Milkwood (*Mimusops zeyheri* Sond.) in South Africa: Diversity, Traditional Knowledge, and Sustainable Utilisation

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DEDICATION

My ancestors walked so I could shine! As the first in my lineage to break what once was a generational curse, I stand as a fulfillment of dreams once deferred~ My ancestors' wildest dreams made manifest. Twaas to them!!!

I dedicate this excellent work to the love of my life and the most amazing woman to have walked the streets of this world, my late mother, Victoria Dorries Mkhonto.

To baby Phenyo, “You were here, you were loved, and I carry you always.” This is for you and the immeasurable strength your beautiful soul gave me daily.

DECLARATION

Considering my familiarity and understanding of the university policy on plagiarism, I declare that the work documented in this thesis is my original independent work and has not been submitted either in part or whole for any degree at any academic institution other than the University of Mpumalanga. All other sources used in this thesis have been duly cited, and the list of references appears accordingly.

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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, nutritional value, and phytochemical properties remains limited. This thesis provides an integrated multi-disciplinary assessment of *M. zeyheri* across two regions, Vhembe in the Limpopo province and Ehlanzeni in the Mpumalanga province using ethnobotanical, ecological, morphological, sensory, nutritional, and chemical approaches. An ethnobotanical survey was conducted to collect data from 116 participants over the age of 18 through semi-structured random face-to-face interviews. Ethnobotanical indices, such as Relative Frequency of Citation (RFC) and Fidelity Level (FL), were used for data analysis. The results demonstrate the multifaceted use of *M. zeyheri* by different ethnic groups to treat various ailments and conditions. A total of 16 other uses of *M. zeyheri* were reported in Limpopo, and 27 reported in Mpumalanga. The RFC values ranged from 0.29 to 1 in Limpopo and from 0.03 to 1 in Mpumalanga. Fruits eaten whole as a snack received an FL value of 100% in both provinces, as recorded from all participants. In Limpopo, the ethnomedicinal use of *M. zeyheri* to treat sexually transmitted infections received an FRC value of 1, recorded from all participants (FL = 100%), with efficacy reported when the species is mixed with other plant species, such as *Aloe vera*, and boiled to form a decoction that is taken orally. Similar use of *M. zeyheri* for treating sexually transmitted infections was also reported (RFC=0.50) in Mpumalanga, highlighting similar use of the same species by different ethnic groups. Other commonly reported uses include the use of *M. zeyheri* for treating skin ailments and conditions, such as ringworm (RFC = 0.57) in Mpumalanga and (RFC = 1) in Limpopo. In Mpumalanga, the use of *M. zeyheri* extends to spiritual and cultural practices, such as using the leaves to remove bad omens (RFC = 0.100) and to collect the spirit of the deceased from where they last took their breath (RFC = 0.05). Different plant parts are utilized in both provinces, with Limpopo having a slightly higher preference for roots (17%), while Mpumalanga has a slightly higher preference for nuts (17%). Both study sites share a similar preference for the use of leaves (22%) and a strong preference for decoction in preparing ethnomedicine (36% and 34%) for Limpopo and Mpumalanga, respectively. Internal and external applications were cited by participants in the study, with oral administration being the most preferred mode of delivery in both Limpopo (75%) and Mpumalanga (55%). Participants in Mpumalanga (40%) identified land expansion and infrastructure development as perceived major threats to the *M. zeyheri* population, while

overharvesting (30%) was identified as a significant concern in Limpopo. These findings are based on community perceptions and are interpreted alongside ecological observations, including size-class distribution patterns, which indicate both active recruitment and potential limitations in the progression of individuals into mature stages. The population dynamics, spatial distribution, tree size variation, and ecological condition of *M. zeyheri* in two distinct regions, the Ehlanzeni District (Mpumalanga) and the Vhembe District (Limpopo), were assessed to understand the species ecology and use patterns. Data collection focused on size-class distribution, with statistical analyses applied to assess population structure and variation using ecological modelling and scoring indices. 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 = 2.68 m), 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 characterization 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 ± 0.61 mm and widths of 24.14 ± 0.55 mm, compared to Limpopo fruits measuring 27.41 ± 0.47 mm in length and 23.22 ± 0.36 mm in width. Nut size followed the same pattern, averaging 2.11 ± 0.36 cm in Mpumalanga and 1.81 ± 0.31 cm in Limpopo. By contrast, leaf morphology remained stable across regions, with overlapping mean leaf lengths (7.23–7.52 cm), widths (3.93–4.03 cm), and leaf areas (21.69–22.80 cm^2). 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, which exhibited 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 nutritional composition of *M. zeyheri* plant parts was determined using standard AOAC methods and elemental analysis by microwave-assisted digestion followed by ICP-OES. Data were analyzed 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. *Mimusops zeyheri* offers a unique combination of energy-dense nuts, protein, mineral-rich leaves, and vitamin-containing pulp, making it a valuable food resource for improving dietary diversity and combating micronutrient deficiencies in rural South Africa. Its climate resilience is suggested by its ability to persist across contrasting agro-ecological zones, including semi-arid and low-input environments, as observed in this study. Lastly, the total phenolics, flavonoids, and alkaloids were quantified using spectrophotometry techniques, and the antioxidant activities using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and Ferric Reducing Antioxidant Power assay. Untargeted secondary metabolites from the different plant parts of *M. zeyheri*, collected in various Agro-ecological zones, were identified using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-QT-MS/MS). Leaves recorded significantly higher phenolic content (19.13 mg GAE g^{-1} in Limpopo; 18.20 mg GAE g^{-1} in Mpumalanga) than nuts (≈14 mg GAE g^{-1}), pulp (≈8 mg GAE g^{-1}), and fibre (≈6 mg GAE g^{-1}). Similar to phenolic content, the total flavonoid content (TFC) of *Mimusops zeyheri* followed a clear pattern across the different plant parts. Leaves exhibit the highest concentration of TFC. .83 ± 0.25 µg QE g^{-1} in Limpopo and 9.30 ± 0.20 µg QE g^{-1} in Mpumalanga. Nuts followed (6.23 ± 0.25 and 5.80 ± 0.15 µg QE g^{-1}), while pulp (around 4 µg QE g^{-1}), with the lowest TFC observed in fruit fibre (around 3 µg QE g^{-1} in both Limpopo and Mpumalanga). Differences in TFC between the WTO provinces are small, emphasizing plant tissue as the main driver of concentration rather than the location where the plant grows. Alkaloid content was again

highest in leaves: 4.67 ± 0.15 mg AE g^{-1} (LLP) and 4.37 ± 0.15 mg GAE g^{-1} (LMP). Nuts followed (3.33–3.10 mg AE g^{-1}), then pulp (~2 mg AE g^{-1}) and fibre (~1.5 mg AE g^{-1}). The pattern mirrors that of phenolics and flavonoids, reflecting the defensive role of alkaloids against herbivores and pathogens, which is strongest in leaves and reproductive tissues. Antioxidant activity differed between plant parts and provinces. Mpumalanga nuts showed the strongest radical-scavenging capacity in the DPPH assay (EC_{50} 0.0581 µg/mL) compared with Limpopo nuts (EC_{50} 0.1767 µg/mL) and much lower than the positive control. Fibre and pulp extracts were more effective in the β-carotene–linoleic acid system, with Mpumalanga fibre showing the lowest EC_{50} (18.24 µg/mL) and pulp extracts ranging between 64.10–77.43 µg/mL. Leaf extracts showed moderate activity in both assays. The heatmap indicated clear tissue- and site-specific patterns, with Mpumalanga samples generally outperforming those from Limpopo. Fruit fibre from Mpumalanga is rich in flavonoids with dominant compounds such as quercetin-3-O-glucoside (Rt 7.82 min, 463.12 m/z) with congeners identified as isoquercitrin (Rt 5.81 min, 463.09 m/z) and rutin (Rt 4.62 min, 609.14 m/z) known for their distinct structural and biological properties while *M. zeyheri* fruit fibre from Limpopo has notable phenolic glycosides such as β-glucogallin (Rt 1.08 min, 367.05 m/z). β-glucogallin has been identified as having potent antioxidant and antimicrobial properties, which also support good gut health function in humans and aid in the preservation of food. *M. zeyheri* fruit pulp from Mpumalanga has a notable array of various flavanol glycosides and nutrient compounds that together create a flavorful and health-promoting profile of tentatively identified compounds. By contrast, the Limpopo pulp is dominated by hydrolysable tannins and phenolic glycosides, reflecting a more therapeutic, preservative-leaning profile. The Mpumalanga nut profile is flavonoid rich with notable quercetin–catechin profile and compounds such as Free quercetin (Rt 5.75 min, 301.03 m/z) alongside its glycosides-isoquercitrin (quercetin-3-O-glucoside; Rt 5.81 min, 463.09 m/z) and a rutin isomer (quercetin-3-O-rutinoside; Rt 5.90 min, 609.14 m/z) known for antioxidant and anti-inflammatory activity relevant to vascular protection, insulin sensitivity and neuroprotection. By contrast, the Limpopo nuts present a tannin-dense fingerprint with higher oligomerization and a striking acylated anthocyanin. A hallmark proanthocyanidin trimer, specifically gallocatechin-(4α→8)-gallocatechin-(4α→8)-gallocatechin (Rt 3.02 min, 913.19 m/z), together with a dimeric feature at 577.14 m/z (identified as endotelon), indicates an extensive condensed-tannin spectrum. In contrast, leaves from Limpopo are characterized by high levels of galloyl phenolics and oligomeric proanthocyanidins (OPCs), associated with vascular and antioxidant benefits, with compounds

such as Glucosyringic acid (Rt 3.08 min, 359.10 m/z) previously reported for its antioxidant and anti-inflammatory effects. This study presents the most comprehensive multidimensional assessment of *Mimusops zeyheri* to date, integrating ethnobotanical knowledge, ecological analysis, morphological and genetic characterization, nutritional profiling, and phytochemical screening. 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. The clear regional structuring observed across ecological, genetic, nutritional, and phytochemical dimensions underscores the importance of context-specific management and utilization strategies. Accordingly, the study recommends the integration of *M. zeyheri* into local conservation and agroforestry programmes, the protection of genetically distinct populations and rare haplotypes, and the promotion of sustainable harvesting practices to reduce pressure from overexploitation and land-use change. 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, and SDG 13 (Climate Action) by highlighting the role of resilient indigenous tree species in supporting adaptation strategies under increasing climate variability. 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. Collectively, these measures will support the sustainable conservation, responsible commercialization, and policy integration of *M. zeyheri* as a climate-resilient indigenous food and medicinal resource.

Keywords: Antioxidants, Biotechnological applications, β-carotene, Chloroplast markers, Culture, Dietary diversity, Domestication potential, Ecological roles, Ethnobotanical, Ethnomedicine, Ethnomedicinal, Genetic diversity, Haplotypes, Indigenous Knowledge, Minerals, Mimusops zeyheri, Morphology, Nutrients, Nutrition, Phytochemical profiling, Proximate analysis, Regional variation, Secondary metabolites, Sensory traits, Tissue-specific variation, Traditional, Therapeutic

CHAPTER ONE
GENERAL INTRODUCTION

1.1 Background

The global population is projected to exceed 9 billion by 2050, with Africa expected to reach approximately 2.5 billion by the same year, while south Africa is projected to reach between 68-70 million. This implies that global food production will need to increase by 70 to 100% (Gunders and Bloom, 2017). In recent years, there has been a global increase in the number of people suffering from hunger, with climate change and variability being major contributors to this trend (Dawson et al., 2016; Lucatello and Sánchez, 2022). The highest Global Hunger Index (GHI) are reported in sub-Saharan Africa and Asia (Ruel-Bergeron et al., 2015). In Africa alone, approximately 20% of the population is chronically undernourished (in terms of dietary and energy supply), making the continent a key and alarming region with a high prevalence of hunger and malnutrition (Mayer and Anderson, 2020).

Another significant aspect of food insecurity is what is known as hidden hunger. This occurs when individuals lack micronutrients, impacting over 2 billion people (Fortin et al., 2021). Hidden hunger can have consequences, including driving people critically ill or even causing death. Sub-Saharan Africa has reported the lowest intake of fruits and vegetables in the world (Hodge, 2016). The lack of fruit intake has been associated with the inability and financial constraints to afford the available commercially sold fruits (Bvenura and Sivakumar, 2017). This problem continues to exist in areas where people primarily rely on a range of monotonous foods, mostly made up of starchy staples and a small selection of legumes (Titcomb and Tanumihardjo, 2019). This imbalanced and poor dietary pattern contributes to high disease rates and mortality.

Sub-Saharan Africa boasts a rich diversity of underutilized indigenous fruit species, which can play a pivotal role in addressing nutrition issues and in medicinal applications for decades. Incorporating underutilized indigenous fruits, which make significant contributions to the food systems of the sub-Saharan African regions, can be a crucial intervention approach to the cropping systems. The potential health benefits associated with underutilized indigenous trees may warrant the development of recipes for use in nutrition interventions and increasing their consumption to combat food security (Snee et al., 2011).

A comprehensive understanding of the distribution, abundance and inclusion of underutilized indigenous fruit trees calls for knowledge generation on their cultivation for sustainable supply. Gaps in knowledge concerning agronomic improvement technologies, such as propagation strategies and nutritional composition, should be addressed to promote and expand their use in cropping systems. The reported benefits are attributed to *Mimusops zeyheri*, a perennial fruit tree and a member of the family *Sapotaceae,* with the potential to alleviate malnutrition, which can be explored and harnessed to improve food security in Africa. Milkwood (*Mimusops zeyheri*) can be used as an integral plant in combating food security challenges and malnutrition, and therefore, there is a need to promote its consumption. The nut meal of *M. zeyheri* has a superior nutrient composition compared to maize meal and can serve as a valuable dietary energy source (Chivandi et al., 2020). Initiatives and innovations, including the incorporation of *M. zeyheri* nut meal into diets, can help reduce micronutrient deficiencies. These solutions hold the potential to meet nutritional requirements for normal growth and disease prevention, contributing to improved food security and nutrition.

The conservation of *M. zeyheri* is becoming increasingly critical due to various challenges, such as habitat loss driven by urbanization and infrastructure development, which poses a significant threat to this species (Mabhaudhi et al., 2017). Additionally, over-harvesting for medicinal and cultural purposes, without sustainable management practices, has led to population decline (Omotayo et al., 2020). Climate change-induced shifts in habitat suitability further compound the challenges faced by this species. Addressing these sustainability challenges requires a multifaceted approach that combines ecological research, community engagement, and conservation efforts.

Understanding its diversity, preserving traditional knowledge, and ensuring its sustainable use are essential components of its conservation. This study aimed to contribute valuable insights into documenting the distribution patterns and varieties of fruit trees in the Agro ecologies of two Provinces in South Africa (Mpumalanga and Limpopo), providing a foundational understanding of their ecological presence. Additionally, the study examined the traditional knowledge of *M. zeyheri* as a food source, considering cultural barriers and opportunities for adoption within communities. Furthermore, it examined the phytochemical composition and secondary metabolites of *M. zeyheri* across various Agro ecologies. As a holistic approach, the study aimed to foster the adoption of *M. zeyheri* among local farmers and communities through capacity-building and

outreach programs, promoting its ecological and economic benefits.1.2 Problem statement Despite the growing recognition of indigenous and underutilized fruit trees as important resources for food security, climate change adaptation and rural livelihoods, *Mimusops zeyheri* remains poorly researched and underutilized 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 utilization of indigenous fruit tree species. Despite increasing global and regional interest in underutilized crops, research on wild fruit trees remains 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.

Underutilized crops play an important role in maintaining biodiversity and offer valuable opportunities for adaptation of climate change, 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.

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 underutilized 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, 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 unrealized.

1.3 Significance of the study

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 health care. In South Africa, the forestry and tree-based sector contribute to national economic activity while also providing essential ecosystem services that support poverty reduction, food production, and household wellbeing. Indigenous fruit trees represent an important yet underutilized 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

unrealized due to limited scientific documentation and weak integration into formal food and agricultural systems.

The significance of the current study lies in its focus on documenting the distribution, abundance, and nutritional composition of the underutilized indigenous fruit tree *M. zeyheri*, thereby addressing key knowledge gaps that limit its broader utilization. Unlocking the nutritional and economic value of this species is particularly important in the context of 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.

Although the significance of this study is grounded on its contribution to food security, biodiversity conservation and rural livelihoods, it is worth noting the integral multidisciplinary of the study integrating ecological, ethnobotanical, nutritional, and phytochemical dimensions. These dimensions are underpinned by key concepts such as diversity, traditional knowledge, and sustainability, which require clear definition and contextualization to guide the research. Furthermore, the study employs a combination of qualitative and quantitative research methodologies to address its objectives in a comprehensive manner. Therefore, a conceptual and methodological framework is necessary to clarify these concepts, illustrate their interrelationships, and demonstrate how the different methodological approaches are integrated to achieve the overall aim of the study. This framework is presented in the subsequent section.

1.4. Conceptual and Methodological frameworks of the study

1.4.1. Overview of the Framework

This study adopts a multidisciplinary and integrative framework to examine *M. zeyheri* within the broader context of biodiversity, indigenous knowledge systems, and sustainable utilization. Given the complexity of the research problem and the diverse nature of the study objectives, it is necessary to clearly define the key concepts underpinning the research and to explain the methodological approach used to address them. The study is conceptually anchored on three interrelated pillars, namely diversity, traditional knowledge, and sustainability, which are examined through a combination of qualitative and quantitative research methodologies. Together, these elements form a coherent framework that guides the investigation and interpretation of findings.

1.4.2. The Concept of Diversity

Diversity, as applied in this study, is understood as a multidimensional concept that captures variation in *M. zeyheri* across ecological, genetic, morphological, and biochemical dimensions. Ecological diversity refers to the distribution, abundance, and population structure of the species across different Agro ecological zones, in this case the Ehlanzeni and Vhembe districts. This dimension provides insight into habitat suitability, regeneration patterns, and the effects of environmental and anthropogenic pressures on the species. Genetic diversity relates to variation at the molecular level, including differences in genetic sequences and the presence of distinct haplotypes among populations. This form of diversity is critical for understanding evolutionary processes, adaptability, and long-term species resilience. Morphological diversity encompasses observable variations in physical traits such as fruit size, leaf dimensions, and tree structure, which may be influenced by both genetic factors and environmental conditions. Finally, biochemical diversity refers to variation in nutritional composition and phytochemical profiles, including differences in macro- and micronutrients as well as bioactive compounds across plant parts and regions. Collectively, these dimensions of diversity provide a comprehensive understanding of the biological and functional potential of *M. zeyheri.*

1.4.3 The concept of Traditional Knowledge

Traditional knowledge is conceptualized in this study as the cumulative body of knowledge, practices, and beliefs developed by indigenous and local communities through long-term interaction with their natural environment. This includes ethnobotanical knowledge related to the use of M. zeyheri as a source of food, medicine, and cultural practices. Traditional knowledge is

inherently context-specific, culturally embedded, and transmitted across generations, often through oral traditions. Importantly, it is not static but evolves in response to changing environmental and socio-economic conditions. In the context of this study, traditional knowledge provides critical insight into how local communities perceive, utilize, and manage M. zeyheri, thereby directly informing the study objectives related to ethnobotanical use, cultural significance, and adoption.

1.4.4. The concept of Sustainability

Sustainability, the third core concept, is understood as the balanced integration of ecological health, economic sustainability, and social acceptability in the utilisation of *M. zeyheri*. Ecological sustainability refers to the conservation of the species, including maintaining viable populations, protecting genetic diversity, and ensuring natural regeneration. Economic sustainability relates to the potential of the species to contribute to livelihoods through income generation, value addition, and integration into local markets. Social sustainability involves the preservation of cultural practices, respect for indigenous knowledge systems, and equitable access to resources. In this study, sustainability is treated as a practical outcome that emerges from the interaction between diversity and traditional knowledge, supported by scientific evidence, and directly linked to the overall aim of promoting conservation, adoption, and sustainable utilisation of the species.

1.4.5. Research methodological approach

To operate the conceptual framework underpinning this study, a mixed-method research design was adopted, integrating both qualitative and quantitative research approaches. The use of mixed methods is justified by the need to capture the complexity of the research problem, which spans both social and natural sciences. Qualitative methods are primarily used to explore traditional knowledge systems, perceptions, and cultural practices associated with *M. zeyheri*, including semi-structured interviews and ethnobotanical surveys. These methods allow for in-depth understanding of local knowledge and practices and are particularly suited to addressing research questions related to ethnobotanical use and cultural significance.

The qualitative component of the study was primarily used to explore traditional knowledge systems, perceptions, and cultural practices associated with *M. zeyheri*. Data were collected through semi-structured, face-to-face interviews conducted with 116 participants across the study

areas in Limpopo (Vhembe District) and Mpumalanga (Ehlanzeni District). These interviews were complemented by ethnobotanical surveys, which facilitated the systematic documentation of plant uses, preparation methods, administration routes, and cultural significance. To enhance analytical rigor, the qualitative data were quantified using ethnobotanical indices, including the Relative Frequency of Citation (RFC) and Fidelity Level (FL), allowing for the assessment of the relative importance, consensus, and reliability of reported uses across communities. This approach ensured that indigenous knowledge was not only documented but also systematically analysed and integrated into the broader scientific framework of the study.

The quantitative component of the study encompassed multiple methodological approaches aligned with the different dimensions of diversity. Ecological assessments were conducted through field-based sampling of *M. zeyheri* populations, where data on tree height, stem circumference, and spatial distribution were collected from sampled individuals across the two Agro ecological zones. Population structure was analysed using size-class distribution, while spatial patterns were examined using Clark–Evans nearest-neighbour analysis to determine whether populations exhibited clustered, random, or dispersed distributions. In addition, an ecological disturbance index was applied to assess the extent of anthropogenic pressures on populations, including land-use change and harvesting intensity.

Morphological variation was assessed using standard morphometric measurements of fruits, leaves, and nuts collected from sampled trees (n = 40; 20 per region), including parameters such as fruit length, width, leaf area, and nut size. Sensory evaluation of fruit quality was conducted with 100 adult participants (50 per site) using a 9-point hedonic scale, allowing for the quantification of consumer preferences across attributes such as taste, aroma, and texture.

Genetic diversity was analysed using DNA barcoding techniques, specifically targeting chloroplast markers *matK* and *trnH-psbA*, which are widely used for plant identification and population differentiation. DNA extraction, amplification, and sequencing were conducted, followed by analysis of genetic distances, haplotype variation, and phylogenetic relationships, enabling the identification of distinct genetic lineages and regional clustering of *M. zeyheri* populations.

Nutritional composition was determined using standard AOAC methods, including proximate analysis (moisture, crude protein, fat, fibre, and ash content) and mineral analysis. Elemental

composition was analysed using microwave-assisted acid digestion followed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to quantify essential macro- and micronutrients such as calcium, magnesium, potassium, and zinc. Statistical analyses, including one-way Analysis of Variance (ANOVA) and Pearson correlation analysis, were performed using R to identify significant differences and relationships among variables.

Phytochemical analysis involved both quantitative and qualitative techniques. Total phenolics, flavonoids, and alkaloids were quantified using spectrophotometric assays, while antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the β-carotene–linoleic acid assay. Furthermore, the identification of untargeted secondary metabolites was conducted using Ultra Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UPLC-MS/MS), enabling detailed characterisation of bioactive compounds across different plant parts and Agro ecological zones.

The integration of qualitative and quantitative methods in this study is inherently complementary. Qualitative data on traditional knowledge provide context-specific insights that inform the interpretation of ecological, nutritional, and phytochemical findings, while quantitative analyses offer empirical validation of the uses and properties identified through indigenous knowledge systems. For example, ethnomedicinal uses reported by participants are supported by the identification of bioactive compounds with known therapeutic properties, while ecological distribution patterns help explain regional differences in utilisation. This integrative approach enhances the robustness, validity, and interdisciplinary relevance of the study and aligns with contemporary research practices in biodiversity, ethnobotany, and sustainability science.

1.5 Aim and Objectives

1.5.1 Aim

The study aims to comprehensively examine *M. zeyheri* in two South African Agro ecosystems (Mpumalanga and Limpopo), focusing on its diversity, traditional knowledge, nutrition and phytochemistry to promote its conservation, adoption, and sustainable utilization.

1.5.2 Specific objectives

i. To investigate the traditional knowledge of *M. zeyheri* as a food source and to understand cultural barriers and opportunities for adoption among indigenous communities.
ii. To assess the distribution and abundance of *M. zeyheri* across two Agro ecologies.

iii. To identify and classify genetic diversity of *M. zeyheri* and assess variations in production, fruit taste, and adaptability in two different Agro ecologies.
iv. To determine the nutritional composition of *M. zeyheri* fruit from two different Agro ecologies in South Africa.
v. To determine the phytochemical composition of *Mimusops zeyheri* fruit from two different Agro ecologies in South Africa.

1.5.3 Research questions

i. What is the population structure and distribution pattern of *M. zeyheri* across the two provinces?
ii. What are the traditional uses of *M. zeyheri* in Limpopo and Mpumalanga provinces?
iii. Are there any morphological, sensory and genetic variations among *M. zeyheri* trees across the two provinces?
iv. Are there differences in nutritional composition of *M. zeyheri* plant parts across Limpopo and Mpumalanga
v. Are there differences in the phytochemical composition of *M. zeyheri* between the two provinces?

1.6 Structure of the thesis

Chapter 1: General Introduction

Chapter 2: Literature Review

Chapter 3: Review of *Mimusops zeyheri* Sond. (Milkwood): Distribution, Utilisation, Ecology, and Population Genetics (Published)

Chapter 4: Traditional and ethnomedicinal knowledge of *Mimusops zeyheri* Sond., as A source of food and medicine in Limpopo and Mpumalanga

Chapter 5: Population and Distribution of *Mimusops zeyheri* in Two Agro ecologies: A Comparative Ecological Study of Ehlanzeni and Vhembe Districts of South Africa

Chapter 6: Morphological, Sensory, and Genetic Assessment of *Mimusops zeyheri* (Sond.) Populations from Limpopo and Mpumalanga, South Africa

Chapter 7: Nutritional composition of *M. zeyheri* plant parts from two different Agro ecologies of South Africa

Chapter 8: Phytochemical Profiling and Identification of Untargeted Secondary Metabolites of *Mimusops zeyheri* Sond (Milkwood) Plant Extracts from Two Distinct Agro-Ecological Zones in South Africa

Chapter 9: Conclusion and Recommendations.

1.7 References

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CHAPTER TWO
LITERATURE REVIEW

2.1. Origin and evolutionary history of *Mimusops zeyheri* Sond.

Mimusops zeyheri Sond is an indigenous wild fruit tree native to Africa, treasured in many Agro ecologies for its cultural, ecological, and nutritional value (Omotayo et al., 2020b). The tree is distributed in South and East Africa, with evidence indicating its origin in the woodland savannas of southern Africa during the Miocene epoch, dating back to between 23 and25 million years ago (Jacobs, 2004). Fossil evidence from Egypt with pollen samples and leaf impressions suggests the presence of the Sapotaceae family in the African continent dating back to the Oligocene era, which was approximately 33-23 million years ago (El Atfy et al., 2022). Paleontological results have reported no alterations over the years, suggesting evolutionary stability within the Sapotaceae family. Species from the Mimusops genus, including *M. zeyheri,* have evolved substantial tolerance to diverse conditions, including drought, with nuts developing a hard coat that remains dormant until favorable conditions (Sinasson et al., 2021).

2.2. Botanical description

Mimusops zeyheri exhibits distinct structural and morphological adaptations. The tree is a medium-sized, wild, perennial fruit tree that reaches 15 meters in height at full maturity, with some exceptional trees exceeding 25 meters (Figure 2.1A), characterized by a dense, rounded canopy (Chivandi et al., 2020). The tree's growth form is characterized by a straight, dark grey to brown trunk that is rough-textured and fissured, with horizontally spreading branches. Although direct measurements of *M. zeyheri* are limited, comparable studies suggest the tree’s thick-textured trunk serves as a good defense mechanism against herbivores (Tomlinson et al., 2016). The tree thrives well in diverse soil conditions, including sandy loam soils and sandy-loamy soils, with optimum growth reported in loamy and clay soils (Mashela et al., 2013b). Tree leaves (Figure 2.1B) are simple, glossy, with alternate and leathery textures, with a 5-12 cm obovate shape. The leaf venation is pinnate, similar to that of *M. laurifolia* (Forssk.) Friis aligns with reported adaptations to semi-arid environments (Mostafa et al., 2023). Flowers are bisexual and arranged in auxiliary clusters essential for facilitating cross-pollination by insects (Kamga-Simo III et al., 2024). *Mimusops zeyheri* belongs to the Sapotaceae family, renowned for its diversity and application in traditional and modern conventional medicine. *Mimusops zeyheri* is distributed in southern Africa,

particularly in South Africa, in Mpumalanga, Limpopo, and KwaZulu-Natal, well known by different vernacular names (Table 2.1).

Figure 2. 1: *Mimusops zeyheri* tree (A), *Mimusops zeyheri* leaves (B). *Photo by Mkhonto C., 2024.* Fruits are between 2-3 cm and resemble an ovoid berry that is green and turns orange-red when ripe (Figure 2.2A-B).

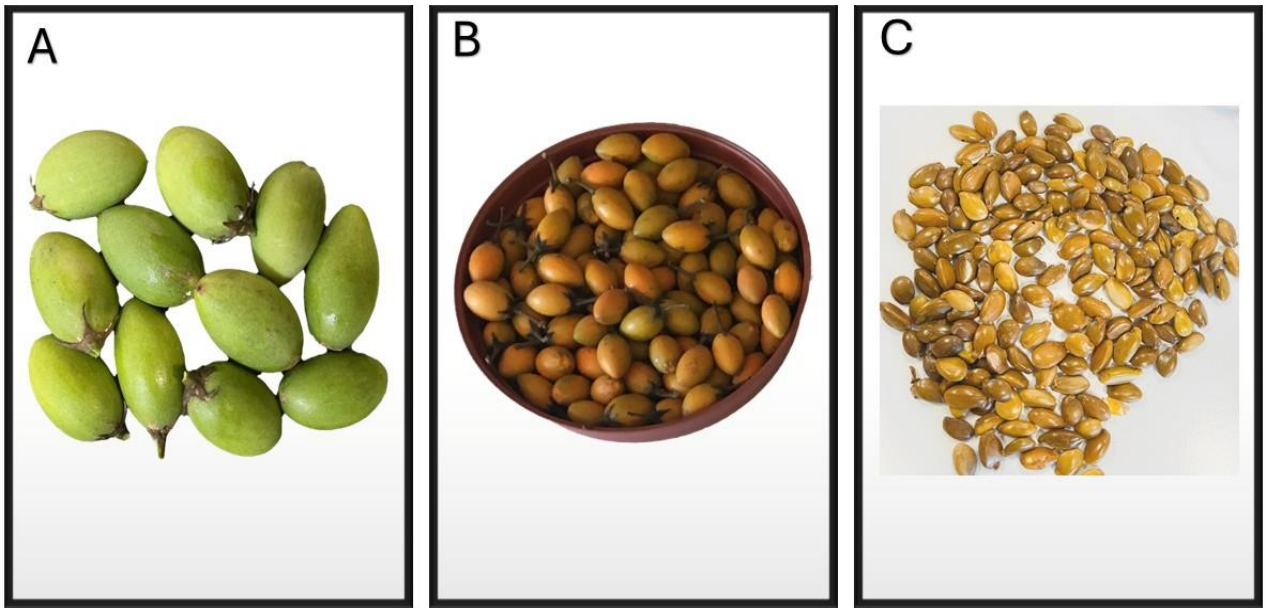

Figure 2. 2: Unripe fruits (A), Ripe fruits (B) and Nuts (C) of *M. zeyheri*. The fruit mesocarp is fleshy and edible, known for its sweet taste, while the endocarp has 1-2 nuts inside (Chivandi et al., 2020). The nuts are dark brown (Figure 2.2 C) and are in a fibrous arrangement.

Table 2.1:Taxonomic classification of *M. zeyheri.*

Taxonomic rank	Description
Kingdom	Plantae
Order	Ericales
Family	Sapotaceae
Genus	Mimusops
Species	*Mimusops zeyheri* Sond
Authority	Sonder 1850
Common names	Milkwood (English), Moepel (Afrikaans), Umnumba (Zulu), Mmupudu (Sepedi), Mubululu (Venda)
Geographic range	Indigenous to southern Africa, particularly South Africa in Mpumalanga, Limpopo, KwaZulu-Natal, and neighbouring Mozambique (Matlala et al., 2024)
Ecological notes	Thrives in subtropical coastal forests pollinated by insects dispersed by birds and mammals (Dube et al., 2016)
Ecological niches	Savanna woodlands, Riverine forests, forest margins, Rocky outcrops, and coastal forests

2.3. Nutritional composition of Mimusops species

The Mimusops genus is well-renowned for its richness in macronutrients, including carbohydrates that contribute to its sweet taste and energy value. Various species, including *M. elengi* L. *(Ruikar et al., 2009), M. zeyheri* (Mngadi et al., 2019a), and *M. kummel* (Bruce ex A.DC.) Kuntze are traditionally consumed and preferred for fruits and nuts. The critical reports on their nutritional diversity (Table 2.2) reveal essential health benefits from consuming and using fruits. The genera have reported notable levels of vitamin C content comparable to that of popular conventional fruits such as oranges (Mngadi et al., 2019a). The mineral composition of Mimusops species is noteworthy, with considerable concentration levels of calcium, iron, and potassium (Mngadi et al., 2017b).

Table 2.2: Comparative nutritional composition of some Mimusops wild fruit species (Per 100g dry fruit)

Species	**Energy (Kcal)**	**Fibre (g)**	**Vitamin C**	**Ca(mg)**	**Fe(mg)**	**K(mg)**	**Protein(g)**	**References**
Mimusops elengi L.	140.2	4.3	35.7	48.5	1.2	210.5	1.8	(Ruikar et al., 2009)
Mimusops hexandra (Roxb.) Dubard	129.8	3.8	29.3	42.1	0.9	308.7	1.5	(Baky et al., 2022c; Chaudhary et al., 2023)
Mimusops zeyheri Sond	155.3	5.1	42.8	55.3	1.5	368.7	2.1	**(**Mostafa et al., 2023)
Mimusops kummel (Bruce ex A.DC.)	134.7	4.0	31.5	45.2	1.1	325.7	1.7	(Wassie, 2025)
Mimusops laurifolia (Forssk). Friis	138.5	4.2	33.4	47.8	1.0	267.4	1.6	**(**Abdel Maksoud et al., 2019)
Mimusops caffra (E. Mey ex A.DC.)	90.7	4.8	52.4	40.2	2.3	230.8	2.0	(Mngadi et al., 2017b)

Note: All values are expressed on a dry weight basis (per 100 g). Data were obtained from different published sources, and variability measures such as confidence intervals were not consistently reported across studies. As such, values are presented as reported in the original references.

2.4. Biodiversity conservation of *Mimusops zeyheri* Sond

Despite its ecological, cultural, and nutritional role, *M. zeyheri* remains underutilized due to inadequate domestication and conservation efforts. Recent ethnobotanical studies highlight its role in food security, ethnomedicine, and soil stabilization (Matlala et al., 2024). However, sustainable cultivation strategies informed by its adaptability and phenology are lacking. Limited studies have documented the tree's genetic diversity and ecophysiological traits, which can potentially improve rural livelihoods and the economy. There is a need for focused studies on sustainable harvesting practices of *M. zeyheri* cultivation in communities, nurseries, and national botanical gardens.

2.5. Phytochemistry and bioactivity of wild fruit trees

Indigenous wild fruit trees have been the cornerstone of rural livelihoods for centuries. Rooted in ethnomedicine, wild fruit tree species have provided for the dietary requirements and therapeutic needs of many indigenous communities (Pfukwa et al., 2022). Although underutilized, indigenous wild fruit trees from Africa, particularly South Africa, have been reported to be reservoirs of essential nutrients and phytochemical constituents with antimicrobial and antioxidant properties (Achilonu et al., 2023). Wild fruits are rich in anthocyanins and flavonoids with efficacy in mitigating antioxidative stress associated with several pathogens related to gastrointestinal disorders (Dhama et al., 2014), skin disorders (Zafra-Stone et al., 2007) and cardiovascular diseases (Zafra-Stone et al., 2007). As interest in natural antioxidants expands, there is a notable need to fully explore and document the phytochemistry and antioxidant potential of wild fruit trees, such as *M. zeyheri* and other species from the Sapotaceae family, as promising agents for the development of functional foods and pharmaceuticals for disease prevention and management.

2.5.1. Pre-extraction treatment and biomass preparations for phytochemical analysis

The quality and consistency of the biomass to be extracted are crucial and significantly influence the extraction results. Pretreatment of samples and biomass is essential to ensure the efficacy of extraction protocols. The selection of pretreatment method and processing influences the effectiveness of extraction protocols (Ramos et al., 2020). The collection and identification of plant parts influence downstream processes, including the enzymic hydrolysis of biomass. Leaves, stems, fruits, and plant bark should be collected at specific seasons and growth stages to ensure optimal phytochemical content (Duguid et al., 2009; Ramos et al., 2020). Accuracy in plant identification ensures reliability while reducing the probability of adulteration. These protocols

must be adhered to, and they need to be balanced to minimize contamination, microbial growth, and infestation, reduce degradation, and enhance solvent penetration during the extraction process.

Drying is the most popular method for preparing medicinal plants for extraction, preserving phytochemicals, preventing spoilage and efficient storage (Ng et al., 2020). Drying is a crucial step in the preparation of herbal medicine, natural extracts, and natural therapeutic products. Freeze drying and hot air drying are the most used drying methods to reduce the moisture content of collected biomass. Sun and shade drying are the most traditional methods of drying used in many ethnic communities. Both drying methods provide good ventilation and preserve thermolabile compounds compared to higher temperatures (Ng et al., 2020).

Studies have reported a comparable high retention of essential oil concentrations, phenolic compounds, and flavonoids in *Thymus capitatus* L., *Mentha piperita L.* and *Sideritis cypria* Post from sun and shade drying (Pereira and Cardoso, 2013; Xylia et al., 2024). The oven method forces convection of temperatures ranging from 40 to 800 °C with air velocities of 0.5 to 2.0 m/s (Premi et al., 2012; Troisi et al., 2018). This drying method significantly reduces drying time compared to sun and air drying, allowing for the standardization of drying parameters (velocity, temperature, and time) to ensure consistency and quality (Vega-Gálvez et al., 2012). Freeze drying is preferred for drying thermolabile compounds, as it is associated with minimal thermal damage, which is known to preserve the cellular structure and bioactive compounds of plants. The process involves removing water from the sample to protect cellular structures through the sublimation of ice crystals at temperatures ranging from -40 to -80°C (Ciurzynska and Lenart, 2011). Sublimation is the second step in freeze drying, where ice sublimates under a vacuum (≤63 Pa) at -20 to 0 °C to remove over 95% of moisture from the sample (Ng et al., 2020). Lastly, desorption occurs when the remaining moisture is removed by subjecting the material to temperatures ranging between 20 and 40°C, thereby retaining a moisture content between 2% and 5% (Freire et al., 2007). Lower drying temperatures (20-40°C) are often employed for drying leaves and herbs such as *Echinacea purpurea* L to retain volatile compounds. Higher freeze-drying temperatures are used to dry berries (*Fragaria vesca* L) and S*allacca zalacca* (Gaertn.) Voss accelerates the drying process without damaging the porous cell structures (Macias, 2013). Freeze drying is a preferred method in the biotechnology and food industries due to its ability to maintain sample and product quality, colour, and shape while simultaneously removing moisture, compared to traditional methods. Despite its

ability to support cellular structures, freeze-drying is a costly process that requires high energy consumption.

2.5.2. Primary extraction methods for phytochemical analysis

Phytochemical extraction is an essential step in analyzing, identifying, and isolating bioactive compounds in medicinal plants. Extraction and the method directly influence the purity and yield outcomes of the bioactive compounds from the sample (Selvaraj et al., 2022). Conventional methods, such as Soxhlet extraction, maceration, and hydrodistillation (Figure 2.3), have been employed for decades and remain popular to this day. However, advancements in eco-friendly modern extraction techniques have led to improvements, such as enzyme-assisted extraction and ultrasound-assisted extraction (Figure 2.3), which have reportedly enhanced efficiency and yield outcomes from smaller sample sizes (Azmir et al., 2013). Maceration is a simple procedure that traditional healers and Indigenous knowledge holders have used due to its efficiency. The protocol is suitable for extracting heat-sensitive compounds, such as polyphenols, reported in *Malus sylvestris* (L.) Mill. extracts (Stojiljković et al., 2016). Soxhlet extraction is preferred for extracting nonpolar compounds and has successfully yielded 17.5 to 37.1% oil in simi *(e Santos et al., 2015).* The soxhlet method has long extraction cycles and is, therefore, time-consuming, with low selectivity for compounds.

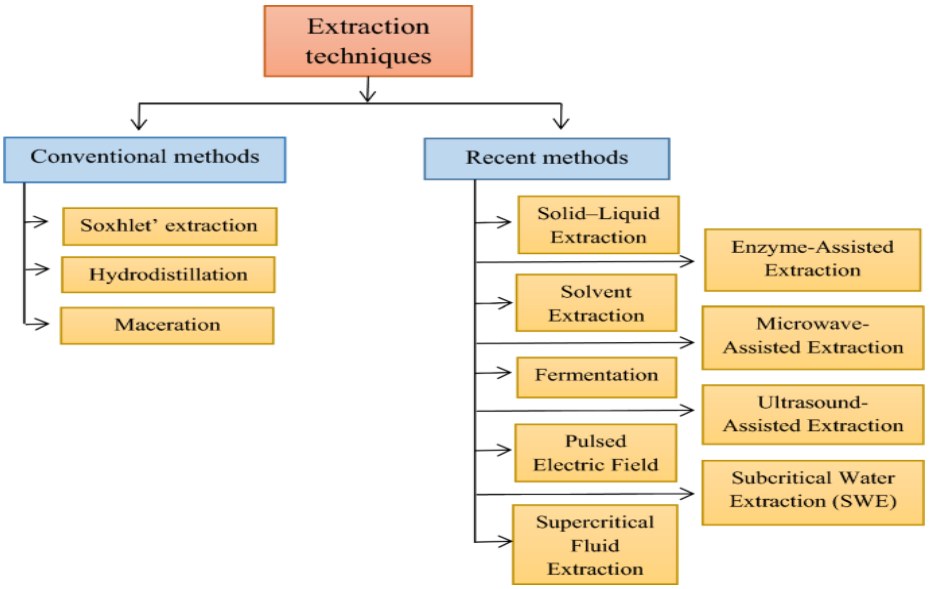

Figure 2. 3: Conventional and Modern plant extraction techniques used for phytochemical analysis.

Table 2.3:Inventory of solvents used for the extraction of plant extracts for phytochemical analysis.

Solvent	Polarity index	Target compounds	Yield (%)	Species extracted	Reference
Water	9.0	Tannins, Saponins, Flavonoids	5-20	*Phoenix dactylifera (*L.*)*	(Djaoudene et al., 2024)
Methanol	5.1	Phenolics, Flavonoids, Alkaloids	15-25	*Blepharis linariifolia (*Pers.)	(Dirar et al., 2019)
Hexane	0.1	Terpenoids, Lipids, Chlorophyll	2-8	*Leea nova-guineensis* (Valeton.*)*	(Awotedu et al., 2019)
Ethanol	4.3	Phenolics, Flavonoids, Terpenoids	12-22	*Andrographis paniculata* (Burm.f.)	(Chao and Lin, 2010))
Acetone	5.1	Phenolics, Alkaloids, Flavonoids	10-18	*Physelous vulgaris* (L.)	(Nawaz et al., 2020)
Chloroform	4.1	Alkaloids, Terpenoids	5-12	*Canscora decussate* (Roth.) Wall	(Sethiya et al., 2010)
Ethyl acetate	4.4	Semi-polar compounds, Flavonoids	8-15	*Centella asiatica* (L.) Urban	(Idris and Mohd Nadzir, 2021)
Ethanol: Water (70:30)	~5.2	Phenolics, Flavonoids	60-80	*Isatis tincoria* (L.)	(Wakeel et al., 2019)
Petroleum ether	0.1	Fatty acids, Terpenoids	3-10	*Abutilon fruticosum* Guill. and Perr.	(Ghaffar and Perveen, 2024)
Glycerol: Alanine NADES	~4.8	Anthocyanins, Polar glycosides	75-85	*Phoenix dactylifera* (L.)	(Djaoudene et al., 2024)
L-Proline: Lactic Acid	6.5	Flavonoids, Saponins, Antioxidants	80-90	*Pronus domestica* (L.)	(Haddadi-Guemghar et al., 2014)
Methanol: Water (80:20)	~6.5	Phenolic acids, Tannis, Flavonoids	70-90	*Melastoma sanguineum* (Sims)	(Zhou et al., 2017)

2.5.3. Techniques used for the analysis of bioactive compounds in crude plant extracts

2.5.3.1. Thin layer chromatography (TLC*)*

This method combines chromatographic separation and in-situ activity measurement, providing rapid results for active chemicals in complex plant matrices (Sasidharan et al., 2011). Xin-Yue et al. (2013) advocate for the use of this method for identifying active chemicals in plants due to its simplicity, high sensitivity, low cost, and widespread use. The technique involves a stationary phase, comprising either silica gel, cellulose, or a solid support such as glass or plastic, and a mobile phase that transports the sample through the column. This technique is easy, provides rapid results, and is preferred for its sensitivity in pharmaceutical analysis, separation of amino acids, and the identification of impurities (Bele and Khale, 2011). Thin Thin-layer chromatography can determine a diverse range of compounds in a sample, verify the identity of the compound, and detect impurities within the sample. It also enables rapid analysis of column chromatography functions.

2.5.3.2. Gas Chromatography-Mass spectrometry (GC-MS)

This technique is beneficial and widely used to separate biological materials from volatile and semi-volatile compounds in essential oils, utilising the unsurpassed peak capacity of GC columns (Mabadahanye, 2020). GC-MS has been beneficial in identifying bioactive terpenes, lipids, and alkaloids in medicinal plants (Al-Rubaye et al., 2017). A study by Ali et al. (2022) effectively identified bioactive compounds in *Periploca hydaspidis* L. and several volatile compounds in quinoa nuts (Karonen and Pihlava, 2022) and 50 volatile compounds in *Mimusops caffra* (Baky et al., 2025).

2.5.3.3. Liquid chromatography-mass spectrometry (LC-MS)

One of the powerful, versatile techniques that is widely used to separate, detect, and identify a wide range of bioactive compounds in crude plant extracts. The technique combines the separation qualities of liquid chromatography (LC) and the structural elucidation capabilities of Mass spectrometry (MS) to provide high resolution and sensitivity, allowing the detection of even trace quantities of bioactive compounds in crude samples (Kumar et al., 2018). Under high pressure, LC-MS separates complex mixtures based on their reaction and activity in the liquid mobile and stationary phases, providing accurate results for a qualitative and quantitative analysis of a wide range of polar and non-volatile bioactive compounds, including flavonoids, alkaloids, fatty acids, and phenolic acids (Ignat et al., 2011). Upon separation, analytes are fed to the mass spectrometer

for ionization and identification using their mass-to-charge (m/z) ratios to identify recognized compounds through comparison with existing standard library data. In metabolomics, this technique has been reported to be beneficial for saliva analysis in the screening and detection of drugs and disease biomarkers. The sensitivity and specificity of this technique make it preferable for detecting low-abundance bioactive compounds (Ignat et al., 2011). The method allows optimization with mass spectrometry LC-MS/MS) which improves the efficiency of identifying compound structures and distinguishing isomers (Kumar et al., 2018). Challenges associated with the technique include the need for careful and specific sample preparation optimization to avoid matrix effects that can otherwise suppress ionization and interfere with compound detection. Additionally, the equipment is expensive and represents a barrier in research-limited facilities and institutions.

2.5.4. Identified gaps in the use of techniques for the analysis of bioactive compounds in the Sapotaceae family.

Studies on the application of analytical techniques for identifying bioactive compounds in the Sapotaceae, including highly medicinally, culturally, and ecologically important genera such as Mimusops and Manilkara, remain fragmented and limited. Despite the increasing recognition and interest in the therapeutic potential of the family, there is a clear gap in the use of techniques for analyzing the phytochemical compounds in the plant species, including *M.s zeyheri,* within the family. While preliminary methods such as TLC and, in some studies, HPLC have been reported, these techniques remain insufficient for the precise identification and structural elucidation of bioactive compounds. Several studies, including results by Teffo et al. (2025b) used basic quantitative methods to detect secondary metabolites in fruits of *M. zeyheri* from different accessions, further isolation and characterization of specific compounds more especially those to be attributed to the biological and therapeutic activity of the plant is lacking limiting the overall understanding of the particular chemical constituents responsible for the pharmacological potential of the species. Numerous studies conclude and report only the crude extract bioactive assay, neglecting the need for further studies aimed at separating and identifying specific active compounds. Lastly, there is a scarcity of phytochemical research on various plant parts, such as the leaves, roots, nuts, and bark of *M. zeyheri,* using metabolomic and chemometric techniques to compare bioactive compounds across different Agro ecologies.

2.6. Oxidative stress and the role of antioxidants from medicinal plants in health

Antioxidative stress occurs because of excess reactive oxygen species (ROS) that tend to overwhelm the body's antioxidant defenses, resulting in cell and tissue damage (Hassan et al., 2017; Kumari, 2023). The damage is responsible, therefore, for pathogen accumulation that is accountable for causing cancer and diabetes, among other conditions. Antioxidants are critical in neutralizing ROS and stabilizing cellular homeostasis. Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA) are the most widely used synthetic antioxidants, preferred mainly due to their low production cost (Yıldız and Çabuk, 2022). There have, however, been reported concerns associated with potential toxicity from BHT and BHA (Shahidi, 2000). While both BHT and BHA are considered carcinogenic by the World Health Organization (WHO), both antioxidant mixtures have previously demonstrated antagonistic antioxidant properties, further questioning their joint usage (Dawidowicz et al., 2015). Another study by Kahl (2019) previously reported tumor-promoting properties associated with BHT and BHA in animal clinical trials. Indigenous wild medicinal species, on the other hand, serve as safer, accessible, and therefore cheaper alternatives to synthetic antioxidants. Wild fruit trees such *as Manilkara kauki* (L.) Dubard *and Pouteria Sapota* (Jacq.) from the Sapotaceae family are reportedly rich in polyphenols and betalains known to prevent ROS by producing electrons, chelating metal ions and regulatory enzymes such as glutathione peroxidase (Kaur, J. et al., 2020). *Mimusops elengi* extracts from the Mimusops genus have, in another study, demonstrated a high antioxidant activity with an IC50 of 1.6 µg/mL recorded over 45 minutes, while isolated tannins from the fruits exhibited remarkable antioxidant properties (Gillani and Shahwar, 2017; Tristantini and Jessica, 2019). Similarly, green leaf extracts of *Mimusops coriacea L.* demonstrated the highest antioxidant efficacy with an IC50 value of 4.99 µg/mL (Bustamante-Pesantes et al., 2023). These results demonstrate the potential effectiveness and ability of plant phytoconstituents in modulating oxidative pathways, thereby underscoring the potential of wild species in preventing and treating ailments and diseases. However, despite the demonstrated and reported antioxidant properties of medicinal wild species associated with good health, there is still a significant lack of scientific and in vitro studies on wild fruit trees native to South Africa, including *M. zeyheri* from the Sapotaceae family. There is, therefore, a need for studies aimed at bridging the existing gap between biomedical research and IKS to validate the Ethnobotanical use of the species within scientific frameworks.

2.7. Common Assays for Evaluating Antioxidant Activity in Plant Extracts

Standardized assays are used to assess the in vitro antioxidant properties of wild fruit tree plant parts by measuring their radical scavenging activities, metal chelating and reducing power activities. Standard employed assays include DPPH (2,2-diphenyl-1-picrylhydrazyl) and ATBS (2,2'-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assays, which work by measuring the hydrogen-donating capacity. Assays such as FRAP (ferric reducing antioxidant power) and ORAC (oxygen radical absorbance capacity) assess the reduction potential and peroxyl radical neutralization, respectively. Several studies on plants from the Sapotaceae family have used different assays to determine and document various antioxidant-related behaviors. The lack of standardized protocols, however, has limited cross-study comparability. In addition, the influence of agro ecological factors such as soil pH, nutrient availability, and climatic conditions on antioxidant assay outcomes in *M. zeyheri* remains poorly understood, as there is a limited body of literature explicitly linking environmental conditions to phytochemical and antioxidant variability in this species. This represents an important research gap that warrants further investigation.

2.7.1. DPPH (2,2-diphenyl-1-piciyihydrazyl) radical scavenging assay

This is a commonly used assay that employs stable free radicals to evaluate a compound's ability to scavenge free radicals. The DPPH radical is purple in colour and changes to yellow, indicating antioxidants have reduced it. The colour change is measured through a spectrophotometer at 512-520nm absorbance (Jefri et al., 2025). Another recent method utilizes a paper-based DPPH device, which offers low reagent consumption and high-throughput analysis (Sirivibulkovit et al., 2018). Compared to other assays, the DPPH assay is the most preferred due to its simplicity and accuracy, yielding rapid and cost-effective results (de Oliveira et al., 2017). The assay is ideal for screening the antioxidant activity of lipophilic and hydrophilic antioxidants in plant extracts. The choice of solvent in the assay is vital as the solvent's polarity influences extraction efficiency and DPPH solubility. Methanol and ethanol are the most used solvents in this assay due to their reported efficiency in dissolving phenolic compounds and the DPPH itself. Solvents such as acetone and ethyl acetate have been used in various studies; however, they have been reported to have less efficacy. The assay has three primary pathways, including Hydrogen Atom Transfer (HAT), which is the most prevalent and functions by donating a hydrogen atom from the antioxidants to the DPPH radical. Phenolics such as flavonoids transfer hydrogen from their hydroxyl (-OH) groups to DPPH to form a non-radical DPPH-H and a stabilized antioxidant radical (ArO) (Bletsa et al.,

2015). For instance, gallic acid donates a hydrogen atom from three of its ortho-positioned hydroxyl groups to achieve rapid scavenging at an IC_{50} value of 0.03mm (Sroka and Cisowski, 2003).

