

Nutraceutical properties of wild fruit plants in Mpumalanga

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Master of Science degree**

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**UNIVERSITY OF
MPUMALANGA**

DECLARATION

I declare that this dissertation titled “Nutraceutical properties of wild fruit plants in Mpumalanga” was carried out at the University of Mpumalanga under the joint supervision of Prof. Wilfred Otang Mbeng, Dr. Tshepiso Ndhlovu, and Dr. Bongisiwe Shelembe. The dissertation presented here results from my original research efforts and has not been offered for any degree or diploma at any other institution. Any references to work conducted by others or materials obtained from external sources have been appropriately cited and referenced.

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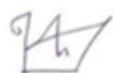


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DEDICATION

To the unwavering resilience, unshakable strength, and unstoppable determination within me, this dissertation is a testament to the challenges I have overcome and the growth I have embraced. I dedicate this work to the dreamer who dared to begin and the achiever who saw it through. Here's to believing in myself, always!!

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ABSTRACT

Nutraceuticals have emerged as reliable substances for managing health conditions by providing both nutrition and phytotherapy. Wild fruit plants, with a long history of use by local communities for nutritional and medicinal purposes, represent promising candidates for nutraceutical development. This study aimed to explore the nutraceutical properties of wild fruit plants harvested from Bushbuckridge local municipality, Mpumalanga Province, South Africa. A comprehensive literature survey on wild fruit plants in Mpumalanga Province identified five key studies documenting a total of 83 wild fruit plant species from 61 genera and 31 families. The review highlighted research gaps, including the need to explore under-documented regions and variations in the cultural use of these plants across different ethnic groups. Based on their medicinal and nutritional uses, frequency of citation, and research gaps, six wild fruit plants were selected for further investigation: *Carissa spinarum* L., *Diospyros mespiliformis* Hochst. ex A.DC., *Euclea crispa* (Thunb.) Gürke, *Ficus thonningii* Blume, *Strychnos spinosa* Lam., and *Strychnos madagascariensis* Poir. The antimicrobial activity of the leaves of these plant species were tested against the bacterial skin pathogens *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Using disc diffusion, the hexane extracts of *D. mespiliformis* and *E. crispa* had an inhibition zone of 28 mm against *P. aeruginosa*. The microdilution assay also revealed that the methanol extract of *D. mespiliformis* exhibited the strongest activity against both pathogens, with a minimum inhibitory concentration (MIC) ranging from 0.781 to 1.563 mg/mL. The antioxidant potential of the plant leaves was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. The methanol extract of *E. crispa* demonstrated potent antioxidant activity, with a half-maximal inhibitory concentration (IC₅₀) of 1.42 µg/mL, comparable to standard ascorbic acid. Other species, including *C. spinarum* and *S. spinosa*, also showed promising antioxidant activity, highlighting their potential to mitigate oxidative stress-related conditions. The nutritional and mineral compositions of *F. thonningii* fruits and the pulp, peel, and seeds of *D. mespiliformis* and *S. spinosa* were analysed. The proximate analysis of the fruits revealed significant protein and fibre content. The elemental analysis of the leaves and fruit showed a wide range of minerals present in the leaves and fruit. Notably, *S. spinosa* peels contained 44.21% fibre, while its leaves were rich in manganese (1,728 mg/kg). Elemental analysis identified essential

minerals like calcium, iron, and magnesium, but also detected heavy metals, such as arsenic, cadmium, and chromium, in the fruits, posing potential toxicity risks. The study demonstrates that wild fruit plants possess significant nutraceutical potential due to their antimicrobial, antioxidant, and nutritional properties. These findings validate their traditional uses and underscore their potential role in addressing food insecurity, managing health disorders, and developing nutraceutical products.

DEFINITIONS OF KEY CONCEPTS

Antibacterial activity- the ability of a substance to suppress the growth or destroy bacteria, helping to prevent or treat bacterial infections (Pancu et al., 2021).

Antioxidant activity- the ability of a substance to inhibit oxidation by neutralising free radicals or reactive oxygen species, thereby reducing oxidative stress and preventing cellular damage (Adeshina et al., 2011).

Bioaccumulation- is the buildup of pollutants in organisms from both dietary sources (trophic transfer) and the abiotic environment (such as air, water, and soil). This process occurs when toxins are absorbed by species faster than they can be eliminated (Nnaji et al., 2023).

Carcinogenic risk- the potential for harmful compounds in the plant to cause cancer, based on the presence of toxins or carcinogenic substances.

Conservation status- describes the information on the preservation and protection of the plant species, including any threats to their survival.

Drug resistance- refers to the reduced effectiveness of a specific drug in preventing or treating a condition (Magiorakos et al., 2012).

Diseases treated – details the specific ailments or health conditions that the plants are known to address.

Elemental analysis- refers to the identification and quantification of minerals and trace elements in the plant, assessing its nutritional and therapeutic potential.

Ferric reducing power- the ability of a substance to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), indicating its potential as an antioxidant (Berker et al., 2007).

Free radical scavenging activity- the ability of substances to neutralise free radicals which cause oxidative stress and associated health risks as well as cellular damage (Adeshina et al., 2011).

Half-maximal inhibitory concentration- a quantitative measure of the concentration of a substance required to produce 50% of its maximum antioxidant effect in a given system (Qureshi et al., 2015).

Medicinal plants- plants are used to maintain health and treat specific ailments, serving diverse purposes in both conventional medicine and traditional healing systems (Smith-Hall et al., 2012)

Medicinal uses- an exploration of how these plants are utilised for therapeutic purposes, including the treatment of specific diseases or health conditions.

Methods of preparation – describes the various ways the plants are processed or prepared for medicinal or nutritional use.

Minimum inhibitory concentration - indicates the lowest concentration of a substance required to suppress the growth of specific bacterial strains (Ryu et al., 2024).

Non-carcinogenic Risk/Target Hazardous Quotient- a measure of the health risks from non-carcinogenic substances in the plant, with the target hazardous quotient indicating the likelihood of adverse effects.

Nutraceuticals- are bioactive substances derived from food sources, including plants, that provide health benefits beyond basic nutrition, contributing to the prevention and treatment of diseases (Aronson, 2017).

Nutritional uses - focuses on the dietary value of the plants, highlighting their role in nutrition, such as their vitamins, minerals, or other beneficial compounds.

Oxidative stress - an imbalance where the production of reactive oxygen species (ROS) exceeds the body's antioxidant defences, resulting in disrupted redox signaling, impaired cellular control, and potential molecular damage (Vo et al., 2024).

Plant parts used - specifies which parts of the plant (e.g., roots, leaves, fruits, bark) are utilized for medicinal or nutritional purposes.

Proximate components- the basic nutritional constituents of the plant, including moisture, protein, fat, carbohydrates, fiber, and ash, that determine its overall nutritional value.

Skin diseases - are a broad category of medical conditions that affect the skin, resulting in symptoms such as inflammation, irritation, infection, or abnormal growths, ranging from mild to severe (Saodat et al., 2024).

Wild plant species- are plants that grow naturally in their native habitats without cultivation or human intervention (Radeva et al., 2023).

PUBLICATIONS

Publication: Chauke, S., Shelembe, B.G., Otang-Mbeng, W. and Ndhlovu, P.T., 2024. Ethnobotanical appraisal of wild fruit species used in Mpumalanga Province, South Africa: A systematic review. South African Journal of Botany, 171: 602-633.

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CONFERENCE OUTPUTS

Chauke S, Tshitshi L, Matlala ME, Otang-Mbeng W, Shelembe BG, Ndhlovu PT., 2024. Antibacterial activity of the leaves of six indigenous wild fruit species against bacterial pathogens of the skin. University of Mpumalanga Research and Innovation Day. Mbombela, South Africa (27th November 2024) (Poster presentation).

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ATCC	American Type of Culture Collection
As	Arsenic
BAF	Bioaccumulation Factor
BHT	Butylated Hydroxytoluene
Ca	Calcium
Cd	Cadmium
CDI	Chronic Daily Intake
Cr	Chromium
CRM	Certified Reference Material
Co	Cobalt
Cu	Copper
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDI	Estimated Daily Intake
Ev	Energy value
Fe	Iron
Fe³⁺	Ferric ion
Fe²⁺	Ferrous ion
FeCl₃	Iron (III) chloride
FRAP	Ferric Reducing Antioxidant Power
IC₅₀	Half-maximal inhibitory concentration
HNO₃	Nitric acid

ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
K	Potassium
Mg	Magnesium
MH	Mueller-Hinton
MIC	Minimum inhibitory concentration
MTPA	Mpumalanga Tourism and Parks Agency
Mn	Manganese
NaOH	Sodium hydroxide
Ni	Nickel
N	Nitrogen
¹O₂	Singlet oxygen
O₂^{•-}	Superoxide anion
OH[•]	Hydroxyl radical
<i>p</i>	Statistical significance
P	Phosphorus
Pb	Lead
<i>r</i>	Correlation coefficient
R_iD	Oral toxicity reference dose
RSA	Free Radical Scavenging Activity
ROS	Reactive Oxygen Species
RSD	Relative Standard Deviation
SANBI	South African National Biodiversity Institute
SD	Standard Deviation
Se	Selenium
SF	Slope Factor
SMME	South African Small, Medium, and Micro Enterprise

SPSS	Statistical Package for the Social Sciences
TCR	Target Carcinogenic Risk
THQ	Target Hazard Quotient
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
Zn	Zinc

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1.1 Introduction

The term “Nutraceutical” arises from both “Nutrition” and “Pharmaceutical” (Rajat et al., 2012). Nutraceuticals represent a rising category of natural products blurring the distinction between medicine and food (Adetuyi et al., 2022). Hence, nutraceuticals are foods which also offer health benefits, such as the prevention and treatment of diseases (DeFelice, 1995). In addition, they are considered medicine due to their physiological effects on the human body (Pandey et al., 2010). A nutraceutical can also be referred to as medical, designer or functional food; phytochemical, or nutritional supplement (Dable-Tupas et al., 2020). According to Prabu et al. (2012), nutraceuticals are classified into dietary supplements, herbals or botanicals, and nutrients. Nutraceuticals are also divided into antioxidants, dietary fibres, polyphenols, and spices (Kaur et al., 2015). They are available as capsules, pills, and tablets (Bernal et al., 2011).

The nutraceutical sector is experiencing significant growth and expansion at a rapid pace since they are regarded as proactive essentials for healthcare (Pandey et al., 2024). Present healthcare markets are leaning towards proactive health approaches, prioritising prevention over treatment and disease control. Furthermore, the nutraceutical industry is presently a significant multi-billion-dollar sector, poised for rapid expansion in the coming decade (Chopra et al., 2022). As highlighted by Jayaweera (2023), the global market of nutraceuticals is predicted to be worth US\$358.5 billion by 2027. However, Habeeba and Mehta (2022) highlighted that the nutraceutical industry is currently in its developmental phase, lacking a universally agreed-upon set of terms and designations recognised legally within this sector. Al-Obaidi et al. (2021) suggests the strict regulation of the nutraceutical industry with evidence supporting minimum safety and quality standards before production approval.

Nutraceuticals have also gained global popularity as alternatives to conventional medicine (Gidde et al., 2022) due to their potential therapeutic activities (Adetuyi et al., 2022; Taroncher et al., 2021). They are beneficial since they increase dietary value while correcting and preventing certain health conditions. Furthermore, they are considered as more natural than traditional western medicine while posing minimal

risks of side effects (Al-Obaidi et al., 2021; Pandey et al., 2010). The popularity of nutraceuticals can be associated with their health benefits including enhanced nutritional value of diets, extended life expectancy, prevention and cure of certain health conditions, lack of side effects, and that they can serve as conventional foods (Shelke et al., 2020). Furthermore, prior research has demonstrated the role of nutraceuticals in addressing various conditions including digestive issues, hypertension, respiratory ailments like colds and coughs, mental health concerns such as depression, cardiovascular diseases like coronary heart disease, weight management, diabetes, cancer, osteoporosis, and other chronic and degenerative disorders like Parkinson's and Alzheimer's diseases among others (Rajat et al., 2012).

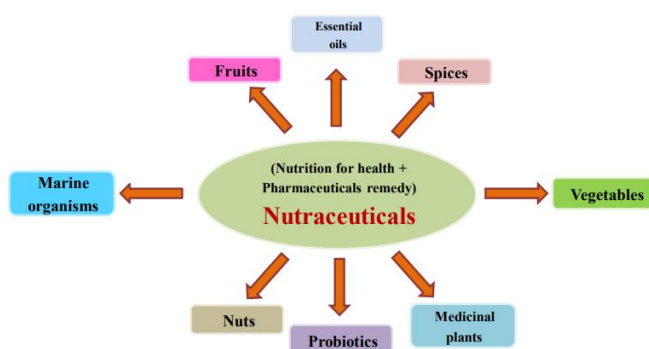


Figure 1.1 Different sources of nutraceuticals (Kaur et al., 2019).

Nutraceuticals can be derived from various sources including plants, and several studies have highlighted the importance of plants as a source of nutraceuticals (Cisneros-Zevallos, 2021; Hayat et al., 2017; Kaur et al., 2019; Prakash et al., 2012; Semwal et al., 2023). Plants are the oldest form of recognised nutraceuticals (Helal et al., 2019). Plant-based nutraceuticals have the potential to enhance human health, address chronic illnesses, and bolster various metabolic functions. The focus on research related to plant nutraceuticals is increasing, driven by their therapeutic benefits and the interest of supplement manufacturers in substituting synthetic ingredients with natural plant-derived molecules, owing to the growing demand for natural products. It is estimated that grains, fruits, and vegetables harbour over 4000 phytochemicals that could potentially be utilised in nutraceutical applications (Al-Obaidi et al., 2021).

Plant-based nutraceuticals have a broad range of bioactive compounds with anti-inflammatory, antioxidant, antimicrobial, and antiproliferative activities (Taroncher et al., 2021). Plant-based nutraceuticals range from carotenoids, coumarins, flavonoids,

lignans, phthalates, plant sterols, polyphenols, saponins, sulphides, and terpenoids (Jain et al., 2022). However, the components of nutraceuticals in plants vary depending on various factors such as climate, humidity, season, soil, and temperature (Bernal et al., 2011). One popular variety of nutraceutical and dietary supplement combines extracts from fruits and medicinal plants like American cranberries, red and blackberries, turmeric, grapevines, and green tea. These blends are renowned for their remarkable detoxifying, anti-aging, and antioxidant properties (Vidal-Casanella et al., 2021).

South Africa is rich in wild fruit plants. Hence, South African diets contain an extensive variety of wild fruits that have been documented since colonial times (Salami et al., 2022). Although South Africa is rich in wild fruit plants, Kimani-Murage et al. (2023) argues that 45% of the population is faced with a lack of food to meet daily nutritional and mineral requirements consequently affecting the health and wellbeing of people. Considering the importance of human health and nutrition for survival, it is imperative to develop new methods to support medical care, which can be found in the vast wild plant ecosystem (Pinela et al., 2016). A wide variety of cultures and societies relied on wild plants as a primary source of their human diet. In addition, native people have used wild plants for centuries to suppress dietary and pathogen-related ailments (Shad et al., 2014). Furthermore, consumption of wild plants remains a tradition in many cultures, both nutritionally and in relation to health (Pinela et al., 2016).

