1.4 ^{a}	93.9±1.2^{a}	318±5 ^{e}	118.3±1.8 ^{b}	168.3±3.0^{a}	100.6±2.0^{b}	94.3±1.3^{a}	1.67± 0.08^{ab}	5.4 ^{b}
FFMP	152.1±2.0^{b}	94.4±1.3 ^{a}	95.1±1.1^{a}	355.3±6^{d}	121.6±2.0^{b}	117.7±2.5^{d}	17.2±0.6 ^{d}	94.2±1.2^{a}	2.16 ±0.10^{a}	6.4 ^{a}
FPLP	126.7±1.90^{d}	94.7±1.5 ^{a}	93.2±1.3^{a}	210.2±5 ^{f}	116.5±1.7 ^{b}	172.5±3.1^{a}	95.5±1.8 ^{c}	94.5±1.4^{a}	1.5 ±0.07^{b}	6.9 ^{a}
FPMP	135.6±2.2 ^{c}	94.5±1.4 ^{a}	93.2±1.2^{a}	380.7±7^{c}	120.9±1.9^{b}	126±2.6^{c}	109.7±2.2^{b}	94.3±1.3^{a}	1.5 ±0.07^{b}	6.6 ^{a}
FNLP	208.7 ±3.0^{a}	94.7±1.3 ^{a}	99.7±1.5^{a}	494.7±8^{a}	141.9±2.5^{a}	153.7±3.0^{b}	128.1±2.4^{a}	95.1±1.4^{a}	Undetected	Undetected
FNMP	214.0±3.2 ^{a}	94.9±1.2 ^{a}	94.3±1.4^{a}	421.3±7^{b}	144.9±2.6^{a}	84.5±2.0 ^{e}	111.3±2.1^{b}	94.9±1.3^{a}	Undetected	Undetected

All analyses are the means of triplicate measurements ± standard deviation. Significant differences among the samples were assessed using the Tukey test. Means that share a superscript letter within a column are not significantly different at p ≤ 0.05. ***Key:*** *FFLP (Fruit fibre Limpopo), FFMP (Fruit fibre Mpumalanga), FPLP (Fruit pulp Limpopo), FPMP (Fruit pulp Mpumalanga), FNLP (Fruit nuts Limpopo), FNMP (Fruit nuts Mpumalanga, LLP (Leaves Limpopo), LMP (Leaves Mpumalanga).*

7.3.2.11 Estimated daily intake (EDI) of elements across the M. zeyheri plant parts

The heat map (Figure 7.1) and radar chart (Figure 7.2) together demonstrate that the estimated daily intake (EDI) of essential minerals from *Mimusops zeyheri* can make a meaningful contribution to adult nutrient requirements when approximately 0.4kg of fresh fruit is consumed daily. Potassium is the most abundant element in the nuts samples (FNLP and FNMP), providing between 2.4 and 2.8mg/kg^{-1} body weight per day^{-1} for a 70 kg adult. This is roughly 170–200mg of potassium per serving. Although this is only around 4% of the World Health Organisation's recommended intake of 4,700mg per day^{1} (Table 7.1), it is a significant addition from a single wild-fruit portion. It becomes more valuable in rural diets where total potassium intake from other foods may be limited. Calcium from nuts ranges from 1.19 to 1.22mg/kg^{-1} body-weight day^{-1}, equivalent to about 80–85mg for a 70kg adult, or roughly 8% of the adult requirement of 1,000mg day^{-1}. While not a complete source on its own, this level is essential in areas where milk or fortified foods are scarce and helps lower the risk of bone-related disorders such as rickets and osteoporosis. Magnesium and phosphorus intakes, estimated at 0.67–0.83 mg/kg^{-1} body-weight day^{-1} and 0.10–0.73mg/kg^{-1} body-weight day^{-1}, respectively, provide about 5-10% of the adult daily needs (310–420mg for magnesium and 700mg for phosphorus), reinforcing their contribution to bone health and energy metabolism.