The Single Electron Transfer (SET) pathway is common in polar solvents, where antioxidants transfer electrons to DPPH, creating a DPPH-anion and a protonated antioxidant (Noipa et al., 2011). This pathway is common in isoflavones with long conjugation systems, metal complexes where metal ions facilitate electron delocalization. SET is influenced by solvent polarity and is often effective with buffers (Xie and Schaich, 2014). A pH>7.0 enhances the deprotonation of phenolic-OH groups. In the RAF pathway, antioxidants create covalent bonds with DPPH through radical-radical coupling. This mechanism, however, is time-dependent on a stoichiometry above the 1:1 molar ratio, which is characterized by reduced reversibility compared to HAT and SET pathways.

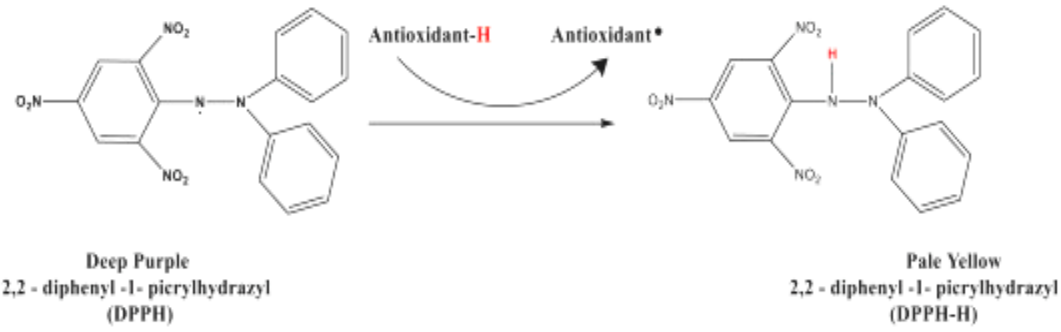

Figure 2. 4: DPPH reaction mechanism interplay between HAT and SET pathways showing hydrogen donation (HAT) and electron transfer routes (SET). Source: (Kumari, 2023)

Although widely used and preferred, the DPPH mechanism has several limitations. Antioxidants perform differently through various mechanisms (HAT, SET, RAT), which the assay fails to distinguish. Reaction kinetics and stoichiometry differ in antioxidants, which often result in inconsistent reactions compared to biological systems (Xie and Schaich, 2014). Secondly, the assay runs on a direct correlation between antioxidants and DPPH Radical Scavenging. The saturation effect and scavenging reactions demonstrate a direct, non-linear, multiphase response at higher concentrations, complicating EC_{50} computations and comparisons. Colored compounds such as anthocyanins can potentially tilt absorbance readings at 515nm, resulting in false positive/negative readings. Some antioxidants may perform poorly if solvents like ethanol are used

as standard protocols, making the assay solvent-specific (Gaikwad et al., 2010). These findings underscore the need for the adoption of standardized assay conditions, as the DPPH assay may not be the most suitable method for ranking antioxidants in natural extracts. The standardized conditions will ensure mechanistic consistency in various studies. Due to the natural structural and broad diversity of plant antioxidants, there is a need for tailored, species-specific mechanistic analyses rather than a universal system that relies on assumptions about DPPH interactions.

2.7.2. 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)

ABTS 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay is widely used for determining the in vitro Total Antioxidant Capacity (TAC) of medicinal plant extracts (Mingle and Newsome, 2020). The reaction in the assay involved the formation of blue green ABTS radical cation. The antioxidants in the sample reduce the radical cation, as evidenced by a decrease in absorbance measured at 734 nm. Methanol and ethanol are commonly used solvents in the assay to dissolve ABTS; however, several other studies have used Phosphate-Buffered Saline (PBS) for targeting water-soluble antioxidants. The limitations associated with the use of solvents in this assay are that they affect and influence the extraction of compounds from medicinal plant extracts. Since ABTS can react with both hydrophilic and lipophilic antioxidants, the solvent system used may result in a competitive challenge that favors one over the other. Nonpolar solvents such as carotenoids are poorly extracted in the aqueous system of the assay, which results in poor reading and an underestimation of the sample antioxidant activity. This assay has reportedly been successful in reporting the dose-dependent ABTS scavenging activity of anthocyanins from *Syzygium cumini,* outperforming synthetic antioxidants with an IC50 of 12 nm (Kotakadi et al., 2024). Similarly, Streptomyces *spp* exhibited a 20-90% ABTS activity correlating with reported phenolic diversity (Law et al., 2019). The pathways involved in this assay include single electron transfer, where antioxidants donate electrons to ATBS, which differs from the mechanism observed in DPPH.

2.7.3. Ferric Reducing Antioxidant Power (FRAP)

This assay measures the antioxidant activity of samples by quantifying their ability to reduce ferric ions to ferrous ions, which produces a blue green colour. This reaction offers the formation of a colored ferrous-tripyridyl triazine complex measured at 593 nm absorbance using a UV-VIS spectrophotometer (Abdolkarimi-Mahabadi et al., 2021). This mechanism is one of the easiest automated methods of measuring the antioxidant activity of plasma. Beyond measuring the

antioxidant activity of plasma, the method is also effective in determining the antioxidant activity of medicinal plant extracts. FRAP is a commonly used measure of the non-enzymatic antioxidant activity of compounds, such as uric acid, which reacts in redox reactions (García-Alcalde et al., 2022). The assay reflects the reducing antioxidant activity of samples under acidic conditions with a pH value of 3.6. FRAP has been widely used in analyzing the antioxidant activity of medicinal plant extracts. Several studies have reported a strong correlation between the antioxidant activity values measured using FRAP and the Phenol Content (TPC) of plant compounds. Although the assay presents contextual constraints, it serves as a viable and effective mechanism for bridging the gap between Indigenous knowledge and evidence-based research in ethnopharmacology. Despite FRAP being automated and easy to follow, it has several limitations, including interference risks due to the potential for overestimating the antioxidant activity of biological samples, particularly when high levels of ascorbic or uric acid are present (Benzie and Devaki, 2018). The pH sensitivity may limit potential in-vitro exploration to only in vivo extrapolation due to the inability to reflect physiological conditions (Benzie and Devaki, 2018). The limitations associated with FRAP, therefore, necessitate the use of complementary methods such as ABTS or ORAC.

2.7.4. Oxygen Radical Absorbance Capacity (ORAC)

The assay measures the inhibition of a free radical, specifically peroxyl radical-induced oxidation. The mechanism utilizes a fluorescent probe that decays during oxidation, and the antioxidants are therefore responsible for slowing the decay through the thermal decomposition of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH). The produced peroxyl radicals resemble the oxidative species found in lipid peroxidation and cellular pathways, making them physiologically significant (Bisby et al., 2008). Phosphate buffer with a pH of 7.4 is used for hydrophilic antioxidants. Organic solvents such as acetone and dimethyl sulfoxide (DMSO) are used to dissolve lipophilic solvents. Fluorescence intensity is measured over time, and the area under the curve influence (AUC) decay is used to quantify the antioxidant activity measured relative to the blank sample without antioxidants and Trolox equivalents (TE) with a water-soluble vitamin E analogue called torrox standard (Beretta et al., 2006). Despite the widespread use of the ORAC assay due to its biological relevance and applicability, the use of fluorescein may result in false-positive or inflated ORAC values, especially in pigmented plant samples, as it is sensitive to temperature, pH, and interference with the fluorescent probe (Prior, 2015). Several studies argue that ORAC does not account for

metabolism, bioavailability, and the in vivo interactions of antioxidants (Prior, 2015; Roy et al., 2010). This is attributed to reports that demonstrate compounds with high ORAC values in vitro have poor absorption and rapid metabolism in the gastrointestinal tract, thereby neglecting their antioxidant effect under physiological conditions. There is a notable lack of a standardized ORAC protocol; therefore, several studies have reported variability in their reaction times, fluorescent probe concentrations, and Radical Generator (AAPH) concentrations. The assay uses a single-factor dilution, which evaluates only free radical scavenging through the HAT pathways (Osakwe and Siegel, 2013), this, therefore, excludes other crucial pathways, such as metal ion chelation, which is critical for inhibiting Fenton reactions, resulting in an underestimation of antioxidant activity in samples and plant extracts where indirect pathways predominate. There is, therefore, a need for a more standardized protocol that incorporates sample matrix effects and efficient calibration using Trolox equivalents. There is also a need for studies that integrate in vivo ORAC results with in vitro cellular antioxidant studies to improve biological relevance. Studies should also focus on the effects of agroecological conditions on ORAC antioxidant values to fully understand the relationship between the plant ecosystem and its influence on the sample's antioxidant potential.

2.8. Antioxidant Studies on Related Mimusops Species or other Sapotaceae family members

The Sapotaceae family has been extensively studied for its antioxidant activity. Various plant parts, such as leaves, roots, bark, and fruits of wild trees from the family, have been reported to exhibit remarkable antioxidant activity through the DPPH and FRAP assays (Kanmani Bharathi and Prakash, 2025; Valvi et al., 2011). *Mimusops elengi, Mimusops caffra* and *Manilkara zapota* (L.) have reported significant antioxidant potential attributed to their high polyphenol, tannin, and flavonoid content (Mohamed et al., 2020). *Mimusops elengi* is the most extensively studied wild fruit tree in the Mimusops genus and has exhibited antihyperglycemic effects in alloxan-induced diabetic mice, suggesting the strong potential of the plant species as an anti-diabetic agent (Ganu et al., 2011). All plant parts of *M. elengi* exhibit a high phenolic content, with methanol and acetone extracts reporting a substantial DPPH radical scavenging activity value IC_{50} ~43.26 µg/mL and a lipid peroxidation inhibition activity through the ferric thiocyanate method (Karmakar et al., 2011). In a study by Mamun et al. (2022) the ethyl acetate fractions of *M. elengi* nuts exhibited the highest ferric reducing power (1.74 ± 0.005) and DPPH scavenging (IC_{50} 39.49 µg/mL), attributed to their high tannin and flavonoid content. Similarly, *M. zapota* fruit pulp and peel have demonstrated

remarkable ABTS scavenging activity, with Pells demonstrating a higher DPPH activity compared to the peel (Gomathy et al., 2013). In a different study, bark ethanol extracts of *M. zapota* demonstrated significant DPPH inhibition activity of IC_{50} = 35.2 μg/mL, surpassing commonly known synthetic antioxidants (Islam et al., 2012).

In Table 2.4, the Sapotaceae family exhibits comparable superior DPPH scavenging activity (75.2%) compared to other families. Its TPC (94.5 mg/GAE/g) is greater than that observed in Ericaceae (52.76 mg/GAE/g) and Luminaceae (45.2 mg/GAE/g), highlighting the family's richness in polyphenols. Compared to Ericaceae and Luminaceae, the Sapotaceae family remains underutilized. However, the high TPC activity underscores the necessity for further studies. These studies collectively highlight the rich and diverse array of antioxidant compounds present in the Sapotaceae family and their potential health benefits. However, several limitations in the findings have been noted, including the variability in extraction protocols and solvent systems that reportedly complicated cross-comparisons (Silva et al., 2024). This, therefore, suggests the need for more animal models for studies dedicated to confirming vitro biological activity and efficacy. Although the reports have attributed the antioxidant activity of the plant extract to phytochemicals such as tannins, the synergistic effect between the two wild fruit trees of the Sapotaceae family remains underexplored. Future research should focus on the industrial applications of these plants, their role in phytochemistry for nutraceuticals, and the preservation of food and beverages.

Table 2.4:Comparative analysis of antioxidant activity across plant families

Family	Key antioxidant species	Notable activity (DPPH scavenging/TPC)	Distinctive compound	Reference
Sapotaceae	*Manikila zapota* (L.)	Peel extract DPPH (75.2%); TPC (94.5 mg/ GAE/g)	Triterpene Phenolic acid Tannins	(Gomathy et al., 2013; Rivas-Gastelum et al., 2023)
Ericaceae	*Vaccinium corymbosium* (L.)	Leaf extract DPPH (IC_{50}= 12.3 µg/mL); TPC (52.76 mg/GAE/g)	Chlorogenic acid Rutin Isoquercetin	(Czernicka et al., 2024)
Fagaceae	*Fagus sylvatica* (L.)	Leaf extracts DPPH (EC_{50}= 0.45 µg/Ml); TPC (78.3 mg/GAE/g)	Catechin epicatechin	(Formato et al., 2021)
Anacardiaceae	*Rhus coriaria* (L.)	Fruit extract DPPH (IC_{50}=18.7µg/Ml); TPC (127.4 mg/GAE/g)	Gallic acid Ellagic acid Anthocyanins	(Mazzara et al., 2023)
Lamiaceae	*Origanum vulgare* (L.)	Essential oil DPPH inhibition (82.4%); TPC (45.2 mg/GAE/g)	Carvacrol Thymol Rosmarinic acid	(Amato et al., 2024)
Rosaceae	*Cydonia oblonga* (Mill.)	Leaf extracts DPPH (89.5 µmol TE/g); TPC (7.8 mg/GAE/g)	Chlorogenic acid Quercetin	(Altuntas and Korukluoglu, 2024)

2.9. Reported Antioxidant Activity of *M. zeyheri* Extracts.

Mimusops zeyheri Sond is reported to be amongst the highly medicinal plants used to treat various ailments and conditions in many rural communities (Lubisi et al., 2024). However, studies on the antioxidant activity of *M. zeyheri* remain sparse. Previous studies have focused on the plant's ethnomedicinal applications and fruit nutritional composition, with no documentation of the plant's phytochemistry and in vitro bio-applicability potential. There is, therefore, a need for research focused on the antioxidant activity of the plant species using standardized protocols, exploring multiple solvents, and including different development stages of the wild fruit tree species to demonstrate variability in antioxidant activity and the influence of the various stages. Future research should also focus on the in vivo bioactivity, toxicity and efficacy of the plant and its extracts on animal models to leverage existing protocols from other *Mimusops* species, such as *M. elengi*. Lastly, there is a need to characterize and isolate the bioactive compounds within the plant species using methods and assays such as such as Electron Spin Resonance (ESR) Spectroscopy, Microfluidic-based LC-MS/MS and UHPLC-QTOF for phytochemical analysis. to better explain and understand their mechanisms of action and, therefore, their therapeutic relevance.

2.10. Relationship between Photochemical Composition and Antioxidant Bioactivity

Several studies have reported a positive correlation between medicinal plant phytochemistry and their antioxidant potential (Nwozo et al., 2023; Srivastava et al., 2012). The relationship is rooted in the presence of phytochemical compounds, including polyphenols, flavonoids, tannins, alkaloids and many classes of secondary metabolites that function as principal forces for redox-modulating activity (Fatima et al., 2021). There is a reported direct relationship between the antioxidant activity of medicinal plant species from various families and their TPC and TFC, as demonstrated using FRAP and DPPH assays (Fatima et al., 2021; Nwozo et al., 2023; Srivastava et al., 2012). Due to the high concentration of bioactive compounds such as betulinic acid and hydroxycinnamic derivatives, *Rhus tripartitum* (DC.) from the Anacardiaceae family (Shahat et al., 2016) and wild physalis spp from the Solanaceae family demonstrated the highest antioxidant activity compared to other cultivated varieties under similar stressful conditions (Kunat-Budzyńska et al., 2022). Similarly, phenolic acids such as gallic acid, chlorogenic acid and flavonoid glycosides work in synergy to enhance the antioxidant activity of medicinal plants such as *Origanum vulgare* (L.) and *Conscora decussate* (Roxb.) by collectively neutralising free radicals and chelating pro-oxidant metal ions (de Torre et al., 2022; Kousalya and Bai, 2016; Martins et al., 2024). This, therefore, means that the

antioxidant qualities of wild fruit trees and plants are enhanced or affected by environmental conditions, such as temperature and water availability, which correlate positively with their bioactive composition (Bautista-Expósito et al., 2018). On the contrary, halophytes such as *Spergularia marina* (L.) Griseb., found in salt marshes, exhibited a reduced concentration of flavonoids and phenolic compounds (Pungin et al., 2023).

The mechanistic functions of bioactive compounds are determined by their structural diversity; for example, the conjugated double bonds in carotenoids quench singlet oxygen, while hydroxyl groups on flavonoid aglycones enhance hydrogen-donating ability. Anthocyanins and condensed tannins in *Fagus* sylvatica (L.) and *Rhus coriaria* (L.) were uniquely characterised in a study by (Aranda et al., 2015). These profiles provide adaptive benefits against oxidative stress, leading to a more pronounced oxidant effect both in vitro and in vivo. Biological activity, however, is influenced by various factors, including extraction techniques, plant health, plant age, and environmental conditions such as pH, altitude, and soil characteristics (Csepregi et al., 2020). Results by Valle-Sánchez et al. (2025), reported a higher phenolic integrity retention in the decoction of Smilax. Spp compared to infusion, which has a direct impact towards their ability to block Advanced Glycation End Products (AGEs), which are linked to causes of chronic illnesses. Critically, the interaction between antioxidant activity and the complexity of plant phytochemistry highlights the importance of conserving plant biodiversity. Similarly, the synergistic interactions of specialized metabolites, such as triterpenoids, drive the reported strong correlation between phytochemical diversity and antioxidant activity in wild fruit trees from the Sapotaceae family (Sayma et al., 2025). Despite the lack of research, plants like *M. zeyheri* have potential phenolic-driven antioxidant potential, which may ultimately open opportunities for the development of nutraceuticals. Metabolomic profiling and in vivo studies must be prioritized in the future to fully harness the antioxidant and therapeutic potential of wild fruit trees such as *M. zeyheri*.

Table 2.5: Comparative analysis of antioxidant activity and phytochemistry of plant species across plant families

Specie	Family	Compound	Antioxidant activity (IC_{50}/EC_{50} / % Inhibition)	Reference
Allium flavum (L.) (**Small yellow onion**)	Amaryllidaceae	Gallic acid, Catechin, & Quercetin derivatives	High radical scavenging (DPPH: 85-92%; ABTS: 78-88%)	(Iwar et al., 2024)
Syzygium cumini (L.) Skeels **(Java plum**)	Myratle	Flavonoids, Phenolic acids	DPPH scavenging: 72-89%; FRAP: 65-78%	(Dos Santos et al., 2024)
*Achyranthes aspera (*L.) (**Devil's horseship)**	Amaranthaceae	Isoquercetin, Quercetin, Rutin	FRAP: 0.8-1.2 mM Fe^{2+}/g; DPPH: 75-88% inhibition	(Abhang et al., 2024)
Camellia oleifera C.Abel **(Tea oil camellia)**	Theaceae	Flavonoids, Phenolic acids	DPPH scavenging: 68-82%	(Zhou et al., 2024)
Dracaena sanderiana Mast **(Lucky bamboo)**	Asparagaceae	Quercetin, Kaempferol, Bilobalide	ABTS: 90-95% inhibition; FRAP: 1.5-2.0 mM Fe^{2+}/g	(Guo et al., 2024)
Ginkgo biloba (L.) **(Ginkgo)**	Ginkgoaceae	Anthocyanins, Procyanidins	ABTS: 92-97% inhibition; FRAP: 1.8-2.3 mM Fe^{2+}/g	(Pagotto et al., 2024)
Aronia melanocarpa (Michx.) Elliot **(Black chokeberry)**	Rosaceae	Hesperidin, Naringin	ORAC: 1200-1500 µmol TE/g; DPPH: 80-87% inhibition	(Saracila et al., 2024)
Citrus reticulata Blanco (**Ponkan)**	Rutaceae	Gallic acid, Catechin, and Quercetin derivatives	High radical scavenging (DPPH: 85-92%; ABTS: 78-88%)	(Liang et al., 2024)

2.11. Potential Impact of Agro-ecological Variation on Antioxidant Activity of *M. zeyheri*

The antioxidant activity of medicinal wild fruit trees varies significantly because of Agro-ecological factors. Plant phytochemistry and, consequently, biological activity is influenced considerably by agro-ecological parameters such as altitude, soil, rainfall, temperature, and solar exposure. Higher and dominant quantities of antioxidants and bioactive compounds have been reported in plants that grow under harsh environmental conditions, which have been linked to improved antioxidant activity. A study by Chivandi et al. (2011c) reported that modulatory enzymic antioxidants in *M. zeyheri* fruits grown in lime-stone soils enhanced ascorbate peroxidase activity by 38% in comparison to sandstone soils. Although anecdotal results and ethnobotanical knowledge suggest significant heterogeneity, no scientific study has assessed how the antioxidant activity of *M. zeyheri* varies across different agro-ecological zones. The only reported metabolomic mapping on the *Mimusops* genus is reported on only 62 volatile metabolites characterised from *M. caffra* (Baky et al., 2025) with no documented comprehensive phytochemical reports on *M. zeyheri*. A study by Chivandi et al. (2011a) reported 25.6% lipids in *M. zeyheri* nuts, which are rich in oleic acid. However, the impact of growth and environmental conditions on the identical lipids and other Fatty Acid Desaturases (FAD) gene expressions remain undocumented. While the study by Teffo et al. (2025b) shows 19% variations in fruit phenolic content from various accessions, genomic markers linking the chemotypes to their respective geographic locations are lacking. There is, therefore, a need to close existing gaps using LC-MS metabolomics profiling to assess *M. zeyheri* across various rainfall gradients. This will validate traditional knowledge associated with the ethnomedicinal use of the plant while also guiding the selection and isolation of target metabolites.

2.12. Propagation of wild fruit trees in Africa

Indigenous wild fruit trees have been a reliable source of food for many rural communities across Africa for centuries; however, their populations are facing decline due to deforestation, unsustainable land-use practices, globalization, and industrialization (Lambin and Meyfroidt, 2011). Plant propagation has been a part of the history of indigenous people since the beginning of plant cultivation, dating back centuries (Balick and Cox, 2020). Different propagation methods have been developed with time and need, including asexual using vegetative techniques and sexual using nuts. The 20th century saw significant advancements in propagation techniques. Auxins for

inducing rooting in cuttings were discovered, and the sterilization of propagation media using steam was adopted (Preece, 2003). Several researchers have highlighted the urgent need for studies that explore and enhance domestication efforts, as well as increased government incentives for cultivation. Efforts to cultivate and conserve indigenous wild fruit trees have been ongoing; however, progress has been limited due to challenges in germplasm availability, knowledge gaps in propagation methods, and reluctance to integrate traditional knowledge systems into conventional food production systems (Salgotra and Gupta, 2016). Successful propagation is essential for conservation, enabling sustainable cultivation and boosting rural livelihoods. Several studies have focused on standardized propagation methods, including asexual and sexual methods, for various wild and minor indigenous fruit trees (Megersa, 2017; Rajpurohit and Jhang, 2016). Nut propagation is the most common and easy propagation method; however, many wild fruit trees show nut dormancy and low germination rates and, therefore, require specific vegetative propagation methods such as cuttings, air layering, grafting and budding (Seglie et al., 2012). The effectiveness of vegetative propagation across wild fruit tree species remains unexplored, necessitating further research. The ongoing conflict in African countries, such as the Democratic Republic of Congo, has placed wild fruit trees under dire threat, emphasizing the need for urgent, effective, and sustainable conservation efforts.

2.12.1. Nut propagation and germination strategies for Wild Fruit Trees (WFT)

Nut propagation is the most traditional technique and is primarily preferred for breeding programs, as it produces genetically diverse and resilient plantlets. This propagation technique is easy and requires low costs; however, constraints such as nut dormancy common in wild fruit trees limit its effectiveness. Species such as *Sclerocarya birrea* and *Uapaca kirkiana* Müll.Arg. have recalcitrant nuts that show nut dormancy, necessitating pre-nut treatment to induce germination (Hamidou et al., 2014; Maliro and Kwapata, 2000). Plants such as *Cynara carduculus* var. scolymus have been successfully propagated through nut, and this approach has reportedly improved genetic diversity, leading to enhanced agronomic resilience (Huarte et al., 2018). Although genetic diversity is crucial, nut propagation is not ideal for domestication objectives that aim to achieve uniformity in fruit characteristics. Offspring usually exhibits different characteristics and, therefore, have different ecological demands, resulting in challenges associated with graftage determination. This technique has a long juvenile period, and consequently, it is less attractive to research on wild fruit trees, as many, such as *M. zeyheri,* are already slow growing by nature. Nut propagation is often

subjected to exposure to foreign pollination and current dichogamy in some wild fruit species (Huarte et al., 2018). Nut propagation is species-specific. Species such as *Carissa carandas* (L.) and *Cardia dichotoma* (L.) have been reported to propagate easily, while species like Ficus *carica* and *Robus ideaus* require specialised pre-nut treatments (Banik et al., 2014; Rahayu et al., 2017). Wild species, such as *Limonia acidissima* (L.) (wood apple), exhibited over 60% germination rates without signs of nut dormancy, with successful propagation achieved through asexual methods, including cuttings and air-layering (Sabarad et al., 2023). Species such as *Flacourtia jangormass* (Lour.) Raeusch exhibited increased germination and biomass after being soaked in cold water for 48 hours, while 3 Butaric acid reportedly (IBN) accelerated rooting in cuttings (Hossain et al., 2011). Successful nut propagation requires knowledge of the structural heredity of the "mother" source of the nuts. Parthenocarpy is also an issue associated with nut propagation, creating a problem as it necessitates the reproduction of the same tree species, including biologically nutless varieties (Maupilé et al., 2024).

Physical nut dormancy is common in some wild fruit trees due to the impermeability of the nut coating. Breaking nut dormancy involves the formation of specialised nut openings, also known as water gaps, in nuts to allow for water permeability (Ferreira and Vieira, 2024). Physiological dormancy due to embryo maturity is a notable issue in nut propagation, as it restricts germination because nuts are at rest. Breaking physiological dormancy can be done by mimicking the natural thermal environment. This can be achieved by regulating the temperature light to facilitate a balance in the release of ABA and gibberellins (GA) (Bewley et al., 2013). Gibberellic acid is synthesized at young plant points, including nuts and is responsible for cell elongation; therefore, it can be applied to induce germination. Ariez et al. (2023) reported the efficacy of GA3 1000MG/L applied for 6 hours in *P. suberosa* (L.), and the success rate was reported at 86% germination. A similar study by Bertsouklis and Papafotiou (2013) reported an 86% germination success rate in *Arbutus andrachne* (L.) nuts treated with 259 mg/L of GA3. These methods underscore the effectiveness of GA^3 in breaking nut dormancy in wild fruit trees. Research on nut germination rates under varying conditions reveals significant influences of moisture, temperature, light exposure, and soil type. A study by Mamani et al. (2018) found that temperature and substrate type significantly affected germination rates of *Eriotheca vargasii (Cuatrec.) A.Robyns*, with optimal germination in prepared media at controlled temperatures, while moisture levels had no effect. Cochrane et al. (2015) demonstrated that tree species in old fields exhibited a bivariate Gaussian

response to interactive temperature and moisture gradients, affecting nutling emergence. Castro (2006) highlighted the importance of high intensity and moisture for the emergence and growth of seedlings of several sub-tropical trees, while soil texture had minimal impact. Nuts in well-lit conditions had high germination success (Castro, 2006). In contrast, those in darkness or buried conditions showed reduced rates, emphasizing the critical role of light and moisture in nut germination across various species.

2.12.2. Vegetative propagation of Wild Fruit Trees (WFT'S)

Vegetative propagation is a very crucial technique for reproducing wild fruit trees that involve the use of plant parts that can develop roots and shoots. This method allows the development of clone offspring that inherit the desirable traits from the mother plant (Awotedu et al., 2021). Standard plant parts used include roots, shoots, stems, and leaves. This method is often preferred and widely regarded as effective in producing rapid multiplication essential for the domestication of WFT'S. Vegetative propagation techniques, such as air layering, grafting, budding, and stem cutting, have reportedly been successful in propagating wild fruit trees, including *Syzygium cumini* and A*langium salvifolium* (L.F.). Wangerin and *Averhoa carambola* (L.) effectively (Bharad and Mahorkar, 2011; Pandey et al., 2022).The historic shift from sexual to vegetative propagation has facilitated the successful domestication of now commercially important wild species, such as olives and grapes. Successful vegetative propagation depends on the type of technique chosen and compatibility with the plant species. Stem cuttings have been reported successful in propagating wild fruit trees such as *Ficus palmata* Forssk, *Dacryodes edulis* (G.Don) H.J.Lam and *Psidium guajava* (L.) *(*Abdullah et al., 2006; Mewar et al., 2018; Mialoundama et al., 2002*)*. This technique involves cutting a stem portion from a mature mother plant and stimulating root and shoot formation in a controlled environment using suitable growing media. Stem cutting is a relatively straightforward and practical technique compared to others; hence, it is widely preferred for the propagation of ornamental plants (Ak et al., 2021).

Table 2.6: An inventory of Wild fruit trees from the Sapotaceae family propagated through nuts and their success rate.

Species	Success rate (%)	Nut preparation method	Nut viability period	Germination period (Days)	Reference
Vitellaria paradoxa C.F. Gaertn. **(Shebe)**	30-45	Depulping and nut sun-drying	2-3 weeks	15-30	(Aderounmu et al., 2020)
Manilkara zapota (L.) P. Royen **(Sapodilla)**	60-75	Fresh nuts	3-4 months	30-45	(Kaur, S. et al., 2020)
Chrysophyllum roxburrghii G. Don **(Wild star apple)**	45-60	Fresh nuts	2-4 months	25-40	(Ramachandran and Aruna, 2019)
Sarcosperma laurinum (Benth.) Hook.f. **(Laurine tree)**	40-55	Soaking	2-3 months	30-45	(Nanyan et al.)
Madhuca longifolia (L.) J.F. Macbr. (**Mahua)**	75-90	Fresh nuts	3-4 months	14-28	(Shirin et al., 2020)
Argania spinosa (L.) Skeels **(Argan)**	35-50	Cracking the woody endocarp	5-6 months	35-60	(Bezzalla et al., 2018)
Chrysophyllum cainito G. Don **(Star apple)**	40-55	soaking	2-3 weeks	21-30	(Parker et al., 2010)
Manilkara hexandra (Roxb.) Dubard **(Khirni)**	55-70	Water soaking	2-3 months	30-45	(Jasani et al., 2024)
Pouteria macrophylla (Lam.) Eyma **(Cutite)**	65-80	Mechanical stratification	3-5 weeks	35-40	(do Nascimento et al., 2024)
Chrysophyllum albidum G. Don **(African star apple)**	45-60	Hot water treatment	2-3 weeks	20=35	(Fredrick et al.)

2.13. Integration of Indigenous knowledge and scientific research in wild fruit trees and plant species.

The integration of Indigenous Knowledge with scientific research has, in recent decades, gained attention and recognition as a valuable approach to mitigating the effects of climate change and associated developmental challenges. Several reports suggest that integrating Indigenous Knowledge (IK) and scientific research can potentially enhance climate adaptation strategies, improve sustainable agricultural production and practices, and support effective land-use practices (Basdew et al., 2017). These indicate an incredibly positive and transformative shift in methodologies used to study indigenous wild plant species and processes. Indigenous Knowledge systems embody holistic, context-specific knowledge gained through generations of environmental interactions, lived experiences, and cultural and spiritual practices. Scientific methodologies, on the other hand, are grounded in empirical methods that have recognized the significance of integrating Indigenous Knowledge Systems to enhance the depth, relevance and sustainability of research outcomes. This aligns with the noticeable global shift in which research methodologies are decolonized to ensure the inclusion of Indigenous people and rural communities as active knowledge co-creators rather than study subjects. Despite the progress and potential benefits associated with this integration, persistent challenges remain in documenting IK, which is fading due to modernization and issues related to changing landscapes (Blose and Gumbo, 2024). There is, therefore, a need for a systematic and well-rounded approach that explores IKS integration with scientific methods to develop well-informed systems and frameworks (Malapane et al., 2024). Several international and national frameworks have laid the foundation for the integrative approach of IKS and scientific research to address historical marginalization and promote equitable practices. Approaches such as “Research is a ceremony; and’ Two-eyed seeing and doing research in a good way have been introduced in Canada, for instance (Greenwood et al., 2022). Other approaches include an indigenous research framework, including the United Nations Declaration on the Rights of indigenous people (UNDRIP) signed in 2007 with international standards for research that mandates the respect for Indigenous people’s rights to control, protect and maintain, among

other things, their cultural heritage (Ignace et al., 2023; Sargent, 2016). The UNESCO local and Indigenous Knowledge Systems (LINKS) programmes, Convention on Biological Diversity (CBD), Nagoya Protocol, and the International Society of Ethnobiology (ISE) Code of Ethics recognize the importance of IKS, promote the integration of IKS, and detail the principles and practices for Ethnobotanical research. Similarly, South Africa's Indigenous Knowledge Systems (IKS) policy (2004), Protection, Promotion and Development Management of Indigenous Knowledge Act (No.6, 2009), were adopted as legislative landmarks to maintain, preserve, improve and legally protect the indigenous knowledge and practices for all South Africans (Mugabe, 2023). Other acts, including the Biodiversity Act (No. 10 of 2004) and associated acts, emphasize the importance of protecting IKS in South Africa, particularly in bioprospecting and biodiversity research (van Zyl et al., 2023). These frameworks, Acts, and policies emphasize multilocality, rationality, and accountability centered around Indigenous people to bridge existing gaps between IKS and scientific research, promoting inclusive and culturally responsive research approaches (Reano, 2020).

2.13.1. Participatory research approaches

The integration of Indigenous Knowledge Systems with Systematic research requires the recognition and acknowledgement of Indigenous people and communities as equal partners in research. Participatory research approaches have evolved to be more inclusive by prioritizing Indigenous communities and their voices to foster true collaboration. Integrated IKS and Systematic participatory approaches strive to power imbalances, enhance developmental outcomes, and promote social action (Kwan and Walsh, 2018). Scholars emphasize that IKS is dynamic and ever-changing and that it should be recognized as such, even when policies are developed and implemented for alignment (Nyahunda, 2024). The alignment of research approaches with IKS principles remains a persistent issue. There is, therefore, a notable increase in demand for IKS-aligned research approaches that recognize the importance of story and narrative relationships in IKS while addressing the ethical concerns and crisis of poor representation in action research to encourage meaningful conversations between different knowledge systems (Keane et al., 2017). Participatory research approaches include

Community-based Participatory Research (CBPR), which has been reported effective in Indigenous knowledge studies (CBPR). In the Eastern Cape province of South Africa, CBPR demonstrated significant ecological knowledge about over 70 indigenous wild fruit trees and other plant species (Shava, 2000) Indigenous communities have demonstrated expertise and skills in identifying and differentiating wild plant species, which have been validated through molecular studies (Gardner et al., 2022). An engagement study by Gardner et al. (2022) with Indigenous knowledge holders of the Eastern Cape province confirmed two distinct species with distinguished characteristics, "*Artocarpus ordoratissmus* and *Artocarpus mutabilis,* which were initially considered a single species in scientific taxonomy. These results underscore and demonstrate how CBPR can potentially advance biodiversity science and Conservation, including a decolonizing approach that challenges the current status quo dominated by Western knowledge systems. The CBPR approach presents challenges related to ethical concerns, complex dynamics in balancing community and individual needs, complex power dynamics and institutional board review requirements (Kwan and Walsh, 2018).

In the Limpopo province, Participatory Rural Appraisal (PRA) has been successfully employed to integrate Indigenous Knowledge Systems (IKS) and systematic research. Many rural communities in the region rely on indigenous species for their livelihood, utilizing PRA techniques such as seasonal calendars, lived experiences, and direct observations. Another study conducted using PRA documented over 92 indigenous wild fruit species from 20 families, 27 of which reportedly had additional uses beyond food and medicinal purposes (Mokganya et al., 2019). Despite the potential of PRA approaches in integrating IKS and systematic research, a significant epistemological gap remains. Scientific research and approaches prioritize quantitative and replicable studies, while IKS is qualitative and orally transmitted. Acknowledging the differences and, therefore, integrating the two systems without devaluing the other remains a challenge. Power imbalances between researchers and Indigenous communities threaten genuine participation and collaboration. Decisions on research priorities in community-based participatory research, including data arrangement and presentation, as well as academic output in terms of publications, are often determined by

external researchers rather than the communities, promoting colonial imbalances and inequalities disguised as collaboration. Despite frameworks and policies, significant inconsistencies persist in the application of fair benefit sharing. Many Indigenous rural communities lack formal education and have limited negotiation experience and resources, therefore leaving them vulnerable to exploitation.

2.13.2. Knowledge co-production models

Knowledge co-creation is developed and implemented based on several key principles that recognize contextual differences, intentionality in involving and engaging Indigenous communities and creating a common understanding for the co-development of shared goals while empowering and promoting participation at every stage of the process. A study by (Ahada et al., 2024) explored the effectiveness of digital storytelling and traditional scientific data from Indigenous and marginalized communities to inform governance and stakeholder discussions and interventions on local food production and security in South Africa. The study emphasizes digital storytelling as a reliable and effective mechanism for knowledge creation, social learning, and detailed knowledge provision. These findings align with other studies that report digital storytelling as a valuable mechanism for creating meaningful participatory spaces, addressing stigma, and enabling agency among participants (Mnisi, 2015) as well as implementing successful community-based projects (Piper et al., 2025).

Developed knowledge creation, such as the “two-eyed” model by Marshall and Bartlett (2004) offers an excellent and promising foundation; however, there is a persistent lack of empirical research aimed at assessing its effectiveness and adaptability in different African indigenous contexts. The lack of empirical results associated with the use of these models in Africa is attributed to the existing structural barriers and inequalities, lack of funding, and poor institutional support for the integration of IKS (Mekoa, 2015). The lack of decolonized and standardized documentation methods for Indigenous knowledge has led to fragmentation in the implementation of knowledge co-creation models in South Africa (Swilling, 2014). This has led to inadequacies in cross-disciplinary training programs that integrate Indigenous knowledge practices (Mosimege, 2020). These challenges hinder the deployment and

promotion of culturally rooted ecological knowledge, specifically knowledge about indigenous wild fruit tree species such as *M. zeyheri,* which is crucial for food security in rural settings and has potential for economic growth through product development, including beverages, snacks, and pharmaceuticals. Advancing Knowledge co-creation requires inclusive and culturally grounded approaches led by Indigenous people. Countries like South Africa need to make funding available for long-term, sustainable research initiatives.

2.13.3. Ethical consideration in IKS research, intellectual property rights and benefits sharing

Historically, marginalized Indigenous communities were exploited, and their rights were not considered. Research on IKS requires careful adherence to ethical protocols; therefore, the integration of IKS and systematic research necessitates recognition of the plurality of IK and the practice of thoughtfulness to achieve cognitive justice. Research, especially research work conducted with Indigenous communities, must have appropriate protocols and frameworks that incorporate a code of conduct that respects Indigenous knowledge systems, transparent and honest terms of dialogue and integration, fairness, and just benefit sharing with all participants. Research objectives and goals must be clearly communicated to Indigenous communities to establish cultural safety, eliminate power imbalances, and ensure that the communities are primary decision-makers in determining the culturally respectful and appropriate level of research, including how the findings are documented and disseminated to the public. The ethical integration of IKS and scientific research requires the protection of Indigenous communities' intellectual property rights (IPR) as a foundational principle. Documenting Indigenous knowledge is essential for protecting community rights and for patent claims

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CHAPTER THREE

REVIEW OF *MIMUSOPS ZEYHERI* SOND. (MILKWOOD): DISTRIBUTION, UTILISATION, ECOLOGY, AND POPULATION GENETICS

Summary

Mimusops zeyheri Sond. (Milkwood) is an indigenous fruit tree species with considerable ecological, cultural, and nutritional significance that remains underexploited. This review synthesizes current knowledge on its distribution, taxonomy, phytochemistry, ethnomedicinal applications, ecological functions, genetic diversity, and biotechnological potential. A systematic literature search, spanning 1949 to April 2024, yielded 87 relevant publications from an initial 155. *M. zeyheri* plays a crucial role in supporting the cultural traditions and economic activities of Indigenous Southern African Communities. Its distribution encompasses South, East, and Southern Tropical Africa, with substantial populations across South African provinces. Ethnomedicinally, various plant parts treat conditions including wounds, gastrointestinal issues, and diabetes. Leaves (34%) and roots (32%) are used, with infusion (33%) and decoction (31%) as primary preparation methods. Oral administration (70%) is the most common, primarily addressing skin conditions (18%). Despite its nutritional richness, a standardized nutrient profile is lacking. Limited genetic diversity studies underscore the need for further research. This review highlights *M. zeyheri's* multifaceted importance and research gaps, particularly in other Southern African countries.

Keywords: Biotechnological applications, Ecological roles, Ethnomedicinal, Genetic diversity**,** Indigenous Knowledge, Nutrients

3.1. Introduction

Mimusops zeyheri Sond., also known as Transvaal red milkwood, is a perennial fruit tree belonging to the Sapotaceae family. The Sapotaceae family from which *M. zeyheri* belongs is more commonly referred to as the Sapodilla family and comprises an estimated 1,250 species and 53 genera of tropical trees, shrubs, and branching vines (Baky et al., 2022a; Pennington, 2004; Swenson and Anderberg, 2005). Researchers have devoted considerable attention to this family on account of its ecological significance, phytochemical diversity, and economic importance (Armstrong et al., 2014; Baky et al., 2022a; Panchal Mital and Jha, 2021).

Sapotaceae species contain an assortment of bioactive compounds, including triterpenoids, saponins, and phenolic compounds, according to phytochemical studies (Chakradhari et al., 2019; Devi and Sangeetha, 2016; Mechqoq et al., 2021). The phytochemical diversity of the Sapotaceae family was illustrated by Dasgupta et al. (2013) who determined the antioxidant and cytotoxic properties of triterpenoids extracted from *Mimusops elengi*, an extant species belonging to the family while, Lim, (2012) also demonstrated the phytochemical and pharmacological properties of *Chrysophyllum cainito*, also known as star apple, with an emphasis on its possible therapeutic uses. Several studies have primarily examined the economic significance of different Sapotaceae species, specifically in relation to their contributions to the production of edible fruits, timber, and traditional remedies (Deklerck et al., 2021; Ojo et al., 2021; Patel and Rao, 2012; Pennington, 2004). The contribution of Sapotaceae species to biodiversity, their interactions with pollinators and nut dispersers, and their function in tropical forest ecosystems have also been the subject of ecological research (Christe et al., 2021).

Mimusops zeyheri is a small to medium-sized evergreen fruit tree reaching a maximum height of 25 m at full maturity (Lemmens, 2005a). This species is characterized by milky latex, and a well-branched, spreading, and rounded crown (Janick and Paull, 2008). The bark ranges in colour from grey to dark brown with a reticulated fissure pattern (Eliton et al., 2011). The tree has an elongated trunk characterized by the absence of planks. The leaves are shiny and arranged in a spiral pattern, lacking stipules, with a petiole length ranging from 0.5 to 3.5 cm (Hamdy et al., 2022; Mashela and Mollel, 2001). The upper leaf surface has prominent vein reticulation, while the lower surface has elevated vein reticulation. The length of the leaf blade is typically 2-3 times more than its width, and its apex is either acute to obtuse or often bluntly apiculate (Hamdy et al., 2022). The lateral veins are arranged in 10-15 pairs.

The distribution of this species spans across South Tropical Africa, including Angola, Malawi, Zambia, Mozambique, and Zimbabwe, extending northward to Tanzania in East Tropical Africa, and southward to Southern Africa, encompassing Botswana, South Africa, Eswatini, Lesotho Namibia (Lemmens, 2005a; Omotayo et al., 2020). In Egypt, *M. zeyheri* heads in the

direction of its variety var. *laurifolia* in terms of morphological characteristics and taxonomy, showing significant similarities and overlap that make the two taxa difficult to definitively distinguish from each other (Hamdy et al., 2022). The tree is native to the northern and eastern parts of South Africa (Beinart and Coates, 2002). In South Africa, *M. zeyheri* is primarily distributed in provinces including the Limpopo, Northwest, Gauteng, Mpumalanga, and KwaZulu Natal Provinces, and therefore, distinctive ethnic groups associated it with distinct vernacular names, for instance, the Afrikaner people call it Moepel, Mmupudu (Northern Sotho), Umpushane (Zulu), Mbubululu (Venda), and Mgamba kapu in Isiswati (Lemmens, 2005a; Lubisi et al., 2023). Evidence shows that *M. zeyheri* typically thrives in habitats including wooded, rocky hillsides or river basins (Lubisi et al., 2023; Venter and Venter, 1996). These species possess a non-invasive lateral root system that is well-suited for rocky habitats with poor-quality soils (Chivandi et al., 2011a; Van Wyk, 2011a). *Mimusops zeyheri* is known to exhibit optimal growth within a temperature range of 12 to 25 ºC, accompanied by an average annual precipitation of 464 mm (Mashela et al., 2013a). *Mimusops zeyheri* can withstand freezing temperatures without harm and needs minimal care, including at least six hours of sunlight every day and a small amount of water to thrive (Hamdy et al., 2022; Mngadi et al., 2019b; Van Wyk, 2011a). This evergreen tree is drought-tolerant, salt-tolerant, and very resistant to root-knot nematodes (Meloidogyne species) and other pests (Monyela, 2021).

Despite the ecological, cultural, and nutritional significance of *M. zeyheri* Sond. in Africa, comprehensive information about this species remains fragmented and underutilized. The lack of synthesized knowledge on its distribution, traditional uses, phytochemical composition, genetic diversity, and potential applications hinders its conservation and sustainable exploitation. Moreover, increasing threats to its natural populations, coupled with limited scientific research, pose challenges to developing evidence-based management strategies. This review aims to consolidate existing knowledge, identify research gaps, and provide a comprehensive overview of *M. zeyheri* to guide future research efforts and inform conservation strategies.

3.2. Materials and Methods

To create a thorough search string, appropriate keywords and their synonyms were identified. Using the proper truncations, alternate spellings were taken into consideration. Relevant articles for the review were found using the following search terms: *Mimusops zeyheri*, Indigenous, ethnomedicinal, nutrients, ecological roles, genetic diversity, and Biotechnological applications; these databases included Scopus, Google Scholar, EBSCO host Academic Search Complete, and ScienceDirect Once the search term was finalized, these databases were searched to find pertinent articles. The search was limited to finding data that matched the goals of the study when the "explode" option was chosen. The search was restricted to Africa, and the period of the literature search was from 1949-April 2024. Studies that fulfilled the requirements for inclusion were downloaded to evaluate their content. Eight themes emerged and were categorized: (i) *M. zeyheri*'s historical and cultural significance among indigenous communities in South Africa; (ii) Traditional medicinal uses and healing properties associated with *M. zeyheri*; (iii) *M. zeyheri*'s ethnomedical uses and modes of administration; (iv) *M. zeyheri* as a source of nutrients; (v) *M. zeyheri*'s ecological importance and ecosystem services; (vi) Conservation status and threats to *M. zeyheri* populations; (vii) Genetic diversity and population genetics in *M. zeyheri*; and (viii) Scientific research and biotechnological applications of *M. zeyheri*. The most thoroughly covered theme in the research questions or findings was used to categorize articles with several themes. Microsoft Word and Excel were used for the article selection, summary, and coding processes, which produced the expected minimal variability.

3.2.1. Literature search results

The systematic review protocol implemented in this study rigorously adhered to the PRISMA framework as described by Moher et al. (2015) and Shamseer et al. (2015) in Figure 3.1. The identification phase encompassed a multifaceted search strategy, utilizing an array of electronic databases. This primary search was augmented by reference list examination and expert recommendations, yielding an additional 11 records from dissertations, theses and books retrieved from the University of Mpumalanga (UMP) Library. The amalgamation of these

search efforts culminated in a corpus of 155 documents for comprehensive full-text assessment. The subsequent screening phase entailed a meticulous examination of titles and abstracts, resulting in the exclusion of 38 documents and the retention of 117 for further scrutiny. The eligibility phase focused on the elimination of duplicate entries, necessitating the removal of 30 documents. Consequently, 87 unique documents progressed to the final inclusion stage. These 87 documents underwent rigorous full-text screening and were incorporated into the systematic review (Figure 3.1). Furthermore, pharmacological studies, in vitro studies, field trials, and ethnobotanical surveys reporting on the traditional use of *M. zeyheri* in Africa accounted for 36.78% of the total literature in the review (Table 3.1). The geographical distribution of research on *M zeyheri* demonstrates a significant concentration in Southern Africa, with South Africa accounting for (75%) of the eligible studies, followed by Botswana and Zimbabwe (6.25% respectively), and Eswatini, East Africa, Egypt, and Namibia (3.13% respectively) as shown in (Table 3.1). The preponderance of South African studies may be attributed to the species' widespread distribution, cultural significance, and established utilization patterns within the country (Wyk and Gericke, 2000). This regional focus suggests a heightened awareness of *M. zeyheri's* potential among South African researchers compared to their counterparts in other African nations. However, this geographical disparity in research intensity may result in other Southern African countries overlooking the potential benefits of *M. zeyheri*, underscoring the need for more collaborative, transnational research initiatives to comprehensively explore the plant's potential and ensure its sustainable utilization (Gordon-Cumming, 2017; Kalaba et al., 2009). Methodologically, ethnobotanical surveys predominate (46.88%) followed by in vitro studies (15.66%) and elemental analyses (12.5%), proximate analysis (6.25%), and various other approaches including field trials, feeding trials, genetic studies, taxonomic revisions, characterisation, and market surveys (3.13% respectively). The prevalence of ethnobotanical surveys is consistent with the importance of documenting traditional knowledge and uses of *M. zeyheri*, which serves as a crucial foundation for understanding the plant's potential and identifying promising research directions (Cotton, 1996). However, the current methodological distribution suggests that research on *M. zeyheri*

is still in its nascent stages, emphasizing the need for more advanced laboratory techniques to elucidate the plant's chemical composition, pharmacological properties, and potential applications across various fields (Gurib-Fakim, 2006).

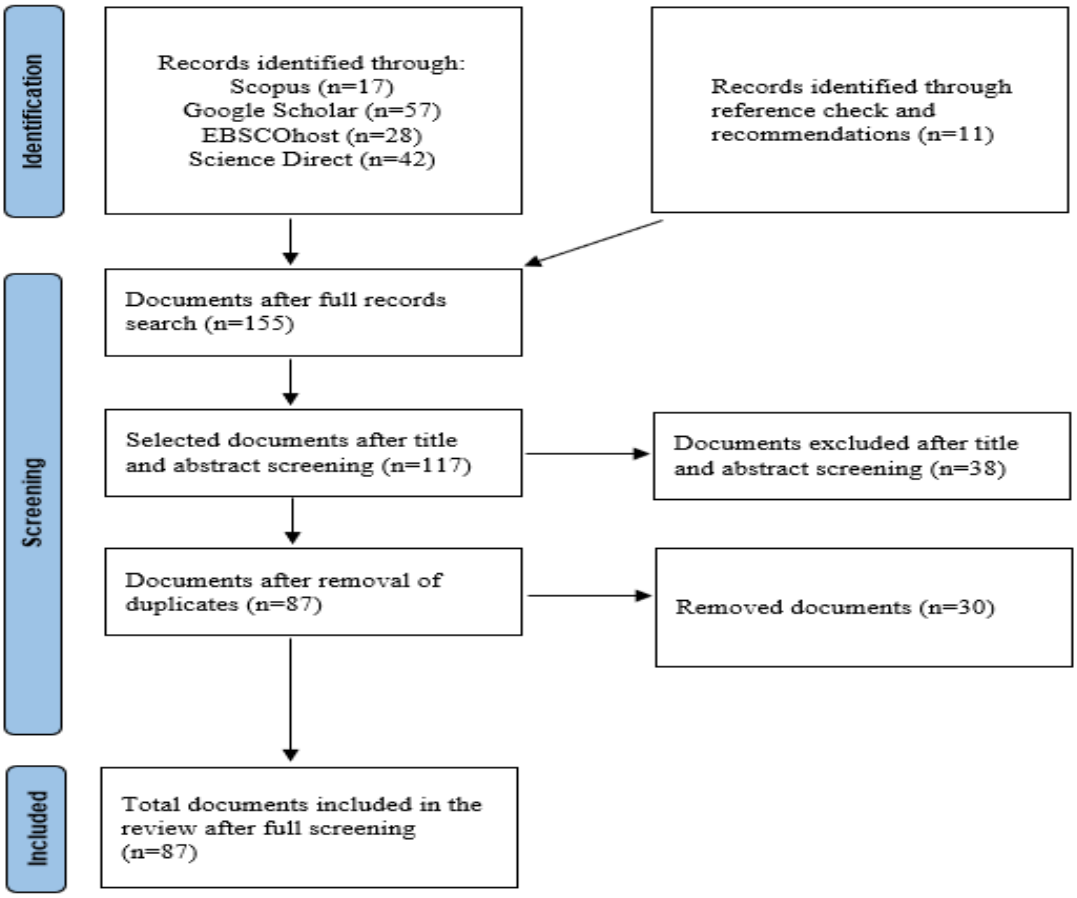

Figure 3. 1: Literature search method used for the selection of articles included in this review.

3.3. Results and discussion

Table 3.1: Summary of Studies on *M. zeyheri* in Sub-Saharan Africa.