Previous research has shown that edible plants are abundant sources of medicinal phytochemicals (Dlamini et al., 2010). Since wild plants serve as a great source of essential nutrients for native people, it is significant to determine the nutritional value of these wild plants (Singh et al., 2017). In recent years, the focus of many researchers has shifted towards plant-based nutraceuticals, primarily because of the abundant array of phytochemicals, some of which remain undiscovered, offering numerous health benefits and properties for preventing diseases (Udeh et al., 2020). Therefore, this study was conducted to review the medicinal and nutritional data on wild fruit plant species used in Mpumalanga Province, South Africa, and to evaluate the antimicrobial, antioxidant, and nutritional potential of selected wild fruit plant species harvested from Bushbuckridge Local Municipality, Mpumalanga Province, South Africa.

1.2 Problem statement

Ethnobotanical studies focusing on the medicinal and nutritional uses of wild fruit species have been conducted in the Bushbuckridge municipality area (High and Shackleton, 2000; Mashile, et al., 2019; Shai et al., 2020; Tshikalange et al., 2016). However, scientific studies to validate the medicinal and nutritional value of the species in the Bushbuckridge area are lacking. Therefore, the present study aims to validate the use of wild fruit plants for medicinal and nutritional benefits. The study focuses on skin diseases to validate the nutraceutical properties of wild fruit plants since skin diseases are ranked as the fourth most common disease among all human diseases (Flohr and Hay, 2021). In addition, skin diseases affect about 70% of the global population and their quality of life (Maddheshiya et al., 2022). The management of skin diseases is a challenge since conventional medicines are associated with undesirable side effects posed by synthetic drugs and the rising resistance of microbes to antimicrobials (Uthaibutra et al., 2023). For instance, *Trichophyton rubrum*, a common fungal pathogen of the skin, has developed resistant strains against terbinafine (Hiruma et al., 2023). Additionally, current antibiotics have disadvantages such as low bioavailability and transdermal capacity, systemic toxicity, and adverse effects that vary with dosage (Teng et al., 2023). The oral administration of allylamines and azoles, for example, is hepatotoxic (Kanimozhi and Rose, 2023). Over the past few years, resistance to other common antibiotics such as erythromycin, kanamycin, neomycin, and tetracycline has increased (Otang and Afolayan, 2016). However, many people in rural areas use medicinal plants for treating skin infections. Furthermore, these medicinal plants are in demand to treat dermatological anomalies (Mabona and Van Vuuren, 2013). Hence, the need to explore the biological activities of these medicinal plants since they can serve as a source of nutrition and novel therapeutic agents that can be used to combat skin diseases and nutrient deficiencies.

1.3 Rationale and justification

The prevalence of skin diseases is on the rise in South Africa (Makwela et al., 2023). Skin diseases can be infectious, inflammatory, and/or cancerous (Kumar, 2021; Marcinkiewicz and Majewski, 2016). Skin diseases represent a significant challenge to both public health and economy across the globe (Phillips et al., 2024). Certain skin ailments result in extended hospital stays and, in severe cases, mortality (Jovic et al.,

2024; Pettit et al., 2023). The health and conditions of the skin are also modulated by nutrition (Piccardi and Manissier, 2009), undernutrition (Demarest-Litchford et al., 2024; Udoh et al., 2024), overnutrition (Morales et al., 2023; Roongpisuthipong and Klangjareonchai, 2024), mineral, and/or vitamin deficiencies (Godswill et al., 2020; Tahir et al., 2023; Xu and Li, 2024). Additionally, deficiencies in energy, protein, vitamins, and trace elements can alter the physiology of the skin (Dupont et al., 2018). Because of the challenges presented by antimicrobial drug resistance and unwanted side effects, the research was driven to explore the antimicrobial properties of medicinal plants against certain pathogens responsible for skin ailments as well as their antioxidant activities. Additionally, recognising the significance of nutrition in preserving skin well-being, the current investigation was inspired to analyse the nutritional content of wild fruit plants. While the primary focus remained on addressing skin diseases, the nutritional evaluation was anticipated to aid in addressing concerns related to food insecurity as well.

Industrial and scientific communities are investing in natural compounds because of their anti-inflammatory, anti-microbial, and antioxidant properties as well as their ability to interact with living cells (Contardi et al., 2021). Plants serve as an abundant source of bioactive substances with a noteworthy impact on the skin. Plant-based products promote skin health through the medical and antioxidant properties they possess (Michalak, 2022). People in the Bushbuckridge area also rely on plants for treatment of various diseases and ailments (Mashile et al., 2019; Rankoana et al., 2015), and to meet nutritional needs (Shai et al., 2020). In comparison to modern pharmaceutical drugs, plant-based natural products possess better efficiency with very low side effects at an affordable rate. Several compounds used to treat skin diseases have been isolated from plants including mangiferin from *Mangifera indica* L., curcumin from *Curcuma longa* L., and embelin from *Embelia ribes* Burm. Other compounds of plant origin such as lutein, lycopene, quercetin, apigenin, gingerol, naringenin, and resveratrol are also used in managing skin diseases (Mohd Zaid et al., 2022). This is proof that plants are a great source of compounds that can be used to treat various diseases. Therefore, it is essential to conduct bioassay studies and nutritional analysis to validate the medicinal and nutritional use of plant species in the Bushbuckridge Local Municipality area.

1.4 Research aim

The aim of the study was to explore the medicinal and nutritional value of wild fruit plants used by the local people in the Bushbuckridge Local Municipality, Mpumalanga province, South Africa.

Specific objectives:

1. To conduct a literature survey of wild fruit plants used by the local people of the Mpumalanga Province, South Africa.
2. To assess the antimicrobial activity of the leaves of *Carissa spinarum* L., *Diospyros mespiliformis* Hochst. ex A.DC., *Euclea crispa* (Thunb.) Gürke, *Ficus thonningii* Blume, *Strychnos spinosa* Lam., and *Strychnos madagascariensis* Poir., collected from the Bushbuckridge Local Municipality, Mpumalanga Province, against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, bacterial pathogens that cause skin diseases.
3. To investigate the antioxidant potential of the leaves of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. spinosa*, and *S. madagascariensis*, collected from the Bushbuckridge Local Municipality, Mpumalanga Province.
4. To analyse the nutritional components of the fruit and the mineral composition of the leaves and fruit of *D. mespiliformis*, *Ficus thonningii*, and *S. spinosa* harvested from the Bushbuckridge Local Municipality, Mpumalanga Province.

1.5 Research questions

1. Are wild fruit plants fully documented with their medicinal and nutritional uses in the Mpumalanga Province?
2. Can the leaves of wild fruit plants be effective against microbial pathogens that cause skin diseases?
3. Do wild fruit plants possess antioxidant activities?
4. Can the wild fruit plants from Bushbuckridge serve as a good source of nutrients?

1.7 Scope and limiting factors

- The study only assessed antimicrobial activity against two bacterial species. Other pathogens, including fungi and viruses, were not evaluated. The antimicrobial testing was conducted *in vitro*, and the results may not fully represent the efficacy of these plant extracts in real-world clinical settings.

- Variability in the chemical composition of plant extracts, due to factors such as harvesting time and environmental conditions, may influence the antimicrobial activity observed.
- The literature survey was based primarily on secondary sources, which may not always provide the most up-to-date or complete information on the medicinal uses of wild fruit species. The availability of literature specific to the Mpumalanga Province was limited, and some species may not have been included due to a lack of documented research in this region.
- The antioxidant assays conducted were limited to two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing power (FRAP) assays, which may not fully capture the diverse range of antioxidant activities present in the plant species. The concentration of antioxidants in the plant samples was not determined for specific bioactive compounds, limiting the understanding of compounds that contribute most to the observed antioxidant effects.
- The antioxidant potential was assessed *in vitro*, and the bioavailability and efficacy of antioxidants when consumed or applied topically may differ. Furthermore, nutritional analysis was conducted on the leaves and ripe fruit of three plant species, and variability in nutrient content due to factors such as growth conditions, ripeness, and processing methods could influence the results. The presence of heavy metals such as arsenic, cadmium, and chromium in some species raised concerns about the safety of consuming these fruits, and further studies are needed to assess the potential risks.

1.8. Ethical consideration

Ethics approval for the study was granted by the University of Mpumalanga's Ethics Committee (Ethics Reference: UMP/Chauke/230013937/MSc/2024; Appendix 1). Permission to collect plant species was obtained from the Mpumalanga Tourism and Parks Agency (Permit Number: MPB. 1465; Appendix 2) and the Mnisi Traditional Council. Voucher samples of the collected plant species were prepared and securely stored at the University of Mpumalanga's Indigenous Flora Research Laboratory for future reference and verification, with the assistance of Dr. L.J Ramarumo. Throughout the study, the University and faculty codes of ethics were rigorously followed, and all

online sources utilised to develop the database of wild fruit plants were properly acknowledged through comprehensive and accurate referencing.

1.9 Dissertation outline

The dissertation is organized into five chapters including the introduction, literature review, antimicrobial activity, antioxidant activity, and nutritional composition as follows:

Chapter 1 -This chapter outlines the thematic background, problem statement and rationale, aim and specific objectives, as well as a brief review on key concepts relevant to the current study such as the structure and functions of the skin, its susceptibility to factors like nutrition, infections, and injuries. It also delves into the current treatments available for skin conditions, highlighting the associated obstacles. Finally, the chapter explores the connection between the skin and nutraceuticals, emphasizing their role in promoting skin health. The chapter further explores the possible solution to current challenges in the treatment of skin conditions via the use of wild fruit plants and the general contribution of plants in the nutraceutical industry.

Chapter 2 – This chapter examines skin diseases, their causes, and current treatment options, highlighting challenges such as antimicrobial resistance and oxidative stress. It provides a systematic review of wild fruit plant species from Mpumalanga Province, South Africa, emphasizing their nutritional and medicinal potential. The chapter also includes botanical descriptions of the plant species selected for biological assays and nutritional analysis and identifies gaps in current research for further exploration.

Chapter 3 – This chapter examines the antibacterial activity of extracts from six select wild species against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using disc diffusion and microdilution assays. Their antioxidant activities were assessed via DPPH and ferric reducing power assays. It outlines the extraction protocols and bioactivity testing methods, results, discussion, their potential health benefits, and applications in skin health.

Chapter 4 – This chapter investigates the nutritional and mineral compositions of *Diospyros mespiliformis*, *Ficus thonningii*, and *Strychnos spinosa*, emphasising their potential to combat food insecurity and support skin health. It includes a detailed

analysis of proximate composition, mineral content, and metal bioaccumulation profiles. The chapter explores the fruits' nutritional benefits, including their protein, fiber, and mineral content, alongside potential risks from metal contamination. Findings are discussed in relation to their implications for food security, health, and safe dietary applications.

Chapter 5 – The final chapter highlights the key findings of the study, discusses their relevance to clinical practice and skin health, and offers recommendations for future research.

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Chapter 2.0: Literature review

Summary

Globally, plants are an essential primary source of livelihood, particularly in rural communities. Food and health security remain pressing concerns in both developed and developing countries, as the rise of antimicrobial drug-resistant microbes and the adverse side effects of conventional drugs increasingly challenge healthcare systems. As a result, alternative sources of nutrition and medicine are urgently needed to mitigate food insecurity and address both long-standing and emerging health issues. The skin, as the body's largest organ, plays multifaceted roles, including acting as a protective barrier, regulating homeostasis, and functioning as a sensory organ. However, various physical and biological disruptions can lead to skin infections or diseases caused by bacterial pathogens like *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Oxidative stress further exacerbates skin damage through reactive oxygen species, impacting DNA and collagen integrity. In this context, traditional nutraceuticals offer promising antimicrobial, antioxidative, and nutritional benefits for skin health. Despite advancements in topical, oral, and intravenous treatments, challenges such as antimicrobial resistance and formulation instability persist, emphasizing the need for innovative approaches in skin disease management. This review critically evaluates existing knowledge on wild fruit species in Mpumalanga Province, South Africa, with a specific focus on their nutritional and medicinal potential. Ethnobotanical information on wild fruits in Mpumalanga was obtained from various scientific databases and relevant literature. A total of 83 plant species, belonging to 63 genera from 31 families, have been documented in the Ehlanzeni District of Mpumalanga. Among the most commonly utilised species are *Carissa spinarum* L., *Strychnos madagascariensis* Poir., and *Strychnos spinosa* L. The findings reveal that 76% of these wild fruit species are used for medicinal purposes, while 51% serve as sources of nutrition. However, ethnobotanical studies conducted in Mpumalanga have been predominantly concentrated in the Ehlanzeni District, highlighting a significant knowledge gap across other regions of the province. Furthermore, while these species hold considerable potential for improving food security and health, their economic significance has not been fully explored. The study underscores the importance of further research to document the nutritional, medicinal, and economic values of wild

fruit species in Mpumalanga Province. Expanding ethnobotanical studies across different ethnic groups and geographic areas can unlock their potential, not only for addressing local health and nutrition challenges but also for contributing to global biodiversity conservation and sustainable development initiatives.

2.1 Introduction

The skin, the largest organ of the human body (Li et al., 2020) is divided into four layers; namely: the stratum corneum, the viable epidermis, the dermis, and subcutaneous tissues (Benson, 2012). This organ forms part of the innate immune system and the first line of defence (Brohi, 2021). It serves as a protective barrier against environmental factors such as ultraviolet radiation, chemicals, allergens, and microorganisms while preventing the loss of moisture and essential nutrients from the body (Abaci et al., 2017; Benson, 2012; Kasemsarn et al., 2016). Moreover, it plays a crucial role in maintaining internal balance (homeostasis) by regulating body temperature and blood pressure. Additionally, the skin functions as a vital sensory organ, detecting environmental stimuli such as temperature variations, pressure, and pain through touch (Benson, 2012).

Any alteration to the skin caused by physical traumas in the form of abrasions, penetrations, cuts and burns, pre-existing dermatoses with impaired barrier states, undernutrition, diabetes mellitus, and various congenital and acquired immunodeficiency syndromes can all lead to skin infections (Bandyopadhyay, 2021). A range of microbial pathogens such as bacteria, fungi, and viruses may affect the skin extending to the soft tissue (subcutaneous tissue, muscles, and fascia), ranging from minor to potentially life-threatening conditions (Eckmann et al., 2024; Pujalte et al., 2023). These skin infections are classified based on the causative pathogen. The pathogens range from gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, to gram-positive bacteria such as *Staphylococcus aureus*, *Corynebacterium* spp., and *Streptococcus* spp., and fungi such as *Candida* spp. and *Aspergillus* spp (Eckmann et al., 2024). Some pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes* are the most common bacterial causes of skin diseases such as furuncles, folliculitis, erythrasma, and impetigo. Impetigo mainly affects children, especially in developing countries (Al-kahfaji, 2022). Fungal pathogens such as *T. rubrum* cause infections such as

onychomycosis, tinea corporis, tinea pedis, tinea manus, tinea capitis, and tinea cruris (Kanimozhi and Rose, 2023).