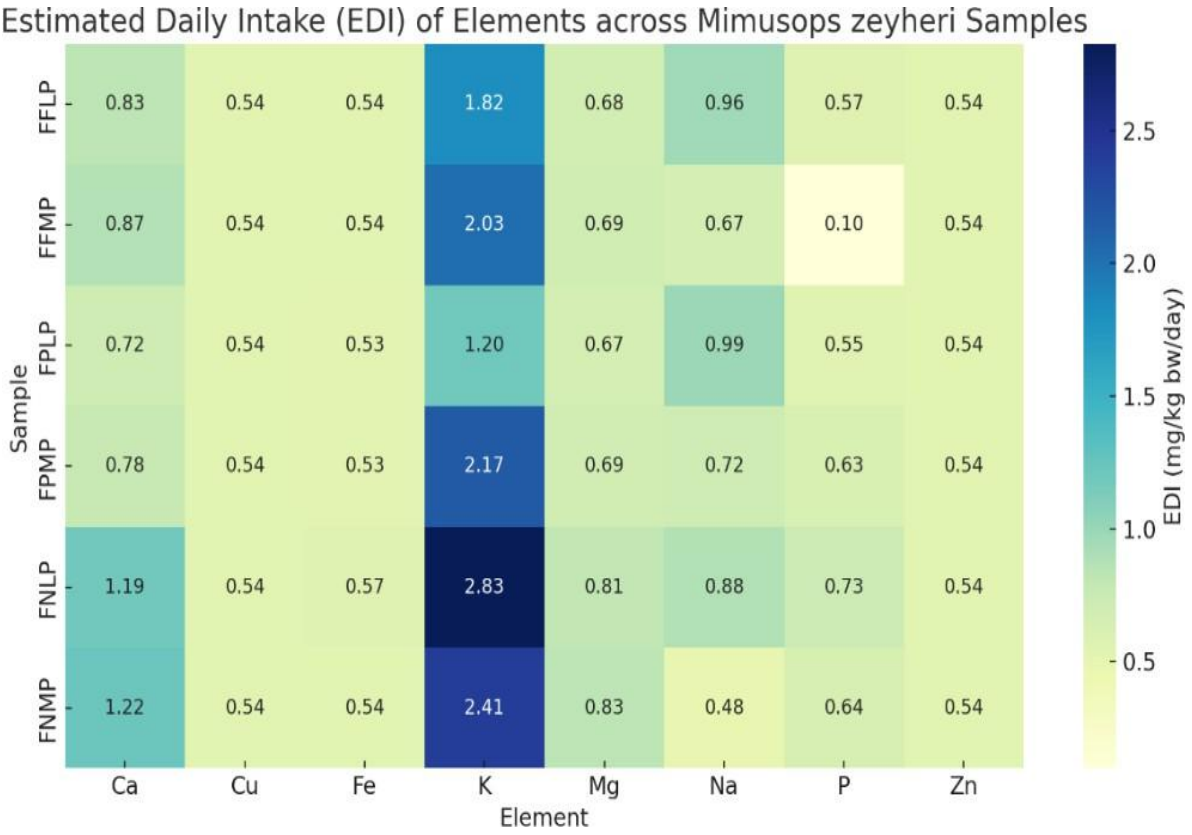


Figure 7-1: Heat map of EDI of elements from *M. zeyheri* samples from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni). Key: FFLP (Fruit fibre Limpopo), FFMP (Fruit fibre Mpumalanga), FPLP (Fruit pulp Limpopo), FPMP (Fruit pulp Mpumalanga), FNLP (Fruit nuts Limpopo), FNMP (Fruit nuts Mpumalanga, LLP (Leaves Limpopo), LMP (Leaves Mpumalanga)