Authors	Country	Method	Major findings
(Cheikhyoussef et al., 2011)	Namibia	Market survey	Identified *M. zeyheri* as an important indigenous fruit tree with potential for commercialization.
(Chivandi et al., 2011a)	South Africa	Proximate analysis	Analyzed the nutritional composition of *M. zeyheri* nuts, including proteins, fatty acids, and vitamins. Reported that *M. zeyheri* nuts are rich in proteins, oleic acid, and vitamin E, indicating potential as a dietary energy supplement and oil source
(Chivandi et al., 2020)	South Africa	Feeding Trial	Evaluated the potential of *M. zeyheri* nut meal as a substitute for maize meal in Japanese quail diets.
(Chivandi et al., 2012a)	South Africa	In vitro assay	Reported the antiproliferative effect of *M. zeyheri* nut oils on Caco-2 and HEK-293 cell lines.
(De Wet et al., 2012)	South Africa	Ethnobotanical Survey	Documented the use of *M. zeyheri* bark, stem, and roots for treating wounds, sores, gonorrhea, and candidiasis.
(Dube, 2023)	South Africa	Ethnobotanical survey	Surveyed and documented the use of *M. zeyheri* leaves for treating tonsillitis.
(Gelfand, 1985)	Zimbabwe	Ethnobotanical survey	Reported the use of *M. zeyheri* nuts as a blood purifier, and leaves for treating dysentery, boils, abscesses, convulsions, and as a sedative.
(Gomes et al., 2019)	South Africa	In vitro assay	*M. zeyheri* nut oil induced cytotoxic effects on MDA-MB-231 breast cancer cells and inhibited the growth of MCF-7 cells.
(Hamdy et al., 2022)	Egypt	Taxonomic revision	Provided a taxonomic revision of the *Mimusops* genus in Egypt, including *M. zeyheri*, and documented its traditional uses for treating wounds and pain relief.
(Hutchings and Scott, 1996)	South Africa	Ethnobotanical survey	Documented the traditional uses of *M. zeyheri* for treating snakebites, scorpion stings, arthritis, dysentery, boils, and abscesses.
(Kokwaro, 2009)	East Africa	Ethnobotanical survey	Documented the use of *M. zeyheri* bark for treating jaundice and as a hepatoprotective agent.
(Kunene et al., 2020)	Eswatini	Ethnobotanical survey	Reported the use of *M. zeyheri* leaves for treating digestive issues.
(Ledwaba, 2008)	South Africa	Genetic diversity analysis	Reported 91% genetic variability among *M. zeyheri* populations in Limpopo Province, South Africa.
(Lubisi et al., 2023)	South Africa	Ethnobotanical survey	Explored local perceptions, utilization, and population status of *M. zeyheri* in the Vhembe Biosphere Reserve.

Authors	Country	Method	Major findings
(Magwede et al., 2019)	South Africa	Ethnobotanical survey	*M. zeyheri* is used traditionally for food, medicine, and construction. The fruit is rich in vitamin C and has the potential for commercialization.
(Mahwasane et al., 2013)	South Africa	Ethnobotanical survey	Reported the use of the whole *M. zeyheri* plant as an aphrodisiac.
(Marobela et al., 2011)	Botswana	Elemental analysis	Analyzed the presence of potentially toxic heavy metals in *M. zeyheri* roots.
(Maroyi, 2011)	Zimbabwe	Ethnobotanical survey	*M. zeyheri* fruits are consumed fresh or processed into jams and jellies. Bark and roots are used in traditional medicine.
(Mashela and Mollel, 2001)	South Africa	Ethnobotanical survey	Identified *M. zeyheri* as an important Indigenous fruit tree with suitable attributes for the semi-arid Northern Province of South Africa
(Mashela et al., 2013a)	South Africa	Field trial	Reported that different essential nutrient elements during and after fruiting in the soil limited the growth of *M. zeyheri* trees and could be used in supplementary fertilization.
(Matlala et al., 2024)	South Africa	Ethnobotanical survey	Investigated the traditional uses of *M. zeyheri* in Gauteng Province, including treating headaches, Hlogwana (sunken fontanelle), boosting immunity, and as a cleansing and purification agent.
(Mngadi et al., 2019b)	South Africa	Elemental analysis	*M. zeyheri* fruits have high levels of chromium and manganese, contributing to their nutritional value.
(Monyela, 2021)	South Africa	Characterization of endophytic fungi	Identified endophytic fungi associated with *M. zeyheri* leaves, including Teratosphaeria and Zeloasperium species.
(Mudau et al., 2022)	South Africa	Ethnobotanical survey	Documented the traditional use of *M. zeyheri* for treating diabetes in the Vhembe District, Limpopo Province.
(Mulaudzi et al., 2012)	South Africa	In vitro assay	Leaf and bark extracts of *M. zeyheri* showed antibacterial activity against various pathogens, including Staphylococcus aureus.
(Neuwinger, 2000)	South Africa	Ethnobotanical survey	Reported the use of *M. zeyheri* roots for treating weight loss.
(Okatch et al., 2012b)	Botswana	Elemental analysis	Analyzed the presence of heavy metals in *M. zeyheri* leaves.
(Ramavhale et al., 2024)	South Africa	In vitro assay	The biological activity was reported for folkloric plants used in the treatment of ‘u wela’ including *M. zeyheri*. The hexane, aqueous, and decoction extracts of *M. zeyheri* showed promising antibacterial activity against *Neisseria gonorrhoeae*, with low minimum inhibitory concentration (MIC) values ranging from 0.02-0.03 mg/mL. The study reported the aqueous extracts of *M. zeyheri* demonstrated noteworthy anti-Candida activity with an MIC value of 0.02 mg/mL against *Candida albicans*.

3.3.1. Historical and cultural significance of M. zeyheri among indigenous communities in Southern Africa

Mimusops zeyheri is an important, underutilized tree species that local people associate with, providing various ecosystem services, including fruits, phytomedicine, cooking oil, and timber (Lubisi et al., 2023). According to Omotayo et al. 2020, *Mimusops zeyheri* holds a significant role and it is largely used by local people in the Southern Africa region. From this context, it is arguable that this species serves as a historical emblem of cultural and ecological importance in South Africa. *M.zeyheri* is not merely a botanical entity in indigenous communities; it is also deeply ingrained in traditional customs, beliefs, and folklore (Lubisi et al., 2023; Monyela, 2021). The tree's strong and tough reddish-brown timber has been highly valued throughout history for its adaptability and was thus used in the creation of traditional equipment (wooden axe), elements and devices of folk music and ceremonial artefacts (Adams, 2021). The tree solid wood which has been believed to possess metaphorical significance commonly used in rites and ceremonies that represent strength, endurance, and ancestral ties (Hutchings and Scott, 1996). In several indigenous cultures, the tree holds profound significance, deeply intertwined with myths, legends, and spiritual beliefs (Table 3.1). It is regarded with utmost reverence as a sacred entity embodying ancestral wisdom and providing protection to those who utilize it according to ancestral guidance (Hutchings and Scott, 1996*)*. The crucial role of this indigenous wild fruit tree in cultural ceremonies, rites of passage, and spiritual rituals like Inthwaso (a Zulu spiritual initiation) underscores its embodiment of cultural continuity and identity among indigenous communities (Hamdy et al., 2022*)*. The tree serves as a living connection to heritage, traditions, and the collective ancestral knowledge passed down through generations (Hamdy et al., 2022; Lemmens, 2005a).

3.3.2. Traditional medicinal uses and healing properties associated with *M. zeyheri*

Traditional medicine has a long and storied history, with most of the world's medicinal plant populations originating from Asia, Africa, and Latin America *(Tilburt and Kaptchuk, 2008)*. Ojewole (2006) estimates that more than 80% of Africans still use traditional medicines made from plants for both personal care and medicinal purposes. According to *Van Wyk (2011a)*, approximately 25% of the world's higher plants, including wild fruit trees are found in Southern Africa. This makes the Southern African region fall amongst the richest plant diversity regions globally. Other species within the genus Mimusops including *Mimusops elengi* Linn., and

Mimusops hexandra (Roxb.), also possess a range of pharmacological properties, including antibacterial, anti-inflammatory, and antiulcer effects (Dutta et al., 2011; Roqaiya et al., 2015). Literature evidence informs that most plant species that share similar genera possess similar medicinal properties (Omotayo et al., 2020*)*. Undoubtedly and arguably so, *M. zeyheri*. could possess similar medicinal properties as other species that belonged to a similar genus. Yet recent scientific evidence revealed that there is still a dearth of studies that have specified the medicinal usage *of M. zeyheri* in the Southern African context (Matlala et al., 2024).

Table 3.2: Ethnomedicinal uses of *M. zeyheri* and modes of administration in Southern Africa.

Ethnobotanical use	**Plant part**	**Method of preparation**	**Administration**	**References**
Treatment of wounds and sores	Stem and Bark	Decoction and pulverization (Bark is dried and crushed into fine powder)	Poultice	(De Wet et al., 2012; Mashela et al., 2013a)
Treatment of wounds (Tilondza)	Fruits and Flowers	Pounded into paste	Poultice	(Mashela et al., 2013a; Matlala et al., 2024)
Treatment of gonorrhea	Roots	Infusion and Decoction	Orally	(De Wet et al., 2012)
Treatment of candidiasis	Bark	Infusion and Decoction	Orally	(De Wet et al., 2012; Ramavhale et al., 2024)
Treatment of ulcers	Bark and roots	Decoction	Orally	(Adhikari et al., 2018)
Treatment of tuberculosis	Roots	Infusion and Decoction	Orally	(Chivandi et al., 2020)
Treatment of weight loss	Roots	Decoction	Orally	(Neuwinger, 2000)
Treatment of headache	Roots	Roots are dried and pulverized into fine powder	Snorted like snuff and inhaled as smoke when burned	(Matlala et al., 2024)
Treatment of womb issues	Roots	Infusion and Decoction	Orally	(De Wet et al., 2012)
Treatment of stomachache	Bark	Decoction	Orally	(Mashela et al., 2013a)
Treatment of menstrual pains	Leaves	Infusion	Orally	(Abd-Elfarag and van Hensbroek, 2019)
Treatment of foul-smelling discharge	Leaves	Infusion	Orally	(Abd-Elfarag and van Hensbroek, 2019)

Ethnobotanical use	Plant part	Method of preparation	Administration	References
Treatment of sunken fontanelle in infants	Leaves	Leaves are dried and later burnt to create ashy powder	Poultice	(Matlala et al., 2024)
Treatment of gastrointestinal issues	Whole plant (Leaves, barks, and nuts)	Decoction	Orally	(Abd-Elfarag and van Hensbroek, 2019)
Treatment of *diabetes mellitus*	Leaves	Decoction	Orally	(Abbet et al., 2014)
Treatment of erectile dysfunction	Whole plant (Leaves, barks, nuts, roots)	Leaf infusion, Nuts are dried and pulverized to mix powder with food. Root decoction	Orally	(Abdillahi and Van Staden, 2012)
Boosting immunity in humans	Roots	Infusion	Orally	(Matlala et al., 2024)
Treatment of gum inflammation	Roots	Decoction	Orally	*(Semenya and Maroyi, 2019)*
Treatment of toothache	Leaves	Infusion	Orally	(Semenya and Maroyi, 2019)
Ethnoveterinary, to improve livestock sexual performance (goats) during the breeding season	Leaves	Leaves are mixed with animal feed	Orally	(Matlala et al., 2024)
Cleansing and purification	Bark and roots	Infusion, Decoction	Orally and bathing	(Matlala et al., 2024)
Tuberculosis	Roots	Infusion Mixed with (spider's web). Pounded and taken orally with warm water. Thrice a day	Orally	*(Semenya and Maroyi, 2019)*
Fever reduction	Roots	Powder	Orally	(Semenya and Maroyi, 2019)
Digestive issues	Leaves	Powder	Orally	(Kunene et al., 2020)
Respiratory problems	Roots	Powder Roots are dried and pulverized into powder and mixed into boiling water.	Inhalation	(Watt and Breyer-Brandwijk, 1962)
Treatment of wounds	Stem and bark	The bark and stem are ponded together to create	Topical application	(Hamdy et al., 2022)

Ethnobotanical use	Plant part	Method of preparation	Administration	References
		a paste with some oil added to it.		
Pain relief	Stem and Bark	Tincture	Orally	(Hamdy et al., 2022)
Tonsils	Leaves	Infusion	Orally Gagle with the leave infusion to relief pain from tonsils	(Dube, 2023)
Malaria	Roots	Decoction	Orally	(Semenya and Maroyi, 2019)
Snake bite	Leaves	Infusion	Topical application	(Hutchings and Scott, 1996)
Scorpion stings	Leaves	Infusion	Topical application	(Hutchings and Scott, 1996)
Typhoid fever	Roots	Decoction	Orally	(Watt and Breyer-Brandwijk, 1962)
Rheumatism	Bark	Powder	Topical application	(Watt and Breyer-Brandwijk, 1962)
Asthma	Stem and bark	Tincture	Inhalation	(Syamsuri et al., 2018)
Complementary medication for Cancer	Leaves	Infusion	Orally	(Syamsuri et al., 2018)
Joint pain	Roots	Pounded into paste	Poultice	(Watt and Breyer-Brandwijk, 1962)
Blood purifier	Nuts	Powder and Infusion	Orally	(Gelfand, 1985)
Treatment of diabetes	Nuts	Powder and Infusion	Orally	(Hutchings and Scott, 1996)
Arthritis	Leaves	Pounded into paste	Topical application	(Hutchings and Scott, 1996)
Aphrodisiac	Whole plant (Leaves, barks, and nuts)	Decoction, infusion, or powder	Orally	(Bhat and Jacobs, 1995; Mahwasane et al., 2013)
Dysentery	Leaves	Infusion and decoction	Orally	(Gelfand, 1985; Hutchings and Scott, 1996)
Treatment of boils and abscesses	Leaves	Pounded into paste	Poultice	(Gelfand, 1985; Hutchings and Scott, 1996)
Ringworm and fungal infections	Bark	Powder or Pounded paste	Topical application	(Watt and Breyer-Brandwijk, 1962)

Ethnobotanical use	Plant part	Method of preparation	Administration	References
Epilepsy	Roots	Infusion and decoction	Orally	(Watt and Breyer-Brandwijk, 1962)
Convulsions	Roots	Infusion and decoction	Orally	(Gelfand, 1985)
Sedative	Leaves	Infusion and decoction	Orally	(Gelfand, 1985)
Kidney infections and Diuretics	Leaves	Infusion and decoction	Orally	(Bhat and Jacobs, 1995)
Jaundice	Bark	Infusion	Orally	(Kokwaro, 2009)
Used as hepatoprotective	Bark	Decoction	Orally	(Kokwaro, 2009)

3.3.3. Plant part used

This review reported the use of 9 distinct plant parts of *M. zeyheri* in Africa for the treatment of various ailments. Based on the critical analysis of Table 3.1, leaves (34%) emerged as the most used plant part of *M. zeyheri* cultivated for ethnomedicinal uses followed by roots (32%) as shown also in Figure 3.2. The predominant use of the two plant parts aligns well with African traditional medicine and can be attributed to several factors including its relative availability all year round, ease of collection or ethnic beliefs of local people (Adeyemi, 2010; Mahwasane et al., 2013; Ndhlovu et al., 2021). In Addition, Alamgeer et al. (Corrigan et al., 2011) suggest that the preference for leaves over other plant parts may be attributed to their role as photosynthetic organs, containing photosynthates that may contribute to medicinal properties. Roots are preferred as they are regarded traditionally as “Strong medicine (Mahwasane et al., 2013). In some cultures, the roots are mixed with a spider’s web. Pounded and taken orally with warm water thrice a day (Table 3.2) for the treatment of tuberculosis. Although regarded as strong, the collection of underground plant parts is not viable, as it has a detrimental impact on the plant's existence and can ultimately be regarded as highly endangered (Maroyi, 2011). According to *Mahomoodally (2013)* the preference of both leaves and roots for ethno-preparations is attributed to their high concentrations of pharmacologically active substances. The significant use of bark (13%) suggests a perceived efficacy, likely due to its concentration of bioactive compounds though this raises sustainability concerns due to potential overexploitation *(Cunningham, 1993)*. Other plant parts, including whole plant (6%), bark and roots combined (4%), and nuts (2%), show moderate usage levels. Lower utilization is noted for stem and bark (8%), fruits and flowers (2%). This diverse usage pattern suggests a comprehensive understanding of *M. zeyheri's* potential benefits among local people and its contribution to livelihood.

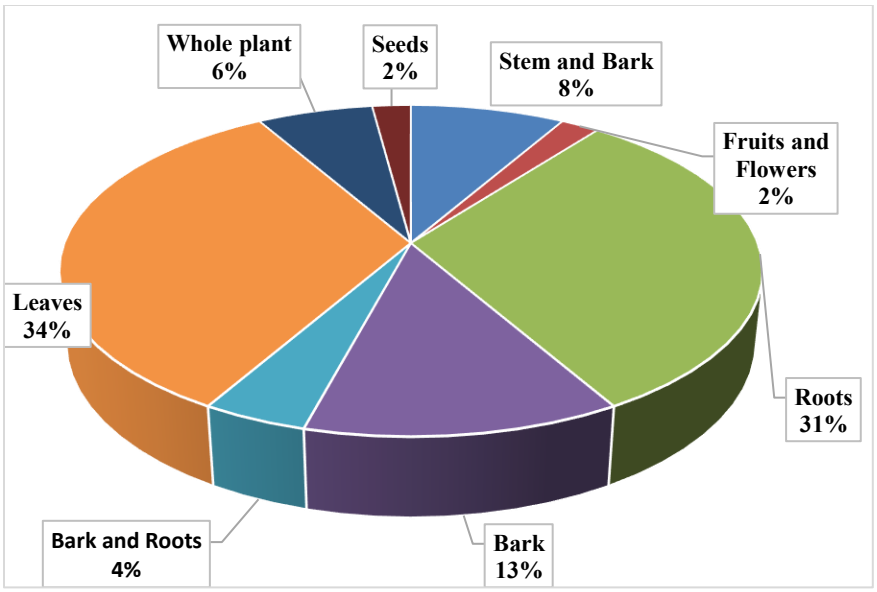

Figure 3.2: *Mimusops. zeyheri* plant parts used for managing different ailments and conditions in Southern Africa.

3.3.4. Methods of preparation

The preparation methods are of paramount importance in ethnomedicine. Preparation methods vary significantly based on the specific plant species, plant parts utilized, geographical location, and cultural context (Halberstein, 2005; Ndhlovu et al., 2021). In this review the most common preparation method is infusion accounting for 33% of all methods (Figure 3.3). The predominance of infusion as a preparatory method aligns with findings from various ethnobotanical studies conducted across diverse African ethnic groups (Arnold and Gulumian, 1984; Corrigan et al., 2011; Hulley and Van Wyk, 2019). The prevalence of this method can be attributed to its simplicity and efficacy in extracting bioactive compounds from plant materials. The ease of implementation and the generally high bioavailability of water-soluble phytochemicals contribute to the method's popularity (Azwanida, 2015). Decoction, closely following at 31%, represents another frequently employed technique. The comparable utilization rates of infusion and decoction are noteworthy, as both methods involve aqueous extraction of plant constituents, though, under different thermal conditions and extraction durations. This predilection for water-based extraction methodologies may be ascribed to their accessibility and deep-rooted cultural significance in traditional therapeutic practices *(T*abuti et al., 2003). Moreover, the preference for these methods may be partly explained by the thermostability of certain phytochemicals and the enhanced extraction efficiency at elevated temperatures (Azwanida, 2015). Powder preparation, constituting 19% of all

procedures, underscores the significant role of desiccated plant material in traditional medicinal applications. This technique offers advantages in terms of extended shelf-life and versatility in administration, which can be particularly beneficial in resource-limited areas (Tahvilian et al., 201*4)*. Additionally, the process of pulverization can increase the surface area of plant material, potentially enhancing the extraction of bioactive compounds during subsequent preparation or administration (Handa et al., 2008). The remaining methodologies comprise pounded paste (8%), tincture (3%), and other unspecified processes (3%). The relatively low prevalence of tinctures is noteworthy, as this method typically involves alcohol-based extraction. This lower representation may be attributed to cultural preferences, resource limitations, or concerns regarding the use of alcohol in certain communities (Sofowora et al., 2013). Furthermore, the efficacy of alcohol-based extractions in preserving certain thermolabile compounds may not outweigh the sociocultural barriers to its widespread adoption in traditional medicine practices (Pandey and Tripathi, 2014).

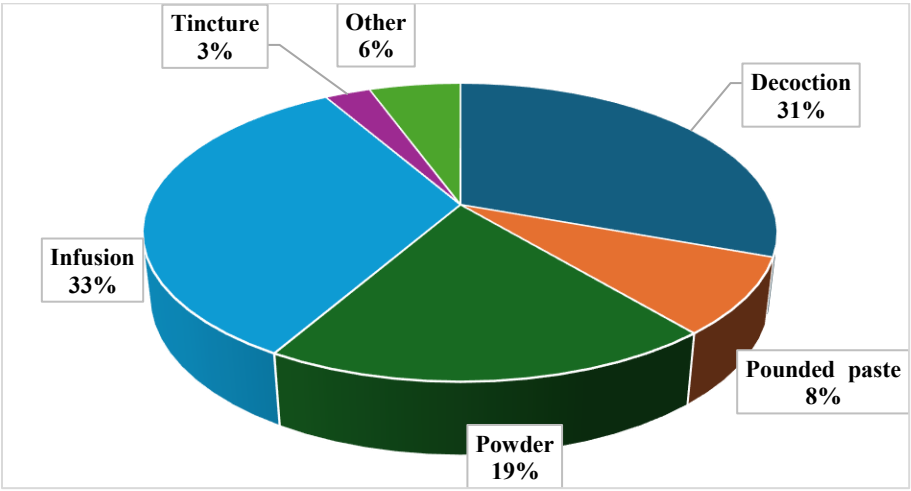

Figure 3. 3: Methods of preparing *M. zeyheri* for treatment of different ailments and conditions in Southern Africa.

3.3.5. Administration method

The distribution of administration methods for *M. zeyheri* in ethnomedicine indicates a high preference for oral administration (70%) in Figure. 3.4. This usage pattern is consistent with several ethnobotanical research on Sapotaceae and other medicinal plants, demonstrating *M. zeyheri's* adaptability in traditional medicine (Cheikhyoussef et al., 2011). The prevalence of oral administration indicates that *M. zeyheri* may contain bioactive substances that are efficacious when consumed. This is consistent with studies from other Sapotaceae species, such as *Mimusops elengi*, which has demonstrated considerable pharmacological activity when taken orally (Kadam et al.,

2012a). *M. zeyheri's* high rate of oral use could be due to the existence of comparable bioactive chemicals that are well absorbed through the gastrointestinal system which could potentially heal a variety of internal disorders (Gupta et al., 2005). Topical application (12%) and poultice use (10%) make up a sizable amount of *M. zeyheri's* traditional use, demonstrating its potential to heal skin diseases and exterior ailments. Shekhawat and Vijayvergia (2010) reported that topical application of plant extracts had considerable wound-healing effects, which were attributed to their antibacterial and anti-inflammatory actions. The usage of *M. zeyheri* in topical and poultice forms implies that it may have similar qualities, making it useful for treating skin infections, wounds, and inflammatory skin diseases (Adewoye et al., 2010). The inhalation method (6%), although uncommon, is an intriguing component of *M. zeyheri's* traditional use. Inhalation as a method of delivery is frequently related to the treatment of respiratory problems or its aromatherapeutic characteristics (Adewoye et al., 2010*)*. While studies on inhaled *M. zeyheri* formulations are few, research on other Sapotaceae species sheds light on potential benefits. In vitro testing of essential oils derived from *Manilkara zapota* leaves showed antibacterial activity against respiratory infections (Nair and Chanda, 2008*)*. *M. zeyheri's* inhalation may be comparable, with its volatile chemicals having good effects on the respiratory system or providing relief through aromatherapy (Osman et al., 2011).

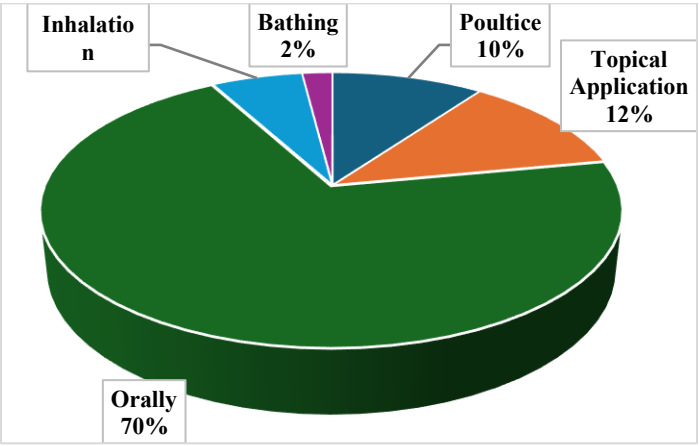

Figure 3. 4: The administration method of ethnomedicine from *M. zeyheri* is used to treat different ailments and conditions in Southern Africa.

3.3.6. Ethnobotanical uses of *M. zeyheri*

Traditional medicine is important in treating many illnesses, especially in rural African areas. *M. zeyheri* has been utilised for generations and is an essential component of healthcare systems in many regions (Maroyi, 2011). The ethnomedicinal use of *M. zeyheri* in Africa shows a wide

spectrum of medicinal and therapeutic applications across several physiological systems (Figure. 3.5). In this review, Skin disorders (18%) are the most reported treated conditions, followed by infections and immune system-related issues (16%). This is consistent with findings from earlier ethnobotanical research, which frequently highlight dermatological and immune-related uses of *M. zeyheri* (Maroyi, 2011; Van Wyk, 2011c). The plant's putative antibacterial and anti-inflammatory qualities, which are found in many medicinal herbs used topically, may explain the plant's high prevalence in the treatment of skin conditions (Maver et al., 2018). The significant prevalence of treated infections and immune system conditions (approximately 17%) suggests that *M. zeyheri* may contain bioactive substances with immunomodulatory properties, necessitating further pharmacological research (Semenya and Maroyi, 2019). Metabolic and organ conditions, as well as other undefined illnesses, account for approximately 13-14% of all ailments reported to be treated with *M. zeyheri*. This wide range of applications demonstrates the plant's adaptability in traditional medicine systems and may reflect its ability to influence different physiological pathways (Street and Prinsloo, 2013). Pain alleviation, reproductive health, fertility, and respiratory difficulties account for roughly 11-12% of recorded uses. The analgesic capabilities suggested by its usage in pain management could be linked to anti-inflammatory chemicals, whilst its use in reproductive health could indicate hormonal or fertility-enhancing activities (Steenkamp, 2003). Rural African people frequently choose traditional herbs such as *M. zeyheri* for a variety of reasons. These include accessibility, as medicinal plants are frequently available in local settings, making them more accessible than modern medications in rural locations (Mahomoodally, 2013). Another issue to consider is cultural relevance, as traditional medicine is strongly ingrained in local cultures and belief systems, making it more accepted and trustworthy. Affordability is also important, as picking native flora is typically more cost-effective than acquiring manufactured medications, particularly in resource-constrained contexts (Van Wyk, 2011a). However, long-term use generates vital research leads but does not ensure safety or efficacy. Street and Prinsloo (2013) emphasise the importance of comprehensive scientific testing of traditional medicines to determine their pharmacological characteristics and potential toxicity. The preference for indigenous herbs in rural African communities, despite the arrival of modern treatment, emphasises the importance of adopting an integrative healthcare system.

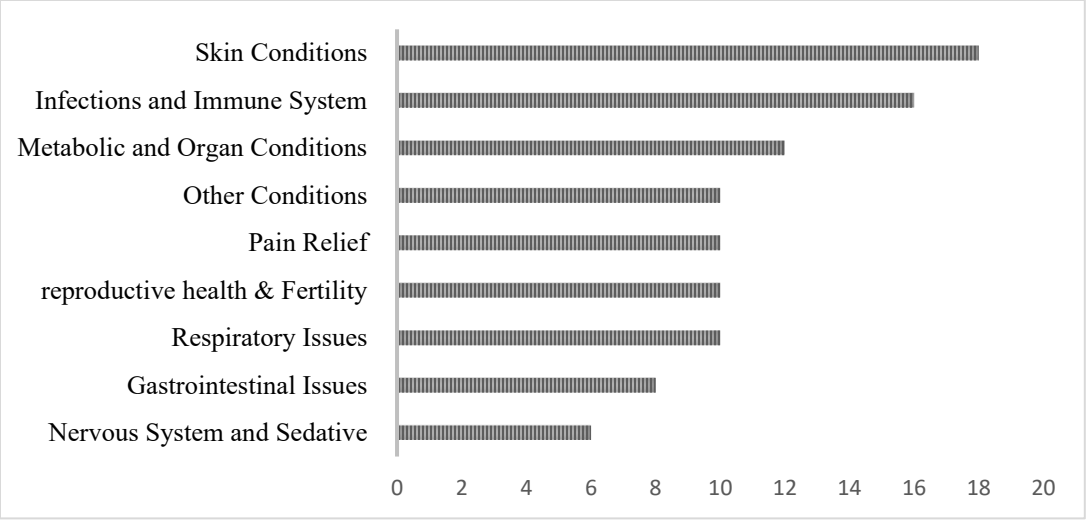

Figure 3. 5: Categories of ailments and conditions treated with *M. zeyheri* in Southern Africa.

3.3.7. *Mimusops zeyheri* as a source of nutrients

Wild fruits are natural store houses of essential macro and micronutrients (Awodoyin et al., 2015). *Mimusops zeyheri* is a valuable fruit tree with a pool of significant nutrients essential to the human body. According to Chivandi et al. (2011a), *M. zeyheri* nuts contain high levels of proteins, oleic acid and vitamin E indicating its potential for effective use as a dietary energy supplement and oil source. A notable vitamin E content ranging between 0.50 to 48.7 µg was reported by Chivandi et al. (2012a). Gomes et al. (2019) reported the highest levels of linoleic acid, measuring 18.87% from the nut meal of *M. zeyheri*. Lipids are crucial to the physiological processes of plant growth, and they have a close association with human metabolism, among other functions *(Li, Y. et al., 2016)*. Gomes et al. (2019) reported a 14.04% lipid yield from the nut oils of *M. zeyheri* and the highest total soluble fatty acid content of 23.902 % compared to *K. africana* and *X. caffra* which yielded 17.15% and 8.57% respectively. The proximate analysis study of the nuts of *M. zeyheri* by Chivandi et al. (2011a) reported a lipid yield of 21.2% with a gross energy value of 24.34 MJ/kg. The nut oil of *M. zeyheri* has the highest reported concentration of glutamic acid in comparison to other amino acids accounting for 1.38% of crude protein found in total fat (Radzuma, 2017). Dehulled nut meal from *M. zeyheri* can substitute a portion of maize meal in Japanese quail diets to provide energy without affecting growth, feed utilisation efficiency, or meat output (Chivandi et al., 2020). At 37.5% diet inclusion, this substitution is economically viable (Chivandi et al., 2020). The use of *M. zeyheri* nut meal may help produce and increase the output of Japanese quail meat. Although there have been studies on the use of *M. zeyheri* nut meal as a feed supplement for

quail and poultry, there is a dearth of studies on its applications in other fields such as the food industry, nutraceuticals, or medicines. There is, therefore, a need for preliminary investigations to discover and assess the uses of *M. zeyheri* in many sectors, such as food fortification, functional foods, organic preservatives, or medicinal substances, leveraging its distinctive nutritional composition and bioactive components.

Venter and Venter (1996) reported plant fresh fruit concentrations ranging between 50-80 mg. g. The dietary benefits of the fruits derived from this tree are notable, mostly attributed to their higher levels of chromium and manganese (Mngadi et al., 2019b). The fruits of *M. zeyheri* have been reported to have a vitamin C content higher than that of guava vitamin C content of approximately 20 mg. g (Venter and Venter, 1996) and oranges (Mashela and Mollel, 2001; Omotayo et al., 2020) suggesting that the fruits can be a valuable addition to the diets of rural people who may especially struggle with limited access to fresh produce containing vitamin C. Wilson and Downs (2012) reported higher levels of carbohydrates (fructose, glucose and sucrose) from the fruits of *M. zeyheri*. These serve as a good energy source and would aid in the active functioning of rural people and school children who might otherwise lack an alternative source of energy. The fruits *of M. zeyheri* exhibit higher levels of dry matter organic matter, protein, and ash content, precisely measuring at 91.1%, 83.3%, 9.3%, and 2.8% respectively, as reported in a study conducted by Chivandi et al. (2020).

Okatch et al. (2012a) determined the composition of *M. zeyheri* leaves which included nitrogen (N) at a concentration of 6.33%, phosphorus (P) at 0.33%, potassium (K) at 1.25%, calcium (Ca) at 0.39%, magnesium (Mg) at 0.06%, zinc (Zn) at 0.0029%, copper (Cu) at 0.0014%, iron (Fe) at 0.0409%, aluminium (Al) at 0.007407% and manganese (Mn) at about 0.005185%. Another research that examined the root of *M. zeyheri* in Botswana discovered trace amounts of chromium (Cr) arsenic (As) and lead (Pb) measuring approximately 73 mg kg 1, 73 mg kg 1 and 20 mg kg respectively (Marobela et al., 2011). All these constituents were found to fall within limits thus confirming the plants' suitability, for medical and dietary purposes. Despite various research examining individual nutrients in *M. zeyheri*, there is still a lack of comprehensive analysis of the plant's complete nutrient profile, which includes fruits, nuts, leaves, and other components such as the roots. This could offer a more comprehensive understanding of the plant's nutritional worth and its uses. There is therefore a need to utilize modern analytical techniques to perform a comprehensive and standardized nutrient analysis of every part of the *M. zeyheri* plant,

encompassing macro and micronutrients, phytochemicals, and bioactive substances. Although the nutrient composition of *M. zeyheri* has been documented, information is scarce regarding the bioavailability and bio accessibility of these nutrients. This knowledge is essential for comprehending the possible health advantages and efficient utilization of these nutrients by the human body.

3.3.8. Ecological importance and ecosystem services provided by *M. zeyheri.*

Wild fruit trees are essential for maintaining a balanced and functional ecosystem. Several wild fruits especially those from the Sapotaceae family including *M. zeyheri* are host to several endophytes (Monyela, 2021*)* and maintain a symbiotic relationship with them (Sieber, 2007b). *Mimusops zeyheri* has a mutualistic relationship with Teratosphaeria, Zeloasperium species, Pezizomycotina endophytes (Monyela, 2021). These endophytes live inside *M. zeyheri* acquiring good nutrition from the fragments, exudates and leachates of the tree without causing harm. In return to what they acquire from the fruit tree, the endophytes provide a physiological impact on the tree by increasing its resistance to environmental and biological stresses (Griffin and Carson, 2018). This relationship serves as an integral component of forest dynamics and influences greatly the survival and regeneration of wild fruit trees (Fu et al., 2003a).

In addition to being a host for endophytes, *M. zeyheri* serves also as a key habitat provider, food source, and soil stabilizer (Braithwaite et al., 1989; Kaiser et al., 2008; Raman, 2001a). Its dense foliage and substantial canopy create a suitable microclimate for various birds and insect species, while its fruits are an important food source for many animals (Raman, 2001a). Although the tree offers these services, there is a lack of information regarding its extensive interaction with other biotic elements of the environment, including insects, birds, and other animal species. A potential approach is to conduct extensive ecological research and biodiversity surveys to gain insight into the complex network of relationships that exist between *M. zeyheri* and different animal species, such as herbivores, pollinators, and nut dispersers. This may shed light on the tree's function in preserving ecosystem balance and encouraging biodiversity.

The tree's extensive root system helps prevent soil erosion, and its fallen leaves contribute to nutrient cycling (Raman, 2001a). However, the tree's role in competition and succession within its ecosystem is not well understood (Raman, 2001a). Conducting extensive ecological studies and field observations to gain insights into the competitive dynamics of *M. zeyheri* with other plant species, as well as its contribution to successional processes within the ecosystem. This might

involve maintaining close monitoring of any changes to the composition of the vegetation, researching the use of resources, and assessing *M. zeyheri 's* effects on other species.

According to England et al. (2020) *M. zeyheri* are essential for maintaining ecosystem services and natural capital in grazed ecosystems. However, it is unknown how specifically *M. zeyheri* contributes to ecosystem services such as soil stabilization, carbon sequestration, and water resource regulation. Potential resolution: Perform field studies and experimental setups to measure the ecosystem services offered by *M. zeyheri*. This could include the measurement of carbon sequestration rates, the evaluation of soil erosion rates in areas with and without *M. zeyheri*, and the examination of the tree's influence on water cycles and hydrological processes.

With its considerable nutritional and commercial potential, *M. zeyheri* is a valuable resource for food security and sustainability (Omotayo et al., 2020). Although Syampungani et al. (2020) reported *M. zeyheri* to play a crucial role in climate change mitigation and adaptation within the Miombo woodlands, but it remains unclear and poorly known how *M. zeyheri* specifically contributes to climate change adaptation and mitigation within its environment. Hence, it is imperative to research to assess the function of *M. zeyheri* in controlling microclimate conditions, regulating temperature and moisture levels, and its capacity to serve as a safeguard against severe weather catastrophes. This may entail doing on-site measurements, utilizing remote sensing methods, and employing ecological modelling instruments. Beyond some of the potential ecosystem services provided by the plant species in the ecosystem, *M. zeyheri* offers a range of ecosystem services that are crucial for rural people, like fuelwood, fodder, and medicinal plants (Dhyani and Dhyani, 2016).

3.3.9. Conservation status of *M. zeyheri* populations

In South Africa, the South African Biodiversity Institute (SANBI) is responsible for evaluating the conservation status of plant species including wild fruit trees in the country (Moraswi et al., 2019; Raimondo, 2011). Assessments by SANBI is crucial in the guide for conservation and they are submitted to the International Union of Conservation of Nature (IUCN). *Mimusops zeyheri* is at present categorized under the “Least Concern” list of indigenous wild fruit trees by the IUCN (Dube et al., 2016). This categorization does not imply that this wild fruit tree species is not facing any threats. The tree has been documented as a host to the Mediterranean fruit fly which has been reported to pose significant harm and threat to the horticulture industry of South Africa (Barnes et al., 2007; Dube et al., 2016; Grové et al., 2017). Although the species is currently not in any

immediate danger of extinction, its distribution and survival are confronted by several threats. The results of the study by Lubisi et al (2023) the Vhembe biosphere reserve highlight that 54.14% of the respondents observed population changes in the tree and noted that the species is steadily declining, scattered in distant areas and around valleys. Local rural people in Africa rely on the exploitation of wild fruits for food, medicine, firewood and timber often used for handicrafts. The exploitation of these wild fruit trees often gets excessive and alongside factors such as climate change, the introduction of alien invasive species, expansion of agricultural land and residential areas, continuous urbanisation poses a great threat to their existence (Cancio et al., 2016; Lubisi et al., 2023; Tiawoun et al., 2019). The effects of such threats, more especially urbanisation are evident in the study by Matlala et al. (2024) who reported a declining and scarce occurrence of *M. zeyheri* as mentioned by residents in the Gauteng province of South Africa often referred to as the country's most urban province.

Historically, local farmers in many African countries such as Botswana (Marobela et al., 2011), Zimbabwe (Gelfand, 1985) and Swaziland (Kunene et al., 2020) carried out the protection of wild fruit trees and plant resources around their forest reserves and farm homelands (Awodoyin et al., 2015). South Africa has established protected areas including national parks, botanical gardens and nature reserves to help conserve biodiversity including wild fruit trees. These areas operate under strict national regulations including acts such as Conservation of Agricultural Resources Act No. 43 of 1983, National Forests Act No. 84 of 1998, National Environmental Management Act No. 107 of 1999, and National Environmental Management: Biodiversity Act No. 10 of 2004 as mentioned several scholars (Paterson, 2006; Ramarumo et al., 2024; Van der Linde, 2006). Botanical gardens and arboreta nurseries play a vital role in ex-situ conservation initiatives, especially for ancient trees such as *M. zeyheri* (Kozlowski et al., 2012). Even though these establishments are deemed useful and progressive, Scientific research argues that they prevent local people from using their surrounding biological resources and receiving direct subsistence advantages (Reid et al., 2004). Furthermore, there are obstacles to overcome with these establishments, such as the absence of a well-established scientific and legal structure for ex-situ operations (Gippoliti, 2012), and the requirement for a synchronized meta-collection infrastructure to monitor and administer living collections (Wood et al., 2020). To successfully tackle these issues, Volis (2017) proposes the establishment of regional conservation priorities, the creation of

genetically diverse collections, and the utilisation of these collections in on-site initiatives. These measures can improve the function of botanical gardens and arboreta in ex-situ conservation.

3.3.10. Genetic diversity and population genetics in *M. zeyheri.*

The genetic and population diversity of various wild fruit tree species have been documented across the globe, however such documentation in South Africa and the African continent remains scanty. Significant genetic structures isolated by distance have been reported in certain wild fruit tree populations (Prasad et al., 2022). Research on genetic variability is important in the survival and evolutionary potential of the species (Gonzalez-Astorga et al., 2008) as it may provide valuable insights into the identification and classification of the plant within breeding programmes hereby providing recommendations for protection and conservation measures (Omotayo et al., 2020). The genetic variability of *M. zeyheri* in South Africa has only been documented in the Limpopo province. A study by Ledwaba (2008) reported 91% genetic variability among *M. zeyheri* populations with 9% population variability. This suggests a research gap exists and therefore prompts a question regarding the presence of comparable genetic patterns or the presence of distinct genetic profiles in other provinces of the country where *M. zeyheri* exists. In the Limpopo Province, *M. zeyheri* is divided into several different clusters with an average genetic similarity estimate that is between 47 and 89% (Ledwaba, 2008) suggesting different levels of genetic relatedness. Further investigation regarding factors that contribute to the formation of such genetic clusters could lead to valuable knowledge generation regarding the historical patterns of tree migration, environmental influences and possible bottlenecks to gene flow within the wild fruit tree species. Such knowledge will also help in identifying populations to prioritize for conservation efforts and the development of corridors for restoration of the plant species and its habitat.

3.3.11. Scientific research, phytochemistry and biotechnological applications of *M. zeyheri*

Although *M. zeyheri* is highly valued as an important traditional wild fruit tree crucial for its medicinal and miscellaneous uses in rural livelihoods, reports of the plant's biological activities are scanty. The first biological activity associated with *M. zeyheri* was reported by Chivandi et al. (2012a) who reported the antiproliferative effect of *M. zeyheri* nut oils on Caco-2 and HEK-293 cell lines. These results indicate a promising avenue for further research on the nut oil mechanism of action and potential application. In a similar study by Gomes et al. (2019), *M. zeyheri* nut oil reportedly induced cytotoxic effects on MDA-MB-231 cells and growth-inhibitory effects on MCF-7 cells. Additional research is required on the in vitro biological activities of different parts

of the plant extracts against various human diseases to scientifically support their use in ethnomedicine. These studies may warrant a potential for the inclusion of the plant in the pharmaceutical industry. Research aimed at exploring the phytochemical constituents of the whole plant is also required. This will create a space for the potential development of novel drugs for the treatment of various health conditions and applications in the cosmetic industry for the formulations of many beauty products such as moisturizers for treating skin conditions. Additional investigation is required to examine the possible biotechnological uses of *M. zeyheri*, in the advancement of functional foods nutrition-derived products.

3.4. Research gaps

The review provides details on the nutritional composition from various studies (Chivandi et al., 2011a; Gomes et al., 2019; Mngadi et al., 2019b) a comprehensive and standardized analysis across all plant parts is lacking. As Omotayo et al. (2020) emphasize, understanding the complete nutrient profile and bioactive compounds is crucial for leveraging the plant's potential. However, the review also notes the scarcity of information on bioavailability and bio accessibility of these nutrients, which (Ramarumo et al., 2024) argue is essential for understanding their efficient utilization and health impacts. This review therefore recommends the utilization of modern analytical techniques to perform a comprehensive nutrient profiling of every part of the *M. zeyheri* plant, encompassing macro and micronutrients, phytochemicals, and bioactive substances. Additionally, co-studies to assess the bioavailability and bio accessibility of these nutrients to better understand their potential health impacts and optimal utilization are necessary.

The lack of clinical studies and potential therapeutic applications is an issue to be addressed around *M. zeyheri*. Several studies primarily focus on the nutritional composition of the plant, with limited information on potential therapeutic applications or clinical studies. Conducting clinical studies could provide valuable insights into the potential medicinal or therapeutic benefits of the plant or its components. Therefore, there is a need to carry out clinical trials and preclinical studies. Designing and conducting preclinical studies to evaluate the potential therapeutic effects of the plant or its extracts on various health conditions or disease models can bridge the existing gap. Based on the preclinical findings, conducting clinical trials to assess the safety, efficacy, and potential therapeutic applications of the plant or its components in human subjects can provide valuable insights into the planned use and its application. There is also a need to explore traditional knowledge and ethnobotanical data by collaborating with local communities and traditional healers

to gather information on the traditional uses and preparation methods of the plant. This traditional knowledge can be Integrated with scientific research to identify potential therapeutic applications and develop evidence-based products or treatments.

Authors like Kozlowski et al. (2012) and Ramarumo et al. (2024) emphasize that conservation and utilization are inextricable, while Omotoyo et al. (Omotayo et al., 2020) caution against unchecked utilisation which may lead to environmental degradation. As argue, that sustainable decision-making requires integrating multidisciplinary knowledge, including indigenous knowledge systems. We need to foster collaborations between researchers, institutions, stakeholders (including local communities), and traditional knowledge holders to facilitate knowledge exchange, resource sharing, and integrated approaches. Multidisciplinary collaborations can effectively address gaps and promote sustainable utilization while preserving traditional practices. While Majeed et al. (2021) report on the biological activities of the Mimusops genus, and the review mentions the potential for biotechnological applications and inclusion in pharmaceuticals, there is a lack of specific research in this area for *M. zeyheri*. This therefore means there is a need for studies aimed at the comprehensive phytochemical analysis of the whole plant and investigation of potential biotechnological uses, including developing functional foods, nutraceuticals, cosmetics, and novel drug candidates, capitalizing on the plant's unique composition and bioactive compounds. Addressing these gaps through future research can contribute to a comprehensive understanding of *M. zeyheri* 's potential benefits, sustainable utilization, and conservation strategies while fostering collaborations between scientific and traditional knowledge systems.

3.5. Concluding remarks

Mimusops zeyheri emerges as a multifaceted indigenous plant species with significant ecological, cultural, and potential economic value. The extensive ethnomedicinal applications documented in some African countries underscore its importance in traditional healthcare systems. The diverse usage of different plant parts for treating a wide range of ailments suggests a rich phytochemical profile worthy of further scientific investigation. The nutritional analysis of *M. zeyheri* fruits and nuts reveals a promising source of essential nutrients, particularly vitamins and minerals, which could contribute to food security and nutrition in rural communities. However, the lack of a standardized nutrient profile across all plant parts indicates a critical area for future research. Ecologically, *M. zeyheri* plays a crucial role in its native habitats, supporting biodiversity and

providing essential ecosystem services, with its endophytic interactions and potential contributions to soil stabilization and climate change mitigation warranting further investigation. Despite its current "Least Concern" conservation status, the species faces threats from overexploitation, habitat loss, and climate change, necessitating more robust, integrated conservation strategies. Limited genetic diversity studies suggest significant variability within populations, highlighting opportunities for targeted conservation and varietal improvement, though substantial knowledge gaps persist across the species' range. Preliminary biological studies have revealed promising cytotoxic and antiproliferative activities in *M. zeyheri* nut oils, opening avenues for potential pharmaceutical applications. However, the scarcity of comprehensive phytochemical analyses and clinical studies underscores significant research opportunities in exploring the full potential of this valuable indigenous species. Notably, this review has highlighted a significant disparity in research in some African countries, with most studies concentrated in South Africa. This geographical imbalance in research intensity may result in overlooking the potential benefits and unique characteristics of *M. zeyheri* populations in other Southern African nations. The limited research in most African countries where *M. zeyheri* is native represents a critical gap in our understanding of its full potential and variability across its natural range. To address the research imbalance, the establishment of collaborative, transnational research initiatives focused on *M. zeyheri* is necessary. Such collaborations should aim to comprehensively explore the plant's potential across its entire natural range, ensuring a more holistic understanding of its variability, uses, and conservation needs. This approach would not only enhance our scientific knowledge but also promote equitable development and sustainable utilization of this valuable resource across all relevant African nations.

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CHAPTER FOUR

TRADITIONAL AND ETHNOMEDICINAL KNOWLEDGE OF *MIMUSOPS ZEYHERI* SOND., AS A SOURCE OF FOOD AND MEDICINE IN LIMPOPO AND MPUMALANGA.

Summary

Approximately 80% of the African population relies on traditional medicine to meet their healthcare needs; however, half of the traditional and ethnomedicine used remains undocumented. Hence, the study aimed to document the traditional and ethnomedicinal use of *M. zeyheri*, including the preparatory and administration methods, and the perceived threats of the species by the locals of the Vhembe district in Limpopo and the Gutshwa village of the Ehlanzeni district in Mpumalanga. An ethnobotanical survey was conducted to collect data from 116 participants through semi-structured, face-to-face interviews. Ethnobotanical indices, such as Relative Frequency of Citation (RFC) and Fidelity Level (FL), were used for data analysis. The results demonstrate the multifaceted use of *M. zeyheri* by different ethnic groups to treat various ailments and conditions. A total of 16 other uses of *M. zeyheri* were reported in Limpopo, and 27 uses were reported in Mpumalanga. The RFC values ranged from 0.29 to 1 in Limpopo and from 0.03 to 1 in Mpumalanga. Fruits eaten whole as a snack received an FL value of 100% in both provinces, as recorded from all participants. In Limpopo, the ethnomedicinal use of *M. zeyheri* to treat sexually transmitted infections received an FRC value of 1, recorded from all participants (FL = 100%), with efficacy reported when the species is mixed with other plant species and boiled to form a decoction that is taken orally. Similar use of *M. zeyheri* for treating sexually transmitted infections was also reported (RFC=0.50) in Mpumalanga, highlighting similar use of the same species by different ethnic groups. Other commonly reported uses include the use of *M. zeyheri* for treating skin ailments and conditions, such as ringworm (RFC = 0.57) in Mpumalanga and (RFC = 1) in Limpopo. In Mpumalanga, the use of *M. zeyheri* extends to spiritual and cultural practices, such as using the leaves to remove bad omens (RFC = 0.100) and to collect the spirit of the deceased from where they last took their breath (RFC = 0.05). Different plant parts are utilized in both provinces, with Limpopo having a slightly higher preference for roots (17%), while Mpumalanga has a slightly higher preference for nuts (17%). Both study sites share a similar preference for the use of leaves (22%). Both study sites demonstrated a strong preference for decoction in preparing ethomedicine (36% and 34%) for Limpopo and Mpumalanga, respectively. Internal and external applications were cited by participants in the study, with oral administration being the most preferred mode of delivery in both Limpopo (75%) and Mpumalanga (55%). Participants in

Mpumalanga (40%) reported land expansion and infrastructure development as a perceived major threat to the *M. zeyheri* population, while overharvesting (30%) was perceived as a significant threat in Limpopo. The study documented the comparative traditional and ethnobotanical use of *M. zeyheri*, including preparation and administration methods, as well as perceived threats, in two provinces. Documenting the ethnobotanical and traditional knowledge of *M. zeyheri* is significant for preserving traditional ancient cultural norms related to specific plant use. It contributes to the understanding of regional traditional knowledge systems.

Keywords: Ethnobotanical, Culture, Indigenous Knowledge, Wild fruits, Traditional, Ethnomedicine

4.1. Introduction

Indigenous Knowledge, deeply rooted in oral traditions and lived life experiences, has for centuries served as an integral foundation of many African communities. Indigenous Knowledge has been a cornerstone for survival and sustainable living, embedded in various practices, including human relationships with plants and the environment (Masenya, 2022). Furthermore, indigenous knowledge details the ways in which indigenous people manage resources to sustain their health and spiritual well-being. Therefore, indigenous knowledge has been a key instrument in the discovery of plants with potent medicinal and antimicrobial properties, which has led to the development of commercial products, including a range of pharmaceuticals (Buragohain et al., 2024). In Africa, research on indigenous knowledge, specifically, the ethnobotanical applications of plants and wild fruit trees have intensified in response to increasing concerns over the loss of biodiversity, ecosystem disruption, cultural erosion and the exclusion of Indigenous Knowledge System (IKS) from recognized core development strategies and agendas due to colonial legacies and power imbalances (Yanou et al., 2023).

Among the thousands of indigenous wild fruit trees with ethnobotanical significance in Africa is *Mimusops zeyheri* Sond., commonly known as Transvaal red milkwood, which has remarkable cultural and ecological importance (Chivandi et al., 2011). *Mimusops zeyheri* belongs to the Sapotaceae family, a tree that grows in various habitats throughout the continent (Williamson-Benavides and Dhingra, 2021), particularly within South Africa, covering provinces of Limpopo, Mpumalanga, Gauteng, KwaZulu-Natal, and Northwest (Van Wyk, 2011b; Venter and Venter, 1996; Williamson-Benavides and Dhingra, 2021). The tree has adapted to diverse ecological zones

in these regions. It is deeply rooted in the culture of many South African rural communities, supporting human health, ecosystem services, food security, and cultural heritage (Chivandi et al., 2011c).

The ethnobotanical practices associated with *M. zeyheri* underscore that traditional knowledge is passed down orally from generation to generation, highlighting the resourcefulness and strong ecological understanding of indigenous communities (Magwede et al., 2019). The known various vernacular names across tribal groups, such as Mmupudu in Northern Sotho, Mububulu in Venda, and Umpushane in Zulu, *M. zeyheri* is woven into the fabric of these communities' lives, representing a key part of their natural heritage (Lemmens, 2005b; Ramarumo and Maroyi, 2020). Over 80% of Africa's population relies on wild fruit trees and medicinal plants, and their use differs from tribe to tribe; therefore, tribes name species respectively based on their usefulness to them (Avakoudjo et al., 2020; Ndhlovu et al., 2023).

Mimusops zeyheri is a vital dietary supplement supplier for communities that rely on forest and woodland resources to boost food security. The fruits from this tree are nutrient-dense, containing vitamins, minerals, and potential bioactive compounds that help in meeting the dietary and health needs of communities with limited access to conventional food markets (Mkhonto et al., 2024). As a reliable food source in both regular and drought-stricken periods, *M. zeyheri* offers a form of dietary resilience for populations vulnerable to food insecurity (Lubisi et al., 2024). This contribution to local food security is particularly valuable in the context of rising food costs, urbanization, and environmental degradation, all of which threaten traditional agricultural systems and rural livelihoods.

The ecological role played by *M. zeyheri* extends beyond its use as food. It is therefore irreplaceable in the ecosystem, contributing to biodiversity and supporting pollinators, as well as soil stabilization, in the ecosystems it inhabits (Sieber, 2007a). This tree has deep roots that are beneficial for rocky and arid regions, helping to retain soil and thereby preventing erosion in sensitive landscapes (Fu et al., 2003b; Raman, 2001b). Additionally, *M. zeyheri* serves a crucial role in providing habitat and nourishment to animals, as well as promoting biodiversity through its interactions with wildlife, including nut-dispersing birds and mammals (Raman, 2001b). These ecosystem services underscore the broader environmental role of *M. zeyheri* and similar wild fruit trees in safeguarding ecosystem functionality and resilience.