2.2 Types of skin diseases



Figure 2.1 Physical manifestation of different skin conditions on the human skin (Raina et al., 2023)

Skin diseases are not only infectious but can be cancerous, congenital, degenerative, and inflammatory (Hay et al., 2015). Non-infectious skin diseases such as dermatitis, and psoriasis (Figure 2.1) are accompanied by an inflammatory response (Mohd Zaid et al., 2022). These inflammatory skin disorders continue to affect people at a global prevalence of 30% per annum (Ohn et al., 2021).

2.3 Bacterial pathogens responsible for skin diseases study.

Two pathogens were selected for the study due to their association with skin diseases and antimicrobial drug resistance (Rashid et al., 2020).

2.3.1 *Klebsiella pneumoniae*

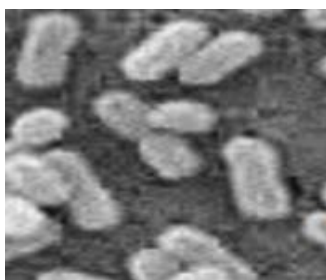


Figure 2.2 Scanning electron microscopy image of *K. pneumoniae* (Kareem and Alsammak, 2017)

Klebsiella pneumoniae (Figure 2.2), a gram-negative bacillus belonging to the Enterobacteriaceae family is prevalent in various environments and can inhabit the intestinal mucosa, skin, and nasopharynx of its host. It has the potential to induce various infections, including those affecting the respiratory system, bloodstream, liver (such as abscesses), and urinary tract (Li et al., 2024). *K. pneumoniae* is recognized as a reservoir for genes associated with multidrug resistance, resulting in limited treatment options due to the emergence of strains resistant to antimicrobial drugs, thereby elevating the pathogen's risk profile (Heng et al., 2024; Verani et al., 2024). Kelishomi et al. (2024) also highlighted that *K. pneumoniae* contributes 13-15% of wound infections which are difficult to treat due to antimicrobial drug resistance. Furthermore, wound infections caused by *K. pneumoniae* can be fatal (Boff et al., 2021).

2.3.2 *Pseudomonas aeruginosa*



Figure 2.3 Scanning electron microscopy image of *P. aeruginosa* (Tyavambiza, 2018).

Pseudomonas aeruginosa (Figure 2.3) is a gram-negative aerobic bacillus (Esfahani et al., 2024), opportunistic in nature with the highest frequency of clinical isolation causing infections in the eyes, ears, urinary tract, respiratory system, and burn wounds (Zhan et al., 2024). *P. aeruginosa* ranks among some of the most prevalent microorganisms responsible for infections in severe skin wounds (Quiñones-Vico et al., 2024).

2.4 Impact of oxidative stress on the skin

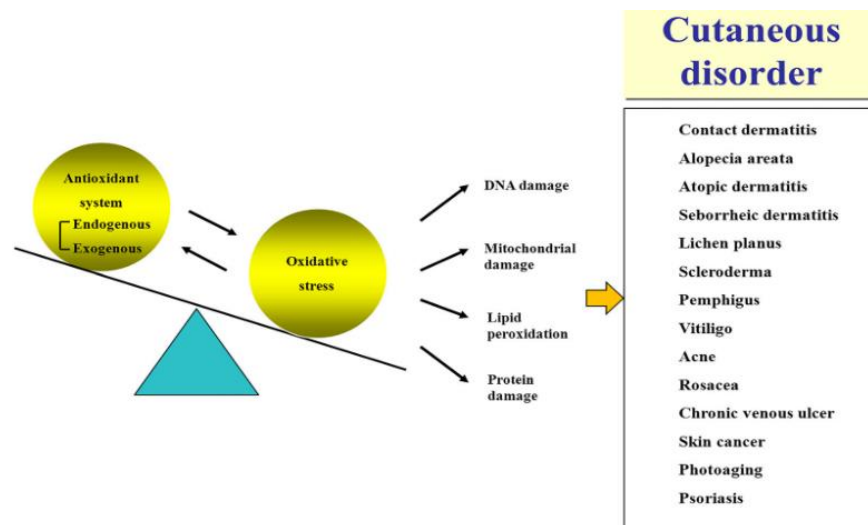


Figure 2.4 Various skin conditions resulting from oxidative stress (Baek and Lee, 2016).

Oxidative stress refers to an imbalance in which the body's tissues struggle to effectively neutralize reactive oxygen species (ROS) originating from either external or internal sources (Trüeb, 2021). Keratinocytes and various skin cells generate ROS like superoxide anion ($O_2^{\circ-}$), peroxides, hydroxyl radical (OH°), and singlet oxygen (1O_2) when stimulated by cytokines, growth factors, environmental pollutants, UV radiation, food additives/preservatives, cosmetics, medications, and physiological stimuli. These ROS may cause damage to the deoxyribonucleic acid (DNA), lipid membranes, collagen structures, and mitochondrial function thereby leading to several skin conditions as shown in figure 2.4 (Baek and Lee, 2016). Moreover, persistent psychological stress can trigger oxidative stress in the skin by diminishing the body's antioxidant defences (Chen et al., 2021).

2.5 Social and psychological impact of skin diseases

Skin diseases are one of the most common diseases that affect people of all age groups. The negative effects of skin diseases on health range from physical incapacity to death (Hay et al., 2014). In addition, they result in psychological and social challenges since the skin is the most visible organ of the human body. Reports indicate that social and psychological distress is more common in patients with skin diseases in comparison with other diseases (Richard et al., 2023). Therefore, skin diseases

affect the quality of life, leading to anger, anxiety, depression, and fear among others (Gebashe et al., 2022; Sanclemente et al., 2017). Depression due to skin diseases can lead to isolation (Li et al., 2020) and suicide in some cases (Hay et al., 2015). Atopic dermatitis, acne vulgaris, and psoriasis are some of the skin diseases that can affect the quality of life (Sanclemente et al., 2017).

2.6 Treatment of skin diseases and challenges

2.6.1 Antimicrobial treatments

Globally, antibiotics are the predominantly prescribed drugs for the protection, maintenance, and restoration of health (Dimma et al., 2023). The treatment of skin infections depends on the type of causative pathogens (Eckmann et al., 2024). Although oral and topical antibiotics are used to treat some skin diseases, there is a rising concern about microbial pathogen resistance development (Li et al., 2020). Kanaujia et al. (2024) reported on the use of antimicrobial peptides from various sources such as amphibians, mammals, and aquatic organisms to treat bacterial, fungal, and viral skin infections and modulate wound healing since they have anti-inflammatory properties and the ability to stimulate cell migration and proliferation. However, some of the antimicrobial peptides are toxic. Other antibiotics such as amoxicillin, vancomycin, erythromycin, azithromycin, and tetracyclines are used (Felgueiras, 2021). However, these antibiotics have limitations such as low transdermal capacity and toxicity (Liang et al., 2024). Over the past ten years, there has been a notable rise in the occurrence of bacterial skin infections, posing significant challenges and burdens on both clinical practice and healthcare finances (Abruzzo et al., 2024). One critical concern associated with these infections is the alarming worldwide dissemination of multidrug-resistant strains, complicating their treatment and presenting a substantial global public health threat (Guo et al., 2024).

2.6.2 Antioxidative treatments

Herbal medicines and nutrition are regarded as the main source of antioxidants for Indigenous people which provide them with protection against oxidative stress (Fahad and Mohammed, 2020). According to Pandel et al. (2013) the antioxidative defence of the skin is also influenced by nutrition. Antioxidants are essential for safeguarding the skin from the detrimental impacts of free radicals produced by UV radiation and environmental pollutants. Antioxidants contribute to skin well-being by thwarting

oxidative stress, lessening inflammation, and stimulating collagen synthesis (Wójciak et al., 2024). Antioxidants such as vitamins C and E, coenzyme Q10, niacinamide, lycopene, and idebenone are applied in topical formulations for the maintenance of skin health (Allemann and Baumann, 2008). Some antioxidants such as vitamins C and E can be taken orally and act synergistically in the prevention of skin DNA damage (Calvo et al., 2024). However, antioxidants are highly unstable and can be challenging to stabilize and ensure that they remain active and produce desired effects when incorporated into a product (Allemann and Baumann, 2008).

2.6.3 Drug administration methods for skin diseases

2.6.3.1 Topical therapy

Most antimicrobial agents are administered topically in the form of creams, gels, ointments, or sprays (Abruzzo et al., 2024). Examples of topical antibiotics include amikacin, benzoyl peroxide, clindamycin, gentamicin, and mupirocin (Dallo et al., 2023). Topical antibiotics are more preferred than systemic antibiotics because topical therapy ensures high and consistent doses directly at the site of infection requiring a smaller amount of antibiotics. This results in improved adherence to treatment, reduced occurrence of side effects, and lowered risks of developing antimicrobial resistance (Ray et al., 2019). However, Bonamonte et al. (2020) and Bandyopadhyay (2021) argue that topical antimicrobials are vulnerable to risks of developing resistance due to how they are stored in a patients' home for extended periods and inappropriate self-administration, leading to contamination, exacerbating the resistance issue. Topical antibiotics are frequently employed to address superficial pyodermas such as impetigo and to manage or prevent infections resulting from minor injuries like cuts, scrapes, burns, and surgical wounds. They are extensively utilized for addressing folliculitides and furuncles. Topical antibiotics find utility in treating secondary bacterial infections associated with skin conditions such as eczema and leg ulcers. However, topical antibiotics have shortcomings such as causing contact dermatitis, wound healing impairment, minimal depth of penetration, and risks of systemic absorption and toxicity (Bandyopadhyay, 2021).

2.6.3.2 Oral therapy

A wide range of oral antibiotics are available in the form of tablets, capsules, liquids, suspensions, and powders (Ebrahimnejad et al., 2024). It has been found that the greatest number and extended use of oral antibiotics is associated with dermal

conditions as compared to other health conditions (Barbieri et al., 2019). Del Rosso and Sachsman (2018) further emphasized that oral antibiotics are commonly prescribed by dermatologists, constituting roughly 20% of all medications prescribed within the field of dermatology. In some skin condition cases such as acne, oral antibiotics are considered when topical antibiotics have been unsuccessful. The most prescribed oral antibiotics include tetracyclines, azithromycin, erythromycin, clindamycin, trimethoprim, cotrimoxazole, and quinolones (Farrah and Tan, 2016). Typically, oral antibiotic agents fail to reach adequate concentrations in the site of infection to effectively eliminate microbial colonization at this site (Hogan et al., 2018). However, for more severe pyoderms such as carbuncles, ecthyma, cellulitis, or erysipelas, systemic antibiotics are typically preferred (Bandyopadhyay, 2021). Administering oral antibiotics can induce resistance in the normal flora present at any site on the body (Farrah and Tan, 2016). Furthermore, the disruptions to the microbiome can lead to the development of inflammatory bowel disease, collagen vascular diseases, and an increased risk of colon and breast cancer (Barbieri et al., 2019).

2.6.3.3 Intravenous therapy

Intravenous administration of antibiotics is not a common practice but only applied in serious skin infections such as cellulitis and necrotizing fasciitis (Sambrano et al., 2012). Antibiotics that are administered intravenously include daptomycin, linezolid, oritavancin, and vancomycin (Jensen et al., 2016). These antibiotics are often administered to patients with prolonged hospitalization and suffering from acute bacterial skin and skin structure infections (Claeys et al., 2015). Intravenous therapy generates a quicker response and ensures full bioavailability, necessitating smaller doses of the drug to achieve the intended effects. But the drawbacks of intravenous medication include swift absorption and heightened bioavailability which consequently increase the likelihood of notable side effects (Castro et al., 2024). Furthermore, obtaining IV access in a highly agitated patient is frequently difficult (Castro et al., 2024).

2.7 Significance of nutraceutical traditional knowledge

Traditional plant-derived nutraceuticals are the primary reservoir of essential nutrients, phytochemicals, and functional foods for over three quarters of the global population

(Al-Obaidi et al., 2021). South Africa is one of the countries enriched with diverse plant life and extensive expertise in the traditional utilization of natural resources across various sectors like cosmetics, medicine, and food. Consequently, there is a high likelihood that South African enterprises or indigenous communities could uncover new commercially valuable nutraceuticals based on their traditional knowledge of plants (Gebashe et al., 2022). Therefore, there is an urgent requirement for the documentation and assessment of traditional knowledge through ethnobotanical research to safeguard both biological and cultural diversity (Sujarwo et al., 2014).

2.8 Nutraceuticals as skincare products

The food and cosmetic sectors are devising fresh approaches to link nutrient intake with skin well-being. As a result, there's a growing trend in utilising food components and supplements purposed to mitigate skin issues or combat skin aging. Incorporating vitamins, minerals, or essential fatty acids into one's diet can potentially enhance skin conditions (Pérez-Sánchez et al., 2018). The study of Sadowski and Sadowski (2020) proved that some skincare products with nutraceutical formulations improve skin elasticity, tone, hydration, and reduce wrinkle formation with no side effects. Nutraceuticals are incorporated into skincare products such as cleansers, toners, serums, moisturisers, sunscreens, and masks (Bell Loo and Le, 2018). Nutraceuticals can be used as oral supplements or incorporated into topical products to maintain skin health (Arora, 2021). Skincare products with nutraceutical ingredients are also known as cosmeceuticals or nutricosmetics since they contain biologically active principles from plants (Raja, 2016; Rangaraj et al., 2023). South African Small, Medium, and Micro Enterprises (SMMEs) have delved into the development of cosmeceutical products, drawing upon the extensive historical knowledge of medicinal plants and scientific studies confirming the effectiveness and safety of plant extracts and essential oils (Gebashe et al., 2022).

Table 2.1 Cosmeceuticals developed from plant species by South African companies (Gebashe et al., 2022).