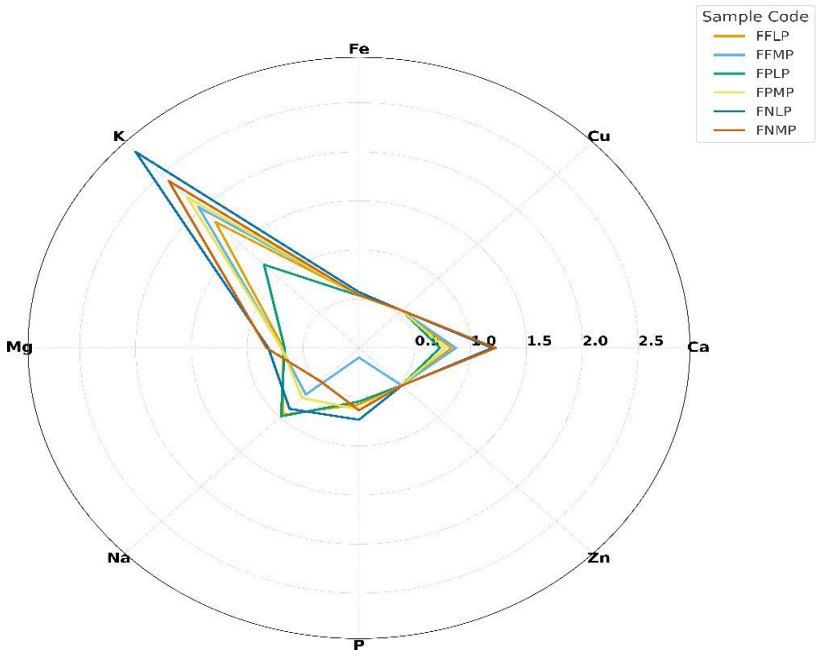


Figure 7-2: Radar chart of EDI of elements from *M. zeyheri* samples from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni). Key: FFLP (Fruit fibre Limpopo), FFMP (Fruit fibre Mpumalanga), FPLP (Fruit pulp Limpopo), FPMP (Fruit pulp Mpumalanga), FNLP (Fruit nuts Limpopo), FNMP (Fruit nuts Mpumalanga, LLP (Leaves Limpopo), LMP (Leaves Mpumalanga)

The radar chart highlights the distinctive nutrient pattern of each tissue type and province. Limpopo nuts exhibit a broader profile, driven by higher levels of calcium, potassium, and phosphorus. In contrast, Mpumalanga pulp extends more strongly on potassium and vitamin C. Copper, zinc and iron show consistent intakes across all samples, with EDI values close to 0.54mg/kg^{-1} body-weight day^{-1} for copper and zinc and about the same for iron for a 70kg adult these correspond to roughly 38mg of each element, far above the adult reference intakes of 0.9mg day^{-1} for copper, 8-18mg day^{-1} for iron and 8- 11mg day^{-1} for zinc (Table 7.1). This confirms that *M. zeyheri* can provide essential trace elements needed for enzyme function, immune support, and haemoglobin production. Vitamin C in the fruit flesh and pulp, averaging 5–7mg per 100 g fresh weight, contributes

about 12–20mg per 0.4kg serving, meeting roughly 15-25% of the adult requirement of 75–90mg day^{-1}. Vitamin C is a primary water-soluble antioxidant that scavenges free radicals and regenerates vitamin E, protecting DNA, proteins, and lipids from oxidative damage. This antioxidant protection contributes to healthy organ development and may lower the risk of chronic disease later in life. Although the vitamin C content of *M. zeyheri* pulp is modest, regular consumption of fresh fruit can help meet daily requirements. International guidelines recommend a daily intake of approximately 15-45mg for children, especially in rural communities where access to commercial fruit sources is limited (Kaur, 2023; Society, 2015). Because vitamin C is not stored in large amounts and is rapidly depleted during infections, seasonal inclusion of *M. zeyheri* fruit in children's diets offers a readily available way to support growth, bone and tooth development, iron utilisation, immune competence, and overall resistance to illness, complementing the mineral-rich nuts to provide a broad spectrum of essential nutrients. Taken together, these results demonstrate that *M. zeyheri* delivers a combination of macro- and micronutrients that compares favourably with other southern African wild fruits, such as *Vangueria infausta*, which generally supply lower levels of calcium and zinc. Regular intake of this fruit as food, therefore, has clear potential to improve dietary quality and reduce hidden hunger in communities where market access is limited, and poverty restricts access to fortified foods.

7.3.3 Correlation and significance

The correlation and significance analysis (Table 7.4) of the elemental and proximate composition of *M. zeyheri* revealed significant relationships. Proximate to proximate correlations revealed notable trends with moisture and dry matter, exhibiting an inverse

relationship (r=-0.99), which aligns with several studies that suggest that as the water fraction decreases, the proportion of solids necessarily increases, reflecting the physiological differences between the fruit tissues, such as the nut fractions, with very low moisture, reported to naturally exhibit the highest dry-matter content (Deng et al., 2019; Frenkel and Hartman, 2012). Dry matter showed a robust positive association with energy value and crude fat ($r>0.95$), a pattern driven by the nut samples (FNLP and FNMP), which contained approximately 94% dry matter and roughly 54g/100 g^{-1} crude fat. Protein content exhibited a strong positive correlation with both energy value and crude fat ($r>0.9$). In contrast, the mineral fraction (ash), which reflects total inorganic matter, showed only weak to moderate correlations with the other proximate constituents ($|r|<0.4$), indicating that overall mineral content varies largely independently of macronutrient composition.