Despite its ecological role and utility contributing to livelihoods, *M. zeyheri's* comparative ethnobotanical and traditional significance has not been fully explored across diverse South Africa's ethnoecological zones. Documented literature reports limited use of this species, leaving gaps in regional use diversity, cultural relevancy, and knowledge, particularly in the context of South Africa's diverse landscape, ethnic groups, cultures, and different socioeconomic statuses, which are known to influence plant use.

Comparative ethnobotanical studies are crucial for understanding regional knowledge systems and contributing to the preservation of cultural and traditional ethnobotanical knowledge, which is at risk of being lost due to changes in land use patterns, modernization, and climate change. This chapter, therefore, aims to document the traditional and ethnobotanical applications of *M. zeyheri* in two distinct South African Agro ecologies: the Vhembe district in the Limpopo Province and the Ehlanzeni district in the Mpumalanga Province. Although the two districts are rich in biodiversity and cultures, they face different environmental and anthropogenic challenges that influence their beliefs and plant use patterns.

4.2. Material and methods

4.2.1. Geographical position of research

The study was conducted in the Vhembe district of Venda, Limpopo Province (Figure 4.1B), and the Gutshwa village of Kabokweni, Ehlanzeni district, Mpumalanga Province (Figure 4.1A), South Africa. The Vhembe district experiences humid climatic conditions with average temperatures ranging between 12.1°C and 27.1°C. The district experiences summer rainfall with annual precipitation ranging between 755 and 798 mm (Table 4.1). Mpumalanga Province experiences a subtropical climate, typified by warm to hot summers and mild winters. Average temperatures range from 16°C in winter to 30°C in summer, although peaks can exceed 35°C during heatwaves (Table 4.1). Rainfall is seasonal, occurring between October and March. Annual precipitation levels typically range between 800 mm and 1,000 mm, supporting various vegetation types and influencing agricultural practices. Winters are dry, with occasional cold fronts from higher-altitude regions influencing local weather. The area is characterized by undulating terrain and its proximity to key watercourses, such as the Crocodile River. The natural vegetation is primarily classified as Lowveld Bushveld, characterised by grasses, shrubs, and scattered deciduous trees such as acacias and marulas. The area encompasses a mix of natural vegetation and human-altered landscapes,

including cultivated fields, residential developments, and patches of degraded land resulting from overgrazing and urban expansion. The surrounding land cover includes a mosaic of subsistence farming plots, commercial plantations (notably citrus and subtropical fruits), and community woodlots.

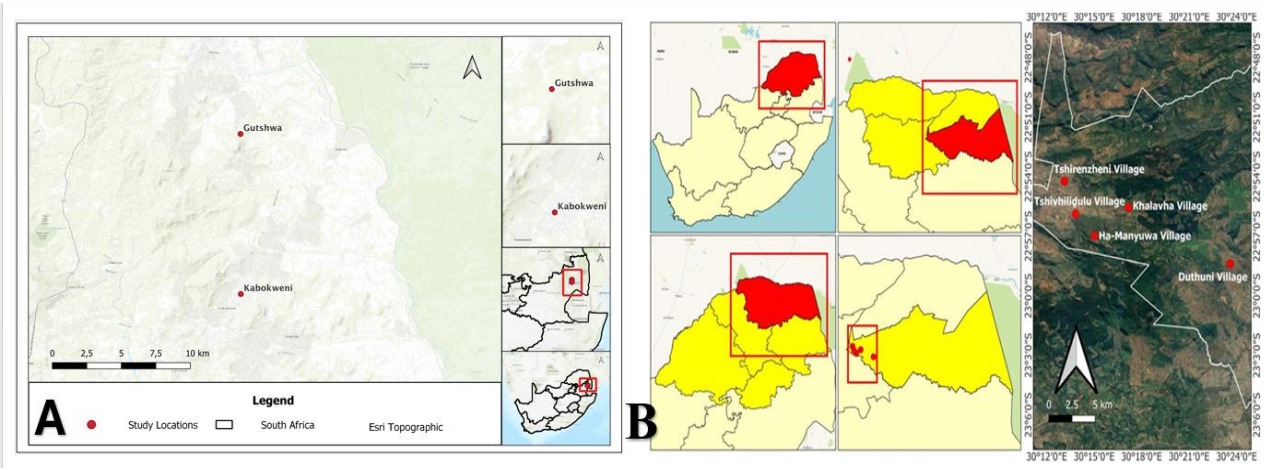

Figure 4.1: Geographic area of Gutshwa in Ehlanzeni district of Mpumalanga Province (A) and Vhembe Limpopo (B), South Africa.

Table 4 1: Soil, Temperature, and Rainfall description of the study sites.

Location	GPS Coordinates	Temperature	Rainfall	Soil texture
Vhembe district	22.7696°S, 29.9741°E	12.1-27.4°C	755-798mm	Sandy, loam & Clay
Ehlanzeni district	29°48′46″ S, 30°38′11″	15.0-28.1°C	7500-860mm	Sandy loam

4.2.2. Ethnomedicinal data collection

The study was conducted for a period of four months in 2024, covering the Vhembe region and Gutshwa in the Ehlanzeni district, Mpumalanga Province. The study sites were purposively selected based on the known occurrence of *M. zeyheri*, their ecological suitability for the species and the presence of communities with established indigenous knowledge systems related to its us. Furthermore, the two regions differ in climatic conditions, vegetation types and socio-cultural contexts, thereby providing a suitable basis for comparative analysis.

The target population comprised adult residents (aged 18 years and above) within the selected communities who were likely to have knowledge of *M. zeyheri*. The population size across these communities is estimated to be large (in the order of several thousand households), thus providing

an adequate sampling frame for statistical inference and participants were randomly selected who were older than 18. A simple random sampling approach was employed at the household level to minimise selection bias, whereby households were approached systematically and one eligible respondent per household was selected for participation. The interviews were conducted in SiSwati in Mpumalanga and Tshivenda in Limpopo, the area's dominant languages. Before data collection, the researcher provided participants with a detailed explanation of the study's objectives and obtained their informed consent for the recording and use of their information. Demographic data of the participants were documented first, followed by information regarding the plant's utilization, its significance in the participants' livelihoods, and their perceptions of concerns surrounding the distribution and abundance of the plant. A total of 116 Participants were interviewed. A total of 116 Participants were interviewed. According to Statistics South Africa (Stats S.A, 2023), the population size of Ehlanzeni and Vhembe districts exceeds several hundred thousand residents, indicating a sufficiently large sampling frame for statistical inference. Therefore, the sample size was determined using standard sample size estimation procedures for large populations, guided by Cochran's (1977) formula, which ensures representativeness at a 95% confidence level and a 5% margin of error. The minimum recommended sample size under these conditions is approximately 96 respondents; therefore, the final sample of 116 participants exceeds this threshold, thereby reducing sampling error and improving the reliability of the findings.

$$n_0 = (Z^2 \times p \times (1 - p)) / e^2$$

Where:

n_0 = required sample size

Z = z-score (1.96 for 95% confidence level)

p = estimated proportion of the population (0.5)

e = margin of error

The study incorporated a focus group discussion to gain more knowledge from village dwellers who had not been interviewed about the uses of this plant. This additional method served as a form of data triangulation, enhancing the depth and validity of the information collected. A photograph of *M. zeyheri* was shown to the participants to confirm a shared understanding between the researcher and participants regarding the specific plant species under discussion.

4.2.3. Ethical considerations

Given that the study involved individuals and their Indigenous knowledge related to *M. zeyheri,* ethical approval was obtained from the University of Mpumalanga before its initiation, with the assigned reference number UMP/MKHONTO201707659/PhD/SBES/2024/01. A random sampling method was used to select participants who were at least 18 years old and willing to participate in the research. Individuals under 18 were excluded, as they are considered minors and cannot provide full legal consent. Prior to the interview sessions, the study's objectives were thoroughly explained to all participants, and permission was obtained from every individual.

4.2.4. Data Analysis

Data were captured into an Excel spreadsheet coded based on themes and analysed thematically using ethnobotanical indices, including Use Value (UV), Fidelity Value (FL), Relative Frequency of Citation (RFC), as described by Leonti (2022) and Tardío and Pardo-de-Santayana (2008) Descriptive statistics(Frequencies and percentages|) were used to summarise the demographic characteristic which were interpreted as pattersns.

Use Value

$$UV = \Sigma \frac{Ui}{N}$$

Use value underscores the importance of the various uses of the *M. zeyheri* plant species, where UV refers to the relative Use Value of the species, *U* is the number of uses mentioned by each participant, and *N* stands for the total number of participants who reported the species.

Fidelity Level

$$FL\ (\%) = \frac{n}{N} x100$$

The fidelity level expresses the response rates per specific use or the degree of consensus between participants (Gouwakinnou et al., 2011), where n is the number of participants related to a particular use of *M. zeyheri* for treating ailments and N is the total number of participants. The FL is significant when above 5% (FL >5%).

Relative Frequency of Citation

$$RFC = \frac{FC}{N}$$

FC is the number of Participants who cited the medicinal uses of *M. zeyheri*, and N is the total number of recruited Participants familiar with the ethnobotanical uses of the species.

4.3. Results and Discussion

4.3.1. Demographic information

The demographic profile of participants in the ethnobotanical survey of *M. zeyheri* revealed a community where traditional knowledge and practices are influenced by age, gender, education, and employment status. The age distribution reveals notable differences between the two provinces. In Limpopo, the majority of the participants fall within the 45-59 age group (47.6%), followed by participants above 60 years of age (19.1%), highlighting that Indigenous knowledge in this province is held by older generations who based on their lived experiences are often regarded as custodians of knowledge (Bihari, 2023). Mpumalanga, on the other hand, shows contrasting results, with a larger proportion of participants falling within the 30-44 years age group (34.5%), followed by those aged 18-29 years (29.3%), with participants aged 45-59 years representing a minor proportion (13.8%). This age distribution patterns suggest that knowledge about *M. zeyheri* is distributed across different generations, with a relatively young and middle-aged population more engaged with the traditional use of *M. zeyheri*, a trend observed in similar ethnobotanical studies where younger generations orally inherit traditional knowledge on the use of wild fruit species from the older generations (Joshi et al., 2011; McGregor, 2004).

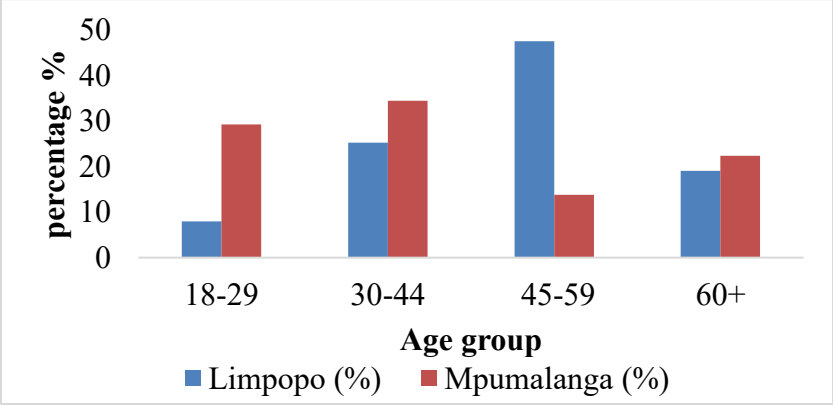

Figure 4.2: Participants' ages from Gutshwa in Ehlanzeni district of Mpumalanga Province and Vhembe district of Limpopo, South Africa.

Gender dynamic reveals a similar pattern, with both provinces showing a higher representation of female participants, 57.1% in Limpopo and 55% in Mpumalanga. These findings highlight the crucial role women play in managing households in both provinces (Kumar et al., 2021; Singh et al., 2022). Furthermore, the role of women as key players in the use of indigenous knowledge related to wild plants has also been reported, which signifies women as custodians of knowledge (Kainer and Duryea, 1992; Ndhlovu et al., 2023; Sood et al., 2015). A larger proportion of the participants in both provinces presented as unmarried, Limpopo (57.1%) and Mpumalanga (53.1%), with a notable proportion of divorced individuals in Limpopo (33.3%) compared to Mpumalanga (19%).

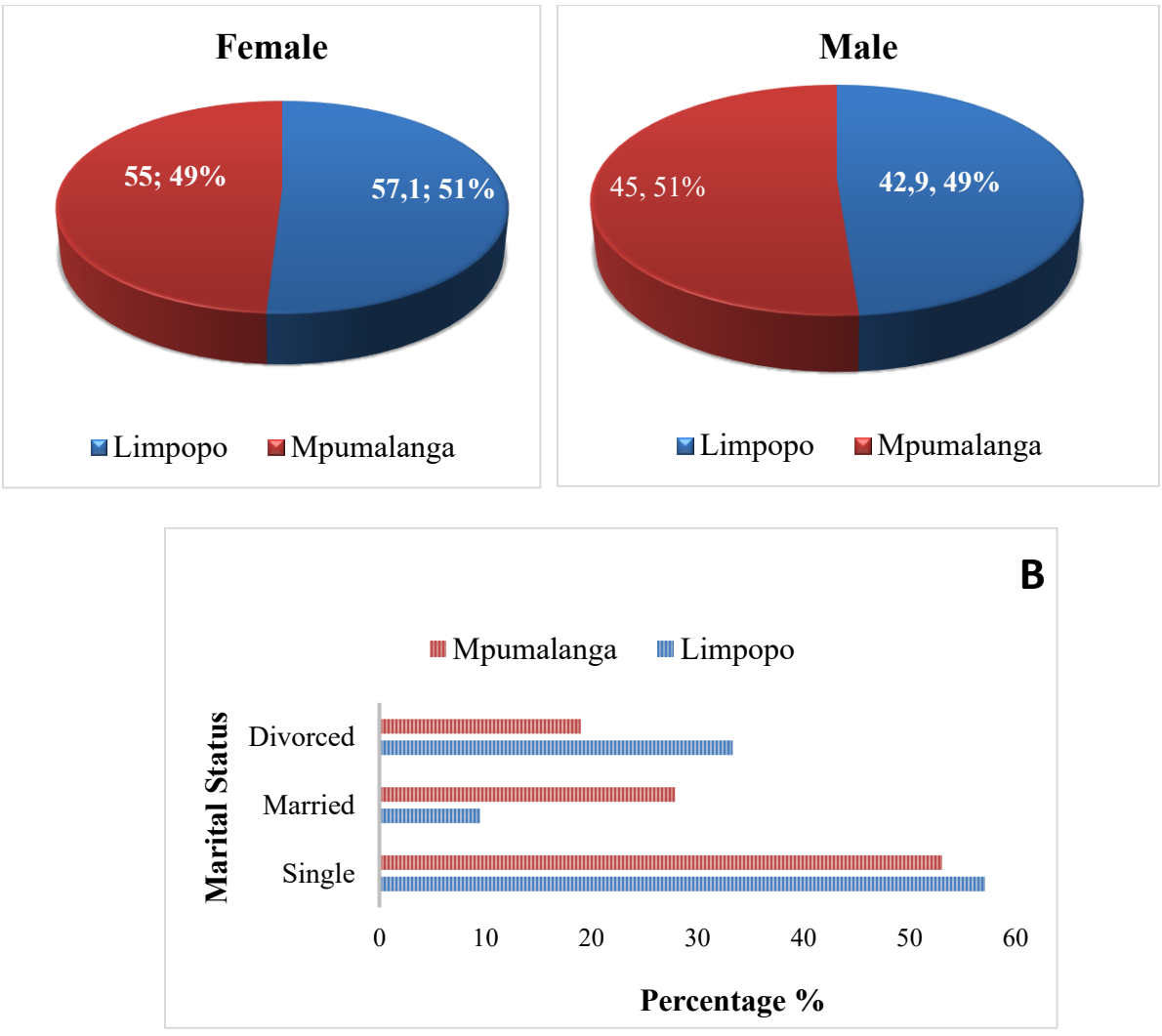

Figure 4.3: Gender and marital status (B) of participants from Gutshwa in Ehlanzeni district of Mpumalanga Province and Vhembe district of Limpopo Province, South Africa.

In Limpopo, most participants had only primary (54.8%) or no formal education (11.8%), whereas a substantial majority of participants in Mpumalanga had attained secondary education (64.8%), while tertiary education holders comprised 23.8%. Several studies have reported a positive correlation between low education levels and greater medicinal plant knowledge and transmission (Corroto et al., 2022; Tamene et al., 2024). The high level of education among participants in Mpumalanga could influence knowledge transmission and preservation, as education has been reported to play a crucial role in the socioeconomic status of participants, including their awareness of environmental health, traditional practices, and sustainability (Arjona-García et al., 2021; Ndhlovu et al., 2023). Lastly, 52.4% and 55.7% of participants from Limpopo and Mpumalanga, respectively, were unemployed, with a considerable number of self-employed participants in Limpopo (28.6%) compared to Mpumalanga (15.2%). This result pattern highlights economic dependency on natural resources, consistent with several reports that have highlighted how communities with limited employment often rely on local biodiversity and natural resources for sustenance (Robinson, 2016).

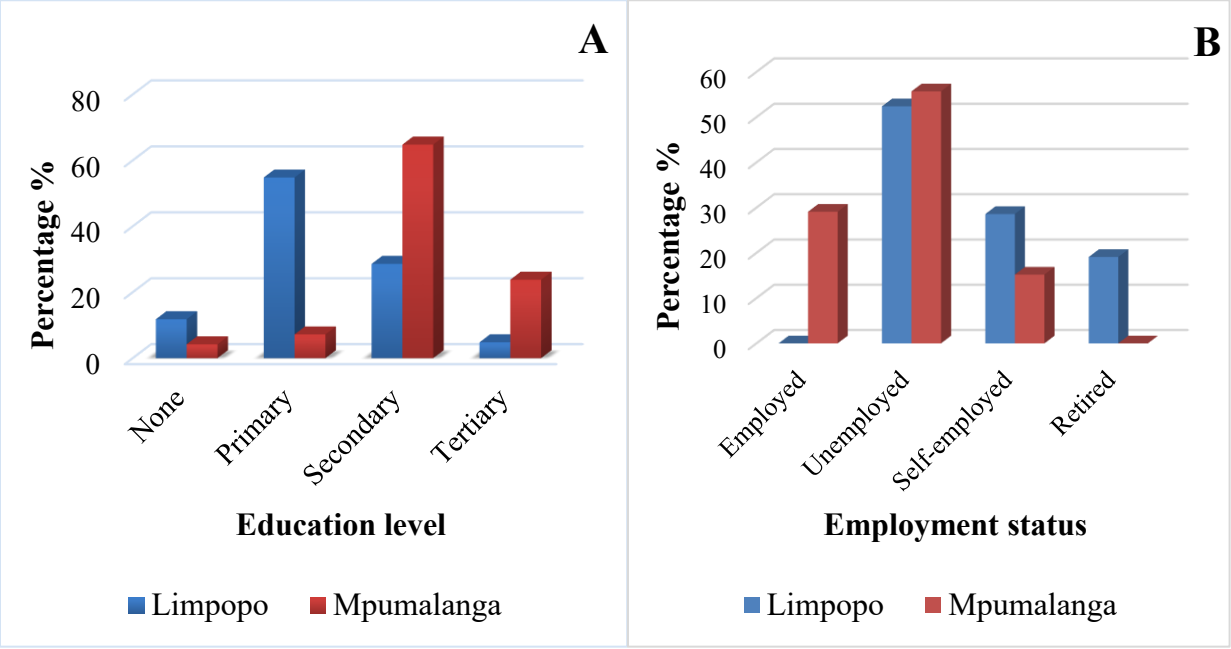

Figure 4.4: Education level (A) and Employment status (B) of participants from Gutshwa in the Ehlanzeni district of Mpumalanga Province and the Vhembe district of Limpopo Province, South Africa.

4.3.2. Relative frequency of citation and use of *M. zeyheri* for traditional and ethnomedicinal purposes**.**

Tables 4.2 and 4.3 document the traditional and ethnomedicinal uses of *M. zeyheri* in Limpopo, Vhembe, and Mpumalanga, specifically in the Ehlanzeni region. The results demonstrate the multifaceted use of *M. zeyheri* by different ethnic groups to treat various ailments and conditions. A total of 16 other uses of *M. zeyheri* were cited in Limpopo (Table 4.3), and a total of 27 uses were mentioned in Mpumalanga (Table 4.4). The FRC values ranged from 0.29 to 1 in Limpopo (Table 4.3) and 0.03 to 1 in Mpumalanga (Table 4.4). Fruits eaten whole as a snack are the most cited traditional use of the wild fruit tree, cited by 58 participants in each province (116 in total). In Limpopo, the ethnomedicinal use of *M. zeyheri* to treat sexually transmitted infections received an FRC value of 1, cited by all participants (FL=100%), with effectivity reported when the species is mixed with other plant species such as *Elephantorrhiza elephantina, Securidaca longepedunculata, Albizia versicolor*, and *Annona senegalensis* boiled to form a decoction that is taken orally. Similar use of *M. zeyheri* for treating sexually transmitted infections is also cited by 29 participants in Mpumalanga, with an RFC value of 0.50, highlighting the similar use of the same species by different tribes. Other commonly cited uses include the use of *M. zeyheri* for the treatment of skin ailments and conditions such as ringworm (RFC=0.57) in Mpumalanga and (RFC=1) in Limpopo. In Mpumalanga, the use of *M. zeyheri* extends to spiritual and cultural practices, such as using the leaves to remove bad omens (RFC = 0.10) and to collect the spirit of the deceased person from where they last took their breath (RFC = 0.05).

Table 4.2: Ethnobotanical and traditional uses of *M. zeyheri*, preparation, and notable modes of administration in Vhembe district, Limpopo Province.

Ethnobotanical & Traditional Use	Plant part	Method of preparation	Administration	N=58	RFC	FL (%)
Treating a Sore throat	Stem, Bark	Stem and bark of *M. zeyheri* are dried and ground into fine powder and combined with the powdered roots and bark of *Zanthoxylum davyi*	Orally The powder is mixed in water and taken orally.	17	0.29	29
Menstrual Cramps	Stem, Bark, Leaves	Stem and bark, or leaves, are boiled and used as a vaginal stem to ease period pains.	Steaming	18	0.31	31
Womb cleansing	Stem, Bark	*M. zeyheri* mixed with the bark of *Cucumis Africanus tender*, and *Warburgia salutaris* are infused in water	Orally The infusion is taken orally twice a day (in the morning and evening after eating) to improve women's fertility.	38	0.65	65
Enhancing weight loss	Fruits	Decoction of ground dried ripe fruits (rough powder) is combined with dried leaves of Moringa oleifera Fruits are dried and ground into fine powder and combined with powdered moringa leaves, boiled (Decoction), and consumed orally in the morning on an empty stomach	Orally	18	0.31	31
Erectile dysfunction	Root, Leaves	The root and bark of *M. zeyheri* are combined with the roots and bark of *Elephantorrhiza elephantina, Securidaca longepedunculata, Albizia versicolor*, and *Annona senegalensis,* boiled to form a decoction that men consume.	Orally The decoction is taken orally as a tea, twice a day, in the morning and evening.	56	0.96	96
Sexually transmitted diseases (Gonorrhea)	Root, Leaves	The root and Bark of *M. zeyheri* are dried and ground into a fine powder, then boiled to form a decoction that is taken orally.	Orally Twice a day (Morning and evening)	58	1	100
Toothache	Root	Root is chewed directly to extract juices to treat pain	Orally	18	0.31	31

Ethnobotanical & Traditional Use	Plant part	Method of preparation	Administration	N=58	RFC	FL (%)
Snakebite	Leaves	Leaves are pounded into a paste and applied to the bite area	Topically	18	0.31	31
Headache	Roots	Roots dried and pulverized to powder, burned and snorted like snuff / inhaled by children	Inhalation	48	0.82	82
Treating Bilharzia	Root, Leaves	The root and Bark of *M. zeyheri* are dried and ground into a fine powder, then boiled to form a decoction that is taken orally.	Orally	58	1	100
Treating external wounds	Stem, Bark	Stem and bark of *M. zeyheri* are dried and ground into fine powder and mixed with the stem and bark of *Zanthoxylum davyi*	Topically	58	1	100
Treating stomach Ulcers	Stem, Bark	Stem or Bark is dried and ground into a fine powder and mixed with water	Orally The decoction is taken three times a day (Morning, Afternoon, and evening before meals	48	0.82	82
Treating painful gums	Roots	Decoction	Orally	24	0.41	41
Fruits as a snack	Fruits	Ripe fruits are eaten as a snack or boiled to soften them.	Orally	58	1	100
Jam	Fruit	Fruits are boiled and mixed with sugar to create jam	Orally	50	0.86	86
Fermented Juice	Fruit	Fruit pulp is mixed with water and left to ferment for over a week to create a mild juice/alcoholic beverage	Orally	29	0.5	50

Table 4.3: Ethnobotanical and traditional uses of *M. zeyheri*, preparation and notable modes of administration in the Ehlanzeni district, Mpumalanga Province.

Ethnobotanical & Traditional Use	Plant part	Method of preparation	Administration	N=58	RFC	FL (%)
Healing a newborn baby's umbilical cord	Bark, Roots, Leaves	Dried and pulverized into powder, and applied directly to the umbilical cord of the newborn baby	Topical	5	0.09	9
Promote sleep in babies	Stem, Fruits	Decoction	Orally (A few drops twice a day) Fruits given fresh	2	0.03	3
Flu and Chest infections.	Stem, Leaves	Stems and leaves are dried and pulverized into powder. Boil the stems and leaves of the plant and drink the decoction	Orally (Morning and Evening)	21	0.36	36
Womb cleaning after giving birth	Stem, Bark	Stem and bark are boiled with other herbs and given to a mother who has just given birth	Orally	11	0.19	19
Boasting libido and fertility in women	Fruits	Fruits are air-dried and consumed with milk as cereal	Orally	17	0.29	29
Treating sexually transmitted infections	Root, Bark	Roots and bark are boiled to form a decoction.	Orally	29	0.50	50
Treating erectile dysfunction in men	Whole plant (Leaves, barks, nuts, roots, Fruits)	Leaf infusion: Nuts are dried and pulverized to create a fine powder that can be mixed with food Root decoction	Orally	4	0.07	7

Ethnobotanical & Traditional Use	Plant part	Method of preparation	Administration	N=58	RFC	FL (%)
Boast fertility and sperm production in men	Nuts, Fruits	The nuts are dried and pulverized to create a fine powder that can be mixed with food.	Orally	14	0.24	24
Treat Ulcers	Bark, Roots	Decoction	Orally	11	0.19	19
To stop Diarrhea	Stem	The stem is chewed to suck the juices, then the pulp is spit out	Orally	4	0.07	7
Cleansing and removal of the bed omen after the death of a family member	Bark, Roots	Infusion, Decoction	Orally, Steaming and bathing	6	0.10	10
Swelling and relief of painful feet	Roots	Decoction	Soak off	9	0.16	16
Treating Asthma	Stem, Bark	Tincture	Inhalation	3	0.05	5
Pain relief	Leaves	Leaves are dried and pulverized. The decoction is used to relieve body pain as a tea and a relaxant	Orally	5	0.09	9
Managing High Blood Pressure	Whole plant	Infusion, Decoction	Orally	26	0.44	44
Treating snake bites	Leaves	Infusion	Topical application	30	0.52	52
Treating skin conditions, i.e., Rash, Ringworms	Bark	Powder or pounded paste	Topical application	33	0.57	57
Treating boils and abscesses	Leaves	Pounded into paste	Poultice	29	0.50	50
Treating painful gums	Roots	Decoction	Orally	37	0.64	64
Headache	Roots	Roots dried and pulverized into powder and snorted like snuff	Snorted	31	0.54	54

Ethnobotanical & Traditional Use	Plant part	Method of preparation	Administration	N=58	RFC	FL (%)
Used to collect the spirit of the deceased person	Stem, Leaves	Leafy branches are used to collect the spirit of the deceased person from their place of death to their place of residence and, eventually, the grave.	-	3	0.05	5
Healing heel cracks	Stem	Latex from the stem is applied as lotion between the cracks on the feet.	Topical	3	0.05	5
Back pain	Whole plant	Decoction	Poultice	3	0.05	5
Boasting Immunity	Fruits	Fruits are dried and ground into a fine powder, which is then added to a soft porridge.	Orally	29	0.50	50
Reducing nausea in infants	Fruits	Fruits are dried and ground into a fine powder and added to baby milk or food.	Oraly	9	0.16	16
Attracting luck during interviews	Bark and Roots	Infusion and decoction remedies are used to bathe before the interview	Soak off	2	0.03	3
Fruits as a snack	Fruits	Ripe fruits are eaten as a snack or boiled to soften them.	Orally	58	1	100

4.3.3. Application of *M. zeyheri* in ethnomedicine for treating ailments and conditions

Mimusops zeyheri is a multifaceted wild tree, used to treat various ailments and conditions, including reproductive health and fertility issues, which is predominantly cited in Limpopo (21%) as demonstrated in Figure 4.5. On the contrary, Mpumalanga reflects a lower usage (14%) of the plant for reproductive health. Skin and wound care show a notable variation between the two provinces, with Mpumalanga registering dominance (32%), which aligns with historical documentation of indigenous wild fruits in traditional African medicine, where such remedies have been fundamental in treating ailments long before modern pharmaceuticals, compared to Limpopo, which registers low usage (11%). These findings are consistent with those reported by Malulekea et al. (2022), who found that skin and wound care are the primary focus in Mpumalanga, with most traditional healers using wild medicinal fruit trees and plants as primary treatments for both human and cattle skin diseases. The diversity in the use of *M. zeyheri* for reproductive health and skin and wound care between the two provinces also underscores the comprehensive region-specific cultural, traditional knowledge, and practices that may be attributed to environmental, cultural norms, beliefs, and region-specific health requirements.

Digestive health (10.5% and 12%) and pain and inflammation (16% and 15%) show a closely uniform use between Limpopo and Mpumalanga, respectively, demonstrating a deep-rooted traditional use regardless of the geographical location. Interestingly, the use of *M. zeyheri* for spiritual and cultural purposes does not appear in Limpopo but records nearly 6% in Mpumalanga, highlighting a holistic understanding of health characteristics of traditional African medicine in the province. This greatly reflects ancient wisdom where plants serve as a bridge between physical healing and spiritual well-being, particularly evident in the 8% of use related to spiritual and cultural practices (Shizha and Charema, 2011).

The high prevalence of skin and wound care applications is crucial, as untreated wounds can lead to severe infections in communities with limited access to conventional antibiotics and antiseptics. The integration of *M. zeyheri* in traditional medicine serves as an example of the broader significance of Indigenous Knowledge Systems (IKS) in rural healthcare systems. Traditional knowledge serves as a sustainable approach to healthcare, where community health needs are met using natural resources without reliance on external and often inaccessible modern products (Kola, 2022). The applicability of *M. zeyheri* in treating respiratory infections (15% and 13%) in Limpopo

and Mpumalanga, respectively, as well as its use for treating "other" conditions, further underscores its versatility in addressing common health issues.

The sustainable utilization of *M. zeyheri* contributes to rural economic resilience in several ways, including providing primary healthcare applications and potential income generation opportunities through the sale of traditional medicinal products, nuts, and fruits (Matlala et al., 2024; Mkhonto et al., 2024). *Mimusops zeyheri* serves as a bridge between Indigenous Knowledge Systems and modern science due to its continued relevance in contemporary healthcare. The importance of incorporating traditional medicine into mainstream formal healthcare systems in regions where traditional practices remain the dominant source of healthcare has been emphasized by the World Health Organization (Bodeker et al., 2017; Payyappallimana, 2010). The significant proportion of dermatological applications suggests possibilities to develop standardized formulations that could enhance both local and international healthcare systems.

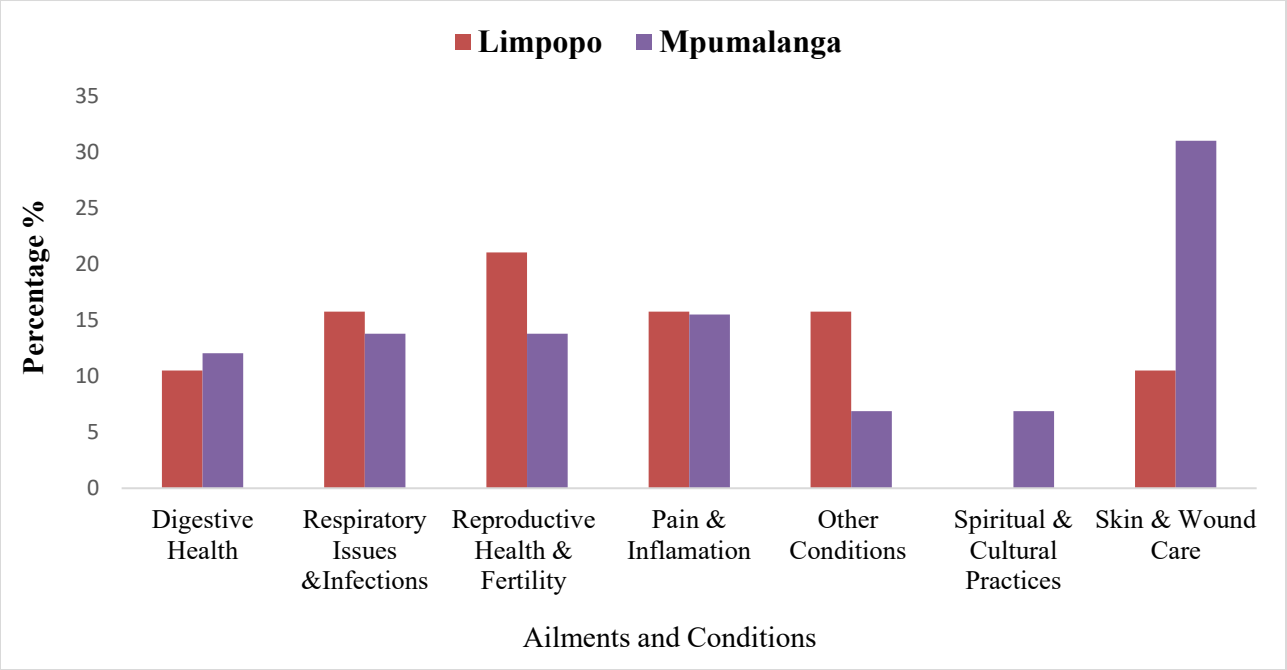

Figure 4.5: Categories of ailments and conditions treated with *Mimusops zeyheri* in Vhembe, Limpopo, and Gutshwa village, Ngodini, Mpumalanga province, South Africa.

4.3.4. Preparation of ethnomedicine from *M.zeyehri*

Traditional preparation methods are crucial in ethnomedicine for the effective extraction of bioactive compounds, which are essential for treating various ailments and conditions. Preparation

methods are mainly region-specific, influenced by local culture, location, and the part of the plant used. Interestingly, the study reveals the prevalence of decoction in both provinces, with slight differences (36% and 34%) in Limpopo and Mpumalanga, respectively (Figure 4.2). These findings are consistent with reported traditional African ethnomedicinal practices that use decoction to extract bioactive compounds by boiling plant parts to break down cellular structures, thereby increasing the bioavailability of compounds (Qihua et al., 2015). Traditional healers in Vhembe often employ decoctions for treating chronic conditions, gastrointestinal disorders, and reproductive health issues such as erectile dysfunction using the roots and bark of *M. zeyheri* (Table 4.2). The high percentage of decoction use suggests a cultural understanding of the need for thorough extraction methods when dealing with woody plant parts, which are common in *M. zeyheri* preparations (Mkhonto et al., 2024).

Infusions are preferred among many African ethnic groups, as shown in the results, with Limpopo citing (21%) and Mpumalanga citing (25%) use of the preparation method. This method is favoured for extracting volatile compounds and heat-sensitive active ingredients that might be degraded by prolonged boiling (Asmaa, 2014). It is worth noting that the use of powder is more prevalent and preferred in Limpopo (21%) compared to Mpumalanga, which underscores the cultural preference for desiccated plant materials in medicinal use.

Interestingly, application methods such as Tinctures and burning (representing 8% and 5%, respectively) are more predominant in Mpumalanga, indicating localized practices that may relate to beliefs and cultural customs. Tincture involves extracting plant materials using alcohol or vinegar as solvents. Traditional healers may prefer tinctures for treating specific conditions where alcohol-soluble compounds are believed to be more effective, such as certain skin conditions or for preserving medicinal preparations (Dlova and Ollengo, 2018). The burning method, used for medical or spiritual purposes, is acknowledged as the most rudimentary technique. Moreover, the creation of medicinal ash or smoke can also be done through this method (Braithwaite et al., 2008). The low percentage suggests this is a specialized preparation method, possibly reserved for specific conditions or ceremonial purposes. The use of burning methods may also indicate an acknowledgment of volatile compounds released through combustion and their potential therapeutic effects (Ningthoujam et al., 2013). The relatively low percentage might suggest that

this method is reserved for specific situations where fresh preparation is necessary for therapeutic efficacy, and its limited use is restricted to external conditions.

The distribution of preparation methods reflects a sophisticated understanding of extraction techniques and their appropriateness for different therapeutic applications (Brusotti et al., 2014). The predominance of water-based methods (decoction and infusion, totalling 59%) suggests a practical approach to medicine preparation, considering water's availability and effectiveness as a solvent for many plant compounds (Rudraswamy et al., 2021). The variety of preparation methods also indicates an understanding of how different extraction techniques might affect the bioavailability and efficacy of medicinal compounds.

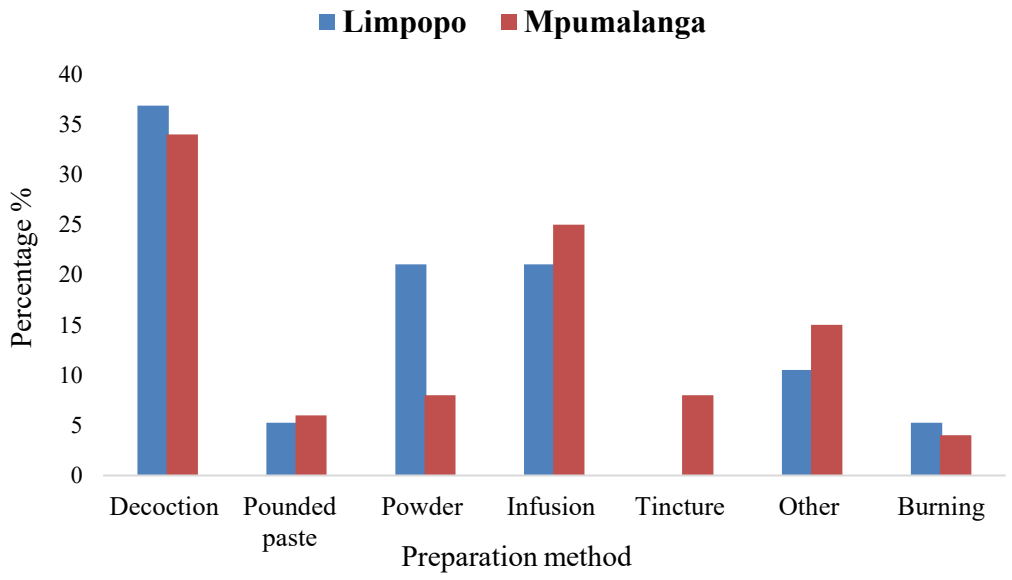

Figure 4.6: Methods of preparing *Mimusops zeyheri* to treat different ailments and conditions in Vhembe, Limpopo Province, and Ehlanzeni, Mpumalanga Province, South Africa.

4.3.5. Administration methods of *M. zeyheri* ethnomedicine

Administration methods in Figure 4.6 shows internal and external applications cited by participants in the study, with oral administration being the most preferred mode of delivery in both Limpopo (75%) and Mpumalanga (55%). These findings are consistent with several studies (Ashu Agbor and Naidoo, 2015; Chakraborty et al., 2022; Kosalge and Fursule, 2009; Rahmatullah et al., 2009) that reported oral administration as a significant and effective delivery mode. Many scholars report that the choice of oral administration is related to the use of solvents, such as water, milk, butter,

and food additives, which facilitate administration. Similarly, the choice of oral administration has been attributed to the easy absorption of bioactive substances from medicinal remedies in the gastrointestinal tract (Gupta et al., 2005). However, there is no consensus on dosage and frequency of use when using oral remedies. Topical applications account for a sizable proportion of application methods in both provinces, with Limpopo (19%) showing a slightly higher preference compared to Mpumalanga (13%), demonstrating the significant potential of *M. zeyheri* in treating skin and wound conditions. These results are consistent with a report from a study by Abbasi et al. (2010) who reported the efficacy of topical administration of 66 plant species against 15 skin diseases. Different administrations, including inhalation (6% and 9%), were cited in Limpopo and Mpumalanga, respectively, demonstrating alternative use of *M. zeyheri* for the treatment of respiratory issues and therapeutic care. Although studies on *M. zeyheri* are scarce, antibacterial findings on other Sapotaceae species, such as Manila zapota essential oils, have demonstrated remarkable respiratory benefits (Gam et al., 2024a).

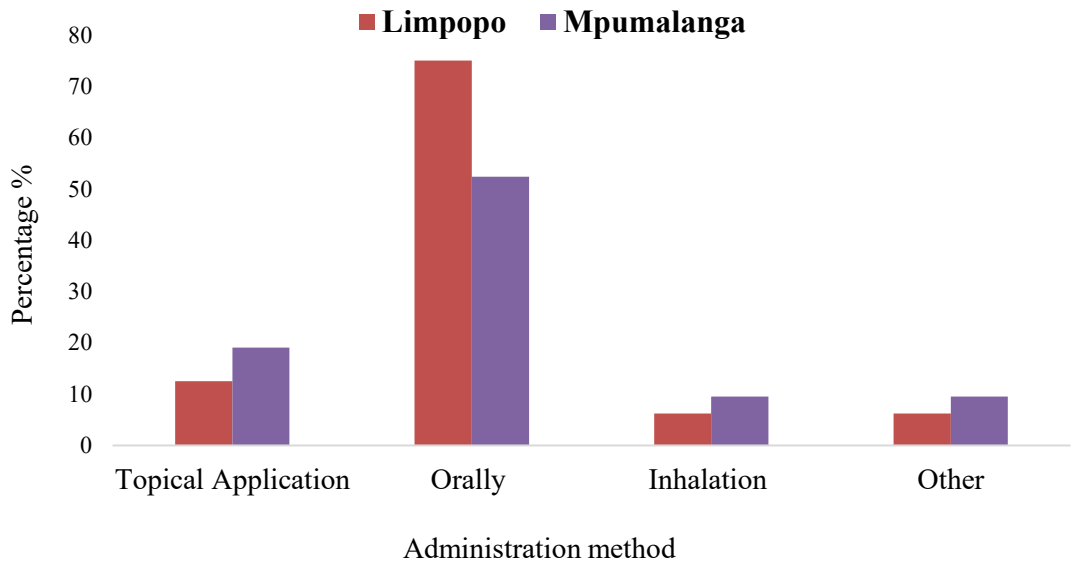

Figure 4.7: The medicinal administration techniques for treating different ailments and conditions in Vhembe, Limpopo Province, and Ehlanzeni, Mpumalanga Province, South Africa.

4.3.6. Mimusops zeyheri plant parts used in ethnomedicine

Different plant parts, including the whole plant, were used for the treatment of various ailments and conditions, as shown in Figure 4.8. The distribution of plant part usage reveals a strong preference for fruits in both provinces, with Mpumalanga exhibiting the highest citation rate

(30%), compared to Limpopo (18%). The predominant use citation of fruits is consistent with findings by other scholars (Deshmukh and Waghmode, 2011; Pachuau and Dutta, 2019; Rana et al., 2021), who reported the use of wild fruits in traditional medicine to treat various ailments and conditions, including ulcers, dysentery, and, at times, as a muscle tonic. Similar to many wild fruits, *M. zeyheri* fruits are rich in vitamin C, antioxidants, and phenolic compounds, which are essential for promoting health and boosting immunity while simultaneously improving food diversity in rural communities. The bifunctional use of *M. zeyeheri* fruits as both food and a source of medicine underscores the species' value and significance in supporting rural healthcare, aligning with Maroyi's (2020) emphasis on the continued integration of wild fruits to leverage their nutritional and medicinal benefits in African traditional medicine.

In Limpopo, roots were among the most frequently cited (17%) plant parts, compared to Mpumalanga (10%). These findings are consistent with those of Mongalo and Makhafola (2018), who reported that roots and rhizomes are the most commonly harvested plant parts in Limpopo, sold in significant quantities by hawkers and vendors across informal markets within the province. The preference for roots is attributed to the variation in concentrations of different active bioactive compounds, which is due to edaphic and climatic factors (Shambharkar et al., 2025). Although slightly low, the uprooting of *M. zeyheri* may pose a threat due to the destructive nature of the practice, which could affect the health and regeneration potential of the plant. Leaves were equally cited in both the Limpopo and Mpumalanga provinces (22%), reflecting a shared understanding of their use and significance in traditional medicine. The shared citation on the use of leaves can be attributed to the presence of a pool of bioactive compounds in leaves that have been reported to be pharmacologically active against various diseases and ailments (Xavier et al., 2015). Nuts usage is cited nearly equally across both provinces, Mpumalanga (17%) and Limpopo (16%), representing a significant component of *M. zeyheri*'s medicinal applications. Research by Baky et al. (2022b) has demonstrated that nuts from the Sapotaceae family contain bioactive compounds with proven antimicrobial and antioxidant properties. These results show that the majority of plant preparations used a single plant part in correspondence with the specific health ailment or condition. Similar findings were also reported for the use of single plants or plant parts for a single health problem (Ganjhu et al., 2015; Ssenku et al., 2022). Some remedies were prepared from the whole plant (11% and 9%) in Limpopo and Mpumalanga, respectively.

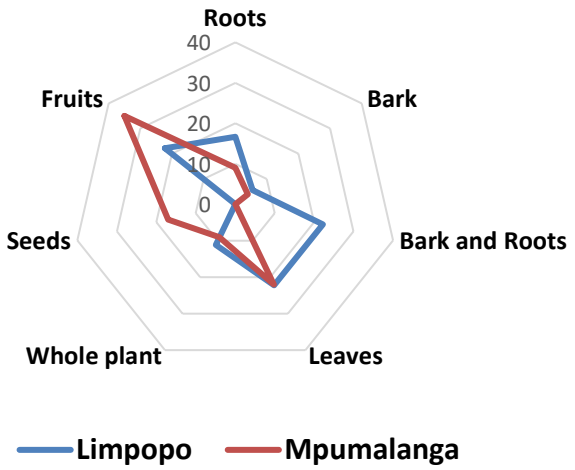

Figure 4.8: *Mimusops zeyheri* plant parts used for managing different ailments and conditions in Vhembe, Limpopo Province, and Ehlanzeni, Mpumalanga Province, South Africa.

4.3.7. Participants' perceptions about threats to *M. zeyheri* populations and distribution

Perceived threats in Figure 4.9 shows that participants in Mpumalanga (40%) reported land expansion and infrastructure development as the primary threat to *M. zeyheri* trees and their distribution, compared to Limpopo (30%), reflecting different perceived socio-economic pressures between the provinces. The results of this study align with those of Alexander et al. (2018), who documented how agricultural expansion and human settlements increasingly fragment the natural habitats of indigenous tree species in South African rural areas. Typically, Mpumalanga has experienced rapid urbanization due to its burgeoning agricultural and mining sectors, as well as population growth. It is undeniable that land expansion leads to the substantial destruction of habitats, with deforestation threatening local indigenous plant species, such as *M. zeyheri* (Dubey et al., 2023; Singh, 2024). Urban and rural expansion can reduce the genetic diversity of wild populations by fragmenting habitats and increasing isolation between populations, potentially affecting long-term species survival (Hending et al., 2023).

Overharvesting presented as the second most significant perceived threat, with Limpopo showing a slightly higher concern (30%) compared to Mpumalanga (25%). Participants reported unsustainable exploitation practices in this region. *Mimusops zeyheri* wastraditionally used for its fruits, timber, and medicinal properties, and local communities often harvested it for food and cultural purposes (Oluoch et al., 2023). In Limpopo Province, roots are the most commonly used

plant parts, resulting in overharvesting threats with minimal potential for restoration. Overharvesting posed a threat to the ecological balance by disrupting the natural fruiting cycle, reducing resource availability due to population decline, decreasing resilience to environmental stressors, and loss of the ability to adapt to climate change (Iyiola et al., 2023).

Participants noted that wildfires in Limpopo Province (20%) were a more concerning threat to the survival of *M. zeyheri* compared to those in Mpumalanga Province (15%). According to Midgley et al. (2010), increased fire frequency and intensity significantly impact tree recruitment and survival, particularly for slow-growing species such as *M. zeyheri*. Though its impacts are species-specific, climate change wasnoted as a threat in both provinces (10% and 15%) in Limpopo and Mpumalanga, respectively. Climate change can interact with impacts such as wildfire, resulting in fragmentation. Both threats can therefore pose severe ecological consequences, such as altering soil composition and altering the soil biome, leading to excessive stress responses in wild fruit trees. In the case of *M. zeyheri*, wildfires may induce the production of defense compounds, such as phenolics and tannins, which serve as a survival mechanism in the tree's phytochemical profile (Wang and Zhang, 2017; Wang et al., 2023). Furthermore, fires can disrupt nutling establishment, reduce fruit availability, and impact *M. zeyheri's* role as a keystone species within its habitat (Pausas and Keeley, 2014). These impacts can exacerbate habitat loss and lower the tree's ability to regenerate, particularly in environments where fire management practices are inadequate.

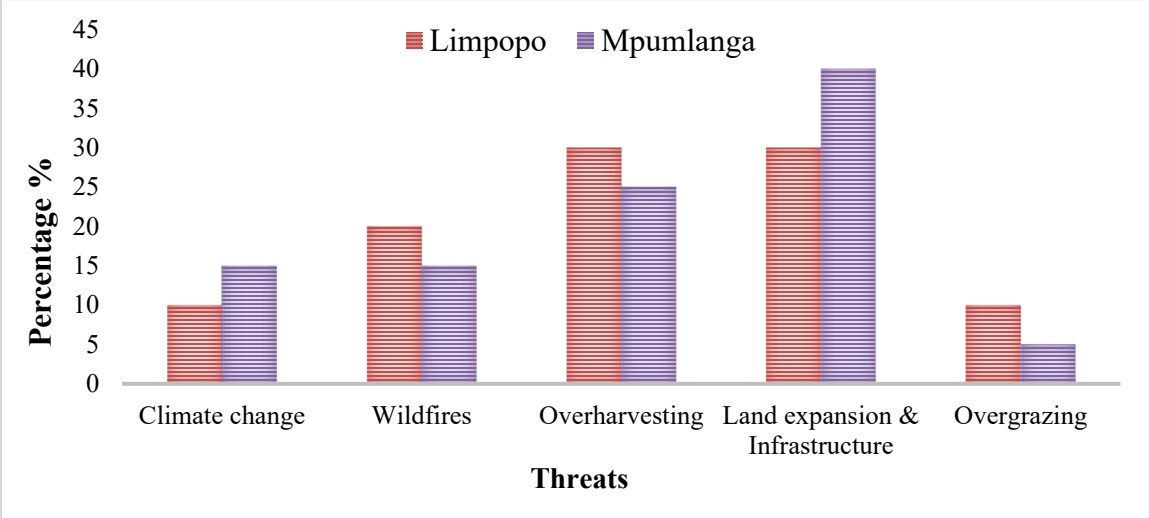

Figure 4.9: Perceived threats to *M. zeyheri* populations and distribution in Vhembe, Limpopo Province, and Ehlanzeni, Mpumalanga Province, South Africa.

4.4. Concluding remarks

This study comprehensively documented the traditional knowledge associated with the medicinal uses of *M. zeyheri.* This included participants' perceptions related to the threats faced by *M. zeyheri* 's populations in the Vhembe district of Limpopo and the Ehlanzeni district of Mpumalanga. The results cemented the traditional relevance and significance of *M. zeyheri* in the dietary requirements and healthcare system of rural people in the Vhembe and Ehlanzeni districts of South Africa. The study revealed the multifaceted uses of *M. zeyheri*, including its application in the treatment of skin and wound ailments and conditions, the treatment of sexually transmitted infections, and its contribution to the spiritual and cultural beliefs of the local people in the two districts. This valuable inventory of the traditional and ethnomedicinal uses of *M. zeyheri* represents a significant step towards the ongoing effort of documenting, preserving, and promoting indigenous knowledge in the study areas. Overall, the study contributes to the knowledge bodies, including ethnobotany, socio-ecological systems, ethnomedicine, and plant sustainability.

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CHAPTER FIVE

POPULATION STRUCTURE, DISTRIBUTION AND SPATIAL PATTERNS OF *MIMUSOPS ZEYHERI* IN TWO AGRO ECOLOGIES: A COMPARATIVE ECOLOGICAL STUDY OF EHLANZENI AND VHEMBE DISTRICTS OF SOUTH AFRICA

Summary

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 ecological condition of *M. zeyheri* in two distinct regions: the Ehlanzeni District (Mpumalanga) and the 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 = 2.68 m), 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 *zeyheri* as a potential agroforestry species, contributing to climate resilience and food security.

Keywords: *Mimusops zeyheri*, population structure, agroecology, ecological disturbance, size-class distribution.

5.1 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 by providing ethnomedicine 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 experiencing population size and density reduction with compromised regeneration capacity due to anthropogenic disturbance. According to Wessels (2021) 81-97% of African wild fruit tree species are projected to experience a reduction in suitable habitats, with 24-42% expected to go extinct by 2085 due to shift or contraction of geographical areas and ecosystems that provide suitable growing conditions for wild tree species. 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, shaped largely by biophysical factors, including topography, soil properties, climate conditions, and land use pattern (Liu et al., 2021). In South Africa, the distribution of wild fruit species is influenced by diverse vegetation and landscape dynamics across the various regions, which show cultivation suitability (Sardeshpande and Shackleton, 2020). The Northern and Western Cape provinces are less suitable for wild fruit growth due to arid to semi-arid climatic conditions including low variable rainfall which limit the establishment potential and productivity of many indigenous wild fruit tree species. These environmental conditions tend to favor the predominance of drought tolerant vegetation such as shrubs and sparse land cover (Salami et al., 2022). In contrast, provinces such as Limpopo, Mpumalanga, KwaZulu-Natal, and the Free State are reported as more favourable for cultivating wild fruit trees due to higher rainfall patterns and suitable temperature regimes (Genis, 2020; Mothupi and Shackleton, 2025; Salami et al., 2022). Several wild fruit species, including milk plum and 29 others prioritized for domestication and commercialization, are found in coastal provinces such as KwaZulu-Natal (Nkosi et al., 2020), with *Strychnos spinosa* ranking the highest. Limpopo and Mpumalanga provinces are recognized 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 utilization patterns of wild fruit trees within the provinces have received insufficient scientific attention, indicating a substantial knowledge gap in research and documentation (Lubisi et al., 2024; Shai et al., 2020).