Plant species (Scientific name)	Formulation type/cosmetic product category	Therapeutic benefit of developed products	Company/brand
African potato (<i>Hypoxis hemerocallidea</i> Fisch. And C. A. Mey.)	Cream, gel cleanser, moisturiser, aftershave soother, soap, toner, body lotion	Athlete's foot, boils, eczema, wounds, psoriasis, acne, rashes, sunburn and minor burns, bed sores, warts, stretch marks, scars, dry skin, cracked heels, boost skin hydration, anti-ageing	Phyto-force, Down to earth, Zinplex, Eskamel, Africology
Aloe (<i>Aloe ferox</i> Mill., <i>A. vera</i> Mill.)	Moisturizer, day and night cream, cleansers, toner, sun lotions, shampoos, conditioner, toothpaste and mouthwash, lightedning cream, soap	Burns, anti-ageing, sun damage/sunburn, acne- prone skin, strengthens and protects hair, insect bites, stings, cuts and bruises wrinkles, rash, eczema, healthy gums	Faithful to nature, Natrалoe, Alcare, Aleounique, Howlistic pets, Aloeferox, Down to earth, Olive handmade soaps
Baobab (<i>Adansonia digitata</i> L.)	Shampoo, conditioner, sheet mask, body wash, hydrotherapy, facial toner, anti-ageing SPF cream, day wash, serum, lightening cream, deep cleansing powder, tonic, powder	Sun damage/sunburn, detoxify and hydrate skin, rejuvenate skin, cell renewal, refines pores, removes impurities, reduce wrinkle depth, smooth fine lines, protect against harmful UV and UVB damage, promotes collagen stimulation, after- shave tonic	Africa organics, Iwori beauty of Africa, Howlistic pets, Isivuno naturals, Skin creamery
Buchu (<i>Agathosma betulina</i> (P. J. Bergius) Pillans)	Soap, moisturiser, and aftershave soother, cream, bath salts, gel, serum, balm, soap, African clay mask	Reduces skin sensitivity, soothe razor-burn and irritation, wounds, bites, burns, eczema, bruising, anti-ageing, hydrate, nourish and brighten skin, itches, scratches, bruises, acne	Antjies Handmade Naturals, Le Naturel, Skimmelberg, Olive handmade soaps, Salty sistas
Bulbine (<i>Bulbine frutescens</i> (L.) Willd.)	Cleanser, sunscreen, day and night cream, moisturiser, toner, soap, soothing spray, bulbine	Discoloration and uneven skin tone, sun damage/sunburn, cuts, insect bites, stimulate collagen production, hydrate skin, grazes, acne,	Afari, Essentially natural, Timola, nautica, African botanicals

	frutescens extract, anti-ageing toner, soap	burns, blisters, cold sores, cracked lips, rashes, ringworm	
Cancer bush (<i>Sutherlandia frutescens</i> (L.) R. Br.)	Cream, soap, lotion, topical gel	Eczema, insect bites, psoriasis, shingles, acne, herpes, lesion, ageing, dark pigmentation, pimples, and marks	Phyto-force, lady K cosmetics, faithful to nature, African botanicals

2.9 Types of nutraceuticals and their impacts on skin health

2.9.1 Antimicrobials

Nutraceuticals are antimicrobials which have been validated through various studies to possess antimicrobial activity against various pathogens (Chanda and Kaneria, 2011). Classes of nutraceuticals with antimicrobial activity include terpenoids (e.g. carnosic acid), polyphenols (e.g. quercetin) and thiols (e.g. allicin) (Gutiérrez-del-Río et al., 2018). However, Costagliola et al. (2021) highlights that despite having direct antimicrobial activity, some nutraceuticals such as vitamin D can stimulate the synthesis of antimicrobial proteins in the immune system.

2.9.2 Antioxidants

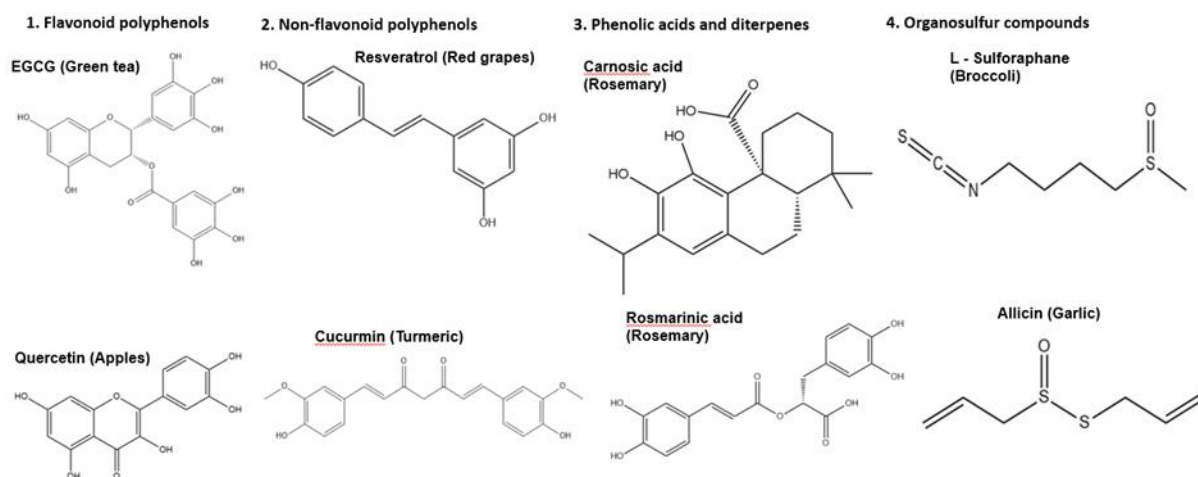


Figure 2.5 Nutraceutical antioxidants from various plant sources (Kelsey et al., 2010).

Antioxidants encompass diverse substances and extracts sourced from a broad array of plants, grains, and fruits (Figure 2.5). They possess the ability to alleviate oxidative stress on the skin or shield from oxidative deterioration (Hoang et al., 2021). Nutraceutical antioxidants operate through various mechanisms and locations, primarily as scavengers for free radicals: 1) they counteract free radicals, 2) mend

oxidized membranes, 3) reduce the production of reactive oxygen species, and 4) neutralize reactive oxygen species (Simioni et al., 2018). Nutraceuticals have been discovered to diminish free radicals by functioning as scavengers, thus averting harm to lipids, proteins, and DNA (Ramli et al., 2020). Foods containing antioxidant-rich nutraceuticals encompass a variety of plant sources, such as fruits (grapes, citrus, blueberries, strawberries, blackberries), vegetables (beans, broccoli, beets, white cabbage, kale, spinach, and soybean), spices (rosemary, oregano, and thyme), herbs (sage), and beverages (tea and wine) among others (Inan, 2019). Anti-oxidative nutraceuticals from plants include carotenoids, polyphenols, phenolic compounds, and vitamin E (Inan, 2019). Several nutraceutical antioxidants have been isolated from various plants including epigallocatechin 3-gallate (EGCG) (Figure 2.5) which was found to possess scavenging properties against superoxide, hydroxyl radical, hydrogen peroxide, and nitric oxide (Kelsey et al., 2010).

2.9.3 Nutrients

Nutrition is essential for cell metabolism and the formation of collagen- the principal structural protein of the extracellular matrix (Stotts and Moulder, 2022). However, both undernutrition and overnutrition can alter the physiology of the skin (Piccardi and Manissier, 2009). Since the skin is a tissue with a high proliferative potential, it is essential to consume sufficient quantities of proteins, carbohydrates, and fats (Michalak et al., 2021). Extreme protein deficiency (Kwashiorkor) causes biochemical changes in the skin while malnutrition (anorexia nervosa) causes skin conditions such as xerosis (Piccardi and Manissier, 2009). Moreover, carbohydrates and fats provide the body with energy while proteins serve their function in the formation of collagen and cell structure (Posthauer et al., 2015). Cao et al. (2020) further elaborated that to construct, repair, and mediate skin physiological functions is amongst the primary functions of proteins. Fatty acid deficiencies are associated with eczema, depigmentation, and impaired wound healing (Krutmann and Humbert, 2011). Dietary fibres can be used to relieve inflammation in psoriasis patients, hence, there is a correlation between the low intake of dietary fibre and psoriasis (Sawada et al., 2021). Other skin defects such as acanthosis nigricans, acrochordons, and keratosis pilaris are attributed to obesity (Yosipovitch et al., 2007). Furthermore, the obese suffer wound-healing complications due to skin folds that provide a moist environment for bacterial growth (Munoz et al., 2020). Low-energy and vegetarian diets are

recommended for the prevention of chronic inflammatory skin diseases such as psoriasis (Piccardi and Manissier, 2009).

2.9.4 Minerals

Trace elements also serve the purpose of maintaining skin health, hence, their deficit may cause skin modifications (Piccardi and Manissier, 2009). Copper (Cu) is a trace mineral found throughout the body (Polefka et al., 2012) that is an essential component of enzymes involved in the synthesis of elastin, collagen, and melanin. Therefore, skin that is deficient in Cu can show signs of sagging and hypopigmentation (Krutmann and Humbert, 2011). Among its many benefits, Cu-peptides have been shown to reduce photodamaged skin, speed wound healing, and calm irritated skin (Polefka et al., 2012). Iron (Fe) is also important for maintenance of skin health since deficiencies in Fe are associated with pale, dry, and scaly skin (Krutmann and Humbert, 2011). As a dietary supplement, chromium (Cr) (III) is acknowledged as an important element in insulin action, protein, fat, and carbohydrate metabolism (Chowdhury et al., 2003). A major cofactor for wound healing enzymes is zinc (Zn) (Polefka et al., 2012) and its deficiency is associated with dermatitis (Krutmann and Humbert, 2011). Crossgrove and Zheng (2004) noted that manganese (Mn) is important for the normal development and function of the body, however, deficiencies in manganese are associated with skin lesions. Lastly, selenium (Se) is essential for the development and function of skin keratinocytes and related to skin antioxidant enzyme activity (Cao et al., 2020).

A concentration gradient of calcium (Ca) ions in the epidermis plays an important role in many skin functions, including keratinocyte differentiation, skin barrier formation, and permeability barrier homeostasis (Lee and Lee, 2018). Additionally, studies have demonstrated that local Ca influences cell proliferation, motility, and maturation as well as the formation of the epidermal lipid barrier through signal transduction and gene expression in cultured keratinocytes, dermal fibroblasts, and reconstructed epidermis (Lansdown, 2002). Healthy skin requires a functional Ca homeostasis in keratinocytes. A disturbance in such can cause skin diseases such as atopic dermatitis, darier and hailey-hailey disease, and psoriasis (Rinnerthaler et al., 2015). Magnesium (Mg) also serves as a co-factor of many enzymes that are associated with protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation in the human body. In addition, the mineral takes part in the active transport of Ca and

potassium (K) ions across cell membranes. Therefore, Mg deficiency lowers calcium and potassium levels which are also important minerals of the skin (Al-Fartusie and Mohssan, 2017). Phosphorus is involved in cell metabolism, and is found in the soft tissues and extracellular matrix of the human skin (Bird and Eskin, 2021).

2.9.5 Concerns of the safety and efficacy of nutraceuticals

There is often insufficient information to validate the effectiveness, safety, and effects of nutraceuticals, posing concerns regarding their use and causing confusion as to how to classify such a product if it is intended for medicinal use (Chopra et al., 2022; Helal et al., 2019). Many claims regarding their medicinal or nutritional benefits remain unsubstantiated (Santini et al., 2018) because the majority of available scientific data originates from animal experiments and laboratory tests, while human clinical trials are limited and inconclusive (Voelkl et al., 2020). Moreover, essential aspects such as the absorption rate, metabolic pathways, dosage effects, and potential toxicity of nutraceuticals remain poorly defined (Paul-Chima et al., 2024). Nutraceuticals may pose potential risks to consumers if these products become contaminated with substances like heavy metals, mycotoxins, or allergens during manufacturing, or with pesticides and fertilizers if applied where the plants are cultivated, or if the plants are naturally toxic (Chopra et al., 2022; Gupta et al., 2018). Furthermore, there is a possibility of adverse interactions between nutraceuticals and therapeutic medications (Andrew and Izzo, 2017).

2.10 A systematic review of wild fruit plant species in the Mpumalanga Province, South Africa

2.10.1 Materials and methods

A literature review spanning from October 2022 to May 2024 was undertaken to document information on wild fruit plant species utilised in the Mpumalanga Province of South Africa. The data was sourced from various online databases including Science Direct, Scopus, Google Scholar, and JSTOR. Additionally, the University of Mpumalanga's library was consulted for theses, dissertations, and books. The search employed keywords such as ethnomedicine, Mpumalanga, wild fruit species, nutraceutical, medicinal, traditional, and nutrition; to identify relevant articles. The inclusion criteria involved studies that recorded wild fruit species throughout the Mpumalanga Province, and from these articles, the nutritional and medicinal uses, and

modes of preparation and plant parts used were extracted. Studies that included the phytochemical analysis of the specific wild fruit plant species were also included. Additional information such as scientific names that were verified using The Worldflora online (<https://www.worldfloraonline.org/>), common names that were cross-referenced with PlantZAfrica (pza.sanbi.org), and conservation statuses based on the South African National Biodiversity Institute (SANBI) Red List of South African Plants (redlist.sanbi.org/species) was extracted from the relevant articles.

2.10.2 Results and discussion

2.10.2.1 Literature search results

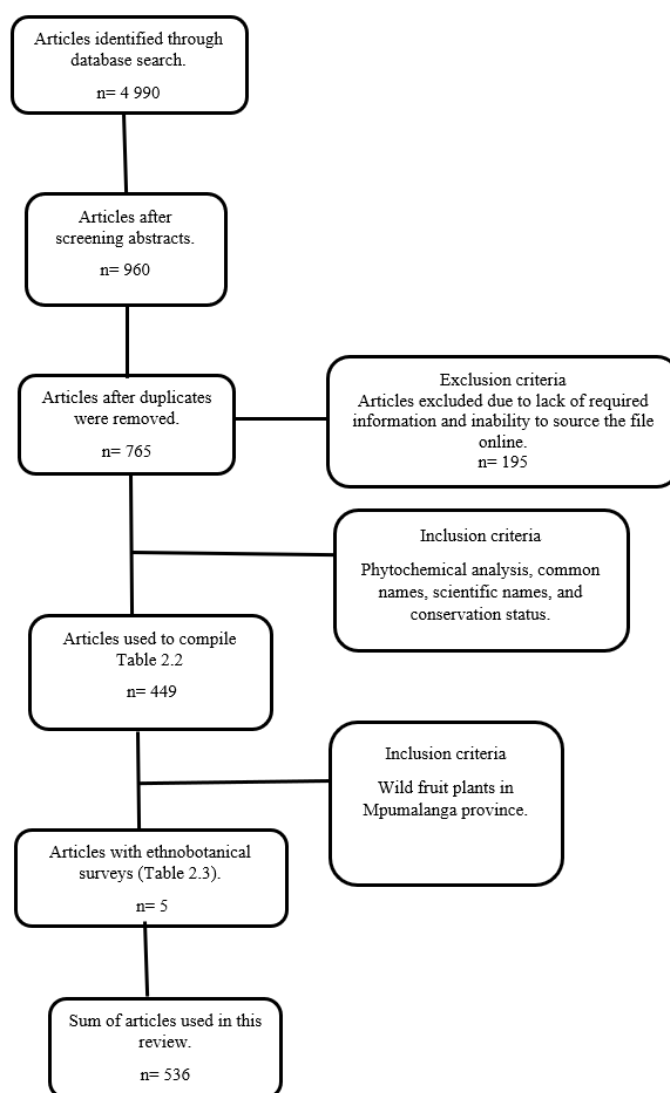


Figure 2.6 Flow diagram indicating the literature search and selection process.