The correlation between proximate content and mineral composition reveals notable patterns. Calcium, magnesium, and zinc displayed exceptionally strong positive correlations with dry matter, crude fat, protein, and caloric content ($|r|\approx0.95–0.99$, $p<0.01$) and, conversely, strong negative correlations with moisture ($|r|\approx0.94–0.99$). For instance, nuts from Limpopo contained approximately 209 mg Ca 100 g^{-1}, 142 mg mg/100 g^{-1}, and 95 mg Zn 100 g^{-1} while having only about 6% moisture. These findings support the well-established and documented dilution/concentration principle, whereby nutrient concentrations increase as water content decreases, and reflect the biochemical tendency of nuts to co-accumulate lipids, proteins, and divalent cations that stabilise membranes and enzymes during desiccation (Lozano, 2009; Samarah et al., 2004).

Potassium, copper, and iron displayed moderate positive relationships with nutrient-dense traits (r≈0.6–0.8). At the same time, sodium exhibited a weak positive association with moisture and negative correlations with the energy-rich variables consistent with sodium's greater mobility and linkage to water status rather than structural storage functions (Rengel et al., 2022).

Mineral-to-mineral correlations revealed a strong association among calcium, magnesium, and zinc, with pairwise correlations exceeding 0.9, indicating coordinated accumulation in the dry, energy-dense nut. Potassium exhibited a moderate correlation with these minerals (r≈0.6–0.7). Similarly, copper and iron exhibited moderate positive correlations with calcium and magnesium. In contrast, sodium correlated negatively with the Ca, Mg, Zn minerals (r≈ –0.4 to –0.5), reflecting its separate physiological pool and link to more hydrated pulp and fibre tissues.

The nutritional composition of *Mimusops zeyheri* exhibits a distinctly tissue-dependent pattern, whereby the nuts, independent of geographic origin, consistently display elevated dry-matter content, mineral abundance, and energy density. At the same time, the pulp and fibre remain markedly higher in moisture and comparatively deficient in minerals. This dominant influence of plant part rather than location accords with findings for other members of the Sapotaceae and related indigenous fruit species. For instance, studies on *Chrysophyllum albidum* (African star apple) and *Manilkara zapota* (sapodilla) report a similar inverse relationship between moisture and nutrient concentration, with nuts and nuts containing substantially greater lipid, protein, and mineral levels than either pulp or peel (Adepoju, 2009; Akinmoladun et al., 2020; Asare et al., 2015). Parallel trends have been documented in *Pouteria campechiana* (Do et al., 2023) and *Vitellaria paradoxa*

(Akoma et al., 2018), where nuts concentrate calcium, magnesium, and zinc, as well as high energy and fat content. In contrast, the fleshy mesocarp remains water-rich and nutritionally dilute. Therefore, the observed accumulation of calcium, magnesium, and zinc with dry matter and protein content in *M. zeyheri* reflects a broader physiological mechanism within Sapotaceae fruits in which desiccated nut tissues function as reservoirs of both macronutrients and divalent cations.

Table 7-4: Correlation coefficient (r) and significance (p) between the elemental composition and proximate components of *Mimusops zeyheri* fruit fibre, fruit pulp, and nuts (n=6) from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni)

	Moisture	Dry Matter	Ash	Crude Fat	Crude Fibre	Protein	Carbohydrates	Energy Value
Ca r	**-0.979***	**0.979***	-0.118	**0.976***	**0.988***	**0.977***	**0.966***	**0.976***
P	0.0006	0.0006	0.8237	0.0008	0.0002	0.0008	0.0018	0.0008
Cu r	-0.758	0.758	-0.597	0.0768	0.617	0.755	0.771	0.766
P	0.0806	0.0806	0.20107	0.0744	0.1918	0.0825	0.0727	0.0754
Fe r	-0.672	0.672	-0.0016	0.665	0.702	0.682	0.660	0.667
p	0.144	0.144	0.9759	0.1498	0.1196	0.1357	0.1538	0.148
K r	-0.771	0.771	-0.057	0.763	0.791	0.772	0.771	0.765
P	0.0729	0.0729	0.09147	0.0775	0.0609	0.0720	0.0726	0.0764
Mg r	**-0.988***	**0.988***	-0.272	**0.987***	**0.951**	**0.986***	**0.988***	**0.987***
P	0.0002	0.0002	0.6016	0.0002	0.0035	0.0003	0.0002	0.0002
Na r	0.414	-0.414	0.014	-0.414	-0.410	-0.404	-0.431	-0.413
P	0.414	0.414	0.978	0.415	0.419	0.427	0.393	0.416
P r	-0.498	0.498	-0.647	0.505	0.351	0.501	0.514	0.506
p	0.314	0.314	0.165	0.307	0.495	0.311	0.296	0.306
Zn r	**-0.942**	**0.942**	-0.494	**0.945**	0.844	**0.946**	**0.948**	**0.946**
p	0.005	0.005	0.320	0.004	0.035	0.004	0.004	0.004