Mimusops zeyheri Sond. (Sapotaceae), recognized as a keystone species, occurs widely in rural communities within Limpopo province particularly the Vhembe District as well as in the Mpumalanga province of South Africa. The ecological distribution of *M. zeyheri* is influenced by soil composition, with reports documenting varying growth responses to clay, loamy and sandy

soils, with clay soil showing remarkable leaf growth patterns (Omotayo et al., 2020). The tree has several reported ecological benefits, including soil stabilization and providing habitat for birds and endophytes (Dube et al., 2016). Although recognized 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 on environmental factors influencing local distribution in different Agro ecologies. While several ethnobotanical studies have documented the traditional and spiritual uses, as well as the regional perceptions of *M. zeyheri,* by local rural communities, detailed ecological studies aimed at quantifying the 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 the rural communities of the Vhembe district in Limpopo, *M. zeyheri* faces mounting pressures attributed to habitat fragmentation, agricultural expansion, and unsustainable harvesting practices (Atanasso et al., 2021; Lubisi et al., 2023). Sustainable utilization and conservation of wild fruit trees require the documentation of their population ecology and distribution patterns.

The sustainable utilisation and conservation of the *M. zeyheri* species require the documentation of their population ecology and distribution patterns. Several studies have shown that wild fruit trees species that occupy larger geographical ranges tend to occur at higher densities and are more widely distributed within those ranges compared to species with more restricted distributions (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 the Vhembe and Ehlanzeni districts of South Africa. The study sought 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.

5.2. Materials and methods

5.2.1. Study site

The study was conducted in two regions of South Africa: the Vhembe district (22.7696°S, 29.9741°E) of Limpopo Province and the 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 influencing the distribution and abundance of wild fruit trees, including *Mimusops zeyheri*. The Vhembe district is the northernmost part of the Limpopo Province and occupies approximately 25,597 km² of total land area, which is nearly 20% of the province. Land use patterns in the Vhembe district are diverse, ranging from livestock rearing, agriculture, mainly maize and sorghum cultivation, and commercial plantations. The district experiences humid climatic conditions, with temperatures ranging from 18 °C in winter to 28 °C in summer and an annual rainfall of 755 mm to 798 mm. summer rainfall is usually between October- and March, with peak precipitation observed mainly in December and February. Additionally. the area is characterized 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. Vhembe is further characterized by the Soutpansberg mountain range, with its highest peak at 1747 m, known to influence the local climate, including the region's temperate and tropical mosaic biota assemblage, which is not found elsewhere in the country. The region is home to various hydrological features, including the Limpopo River, which serves as an international border between Zimbabwe and Botswana, and the 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. The Soutpansberg is home to diverse Afromontane species, including *Zanthoxylum capense* (small knob wood), *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 characterized by grassland species, such as *Panicum maximum* Jacq (guinea grass) and *Themeda triandra* Forssk. (red grass), as well as scattered eragrostis 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 750–860 mm, 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 *Sclerocarrya birrea* (A. Rich) Hoschst (Marula)., *Magnifera indica* L., (Mango) and *Acacia mearnsii* De Wild. (Black wattle) The region is known for its mountainous peaks, which serve as tourist attraction sites, including the Mariepskop mountains with an elevation of 1,944 m, Mohololoholo mountain peak with an elevation of 1,794 m, and the Hebronberg mountain peak with an elevation of 1,767 m. The region is home to several water bodies, including the Olifants River, which borders the Mpumalanga and Limpopo provinces, and the Crocodile River, which is significant for irrigation in citrus farming, aquatic life, and domestic use.

5.2.2. Sampling

The study employed a systematic random sampling method as described by Amini et al. (2015) implemented within a stratified sampling framework. First, each study site was divided into distinct strata based on observable ecological characteristics such as vegetation type, land-use patterns, and topographical variation to ensure representative coverage of the study area. The study employed a stratified systematic random sampling design as described by Amini et al. (2015). First, each study site was divided into distinct strata based on observable ecological characteristics, including vegetation type, land-use patterns, and topographical variation. This stratified sampling approach ensured that all key habitat types within each site were adequately represented and reduced the risk of sampling bias associated with spatial heterogeneity. Within each stratum, systematic random sampling was implemented using transects. At each study site, a total of *7* transects were established, each measuring *[100 m × 10 m]*. Transects were positioned to capture variation within strata and were treated as replicates. Along each transect, a random starting point was determined, after which trees were selected at fixed intervals (*every 5 m*), ensuring equal probability of selection and consistent spatial coverage. Across all transects within a site, a total

of 41 trees were sampled per study site, distributed proportionally across strata. GPS coordinates of each tree were collected for spatial distribution analysis This allocation ensured that sampling intensity reflected the relative size or importance of each stratum while maintaining comparability between sites. The tree circumference was measured using a diameter tape at ground level. Measurements included trees of all sizes, including saplings, seedlings, and mature tree classes. The tree height was measured using the tangent method, with the assistance of 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 described in Table 5.1. Each sampled tree and its immediate surrounding area were visually assessed for signs of disturbance, including bark removal, branch cutting, evidence of fire damage, grazing pressure, and land-use impacts. Based on the observed level of disturbance, each tree was assigned to one of the five predefined classes (A–E), corresponding to increasing levels of ecological impact.

Table 5.1: Ecological disturbance modeling 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%

5.2.3. Data analysis

Field data were coded and entered into a Microsoft Excel 2023 spreadsheet (Version 2301, Microsoft Corporation) for data management and preliminary organization. The coded data were analyzed using a single-factor ANOVA ecological scoring model and a paired t-test for sample means with statistical significance evaluated at 5% probability level ($P<0.05$). Furthermore, some data was analyzed using graphs and percentages.

5.3. Results and Discussion

5.3.1. Tree size class distribution

Figure 5.1 shows different size class frequencies of *M. zeyheri* based on circumference measurements taken at ground level interpreted descriptively as an indicator of population structure. The results show a predominance of trees in the smaller circumference class level at both regions (0-15 cm and 16-31 cm), implying that the population of *M. zeyheri* is under threats in both provinces. This pattern suggests active recruitment and regeneration within populations. However, the low representation of larger size classes may indicate potential limitations in the progress of individual trees into mature stages which can be associated with environmental pressures and anthropogenic disturbances. 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 imply a healthy population structure and future reproduction to avoid extinction risk, while, on the contrary, Tshisikhawe et al. (2012) maintained that a healthy population structure resembles a mix of seedlings 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, it is worth noting that the current study was all about the distribution and population structure of an understudied fruit plant, *M. zeyheri,* in the Vhembe and Ehlanzeni districts of South Africa and never applied Version 3.1 of the IUCN ’s red list Categories and Criteria data gathering.

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, while the absence of larger tree individuals indicates a high tree mortality rate attributed to increased harvesting pressures for food, timber or habitat expansions. Trees in the Ehlanzeni were mainly found within homesteads and in proximity to them, exposing them to human influence as communities have access to the trees for fruits, timber, and other plant parts used for medicinal purposes.

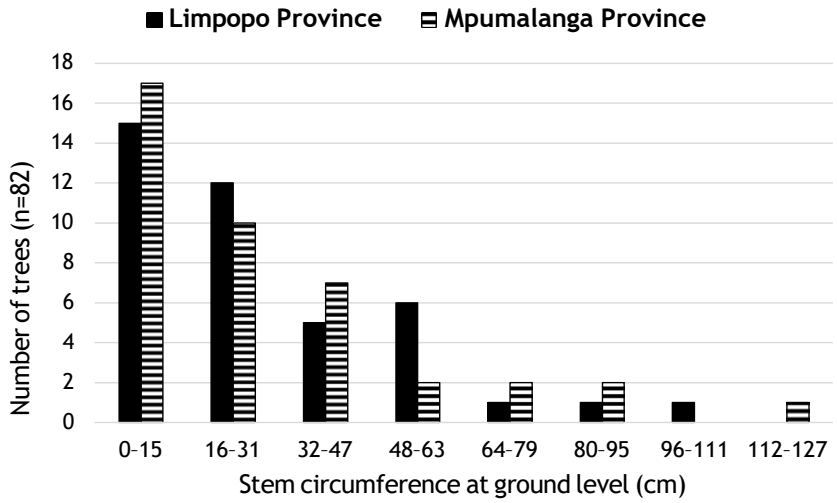

Figure 5. 1: Circumference size-class of *Mimusops zeyheri* in the Limpopo and Mpumalanga Province (s) of South Africa, n=82.

5.3.2. Tree height class distribution

Wild fruit trees exhibit significant variations in height distribution across different regions and altitudes. Figure 5.2 shows the distribution of *M. zeyheri* tree height categories across the two areas. Table 5.2 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) as shown in Table 5.3 with a p-0,017813 and a F-5,853646 (Table 5.2) indicating that the observed statistical difference is driven by taller individuals in Mpumalanga. *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. The Ehlanzeni region of Mpumalanga receives high rainfall and has more humid conditions that support larger tree individuals (Greyling, 2018), conversely, the Vhembe region of the Limpopo province is drier and receives 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 tree heights, necessitating the development of adapted wild tree management strategies. Climatic conditions often influence soil fertility and soil moisture availability, which are critical

determinants of tree growth and productivity. The differences in the two agree-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 Vigor (Kreuzwieser and Gessler, 2010; Lévesque et al., 2016; Ravhuhali et al., 2022). Some trees, such as *Moringa oleifera,* can adapt very well to varying soil conditions without compromising tree growth and height (Bopape-Mabapa et al., 2021). These findings underscore the complex interactions between climate variables and tree growth in the South African ecosystem, which emphasises the need for Soil physiochemical analysis between the two Agro ecologies to validate the observed patterns.

Table 5. 2: Statistical comparison for height between *Mimusops zeyheri* in the Limpopo and Limpopo Province.

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

Table 5.3: Mean height comparison of *Mimusops zeyheri* between Limpopo and Mpumalanga Provinces

Province	Sample size (n)	Mean height (m)	Variance	Standard deviation
Limpopo	41	2.72	2.94	1.72
Mpumalanga	41	5.63	56.57	7.52

Figure 5.2 emphasises the differences noted in Table 5.2 marked divergence in height structure between provinces, with Mpumalanga showing a higher proportion of taller individuals (>6 m) compared to Limpopo. In Limpopo, most trees fall within shorter height categories, pointing to 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 observed in subsequent figures showing disturbances. Studies have reported a positive correlation between mean tree height, diameter, and canopy cover for wild fruit trees (Ekasari and Kurnia, 2023; Gopal et al., 2020; Li, et al., 2021; Woldegebriel, 2025). Tree height is influenced by several factors, including altitudes, which affect the morphological and biological characteristics of wild fruit tree services (Oršanić et al., 2009). According to Schäfer et al. (2000), taller trees exhibit a low stomatal conductance, with a 60% decrease reported in *Fagus sylvatica* trees that were over 30 m 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 height has been proposed as an alternative productivity indicator, useful more especially in natural and mixed species forests and 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 observed in the study indicate a lack of larger individuals in both Agro ecologies, suggesting a low recruitment pattern from younger to more mature size class individuals. This, therefore, 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.

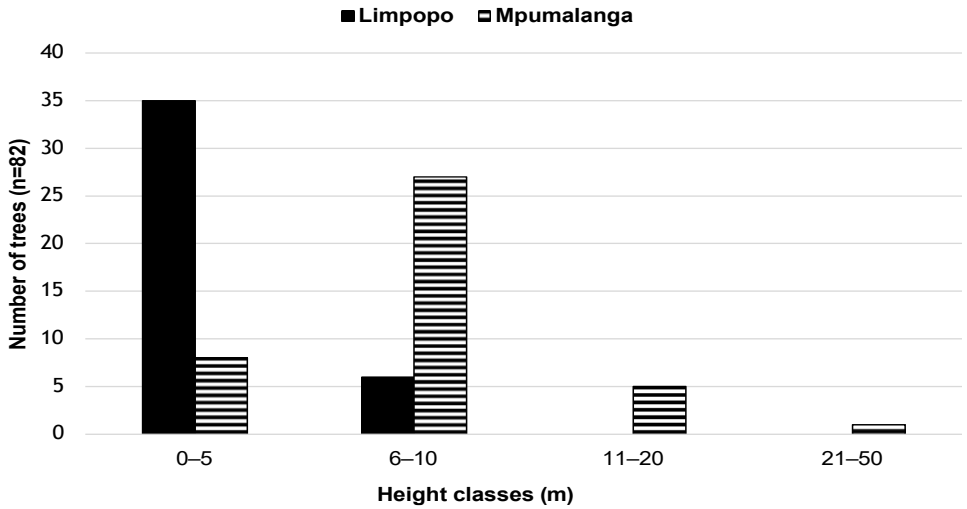

Figure 5. 2:Height size-class comparison of *Mimusops zeyheri* found in the Limpopo Province and Mpumalanga Province.

5.3.3. Tree- distance and spatial distribution patterns

The results in Table 5.4 shows a significant difference in the distribution distance of *M. zeyheri* trees between the two Agro ecologies based on the Universal Transverse Mercator coordinates (UTM). The mean distribution distance is notably greater in Vhembe (5.54m) than it is in Mpumalanga (2.68 m), suggesting a dispersed tree population structure in the Vhembe region. Tree-to-tree distance is influenced by several factors in the ecosystem, including intra-competition among trees. Nut dispersal mechanisms, including anemochory and zoochory, are everyday influencers of wild fruit tree distribution (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 the Vhembe region. A higher degree of variance (29.89) in the area suggests a greater degree of randomness in nut dispersal patterns, which can be attributed to a significant extent to wind dispersal. Given the small and lightweight size of *M. zeyheri* nut, wind dispersal over long distances is possible, which explains the larger tree spacing patterns in the region. The low variance (10.28) in the distance between trees in Ehlanzeni (Table 5.3) indicates a dominance of zoochory. These results are further emphasised by the data shown in Table 5.4 which reflects a clustered distribution pattern (also visible in figure 5.3) 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 tend to grow more slowly and conserve resources while smaller trees grow faster and use resources more actively, showing different growth strategies within the same species. This is evident in the study, as *M. zeyheri* trees in Vhembe exhibit a conservative strategy, while *M. zeyheri* trees in Ehlanzeni show a more acquisition-oriented strategy due to the clustered patterns. 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 factor independent of the distribution of their diameter. 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 5. 4: 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	
Hypothesized 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 5. 3: Clark–Evans nearest-neighbour statistics for *Mimusops zeyheri* populations in Limpopo and Mpumalanga.

Region	U (Unique tree positions)	Area (m²)	Estimated 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

Area (m²) represents the spatial extent derived from recorded UTM (Universal Transverse Mercator) coordinates of sampled tree positions within each study site. Differences in area between Limpopo and Mpumalanga reflect variation in sampling extent rather than a standardised plot size. In this study, the analysis focuses on spatial distribution patterns (Clark–Evans index) rather than direct comparison of density values between sites.

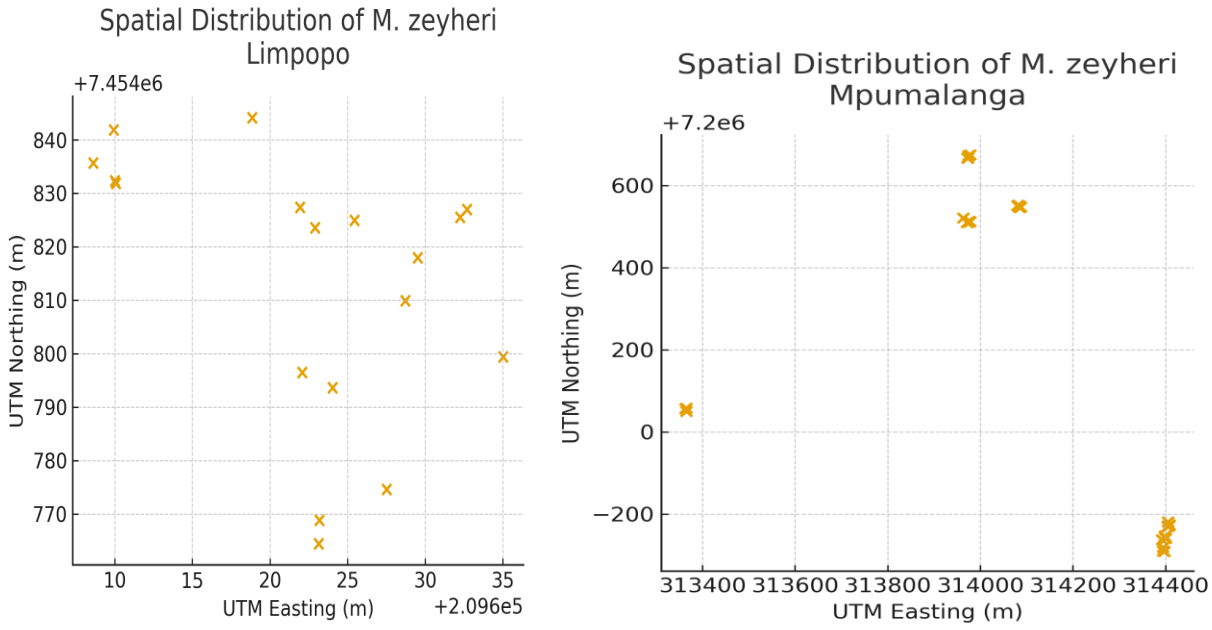

Figure 5. 3:Spatial distribution of *Mimusops zeyheri* based on UTM (Universal Transverse Mercator coordinates in Limpopo and Mpumalanga.

5.3.4. 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, and 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: repeated branch cutting, stem debarking, or selective removal of large limbs as seen in Figure 5.4. Studies of tree populations subjected to such pressure show that these actions can severely weaken individuals — reducing canopy cover, damaging vascular tissue, increasing susceptibility to pests or pathogens, and ultimately 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 likely not 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 5.4. 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, supports soil stability, offers shade, and contributes to microclimate

regulation (Mkhonto et al., 2024b). 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), and thereby weaken 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.

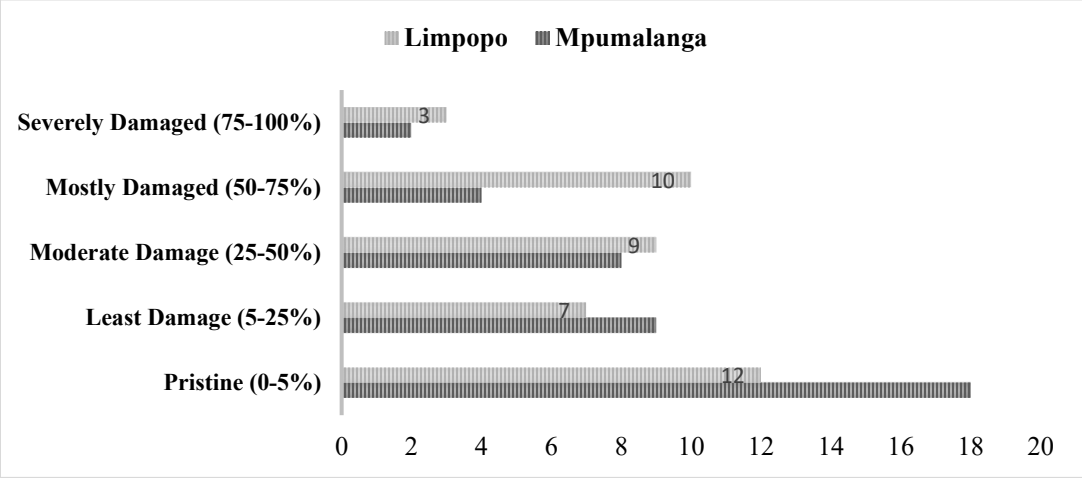

Figure 5. 4: Disturbance Scores of *Mimusops zeyheri* Populations in Limpopo Province and Mpumalanga Province, South Africa.

5.3.5. Observed Damages and Disturbances

The removal of *M. zeyheri* for residential development, noted in Figure 5.6, significantly contributes to population decline by entirely 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 like *Sclerocarya birrea* and *Adansonia digitata*

indicate that the removal of mature trees diminishes fruit availability, modifies nut dispersal, and hinders recruitment, particularly in slow-growing species dependent on substantial, long-lived adults.

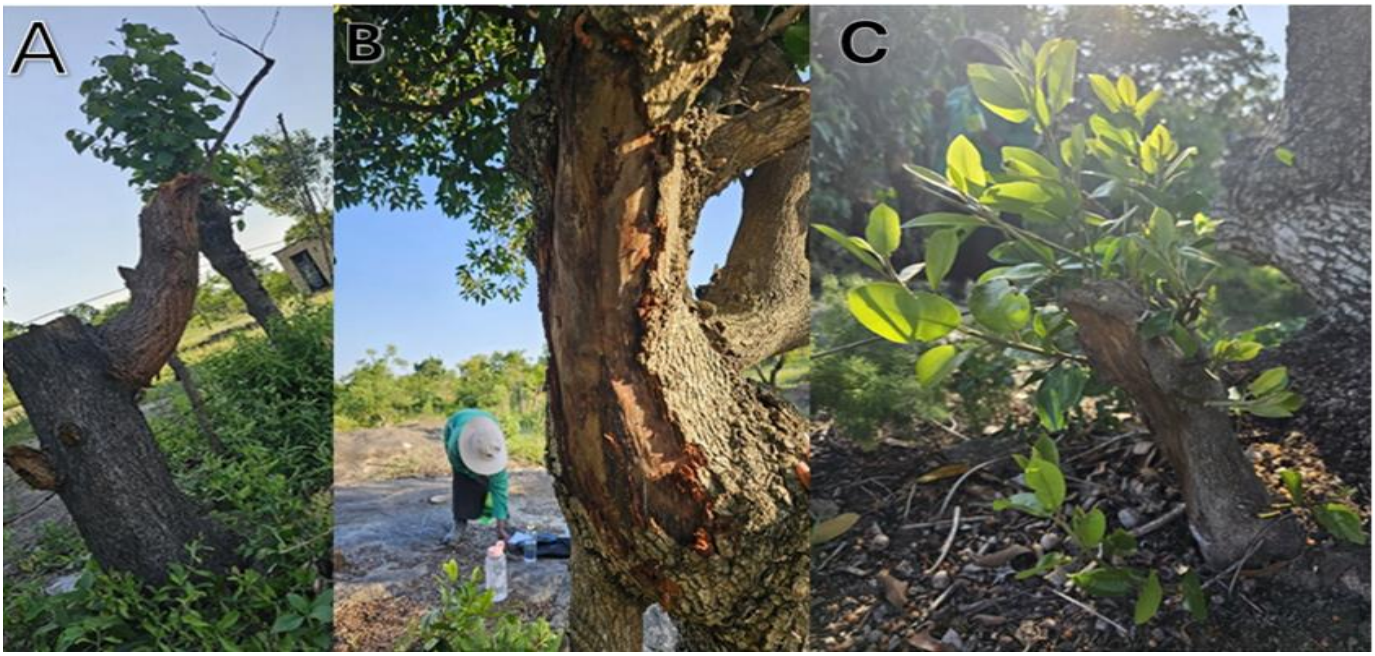

Figure 5.5: *Mimusops zeyheri* stem from cut-off tree (A), Damaged bark(B) and regeneration from a cut branch (C).

Figure 5.6: Cleared landscape at one of the study sites in Limpopo, showing cut or burnt woody vegetation, including former populations of *M. zeyheri*, for residential stands and settlement expansion.

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 practices that have been shown to reduce long-term damage when properly managed (Ryan et al., 2016). Second, community awareness and participatory management programs 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. Third, active propagation and domestication of *M. zeyheri* should be encouraged: establishing woodlots or agroforestry plots so that dependence on wild populations is reduced, while providing a sustainable supply of fruit, medicine and wood.

5.4 Concluding remarks.

The study provides valuable insights into the ecology and population dynamics of *M. zeyheri* in the two study Agro ecologies of 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-sized trees across both regions reflects active recruitment and regeneration within the populations, indicating that younger individuals are establishing successfully. However, the relatively low representation of larger size classes may suggest potential limitations in the progression of individuals into mature stages, which could have implications for long-term population stability. While no direct evidence of widespread or systematic cutting of mature trees was quantified in this study, field observations and the proximity of trees to human settlements, particularly in Ehlanzeni, suggest that selective harvesting and anthropogenic pressures may contribute to the reduced presence of larger individuals. Therefore, although the presence of smaller trees is ecologically favourable in terms of regeneration, the imbalance in size-class distribution warrants attention from a sustainability perspective.

Local municipalities and traditional leaders need to prioritise collaborative efforts aimed at establishing protected areas within their respective territories. Such conservation strategies should incorporate community-based management approaches that allow for controlled and sustainable

utilisation of the species, ensuring that local communities continue to benefit from its ecological, nutritional, and cultural value while maintaining long-term population viability. The results indicate the potential regeneration rate of the tree, making it an ideal candidate for domestication. There is, therefore, a need for the development of species-specific propagation trials of *M. zeyheri* under greenhouse conditions using different methods, including 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 and awareness programmes where communities are educated and trained on the importance and sustainability of plant species. There is a need for more research focused on assessing the economic diversity of species, evaluating susceptibility to climate change, and conducting in-depth analysis of the species' reproductive biology to inform the development and application of tailored interventions for conservation. This will ensure the safeguarding of critical species, along with the indigenous knowledge associated with them, while simultaneously supporting the economies of local communities and their cultural traditions.

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CHAPTER SIX

Morphological, Sensory, and Genetic assessment of *Mimusops zeyheri* (Sond.) Populations from Limpopo and Mpumalanga, South Africa

Summary

Understanding variation within *M. zeyheri* is essential for guiding its conservation, domestication, and potential development as a high-value indigenous fruit tree. Despite its ecological and socio-economic importance, existing studies have largely focused on either morphological traits or ethnobotanical uses, with limited integration of sensory attributes and genetic variation across geographically distinct populations. Furthermore, comparative studies examining multiple dimensions of diversity (morphological, sensory, and genetic) within and between populations in Limpopo and Mpumalanga remain scarce, creating a gap in knowledge necessary for informed selection and breeding programs This chapter examined the morphological, sensory, and genetic diversity of *M. 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 ± 0.61 mm and widths of 24.14 ± 0.55 mm, compared to Limpopo fruits measuring 27.41 ± 0.47 mm in length and 23.22 ± 0.36 mm in width. Nut size followed the same pattern, averaging 2.11 ± 0.36 cm in Mpumalanga and 1.81 ± 0.31 cm in Limpopo. By contrast, leaf morphology remained stable across regions, with overlapping mean leaf lengths (7.23–7.52 cm), widths (3.93–4.03 cm), and leaf areas (21.69–22.80 cm^2). 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 *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 displayed 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.

Keywords: *Mimusops zeyheri*; morphology; sensory traits; genetic diversity; chloroplast markers; haplotypes; regional variation; domestication potential

6.1. Introduction

Variation within a species forms the basis of its capacity to adapt, persist, and evolve under 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 continues to be 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 underutilized fruit trees like *M. zeyheri*, as they seek hardy, nutrient-rich fruits suitable for semi-arid and changing climates (Chivandi 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 realized 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 compartmentalized 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 recognized for its nutritional and ecological value, molecular-level studies remain 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 program, progress depends on the extent of genetic variation and how it can be utilized. 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 the differences in fruit sweetness, color, or antioxidant activity seen 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, which contribute 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 toward its potential for selection, propagation, and eventual domestication as a high-value indigenous fruit tree.

6.2. Materials and methods

6.2.1. Morphological analysis

6.2.1.1. Morphological sample collection and analytical procedure

Morphological data were obtained from *M.s zeyheri* trees growing in the same sites where leaf samples for DNA analysis were collected, namely the Vhembe District of Limpopo and the 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 chosen to ensure comparability between regions and to minimize developmental bias. Bark characteristics were not sampled destructively, instead high-resolution photographs of the bark were taken in situ to allow for morphological comparison between study sites.. After collection, the fruits were placed in labelled paper bags, transported to the laboratory, and processed within seventy-two hours to

minimize 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 ±0.01 mm. 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. Leaf width was measured as the maximum distance across the leaf lamina to its apex and length was measured from the base of the lamina to the apex along the midrib using. Leaf area was estimated using leaf length and width measurements, adjusted by a correction factor of 0.66 which was obtained from established reported leaf area estimation approaches from broad non-rectangular leaves, were the leaf area is the approximated as the proportion of the product of length and width to account for taper and shape. 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.

6.2.1.2. Data analysis

All quantitative data were first entered and cleaned in Microsoft Excel before being analyzed 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 provide a summary of 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.

6.2.2. Sensory evaluation

6.2.2.1. 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 the Vhembe District (Limpopo) and the Ehlanzeni District (Mpumalanga), followed by the steps shown in Figure 6.1. 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 sterilized utensils. Each portion was placed in identical, coded, non-transparent containers to minimize visual bias, and all samples were served in a randomized sequence.

The sensory assessment was carried out separately in Limpopo and Mpumalanga, with fifty willing participants recruited in each region. Participants were adults (≥18 years), and basic demographic information, including age group and gender, was recorded to assess potential sources of bias in sensory preferences. All participants took part voluntarily after being informed about the purpose and nature of the study. To improve objectivity and minimize visual bias, fruit samples were presented in closed, coded containers, preventing participants from making selections based on appearance prior to evaluation. 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. A blind tasting protocol was applied in which samples were anonymized using random codes. Between samples, participants rinsed their mouths with clean water to reduce carry-over effects. Each participant evaluated eight sensory attributes (appearance, aroma, taste, sweetness, texture, mouthfeel, aftertaste, and overall acceptability) using a standardized 9-point hedonic scale, where 1 = "Dislike" and 9 = "Very Liked" (Appendix 9.1). Completed score sheets were checked for completeness before collection. To ensure data quality, incomplete or inconsistent responses were excluded from analysis. In addition, any missing or failed observations (e.g., due to participant non-response or sample issues) were documented and handled using appropriate statistical procedures to avoid bias in the results.

6.2.2.2. Data analysis

Data from the sensory evaluation were entered into a cleaned spreadsheet and analyzed 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 visualize attribute profiles across sites, while Principal Component Analysis (PCA) was performed to identify the attributes contributing 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.

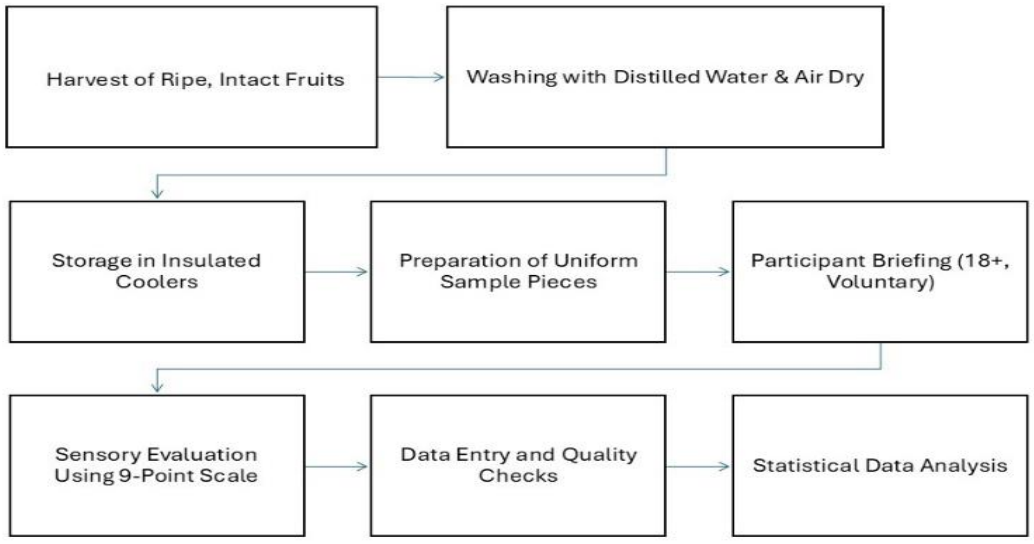

Figure 6.1:Flow Diagram of the Sensory Evaluation Process

6.2.3. Genetic Analysis

6.2.3.1. Leaf sample collection

Leaf samples of *M. 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, twenty individual trees were randomly selected, and one mature, healthy leaf was sampled from each tree, resulting in a total of forty 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 according to its collection site and number (M1–M20 for Mpumalanga and LP1–LP20 for Limpopo) as shown in figure 6.2. The bags were kept in a portable cooler during transport from the field to the laboratory to minimize degradation of genetic material. Upon arrival, samples were air-dried and stored at 4 °C until DNA extraction and subsequent molecular analyses.

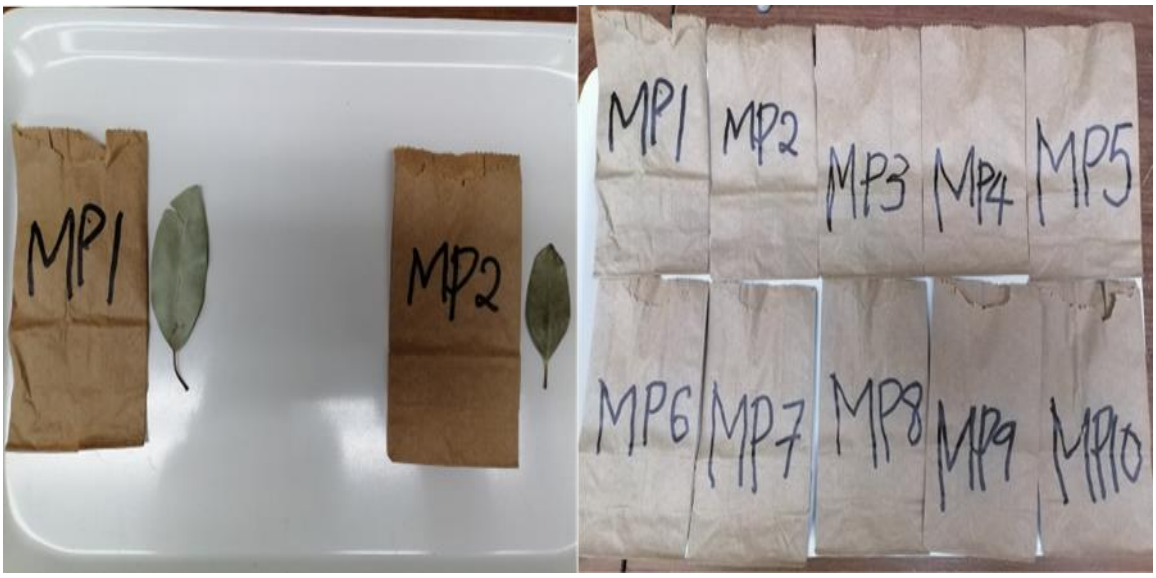

Figure 6.2:Collected leaf samples from Mpumalanga. *(Photo by Mkhonto C., 2025).*

6.2.3.2. 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 recognized 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 was performed using the CTAB (cetyltrimethylammonium bromide) method of Doyle and Doyle, 1987. The DNA extracted was processed using a Polymerase Chain Reaction (PCR), an internationally recognized technique used to amplify DNA fragments through a process of temperature cycling, thereby generating thousands to millions of copies of a particular 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 instrument used for PCR was a Gene Amp® PCR System 9800 Fast Thermal Cycler (Applied Biosystems). PCR products were visualized on 1% agarose gels, and visible products were selected for DNA sequencing.

The amplified fragments were then bi-directionally sequenced using an internationally recognised method of Sanger cycle sequencing that provides the actual DNA code of the amplified regions. The method 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 visualized 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 analyzed. The algorithm Basic Local Alignment Search Tool (BLAST) was used to compare query sequences to GENBank (NCBI) and the Barcode of Life Database (BOLD), both of which are internationally recognized 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.

6.3. Results and Discussion

6.3.1. Bark morphology

Bark morphology of *M. zeyheri* showed noticeable variation between the two study sites (Figure 6.3). In Mpumalanga, individuals were characterised by dark brown to blackish bark that was deeply fissured and coarse, with visible lichen growth on the bark surface. In contrast, trees from Limpopo exhibited lighter grey-brown bark that was comparatively thinner and smoother, with minimal fissuring. These observed differences suggest site-specific morphological variation associated with contrasting environmental conditions between the two regions. Trees in Mpumalanga are typically found in more humid and shaded environments such as forest margins and moist valleys, whereas Limpopo populations occur in relatively drier conditions, often associated with rocky outcrops and semi-arid savanna landscapes. These findings are consistent with patterns reported in the literature, where bark characteristics such as fissuring, thickness, and pigmentation are influenced by environmental factors. For example, studies within the Sapotaceae indicate that greater bark fissuring, thicker periderm layers, and darker pigmentation are often associated with higher moisture availability, enhanced cambial activity, and sustained secondary growth (Pumijumnong et al., 2023; Rahman et al., 2019). Conversely, lighter and smoother bark has been linked to drier environments, where reduced fissuring and thinner bark may help limit water loss and reflect solar radiation.

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, emphasizing 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.

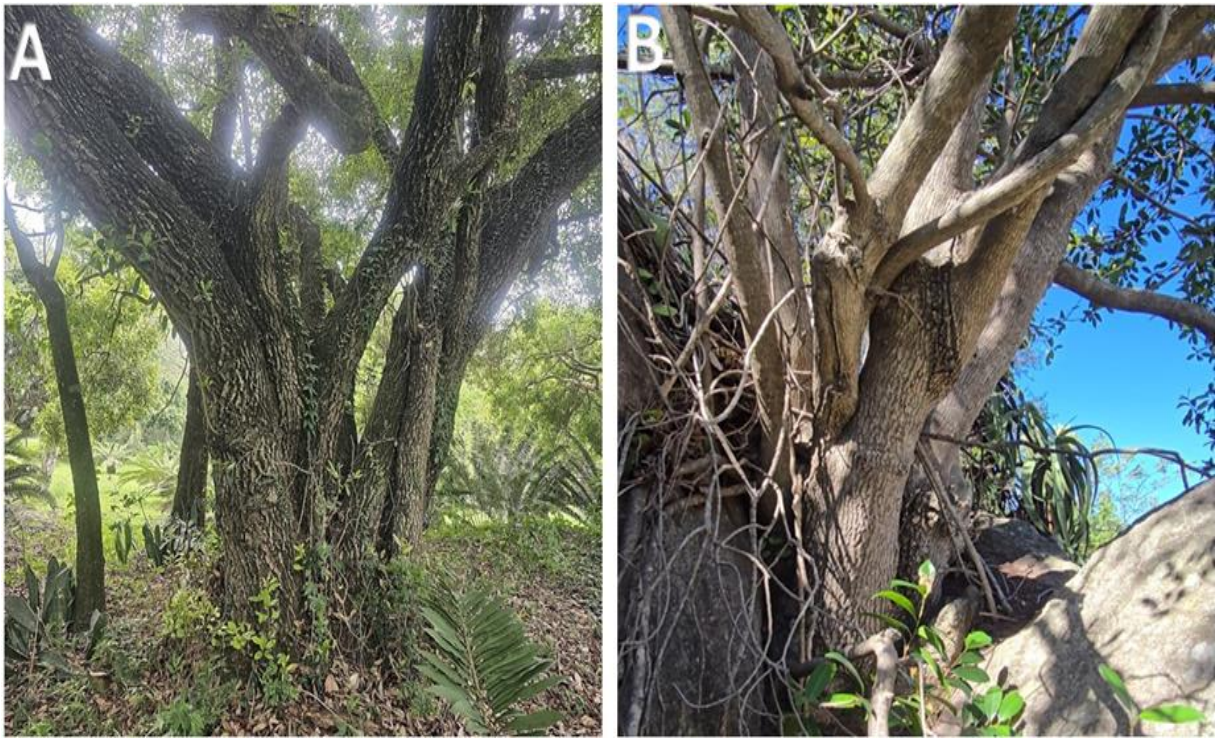

Figure 6.3: Comparative bark morphology of *M. zeyheri* populations from two regions in South Africa, Mpumalanga, Ehlanzeni district (A) and Limpopo, Vhembe district (B).*(Photo by Mkhonto C., 2025).*

6.3.2. Nut and fruit morphology

Nut and fruit size results of *M. zeyheri* from Limpopo and Mpumalanga are shown in Table 6.1. 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 ± 0.36 cm) and larger fruits, both in length (29.41 ± 0.61 mm) and width (24.14 ± 0.55 mm), whereas Limpopo individuals had smaller nuts (1.81 ± 0.31 cm) and smaller fruit dimensions overall. 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, the 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 characterizing the Mpumalanga population, typically contain more 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 give rise to seedlings 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-nuted morphotypes often germinate more slowly but sustain emergence over a longer period, whereas 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 seedlings, while 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 colonization 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 fruit size differences 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 emphasizes 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 6.4:comparison of *M. zeyheri* nut morphology from two regions in South Africa, Mpumalanga, Ehlanzenidistrict (D) and Limpopo, Vhembe district (C). *(Photo by Mkhonto C., 2025).*

Table 6.1: Nut size and fruit morphological traits of *Mimusops zeyheri* from Mpumalanga and Limpopo, including correlations between nut size and fruit dimensions.

Province	Nut length (mm)	Fruit Length (mm)	r (Nut–Fruit Length)	Fruit Width (mm)	r (Nut–Fruit Width
Mpumalanga	2111 ± 0.36[a]	29.41 ± 0.61[a]	0.117	24.14 ± 0.55[a]	–0.078
Limpopo	18.1 ± 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 ($p < 0.05$) according to Turkeys multiple range test. r represents the Pearson correlation coefficient indicating the strength and direction of the linear relationship between the nut size and the fruit dimensions.

6.3.3. Leaf Morphology

The leaf morphometric results in Figure 6.5 revealed minimal differences in leaf size between Limpopo and Mpumalanga populations of *M. zeyheri*. Mean leaf length was 7.23 cm in Limpopo and 7.52 cm in Mpumalanga, while mean leaf width measured 3.93 cm and 4.03 cm, respectively. Estimated leaf area also showed strong similarity, with averages of 21.69 cm² (Limpopo) and 22.80 cm² (Mpumalanga). The narrow range of differences, typically less than one centimetres, 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 characterized by thick, leathery leaves that maintain structural consistency across environmental gradients (Chivandi et al., 2012b; Mkhonto et al., 2024b). The slight tendency toward 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.

Importantly, 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.

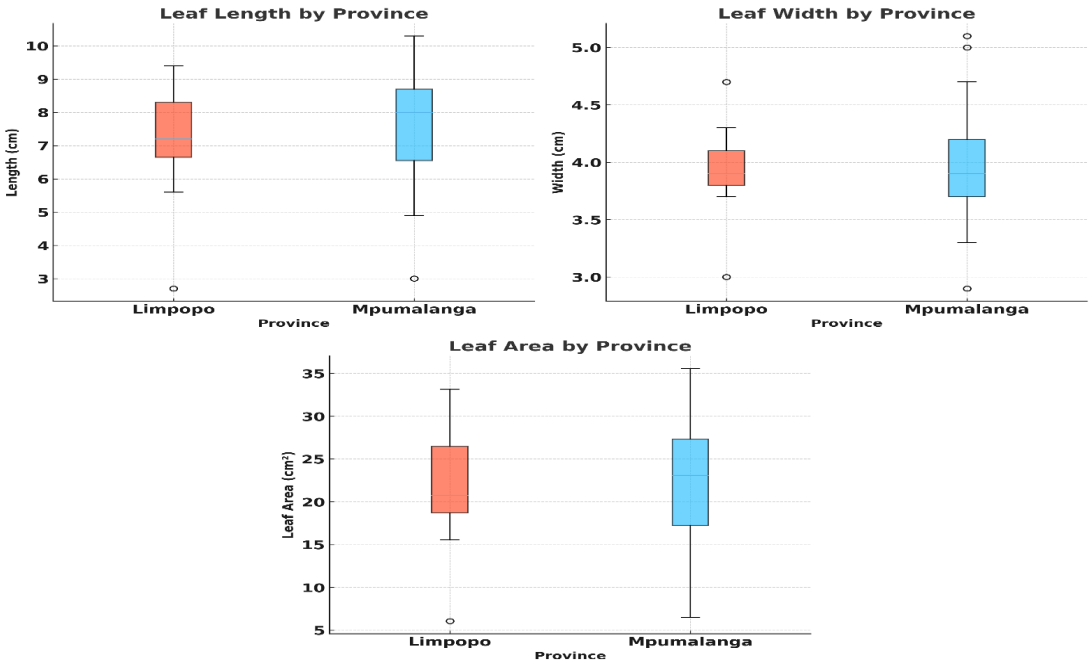

Figure 6.5:Variation in leaf length, leaf width, and estimated leaf area of *M. zeyheri* from Limpopo and Mpumalanga.

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 6.6A). 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 6.6B). 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 6.6:Early reproductive (A) and Vegetative (B) morphology of *M. zeyheri* from the Mpumalanga populations. *(Photo by Mkhonto C., 2025).*

The Limpopo population of *Mimusops zeyheri* showed visible signs of reproductive activity during the sampling period, with several branches bearing compact clusters of immature axillary flower buds (Figure 6.7A). This observation is based on field visual assessment rather than quantitative measurements of phenological stages such as days to flowering or fruiting. The presence of these structures indicates that individuals in this population were actively entering a reproductive phase at the time of sampling. This finding is presented as a descriptive observation and is not directly linked to the measured leaf morphological traits in this study.

. 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 6.7.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 characterized by early-stage reproductive activity and moderate biotic stress while maintaining the general vegetative morphology typical of the species.

Figure 6 7: Early reproductive (A) and vegetative (B) morphology of *Mimusops zeyheri* from the Limpopo populations. *(Photo by Mkhonto C., 2025).*

6.3.4. Sensory evaluation

6.3.4.1. Participant's Sensory responses to fruit attributes

The descriptive statistics in Table 6.3 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 Mpumalanga, Ehlanzeni (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 6.8) and PCA (Figure 6.9) together provide a comprehensive understanding of how respondents from both sites perceived the sensory qualities of *M. zeyheri*. The radar chart offers a direct visual comparison of mean sensory scores across attributes, clearly illustrating differences in how the fruit was perceived between Limpopo and Mpumalanga. In particular, it highlights that samples from Limpopo consistently received higher ratings across most attributes, including appearance, aroma, taste, and mouthfeel, thereby providing an intuitive overview of sensory preference patterns. The PCA complements and extends these observations by illustrating how the sensory attributes collectively contribute to overall perception. 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 perceived.

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 recognized *M. zeyheri* as a palatable fruit. Across both sites, aroma emerged as one of the key sensory drivers of acceptance. Literature consistently shows that aroma plays a decisive role in shaping consumer expectations because it results from the combined effect 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 underutilized 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 emphasize that even modest differences in aroma intensity can shape how consumers perceive freshness and flavor richness.

Appearance played an important role in shaping how respondents from both sites viewed the fruit. Colour and surface characteristics are widely recognized as early indicators of ripeness and quality compounds (Kilinçoğlu et al., 2025) and this was reflected in the similar and 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., 2023; 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*.

It is worth noting that 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 influence 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 recognize *M. zeyheri* as a fruit with appealing sensory traits. Furthermore, maturity and harvest timing are crucial factors that greatly affect the perceived flavor, aroma, and texture. A study by Ilhan (2024) highlights that soluble solid content and flavor compounds undergo substantial changes as the fruit matures, which influence the senses in individuals. 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 6. 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

Nutiness	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

Note: LV = Limpopo; MPE = Mpumalanga; Diff = difference between mean scores of Limpopo and Mpumalanga; std = standard deviation; Min = minimum observed score; Max = maximum observed score.

. Table 6.2 reports the mean, standard deviation, minimum, and maximum values for each attribute, together with the mean differences between sites. Sensory ratings were captured using a 9-point hedonic scale, where 1 indicated "very poor" and 9 indicated "excellent." Comparisons between LV and MPE were performed using Welch's independent samples t-test, and the results indicated statistically significant differences ($p < 0.05$) for all attributes.

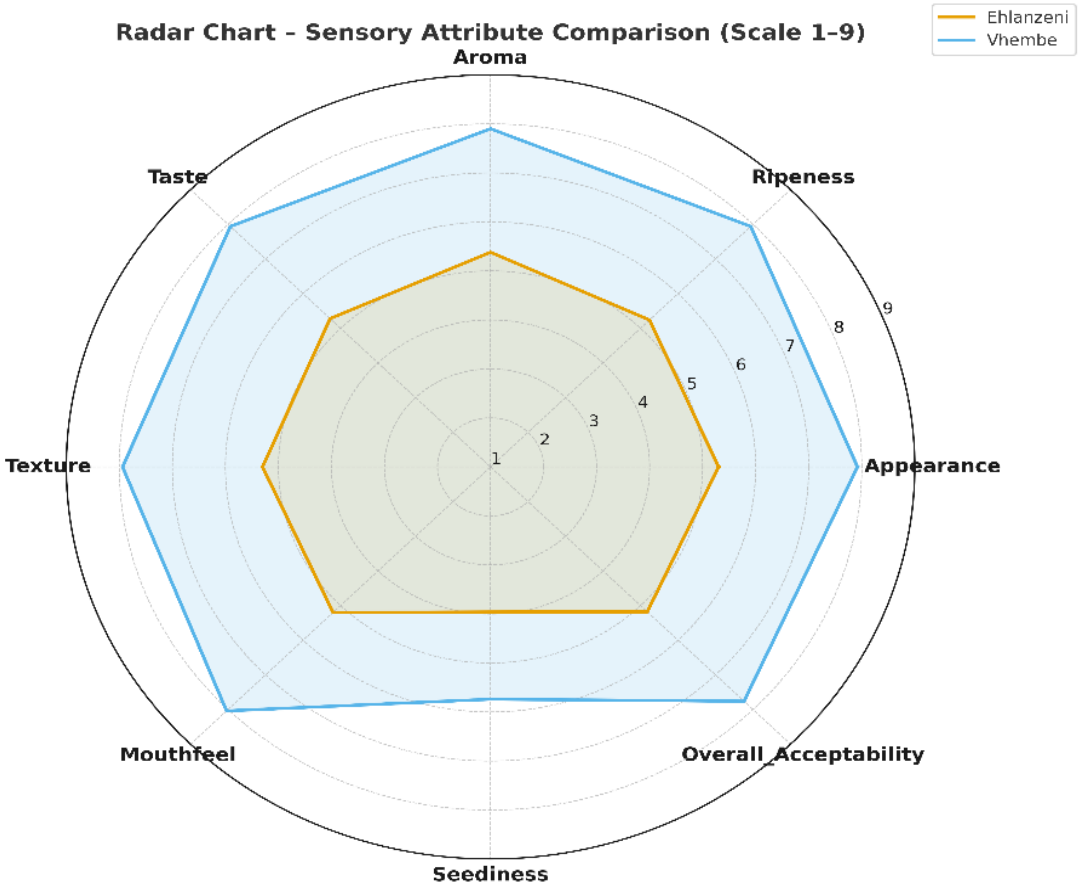

Figure 6.8: Radar plot showing the mean sensory evaluation scores of *Mimusops zeyheri* fruits across the two study sites, LV (Limpopo, Vhembe) and MPE (Mpumalanga, Ehlanzeni)

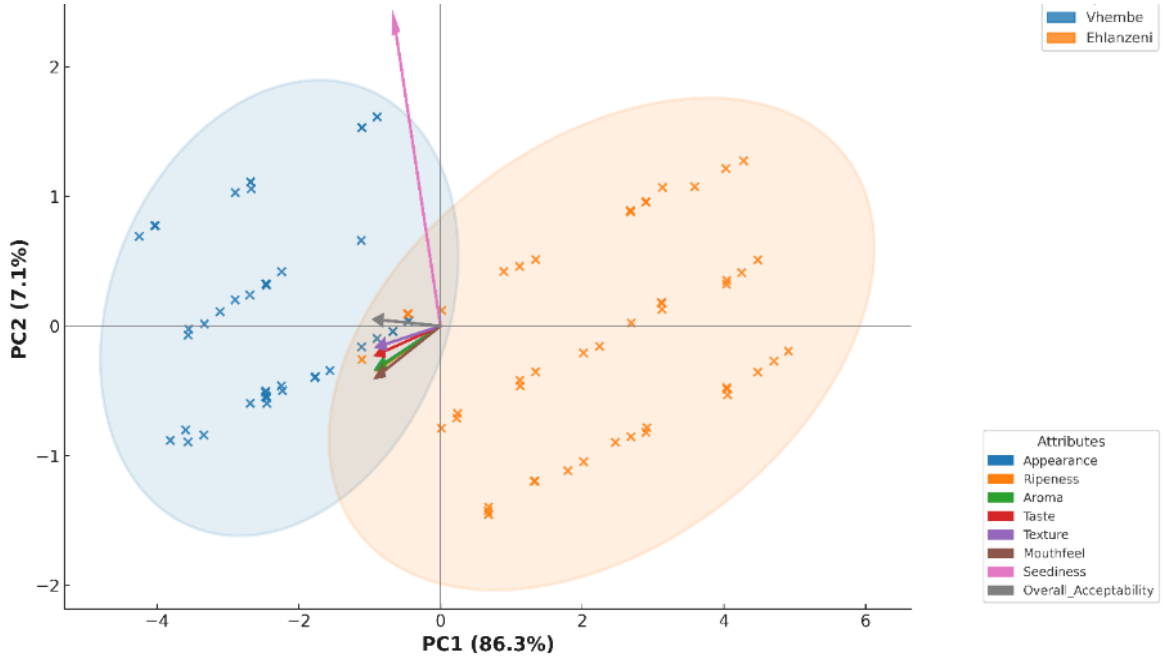

Figure 6.9: Principal Component Analysis (PCA) biplot illustrating respondents' overall sensory perception patterns for *Mimusops zeyheri* fruits from LV (Limpopo–Vhembe) and MPE (Mpumalanga, Ehlanzeni). Points represent individual respondents; shaded ellipses depict 95% confidence regions for each study site. Colour-coded loading vectors represent the contribution of sensory attributes to PC1 and PC2, with corresponding attribute colours shown in the legend.