The data gathered from various scientific databases constituted 4990 studies. However, abstract screening resulted in the identification of 960 articles relevant to the study (Figure 2.6). After excluding articles devoid of the required information,

inaccessible full text, and duplicates, 765 articles remained. Therefore, to complete Table 2.2, a total of 449 articles was used to document various wild fruit species in Mpumalanga Province, including their distribution, medicinal and nutritional uses, plant parts used and modes of preparation, phytoconstituents, pharmacological activities, and nutritional compositions. Five studies have reported the use of wild fruit species in the Mpumalanga Province, primarily in the Ehlanzeni District (Table 2.3). A total of 536 articles were used in the study including references that were used in the discussion of the results. The most dominant region was Bushbuckridge, followed by Mbombela and Thaba Chweu. Other districts such as Umjindi, Nkomazi, Gert Sibande, and Nkangala need further exploration (Tshikalange et al., 2016). Rasethe et al. (2013) noted a considerable difference of the use and reliance on wild plants between two villages in the Limpopo Province indicating that the ethnobotanical knowledge and use of plants may vary between different areas.

2.10.2.2 Diversity of wild fruit species

In this study, 83 wild fruit plant species belonging to 63 genera and 31 different families were identified (Table 2.1). Fabaceae and Anacardiaceae were the most dominant plant families, each contributing 15% and 9% respectively. Fabaceae species are widely distributed in South Africa. Lötter et al. (2014) revealed that the Fabaceae family was the second most common family of plants in the forests of the Mpumalanga Province. Unsurprisingly, the family Fabaceae is considered among the most significant plant families contributing to food and nutrition security globally and has the ability to symbiotically fix nitrogen (Alimi et al., 2021; Talukdar, 2013). Fabaceae species hold substantial economic and cultural importance, serving as sources of traditional medicines, food, timber, dyes, firewood, ornamental plants, and gums (Van Wyk, 2019). The current study is in contrast with previous studies which revealed that the Anacardiaceae family accounted for the greatest number of species (Mashile et al., 2019b; Shackleton et al., 2000; Shai et al., 2020). The most cited plant species include *Carissa spinarum* L., *Strychnos madagascariensis* Poir., and *Strychnos spinosa* L. Similarly, Shackleton et al. (2000) found that *C. spinarum* and *S. spinosa* were some of the most commonly used plant species in Mpumalanga Province. Shai et al. (2020) found that *S. madagascariensis* had the highest use value. Plant species like *S. madagascariensis* and *S. spinosa* were identified in various regions of South Africa as valuable for nutrition and household income, as documented by Akweni et

al. (2020). Similarly, they were recognized in Mozambique (Chemane et al., 2022; Mause et al., 2021a) and Zimbabwe (Ngadze et al., 2017) for their economic and nutritional significance.

Table 2.2 Ethnobotanical knowledge of wild fruit plant species in Mpumalanga Province, South Africa. The scientific names were verified using The World Flora Online (www.worldfloraonline.org) and conservation statuses were verified using the South African Red data list (redlist.sanbi.org).

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
<i>Lannea edulis</i> (Sons.) Engl. Family: Anacardiaceae Habit: Shrublet	Diphiroku (P); nchunchungwa (XT); wild grape (E); wildedruif (A); umgabunk homo (Z); mutsambatsi (W); phepo (T)	Least concern	Free State, Gauteng, KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit	Not specified	Drupe	Leaves- antihyperglycemic, antihyperlipidemic Bulb- pro-inflammatory, anti-HIV, toxicity	Root bark- Dihydroalkylhexenones, alkylphenols, cardanol	Fruit-Ash, fibre, protein, carbohydrates, Ca, Fe, P	(Banda et al., 2018; Herrera et al., 2018; Malaisse and Parent, 1985; Mashile et al., 2019b; Queiroz et al., 2003; Shai et al., 2020; Sigidi et al., 2017)
<i>Lannea schweinfurthii</i> Engl. Family: Anacardiaceae Habit: Tree	Morulanop sane (P); False Marula (E); Mulivhadza (V); Umganunkomo (Z); Valsmaroela (A)	Least concern	Mpumalanga, Limpopo, and KwaZulu-Natal	Edible fruit	Root decoction- body aches	Drupe	Stem bark- antibacterial, antifungal Root bark- anti-inflammatory, antioxidant, cytotoxicity Leaves- antiplasmodial, cytotoxicity	Stem bark- Fatty acids and derivatives, terpenoids, phenolics, polyketide derivatives, steroids, lupeol, epicatechin Roots- catechin	Leaves and root bark- ash, moisture, fibre, protein, fat, Cu, Fe, Zn, Pb, Mn, Cd	(Gathirwa et al., 2011; Herrera et al., 2018; Lawal et al., 2019; Maregesi et al., 2008; Mashile et al., 2019b; Okoth et al., 2014; Oyugi, 2016; Shai et al., 2020; Tshikalange et al., 2016; Wamuyu et al., 2020; Yaouba et al., 2018)
<i>Sclerocarya birrea</i> (A.Rich.) Hochst. subsp. <i>caffra</i> (Sond.) Kokwaro	Morula (PI); marula (E); mufula (V); and ukanyi (XT)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit- jam, beverage, or wine. Nuts	Bark- sexually transmitted diseases/infections, diarrhoea	Drupe	Leaves- antioxidant, cytotoxicity, antibacterial, antidiabetic, antidiarrhoeal,	Stem- Catechin, 56uinic acid, epigallocatechin gallate, epicatechin gallate	Fruit- moisture, fat, fibre, Ca, Fe, Zn, Vitamin C	(Armentano et al., 2015; Baba et al., 2014; Belemtougri et al., 2016; Do et al., 2020; Eloff, 2001; Kamanula et

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Family: Anacardiaceae Habit: Tree							antisecretory, antiplasmodial Bark- antioxidant, toxicity, anti-convulsant, antibacterial, anti-inflammatory Roots- antibacterial, antioxidant, proapoptotic			al., 2022; Maharaj et al., 2022; Manzo et al., 2017; Mashile et al., 2019a, b; Ojewole, 2003, 2007; Russo et al., 2018; Shackleton et al., 2000; Shai et al., 2020; Shoko et al., 2018)
<i>Ozoroa sphaerocarpa</i> R.Fern. & A.Fern. Family: Anacardiaceae Habit: Tree	Currant resin tree (E); korentehar puisboom (A); imfuce lemnyamma (W); monoko (S)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Not specified	Whole plant decoction/ Infusion-wounds, induces lactation	Drupe	Bark- antibacterial, antifungal, antioxidant, haemolytic, toxicity Leaves- antibacterial, haemolytic, toxicity, antioxidant, antifungal Fruit- antibacterial, antifungal, antioxidant, haemolytic, toxicity	Fruit, leaves, and bark- phenolics, flavonoids	Not available	(Papo et al., 2022; Sibandze, 2018; Sibandze et al., 2009, 2010; Teichman and Van Wyk, 1993; Tshikalange et al., 2016)
<i>Searsia leptodictya</i> (Diels) T.S.Yi, A.J.Mill. & J.Wen Family: Anacardiaceae Habit: Tree	Mountain karee (E); bergkaree (A); inHlangushane (W); Mohlwehlwe (S)	Least concern	Free State, Gauteng, Limpopo, Mpumalanga, and North West	Edible fruit	Not specified	Drupe	Leaves- cytotoxicity, antioxidant, and antimicrobial activities	Leaves- phenolics, tannins, flavonoids, flavonols	Leaves- P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn	(Ahmed et al., 2014; Mashile et al., 2019b; Ravhuhali et al., 2023; Yang et al., 2016)
<i>Searsia pendulina</i> (Jacq.) Moffett Family: Anacardiaceae Habit: Tree	White Karee(E); witkaree(A); mosilabele (S); Botlhotlho (PI)	Least concern	Free State, Northern Cape, and Mpumalanga	Edible fruit- alcoholic beverage.	Not specified	Drupe	Leaves- cytotoxicity, antioxidant, and antimicrobial activities	Leaves- phenolics, tannins, flavonoids, flavonols	Not available	(Ahmed et al., 2014; Shai et al., 2020; Yang et al., 2016)

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<i>Annona senegalensis</i> Pers. Family: Annonaceae Habit: Tree	Motilepo (PI); African Custard-apple (E); Amamense (Nd); Wildesuike rappel (A); Isiphofu (Z); Muembe (V)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit	Decoction of leaves, roots- cosmetics and sexually transmitted diseases/infections	Etaerio berry	Leaves- cytotoxicity, analgesic, antisickling, antipyretic, antioxidant, anticonvulsant, central depressant, anxiolytic, antibacterial, antifungal, antimalarial Stem bark- antidiarrhoeal Root bark- cytotoxicity, anti-inflammatory, antibacterial, antiulcer	Stem bark- Hexadecane, oleic acid, squalene, tetratriacontane Leaves- saponins, tannins, and phlobatannins Root wood- sterols and triterpenes, polyphenols, reducing compounds, and flavonoids	Leaves and fruits- moisture, ash, fibre, fats, proteins Flower- moisture, ash, protein, carbohydrate, lipid, fiber, Na, K, Mg, P, Ca, N Roots- moisture, ash, fibre, protein, carbohydrate, fats	(Ajaiyeoba et al., 2006; Awa et al., 2012; Bongo et al., 2017; Donhouedé et al., 2023; Hamisu et al., 2023; Ilboudo et al., 2019; Konaté et al., 2021; Lino and Deogracious, 2006; Mashile et al., 2019a, b; Megwas et al., 2020; Mubarak et al., 2022; Ogbadoyi et al., 2007; Okoli et al., 2010; Shackleton et al., 2000; Shai et al., 2020; Suleiman et al., 2008; Tukur et al., 2020)
<i>Carissa spinarum</i> L. Family: Apocynaceae Habit: Shrub	Mothokolo (PI); ntshuguru (XT); climbing num-num (E); ranknoemn oem (A); murungulu (V); umlugulu (Nd)	Least concern	Limpopo and Mpumalanga	Edible fruit- beverages/wine	Root decoction- body pains, ear problems, vomiting blood	Berry	Leaves- antinociceptive, antibacterial Roots- anthelmintic, antibacterial, antifungal, wound healing Root bark- antioxidant, anti-inflammatory, hepatoprotective	Leaves- Tannins, saponins, alkaloids, flavonoids, glycosides, terpenoids, Anthraquinones	Fruit- moisture, protein, fat, ash, fiber, carbohydrate, energy, vitamin C, K, Ca, Fe, Zn, Mn	(Ahmad and Mir, 2022; Ali and Engidawork, 2022; Ayalew Tiruneh et al., 2022; Feyissa and Melaku, 2016; Harwansh et al., 2010; Liu et al., 2021; Mashile et al., 2019a, b; Mworio et al., 2015; Sanwal and Chaudhary, 2011; Shackleton et al., 2000; Siyum and Meresa, 2021; Tshikalange et al., 2016)

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<i>Asparagus exuvialis</i> Burch. Family: Asparagaceae Habit: Shrub	Nkwangula tilo lowuntsongo (XT)	Least concern	Mpumalanga	Not specified	Root decoction- Back pains Fatigue	Berry	Mucilage- anti-ulcer, toxicity	Mucilage-alkaloids, saponins, tannins, flavonoids, and glycosides	Mucilage-carbohydrates, proteins	(Shatri, 2023; Tshikalange et al., 2016)
<i>Laggera crispata</i> (Vahl) Hepper & J.R.I.Wood Family: Asteraceae Habit: Herb	Xikhwaxa (XT)	Least concern	Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West	Not specified	Root decoction-swollen stomach	Drupe	Aerial part-antioxidant, antibacterial	Aerial part-Guaiol, phytol, erucylamide	Not available	(Daubotei, 2021; Tshikalange et al., 2016)
<i>Cordia monoica</i> Roxb. Family: Boraginaceae Habit: Tree	Sandpaper Saucer-berry (E), Mpungwana (XT)	Least concern	KwaZulu-Natal, Limpopo, Mpumalanga	Not specified	Bark infusion-good luck	Berry	Roots- antibacterial	Leaves- Phytol acetate, n-hexadecanoic acid, neophytadiene, neopentyl hydroxyl acetate and nonacosane	Not available	(Omwenga et al., 2009; Sivakumar and Dhivya, 2015; Tshikalange et al., 2016)
<i>Elaeodendron transvaalense</i> (Burt Davy) R.H.Archer Family: Celastraceae Habit: Tree	bushveld saffron (E); lepelhout (A); uMgugudo (Z); shimapana (XT); monamane (S)	Near threatened	KwaZulu-Natal, Limpopo, Mpumalanga, North West	Not specified	Stem decoction-induce vomiting and goodluck	Drupe	Stem bark-antibacterial, anti-HIV, antifungal, pro-inflammatory Rootbark-hypoglycaemic, toxicity	Stem bark- lup-20(30)-ene-3 α ,29-diol; 6 β -hydroxylup-20(29)-ene-3-one; and 30-hydroxylup-20(29)-ene-3-one; 4'-O-methylepigallocatechin	Not available	(Deutschländer et al., 2009; Khumalo et al., 2019; Mamba et al., 2016; Tshikalange et al., 2016)
<i>Gymnosporia buxifolia</i> (L.) Szyszyl. Family: Celastraceae Habit: Tree	Stinking spike-thorn (E); gewone pendoring (A); ingqwanga	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo,	Not specified	Leaves and root decoction/infusion-epilepsy	Capsule	Cell biomass-antibacterial Leaves-antioxidant	Cell biomass-phenolics, flavonoids Leaves- D-mannitol or dulcitol, dihydro- β -	Not available	(Killian, 2009; Kumari et al., 2018; Tshikalange et al., 2016)