Sample types: Fruit fibre, Fruit Pulp, Nuts (N=6 to provinces). Elements Ca= Calcium, Cu=Copper, Fe=Iron, Mg=Magnesium,= Na= Sodium, P=Phosphorus, Zn=Zinc: Stars indicate significance: p<0.05, p<0.01**, p<0.001***: Bold indicates Very strong correlation: |r|≥ 0.94.*

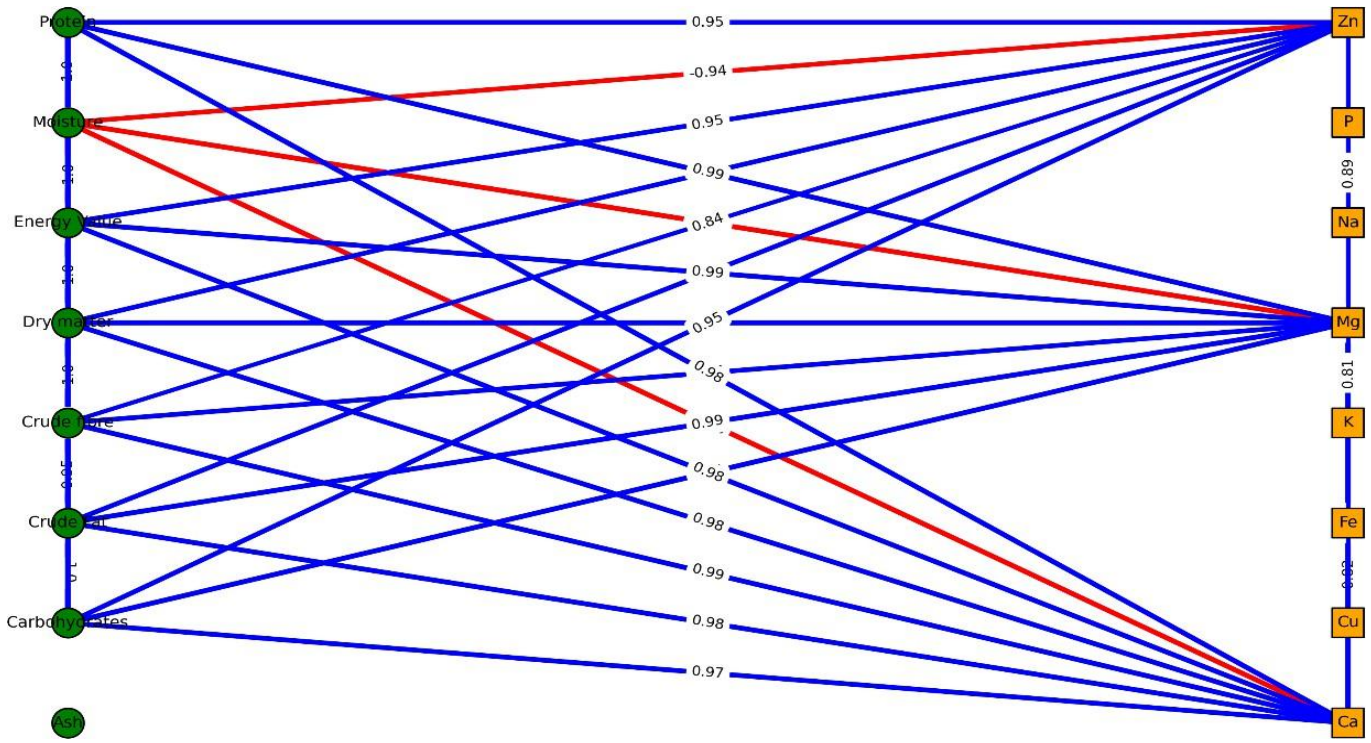


Figure 7-3: Correlation network of the significant associations (|r| ≥ 0.8; p<0.05) among proximate constituents and mineral elements of *M. zeyheri* fruits. Proximate variables are shown as green circles and minerals as orange squares; blue lines denote positive correlations and red lines negative correlations, with edge thickness proportional to the strength of the relationship