6.3.5. DNA analysis

DNA barcoding was employed to assess the level of genetic diversity and relatedness among *Mimusops zeyheri* populations collected from 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 showing 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 6.3: PCR amplification and sequencing success of *Mimusops zeyheri* samples from Limpopo (LP) and Mpumalanga (MP) using *matK* and *trnH–psbA* markers (green = successful amplification/sequencing; red = failed amplification/sequencing).

		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

6.3.5.1. Sequence Alignment and Marker Efficiency

Figure 6.10 presents the DNA barcode profiles of *M. 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 the different nucleotide (A, T,C and G) at aligned positions along the sequence. The distinct vertical colour shifts indicate polymorphic sites, while uniform colour stretches denote conserved regions. Samples LP1–LP9 and MP3–MP10 exhibit clear 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, which underscores why this marker is effective in distinguishing between closely related populations. The mix of colored segments and lack of uniformity between individuals confirm that trnH-psbA picks up mutations more quickly than coding regions like matK, a phenomenon that researchers have extensively documented in flowering plants (Pang et al., 2012; Fazekas et al., 2008). This higher variability proves useful when studying populations, since it reveals subtle genetic differences tied to where populations live and how they've adapted to local conditions. The

observed clear 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.

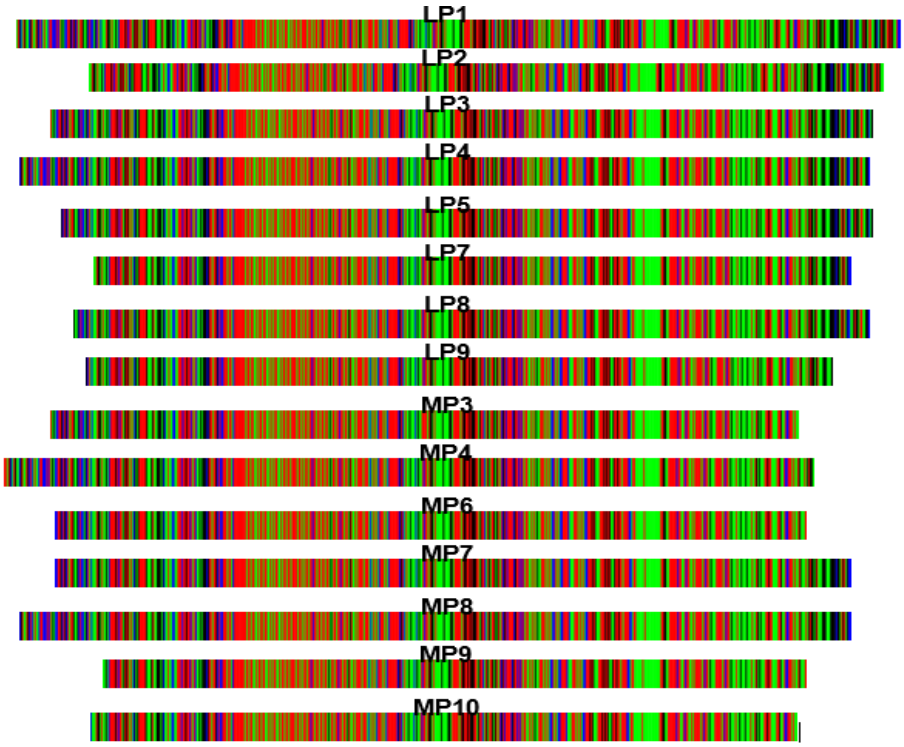

Figure 6.10: DNA barcode representation of *M. zeyheri* samples from Limpopo (LP) and Mpumalanga (MP) based on the *trnH–psbA* marker. Each horizontal bar represents an individual sample, with coloured segments indicating nucleotide variation along the sequence, thereby illustrating genetic similarities and differences among individuals.

6.3.5.2. Genetic Distance and Clustering

The heatmaps and clustering thresholds in figure 6.11 provide a coherent picture of how *M. zeyheri* individuals group genetically across the two regions. The patterns in Table 6.5 reinforce what the heatmaps visualize 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, which correspond closely with the warm and cool colour gradients in the heatmaps. The presence of two singletons in the matK results (LP3 and MP5) suggests that certain 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 optimized threshold of 0.0005, implies a lineage split that is both shallow and subtle. 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 important 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 localized trait complexes linked to growth rhythm, fruit chemistry, and nutling vigour (Chibuzor et al., 2017) .

Such structuring also has implications for 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 emphasize 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, provides 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.

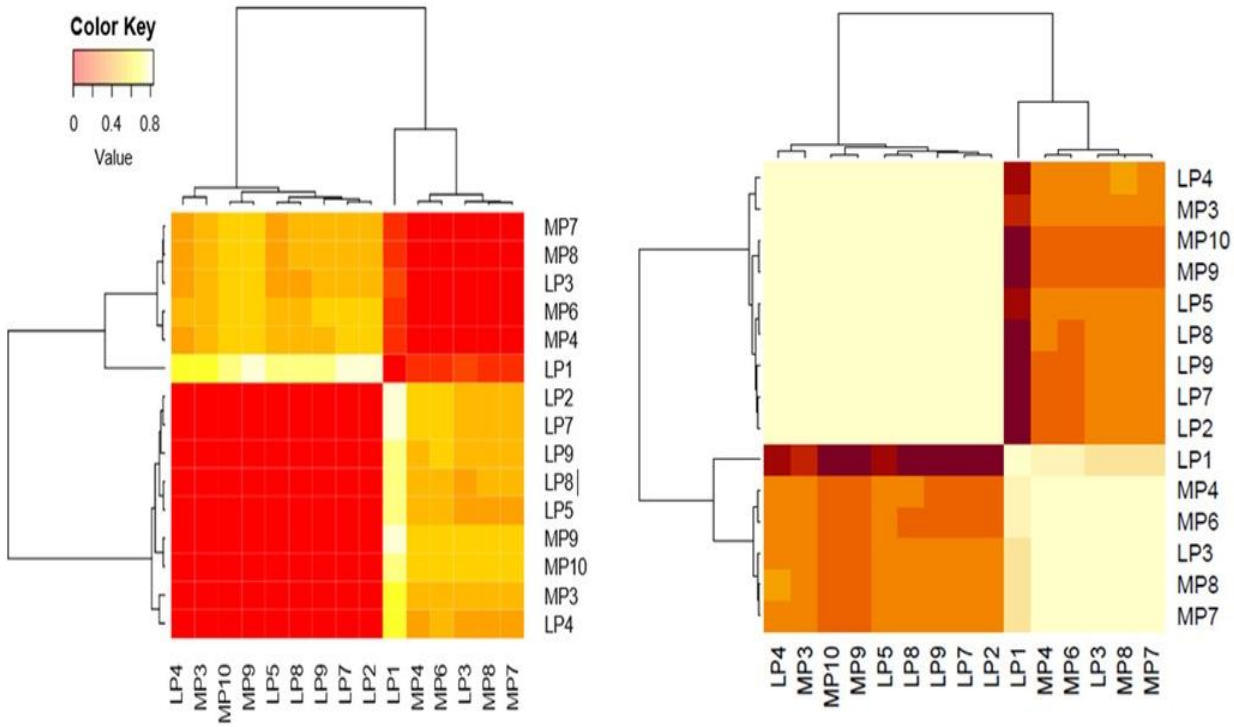

Figure 6.11:Heatmap showing pairwise genetic distances among *Mimusops zeyheri* samples from Limpopo and Mpumalanga based on combined chloroplast sequences. LP1-10 = Are all the Limpopo samples, while MP1-10= are all the Mpumalanga samples.

Table 6.4: Cluster composition and optimized genetic distance (K80) thresholds for *M. zeyheri* based on matK and trnH-psbA markers

Marker	Optimized threshold (K80)	Number ofclusters	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

Note: Cluster ID (C1, C2, etc.) denotes genetically distinct groups identified based on sequence similarity using the specified marker and optimized threshold. Samples grouped within the same cluster share high genetic similarity, whereas singleton clusters (e.g., C3, C4) represent unique or highly divergent sequences. Sample codes (LP and MP) correspond to individual samples from Limpopo and Mpumalanga, respectively.

6.3.5.3. Cluster Optimization and Grouping

The silhouette analysis reveals a two-cluster model corresponding to the geographic separation of sampling regions (Figure 6.12), 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, signaling 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 genetic structure observed also corresponds with regional differences in fruit traits. Studies on *Pouteria caimito* and *Synsepalum dulcificum* show that chloroplast haplotypes can align with differences in carotenoids, phenolics, and flavor 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.as it enables the identification of genetically distinct groups that can be conserved separately to maintain overall genetic diversity while also guiding the selection of representative individual for domestication and breeding programmes. 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 utilization potential of *M. zeyheri* across varied agricultural and ecological settings.

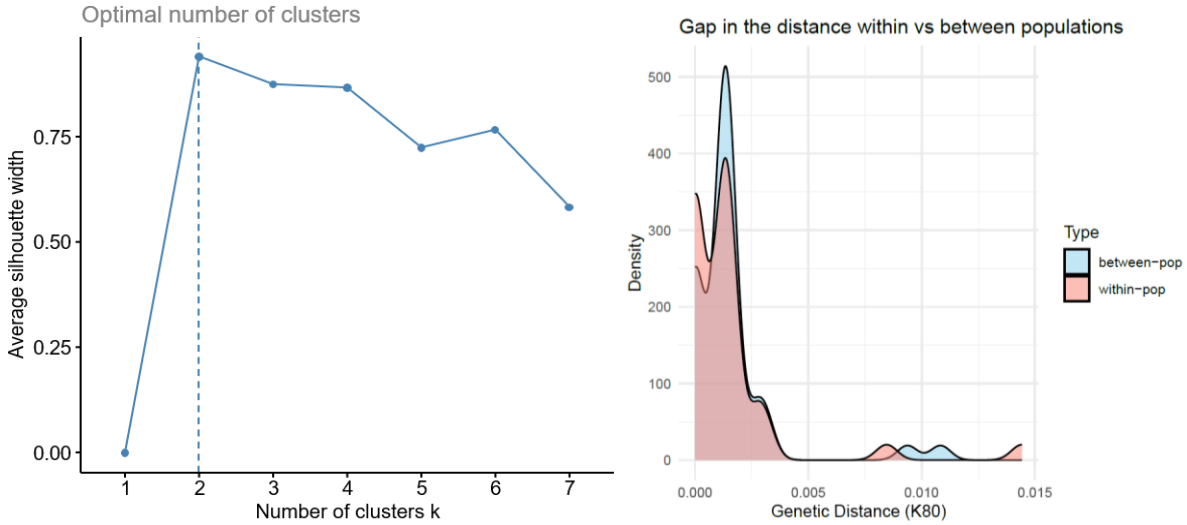

Figure 6.12: Optimal number of genetic clusters and distribution of genetic distances within and between populations of *Mimusops zeyheri.*

6.3.5.4. Phylogenetic Tree and Population Grouping

The phylogenetic network in Figure 6.13 provides additional insight into the genetic structure of *M. zeyheri*, illustrating the relationships and genetic distances among individuals, 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, the clustering of several Limpopo and Mpumalanga individuals along 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-C 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-C 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, altered mineral composition, or distinctive fruit quality characteristics (Achigan-Dako et al., 2015; Ugese et al., 2008). 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 programs, 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.

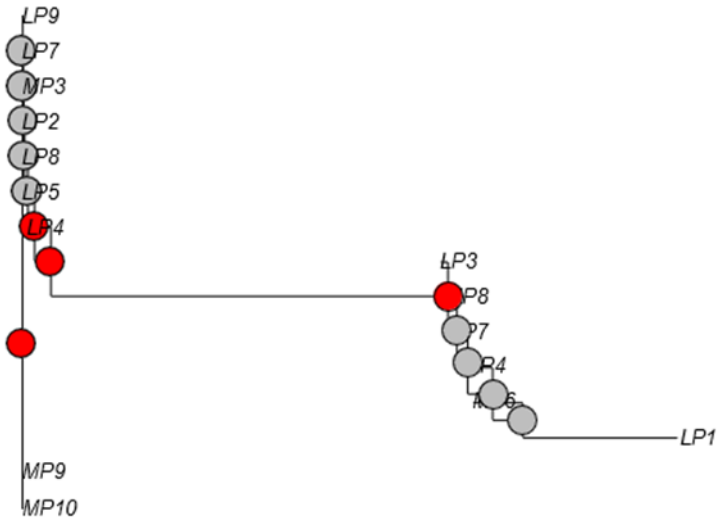

Figure 6.13: Neighbour-joining phylogenetic tree showing genetic relationships among *Mimusops zeyheri* samples from Limpopo and Mpumalanga.

6.4. Concluding remarks

This study demonstrates that *M. 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, with Mpumalanga trees producing 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. Genetic analyses confirmed two broad lineages with additional rare haplotypes, indicating long-term divergence, limited maternal gene flow, and the persistence of localized genetic variants.

Several gaps temper the interpretation of these findings. Morphology was limited to external traits, leaving the chemical basis of sensory differences unexplored. Sensory scoring, though useful, was not supported by instrumental assessments of sugars, volatile compounds, or secondary metabolites. The molecular analysis relied solely on chloroplast markers, which capture only maternal inheritance and do not fully resolve population structure or adaptive variation. Additionally, the study was confined to two provinces, yet *M. zeyheri* occurs across a wider environmental gradient that may hold additional diversity. The sample size of 40 trees, while adequate for exploratory analysis, may limit the extent to which the findings can be considered a full representation of the species across its range.

Future work should extend genetic analyses to nuclear markers or genome-wide SNPs, incorporate detailed phytochemical profiling, and broaden geographic sampling, including increased sample sizes, to establish a fuller understanding of variation within the species. For conservation and utilization, the two primary genetic lineages, together with the rare haplotypes identified, should be treated as distinct resources. Domestication efforts may benefit from combining the favorable 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 SEVEN

NUTRITIONAL COMPOSITION OF *M. ZEYHERI* PLANT PARTS FROM TWO DIFFERENT AGRO ECOLOGIES OF SOUTH AFRICA

Summary

Indigenous Wild Fruit trees are receiving growing attention for their ecological roles and their nutrient-rich qualities, which make them important in efforts to reduce hidden hunger and build climate-resilient food systems. One notable species is *M. zeyheri*, a drought-tolerant wild fruit native to southern Africa. Despite is ecological significance and reported contribution to rural livelihoods, its nutritional value, and broader contribution to diets remain only loosely documented. The study on the nutritional composition of *M. zeyheri* plant parts hypotheses: (i) that *Mimusops zeyheri* fruits possess significant nutritional value, particularly in terms of micronutrients and antioxidant properties; (ii) that nutritional composition varies across populations due to environmental and/or genetic influences; and (iii) that the species has the potential to contribute meaningfully to food security and dietary diversity in rural communities.

Ripe fruits and leaves were collected from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni) provinces. Proximate composition was determined using standard AOAC methods and elemental analysis by microwave-assisted digestion followed by ICP-OES. Data were analyzed 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/100 g^{-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), reflecting nutrient concentration as moisture decreases. Although the two agro ecological zones differ environmentally, only minor provincial differences were observed in nutrient composition, suggesting that the nutritional profile of *M. zeyheri* is relatively stable across these agro ecological conditions. This indicates a level of ecological resilience in nutrient expression, enhancing its suitability as a reliable food resource across diverse environments

. *M. zeyheri* offers a unique combination of energy-dense nuts, protein and 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.

Keywords: Nutrition, Proximate analysis, Minerals, β-carotene, Dietary diversity, ecological

7.1. Introduction

Global food systems face increasingly significant pressures from population growth, climate change, and environmental degradation that threaten the capacity of conventional agriculture to sustainably meet future nutritional needs. More than 700 million people experience chronic hunger, while billions suffer "*hidden hunger*" from 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, characterized 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 toward 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-utilized 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, in addition to containing 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, the region continues to experience some of the highest levels of food insecurity. According to (Akinnifesi et al., 2007). This is partly due to low cultivation of edible plant species, only a small commercial trade levels (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, stabilize 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 utilized. The yellow-orange fruits are eaten fresh or fermented into beverages; leaves and bark are used in remedies for diarrhea, 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* is 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* form Limpopo (Vhembe) and Mpumalanga (Ehlanzeni).

7.2. Material 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 the Vhembe district of the Limpopo Province (23.9000° S, 29.4500° E), and the Ehlanzeni district of the 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. To ensure adequate biological replication, sampling was conducted using a stratified field-based approach, where representative trees were selected across the study sites based on prior ecological survey and spatial distribution (transect-based sampling). A total of twenty (n = 20) individual trees per province were sampled, and each tree was treated as an independent biological replicate. From each selected tree, leaves and fruits were collected to capture within-population variability and ensure representativeness of the sampled populations. Leaves were collected from multiple positions on each tree, including both inner and outer canopy regions, and from different orientations (sun-exposed and shaded sides) to account for potential variation in fibre and tannin content associated with leaf position. Only fully expanded, mature, and healthy leaves were selected to ensure consistency across samples. The plant leaves and ripe fruits were separated from the other plant parts and washed thoroughly with tap water and again with distilled water to remove any further debris that may cause contamination Voucher specimen (C003) from the leaves were prepared through drying and pressing 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 and again with distilled water to remove any further debris that may cause contamination. The leaves were oven dried for 48 hours at 40°C. The fruits were subjected to separation of the fiber and pulp and

freeze dying at -80 degrees using FD-12 Series freeze dryer (Lyophilizer). The dried plant parts were subjected to pulverization using Waring TNNK160K commercial blender at low speed. The powdered samples were stored in airtight containers to preserve the biomolecules present in the plant and stored at a -80 degrees 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 equation (1):

$$\%\ Moisture\ content = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (1)$$

where $W_1$is the initial weight of the sample and W_2 is the weight after drying. Dry matter was expressed by difference as equation (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 equation (3):

$$\%\ Ash = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (3)$$

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 then exposed to Soxhlet extraction using petroleum ether (boiling range: 45–60 °C) for five 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 using equation (4):

$$\%\ Fat = \frac{W_1 - W_2}{W_1} \times 100 \quad (4)$$

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 and treated with 200 mL of 1.25 % sodium hydroxide, again boiled for 30 minutes, 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 using equation (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 was measured using the Kjeldahl method described by Goyal et al. (2022) 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 (metabolizable 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 *M. 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 nebulizer and cyclonic spray chamber. Calibration curves were prepared from multi-element standards spanning the expected concentration ranges, and verification standards were analyzed 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 normalized 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 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. Triplicate measurements were averaged for each biological replicate (individual tree), and these mean values were used in subsequent statistical analyses. One-way ANOVA followed by Tukey's HSD test was applied to assess

differences between groups at $p \leq 0.05$.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 *M. 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 ($p < 0.05$), emphasizing that intrinsic plant tissue and physiology, rather than geographic or regional factors, are the primary determinants of moisture variation in *M. 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 stabilizes 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 hold the highest levels 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 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. Since there is little water. 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 *M. 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 current study, earlier work on *M. zeyheri* foliage found calcium levels above 2 g 100 g^{-1} and potassium 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 capable of complementing cereal-based diets that are 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). Evident from this current 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 among different organs of *Pinus massoniana*, while Bakker and Elbersen (2005) observed that wetland species often exhibit higher ash content than C4 grasses, demonstrating how 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 developmental stage of the fruit all affect how lipids are produced and stored, accounting for the substantial variation observed both within a single species and across different species (Bacelar et al., 2024). This is consistent with the current findings of this study, as *M. 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, 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 fiber, making them valuable nutritional resources 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 meals 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 forms a very important part of a healthy diet since it is responsible for building and repairing muscles and bones and making 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 it 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 defense 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 recognized 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 *M. 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 of 12-18 % on a fresh-weight basis of *Strychnos spinosa* higher than that 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 labor-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 World Health Organization (Ahsan, 2021) and Food and Agriculture Organization guidelines (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 caloric sources but cultural anchors of 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 where wild fruits like *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 nut mirrors the multipurpose roles of these other wild fruits, strengthening household food security and providing an emergency food reserve during periods of staple-crop shortage.

7.3.1.8. Energy value

Energy content in *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 true 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 that add 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 risks of over-consumption, while still supplying vitamins and phytochemicals.

Nutrition studies further show that high-energy foods dominated by 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 offers a hydrating snack that contributes to 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 $p \leq 0.05$. 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 selected study area. Elements analyzed include calcium, copper, iron, potassium, magnesium, sodium, phosphorus, zinc, β-carotene and vitamin C. The results show clear variation by plant part, with nuts and leaves generally richer in minerals than the pulp and fibre, while provincial differences are minor, indicating that nutrient levels are largely 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, where developing radicle (nut embryos) accumulate structural and signaling 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), a value close to that noted in the nuts of *M. zeyheri* in the study and substantially greater than that of most cultivated fruits. Adequate dietary calcium is fundamental for skeletal development, bone mineralization, neuromuscular excitability, blood clotting, and cell signaling. 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 to destabilize bacterial cell walls and 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 report copper concentrations ranging from about 82 to 105 mg kg^{-1} dry weight (Lim, 2012), depending on maturity stage and soil conditions. Similarly, *Mimusops caffra* nut studies show copper values between roughly 88 and 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 and 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 anemia, neutropenia, connective-tissue disorders, and impaired immune responses. The consistently high copper concentrations measured in *M. 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 recognized 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 the 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 the pulp, flesh, leaves, or nuts from either province, they obtain a comparable iron contribution. 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 hemoglobin synthesis, oxygen transport, and the functioning of numerous enzymes that utilize oxidative processes. Iron deficiency remains the most common micronutrient disorder worldwide, leading to iron-deficiency anemia characterized 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 anemia prevalence remains high.

7.3.2.4. Potassium

Potassium showed the most significant variability of all 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 favorable 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 calcium and potassium, with the highest concentrations in the nut and only slight differences in agroecology. 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), and *M. zeyheri* leaves analyzed 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 in the diet.

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 favorable 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 occurred in the mid-portion 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 both as a potent antioxidant and as 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 synthesized 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 *M. 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 important, 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 stabilize 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 in teeth 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 defenses are still maturing. It accumulates in white blood cells and enhances neutrophil migration, phagocytosis

and the oxidative burst that destroys invading microbes, reducing both 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 $p \leq 0.05$.**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 *M. zeyheri* plant parts

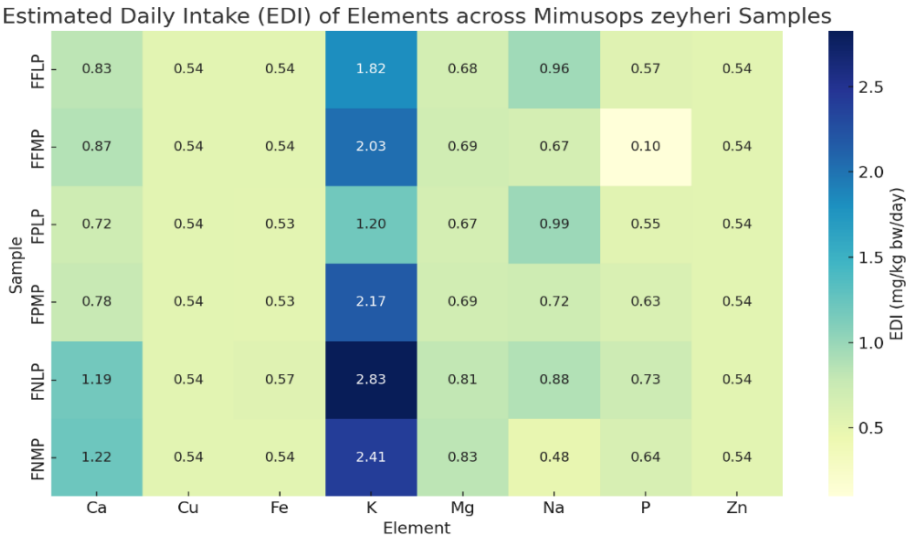

Figure 7.1: Heat map of EDI of elements from *M. zeyheri* samples from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni).Key: FFLP (Fruit fibre Limpopo), FFMP (Fruit fibre Mpumalanga), FPLP (Fruit pulp Limpopo), FPMP (Fruit pulp Mpumalanga), FNLP (Fruit nuts Limpopo), FNMP (Fruit nuts Mpumalanga, LLP (Leaves Limpopo), LMP (Leaves Mpumalanga).

The heat map (Figure 7.1) and radar chart (Figure 7.2) together demonstrate how the estimated daily intake (EDI) of essential minerals from *M. zeyheri* can make a meaningful contribution to adult nutrient requirements when approximately 0.4 kg of fresh fruit is consumed daily. Potassium is the most abundant element in the nuts samples (FNLP and FNMP), providing between 2.4 and 2.8 mg/kg^{-1} body weight per day^{-1} for a 70 kg adult. This is roughly 170–200 mg of potassium per serving. Although this is only around 4% of the World Health Organization's recommended intake of 4,700 mg/per day^{-1} (Table 6.1), 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 1.22 mg/kg^{-1} body-weight day^{-1}, equivalent to about 80–85 mg for a 70 kg adult, or roughly 8% of the adult requirement of 1,000 mg day^{-1}. While not a complete source on its own, this level is important in areas where milk or fortified foods are scarce and help 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 0.10–0.73 mg/kg^{-1} body-weight day^{-1}, respectively, provide about 5-10% of the adult daily needs (310–420 mg for

magnesium and 700 mg for phosphorus), reinforcing their contribution to bone health and energy metabolism.

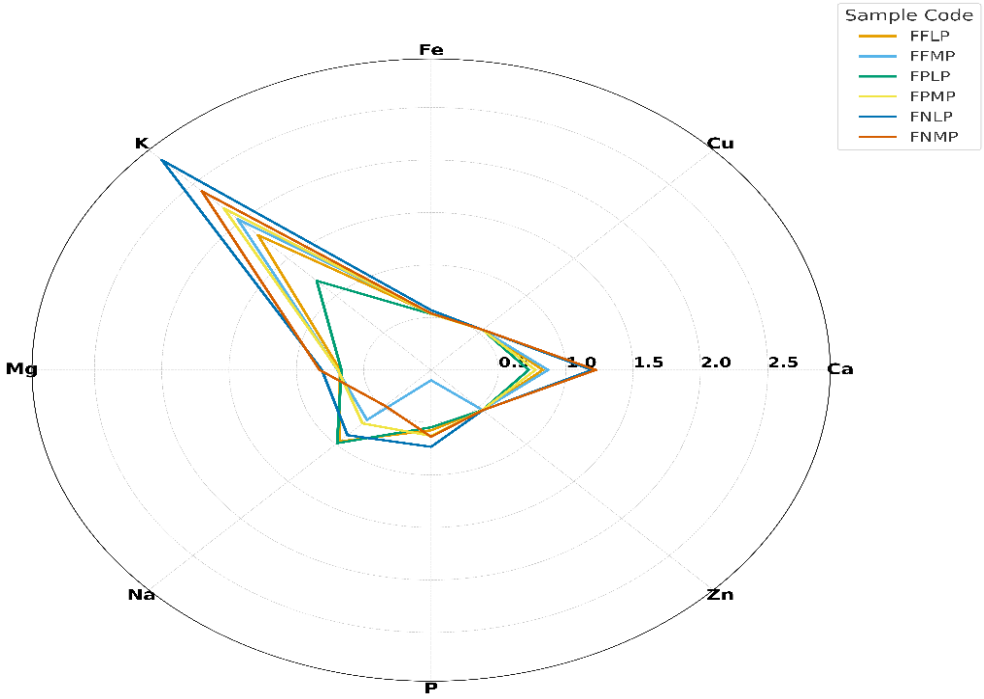

Figure 7.2:Radar chart of EDI of elements from *M. zeyheri* samples from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni).Key: FFLP (Fruit fibre Limpopo), FFMP (Fruit fibre Mpumalanga), FPLP (Fruit pulp Limpopo), FPMP (Fruit pulp Mpumalanga), FNLP (Fruit nuts Limpopo), FNMP (Fruit nuts Mpumalanga, LLP (Leaves 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 0.54 mg/kg^{-1} body-weight day^{-1} for copper and zinc and about the same for iron for a 70 kg adult these correspond to roughly 38 mg of each element, far above the adult reference intakes of 0.9 mg day^{-1} for copper, 8-18 mg/ day^{-1} for iron and 8-11 mg/day^{-1} for zinc (Table 7.1). This confirms that *M. zeyheri* can provide important trace elements needed for enzyme function, immune support, and hemoglobin production. Vitamin C in the fruit flesh and pulp, averaging 5–7 mg per 100 g fresh weight, contributes about 12–20 mg per 0.4 kg serving, meeting roughly 15-25% of the adult

requirement of 75–90 mg/day^{-1}. Vitamin C serves as a primary water-soluble antioxidant, scavenging free radicals and regenerating vitamin E to protect 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 15-45 mg 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 utilization, 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* provides a combination of macro- and micronutrients that compares favorably 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 the availability of 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 notable relationships with statistical significance. 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 very strong 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 54 g/100 g^{-1} crude fat. Consistent with the recognized storage role of nuts, in which proteins and lipids are jointly accumulated (Khalid et al., 2023). 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 ($|r| \approx 0.95–0.99$, $p < 0.01$) and, conversely, strong negative correlations with moisture ($|r| \approx 0.94–0.99$). 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 stabilize membranes and enzymes during desiccation (Lozano, 2009; Samarah et al., 2004). Potassium, copper and iron displayed moderate positive relationships with nutrient-dense traits ($r \approx 0.6–0.8$) 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 close degree of association between calcium, magnesium, and zinc with pairwise correlations exceeding 0.9, indicative of their coordinated accumulation in the dry and energy-dense nut. Potassium exhibited a moderate correlation with these minerals ($r \approx 0.6–0.7$). Similarly, copper and iron likewise exhibited moderate positive correlations with calcium and magnesium. In contrast, sodium correlated negatively with the Ca, Mg, Zn minerals ($r \approx –0.4$ to $–0.5$), reflecting its separate physiological pool and link to more hydrated pulp and fibre tissues.

The nutritional composition of *M. zeyheri* exhibits a distinct tissue-dependent pattern, whereby the nuts, independent of geographic origin, consistently display elevated dry-matter content, mineral abundance, and energy density, while 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, along with high energy and fat content. In contrast, the fleshy mesocarp remains water-rich and nutritionally diluted. The observed accumulation of calcium, magnesium, and zinc with dry matter and protein content in *M. zeyheri* therefore 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, 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 -value	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 - value	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- value	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-value	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 -value	**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-value	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-value	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-value	0.005	0.005	0.320	0.004	0.035	0.004	0.004	0.004

Note: r = Pearson correlation coefficient; p = significance value. Sample types: Fruit fibre, fruit pulp, and nuts (N = 6 per province). Elements: Ca = Calcium, Cu = Copper, Fe = Iron, Mg = Magnesium, Na = Sodium, P = Phosphorus, Zn = Zinc. Asterisks indicate statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001*). *Bold values indicate very strong correlations (|r| ≥ 0.94).*

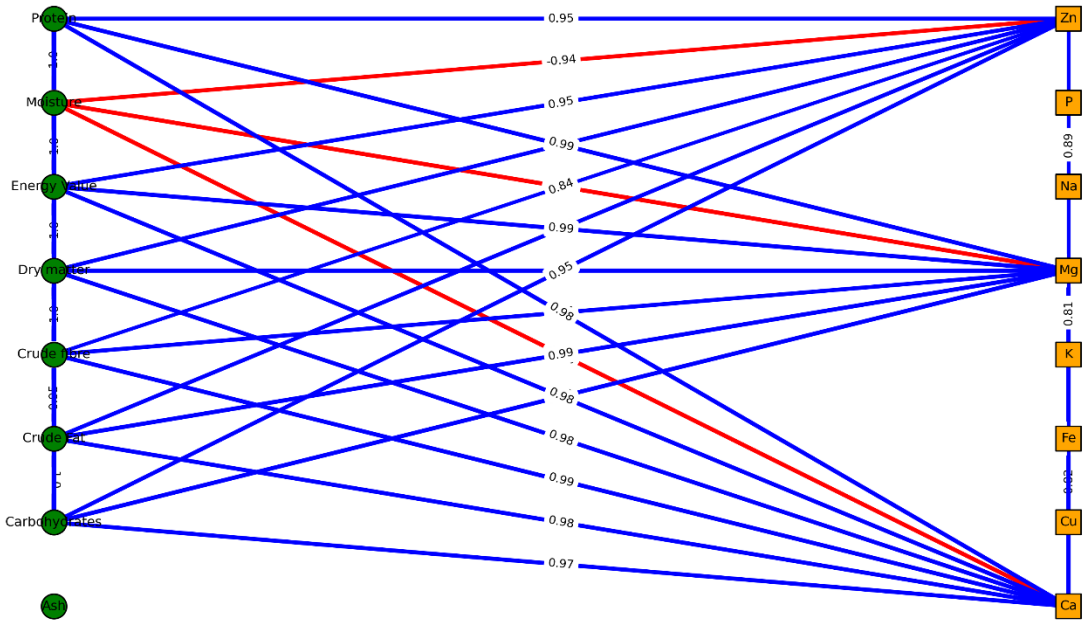

Figure 7. 3: Correlation network of the significant associations ($|r| \geq 0.8$; $p < 0.05$) among proximate constituents and mineral elements of *M. zeyheri* fruits. Proximate variables are shown as green circles and minerals as orange squares; blue lines denote positive correlations and red lines 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, whereas the pulp and fibre components occupy the outer margins of the network.

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 contributes natural sugars, vitamin C, and β-carotene that 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 the 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 toward resilient diets and poverty reduction in rural South Africa.

7.5. References

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CHAPTER EIGHT

PHYTOCHEMICAL PROFILING AND IDENTIFICATION OF UNTARGETED SECONDARY METABOLITES OF *MIMUSOPS ZEYHERI* SOND (MILKWOOD) PLANT EXTRACTS FROM TWO DISTINCT AGRO-ECOLOGICAL ZONES IN SOUTH AFRICA

Summary

Mimusops zeyheri is traditionally used in ethnomedicine and for therapeutic applications in Southern Africa, particularly in the Limpopo and Mpumalanga provinces of South Africa. The fruit tree parts contain active bioactive constituents that contribute to its medicinal and nutritional value; however, there is limited scientific literature documenting the phytochemical profiling and, therefore, comparative chemical composition of these wild fruit tree parts across different Agro-ecological zones of South Africa. The study quantified the total phenolics and flavonoids using spectrophotometry techniques and further identified untargeted secondary metabolites from the different plant parts of *M. zeyheri* collected in different Agro-ecological zones using Ultra Performance Liquid chromatography mass spectrometry (UPLC-QT of-MS/MS). Furthermore, the study evaluated the antioxidant activities of *M. zeyheri* plant parts using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and Ferric Reducing Antioxidant Power assay. Leaves recorded significantly higher phenolic content (19.13 mg GAE g^{-1} in Limpopo; 18.20 mg GAE g^{-1} in Mpumalanga) than nuts (≈14 mg GAE g^{-1}), pulp (≈8 mg GAE g^{-1}), and fibre (≈6 mg GAE g^{-1}). Similar to phenolic content, the total flavonoid content (TFC) of *Mimusops zeyheri* followed a clear pattern across the different plant parts. Leaves exhibit the highest concentration of TFC. .83 ± 0.25 µg QE g^{-1} in Limpopo and 9.30 ± 0.20 µg QE g^{-1} in Mpumalanga. Nuts followed (6.23 ± 0.25 and 5.80 ± 0.15 µg QE g^{-1}), while pulp (around 4 µg QE g^{-1}), with the lowest TFC observed in fruit fibre (around 3 µg QE g^{-1} in both Limpopo and Mpumalanga). Differences in TFC between the WTO provinces are small, emphasizing plant tissue as the main driver of concentration rather than the location where the plant grows. Alkaloid content was again highest in leaves: 4.67 ± 0.15 mg AE g^{-1} (LLP) and 4.37 ± 0.15 mg GAE g^{-1} (LMP). Nuts followed (3.33–3.10 mg AE g^{-1}), then pulp (~2 mg AE g^{-1}) and fibre (~1.5 mg AE g^{-1}). The pattern mirrors that of phenolics and flavonoids, reflecting the defensive role of alkaloids against herbivores and pathogens, which is strongest in leaves and reproductive tissues. Antioxidant activity differed between plant parts and provinces. Mpumalanga Nuts showed the strongest radical-scavenging capacity in the DPPH assay (EC_{50} 0.0581 µg/mL) compared with Limpopo Nuts (EC_{50} 0.1767

μg/mL) and much lower than the positive control. Fibre and pulp extracts were more effective in the β-carotene–linoleic acid system, with Mpumalanga fibre showing the lowest EC_{50} (18.24 μg/mL) and pulp extracts ranging between 64.10–77.43 μg/mL. Leaf extracts showed moderate activity in both assays. The heatmap indicated clear tissue- and site-specific patterns, with Mpumalanga samples generally outperforming those from Limpopo. Fruit fibre from Mpumalanga is rich in flavonoids with dominant compounds such as quercetin-3-O-glucoside (Rt 7.82 min, 463.12 m/z) with congeners identified as isoquercitrin (Rt 5.81 min, 463.09 m/z) and rutin (Rt 4.62 min, 609.14 m/z) known for their distinct structural and biological properties while *M. zeyheri* fruit fibre from Limpopo has notable phenolic glycosides such as β-glucogallin (Rt 1.08 min, 367.05 m/z).β-glucogallin (Rt 1.08 min, 367.05 m/z) has been reported in the literature as a bioactive phenolic compound with notable antioxidant properties and emerging evidence suggesting antimicrobial potential, alongside its role in supporting gut health and contributing to food preservation. *Mimusops zeyheri* fruit pulp from Mpumalanga has a notable array of various flavanol glycosides and nutrient compounds that together create a flavorful and health-promoting profile of tentatively identified compounds. By contrast, the Limpopo pulp is dominated by hydrolysable tannins and phenolic glycosides, reflecting a more therapeutic, preservative-leaning profile. The Mpumalanga nut profile is flavonoid rich with notable quercetin–catechin profile and compounds such as Free quercetin (Rt 5.75 min, 301.03 m/z) alongside its glycosides—isoquercitrin (quercetin-3-O-glucoside; Rt 5.81 min, 463.09 m/z) and a rutin isomer (quercetin-3-O-rutinoside; Rt 5.90 min, 609.14 m/z) known for antioxidant and anti-inflammatory activity relevant to vascular protection, insulin sensitivity and neuroprotection. By contrast, the Limpopo nuts present a tannin-dense fingerprint with higher oligomerization and a striking acylated anthocyanin. A hallmark proanthocyanidin trimer, specifically gallocatechin-(4α→8)-gallocatechin-(4α→8)-gallocatechin (Rt 3.02 min, 913.19 m/z), together with a dimeric feature at 577.14 m/z (identified as endotelon), indicates an extensive condensed-tannin spectrum. In contrast, leaves from Limpopo are characterized by high levels of galloyl phenolics and oligomeric proanthocyanidins (OPCs), associated with vascular and antioxidant benefits, with compounds such as Glucosyringic acid (Rt 3.08 min, 359.10 m/z) previously reported for its antioxidant and anti-inflammatory effects.

Keywords: Phytochemical profiling, Antioxidants, Secondary metabolites, Tissue-specific variation, Therapeutic

8.1. Introduction

Wild fruit trees are the cornerstones for traditional medicine and food security contributors in many African countries, especially in Sub-Saharan Africa. These indigenous species frequently yield fruits and plant parts rich in nutrients and bioactive secondary metabolites and thus offer a promising and reliable supply to mitigate malnutrition, chronic disease, and livelihood vulnerability in rural communities. Wild fruit trees have different classes of phytochemicals, including tannins, Flavonoids, phenolics, and alkaloids, with significant antimicrobial, antioxidant and anti-inflammatory properties, with the potential to enhance human health beyond basic nutritional needs (Bachheti et al., 2023; Bvenura and Kambizi, 2024)

Mimusops zeyheri Sond is one of the essential underutilized species of the Sapotaceae family, consisting of 53 genera and around 1250 species, employed in traditional medicine for their anthelmintic, tonic, and astringent properties (Baky et al., 2022d; Gam et al., 2024b). These plants are known to produce biochemical compounds in response to stress, herbivory, and to defend themselves against piercing sucking insects such as whiteflies. The released compounds vary, belonging to different groups and classes, and are valued mainly for their allelopathic, antimicrobial, antioxidant, and anti-inflammatory properties (Bano and Ahmed, 2017). Hence, the potential effects of wild fruit tree phytochemistry on human health, food security, and pharmaceutical development of these wild fruit trees, including *M. zeyheri*, have gained considerable interest over the last decades due to the large pool of secondary metabolites. These secondary metabolites are a great novel source of pharmaceuticals used in commercial healthcare systems and food additives, responsible for their antioxidant, antibacterial, and other advantageous characteristics (Khade et al., 2023). The bark, leaves, and fruits of *M. zeyheri* have long been used in therapeutic formulations to treat skin and infectious conditions, gastrointestinal ailments and to remedy pain due to their abundant phytochemical content (Matlala et al., 2024; Mkhonto et al., 2024).

While several studies have documented the ethnomedicinal and therapeutic uses of *M. zeyheri*, there is a significant gap in scientific literature on the phytochemical profiling and, therefore, chemical composition of this wild fruit tree. Existing studies have had a skewed focus on commercially popular species from the Sapotaceae family, such as *Pouteria sapota (Yahia et al., 2011)*, *Mimusops elengi (Sayed et al., 2023) and Madhuca indica* (Gujjeti and Mamidala, 2013)

while *M. zeyheri* remained overlooked. Moreover, the phytochemical profiles of plants are significantly influenced by environmental factors, including soil composition, climate, altitude, and other ecological variables (Ogwu et al., 2025). This is especially pertinent in the South African context, since the presence of distinct Agro-ecological zones gives rise to a wide range of growing circumstances for species such as *M. zeyheri*. Their comparative profiling across different provinces is significant for identifying biologically rich populations of the same species, which is essential for domestication efforts and bioprospecting purposes, thereby strengthening food security and developing antimicrobial drugs that could combat the increasing problem of antibiotic resistance.

South Africa is rich in biodiversity and unique terrains comprising coastal, inland, and mountainous regions shaped by varying climates and soil characteristics, which offer a special and distinctive opportunity to intraspecific variations in the phytochemistry and secondary metabolism of Indigenous plant populations of the same species. Hence, this study aims to provide a comprehensive phytochemical profiling and untargeted secondary metabolites constituents of *M. zeyheri* plant parts from Limpopo and Mpumalanga using quantitative methods and Ultra-performance liquid chromatography (UPLC–QTOFMS/MS).

8.2. Materials and methods

8.2.1. 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 the Vhembe district of the Limpopo province (23.9000° S, 29.4500° E) and the Ehlanzeni district of the Mpumalanga province (25.4652° S, 30.9785° E). These two aro ecologies were chosen to provide a comprehensive representation of the plant's natural habitat across different regions. Voucher specimens (C003) from the leaves were prepared through drying and pressing (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 and again with distilled water to remove any further debris that may cause contamination. The leaves were oven-dried for 48 hours at 40 °C. The fruits were subjected to separation of the fiber and pulp and freeze drying at -80 degrees using the FD-12 Series freeze dryer (Lyophilizer). The dried plant parts were subjected to pulverization using a

waring TNNK160K commercial blender at low speed. The powdered samples were stored in airtight containers to preserve the biomolecules present in the plant and stored at room temperature. The crude plant extract was prepared following the Soxhlet extraction method as described by (Redfern et al., 2014). Approximately 50 g of the powdered plant was extracted separately in 300 ml of 70% ethanol and acetone (99.99%) on an orbital shaker (Labcon laboratory service [Pty], South Africa) for 24 hours. Acetone as an extraction solvent was chosen based on its ability to extract both polar and non-polar solvents, and the use of ethanol was influenced by its availability. The extracts were thereafter filtered using a Buchner funnel and Whatman No. 1 filter paper, and then the filtrate was concentrated to dryness using a rotary evaporator (Heidolph Laborata 4000, Heidolph instruments, GmbH and Co, Germany) at 40 °C (Sagbo et al., 2020). Each extract was exposed to fan air for solidification (Alara et al., 2018)

8.2.2. Determination of Total Phenolics, Flavonoids, and Alkaloids

The total phenolics were determined using the Folin-Ciocalteu colourimetric method as described by (Phuyal et al., 2020). 0.5 mL of the extract was diluted with 2.5 mL of distilled water, followed by 0.5 mL of Folin Ciocalteu reagent. After a 5-minute reaction, 1.5 mL of 7.5 % sodium carbonate was added. Samples were incubated in darkness for 60 min at room temperature to allow complete colour development. Absorbance was measured in triplicate at 760 nm using a UV–Vis spectrophotometer. Results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE g^{-1}).

Total flavonoid content was estimated using the aluminum chloride ($AlCl_3$) method as described by Pękal and Pyrzynska (2014) with slight modifications. 1 mL of plant extract was combined with 4 mL of distilled water, 0.3 mL of 5 % sodium acetate, and 0.3 mL of 2 % $AlCl_3$ in methanol. After 15 min incubation at room temperature, the absorbance was measured in triplicate at 430 nm. Quercetin was used as the standard (0–100 µg mL^{-1}), and results were expressed as milligrams quercetin equivalents per gram dry weight (mg QE g^{-1}).

The total alkaloid content was analyzed using a Dragendorff-based method as described by Sreevidya and Mehrotra (2003) with modifications. 5 g of the powdered sample was extracted with 0.2 M sulfuric acid under constant shaking for 2 h. The filtrate was adjusted to pH 9–10 with 0.1 M sodium hydroxide and partitioned three times with 25 mL chloroform. Combined chloroform

extracts evaporated to dryness, and the residue was re-dissolved in 5 mL of methanol. The addition of Dragendorff reagent produced a visible orange-red precipitate, which fluoresced distinctly under UV light, serving as a qualitative indicator of alkaloid presence. 1.0 mL of the methanolic alkaloid solution was then measured in triplicate at 435 nm using a UV–Visible spectrophotometer. Results were expressed as mg atropine equivalents per g dry weight (mg AE g^{-1}).

8.2.2.1. Data analysis

Results were reported as mean ± standard deviation (n = 3). Statistical significance among plant fractions and collection sites was assessed using one-way ANOVA followed by Tukey's HSD test at $p \leq 0.05$.

8.2.3. Determination of Total Antioxidant Activities Using DPPH Assay

The antioxidant activity of *M. zeyheri* was determined following the 2, 2′-diphenyl1-picrylhydrazyl (DPPH) radical scavenging method described by (Mokgehle et al., 2019). 15 µL of leaf, fruit pulp and fruit fiber extract were diluted with 735 µL absolute methanol at varying doubled subsequent concentrations (O.39, 0.78, 1.56, 3.12, 6.24, 12.5, 25, 50 mg/mL^{-1}) to create a reaction mixture. The final mixture volume was 1.5ml after the prepared methanol DPPH solution (750 µL; 50 µM) was added. The experiment was conducted in three replicates. To complete the reaction mixture, plates containing the reaction solution were left at room temperature in the dark for half an hour, and the actual assay was performed in a dimmed light. A UV/VIS spectrophotometer (Thermo Scientific™ NanoDrop™ Eight Microvolume, News Medical & Life Sciences, Manchester, United Kingdom) was used to test the solutions absorbance at 517 nm with methanol as a blank. As a positive control, Ascorbic acid, a common antioxidant, was used. The percentage of DPPH scavenging activity was calculated as percentage inhibition of DPPH, where Abs517nm sample is the absorbance of the reaction mixture, which contains the resuspended extract or positive control, and Abs517nm Neg control is the absorbance of the negative control.

8.2.3.1. Data analysis

The DPPH radical-scavenging assay for each plant part was conducted in triplicate, and the absorbance values were used to calculate mean inhibitory activity and corresponding standard deviations. One-way ANOVA was performed to determine statistical differences among plant

parts, followed by a post-hoc test to separate significantly different mean's. The results were presented in a table with superscripts indicating groups that differed significantly at $p < 0.05$.

8.2.4. Determination of Total Antioxidant Activities using Frap Assay

The Ferric Reducing Antioxidant Power of *M. zeyheri* plant parts was determined following the method described by (Mokgehle et al., 2019). 30 µL of Methanol and 30 µL plant part extract at a concentration of 50 mg/mL were added into 96 microtiter plates, with butylated hydroxytoluene [BHT] dissolved in methanol added as a positive control. Triplicates of two-fold dilution were used. 40 µL potassium phosphate buffer (0.2M, pH 7.2) and 40 µL potassium ferricyanide (1% in phosphate buffer, w/v) were added to each well. Aluminum was used to cover the plates for 20 minutes of incubation. 40 µL trichloroacetic acid (10% in phosphate buffer, w/v), 150 µL distilled water and 30 µL FeCl3 (0.1% in phosphate buffer, w/v) were added after incubation. Foil was used to cover the plates, which were later incubated again at room temperature for half an hour to complete the reaction. The assay was repeated twice with absorbance read at 630 nm using a microtiter plate reader (Multiscan skyhigh Microplate Reader, Them Fischer Scientific, South Africa). The reducing antioxidant power of the extract and ascorbic acid was expressed by graphically plotting absorbance against concentration.

8.2.4.1. Data analysis

The FRAP assay for all plant parts was performed in triplicate, and the resulting absorbance readings were used to compute mean reducing power values and their standard deviations. Statistical differences among plant parts were assessed using one-way ANOVA, followed by a suitable post-hoc test to distinguish significantly different groups. The results were summarized in a table, with superscripts indicating mean values that differed significantly at $p < 0.05$.

8.2.5. Ultraperformance liquid chromatography–quadrupole time-of-flight mass spectrometry profiling

A 0.22-µm polytetrafluoroethylene filter was used to filter the supernatants. A Quadrupole 120 time-of-flight (QTOF) mass spectrometer UPLC–QTOF/MS (Waters, Milford, Massachusetts, United States) was used to identify and quantify predominant secondary metabolites. The instrument Shimpack C18, 2.1×100 mm, 2.7-µm column from Shimadzu (Honeydew, South Africa), where the mobile phase consisted of formic acid (0.1%) in deionized water (solvent A) and methanol with 0.1% formic acid (solvent B and the temperatures of the column and auto-

sampler were maintained at 30 °C and 10 °C, respectively. Chromatographic separation was achieved using a 30-minute gradient elution method consisting of the following settings: the initial conditions were 5% solvent B at a flow rate of 0.4 mL min−1 and held constant for 3 min. Conditions were then changed to 45% solvent B at 9 min, increased slightly to 50% solvent A at 21 min, and then quickly ramped up to 90% solvent B at 22 min and kept constant for 3 min.

Data were analysed in both negative and positive ionisation modes. The MS was configured to scan the range of 100–1000 Da with a scan time of 0.2 s. The following optimal settings were used as described by (Mokgehle et al., 2023). Capillary voltage of 4.5 eV, sample cone potential of 30 V, source temperature of 120 °C, desolvation temperature of 450 °C, desolvation gas flow of 550 L h−1, and multichannel plate detector potential of 1600 V. during identification, the mass spectrometry data were collected using a collision energy ramp of 10–30 eV and, when necessary, a higher collision energy ramp of 60–165 eV was also used r to achieve efficient fragmentation to aid.

8.2.5.1. Data analysis

Data were processed using the Sum formula, and identification by ranking isotope patterns using mass spectrometry on the SIRIUS software. SIRIUS Functions 1 (unfragmented channel) and 2 (fragmented channel) of the Waters MSe data were processed to produce MS1 and MS2 spectra as well as extracted ion chromatograms with associated peak height intensity data, molecular formula and fragmentation trees. Based on the accurate mass elemental compositions, compounds were identified from the listed databases and then subjected to in silico fragmentation using tree score and library database matches. According to the spectral match between the in silico and measured spectra, a score (out of 10) is assigned to each of the possible compound matches, with the highest score and likely the first compound on the list being accepted as the most likely match (assuming a score of at least 4).

8.3. Results and Discussion

8.3.1. Phytochemical profiling of Total phenolics, Flavonoids and Alkaloids

Table 8.1:Total phenolics, Flavonoids, and Alkaloids content of *M. zeyheri* Fruit fibre, Fruit pulp, Nuts, and leaves from Limpopo (Vhembe) and Mpumalanga (Ehlanzeni).

	Total phenolics (mg GAE g−1)	Total flavonoids (µg QE g−1)	Alkaloids mg AE g−1
FFLP	6.50 ± 0.20^{d}	3.23 ± 0.15^{c}	1.50 ± 0.10^{c}
FFMP	6.00 ± 0.20^{d}	3.00 ± 0.10^{c}	1.40 ± 0.10^{c}
FPLP	8.23 ± 0.25^{c}	4.13 ± 0.15^{c}	2.10 ± 0.10^{b}
FPMP	7.80 ± 0.20^{c}	3.90 ± 0.15^{c}	2.00 ± 0.10^{b}
FNLP	14.53 ± 0.25^{b}	6.23 ± 0.25^{b}	3.33 ± 0.15^{a}
FNMP	13.50 ± 0.20^{b}	5.80 ± 0.15^{b}	3.10 ± 0.10^{a}
LLP	19.13 ± 0.4^{a}	9.83 ± 0.25^{a}	4.67 ± 0.15^{a}
LMP	18.20 ± 0.25^{a}	9.30 ± 0.20^{a}	4.37 ± 0.15^{a}

All analyses are the means of triplicate measurements ± standard deviation. Means that share a superscript letter within a column are not significantly different at $p \leq 0.05$. GAE = gallic acid equivalents, QE = quercetin equivalents 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).

Table 8.1 show clear variation in total phenolics, flavonoids, and alkaloids across different plant parts and between the two provinces. Leaves recorded the highest concentrations of all three phytochemical groups in both Limpopo and Mpumalanga, followed by nuts, pulp, and fibre. Total phenolic content ranged from 6.00–6.50 mg GAE g^{-1} in fruit fibre, 7.80–8.23 mg GAE g^{-1} in pulp, 13.50–14.53 mg GAE g^{-1} in nuts, and 18.20–19.13 mg GAE g^{-1} in leaves. A similar trend was observed for total flavonoids, which ranged from 3.00–3.23 µg QE g^{-1} in fibre, 3.90–4.13 µg QE g^{-1} in pulp, 5.80–6.23 µg QE g^{-1} in nuts, and 9.30–9.83 µg QE g^{-1} in leaves. Total alkaloid content followed the same pattern, ranging from 1.40–1.50 mg AE g^{-1} in fibre, 2.00–2.10 mg AE g^{-1} in pulp, 3.10–3.33 mg AE g^{-1} in nuts, and 4.37–4.67 mg AE g^{-1} in leaves.