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	ne (Z); mnqaqoba, umhlongwe (X); sephatwa (S); motlhonu (T); sihlangu lesimnyama (W) ; tshiphandwa (V)		Mpumalanga , North West, Northern Cape, Western Cape					agarofuran sesquiterpenoid		
<i>Parinari curatellifolia</i> Planch. ex Benth. Family: Chrysobalanaceae Habit: Shrub	Mphola (PI); mbulwa (XT); hissing tree (E); grysappel (A); mobola (T); muvhula (V)	Least concern	Limpopo and Mpumalanga .	Edible fruit-syrups, soft porridge	Roots- pneumonia, sore eyes, ear problems	Drupe	Leaves- toxicity, anti-inflammatory, antibacterial, analgesic Stem- antibacterial Stem bark- antiplasmodial Root- antibacterial	Leaves- tannins, saponins, terpenoids, alkaloids, cardiac glycosides, flavonoids	Fruit- dry matter, ash, fibre, fat, protein, carbohydrate, K, Mg, Mn, Cu, Zn, Fe, P Leaves- K, Na, Ca, Al, Mn, Cr, Pb, Cu, Co, Cd, Ni	(Chatepa et al., 2018; Fakai et al.; Gororo et al., 2016; Ibibia et al., 2023a, b; Kahimbi et al., 2023; Mashile et al., 2019a, b; Mawire et al., 2021; Omoniwa et al., 2021; Peni et al., 2010; Shai et al., 2020; Sylvanus et al., 2014)
<i>Parinari capensis</i> Harv. Family: Chrysobalanaceae Habit: Shrublet	Mmolofasane (P); Dwarf Mobolaplum (E); Gruisappel (A)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga , and North-West.	Edible fruit-soft porridge, syrups	Roots- ear problems, sore eyes	Drupe	Whole plant- anticancer Stem- antimalarial Roots- antibacterial	Whole plant- 10 α -hydroxy-9 α -methyl-15-oxo-20-nor-kaur-16-en-19-oic acid γ -lactone (15-oxozoapatlin), diterpene lactones Stem- 13-methoxy-16,17-dibromide-15-oxozoapatlin	Fruit- moisture, protein, amino acids Leaves- dry matter, moisture, Ca, P, Mg, N, K, C	(Baumgärtel et al., 2022; Fouché et al., 2008; Garo et al., 1997; Gomes et al., 2019; Khalid, 2009; Mashile et al., 2019b; Mulyangote, 2016; Shai et al., 2020; Uys et al., 2002)

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<i>Combretum imberbe</i> Warwa. Family: Combretaceae Habit: Tree	Leadwood (E); hardekool (A); motswiri (S); mondzo (XT); motswere (T); umbondwe (Z); muhiri (V)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West	Not specified	Root/ stem infusion-menstruation	Nut	Leaves, stem-antioxidant Wood ash-antifungal	Leaves- tannins, saponins, terpenoids, steroids, cardiac glycosides, flavonoids	Leaves, stem-moisture, protein, energy, Ca, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Zn Leaves- dry matter, protein, fibre, P	(Dambe et al., 2015; Mathipa et al., 2022; Pelowetse et al., 2007; Tshikalange et al., 2016)
<i>Terminalia sericea</i> Burch. ex DC. Family: Combretaceae Habit: Tree	Mususu (V); silver cluster-leaf (E); vaalboom (A)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West, Northern Cape	Not specified	Decoction of tumours-tonsils	Nut	Leaves- antioxidant, hepatoprotective, antibacterial Fruit- antioxidant Roots- antibacterial, antifungal, anti-inflammatory, antioxidant, cytotoxicity Stem bark-antidiabetic, antioxidant	Root bark- Sericoside, sericic acid, resveratrol-3-O- β -rutinoside Fruit, root, leaves, stem- phenolics	Seeds- dry matter, organic matter, protein, fat, amino acids, fatty acids, fibre, ash, Ca, Mg, P, vitamin E, squalene	(Anokwuru et al., 2018; Chivandi et al., 2013; Mochizuki and Hasegawa, 2007; Moshi and Mbawambo, 2005; Mulaudzi et al., 2021; Nel et al., 2020; Nkobile et al., 2011; Sobeh et al., 2019; Tshikalange et al., 2016)
<i>Ipomoea oblongata</i> E.Mey. ex Choisy Family: Convolvulaceae Habit: Herb	Wild morning glory (E); krismisblossom (A); ubhoqo (Z); mothokgo (S)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West, Northern Cape	Not specified	Bulb decoction-asthma, high blood pressure	Capsule	Roots- antibacterial, antidiabetic, toxicity, antioxidant Leaves, stem-antibacterial, toxicity	Roots- saponins, steroids, triterpenoids, alkaloids, flavonoids, tannins	Roots- carbohydrates, protein	(Kose et al., 2021; Polori et al., 2021; Semenya et al., 2018; Tshikalange et al., 2016)

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<i>Diospyros lycioides</i> Desf. subs <i>Lycioides</i> Family: Ebenaceae Habit: Shrub	Bluebush, star-apple (E); bloubos (A); Muthala (V); Lethanyu (T); Monkga-nku (S); Umbhongisa (X); Umbulwa (Z); Umcafudane (W)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West, Northern Cape	Not specified	Root decoction- sexually transmitted diseases	Berry	Stem- antibacterial, anti-inflammatory Roots- antibacterial Leaves- antibacterial, antioxidant, antimetastatic, toxicity, anti-inflammatory	Leaves- flavonoids, tannins, saponins, terpenoids, cardiac glycosides, alkaloids, coumarin, triterpenoids, anthraquinone, phenolics Root, twigs- coumarin, triterpenoids, anthraquinone, tannins, alkaloids, steroids, flavonoids	Leaves- Ca, P, K, protein, Fruit- Ca, Cu, Fe, P, Mg, K, Na, Zn, carbohydrates, fibre, energy, flavonoids, protein,	(Bagla et al., 2016; Fawole et al., 2009; Kanyemba et al., 2022; Mbanga et al., 2013; Tshikalange et al., 2016; Wehmeyer, 1986)
<i>Diospyros mespiliformis</i> Hochst. ex A.DC. Family: Ebenaceae Habit: Tree	Ditsoma (PI); ntoma (XT); jackal-berry (E); jakkalsbesie (A); musuma (V)	Least concern	Limpopo and Mpumalanga	Edible fruit	Branches- epilepsy	Berry	Fruit- antioxidant, anti-inflammatory, antispasmodic, cytotoxicity, antiplasmodial, mito-protective Leaves- antioxidant, anti-inflammatory, toxicity, spasmolytic, wound healing Stem bark- antipyretic, anti-inflammatory, cytotoxicity, antioxidant, wound healing Roots- antioxidant, wound healing	Bark, leaf, root- Anthraquinones, alkaloids, flavonoids, saponin glycosides, steroids, tannins, volatile oils	Fruit- ash, carbohydrate, protein, moisture, fat, Ca, K, Mg, Na, P, S, Mn, Cu, Fe, Se, Zn Roots, leaves, and bark- moisture, ash, lipid, fibre, protein, N, carbohydrate	(Adzu et al., 2002; Ebbo et al., 2014, 2019b, 2022; Mashile et al., 2019a, b; Nitiéma et al., 2023a, b; Nyambe et al., 2019a; Olanlokun et al., 2021; Shackleton et al., 2000; Shai et al., 2020)

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
<i>Euclea crispa</i> (Thunb.) Gürke Family: Ebenaceae Habit: Tree	Motlhakola swifi (PI); xintomanto mane (XT), blue guarri (E); bloughwarr ie (A); umgwali (X); motlhalets ogane (T); idungamuz i (Z)	Least concern	Mpumalanga, Eastern Cape, and KwaZulu-Natal.	Edible fruit Leaves-herbal tea	Not specified	Berry	Leaves- antifungal, antibacterial, antioxidant, antiproliferative Stem bark- antibacterial, antidiarrhoeal	Stem bark- Tannins, saponins, flavonoids, cardiac glycosides, steroids Leaves- tannins, flavonoids, steroids, saponins, reducing sugars, cardiac glycosides	Leaves- energy, ash, protein	(Alayande et al., 2016, 2017b, 2018; Kujoana et al., 2023; Magama et al., 2003a; Mashile et al., 2019b; Palanisamy et al., 2019, 2020; Rademan et al., 2019; Tshikalange et al., 2016; Wallnöfer, 2001)
<i>Euclea divinorum</i> Hiern. Family: Ebenaceae Habit: Tree	Motlhakolane (PI); nhlangua (XT); magic guarri (E); towerghwarrie (A); umhlangula (Z); umdledany amatane (T); mutangule (V)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga.	Edible fruit	Not specified	Berry	Leaves- antifungal, antibacterial, antioxidant Stem- antibacterial, antifungal Chewing sticks- antibacterial, cytotoxicity Root- antifungal, antioxidant, cytotoxicity, diuretic, antimalarial	Root bark- alkaloids, cardiac glycosides, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenes, volatile oils Tender stems- cardiac glycosides, phenols, quinones, saponins, steroids, tannins, terpenes, volatile oils Leaves- volatile oils, terpenes, tannins, steroids, saponins, quinones, cardiac glycosides	Leaves- fibre, protein, ash, organic matter, P, N	(Al-Fatimi, 2019; Bogari et al., 2022; Girmaw and Engdawork, 2022; Habte et al., 2021; Kilonzo et al., 2019; Mashile et al., 2019a, b; Mbabazia et al., 2020; Ngari et al., 2013; Scogings et al., 2015; Shai et al., 2020; Wallnöfer, 2001; Woldemedhin et al., 2017)

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<i>Euclea natalensis</i> A.DC. Family: Ebenaceae Habit: Tree	Natal ebony, (E); natalghwarrie (A); umKhasa (X); umAnyathi (Z); umHlangula (XT)	Least concern	Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and Western Cape	Not specified	Roots, stem- Skin care, STI	Berry	Leaves- antiplasmodial, cytotoxic Roots- antifungal, antibacterial, antiviral, antischistosoma cytotoxic, antitubercular	Seeds and seedlings- naphthoquinones Root bark- Naphthoquinones, triterpenoids	Not available	(Bapela et al., 2008; Lall et al., 2005a, b, 2006; Lima et al., 2022; Sparg et al., 2000; Tajuddeen et al., 2022; Tshikalange et al., 2016; Wallnöfer, 2001; Weigenand et al., 2004)
<i>Jatropha zeyheri</i> Sond. Family: Euphorbiaceae Habit: Herb	Verfbol (A); sefapabadi (S); xidomeja (XT); mafuredonga (V); ugodide (Z)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West	Not specified	Chew bulb- miscarriage, testicle sores	Capsule	Roots- antibacterial, antioxidant, cytotoxicity, anticancer Leaves- antioxidant, antibacterial	Leaves- tannins, flavonoid, phenolics	Leaves- K, Ca, Mg, S, Ni, P, Al, Co, Cr, Na	(Bango and Mphosi, 2023; Mongalo et al., 2013, 2019; Mutshekwa et al., 2019; Sehlapelo et al., 2021; Tshikalange et al., 2016)
<i>Abrus precatorius</i> L. subsp. africanus Verdc. Family: Fabaceae Habit: Liana	Bead vine (E); amaboep (N); nsimani (XT); umkhokha (Z)	Least concern	Eastern Cape, KwaZulu-Natal, Limpopo, Mpumalanga	Not specified	Decoction of whole plant- Kidney problems Blood in urine	Pod	Seed, leaves, stem- antibacterial, antifungal, antioxidant	Seed, root, leaves- tannin, saponins, alkaloids, flavonoids, terpenoids, steroids, phenols	Seeds- ash, fibre, fat, moisture, Na, K, Ca, Mn, P, Fe, Zn, Cu, Mn Leaves- Moisture, ash, fibre, protein, fat, carbohydrate, Na, Fe, Zn, Ca, K, Mg	(Adelowotan et al., 2008; Palvai et al., 2014; Paul et al., 2013; Sunday et al., 2016; Tresina and ramasamy Mohan, 2012; Tshikalange et al., 2016)
<i>Albizia harveyi</i> E.Fourn. Family: Fabaceae Habit: Tree	Bosveld-vals doring (A), bushveld false-thorn	Least concern	Limpopo, Mpumalanga	Not specified	Root decoction- cleansing ceremonies	Pod	Leaves- antibacterial Roots- toxicity Stem bark- antioxidant,	Leaves- Tannins, saponins, flavonoids Steroids,	Leaves- dry matter, protein, ash, fibre	(Makoye et al., 2020; Moshi et al., 2003; Shayo and Udén, 1999; Sobeh et al., 2017;

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	(E), mohlalag akga (S), muvhola (V), Umbola (Nd)						antidiabetic, hepatoprotective	terpenoids, phenolics Stem bark- tannins, flavan-3-ol derivatives		Tshikalange et al., 2016)
<i>Chamaecrista capensis</i> (Thunb.) E.Mey. var. <i>capensis</i> Family: Fabaceae Habit: Herb	Mahlakule (XT)	Least concern	Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West	Not specified	Root decoction- witchcraft	Pod	Not available	Not available	Not available	(Tshikalange et al., 2016)
<i>Dalbergia melanoxylon</i> Guill. & Perr. Family: Fabaceae Habit: Tree	African ebony (E), sebrahout (A); mogôrôgôr ô (S); xipalatsi (XT); muuluri (V); umphhingo (Z)	Least concern	Limpopo, Mpumalanga	Not specified	Root decoction- rash	Pod	Leaves- antioxidant Bark- antibacterial, antifungal, antioxidant	Leaves- tannins, saponins, glycosides, flavonoids, anthroquinones Bark- alkaloids, flavonoids, triterpenes, tannins, saponins, glycosides	Leaves- proteins, moisture, ash, fibre	(Najeeb et al., 2018; Swetha, 2017; Tshikalange et al., 2016; Vyas and Naik, 2019)
<i>Dichrostachys cinerea</i> subsp. <i>nyassana</i> (Taub.) Brenan Family: Fabaceae Habit: Shrub	Grootblaar-sekelbos (A); Kalahari Christmas Tree (E); Muunga (V); Ugagane (Z)	Least concern	Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga	Not specified	Root and pods decoction/ infusion- snake bite, wounds	Pod	Root- antioxidant, antibacterial Leaf- analgesic, anti-inflammatory, antioxidant, antibacterial Stem bark- analgesic, anti-inflammatory, antioxidant, antiparasmodial, toxicity, antibacterial	Leaves- Alkaloids, glycosides, flavonoids, saponins, steroids Fruit- tannins, phenolics	Fruit- dry matter, organic matter, fibre, N Root- ash, proteins, carbohydrates	(Bolleddu et al., 2019; Kweyamba et al., 2019; Mlambo et al., 2004; Susithra and Jayakumari, 2018; Tshikalange et al., 2016)

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<i>Macrotyloma maranguense</i> (Taub.) Verdc. Family: Fabaceae Habit: Tree	Xikondlo (XT), Mokorola kgogo (PI)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit	Bulb-chewed to treat swollen testicles	Pod	Not available	Not available	Not available	(Shai et al., 2020; Tshikalange et al., 2016)
<i>Mundulea sericea</i> (Willd.) A.Chev. Family: Fabaceae Habit: Tree	Rhodesian silver-leaf (E); kurkbos (A); mosetla-thlou (S); umSindandlovana (W); ntsandzandlopfu (XT), moswaatlou (T); mukunda-ndou (V); umHlalante the, umSindandlovu (Z)	Least concern	Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North West	Not specified	Roots-added to bath water to release nervous tension	Pods	Leaves- antioxidant, antiparasitodal, antileishmanial, antifungal, antibacterial Bark- anticancer, antidiabetic, anti-inflammatory, antioxidant, antifungal, antibacterial, toxicity Twigs- antibacterial, antifungal	Leaves- morphinan, agarospirol, cinnamic acid Leaves, stem bark, twigs- 5-methoxy mundulin	Not available	(Chepkirui et al., 2021; Gangadevi et al., 2020, 2021a, b; Gangadevi et al., 2020; Khyade and Waman, 2017; Langat et al., 2012; Mazimba et al., 2012a, b; Tshikalange et al., 2016)
<i>Ormocarpum trichocarpum</i> (Taub.) Engl. Family: Fabaceae Habit: Tree	Caterpillar Bush (E); Isithibane (Z); Mosepe (S); muthari (V), rusperboontjie (A), umsindandlovana (Z)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Not specified	Inner bark of roots infusion-erectile dysfunction	Pods	Aerial parts- antiparasitodal, antibacterial, cytotoxicity Leaves- antibacterial, antioxidant, mutagenic and antimutagenic	Leaves- Terpenoids, flavonoids	Not available	(Chukwujekwu et al., 2012, 2013; Jacqueline et al., 2018; Tshikalange et al., 2016)