Figure 7.3 presents a visual summary of the significant correlations listed in Table 7.3. A compact group of proximate parameters, including dry matter, crude fat, protein, and energy value, exhibits strong positive correlations with calcium (Ca), magnesium (Mg), and zinc (Zn), closely matching the high coefficients observed in the numerical analysis. Moisture is linked to this group only through negative correlations, reflecting its clear inverse association with these energy and mineral-rich traits. Sodium (Na) and phosphorus (P) display only a few weak connections, and ash remains completely unconnected, indicating their limited roles in the nutrient-dense cluster. The figure complements the correlation table by illustrating how the nut tissues of *M. zeyheri* form a distinct high-energy, high-mineral core. In contrast, the pulp and fibre components occupy the network's outer margins.

7.4 Concluding remarks

The findings of this chapter demonstrate that *M. zeyheri* provides an unusually balanced source of essential nutrients that can significantly enhance everyday diets in southern Africa. Across both Limpopo and Mpumalanga, the nuts stand out as dense stores of protein, energy, and key minerals such as calcium, magnesium, zinc, and potassium. These nutrients are required for bone formation, immune function, and expected growth, yet they are often lacking in rural households where access to fortified foods and animal protein is limited. The leaves provide additional value, with high levels of protein and dietary fibre. Furthermore, the fruit pulp provides natural sugars, vitamin C, and β-carotene, which support iron absorption, promote healthy skin, and enhance resistance to infection. Because these nutrient levels remain consistent across different agro-ecological zones, the tree is reliable even under climate variability and drought

common in sub-Saharan Africa. Regular consumption of *M. zeyheri* nuts, leaves, and fresh fruit can help families meet a significant portion of their daily requirements for calcium, iron, zinc, and vitamin C, directly addressing the "*hidden hunger*" of micronutrient deficiency. By strengthening food security, reducing reliance on purchased staples, and supporting local livelihoods, *M. zeyheri* aligns closely with Sustainable Development Goals 2 (Zero Hunger) and 1 (No Poverty). Promoting its conservation, domestication, and integration into community food systems, therefore, represents a practical step towards resilient diets and poverty reduction in rural South Africa.

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CHAPTER EIGHT

STUDY LIMITATIONS, RECOMMENDATIONS AND CONCLUSIONS

This study makes a substantial contribution to both academia and society by providing the first comprehensive, multi-dimensional investigation of *Mimusops zeyheri* that spans ethnobotany, ecology, morphology, sensory science, genetics, water use and nutrition. Academically, the study fills critical knowledge gaps in indigenous fruit tree research by generating novel baseline data on population structure, genetic diversity, water use and nutritional profiles, areas that have been previously underexplored, despite the species' widespread traditional use. The integration of scientific analyses with community knowledge strengthens interdisciplinary scholarship and demonstrates how ethnobotanical insights can be validated and extended through laboratory and field-based methods. Methodologically, the thesis adopts a holistic approach that can be replicated for other neglected and underutilised species across Africa, thereby establishing a valuable framework for future researchers.

Societally, this study contributes to the preservation of indigenous knowledge systems at a time when they are threatened by land-use change, cultural shifts, and biodiversity loss. By documenting traditional medicinal and cultural uses, identifying threats to wild populations, and demonstrating the species' nutritional and phytochemical potential, the research provides evidence-based pathways for community empowerment, food security initiatives, and biodiversity-driven livelihood development. The findings demonstrate that *M. zeyheri* is not only culturally significant but also a scientifically validated resource with potential for rural enterprise development, improved nutrition, and innovative natural

product development. Through these contributions, the project advances national priorities in conservation, indigenous food systems, bioeconomic development, and the promotion of African-led scientific research.