In general, the highest concentration of all three classes of secondary metabolites was observed in leaves, followed by nuts, pulp, and fibre. These results are consistent with several reports on the physiological role of leaves as primary sites for photosynthesis and secondary metabolite biosynthesis (Bocso and Butnariu, 2022; Pagare et al., 2015; Patra et al., 2013) due to active leaf tissue activity against ultraviolet radiation and oxidative stress

8.3.1.1. Total phenolics

Leaves recorded significantly higher phenolic content (19.13 mg GAE g^{-1} in Limpopo; 18.20 mg GAE g^{-1} in Mpumalanga) than nuts (≈14 mg GAE g^{-1}), pulp (≈8 mg GAE g^{-1}), and fibre (≈6 mg GAE g^{-1}). Despite the differences in TPC units among the different plant parts, the rank order (Leaves-Nuts-Pulp-Fibre) is consistent with reported species within the Sapotaceae family, showing conserved biosynthetic regulation. Differences in TPC between the two regions were not statistically significant and are comparable to the TPC results reported in the leaves of *Mimusops elengi* (20–25 mg GAE g^{-1}) in a study by (Kadam et al., 2012b). Furthermore, the regional TPC uniformity can be attributed to comparable edaphic conditions, as both the Vhembe district of Limpopo and the Ehlanzeni district of Mpumalanga have semi-tropical climates with similar altitudinal gradient levels, reducing differences in environmental stress levels. Similarly, M. elengi fruits have reported a 7-10 mg GAE g^{-1} TPC range, aligning closely with the fruit pulp and Fruit fibre of *M. zeyheri* observed in the study (Sayed et al., 2023). Such alignment underscores the effects of the role exhibited by the phenolic biosynthetic pathway observed within the Mimusops genus and more generally within the Sapotaceous family in species such as the *Chrysophyllum albidum*, where fruit phenolics reportedly averaged 7–12 mg GAE g^{-1}(Imaga et al., 2023). Phenolic-rich extracts are widely used as natural food preservatives due to their astringent flavour that deters pests and some microbes, in addition to their applications as leads of pharmaceutical antioxidant and anti-inflammatory agents (Beya et al., 2021; Oulahal and Degraeve, 2022). These natural preservatives can be incorporated directly into food formulations or delivered through edible coatings and active packaging materials to improve stability and controlled release (Bouarab Chibane et al., 2019).

8.3.1.2. Total Flavonoids content

Similar to phenolic content, the total flavonoid content (TFC) of *M zeyheri* followed a clear pattern across the different plant parts. Leaves exhibit the highest concentration of TFC. .83 ± 0.25 µg QE g^{-1} in Limpopo and 9.30 ± 0.20 µg QE g^{-1} in Mpumalanga. Nuts followed (6.23 ± 0.25 and 5.80 ± 0.15 µg QE g^{-1}), while pulp (around 4 µg QE g^{-1}), with the lowest TFC observed in fruit fibre (around 3 µg QE g^{-1} in both Limpopo and Mpumalanga). Differences in TFC between the WTO provinces are small, emphasizing plant tissue as the main driver of concentration rather than the location where the plant grows. This is consistent with the report by Sampio et al. (2016, who emphasized that differences in phytochemical content and specifically flavonoid content are attributed to various factors, including plant part composition, including their various solubility

and polarity (Kumar et al., 2017). The observed distribution of TFC is commonly reported in many woody wild fruit trees (Phuyal et al., 2020; Samatha et al., 2012). Leaves tend to accumulate higher levels of flavonoids due to exposure to environmental stressors, including sunlight, hence the increased release of flavonoids that actively act as "Sunscreen" and antioxidants (Agati et al., 2009; Saewan and Jimtaisong, 2013). Flavonoids scavenge reactive oxygen species and aid in cell wall preservation. Nuts protect the Nuts during storage and germination and additionally benefit greatly from the high levels of microbial and oxidative resistance. Pulp and fiber serve largely as dispersal tissue, attracting animals for Nut dispersal; therefore, their defensive demands are reduced, as seen by their lower flavonoid contents. Compared to related species, *M. zeyheri* leaves fall under moderate TFC levels. *M. elengi*, a well-studied congener from the Sapotaceae family, contains 8-10 µg QE g^{-1} TFC (Sehgal et al., 2011), which is closely within a similar range to the findings of *M. zeyheri* in this study. Another comparable range was reported in Chrysophyllum albidum, a Sapotaceae member, with TFC between 7 and 12 µg QE g^{-1}. Flavonoid contents (Adepoju-Bello et al., 2019)similar to those observed in the pulp and fibre have been reported in the fruits of *Synsepalum dulcificum*, ranging from 3-5 µg QE g^{-1} (Obafemi et al., 2017). Contrary to the findings, some Sapotaceae members, such as *Mailkara zapota*, have reported higher levels of TFC in leaves reaching 20 µg QE g^{-1} or more when grown under extreme exposure to sunlight and water-stressful conditions (Tamsir et al., 2020), demonstrating how habitat and climate interact with genetics to influence the phytochemical profiling of plant parts. Overall, the different *M. zeyheri* plant parts, especially the leaves from both provinces, suggest a strong potential to release various compounds both to deter or attract insects and also play a role in various beneficial health properties (BANGO et al., 2025). A previous report by Mohammed et al. (2023) associated high quantities of flavones, which play essential roles in signaling and defence, and are important nutraceuticals in the human diet. Furthermore, flavonoids act as agents for antioxidant activity because of their ability to act as enzymes and pathways involved in anti-inflammatory processes (Mokgehle et al., 2019; Ysrafil et al., 2023). Diets rich in flavonoids are reported to improve cardiovascular health and reduce the risks of metabolic syndromes; hence, the decoction and infusion of *M. zeyheri* leaves for colds and oral health potentially harness the anti-inflammatory effects of this compound class. The notable flavonoid content in *M. zeyheri* leaves underscores its potential as a raw material for standardized flavonoid extracts applicable in cosmeceuticals or dietary supplements.

8.3.1.3. Total Alkaloid content

Alkaloid content was again highest in leaves: 4.67 ± 0.15 mg AE g^{-1} (LLP) and 4.37 ± 0.15 mg GAE g^{-1} (LMP). Nuts followed (3.33–3.10 mg AE g^{-1}), then pulp (~2 mg AE g^{-1}) and fibre (~1.5 mg AE g^{-1}). The pattern mirrors that of phenolics and flavonoids, reflecting the defensive role of alkaloids against herbivores and pathogens, which is strongest in leaves and reproductive tissues. The TAC observed in the leaves of *M. zeyheri* is comparable to that reported by (Muhammad and Abubakar, 2016) in *Chrysophyllum albidum* (3–5 mg g^{-1}) and slightly in a report by (Yong and Shukkoor, 2020) of *Manilkara zapota* fruit (1–2 mg g^{-1}), demonstrating *M. zeyheri's* richness in the compound class. Similar results have been reported in *Mimusops elengi* bark and leaves, used in Ayurvedic medicine for potential antimicrobial activity (Sircar and Mandal, 2016). Alkaloids remain a cornerstone of modern drug discovery, and regulated amounts exhibit notable pharmacological, analgesic, anti-malarial, and antimicrobial properties (Sircar and Mandal, 2016). Rural households often rely on alkaloid-containing plants (Schläger and Dräger, 2016), including the bark and leaves of *M. zeyheri,* for traditional remedies against fever, gastrointestinal infections, and wound healing. Economically, this underpins a small but growing trade in herbal preparations and contributes to household income.

8.3.2. Antioxidant activity of *M. zeyheri* plant parts from Vhembe and Ehlanzeni districts

DPPH assay

8.3.2.1. DPPH Radical Scavenging EC_{50} (µg/mL)

The DPPH assay reveals a distinct hierarchy among *M. zeyheri* plant parts, with the nuts standing out (Table 8.3). The Mpumalanga nut extract (MPN) has an exceptionally low EC_{50} of 0.0581 ± 0.0024 µg/mL, followed by the Limpopo nut extract (LPN) at 0.1767 ± 0.0070 µg/mL, both of which outperform the ascorbic acid/BHT positive control (0.2458 ± 0.0017 µg/mL). This indicates that relatively small amounts of nut extracts are sufficient to quench 50% of DPPH radicals, suggesting a very high concentration of efficient hydrogen-donating compounds. Similar patterns have been reported within the genus, in bark and nut extracts of *Mimusops elengi*, which show stronger DPPH scavenging than ascorbic acid, with activity closely linked to high phenolic content (Rao et al., 2011; Shahwar and Raza, 2012).

Leaves occupy an intermediate position in the DPPH assay, with Mpumalanga and Limpopo leaf extracts (MPL and LPL) showing EC_{50} values of 3.060 ± 0.13 µg/mL and 3.314 ± 0.151 µg/mL,

respectively. These values are clearly higher (weaker activity) than those of the nuts but still markedly lower than those of the pulp and Fibre, confirming leaves as a secondary but meaningful antioxidant reservoir. Comparable findings have been reported for other Sapotaceae species. Methanol extracts of *Mimusops caffra* and *M. elengi* typically show strong, but not necessarily maximal, DPPH activity (Abdelmohsen et al., 2020; Dabadi et al., 2021) when compared to more lignified tissues such as bark or highly phenolic nut fractions observed in this study. In this context, the leaf data for *M. zeyheri* closely follow the general pattern of the genus, where leaves contain significant levels of pharmacologically active phenolics and flavonoids but do not always exhibit the same strength as nuts or bark.

By contrast, the edible pulp and the Fibre are the least effective radical scavengers in the DPPH system. Pulp from Mpumalanga (MPF) and Limpopo (LPF) shows EC_{50} values of 4.570 ± 0.028 µg/mL and 8.564 ± 0.18 µg/mL, respectively, while the fibre extracts are weaker still, with MPP at 11.41 ± 0.15 µg/mL and LPP at 21.26 ± 0.10 µg/mL. This ranking - nuts > leaves > pulp > fibre -is noteworthy because, in many other wild and cultivated fruits such as *Vangueria infausta*, fibre typically rivals or even surpasses the pulp in DPPH activity due to its high phenolic concentration (Fernandes et al., 2023; Núñez-Gómez et al., 2023). In marula (*Sclerocarya birrea*), another key southern African wild fruit, Fibre and nut fractions have reportedly high radical-scavenging capacity, although patterns vary with extraction solvent and cultivar (Rama et al., 2023). The noted relatively weak DPPH response of *M. zeyheri* Fibres in this study, therefore, may suggest either lower levels of hydrophilic phenolics in this tissue or a larger contribution of more lipophilic antioxidants that are less optimally captured in the DPPH assay (Marinas et al., 2020; Shahidi and Hossain, 2023).

8.3.2.2. *β-carotene*–linoleic acid

The β-carotene–linoleic acid assay paints a complementary picture by focusing on inhibition of lipid peroxidation. Here, the Mpumalanga Fibre extract (MPP) is clearly dominant, with an EC_{50} of 18.24 ± 1.16 µg/mL, substantially lower than all other plant parts and far below the positive control (229.04 ± 3.08 µg/mL). Pulp extracts evidently perform well in this analysis, with LPF and MPF showing EC_{50} values of 64.10 ± 1.33 µg/mL and 77.43 ± 1.01 µg/mL, respectively. In contrast, nut and leaf extracts exhibit more moderate activity, for example, MPN and LPN at 114.73 ± 1.22 µg/mL and 559.00 ± 23.39 µg/mL, and MPL and LPL at 178.78 ± 1.92 µg/mL and

117.58 ± 7.90 µg/mL. This pattern suggests that fibres and pulps are particularly effective in protecting against lipid oxidation, likely due to their higher proportion of lipophilic antioxidants, such as carotenoids, certain flavonoid aglycones, and possibly tocopherols (Palafox-Carlos et al., 2011). Similar results have been documented for Sapotaceae such as sapodilla and other wild fruits, where fruit Fibres, despite sometimes modest DPPH values, demonstrate strong β-carotene–linoleic acid activity (Kulkarni et al., 2007).

The strong performance of *M. zeyheri* Fibre in this assay fits well within broader work on indigenous fruits and their by-products. Reviews of southern African wild fruits highlight that Fibers and pomace often possess high antioxidant potential in lipid systems and are promising sources of natural food preservatives (Lubisi et al., 2025; Mnisi et al., 2022) In marula, for example, processing methods that retain Fibre and outer tissues tend to enhance the overall antioxidant capacity of juice and derived products, reflecting the contribution of lipophilic phytochemicals in those tissues (Fernandes et al., 2023; Rama et al., 2023). β-carotene–linoleic acid data for *M. zeyheri* suggest a similar opportunity, while nuts may be more suitable as concentrated radical-scavenging ingredients. Fibre and pulp appear particularly attractive for applications where protection of lipid-rich matrices is required.

Differences between provinces are also evident in the β-carotene–linoleic acid assay and broadly mirror the trends observed in the DPPH assay. Mpumalanga samples tend to exhibit better lipid-phase antioxidant performance than their Limpopo counterparts. The contrast is especially pronounced in the nuts, where MPN (114.73 µg/mL) is far more effective than LPN (559.00 µg/mL), and in Fibre, where MPP (18.24 µg/mL) is clearly superior to LPP (233.63 ± 1.68 µg/mL). These inter-site differences are consistent with recent work on *M. zeyheri* accessions, which shows that maturity stage, genotype, and environmental conditions (such as rainfall and temperature) significantly affect the profiles of primary and secondary metabolites, including phenolics and carotenoids (Teffo et al., 2025a). Comparable provenance-linked variation has also been reported for other wild species, including *Spondias tuberosa*, underlining that antioxidant potential in Sapotaceae and associated wild fruit trees is strongly modulated by agro-ecological context (de Sousa Araújo et al., 2012; Ramírez-Briones et al., 2019a).

In contrast to the DPPH assay, where nut extracts exhibited the strongest radical scavenging activity, the β-carotene–linoleic acid assay revealed superior performance in fibre and pulp

extracts. This shift in antioxidant effectiveness highlights the influence of differing assay mechanisms and compound polarity. While the DPPH assay primarily reflects the activity of hydrophilic antioxidants capable of donating electrons or hydrogen atoms, the β-carotene–linoleic acid system evaluates the inhibition of lipid peroxidation within an emulsion, thereby favouring lipophilic or amphiphilic compounds. The enhanced activity observed in fibre and pulp extracts therefore suggests the presence of compounds more effective in lipid environments. Collectively, these results indicate that different plant parts of *M. zeyheri* exhibit complementary antioxidant roles, with certain tissues better suited for aqueous radical scavenging and others more effective in protecting lipid-based systems against oxidative damage.

Table 8.2: Radical Scavenging and Lipid Peroxidation Inhibition of *M. zeyheri* Plant Parts Determined by DPPH and β-Carotene–Linoleic Acid Methods

Sample	**DPPH Radical Scavenging EC_{50} (µg/mL)**	**β-Carotene Linoleic Acid EC_{50} (µg/mL)**
MPN	0.0581 ± 0.0024^{a}	114.73 ± 1.22^{c}
LPN	0.1767 ± 0.0070^{b}	559.00 ± 23.39^{e}
Ascorbic / BHT (PC)	0.2458 ± 0.0017^{c}	229.04 ± 3.08^{d}
MPL	3.060 ± 0.13^{d}	178.78 ± 1.92^{d}
LPL	3.314 ± 0.151^{d}	117.58 ± 7.90^{c}
MPF	4.570 ± 0.028^{e}	77.43 ± 1.01^{b}
LPF	8.564 ± 0.18^{f}	64.10 ± 1.33^{b}
MPP	11.41 ± 0.15^{g}	18.24 ± 1.16^{a}
LPP	21.26 ± 0.10^{h}	233.63 ± 1.68^{d}

PC (positive control), 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 8.1 provides a concise visual summary of the antioxidant profiles across plant parts and sites, clearly clustering high-activity samples. Extracts from Mpumalanga, particularly the nut (MPN) and Fibre (MPP), occupy the most intense regions of the colour scale, reflecting their markedly low EC_{50} values in both assays. Weaker-performing tissues, such as the Limpopo nut (LPN) and pulp (LPP), appear in lower-intensity zones, visually affirming the pronounced inter-

site variation observed numerically. When the two assays are considered together, a complementary spatial pattern also becomes apparent: nuts, particularly from Mpumalanga, are outstanding in hydrophilic radical scavenging, with DPPH EC_{50} as low as 0.0581 µg/mL, whereas Fibres and pulps, especially Mpumalanga Fibre, dominate in the β-carotene–linoleic acid system, with EC_{50} values between 18.24 and 77.43 µg/mL. Leaves consistently occupy an intermediate position in both assays, which is clearly reflected in their mid-range colour intensity on the heatmap. Such differentiation across tissues is consistent with reports in Sapotaceae and other wild fruit trees, where variation in accumulation of distinct phenolic and lipophilic compounds leads to tissue-specific antioxidant strategies rather than uniform activity across the plant (Ramírez-Briones et al., 2019b).

The functional complementarity highlighted by the heatmap aligns with literature suggesting that different tissues in wild fruit species specialize in distinct antioxidant niches (Medina-Medrano et al., 2015). Nuts and other lignified plant parts tend to concentrate phenolics that excel in DPPH-type assays, while pigmented or oil-rich outer tissues contribute more strongly to lipid-phase protection due to the presence of carotenoids, tocopherols and less polar flavonoid aglycones (Bolling et al., 2010). Studies on *Mimusops elengi* and other members of the family describe similar partitioning of antioxidant activity, where bark, nut and kernel fractions demonstrate exceptional radical-scavenging capacity, while Fibers and fruit tissues are more effective at delaying lipid oxidation in model systems (Shahwar and Raza, 2012). In the context of *M. zeyheri*, the heatmap therefore reinforces that no single plant part is universally "best"; instead, each tissue displays a distinctive antioxidant profile shaped by its phytochemical composition and ecological function. This situates *M. zeyheri* among the more promising indigenous fruits in southern Africa from an antioxidant perspective.

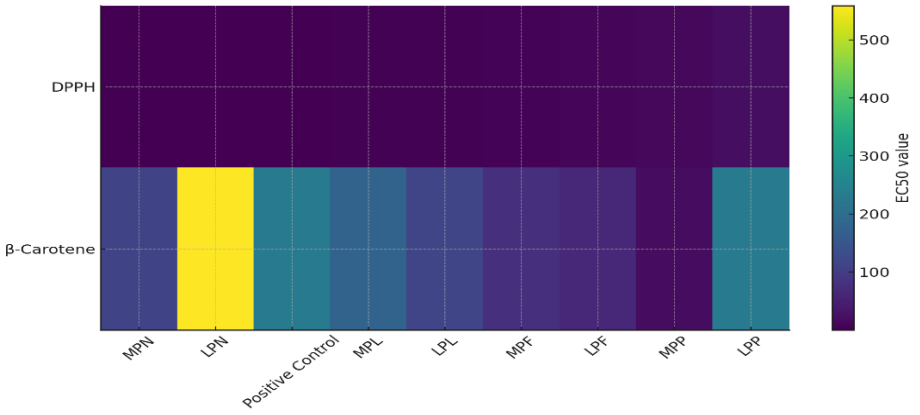


Figure 8.1:Heatmap Visualization of DPPH and β-Carotene–Linoleic Acid Assay Results for *M. zeyheri* plant part Extracts.

8.3.3. Secondary metabolites tentatively identified in *M. zeyheri* plant parts from Vhembe and Ehlanzeni districts

8.3.3.1. Fruit fibre

Table 8.3 shows 37 compounds tentatively identified from *M. zeyheri* fruit fibre from Vhembe, Limpopo, while Table 8.4 shows 17 compounds tentatively identified from *M. zeyheri* fruit fibre from Ehlanzeni, Mpumalanga. Both provinces contain a notable diversity of compound classes and a rich spectrum of polyphenols. Fruit fibre from Mpumalanga is rich in flavonoids with dominant compounds such as quercetin-3-O-glucoside (Rt 7.82 min, 463.12 m/z) with congeners identified as isoquercitrin (Rt 5.81 min, 463.09 m/z) and rutin (Rt 4.62 min, 609.14 m/z) known for their distinct structural and biological properties. These compounds are renowned for their antioxidant and anti-inflammatory properties that aid in the reduction of cardiovascular risks and modulating immune system responses in addition to lending colour and stability to food and fruit products (Valentová et al., 2016). Similar quercetin derivatives occur in *Manilkara zapota* (Bashir, 2019) and *Chrysophyllum albidum (Adebayo, Abiodun Humphrey et al., 2011)*, where they are valued for both medicinal teas and functional beverages. Mpumalanga fibre further contains catechin (Rt 6.10 min, 289.07 m/z) and epigallocatechin (Rt 6.20 min, 305.07 m/z), flavan-3-ols with well-established roles in vascular protection and microbial inhibition, important for both human health and natural food preservation due to improved water solubility and subsequent bioavailability, with plasma detection of active metabolites following oral administration (Di Pede et al., 2023; Favari et al., 2020). In addition to the notable flavonoids, notable compounds identified in the fruit fibre of *M zeyheri* in Mpumalanga include pentacyclic triterpenes such as lupeol (Rt 7.44 min, 425.38 m/z), β-amyrin (Rt 7.44 min, 425.38 m/z), oleanolic acid (Rt 7.60 min, 455.35 m/z), and hederagenin (Rt 7.60 min, 471.35 m/z) which are some of the popular Sapotaceae triterpenoids used in traditional skin care. These compounds have been reportedly isolated from *Vitellaria paradoxa* (Catteau et al., 2021) and *Pouteria* species (Rodrigues et al., 2017) and are renowned for their powerful anti-inflammatory properties, wound healing, and dermo-protective effects commonly applied in topical and herbal cosmetics. Their presence in the fibre of *M. zeyheri* fruits underscores their role in rural health and indicates a good potential for supporting the nutraceutical and cosmeceutical value chain.

Although also rich in flavanols such as isohyperoside (Rt 5.50 min, 463.09 m/z) and Laricitrin-3-galactoside (Rt 5.52 min, 493.10 m/z), *M. zeyheri* fruit fibre from Limpopo has notable phenolic glycosides such as β-glucogallin (Rt 1.08 min, 367.05 m/z). β-glucogallin has been identified and reported in *M. elengi* with potent antioxidant and antimicrobial properties that also support good gut health function in humans and aid in the preservation of food (Rao et al., 2011). Minor cucurbitacin derivatives (Rt 6.80 min, 559.33 m/z) have been identified in the fruit fibre of *M. zeyheri* in Limpopo, which are of pharmacological value due to their cytotoxic and anti-inflammatory properties (Chen et al., 2012). Additionally, dihydrocucurbitacin B/ dihydroisocucurbitacin B (Rt 6.80 min, 559.33 m/z) has been identified, indicating a triterpenoid stream with potential bioactivities (cytotoxic/anti-inflammatory) suitable for lead-finding once safety is profiled.

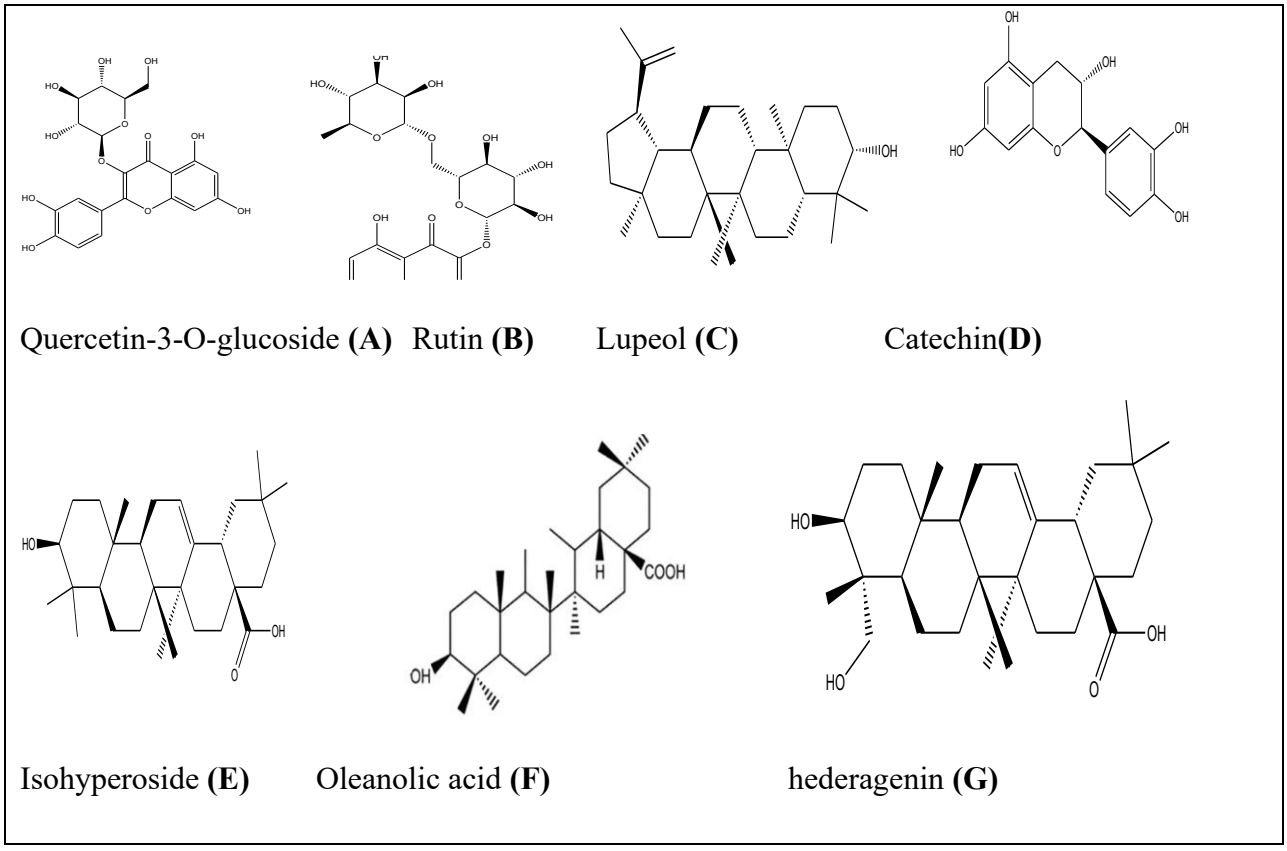


Figure 8.2: Compounds identified in the fruit fibre of *M. zeyheri* from Vhembe district, Limpopo Province (E, F,G) and Ehlanzeni district, Mpumalanga Province (A, B, C, D)

Table 8. 3:Characterisation and Identification of Secondary Metabolites Present in *M. zeyheri* Fruit Fibre collected from Vhembe, Limpopo Province

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
1	0.90	484.87	484.8729; 112.09859	$C_{17}H_8C_{12}N_2O_3S_4$	N.i	3.08	N.i
2	0.92	371.12	371.1210; 325.1157; 117.0554; 161.0458; 143.0358; 119.0351; 101.0251; 113.0240; 163.0619	$C_{13}H_{24}O_{12}$	4-O-beta-D-glucopyranosyl-L-glycero-alpha-D-manno-heptopyranose	2.53	Carbohydrates (Glycoside sugar)
3	1.10	377.08	377.0873; 221.0882; 179.0573; 161.0472; 119.0355; 101.0243; 113.0242	$C_{18}H_{18}O_9$	6-[(3R;4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl[furo[3,2-g]chromen-7-one	4.50	Flavonoid (Coumarin glycoside derivative)
4	1.10	533.17	533.1749; 191.0565; 173.0458; 127.0396	$C_{19}H_{34}O_{17}$	2-O-(6-O-glycero-manno-Heptopyranosyl-glucopyranosyl)glucopyranose	1.61	Carbohydrate (Oligosaccharide)
5	1.34	341.11	241.1103; 237.0624; 191.0576; 179.0561; 161.0458; 143.0359; 119.0360; 101.0243; 113.0236	$C_{12}H_{22}O_{11}$	Brachiose	2.23	Carbohydrate (Disaccharide)
6	1.34	281.09	281.0888; 129.0206; 113.0244; 112.0170; 111.0081	$C_{10}H_{18}O_9$	N.i	1.997	N.i
7	1.34	317.06	317.0561; 225.0083; 206.9950; 152.9859; 134.9749; 106.9804; 164.9867; 136.9916; 149.9918; 122.9784; 127.0403; 125.0249; 101.0251	$C_9H_{18}O_{10}S$	6-(2,3-dihydroxypropoxy)-3,4,5-trihydroxyoxan-2-yl]methanesulfonic acid	1.737	Sulfonated sugar derivative
8	1.08	367.05	367.0453; 271.0463; 241.0363; 223.0260; 197.0470; 211.0254; 183.0305; 188.0067; 124.0167; 169.0145; 151.0043; 123.0088; 125.0244; 107.0136; 113.0234; 101.0239	$C_{13}H_{16}O_{10}$	Beta-Glucogallin	1.455	Hydrolyzable tannin (Gallotannin derivative)

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
9	3.93	345.08	345.0842; 183.0309; 168.0066; 166.9986; 139.0404; 124.0170; 107.0147; 138.0320; 123.0094; 137.0255	$C14H_{18}O_{10}$	Methyl Gallate 3-O-Beta-D-Glucopyranoside	3.681	Phenolic glycoside
10	3.93	359.10	359.1000; 197.0457; 182.0223; 166.9989; 181.0142; 153.0556; 138.0323; 123.0087; 123.0458; 152.0494	$C_{15}H_{20}O_{10}$	Glucosyringic acid	1.99	Phenolic glycoside
11	4.20	461.13	461.1323; 167.0351; 125.0239; 123.0444; 163.0418	$C_{19}H_{26}O_{13}$	Saccharumoside D	1.560	Phenolic glycoside
12	4.69	461.13	461.1319; 209.046; 191.0358; 163.0407; 181.0506; 167.0354; 151.0044; 123.0094; 149.0238; 121.0296; 125.0247; 123.0447; 101.0245	$C19H_{26}O_{13}$	Saccharumoside C	2.270	Lignan
13	4.80	401.15	401.1470; 269.1030; 193.0503; 178.0286; 125.0235; 161.0459; 159.0311; 112.0241; 101.0245	$C_{18}H_{26}O_{10}$	Baxgp	0.361	Phenolic glycoside
14	4.98	519.25	519.2564; 387.2053; 191.067; 161.0460; 143.0355; 101.0246; 149.0467; 113.0262	$C_{24}H_{40}O_{12}$	Platanionoside C	3.295	N.i
15	5.02	553.21	552.2052; 433.1123; 212.0121; 271.1810; 222.0515; 227.0715; 201.0726; 292.0885; 233.0672; 191.0550; 142.0452; 131.0332; 101.0051; 205.1231; 122.6916; 152.0841	$C_{24}H_{38}O_{12}$	N.i	1.086	Flavonoid glycoside
16	5.32	499.07	499.0670; 317.0305; 316.0242; 270.0185; 178.9984	$C_{21}H_{20}O_{12}$	Hypertin	2.980	Terpenoid glycoside
17	5.40	509.23	509.2260; 463.2226; 331.1805; 161.0463; 119.0352; 101.0247;	$C_{22}H_{38}O_{13}$	Hexosyl-hexosyl-geraniol	2.955	N.i

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
			317.0294; 316.0237; 149.0458; 113.0242				
18	5.41	531.08	531.0780; 4633.0909; 317.0327; 316.0244	$C_{24}H_{20}O_{14}$	N.i	3.088	Flavonoid glycoside
19	5.50	463.09	463.0904; 37.0310; 316.0234; 270.0168; 299.0207; 271.0296; 243.0305; 178.9909; 151.0039; 300.0289; 272.0288; 254.0226; 283.0258; 273.0412; 255.0308; 227.0354	$C_{21}H_{20}O_{12}$	Isohyperoside	0.768	Sucrose ester
20	5.52	509.23	509.2260; 463.2206; 191.0563; 161.0450; 125.0244; 119.0358; 101.0235; 149.0449; 131.0367; 113.0249	$C_{22}H_{38}O_{13}$	2,4-Di(isovaleryl)sucrose	2.247	Flavonoid glycoside
21	5.52	493.10	493.1011; 331.0470; 330.0396; 315.0157; 287.0219; 316.0246	$C_{22}H_{22}O_{13}$	Laricitrin 3-Galactoside	1.857	Flavonoid glycoside
22	5.58	435.23	435.1315; 301.0335; 271.0233; 255.0294; 179.0352; 125.0245; 123.0450; 272.0303; 167.0343; 151.00039; 149.0253; 119.0514	$C_{21}H_{24}O_{10}$	Phlorizin	1.785	Flavonoid glycoside
23	5.59	301.00	301.0000; 252.0069; 216.0019; 226.0062; 210.0117; 242.0074; 154.0155; 272.0051; 254.0942; 245.0100; 227.0001; 217.0149; 199.0042; 182.0155; 213.0207; 184.0242; 157.0222; 129.0241; 101.0224; 237.6023; 222.0145; 201.0201; 172.0247; 145.0201; 117.0244	$C_{14}H_6O_8$	Gallogen	1.098	Anthraquinone
24	5.60	433.08	433.0795; 301.0364; 300.0285; 254.0218; 283.0275; 255.0314;	$C_{20}H_{18}O_{11}$	Reinutrin	0.986	Flavonoid

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
			178.9995; 151.0034; 135.0080; 271.0280; 243.0294; 271.0622				
25	5.63	263.02	263.0206; 191.0354; 190.0285; 173.0240; 145.0299; 163.0394; 135.0458; 117.0342; 107.0503; 147.0452; 129.0338; 119.0500	$C_{12}H_8O_7$	Ancistroquinone E	0.363	Cyclic peptide
26	5.81	483.07	483.0721; 301.0255; 300.0285; 283.0254; 273.0414; 271.0257; 243.0311; 178.9962; 151.0044; 256.0315	$C_{21}H_{20}O_{11}$	Quercetin	3.544	Flavonoid glycoside
27	5.81	564.42	564.4157; 546.4044;450.3478; 337.2633; 224.1771; 451.3327; 338.2474; 320.2373; 225.1616; 207.1505; 137.0715; 129.1030; 130.0879; 112.0771	$C_{30}H_{55}N_5O_5$	Clavatustide C	3.875	Norisoprenoid (Plant hormone – ABA derivative)
28	5.84	477.11	477.1060; 449.1085; 331.0466; 320.0405; 316.0240; 315.0151; 314.0440; 299.206; 271.0248; 243.0299; 151.0034; 285.0420; 285.0420; 315.0527; 200.0283	$C_{22}H_{22}O_{12}$	Tamarixin	2.493	Flavonoid
29	6.01	791.49	791.4940; 723.5013; 677.5000	$C_{44}H_{72}O_{12}$	Sapimukoside J	2.605	Flavonoid
30	6.05	263.13	263.1398; 204.1162; 189.0923; 163.0770; 146.0524; 122.0366; 159.1198; 152.0847; 138.0877; 203.1074; 151.0765; 136.0533; 125.0610; 119.0885	$C_{15}H_{20}O_4$	Abscisin li		Flavonoid
31	6.09	331.05	331.0472; 316.0237; 299.0228; 271.0254; 243.0326;287.0204; 259.0252; 193.0144; 192.0089; 163.0036; 164.0120; 178.9995;	$C_{16}H_{12}O_8$	Mearmsetin	1.184	Flavonoid

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
			169.0151; 152.0132; 124.0159; 151.0036; 125.0238; 107.0137				
32	6.09	331.05	331.0472; 316.0237; 299.0228; 271.0254; 243.0326;287.0204; 259.0252; 193.0144; 192.0089; 163.0036; 164.0120; 178.9995; 169.0151; 152.0132; 124.0159; 151.0036; 125.0238; 107.0137	$C_{16}H_{12}O_8$	Annulatin	0.974	Flavonoid
33	6.09	331.05	331.0472; 316.0237; 299.0228; 271.0254; 243.0326;287.0204; 259.0252; 193.0144; 192.0089; 163.0036; 164.0120; 178.9995; 169.0151; 152.0132; 124.0159; 151.0036; 125.0238; 107.0137	$C_{16}H_{12}O_8$	Latricitin	0.974	Flavonoid
34	6.09	331.05	331.0472; 316.0237; 299.0228; 271.0254; 243.0326;287.0204; 259.0252; 193.0144; 192.0089; 163.0036; 164.0120; 178.9995; 169.0151; 152.0132; 124.0159; 151.0036; 125.0238; 107.0137	$C_{16}H_{12}O_8$	Europetin	0.974	Triterpenoid (Cucurbitacin derivative)
35	6.56	315.05	315.0522; 300.0288; 148.0180; 136.9152; 108.020; 283.0269; 227.0356; 163.0033; 135.0101; 151.0036; 149.9951; 125.0252; 124.0153; 10.0137; 271.0259	$C_{16}H_{12}O_7$	Rhamnetin	1.082	Triterpenoid (Cucurbitacin derivative)
36	6.80	559.33	559.32295; 541.3193; 499.3082; 481.2969; 497.327; 455.3171; 389.2837; 371. 2694; 323.2386; 497.2959; 515.3422; 473.3318	$C_{32}H_{48}O_8$	Dihydrocucurbitacin B	2.309	Triterpenoid

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
37	6.93	517.32	517.3192; 499.3078; 353.2480; 469.3004; 437.3095; 407.2989; 383.2625; 309.2117; 291.1977	$C_{30}H_{46}O_7$	Barringtonic Acid	3.341	N.i

Note: Peak no. = sequential number assigned to each detected compound; Rt = retention time (minutes) indicating the time taken for a compound to elute from the chromatographic column; $[M–H]^-$ Observed = deprotonated molecular ion detected in negative ion mode; Product Ions (MS/MS) = fragment ions obtained after tandem mass spectrometry used for compound identification; Empirical Formula = molecular formula of the detected compound; Putative Name = tentatively identified compound based on mass spectral data and database comparison; Ppm (error) = mass accuracy expressed as parts per million, indicating the difference between observed and theoretical mass; Chemical Class = classification of compounds based on their chemical structure or functional group.

Table 8.4:Characterisation and Identification of secondary metabolites present in *M. zeyheri* fruit Fibre collected from Ehlanzeni, Mpumalanga Province.

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
1	0.86	285.04	286.0; 99.99; 85.0; 21.80; 69.0; 17.30; 65.0; 8.30; 257.0; 8.30; 287.055; 44.56; 241.0495 17.24; 213.0544; 11.46; 165.0183; 5.67; 258.0523; 5.54	$C_{15}H_{10}O_6$	Kaempferol	2.348	Flavonol
2	0.88	317.03	147.0; 100; 133.0; 62.56; 193.0; 27.33; 207.0; 25.13; 179.0; 24.92	$C_{15}H_{10}O_8$	Myricetin	3.567	Flavonol
3	4.65	315.051	316.0; 99.99; 317.0; 16.70; 315.0; 13.70; 287.0; 7.20; 149.0; 6.40; 153.01678; 100; 302.04175; 84.60; 229.04778; 54.40; 274.05161; 37.80; 317.05765; 32.60	$C_{16}H_{12}O_7$	Isorhamnetin	1.342	Flavonol (O-methyl)
4	4.62	609.146	611.0; 100; 610.0; 23.75; 303.0; 17.17; 302.0 ;12.51; 612.0 9.16; 609.1472; 999; 607.1321; 506; 610.1508; 244; 608.1357; 131; 1217.2849; 71	$C_{27}H_{30}O_{16}$	Rutin	3.478	Flavonol diglycoside
5	6.65	447.093	413.0872; 38.40; 303.0498; 22.53; 431.0976; 20.21; 345.0604; 14.33; 369.0601; 4.53	$C_{21}H_{20}O_{11}$	Quercetin-3-O-rhamnoside	2.417	Flavonol glycoside
6	6.86	463.088	399.0713; 25.68; 447.0928; 24.94; 369.0605; 19.74; 345.0604; 12.41; 429.0826; 6.45	$C_{21}H_{20}O_{12}$	Hyperoside	1.345	Flavonol glycoside
7	6.68	447.093	-	$C_{21}H_{20}O_{11}$	Kaempferol-3-O-glucoside	4.876	Flavonol glycoside
8	6.85	463.088	-	$C_{21}H_{20}O_{12}$	Myricitrin (Myricetin-3-O-rhamnoside)	3.026	Flavonol glycoside
9	6.86	271.061	473.0; 100; 179.0; 69.77; 296.0; 69.67; 474.0; 47.85; 177.0; 40.74	$C_{15}H_{12}O_5$	Naringenin	1.672	Flavanone

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
10	7.40	269.046	471.0; 100; 472.0; 55.36; 470.0; 26.23; 473.0; 25.53; 228.0; 23.82; 117.0336; 100; 118.0351; 8.10; 182.04207; 3.10; 66.00433; 2.80; 89.03706; 2.60	$C_{15}H_{10}O_5$	Apigenin	4.478	Flavone
11	7.40	285.04		$C_{15}H_{10}O6$	Luteolin	1.417	Flavone
12	7.44	425.379	-	C30H50O	Lupeol	4.568	Triterpenoid
13	7.44	425.379	-	$C_{30}H_50O$	Î²-Amyrin	2.245	Triterpenoid
14	7.60	455.353	455.352, 100, 456.3546, 36.87, 407.3302, 5.52, 457.3596, 4.47, 408.3362, 2.68	$C_{30}H_{48}O_3$	Oleanolic acid	2.761	Triterpenoid acid
15	7.60	471.348	471.3573, 999, 472.3577, 338, 407.3367, 152 473.3643, 81	$C_{30}H_{48}O_4$	Hederagenin	1.091	Triterpenoid (sapogenin)
16	6.80	559.33	559.32295, 541.3193, 499.3082, 481.2969, 497.327, 455.3171, 389.2837, 371. 2694, 323.2386, 497.2959, 515.3422, 473.3318	$C_{32}H_{48}O_8$	Dihydroisocucurbitacin B	2.309	Triterpenoid
17	6.99	519.34	519.3551, 475.2454 44.1126	$C_{30}H_{48}O_7$	Ballericagenin B	4.601	Carbohydrate (Glycoside sugar)

Note: Peak no. = sequential number assigned to each detected compound; Rt = retention time (minutes) indicating the time taken for a compound to elute from the chromatographic column; $[M–H]^-$ Observed = deprotonated molecular ion detected in negative ion mode; Product Ions (MS/MS) = fragment ions obtained after tandem mass spectrometry used for compound identification; Empirical Formula = molecular formula of the detected compound; Putative Name = tentatively identified compound based on mass spectral data and database comparison; Ppm (error) = mass accuracy expressed as parts per million, indicating the difference between observed and theoretical mass; Chemical Class = classification of compounds based on their chemical structure or functional group.

8.3.3.2. Compounds identified in *M. zeyheri* Fruit pulp

Table 8.5 shows 21 compounds identified from the fruit pulp of *M. zeyheri* from Ehlanzeni, Mpumalanga, while Table 8.6 shows 25 compounds identified from the fruit pulp of Vhembe, Limpopo. *M. zeyheri* fruit pulp from Mpumalanga has a notable array of various Flavonol glycosides and nutrient compounds that together create a flavorful and health-promoting profile. Myricitrin (Rt 5.37 min, 499.07 m/z) is a rhamnoside of myricetin widely documented in *Chrysophyllum albidum* (Idowu et al., 2016) and *Mimusops elengi* (Abdelmohsen et al., 2020) and exhibits antioxidant, anti-inflammatory, and antidiabetic effects that support glucose control and redox balance. Hyperin (Rt 5.50 min, 463.09 m/z), a quercetin derivative also reported in *Manilkara zapota*, is reported widely for its neuro- and cardio protection properties (Tamsir et al., 2020). Kaempferol rhamnoside (Rt 6.71 min, 431.4 m/z) contributes additional antioxidant and vascular-protective effects and is consistent with signals observed in Sapotaceae bark and edible berries (M Calderon-Montano et al., 2011). The pulp further contains abundant organic acids—malic (Rt 6.78 min, 133.014 m/z), quinic (Rt 6.78 min, 191.056 m/z), and *citric* (Rt 7.05 min, 191.02 m/z) which enhance flavour, lower pH to inhibit spoilage organisms, and improve mineral bioavailability, thereby supporting probiotic beverage development and gut health (Shi et al., 2022) Minor phenolic acids such as gallic (Rt 7.20 min, 169.014 m/z), protocatechuic (Rt 7.20 min, 153.019 m/z), vanillic (Rt 7.20 min, 167.035 m/z), syringic (Rt 7.60 min, 197.046), caffeic (Rt 7.60 min, 179.035 m/z), p-coumaric (Rt 7.60 min, 163.04 m/z), and ferulic (Rt 7.60 min, 193.051m/z) provide further antioxidant and antimicrobial capacity consistent with other Sapotaceae fruits (Baky et al., 2016). Complementing these are key amino acids alanine (Rt 6.03 min, 89.09 m/z), tyrosine (Rt 6.22 min, 181.90 m/z), aspartic acid (Rt 6.22 min, 133.05 m/z), serine (Rt 6.50 min, 105.10 m/z), and proline (Rt 6.71 min, 142.00 m/z), which add umami/sweet notes, contribute to protein nutrition, and act as metabolic precursors (e.g., tyrosine → catecholamines) (Li, P. et al., 2021). Taken together, this flavonoid–acid–amino matrix enhances palatability, aids mineral chelation, and favours microbiome-friendly fermentation, well-suited to functional beverages, youth-nutrition porridges, and fermented fruit products (Anumudu et al., 2024; Septembre-Malaterre et al., 2018).

By contrast, the Limpopo pulp is dominated by hydrolysable tannins and phenolic glycosides, reflecting a more therapeutic, preservative-leaning profile. β-Glucogallin (Rt 2.89 min, 367.05 m/z) is a gallic-acid glucoside reported in *Mimusops elengi* and *Manilkara hexandra* with

hepatoprotective and antimicrobial properties and the ability to stabilize foods via metal chelation and inhibition of lipid peroxidation (Baky et al., 2022d). Glucosyringic acid (Rt 3.08 min, 359.10) is well known for its antioxidant and anti-inflammatory activity akin to syringic-acid derivatives in Sapotaceae leaves (Srinivasulu et al., 2018). Saccharumoside C (Rt 3.89 min, 461.13) and penstemide (Rt 4.42 min, 443.19) contribute antimicrobial and gastroprotective effects, while petiolaroside (Rt 4.76 min, 609.15), a phenolic diglycoside also detected in *Chrysophyllum* spp., supports antioxidant capacity and aligns with traditional gastrointestinal remedies (Srinivasulu et al., 2018). These galloyl-rich hydrolysables impart astringency that naturally suppresses spoilage microbes (Widsten et al., 2014), making the Limpopo pulp ideal for decoctions, spreads, and preservative-leaning syrups that extend household shelf-life and reduce reliance on synthetic additives.

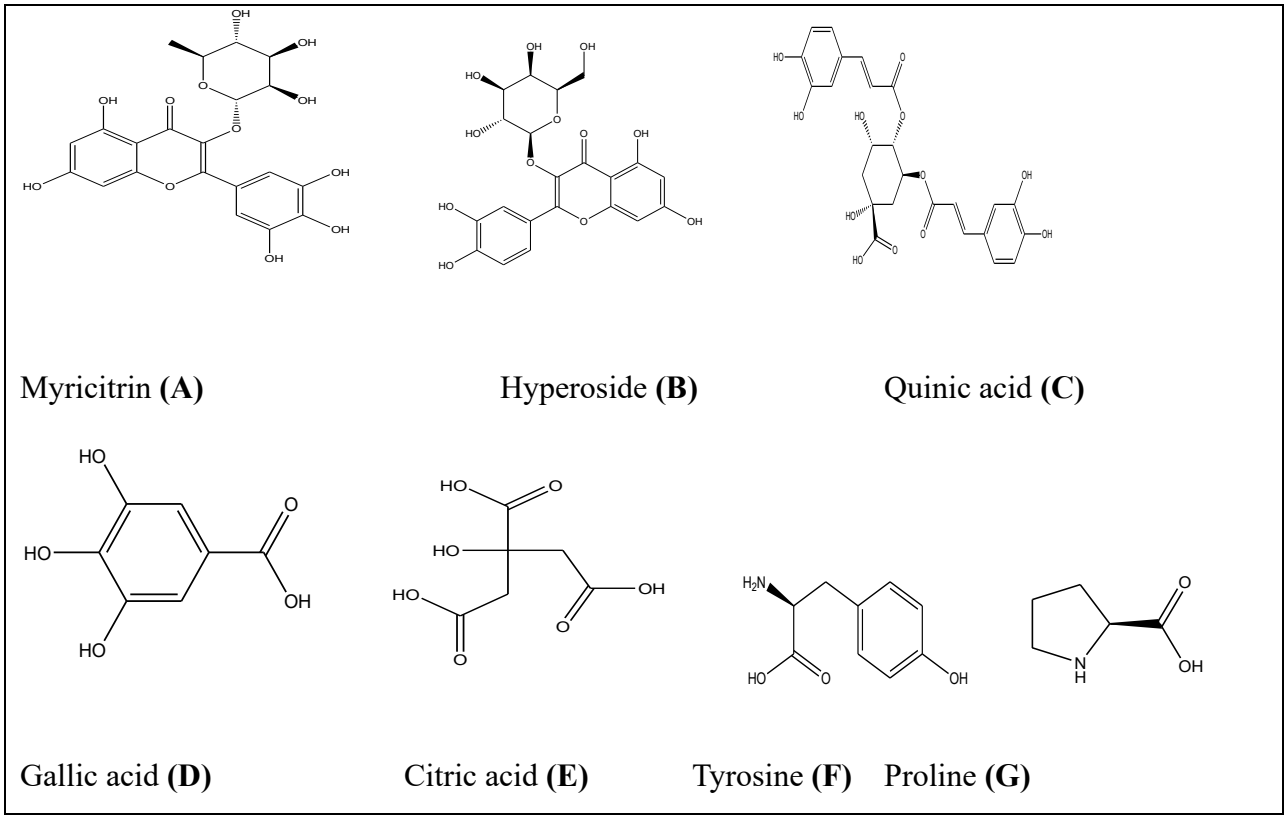


Figure 8. 3: Compounds identified in the fruit Pulp of *M. zeyheri* from Vhembe district, Limpopo (D,E,F,G) and Ehlanzeni district Mpumalanga province (A,B,C)

Table 8.5:Characterization and Identification of secondary metabolites present in *M. zeyheri* fruit pulp collected from Ehlanzeni, Mpumalanga.

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
1	5.35	557.23	557.2275; 539.2170; 495.2264	$C_{26}H_{38}O_{13}$	Lobetyolinin	4,71	Glycoalkaloid
2	5.37	499.07	499.0670; 317.0305; 316.0242; 270.0185; 178.9984	$C_{21}H_{20}O_{12}$	Myricitrin	3.823	Flavonoid glycoside
3	5.50	463.09	463.0994; 317.0310; 316.0234; 270.0168; 299.0207; 271.0256; 243.0305; 178.9989; 151.0039; 301.0354; 300.0285; 2720331; 254.0226; 283.0296; 273;0412; 255.0308; 227.0354	$C_{21}H_{20}O_{12}$	Hyperin	4.202	Flavonoid glycoside
4	6.03	89.09	89.0920	$C_3H_7NO_2$	Alanine	2.345	Amino acid
5	6.22	181.90	133.45; 59.00; 129.52; 157.75; 38.39; 118.63; 177.06	$C_{19}H_{11}NO_3$	Tyrosine	4.876	Amino acid
6	6.22	133.05	180.20:43.16; -0.00:21.77; 55.09:65.98; 39.30:90.44; 176.91:38.11	$C_4H_7NO_4$	Aspartic acid	1.026	Amino acid
7	6.50	105.10	116.1; 100; 132.1 91.46; 103.05; 17.33;144.05 14.65; 147.1; 14.22	$C_3H_7NO_3$	Serine	3.723	Amino acid
8	6.71		142.0; 1;143.0; 0.13; 72.0; 0.04; 144.0; 0.04; 216.0; 0.04; 166.0; 1; 77.0; 0.71; 71.0 0.59;170.0; 0.53; 96.0; 0.52	$C_5H_9NO_2$	Proline	2.345	Amino acid
9	6.72	347.24	-	$C_{18}H_{36}O_6$	Salvic acid	4.876	Diterpenoid/organic acid
10	6.71		-	$C_{21}H_{20}O_{10}$	Kaemferol rhamnoside	4.026	Flavonoid glycoside
11	6.78	133.014	147; 99; 233; 265; 133;195; 148;161; 101; 159	$C_4H_6O_5$	Malic acid	2.723	Organic acid
12	6.78	191.056	157.048050; 100; 139.036621; 94.47;129.052505; 90.53; 147.063400; 79.22; 193.069946; 46.01	$C_7H_{12}O_6$	Quinic acid	2.345	Organic acid

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
13	7.05	191.02	273.0; 1; 274.0; 0.25; 211.0; 0;.24;347.0; 0.23 133.0; 0.21; 147.0; 100; 273.0; 51.85; 133.0; 18.12; 149.0; 18.02; 148.0; 16.92	$C_6H_8O_7$	Citric acid	4.876	Organic acid
14	7.05	173.046	204.05; 100; 147.05; 18.15; 205.0; 17.90; 206.0; 8.62; 255.057; 129.0; 100; 157.0; 62.05 175.0; 56.59; 130.0; 10.95; 142.0; 10.41	$C_7H_{10}O_5$	Shikimic acid	1.191	Organic acid
15	7.20	169.014	281.0; 100; 282.0; 23.52; 179.0 23.22; 133.0 17.92; 458.0; 17.72; 169.01321; 100;125.0236 59.80; 126.02489; 5.40; 123.01162;124.01428 1.60	$C_7H_6O_5$	Gallic acid	0.993	Phenolic acid
16	7.20	153.019	193.0 1; 77.0; 0.27; 370.0; 0.18; 87.0 0.17; 194.0; 0.16	$C_7H_6O_4$	Protocatechuic acid	1.560	Phenolic acid
17	7.20	167.035	297.0; 1; 267.0; 0.94; 223.0; 0.89; 253.0; 0.61; 126.0; 0.59	$C_8H_8O_4$	Vanillic acid	3.667	Phenolic acid
18	7.60	197.046	140.0; 100; 155.0; 94.89; 199.0; 25.27; 123.0; 22.50; 95.0; 8.68	$C_9H_{10}O_5$	Syringic acid	2.66	Phenolic acid
19	7.60	179.035	219.0; 1; 396.0; 0.29; 191.0; 0.19; 220.0; 0.18; 397.0; 0.11	$C_9H_8O_4$	Caffeic acid		Phenolic acid
20	7.60	163.04	164.0; 99.99; 147.0 44; 163.0 37.60; 91.0 27.20; 65.0; 17.60	$C_9H_8O_3$	p-Coumaric acid	4.876	Phenolic acid
21	7.60	193.051	338.0; 1;249.0; 0.84; 323.0; 0.75; 308.0; 0.75; 293.0; 0.59	$C_{10}H_{10}O_4$	Ferulic acid	4.026	Phenolic acid

Note: Peak no. = sequential number assigned to each detected compound; Rt = retention time (minutes) indicating the time taken for a compound to elute from the chromatographic column; $[M–H]^-$ Observed = deprotonated molecular ion detected in negative ion mode; Product Ions (MS/MS) = fragment ions obtained after tandem mass spectrometry used for compound identification; Empirical Formula = molecular formula of the detected compound; Putative Name = tentatively identified compound based on mass spectral data and database comparison; Ppm (error) = mass accuracy expressed as parts per million, indicating the difference between observed and theoretical mass; Chemical Class = classification of compounds based on their chemical structure or functional group.