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<i>Philenoptera violacea</i> (Klotzsch) Schrire Family: Fabaceae Habit: Tree	Apple-leaf (E); appelblaar (A); umBhandu (Z)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Not specified	Root infusion- induce vomiting	Pods	Leaves, flower, twigs- anticancer, antioxidant, antibacterial	Twigs, leaves, flowers- tannins, flavonoids, steroids, terpenoids, alkaloids, cardiac glycosides	Not available	(Manduna et al., 2014; Mfengwana and Mashele, 2016; Ntsoelinyane, 2014; Tshikalange et al., 2016)
<i>Peltophorum africanum</i> Sond. Family: Fabaceae Habit: Tree	African blackwood (E); boerboon (A); umthobo (Z); mosese (S); mosêtlha (W); musese (V); ndzedze (XT)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North West	Not specified	Root decoction- body pains	Pods	Leaves- hepatoprotective, antioxidant, anti-inflammatory Stem bark- antibacterial, anticancer, antifungal, antioxidant, antiviral, cytotoxicity Roots- antioxidant	Leaves- saponins, flavonoids, tannins, phenols, glycosides, terpenoids, phlobatannins Stem bark- polyphenols	Leaves- ash, fibre, protein, energy, fat, amino acids Stem bark- Al, Cl, Na, N, S, C, O, Ti, Si, Au, Cu, Zn, K	(Adebayo et al., 2017; Bizimenyera et al., 2007; Ebada et al., 2008; Mudau et al., 2021, 2022; Okeleye et al., 2013, 2015, 2017, 2019; Theo et al., 2009; Tshikalange et al., 2016)
<i>Pterocarpus angolensis</i> DC. Family: Fabaceae Habit: Tree	Transvaal teak (E); wilde-kiaat (A); morôtô (S); mokwa, morotômad i (Tswana); umvangazi, umbilo (Z)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Not specified	Root decoction- Heartburn, stomach problems, induces vomiting	Pods	Leaves- antibacterial, antifungal Bark- antibacterial, antifungal, antioxidant, antiplasmodial, anti-inflammatory, cytotoxicity Fruit- antibacterial, antifungal Root- antibacterial, antifungal	Stem bark- glycosides, ketone, saturated and unsaturated fatty acids, alcohols, sterols Stem bark- tannins, saponins Leaves- flavonoids, tannins, terpenes Fruit- tannins Root- saponins	Leaves- protein, fibre, Na Mg K Ca P	(Abubakar and Majinda, 2016; Anokwuru et al., 2017; Chipinga, 2018; Holdo, 2003; Sigidi et al., 2016; Tshikalange et al., 2016; Zininga et al., 2017)
<i>Pterocarpus rotundifolius</i> (Sond.) Druce	Muhataha (V); round-leaved	Least concern	Gauteng, KwaZulu-Natal,	Not specified	Ground root- treat fertility in cows	Pods	Not available	Dalbergieae lectins	Leaves-protein, fibre, Na, Mg, K, Ca, P	(Holdo, 2003; Nascimento et al.,

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Family: Fabaceae Habit: Tree	bloodwood (E); dopperkiaat (A)		Limpopo, Mpumalanga, and North West							2020; Tshikalange et al., 2016)
<i>Schotia brachypetala</i> Sond. Family: Fabaceae Habit: Tree	African walnut (E); huilboerboon (A); umfofofo (X), ihluze (Z), molohe (S); mulibi (V); nwavilombe (XT); umutwa (T); uvovovo (W)	Least concern	Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, and Mpumalanga	Not specified	Seeds, roots decoction- Shoulder and sternum pains	Pods	Leaves- antibacterial, antioxidant, anti-inflammatory Bark- antibacterial Aril of seeds- antioxidant, antibacterial, antimalarial Root bark- antibacterial Roots- antibacterial Seed pods with seeds- antibacterial Flowers- antibacterial Young stems- antibacterial	Leaves- polyphenols, flavonoids, gallic acid Aril of seeds- quercetin glucoside derivatives	Leaves- ash, fibre, protein, carbohydrates, energy, fat	(Du, 2011; Du et al., 2014; El-Hawary et al., 2015; McGaw et al., 2002; Mudau et al., 2021; Sobeh et al., 2016; Tshikalange et al., 2016)
<i>Senna italica</i> subsp. <i>arachoides</i> (Burch.) Lock Family: Fabaceae Habit: Shrub	Eland's pea, wild senna (E); swartstorm, wilde ertjies (A)	Least concern	Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, and North West	Not specified	Root decoction- sexually transmitted infections	Pods	Aerial parts- antibacterial, antifungal, anticancer, antioxidant Leaves- antinociceptive Roots- antioxidant, antibacterial, antiproliferative	Roots- flavonoids, prosalins, caretenoids	Leaves- fibre, protein	(Hembapu et al.; Madkour et al., 2017; Masoko et al., 2010a; Olorukooba et al., 2023; Tshikalange et al., 2016)
<i>Piliostigma thonningii</i> (Schumach.) Milne-Redh. Family: Fabaceae Habit: Tree	Camel's foot (E); kameelspoor (A); mukolokote (V); mokgoropo	Least concern	Limpopo and Mpumalanga	Edible fruit	Not specified	Pod	Leaves- antibacterial, analgesic, antimalarial, anti-inflammatory Stem bark- cytotoxic,	Leaves- saponins, alkaloids, tannins, flavonoids Stem bark- Alkaloids, flavonoids, steroids,	Leaves- carbohydrate, protein, ash, fat, energy, moisture, fibre Stem bark and roots- moisture,	(Alagbe, 2019; Bunney et al., 2019; Igbe et al., 2012; Ighodaro et al., 2012; Jimoh and Oladiji, 2005; Kwaji et al., 2010;

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	(S); nkolokotso (Z)						antioxidant, anti-inflammatory, analgesic, lipoxygenase inhibition	terpenoids, saponins, carbohydrates Seeds- saponins, flavonoids, phenolics, glycosides, anthraquinone, cardiac glycosides	dry matter, protein, fibre, carbohydrate, energy, Ca, P, K, Mg, Zn, Mn, Fe, Na, Cu, Co, Cr, Se, Cd, Pb Seeds- moisture, ash, fibre, protein, fat, Fe, Se, Zn, Mn, P, Ca	Marquardt et al., 2020; Mashile et al., 2019b; Noufou et al., 2016; Olela et al., 2020; Seyi et al., 2022)
<i>Senegalia nigrescens</i> (Oliv.) P.J.H. Hurter Family: Fabaceae Habit: Tree	Knob thorn (E); knoppiesdoring (A); muunga (V); mooka (S); umKhaya (Zulu)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North West	Not specified	Stem infusion-diarrhoea	Pod	Stem bark- antibacterial, antifungal, cytotoxicity, antioxidative, anticancer Roots, leaves- antibacterial	Stem bark- 3 β -hydroxy-20(29)-en-lupan-30-al; melanoxetin; quercetin; quercetin-3-O-methyl ether	Leaves- fibre, protein, ash, fat, carbohydrates, energy	(Bodede et al., 2018, 2021; Mudau et al., 2021)
<i>Vachellia karroo</i> (Hayne) Banfi & Glasco Family: Fabaceae Habit: Tree	Sweet thorn (E); soetdoring (A); mookana (S); mooka (W); umuNga (Z)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, and Western Cape	Not specified	Root decoction- sexually transmitted infections	Pod	Leaves- antifungal, antioxidant, cytotoxicity, anti-inflammatory, Pods- toxicity	Leaves- catechin, epicatechin, biocalcin, quercetin	Leaves- organic matter, protein, ash	(Maposa et al., 2020; More et al., 2021a, b; Ravhuhali et al., 2022)
<i>Vachellia nilotica</i> (L.) P.J.H.Hurter & Mabb. Family: Fabaceae Habit: Tree	Scented-pod acacia (E); lekkerruikp eul (A); Mogohlo	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga	Edible fruit	Not specified	Pod	Bark- antiviral, toxicological, antibacterial Leaves- toxicological, antibacterial	Leaf, stem bark, pods- phenols, flavonoids	Seed pods and shoots- protein, fibre, energy, N, K, Ca, P, Na, Fe, Mn, Zn, Cu	(Abdallah et al., 2021; Donalisio et al., 2018; Manganyi et al., 2023; Mashile et al., 2019a, b; Okoro et al., 2014)

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	(S); umNcawe (W); Motsha (T); umNqawe (Z)		, and North West				Roots- antibacterial, toxicological			
<i>Hypoxis hemerocallidea</i> Fisch. & C.A.Mey. Family: Hypoxidaceae Habit: Herb	Gifbol (A), Inkomfe (Z), Star-flower (E), Mbhumbhunu (XT)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West	Not specified	Bulb decoction-high blood pressure	Capsule	Bulb- antibacterial, antifungal, antioxidant, toxicity	Bulb- Undecane, benzothiazole, heptacosane, 2,7-Octanedione, α -Myrcene	Leaf, corm, peels, roots- protein, fat, ash, fibre, dry matter, Ca, Mg, K, Na, P, Zn, Cu, Mn, Fe, Ce, Fe, Si	(Afolayan and Otunola, 2014; Mwinga et al., 2019a; Otunola and Afolayan, 2019; Tshikalange et al., 2016)
<i>Strychnos madagascariensis</i> Poir. Family: Loganiaceae Habit: Tree	Mogwagwa (P); nkwaka (XT); black monkey orange (E); swartklapper (A); mogorwagorwana (T); mukwakwa (V); umKwakwa (Z)	Least concern	Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North West.	Edible fruit	Decoction/ infusion of leaves and roots-wounds Roots-induce vomiting	Berry	Fruit- cytotoxicity, antibacterial, antidiabetic, antioxidant Bark- antifungal Leaves- antibacterial, antifungal Seed coat- antioxidant, antidiabetic	Leaves- Alkaloids, flavonoids, steroids, triterpenoids, saponins, tannins Seeds- pyrazole, vinyl acrylate, ethylene acetal, furfuryl alcohol, furaneol, butanoic acid	Seeds- fat, fibre, sugar, starch, protein, Ca, Mg, K, P, Na, Zn, Mn, Fe, Cu Fruit- moisture, ash, protein, fibre, fat, carbohydrate, K, Mg, N, Ca, Na, P, Fe, Zn, Cu	(Bunney et al., 2019; Mashile et al., 2019a, b; Nciki et al., 2016; Nhaca et al., 2020; Oboh et al., 2020a, 2023; Shackleton et al., 2000; Shai et al., 2020; Tshikalange et al., 2017; Van Rayne et al., 2020; Van Vuuren et al., 2015)
<i>Strychnos spinosa</i> Lam. Family: Loganiaceae Habit: Tree	Moshala (P); nsala (XT); spiny monkey orange/green monkey orange (E); doringklap	Least concern	Eastern Cape, KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit	Not specified	Berry (Bunney et al., 2019)	Fruit- antioxidant Leaves- antifungal, antioxidant, antibacterial, antitrypanosomal Root bark- antibacterial,	Leaves- Alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, steroids, tannins	Fruit- dry matter, ash, protein, fat, fibre, vitamin C, P, Ca, Mg, Fe, K, Na, Zn, Cu, Mn	(Amarteifio and Mosase, 2006; Hoet et al., 2006; Isa et al., 2014; Lawal et al., 2019; Mashile et al., 2019a, b; Mause et al., 2021b; Mbunde et

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	per (A); umKwakwa (T); muramba (V)						antifungal, antioxidant	(Nhaca et al., 2020)		al., 2019; Shackleton et al., 2000; Shai et al., 2020; Tor-Anyiin et al., 2015)
<i>Abutilon fruticosum</i> Guill. & Perr. Family: Malvaceae Habit: Shrub	Shrubby Abutilon (E)	Least concern	Limpopo, Mpumalanga	Not specified	Root decoction- Cramps Muscle pulls	Capsule	Aerial parts- antibacterial, antifungal	Aerial parts- flavonoids, tannins, sterols, terpenes, quercetin, rutin, synaptic acid, diosmin Leaves- Palmitic acid, neophytadiene, tetradecanoic acid, Azulene	Aerial parts- carbohydrates Leaves- Carbohydrates, proteins, fat	(Bano and Deora, 2020; Gouda et al., 2022; Tshikalange et al., 2016)
<i>Grewia flavescens</i> Juss. Family: Malvaceae Habit: Tree	Mopharats hena (PI); nsihana (XT); square-stemmed raisin (E); skurweblaa rrosyntjie (A); loklolo (W); motuu (T); mupharash eni (V); ilalanyathi (Z)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and Northwest.	Edible fruit-beverage or juice	Not specified	Drupe	Leaves- antidiabetic, antibacterial, toxicity Stem bark- immunomodulatory, cytotoxic Whole plant- anthelmintic	Leaves, fruit- Triterpenes	Fruit- K, Ca, Mn, Fe, Cu, Zn, amino acids, moisture, fibre, ash, protein, fat, Carbohydrate, sugar, starch	(Elhassan and Yagi, 2010; Elhassan et al., 2021; Kumar et al., 2022; Mashile et al., 2019b; Motlhanka et al., 2008; Sana et al., 2023; Shai et al., 2020; Yanadaiah, 2013)
<i>Grewia occidentalis</i> L. Family: Malvaceae Habit: Shrub	Ross-berry (E), kruisbessie (A), mogwane (S),	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal,	Not specified	Stem-magic	Berry	Bark- antioxidant, toxicity Shoots- antibacterial Leaves and twigs- antifungal	Bark and wood- Sinapaldehyde (E)-4-hydroxy-3,5-dimethoxycinnam aldehyde); coniferaldehyde	Leaves- protein, crude fibre, ash, fibre, Ca, Na, Fe, Cu, Zn, Mn, Mg, K	(Afolayan et al., 2002; Grierson and Afolayan, 1999; Kumar et al., 2022; Mthi et al., 2016; Steenkamp et al.,