8.1 Conclusion

This study presents the most comprehensive multidimensional assessment of *Mimusops zeyheri* to date, integrating ethnobotanical knowledge, ecological analysis, morphological and genetic characterisation, water use efficiency using sap flows and nutritional profiling. Together, the findings reveal *M. zeyheri* as a culturally valued, nutritionally important, and chemically rich indigenous fruit tree with significant potential for conservation, domestication, and rural economic development. Ethnobotanical surveys across Limpopo and Mpumalanga show that local communities rely on *M. zeyheri* for a wide range of medicinal, nutritional, cultural, and spiritual uses. Its high citation and fidelity levels for treating skin ailments, sexually transmitted infections, and other conditions reflect longstanding traditional knowledge systems and demonstrate the continued relevance of the species in primary healthcare. However, participants also identified land transformation in Mpumalanga and overharvesting in Limpopo as key threats, indicating that the species faces uneven human pressure and requires targeted conservation support. Ecological assessments further reveal regionally distinct population structures and spatial patterns: dispersed trees in Vhembe linked to wind dispersal, and clustered trees in Ehlanzeni driven by zoochory. High disturbance levels in Vhembe contrast with the relatively intact stands in Mpumalanga. Although regeneration is present in both sites, the dominance of smaller size classes suggests recruitment bottlenecks likely tied to harvesting pressures. These results highlight that *M. zeyheri* is ecologically resilient but

vulnerable to human disturbance, underscoring the need for proactive conservation and domestication strategies.

Morphological and sensory analyses show pronounced regional variation. Mpumalanga fruits and nuts tend to be larger, yet Limpopo fruits are consistently preferred for taste, aroma, and overall acceptability. Genetic analyses confirm the existence of two distinct lineages with unique haplotypes, demonstrating that environmental gradients and historical divergence have shaped population differentiation. These findings are valuable for future breeding, propagation, and domestication initiatives. Sap flow measurements showed the importance of considering diurnal and seasonal weather variability when interpreting sap flow and transpiration rates in indigenous fruit trees. Although this study forms part of valuable baseline information on the water-use behaviour of *Mimusops zeyheri*, it can be used as a foundation for future research to manage and promote underutilised trees and possible integration into water-efficient agroforestry systems. Nutritional profiling demonstrates that *M. zeyheri* is an exceptional food resource. Nuts are highly energy dense (~595 kcal/100 g), rich in fat (≈54%), protein (≈19%), and essential minerals such as calcium (≈210 mg/100 g) and magnesium (≈143 mg/100 g). Leaves provide high protein (~24%) and fibre (~22%), while fruit pulp contains modest vitamin C (5–7 mg/100 g) and β-carotene (~1.5–2.1 mg/100 g). These attributes position *M. zeyheri* as a nutritious, climate-resilient species that can support dietary diversification and micronutrient security in rural households. Overall, the project demonstrates that *M. zeyheri* is not only a culturally and medicinally important resource but also a nutritionally rich and chemically diverse species with strong potential for value addition, product development, and livelihood enhancement if sustainably managed.

8.2 Study limitations

While this study offers a comprehensive and multi-layered assessment of *Mimusops zeyheri*, certain boundaries were inherent to the scope and design of the research. First, the ecological and ethnobotanical assessments were conducted in two selected provinces because they represent distinct ecological zones and culturally diverse communities where the species is abundant. These sites provided sufficient variation to meet the research objectives, although they do not represent every region where *M. zeyheri* occurs. Second, the genetic analysis employed widely accepted chloroplast markers that are standard in plant phylogeography and adequately resolved population differentiation for this study, even though additional nuclear markers may offer finer detail in future work. Third, sensory evaluation focused on adult community members familiar with the fruit, ensuring culturally meaningful responses. Although future studies may broaden demographic representation as part of product development initiatives, this approach ensured culturally relevant responses. Fourth, the project had an objective on the propagation strategies of *M. zeyheri*. However, during the research, seed propagation was limited by low and inconsistent germination rates, commonly associated with hard seed coats, possible dormancy, and variability in seed viability. Furthermore, the availability of mature, viable seeds was seasonal, restricting continuous propagation efforts. Fifth, the propagation using cuttings also proved difficult, as *M. zeyheri* showed low-rooting success under standard glasshouse conditions. Cuttings were slow to develop roots and, in many instances, failed to establish successfully, even when treated with rooting hormones. These limitations suggest that conventional seed and cutting propagation methods may be insufficient for the efficient domestication of *M. zeyheri*.

Future work should therefore explore alternative or improved propagation approaches, such as refined pre-treatment techniques, grafting, or tissue culture, to support sustainable cultivation and conservation of the species. These limitations do not undermine the validity of the findings. Instead, they define the study boundaries and provide direction for future research that may extend this work into broader ecological regions, molecular datasets, consumer groups, and seasonal cycles.