Table 8.6:Characterization and Identification of secondary metabolites present in *M. zeyheri* Fruit pulp collected from Vhembe; Limpopo

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
1	1.22	484.87	484.8729; 112.9859	$C_{17}HBC_{12}N_2O_3S_4$	N.i	4.89	Not identified
2	1.45	533.17	533.1750; 353.1125; 191.0563	$C_{20}H_{30}N_4O_{13}$	Boc-Asp-Ala-Asp-Asp-OH	1.120	Peptide
3	1.98	341.11	341.1103; 237.0824; 191.0578; 179.0581; 181.0458; 143.0359; 119.0380; 113.0238; 101.0243	$C_{12}H_{22}0_{11}$	Laminariaceae	2.530	Polysaccharide (algal polysaccharide; Laminariaceae source)
4	2.01	281.09	281.0888; 129.0206; 113.0244; 111.0081; 112.0170	$C_{10}H_{18}0_9$	N.i	1.997	Not identified
5	2.05	317.06	317.0581; 225.0083; 206.9950; 152.9859; 134.9749; 106.9904; 184.9887; 138.9918; 148.9916; 122.9784; 127.0403; 125.0249; 101.0251	$C_9H_{18}O_{10}S$	[6-(2;3-dihydroxypropoxy)-3;4,5-trihydroxyoxan-2-yl]methanesulfonic acid	0.34	Sugar derivative (sulfonated glycoside)
6	2,56	523.13	523.1231; 221.055; 271.0486; 241.0282; 223.0233; 191.0265; 211.0247; 191.0574; 151.009; 125.0245; 107.0120	$C_{20}H_{28}O_{16}$	Jbir-101	1.482	Alkaloid/heterocyclic compound
7	2.89	367.05	367.0453; 271.0483; 241.0363; 223.0260; 197.04.70; 211.0254; 183.0305; 188.0087; 124.0167; 168.0145; 151.0043; 123.0098; 125.0244; 107.0138; 113.0234; 101.0239	$C_{13}H_{16}O_{10}$	Beta-Glucogallin	1.455	Phenolic glycoside (hydrolysable tannin)
8	3.08	359.10	359.1000; 197.0457; 182.0223; 166.9989; 181.0142; 153.0558; 138.0323; 123.0087; 123.0458; 152.0494	$C_{15}H_{20}O_{10}$	Glucosyringic acid	1.199	Phenolic glycoside

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
9	3.80	329.09	329.0892; 167.03.51; 125.0240; 123.0457	$C_{14}H_{18}O9$	3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl 4-hydroxy-3-methoxybenzoate	0.933	Phenolic glycoside
10	3.89	461.13	461.1323; 167.0351; 125.0239; 123.0444; 163.0418	$C_{19}H_{26}O13$	Sacharumoside C	1.580	Phenolic glycoside
11	4.42	443.19	443.1949; 237.1512; 219.1403; 189.1285; 171.1185; 161.0461; 143.0364; 119.0355; 101.0248; 113.0247	$C_{21}H_{32}0_{10}$	Penstemide	3.717	Phenolic glycoside
12	4.75	539.14	539.1465; 466.1274; 449.1135	$C_{31}H_{20}N_6O_4$	N.i	2.258	Not identified
13	4.76	609.15	609.1494; 447.0970; 343.0480; 301.0361; 299.0216; 271.0268	$C_{27}H_{30}O_{16}$	Petiolaroside	4.90	Phenolic glycoside
14	4.76	437.12	-	$C_{18}H_{26}O_{10}$	N.i	5.0	Not identified
15	4.80	401.15	401.1470; 269.1030; 193.0503; 178.0286; 125.0235; 161.0459; 159.0311; 113.0241; 101.0245	$C_{18}H_{26}0_{10}$	Icaraside F2	0.361	Flavonoid glycoside (prenylated)
16	4.95	555.21	555.2123; 337.2005; 511.2227; 457.2293; 257.2019; 205.1777	$C_{26}H_{36}O_{13}$	Syveroside B	3.322	Phenolic glycoside
17	4.97	519.25	519.2464; 387.20.53; 191.0587; 161.0480; 143.0355; 101.0246; 149.0467; 113.0262	$C_{24}H_{40}O_{12}$	Platanionoside	3.395	Phenolic glycoside
18	4.98	421.17	421.1652; 277.1202; 146.0818; 255.0893; 217.1209	$C_{17}H_{30}N_4O_4S$	9-[3-isopropyl-1,2,4-oxadiazol-5-yl]-5-(propylsulfonyl)-1,5-diazacycloundecan-2-one	2.959	Heterocyclic compound (oxadiazole derivative)
19	5.00	465.11	465.1082; 313.0958; 275.0558; 259.0607; 217.0607; 217.0516; 151.04.03; 125.0245; 151.0042	$C_{21}H_{22}O_{12}$	Isoglucodistylin	1.238	Stilbene glycoside
20	5.02	553.21	553;2052; 432.1125; 312.0721; 201.0726; 292.0025; 205.1231	$C_{24}H_{38}O_{12}$	N.i	1.086	Not identified

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
21	5.04	338.25	338.2463; 224.1771; 129.1026; 112.0757	$C_{18}H_{33}N_30_3$	3,6,9-tris(2-methylpropyl)-1,4,7-triazanane-2,5,8-trione	2.077	Alkaloid derivative (cyclic tripeptide-like)
22	5.28	573.22	573.2216; 555.2108; 529.2306; 482.1780; 375.1507; 331.1420; 191.0577; 149.0463; 131.0375; 101.0254; 151.0751	$C_{26}H_{38}O_{14}$	Yadanzigan	4.770	Alkaloid / limonoid-type terpenoid
23	5.27	615.10	615.1020; 462.0923; 313.0584; 301.0359; 300.0280; 169.0157	$C_{28}H_{24}O_{16}$	2"-O-Galloylhyperin	5.1	Phenolic glycoside (galloylated flavonoid)
24	5.31	585.09	585.0914; 439.0318; 421.0216; 313.0011; 285.0048; 259.0256; 178.9980	$C_{27}H_{22}O_{15}$	Quercetin 3-(2"-Galloyl-Alpha-L-Arabinopyranoside)	4.45	Flavonoid glycoside (galloylated quercetin derivative)
25	5.32	569.10	569.23099; 481.0927; 463.0769; 417.0878; 399.0781; 301.0235; 355.0500; 319.0284; 151.0032; 192.9975; 125.0243; 329.0713; 167.0174	$C_{24}H_{26}O_{14}S$	N.i	3.687	Not identified

Note: Peak no. = sequential number assigned to each detected compound; Rt = retention time (minutes) indicating the time taken for a compound to elute from the chromatographic column; $[M–H]^-$ Observed = deprotonated molecular ion detected in negative ion mode; Product Ions (MS/MS) = fragment ions obtained after tandem mass spectrometry used for compound identification; Empirical Formula = molecular formula of the detected compound; Putative Name = tentatively identified compound based on mass spectral data and database comparison; Ppm (error) = mass accuracy expressed as parts per million, indicating the difference between observed and theoretical mass; Chemical Class = classification of compounds based on their chemical structure or functional group.

8.3.3.3. Compounds identified in M. zeyheri Nuts.

The Mpumalanga nut profile is flavonoid-rich and reads as a quercetin–catechin profile with moderate oligomerization (Table 8.7). Free quercetin (Rt 5.75 min, 301.03 m/z) alongside its glycosides isoquercitrin (quercetin-3-O-glucoside; Rt 5.81 min, 463.09 m/z) and a rutin isomer (quercetin-3-O-rutinoside; Rt 5.90 min, 609.14 m/z) known for their antioxidant and anti-inflammatory activity relevant to vascular protection, insulin sensitivity and neuroprotection (Guo et al., 2013; Li, X. et al., 2016). Glycosylation improves quercetin's aqueous solubility and intestinal uptake via lactase-phlorizin hydrolase and SGLT1-linked pathways (Day et al., 2003), which helps explain the prominence of isoquercitrin/rutin in functional foods such as *M. zeyheri*. In Sapotaceae, quercetin glycosides are frequently reported in *Chrysophyllum albidum* Nut and fibre extracts (Muhammad and Abubakar, 2016) and in *Mimusops elengi (Srivastava et al., 2024)*, where they are associated with traditional uses for oral and gastrointestinal complaints. From a food perspective, quercetin and its glucosides retard lipid peroxidation and can improve the shelf-life of nut flours or expressed Nut oils, supporting "clean-label" presser.

The Mpumalanga nuts also show catechin (Rt 6.10 min, 289.07 m/z) and an epicatechin/epigallocatechin proxy (Rt 6.20 min, 305.07 m/z) together with a procyanidin dimer (Rt 6.02, 577.13). Monomeric flavan-3-ols (catechin/epicatechin) provide rapid antioxidant quenching and endothelial nitric-oxide–mediated benefits; B-type procyanidin dimers extend these effects through inhibition of LDL oxidation and modulation of gut microbiota-derived phenyl-γ-valerolactones, compounds implicated in cardiometabolic risk reduction (Mena et al., 2019; Wiese et al., 2015) (Corti et al., 2009; Ottaviani et al., 2016). Comparable flavan-3-ol signatures have been documented in edible Sapotaceae such as *Manilkara zapota* Nuts (Bangar et al., 2022; Kumar Sahu et al., 2019) and *M. elengi* kernels (Chakradhari et al., 2019). Mpumalanga nuts present a quercetin glycoside-rich spectrum and low-DP procyanidin, which fits applications where bitterness/astringency must be controlled (Hong et al., 2006) (e.g., fortified nut porridges, extruded grain-nut blends), while still delivering potent radical-scavenging capacity and mild antimicrobial action useful for extending product freshness (Monagas et al., 2009). In traditional contexts across the Sapotaceae family, astringent Nut or bark decoctions have been used for diarrhoea, oral ailments and wound care uses that mechanistically align with the protein-precipitating and antimicrobial properties of catechins and quercetin glycosides (Bano and Ahmed, 2017; Bashir, 2019; Gam et al., 2024b).

By contrast, the Limpopo nuts present a tannin-dense fingerprint with higher oligomerization and a striking acylated anthocyanin. A hallmark proanthocyanidin trimer, specifically gallocatechin-(4α→8)-gallocatechin-(4α→8)-gallocatechin (Rt 3.02 min, 913.19 m/z), together with a dimeric feature at 577.14 m/z (identified as endotelon), indicates an extensive condensed-tannin spectrum. Higher-DP proanthocyanidins have lower immediate bioavailability but stronger metal-chelating, protein-binding and oil-stabilizing actions, which are advantageous for protecting Nut oils and nut flours against rancidity and for inhibiting spoilage enzymes (Friedman and Jürgens, 2000; Santos-Buelga and Scalbert, 2000; Vazquez-Flores et al., 2018). Health-wise, these polymers still contribute via colonic biotransformation to phenolic metabolites that improve endothelial function and glycaemic control (Álvarez-Cilleros et al., 2018). Within Sapotaceae, Nut and bark fractions of *M. elengi (Kadam et al., 2012b)* and *Manilkara* spp. (Alamgir et al., 2024) are repeatedly described as rich in condensed tannins used traditionally as astringents and for oral care, consistent with the Limpopo nut chemistry.

The Limpopo profile is further distinguished by an intensely acylated anthocyanin (Table 8.8), cyanidin 3-(6"-p-coumarylglucoside)-5-4"',6"'-dimalonylglucoside (Rt 5.45 min, 927.19 m/z). Acylation with *p*-coumaric and malonyl groups increases colour stability against heat, light and pH shifts, which is crucial if the Nut fraction is repurposed as a natural colorant in baked goods or beverages (Andersen and Jordheim, 2013; Yañez-Apam et al., 2023). Cyanidin derivatives are associated with neuroprotective, anti-inflammatory and vasodilatory effects, and epidemiology links higher anthocyanin intake to reduced hypertension and type-2 diabetes risk (Cassidy et al., 2013; Mattioli et al., 2020). Reports of acylated cyanidins exist in several tropical fruits; although less common in Sapotaceae Nuts, pigmented tissues in *Chrysophyllum* spp. (Luo et al., 2002) and *Manilkara* fibres (Bano and Ahmed, 2017) contain cyanidin-type anthocyanins that underpin traditional uses as tonics and coloring agents. In community food systems, compounds such as anthocyanin offers dual value, colour plus antioxidant protection, supporting clean-label product development for small bakeries and household-level with cereal–nut mixes.

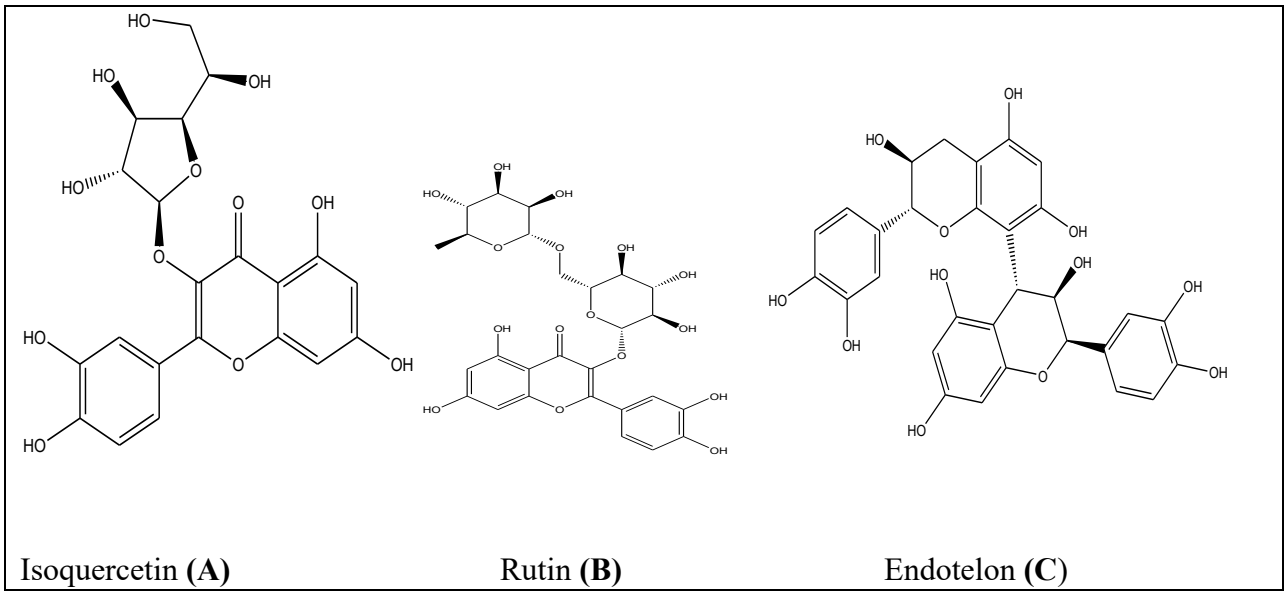


Figure 8. 4:Compounds identified in the Nuts of *M. zeyheri* from Vhembe district, Limpopo province (C) and Ehlanzeni district, Mpumalanga province (A,B).

Table 8.7: Characterization and Identification of secondary metabolites present in *M. zeyheri* fruit nuts collected from Ehlanzeni, Mpumalanga.

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
1	5.75	301.03	301.0348; 179.0201; 151.0049; 559.0 1 133.0 0.41; 193.0 0.38;77.0 0.33	$C_{15}H_{10}O_7$	Quercetin	0.95	Flavonoid
2	5.81	463.09	463.0887; 301.0345; 179.0206	$C_{21}H_{20}O_{12}$	Isoquercitrin	1.85	Flavonoid glycoside
2	5.9	609.14	609.1467; 301.0349; 179.0203	$C_{27}H_{30}O_{16}$	Rutin isomer	2.44	Flavonoid glycoside
4	6.02	577.13	577.1350; 289.0712; 245.0453	$C_{30}H_{26}O_{12}$	Procyanidin dimer (B-type)	1.32	Flavonoid oligomer
5	6.1	289.07	289.0713; 245.0451; 205.0342	$C_{15}H_{14}O_6$	Catechin	0.87	Flavan-3-ol
6	6.2	305.07	305.0669; 179.0345; 125.0243	$C_{15}H_{14}O7$	Epigallocatechin	1.2	Flavan-3-ol
7	6.34	633.16	633.1571; 301.0350; 151.0052	$C_{28}H_{34}O_{17}$	Quercetin-3-O-galactoside-hexoside	2.03	Flavonoid glycoside
8	6.42	447.09	447.0932; 285.0404; 179.0201	$C_{21}H_{20}O_{11}$	Kaempferol-3-O-glucoside	1.15	Flavonoid glycoside
9	6.5	285.04	285.0400; 151.0048; 133.0287	$C_{15}H_{10}O_6$	Kaempferol	0.98	Flavonoid
10	6.66	431.1	431.0984; 269.0457; 151.0049	$C_{21}H_{20}O_{10}$	Apigenin-7-O-glucoside	1.75	Flavonoid glycoside
11	6.74	271.06	271.0601; 151.0048; 117.0332	$C_{15}H_{10}O_5$	Apigenin	1.02	Flavonoid
12	6.82	611.16	611.1603; 305.0671; 179.0206	$C_{27}H_{30}O_{16}$	Gallocatechin gallate	2.11	Flavan-3-ol gallate
13	6.93	355.1	355.1012; 193.0505; 133.0287	$C_{16}H_{18}O_9$	Chlorogenic acid	1.36	Phenolic acid
14	7.02	353.09	353.0885; 191.0563; 179.0345	$C_{16}H_{18}O_9$	Caffeoylquinic acid	0.95	Phenolic acid
15	7.12	337.09	337.0921; 191.0566; 163.0401	$C_{15}H_{14}O9$	Feruloylquinic acid	1.22	Phenolic acid
16	7.25	609.18	609.1812; 301.0351; 151.0046	$C_{27}H_{30}O_{16}$	Quercetin-3-O-galactoside	2.04	Flavonoid glycoside
17	7.4	447.13	447.1299; 285.0403; 151.0049	$C_{21}H_{20}O_{11}$	Kaempferol-3-O-rutinoside	1.63	Flavonoid glycoside
18	7.55	473.12	473.1221; 311.0710; 151.0048	$C_{21}H_{20}O_{12}$	Isorhamnetin-3-O-glucoside	2.34	Flavonoid glycoside
19	7.7	317.03	317.0301; 151.0045; 119.0342	$C_{15}H_{10}O_8$	Myricetin	0.88	Flavonoid
20	7.82	463.12	463.1231; 301.0347; 179.0202	$C_{21}H_{20}O_{12}$	Quercetin-3-O-glucoside	1.55	Flavonoid glycoside
21	7.95	595.17	595.1712; 285.0400; 151.0049	$C_{27}H_{30}O_{15}$	Kaempferol-3-O-rhamnoside-hexoside	2.47	Flavonoid glycoside
22	8.05	325.09	325.0932; 163.0400; 119.0347	$C_{15}H_{10}O_8$	Coumaroylquinic acid	1.41	Phenolic acid
23	8.15	609.15	609.1503; 301.0348; 179.0201	$C_{27}H_{30}O_{16}$	Quercetin-3-O-arabinoside	2.22	Flavonoid glycoside

Table 8.8:Characterization and Identification of secondary metabolites present in *M. zeyheri* nuts collected from Vhembe, Limpopo.

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
1	1.02	704.81	533.1750; 353.1125; 191.0563	$C_{20}H_{30}N_4O_{13}$	Boc-Asp-Ala-Asp-Asp-OH	0.196	Peptide
2	1.76	377.09	377.08.73; 221.0882; 179.0572; 161.0472; 119.0355; 113.0342; 101.0243	$C_{18}H_{18}O_9$	6-[[{(3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl}oxymethyl]furo[3,2-g]chromen-7-one	0.836	Phenolic / coumarin glycoside
3	3.02	913.19	305.01; 611.0; 575.0; 695.0; 739.00; 707.00	$C_{45}H_{38}O_{21}$	Gallocatechin-(4alpha->8)-gallocatechin-(4alpha->8)-gallocatechin	2.077	Flavonoid (proanthocyanidin trimer)
4	3.03	533.17	533.1749; 191.0565; 173.0458; 127.0396	$C_{19}H_{34}O_{17}$	2-O-(6-O-glycero-manno-Heptopyranosyl-glucopyranosyl)glucopyranose	0.289	Sugar (trisaccharide)
5	3.03	663.20	663.2024; 321.0847; 303.0730; 217.0359; 175.0253; 169.0164; 157.0138; 103.0409	$C_{24}H_{49}O_{21}$	3-[(3,4-dihydroxy-6-(hydroxymethyl)-5-[(3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy]oxan-2-yl)oxy]-4,5-dihydroxy-6-[(4,5,6-trihydroxy-2-methyloxan-3-yl)oxy]oxane-2-carboxylic acid	0.653	Sugar derivative (acidic oligosaccharide)
6	3.80	191.06	191.0566; 171.0263; 137.0246; 127.0405; 109.0297; 109.0213	$C_7H_{12}O_6$	Kinic acid	0.184	Organic acid (cyclitol derivative)
7	3.80	281.09	281.0888; 129.0206; 113.0244; 111.0081; 112.0170	$C_{10}H_{18}O_9$	N.i	0.224	Not identified
8	3.80	465.09	465.0906; 331.0883; 271.0476; 241.0352;	$C_{17}H_{22}O_{15}$	(3R,4S,5R)-4,5-bis(carboxymethyl)-3,4,5-	0.379	Organic acid derivative

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
			211.0254; 193.0141; 169.0146; 169.0079		trihydroxy-3-[(1R)-1-hydroxy-2-prop-2-enoyloxyethyl]-6-oxooctanedioic acid		
9	4.40	577.14	577.1376; 407.0766; 289.0732	$C_{30}H_{26}O_{12}$	Endotelon	1.733	Flavonoid oligomer (proanthocyanidins)
10	4.80	519.25	519.2484; 387.2053; 191.05.67; 161.0460; 143.0355; 101.0246; 149.0487; 113.0262	$C_{24}H_{40}O_{12}$	N.i	3.295	Not identified
11	4.80	401.15	401.1470; 269.1030; 193.0503; 161.0459; 178.0286; 125.0235; 159.0311; 113.0241; 101.0245	$C_{18}H_{26}O_{10}$	Icariside F2	0.361	Flavonoid glycoside (prenylated)
12	4.85	539.15	539.1465; 566.1274; 449.1135	$C_{31}H_{20}N_6O_4$	N.i	2.25	Not identified
13	4.85	443.19	443.1940; 237.1512; 161.0461; 143.0364; 119.0355; 113.0247; 219.1403; 189.1285; 171.1185	$C_{21}H_{32}O_{10}$	Dihydrophaselic Acid 4'-O-Beta-D-Glucopyranoside	3.717	Phenolic glycoside
14	4.89	461.13	461.1323; 167.0351; 163.0418; 125.0239; 123.044	$C_{19}H_{26}O_{13}$	Saccharumoside C	2.342	Phenolic glycoside
15	4.99	553.21	553.2032; 433.1125; 312.0721; 271.6810; 222.0516; 227.0715; 201.0726; 292.6225; 265.1231; 133.0216; 132.0841; 232.0872; 191.0880;	$C_{24}H_{38}0_{12}$	N.i	1.086	Not identified

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
			142.0432;131.0352; 101.0351				
16	5.07	479.08	479.0853; 317.0309; 316.02.36; 288.03.8; 270.0176; 214.0275; 287.0213; 2710262; 178.9992; 151.0058	$C_{21}H_{20}O_{13}$	Isomytricin	3.972	Polyketide/antibiotic (macrolide-type)
17	5.16	615.23	615.2322; 407.1723; 359.1529; 229.0525; 195.0644; 165.0549	C28H40O15	Laciniatoside VI	2.763	Iridoid glycoside
18	5.34	617.12		C28H26016	Taxillusin	4.21	Flavonoid glycoside
19	5.45	927.19	927.1885; 462.0902; 301.0348; 300.0280	C42H40O24	Cyanidin 3-(6"-p-coumarylglucoside)-5-4"';6"'-dimalonylglucoside)	1.425	Anthocyanin (glycosylated; acylated)
20	5.54	765.10	765.0983; 463.0931; 300.9999	C36H22N4016	7-[2-carboxy-5;6-dihydroxy-1H-indol-4-yl]-4-(2-carboxy-5;6-dihydroxy-1H-indol-7-yl)-5;5,6,6-tetrahydroxy-1H,1'H-[4,7'-biindole]-2,2'-dicarboxylic acid	2.897	Alkaloid derivative (indole-based)
21	5.58	509.23	509.2260; 463.2228; 331.1805; 161.0463; 119.03.52; 101.02.47; 317.0294; 316.0237; 149.04.58; 113.0242	C22H38O13	Hexosyl-hexosyl-geraniol	2.955	Terpenoid glycoside (monoterpenoid glycoside)

Note: Peak no. = sequential number assigned to each detected compound; Rt = retention time (minutes) indicating the time taken for a compound to elute from the chromatographic column; $[M–H]^-$ Observed = deprotonated molecular ion detected in negative ion mode; Product Ions (MS/MS) = fragment ions obtained after tandem mass spectrometry used for compound identification; Empirical Formula = molecular formula of the detected compound; Putative Name = tentatively identified compound based on mass spectral data and database comparison; Ppm (error) = mass accuracy expressed as parts per million, indicating the difference between observed and theoretical mass; Chemical Class = classification of compounds based on their chemical structure or functional group.

8.3.3.4. Compounds identified in *M. zeyheri* leaves

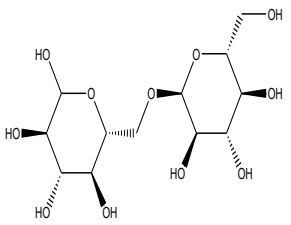
Leaves from Mpumalanga display a broad mix of small sugars, phenolics, and triterpenoids that together suggest strong nutritional and medicinal value (Table 8.9). Myricitrin (Rt 5.37 min, 499.07 m/z) , a rhamnoside of myricetin also identified in *Chrysophyllum albidum (Adebayo, Abiodun H et al., 2011)* and *Myrica rubra* (Song et al., 2021). It provides strong antioxidant, anti-inflammatory, and antidiabetic effects by improving glucose metabolism and reducing oxidative stress (Adebayo, Abiodun H et al., 2011). Hyperin (Rt 5.50 min, 463.09 m/z), a quercetin glycoside reported in *Manilkara zapota (Gam et al., 2024b)* and *Prunus domestica* (Treutter et al., 2012) with neuroprotective and cardioprotective properties. Kaempferol rhamnoside (Rt 6.71 min, 447.01 m/z) is also reported in *Chrysophyllum cainito*, where it contributes vascular protection and anti-cancer potential (Luo et al., 2002). Identified early eluting carbohydrates such as 1-O-β-D-glucopyranosyl-4-glyceromanno-heptopyranose (Rt 0.74; 371.12 m/z) and brachiose (Rt 0.85; 341.11 m/z) serve not only as energy reserves but also as precursors for glycosylation, a process that increases the solubility and stability of many secondary metabolites (Tiwari et al., 2016). Quinic acid (Rt 0.88; 191.06 m/z), identified also in Mpumalanga, is a clohexanecarboxylic acid naturally found in various medicinal plants and fruits that exhibits diverse biological activities, plays a crucial role in shikimate-pathway metabolism, and acid biosynthesis, including tryptophan, phenylalanine, and tyrosine (Benali et al., 2024; Ercan and Doğru, 2022). Human gastrointestinal microflora metabolize quinic acid through this pathway, converting it to hippuric acid and producing efficacious antioxidant amino acids and vitamins (Pero, 2010). The detection of a coumarin-type phenolic (Rt 0.86; 377.09 m/z) and a jasmonate-like oxylipin (Rt 4.65; 387.17 m/z) points to leaf chemistry with both oxidative defence and pest resistance, traits that can enhance the durability of harvested material and add to its dietary benefits.

Additionally, Mpumalanga leaves exhibit a strong presence of triterpenoid saponins and flavanol glycosides. Myricetin 3,3′-digalactoside (Rt 4.93; 641.14 m/z) and Icariside F2 (Rt 4.80; 401.15 m/z), which provide well-documented antioxidant and anti-inflammatory activity. Cucurbitacin C (Rt 6.82; 559.32 m/z), platycodon saponin 2 (Rt 6.74; 843.44 m/z), and bellericagenin B (Rt 7.00; 519.33 m/z) further show the strong triterpenoid and saponin signature typical of medicinal leaves used as tonics or expectorants (Petrović et al., 2022). These compounds, familiar from species such as *Platycodon grandifloras (Ji et al., 2020)* and *Terminalia bellerica (Kadian et al., 2014)*, support

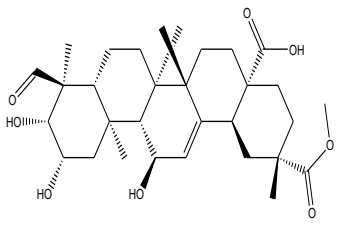
both traditional uses and potential nutraceutical or cosmetic applications if safety and dosage are established.

In contrast, leaves from Limpopo are characterized by high levels of galloyl phenolics and oligomeric proanthocyanidins (OPCs), associated with vascular and antioxidant benefits (Table 8.10). Glucosyringic acid (Rt 3.08 min, 359.10 m/z), previously identified in *Syzygium aromaticum* (Fathoni et al., 2017) , is known for its antioxidant and anti-inflammatory effects. Identified B-Glucogallin (Rt 1.59; 367.05 m/z), also reported in *Mimusops elengi* bark (Akhtar et al., 2010) and *Manilkara hexandra* (Worowounga et al., 2022) is greatly recognized for hepatoprotective, anti-diarrhoeal, and antimicrobial actions. Syringic acid β-glucopyranosyl ester (Rt 3.93; 359.10 m/z) is identified, indicating strong hydrolysable tannin chemistry that enhances astringency and natural preservative qualities (Chen et al., 2023). OPCs such as endotelon (Rt 1.42 min; 577.14 m/z), theasinensin A (Rt 4.02; 913.15 m/z), and several galloylated dimers and trimers (Rt 4.06–4.39 min; 745.15–865.20 m/z) reinforce this antioxidant profile and offer antimicrobial potential for dried-leaf products or herbal teas (Alfke et al., 2021). Phenolic glycosides, including saccharumoside C (Rt 4.20 min; 461.13 m/z), contribute additional antioxidant and antiproliferative effects.

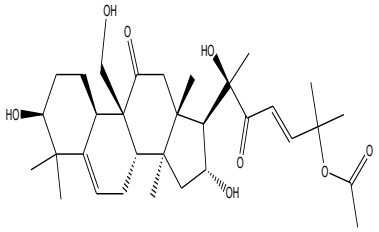
Flavonol glycosides such as isoglucosidistylin (Rt 5.02 min; 465.11 m/z), laricitrin 3-galactoside, and patuletin-3-O-β-D-glucoside complement the tannin chemistry with anti-inflammatory and photoprotective properties (de Souza et al., 2016). Acinospesigenin C (Rt 6.68 min; 545.31 m/z) and Peniciside (Rt 6.86 min; 677.43 m/z) point to triterpenoid saponins that, while less diverse than in Mpumalanga, still provide surfactant and antimicrobial potential. Overall, Limpopo leaves show a stronger emphasis on hydrolysable tannins and condensed proanthocyanidins, supporting their use as astringent infusions and highlighting their value for antioxidant nutraceuticals.



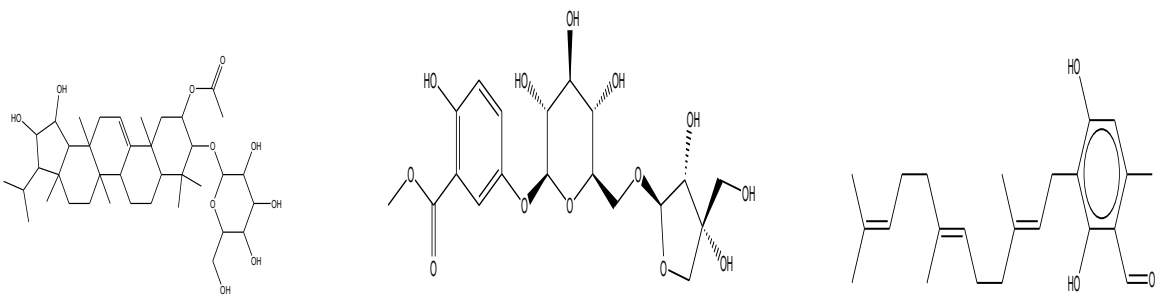
Brachiose **(A)**



Acinospesigenin C **(B)**



Cucurbitacin C **(C)**



Peniciside **(D)** saccharumoside C **(E)** isoglucosidistylin **(F)**

Figure 8.5: Compounds identified in the fruit fibre of *M. zeyheri* from Vhembe district, Limpopo province (D,E,F) and Ehlanzeni district ,Mpumalanga province (A,B,C)

Table 8. 9: Characterization and Identification of secondary metabolites present in *M. zeyheri* Leaves collected from Ehlanzeni, Mpumalanga.

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
1	0.74	371.12	371.1218; 325.1157; 191.0565; 163.0619; 161.0460; 143.0353; 113.0249; 145.0500; 1990356; 101.0244	$C_{13}H_{24}O_{12}$	1-O-beta-D-glucopyranosyl-4-glycero-alpha-D-manno-heptopyranose	2.661	Sugar (disaccharide)
2	0.85	341.11	341.1104; 163.0608; 131.0351; 113.0244; 119.0357; 101.0241	$C_{12}H_{22}O_{11}$	Brachiose	0.358	Sugar (trisaccharide)
3	0.86	377.09	377.0874; 221.0674; 179.0575; 161.0463; 125.0237; 119.0341; 101.0243; 113.0245; 149.0473	$C_{18}H_{18}O_9$	6-{[(3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl}furo[3,2-g]chromen-7-one	1.974	Phenolic
4	0.88	191.06	191.0566; 127.0390; 109.0294; 108.0222; 11.0454	$C_7H_{12}O_6$	Kinic acid	2.341	Organic acid
5	4.65	387.17	387.1676; 207.1012; 163.1128; 192.0439; 177.0185; 199.0354; 101.0243	$C_{18}H_{28}O_9$	2-[3-oxo-2-(5-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}pent-2-en-1-yl)cyclopentyl]acetic acid		Organic acid
6	4.65	609.28	609.2789; 507.2836; 477.2351; 471.2637; 447.2612; 327.2187; 309.2112; 253.0933; 221.0672; 191.0559; 149.0462; 131.0352; 113.0237; 179.0574; 161.0459; 143.0354; 125.0243; 101.0240; 447.2258	$C_{27}H_{46}O_{15}$	[(2S)-1-pentanoyloxy-3-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-[[(2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxypropan-2-yl] heptanoate	2.828	Glycolipid
7	6.82	559.32	559.3296; 541.3211; 515.3425; 49.3998; 481.29.60; 289.2833	$C_{32}H_{48}O_8$	Cucurbitacin C	4.89	Triterpenoid

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
8	6.39	849.36	849.3607; 817.3335; 771.2904; 803.3212	$C_{35}H_{62}O_{23}$	6-O-dodecanoylstachyose	0.088	Sugar (tetrasaccharide derivative)
9	6.74	843.44	843.4432; 797.4377; 293.0894; 233.0671; 191.0560; 149.0468; 125.0224; 503.3402	$C42H_{68}O_{17}$	Platycodon saponin 2	1.342	Triterpenoid
10	7.0	519.33	519.3348; 501.3243; 483.3137; 455.3168; 437.3088; 435.2903; 417.2809; 389.2851; 381.2790; 363; 2693; 337.2543; 409.3131; 393.2814; 391.3025; 373.2914; 357.2603; 441.3395; 425.3070; 407.2988; 379.3030; 475.3444; 459.3115; 457.3344; 439.3241	$C_{20}H_{48}O7$	Bellericagenin B	3.478	Triterpenoid
11	7.08	365.21	365.2114; 293.2127; 211.1345; 171.1031; 139.1123; 127.1125	$C_{24}H_{30}O_3$	Drospirenone	1.417	Steroid
12	7.42	547.33	547.3301; 501.3244; 483.3140; 481.2954; 465.3025; 455.3178; 409.3101; 471.3125; 453.3029; 435.2921; 457.3349; 439.3244; 421.3159; 391.3036; 373.2909	$C_{31}H_{48}O_8$	(2S,8S,9R,10R,13R,14S,16R,17R)-17-[(2R,5R)-2,6-dihydroxy-5-methoxy-6-methyl-3-oxoheptan-2-yl]-2,16-dihydroxy-4,4,9,13,14-pentamethyl-2,7,8,10,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthrene-3,11-dione	2.345	Steroid
13	7.44	545.31	545.3140; 527.3944; 499.3073; 439.3220; 465.3047; 449.2724; 437.3087; 451.2897; 433.2750; 423.943; 407.2989	$C_{31}H_{46}O_8$	Narizoside	4.876	Triterpenoid
14	7.47	529.22	529.3196; 511.3085; 467.3201; 391.3040; 435.2921; 407.2994	$C_{31}H_{46}O_7$	Heteronemin Acetate	4.026	Terpenoid

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
15	7.47		768.5274; 733.3848; 731.3695; 550.4856; 326.2710; 279.2349; 271.2286; 225.2236; 476.3262; 179.0563; 161.0453; 143.0355; 119.034; 101.0237; 149.0459; 131.0356; 113.0239	$C_{42}H_{75}NO_{11}$	20-[3-[4-[6-[(4R)-4-carboxy-2,7-dioxooctoxy]-5-oxohexoxy]butoxy]propylamino]-20-oxoicosanoic acid	2.723	Fatty acid

Note: Peak no. = sequential number assigned to each detected compound; Rt = retention time (minutes) indicating the time taken for a compound to elute from the chromatographic column; $[M–H]^-$ Observed = deprotonated molecular ion detected in negative ion mode; Product Ions (MS/MS) = fragment ions obtained after tandem mass spectrometry used for compound identification; Empirical Formula = molecular formula of the detected compound; Putative Name = tentatively identified compound based on mass spectral data and database comparison; Ppm (error) = mass accuracy expressed as parts per million, indicating the difference between observed and theoretical mass; Chemical Class = classification of compounds based on their chemical structure or functional group.

Table 8. 10:Characterization and identification of secondary metabolites present in *M. zeyheri* Leaves collected from Vhembe, Limpopo Province

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
1	0.84	371.12	371.1210; 325.1157; 179.0554; 161.0456; 143.0356; 119.0351; 101.0251; 113.0240; 163.0619	$C_{13}H_{24}O_{12}$	**1-O-beta-D-glucopyranosyl-L-glycero-alpha-D-manno-heptopyranose**	2.539	Disaccharide (heptose–glucose)
2	0.86	533.17	533.1750; 353.1125; 191.0563	C20H30N4O13	Boc-Asp-Ala-Asp-Asp-OH	1.120	Protected oligopeptide (
3	0.92	377.09	3.77.0872; 221.0882; 179.0572; 161.0472; 119.0055; 101.0242; 112.0242	$C_{18}H_{18}O_9$	Tris(oxiranylmethyl) benzene-1,3,5-tricarboxylate	5.0	Epoxide-functional triester (glycidyl ester; epoxy monomer)
4	0.92	533.17	533.1749; 191.0565; 173.0458; 127.0396	$C_{19}H_{34}O_{17}$	2-O-(6-O-glycero-manno-Heptopyranosyl-glucopyranosyl)glucopyranose	1.621	Trisaccharide
5	1.01	191.06	191.0566; 171.0293; 137.0246; 127.0405; 109.0297; 108.0213	$C_7H_{12}O_6$	Kinic acid	1.538	carboxylic acid
6	1.11	345.08	345.0842; 183.0309; 168.0066; 166.9986; 139.0404; 124.0170; 107.0147; 138.0320; 123.0094; 137.0255	$C_{12}H_{18}O_{10}$	4-hydroxy-3-methoxy-5-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}benzoic acid	3.681	glycoside
7	1.11	345.08	345.0842; 183.0309; 168.0066; 166.9986; 139.0404; 124.0170; 107.0147; 138.0320; 123.0094; 137.0255	$C_{12}H_{18}O_{10}$	Methyl Gallate 3-O-Beta-D-Glucopyranoside	3.681	glycoside

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
8	1.42	577.14	577.1376; 407.0766; 289.0732	$C_{30}H_{26}O_{12}$	Endotelon	1.733	Oligomeric proanthocyanidins (OPCs)
9	1.42	577.14	577.1376; 407.0766; 289.0732	$C_{30}H_{26}O_{12}$	Laricitrin 3-Galactoside	1.733	glycoside
10	1.42	577.14	577.1376; 407.0766; 289.0732	$C_{30}H_{26}O_{12}$	Patuletin 3-O-Beta-D-Glucoside	1.733	glycoside
11	1.59	367.05	367.0453; 271.0463; 241.0363; 223.0260; 197.0470; 211.0254; 183.0305; 188.0067; 124.0167; 169.0145; 151.0043; 123.0088; 125.0244; 107.0136; 113.0234; 101.0239	$C_{13}H_{16}O_{10}$	Beta-Glucogallin	1.455	Tannin
12	3.61	583.26	-	$C_{25}H_{44}O_{15}$	Lunaroside	2.031	glycoside
13	3.93	359.10	359.1000; 197.0457; 182.0223; 166.9989; 181.0142; 153.0556; 138.0323; 123.0087; 123.0458; 152.0494	$C_{15}H_{20}O_{10}$	lucosyringic Acid	1.199	glycoside
14	3.93	359.10	359.1000; 197.0457; 182.0223; 166.9989; 181.0142; 153.0556; 138.0323; 123.0087; 123.0458; 152.0494	$C_{15}H_{20}O_{10}$	Syringic Acid Beta-Glucopyranosyl Ester	1.199	Phenolic acid glycosyl ester
15	4.02	913.15	-	$C_{44}H_{34}O_{22}$	Theasinensin A	4.523	Dimeric flavan-3-ol (catechin dimer)
16	4.06	745.15	745.1447; 593.1360; 423.0722; 407.0780; 289.0722; 271.0631;	$C_{37}H_{30}O_{17}$	8-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2H-1-benzopyran-4-yl]-5,7-	3.191	Galloylated proanthocyanidin dimer

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
			177.0193; 124.0235; 169.0149		dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate		
17	4.06	745.15	745.1447; 593.1360; 423.0722; 407.0780; 289.0722; 271.0631; 177.0193; 124.0235; 169.0149	$C_{37}H_{30}O_{17}$	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-yl]-3,4-dihydro-2H-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate	3.191	Galloylated proanthocyanidin dimer
18	4.09	329.09	329.0892; 167.0351; 125.0240; 123.0457	$C_{14}H_{18}O_9$	3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl 4-hydroxy-3-methoxybenzoate	0.993	glucoside
19	4.20	461.13	461.1323; 167.0351; 125.0239; 123.0444; 163.0418	$C_{19}H_{26}0_{13}$	Saccharumoside C	1.560	glycoside
20	4.39	865.20	-	$C_{45}H_{38}O_{18}$	4-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2H-1-benzopyran-8-yl]-8-[3,5,7-trihydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-yl]-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol	4.667	Proanthocyanidin trimer (flavan-3-ol oligomer)
21	4.39	865.20	-	$C_{45}H_{38}O_{18}$	2-(3,4-dihydroxyphenyl)-8-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2H-1-benzopyran-4-yl]-4-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2H-1-	4.66	Proanthocyanidin trimer

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
					benzopyran-8-yl]-3,4-dihydro-2H-1-benzopyran-3,5,7-triol		
22	4.39	865.2O	-	$C_{45}H_{38}O_{18}$	2-(3,4-dihydroxyphenyl)-8-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2H-1-benzopyran-4-yl]-4-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2H-1-benzopyran-8-yl]-3,4-dihydro-2H-1-benzopyran-3,5,7-triol	4.66	Proanthocyanidin trimer
23	4.71	387.17	387.1677; 207.1019; 163/1131; 113.0239	$C_{18}H_{28}O_9$	2-[3-oxo-2-(5-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}pent-2-en-1-yl)cyclopentyl]acetic acid	2.451	glucoside
24	4.71	37.17	387.1677; 207.1019; 163/1131; 113.0239	$C_{18}H_{28}O_9$	2-[3-oxo-2-(5-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}pent-1-en-1-yl)cyclopentyl]acetic acid	2.451	Oxylipin
25	4.71	387.17	387.1677; 207.1019; 163/1131; 113.0239	$C_{18}H_{28}O_9$	(3r,7s)-12-oxic-ja	2.451	Jasmonate (plant hormone; oxylipin)
26	4.79	437.12	437.1238	C18H26O10	N.i	0.913	
27	4.80	401.15	401.1470; 269.1030; 193.0503; 178.0286; 125.0235; 161.0459; 159.0311; 113.0241; 101.0245	C18H26O10	Icariside F2	0.361	flavonoid
28	4.93	641.14	641.1401; 479.0823; 3170321; 316.0237; 178.9991	$C_{27}H_{30}O_{18}$	Myricetin 3,3'-Digalactoside	3.068	diglycoside
29	5.02	553.21	-	$C_{24}H_{38}O_{12}$	N.i	1.086	

Peak no	Rt	[M-H]- Observed	Product Ions (MS/MS)	Empirical Formula	Putative Name	Ppm (error)	Chemical Class
30	5.02	465.11	465.1062; 313.0958; 275.0558; 259.0807;217.0516; 151.0403; 125.0245; 151.0042	$C_{21}H_{22}O_{12}$	Isoglucosidistylin	1.238	glycoside
31	5.16	615.23	615.2322; 407.1723; 359.1529; 229.0525; 195.0644; 165.0549	$C_{28}H_{40}O_{15}$	Laciniatoside VI	2.763	glycoside
32	5.59	301.00	-	$C_{14}H_6O_8$	Gallogen	0.557	tannin
33	6.01	677.50	677.5005; 659.4895; 451.3325; 338.2448;320.2329; 225.1619; 130.0881	$C_{36}H_{66}N_6O_6$	-	2.138	
34	6.68	545.31	545.3143; 527.3037; 451.2861; 433.2726; 323.2024	$C_{31}H_{46}O_8$	Acinospesigenin C	2.447	Triterpenoid sapogenin (aglycone)
35	6.75	827.45	827.4510	$C_{24}H_{68}O_{16}$	-	1.057	
36	6.86	677.43	677.4274; 349.2007; 261.1118; 233.1167 2;22.0897; 193.0856; 327.2186	$C_{38}H_{62}O_{10}$	Peniciside	3.456	Glycoside

Note: Peak no. = sequential number assigned to each detected compound; Rt = retention time (minutes) indicating the time taken for a compound to elute from the chromatographic column; $[M–H]^-$ Observed = deprotonated molecular ion detected in negative ion mode; Product Ions (MS/MS) = fragment ions obtained after tandem mass spectrometry used for compound identification; Empirical Formula = molecular formula of the detected compound; Putative Name = tentatively identified compound based on mass spectral data and database comparison; Ppm (error) = mass accuracy expressed as parts per million, indicating the difference between observed and theoretical mass; Chemical Class = classification of compounds based on their chemical structure or functional group

8.4. Concluding remarks

This chapter provides the first comprehensive phytochemical and untargeted metabolite profile of *M. zeyheri* plant parts collected from two distinct South African agro-ecological zones. Across all assays, leaves consistently exhibited the highest concentrations of total phenolics, flavonoids, and alkaloids, followed by nuts, pulp, and fibre. The antioxidant potential of *M. zeyheri* is not uniform across its tissues, with Nuts performing best in the radical-scavenging assay and peel and pulp offering stronger protection against lipid oxidation. Provincial differences were also evident, particularly in the Nut and peel extracts, with Mpumalanga material generally outperforming Limpopo. These findings suggest that different plant parts may be suited to different applications, and that provenance plays an important role in determining their chemical value. Ultra-performance liquid chromatography coupled with high-resolution mass spectrometry revealed a diverse suite of secondary metabolites, including flavanol glycosides (e.g., quercetin-3-O-glucoside, rutin, hyperoside), triterpenoids (e.g., lupeol, oleanolic acid, hederagenin), hydrolysable tannins (e.g., β-glucogallin), and phenolic acids, many of which are recognized for antioxidant, antimicrobial, anti-inflammatory, and nutraceutical properties.

Notably, phytochemical patterns were driven more by plant tissue type than by geographical origin, as reflected in the comparable metabolite profiles of Limpopo and Mpumalanga collections. This indicates that *M. zeyheri* maintains a stable secondary metabolism across different environmental conditions, reinforcing its potential as a reliable source of bioactive compounds for food, pharmaceutical, and cosmetic applications. At the same time, subtle regional differences such as the tannin-rich Limpopo pulp and the flavonoid-rich Mpumalanga fibre highlight opportunities for site-specific value-addition and targeted product development. Overall, these findings confirm *M. zeyheri* as a high-value indigenous species with strong prospects for domestication, functional food formulation, and the discovery of natural products. Future work should focus on validating the bioactivities of the identified metabolites through vivo and clinical studies, optimizing sustainable harvesting or cultivation practices, and exploring commercialization pathways that also support local livelihoods and biodiversity conservation.

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CHAPTER NINE
SIGNIFICANCE OF THE FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

9.1. Significance of the study

This study makes a substantial contribution to both academia and society by providing the first comprehensive, multi-dimensional investigation of *M. zeyheri* that spans ethnobotany, ecology, morphology, sensory science, genetics, nutrition and phytochemistry. Academically, the study fills critical knowledge gaps in indigenous fruit tree research by generating novel baseline data on population structure, genetic diversity, nutritional profiles, and bioactive compounds, 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 underutilized 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 thesis advances national priorities in conservation, indigenous food systems, bioeconomic development, and the promotion of African-led scientific research.

9.2. Conclusion

This study presents the most comprehensive multidimensional assessment of *M.zeyheri* to date, integrating ethnobotanical knowledge, ecological analysis, morphological and genetic characterization, nutritional profiling, and phytochemical screening. 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. 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. Phytochemical screening and UPLC-Q-tof-MS profiling further confirm the species' strong biological potential. Leaves consistently contain the highest phenolic, flavonoid, and alkaloid content, while nuts particularly from Mpumalanga exhibit notable antioxidant activity. Distinct compounds such as quercetin glycosides, rutin, β-glucogallin, and diverse hydrolysable tannins demonstrate therapeutic potential aligned with many of the traditional uses documented. Tissue-specific chemical signatures and regional variation reinforce the value of preserving genetic diversity across landscapes. Overall, the thesis 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. The combined evidence supports the advancement of conservation,

domestication, and community-centered utilization pathways that recognize both ecological constraints and socio-economic opportunities.

9.3. Study Limitations

While this study offers a comprehensive and multi-layered assessment of *M. zeyheri*, certain boundaries were inherent to the scope and design of the research. First, the ecological and ethnobotanical assessments were conducted in two provinces selected because they represent distinct ecological zones and culturally diverse communities where the species is abundant. These sites provided sufficient variation to meet the study 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 the purposes of 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 was employed to ensure culturally relevant responses. Fourth, phytochemical analyses were performed on samples collected at a uniform phenological stage to ensure comparability across sites; while seasonal variation may exist, this controlled sampling approach was sufficient for establishing baseline chemical profiles. 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.

9.4. Future Research

- 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.
- 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.

- 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.
- 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.
- 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.
- Expanded sensory and consumer studies:

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

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 *M. zeyheri* as an indigenous resource of ecological, nutritional, medicinal, and economic importance. Policies should prioritize 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 programs, promoting its domestication and cultivation as part of food security interventions. Furthermore, policies must recognize 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.

LIST OF APPENDICES

Appendix 9.1: *Mimusops zeyheri* sond (Milkwood) Fruit Sensory Evaluation Form

Panelist 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	Panelist Comments
Appearance	Color 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 odors	1 (Very Poor) – 9 (Excellent)	
Taste	Sweetness, tartness, bitterness, overall flavor 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 (9-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 Extremely

Appendix 9.2: Plant Authentication Letter

SANBI
Biodiversity for Life
South African National Biodiversity Institute

National Herbarium (PRE)
South African National Biodiversity Institute

Private Bag X101, Pretoria, 0001
South Africa
Tel: +27 12 8435000, Fax: +27 12 8043211
Email: PREherbarium@sanbi.org.za

Ref: Batch 25054C

Plant Identification Dispatch List

5 August 2025

Client: C. Mkhonto

Address: 56 Barbet Street, Stonedge, Nelspruit, 1200

Tel: 00

Cell: 0630885659

Email: 201707659@ump.ac.za

ID CODES:

1 = Specimen too poor to ID
2 = Label information inadequate
3 = Cannot match specimen in herbariu
4 = Specialist not available to do ID
5 = Genus requiring/under revision
6 = Specimen closest to name listed (cf)
7 = Please send more material
8 = Please refer to attached note/letter
9 = New record
K = specimen kept for herbarium
S = specimen scrapped
R = specimen returned

Collector	No.	Plant Name	Det. By	Det. Notes	ID Code	Fate
Mkhonto, C.	02	Mimusops zeyheri Sond.	Maswoliedza, M -- 9/2025		0	K

Curator
National Herbarium (PRE)