8.3 Future research

8.3.1 Spatial mapping using GIS

There is a need to develop high-resolution distribution maps to assess habitat suitability, fragmentation patterns, and population connectivity. Given the documented regional differences in population structure and disturbance levels, spatial modelling would support targeted conservation planning.

8.3.2 Propagation and domestication studies

Future research needs to focus on investigating nut germination, vegetative propagation, and nursery establishment techniques. The study reveals clear morphological and genetic variation between provinces, highlighting the importance of selecting suitable germplasm for cultivation to promote a wider distribution of the plants.

8.3.3 Biological assays and compound isolation

Given the notable phytochemistry of *M. zeyheri*, there is a need to conduct antimicrobial, anti-inflammatory, and antioxidant assays using purified compounds. The presence of quercetin derivatives, β-glucogallin, proanthocyanidins, and related metabolites provides a scientifically grounded basis for pharmacological exploration.

8.3.4 Development of community-based value-added products

Future studies should explore the commercial potential of nutraceutical, medicinal, and dietary products. For example, the fruit pulp's vitamin C content (5–7 mg/100 g) and β-carotene levels support its use in functional foods, while the nutrient- dense nuts (~595 kcal/100 g) offer potential for high-energy snacks or fortified products. Community involvement will ensure culturally appropriate and economically equitable benefit-sharing.

8.3.5 Long-term ecological monitoring

There is a need to establish permanent plots to track regeneration, mortality, and the effects of harvesting pressure, particularly in highly disturbed areas such as Vhembe.

8.3.6 Expanded sensory and consumer studies

There is a need to evaluate preferences across diverse demographic groups to guide cultivar selection and future product development.

8.4 Policy and conservation recommendations

The findings of this study highlight the need for coordinated policy action to ensure the sustainable use, conservation, and development of *Mimusops zeyheri* as an indigenous resource of ecological, nutritional, medicinal, and economic importance. Policies should prioritise the integration of *M. zeyheri* into national and provincial biodiversity conservation strategies, particularly in regions such as Vhembe, where high disturbance and regeneration bottlenecks were observed. Sustainable harvesting guidelines must be developed in collaboration with local communities to protect vulnerable size classes and ensure long-term population stability. Given the species' nutritional value and phytochemical richness, policymakers should support the inclusion of *M. zeyheri* in agroforestry and climate-resilient agricultural programmes, promoting its domestication and cultivation as part of food security interventions. Furthermore, policies must recognise and protect indigenous knowledge associated with the species, ensuring that community custodianship is upheld through benefit-sharing frameworks and fair intellectual property mechanisms. Support for emerging community enterprises that process *M. zeyheri* into value-added medicinal, nutraceutical, and dietary products will promote rural economic development while encouraging biodiversity-based livelihoods.

APPENDIX 1: *Mimusops zeyheri* sond (milkwood) fruit sensory evaluation form

Panellist Name: _____ **Date:** _____
Sample Code: _____ **Location:** _____

Instructions: Please evaluate each attribute carefully and provide your ratings and comments based on your sensory perceptions of the fruit sample provided.

Attribute	Description	Rating Scale	Panellist Comments
Appearance	Colour uniformity, brightness, visual appeal, blemishes	1 (Very Poor) – 9 (Excellent)	
Ripeness	Maturity, readiness for consumption	1 (Very Poor) – 9 (Excellent)	
Aroma	Fruitiness, freshness, presence of off odours	1 (Very Poor) – 9 (Excellent)	
Taste	Sweetness, tartness, bitterness, overall flavour balance	1 (Very Poor) – 9 (Excellent)	
Texture	Juiciness, firmness, smoothness, fibrousness	1 (Very Poor) – 9 (Excellent)	
Mouthfeel	Smoothness, astringency, stickiness, aftertaste	1 (Very Poor) – 9 (Excellent)	
Nuttiness	Quantity and size of nuts, ease of consumption	1 (Very Poor) – 9 (Excellent)	
Overall Acceptability	Overall liking and preference of the fruit	1 (Dislike Extremely) – 9 (Like Extremely)	

Scale Key (G-Point Hedonic Scale): Circle around
1 – Dislike Extremely 2 – Dislike Very Much
3 – Dislike Moderately 4 – Dislike Slightly 5 – Neither Like nor Dislike 6 – Like Slightly
7 – Like Moderately; 8 – Like Very Much 9 – Like Extreme