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	iLalanyathi (Z), umSipane (W), umNqabaza (X), Mulembu (V), Nsihana (XT)		Limpopo, Mpumalanga, North West, Northern Cape, Western Cape							2005; Tshikalange et al., 2016)
<i>Sida rhombifolia</i> L. Family: Malvaceae Habit: Shrub	Ivivane (W), Pretoria Bossie (A), Pretoria Sida (E), Tihoveta (XT)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West	Not specified	Ground whole plant- anti dandruff	Capsule	Whole plant- anti-inflammatory, toxicity, anti-cholinergic, antiplasmodial Leaves- analgesic, hypoglycaemic, antidiabetic Fruit- antibacterial Root- anti-inflammatory	Whole plant- rhombifoliamide, xylitol dimer, oleanolic acid, b-amyryn glucoside, ursolic acid, b-sitosterol glucoside, tiliroside	Aerial parts and roots- moisture, ash, Na, Ca, K, Co, Cu, Mg, Zn	(Gangadhar et al., 2019; Kamdoum et al., 2022; Logeswari et al., 2013; Mah et al., 2017; Rao and Mishra, 1997; Sarangi et al., 2010; Shaheen et al., 2017; Tshikalange et al., 2016)
<i>Trichilia dregeana</i> Harv. & Sond. Family: Meliaceae Habit: Tree	Forest mahogany (E); rooiessenhout (A); uMathunzi ni (Z); umKhuhlu (Xhosa); mmaba (S); mutshikili (V)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit	Not specified	Capsule	Leaves- antibacterial, antifungal, antioxidant, anti-cholinesterase mutagenic, anti-inflammatory, wound healing, toxicity	Leaves- Cycloart-23-ene-3,25-diol, lyoniresinol, maslinic acid, asperphernamate	Seeds- protein, fat, ash, moisture, K, P, S, Mg, Ca, Cu, Mn, B, Fe, Zn Fruit- sugar, moisture, protein, fat	(Eldeen et al., 2007; Fotso Tatso et al., 2023; Mashile et al., 2019a, b; Shewaye et al., 2023; Tsomele et al., 2021; Wilson and Downs, 2012)

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<i>Trichilia emetica</i> Vahl. Family: Meliaceae Habit: Tree	Natal mahogany (E); rooiessehnout (A); mamba (S); umathunzi ni (Z); umkuhlu (W); umkhuhlu (X); nkulu (XT); mutuhu (V)	Least concern	KwaZulu-Natal and Mpumalanga	Edible fruit-beverage/juice	Roots, stem infusion-painful feet, stomach cleansing Bark, roots-kidney problems	Capsule	Leaves- antidiarrheal, antiparasitodal, antibacterial Seeds- antioxidant, cytotoxic, antifungal, antiparasitodal, antitrypanosomal Stem bark- antibacterial, antifungal, antiparasitodal, antitrypanosomal	Bark- Trichirokin, rohituka-3, rohituka-9 Leaves- phenolics, flavonoids Seeds- alkaloids, cardiac glycosides, flavonoids, phenols, sterols, terpenoids	Seeds- dry matter, fat, fibre, ash, protein, energy, carbohydrate, protein Seed oil- Ca, Cr, Cd, Zn, Cu	(Adinew, 2014, 2015; Atindehou et al., 2004; Babalola and Adelakun, 2018; Konaté et al., 2015; Perumal et al., 2020a, b; Rukayyah et al., 2015; Shackleton et al., 2000; Shai et al., 2020; Tshikalange et al., 2016; Tsopgni et al., 2019)
<i>Ficus sur</i> Forssk. Family: Moraceae Habit: Tree	Mogo (PI); broom cluster fig (E); besem-trosvy (A); umkhiwane (X and Z)	Least concern	Eastern Cape, KwaZulu-Natal, Limpopo, Mpumalanga, and Western Cape.	Edible fruit-jam	Bark- chest problems	Drupe	Leaves- toxicity, antioxidant, diuretic Stem bark- toxicity, anti-inflammatory, antioxidant Fruit- antibacterial, antioxidant Roots- antioxidant	Fruit and leaves- β -sitosterol, lupeol, epicatechin, phaeophytin A	Bark and leaves- ash, fibre, fat, moisture, protein, carbohydrate, Mg, Mn, Fe, K, Cu, Ca, Na, Zn, Ni Fruit- Mg, Mn, Fe, Cu, Zn, Cd, K, Ca, Pb	(Akesa et al., 2017; Akoto et al., 2020; Ayele et al., 2020; Mashile et al., 2019a, b; Odusanmi, 2017; Ogunlaja et al., 2022; Pawlos et al., 2021; Saloufou et al., 2018; Shai et al., 2020; Sieniawska et al., 2022)
<i>Ficus thonningii</i> Blume Family: Moraceae Habit: Tree	Xirhombe, xirhomberh ombe, nhlulawu mbe (XT); mokumo (PI)	Least concern	Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North-West.	Edible fruit-jam	Root infusion- eye problems	Drupe	Leaves- antimicrobial, antioxidant, analgesic, anti-inflammatory, cytotoxicity, antimalarial Stem bark- antiulcer Fruits- antiulcer	Fruit and roots- Thonningiol, thonningiisoflavan, β -sitosterol	Leaves- protein, fibre, Al, N, K, Ca, Mg, Na, Mn, Fe, S	(Akesa et al., 2017; Falade et al., 2014; Fokunang et al., 2019; Fongang et al., 2015; Mashile et al., 2019a, b; Otimenyin et al., 2004; Shai et al., 2020; Tegbe et al., 2006; Tegegne,

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										2008; Tshikalange et al., 2016; Uku et al., 2020; Yahaya et al., 2021b)
<i>Syzigium cordatum</i> Hochst. Ex Krauss Family: Myrtaceae Habit: Tree	water berry (E); waterbessie (A); umdoni (Z); umjomi (X), mawthoo (Southern Sotho), mutu (V); muhlwa (XT)	Least concern	KwaZulu-Natal, Mpumalanga, and Western Cape.	Edible fruit-alcoholic beverage.	Roots-tuberculosis	Berry	Leaves- antidiabetic, antidiarrhoeal, antibacterial, antifungal, anti-inflammatory, mutagenic Bark- antioxidant, cytotoxicity	Bark- alkaloids, flavonoids, glycosides, phenolic acids, terpenoids, coumarins Fruit- phenolics, flavonols Leaves- toluene, triacetin, silane, ledol	Fruit and seeds- moisture, fat, fibre, ash, protein, carbohydrates, energy, K, Ca, Na, Zn, Fe, Ag, B, Al	(Chalannavar et al., 2011; Cordier et al., 2013; Deliwe and Amabeoku, 2013; Maliehe, 2015; Mashile et al., 2019b; Mulaudzi et al., 2012; Ndhlela, A. et al., 2008; Shai et al., 2020)
<i>Syzygium intermedium</i> Engl. & Brehmer Family: Myrtaceae Habit: Tree	Intermediate Waterberry (E), Dithlo (PI)	Least concern	Mpumalanga	Edible fruit	Not specified	Berry	Not available	Not available	Not available	(Shai et al., 2020)
<i>Syzygium guineense</i> DC. Family: Myrtaceae Habit: Tree	Waterberry (E); waterpeer (A); umdoni (Nd)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit	Not specified	Berry	Leaves- antimalarial, antiulcer, analgesic, anti-inflammatory, antioxidant, antidiarrhoeal, antihypertensive, vasodepressor Stem bark- anti-tuberculosis, antibacterial	Leaves- Fatty acids, Hydrocarbons, terpenes, organic acids	Fruit- Ca, K, P, S, Mn, Fe, Ti, Zn, Rb, Sr, Zr, protein, fibre, ash, fat	(Abok and Manulu, 2017; Ayele et al., 2010; Edewor et al., 2021; Ezenyi and Igoli, 2019; Ezenyi et al., 2019; Ior et al., 2012; Koval et al., 2018; Maregesi et al., 2016; Mavanza et al.; Oladosu et al., 2017; Shackleton et al., 2000; Sibiya et al., 2021; Tadesse and Wubneh, 2017)

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
<i>Ochna natalitia</i> Walp. Family: Ochnaceae Habit: Tree	Natal plane (E); natalrooiho ut (A); mbovu (Z); tshipfure (V); isibomvu (X)	Least concern	Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North West	Not specified	Root decoction-painful joints	Drupe	Leaves-antibacterial, antifungal, antioxidant, mutagenicity, cytotoxicity, genotoxicity,	Leaves-phytosterols, anthraquinones, tannins, flavonoids, terpenoids	Not available	(Chemane, I. et al., 2022; Makhafola, 2012; Makhafola et al., 2014; Ngubane et al., 2024; Shah et al., 2018; Suleiman et al., 2010; Tshikalange et al., 2016)
<i>Jasminum abyssinicum</i> Hochst. ex DC. Family: Oleaceae Habit: Shrub	Mthundang azi (Z)	Least concern	KwaZulu-Natal, Limpopo, Mpumalanga	Not specified	Root decoction-bladder cleaner	Berry	Leaves- antioxidant, anti-proliferative, antibacterial Root- analgesic, anti-inflammatory	Root- flavonoids, glycosides, saponins, secoiridoid glucosides, terpenoids, triterpenes	Not available	(Gallo et al., 2006; Habtamu et al., 2010; Tadiwos et al., 2017; Tauchen et al., 2015; Tshikalange et al., 2016)
<i>Jasminum fluminense</i> subsp. <i>Fluminense</i> Family: Oleaceae Habit: Shrub	Maloyana (XT)	Least concern	KwaZulu-Natal, Limpopo, Mpumalanga	Not specified	Root decoction-sexually transmitted infections	Berry	Leaves, flower-antiplasmodial	Leaves- Phytol, hexadecanoic acid, linolenic acid, squalene Flower- Phenylethanolamine, cinnamic acid	Leaves, flowers-carbohydrates, proteins	(Arivoli et al., 2018; Tshikalange et al., 2016)
<i>Antidesma venosum</i> E.Mey. ex Tul. Family: Phyllanthaceae Habit: Tree	Tasselberry (E); Tosselbessie (A); Isiqutwane (Z); Segagama (T); Mufhala-khwali (V); Umtiyongi (X)	Least concern	Eastern Cape, KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit	Root decoction-fertility in women	Berry	Leaves-antibacterial Roots- antibacterial, antifungal, toxicity Stem bark-antibacterial, antifungal, toxicity	Stem, leaves-Loliolide, β -sitosterol 3-O- β -D glucoside, lutein, 4-hydroxyphenylethyl trans-ferulate, pheophytin A, pheophytin B	Fruit- moisture, protein, amino acid content, sugars, fat, vitamin C, vitamin E, vitamin B ₁ and B ₂	(Adegoke et al., 2013; Baumgärtel et al., 2022; Hlengwa, 2018; Mashile et al., 2019a, b; Mwangomo et al., 2012; Shackleton et al., 2000; Tshikalange et al., 2016; Wilson and Downs, 2012)
<i>Bridelia micrantha</i> (Hochst.) Baill.	Motsere (PI); ndzerhe (XT); bruin	Least concern	Eastern Cape, KwaZulu-Natal,	Edible fruit	Bark-headache, sore eyes, toothache,	Berry	Bark- antioxidant, antibacterial, anticonvulsant, sedative,	Leaves- Phosphoric acid, Decanal, phenyl salicylate.	Fruit- sugar, moisture, protein, fat	(Adesina et al., 2020; Asumang et al., 2021; Bum et al., 2012; Mashile et

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
Family: Phyllanthaceae Habit: Tree	stinkhout (A); motsere (S); ndzerhe (T)		Limpopo, and Mpumalanga		and diarrhoea		antioxidant, antinociceptive, toxicity Leaves- antioxidant, antibacterial, antifungal, wound healing		Leaves- fibre, protein, Ca, P, Mg, Na, S, Fe, Mn, Cu, Mo, Co, Zn, Se	al., 2019a, b; Ngane, 2019; Ondiek et al., 2010; Onoja et al., 2014; Shai et al., 2020; Shelembe et al., 2016; Steenkamp et al., 2009; Wilson and Downs, 2012)
<i>Flueggea virosa</i> (Roxb. ex Willd.) Royle Family: Phyllanthaceae Habit: Tree	Motlhalabu (PI); white berry-bush (E); witbessies bos (A); mutangahuma (V); mpfalambati (XT); Muhlakau me (S)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit	Bark- chest problems	Berry	Leaves- antimalarial, analgesic, antiviral, toxicity, anti-inflammatory Stem- analgesic, anti-inflammatory Twigs- antiviral Roots- toxicity, anti-inflammatory, antipyretic	Roots- trinorditerpenes Fruit- flueindoline A, B, and C, norsecurinamines A and B	Not available	(Chao et al., 2014; Dénou et al., 2021; Ezeonwumelu et al., 2012; Mashile et al., 2019a, b; Qiu-Jie et al., 2020; Shai et al., 2020; Singh et al., 2017; Zhang et al., 2015)
<i>Phyllanthus reticulatus</i> Poir. Family: Phyllanthaceae Habit: Shrub	Potato plant (E); aartappelbos (A); umchumelo (Z); thethenya (XT)	Least concern	KwaZulu-Natal, Limpopo, Mpumalanga	Not specified	Root decoction- blood problems	Berry	Fruit- anti-inflammatory Leaves- antibacterial, antioxidant, analgesic, anti-inflammatory, CNS depressant Stem bark- antioxidant	Leaf, stem bark- tannins, alkaloids, reducing sugars, flavonoids, steroids, saponins, anthraquinones	Leaf, stem bark- ash, moisture, protein	(Begum and Rahaman, 2021; Haque et al., 2016; Khatun et al., 2013; Kumar et al., 2012; Tshikalange et al., 2016)
<i>Faurea saligna</i> Harv. Family: Proteaceae Habit: Tree	African beech (E); Bosveldboekenhout (A); iSefu (Z); umOnyeli (N);	Least concern	KwaZulu-Natal, Mpumalanga	Not specified	Leaf decoction- epilepsy	Nut	Stem bark- antioxidant, anti-inflammatory, toxicity Bark- antibacterial	Leaves- tannin Bark- tannin	Leaves- Ca, Cl, Cu, Fe, K, Mg, Mn, Na, Ni and P	(Ernst, 1975; Lawal et al., 2019; Mthethwa, 2009; Tshikalange et al., 2016; Würger et al., 2014)

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
	mohlako (S); muTango (V)									
<i>Berchemia discolor</i> Hemsl. Family: Rhamnaceae Habit: Tree	Mogokgom a (P); nyiri (XT); birdplum (E); voëlpruim (A); motsintsila (T); munie (V); uvuku (Z)	Least concern	KwaZulu-Natal, Limpopo, and Mpumalanga	Edible fruit-alcoholic beverage, porridge.	Bark, roots-wounds, bleeding gums	Drupe	Leaves- antioxidant, antifungal, antibacterial, antituberculosis Bark- antibacterial, antituberculosis	Bark, fruit, leaves- Alkaloids, anthraquinone, phlobatanins, saponins, steroids, tannins, terpenoids, phenols, cardiac glycoside, flavonoids	Leaves and shoots- fibre, protein, ash, P, K, Ca, Mg, Cu, Mn, Fe	(Cheikhoussef et al., 2010; Green et al., 2010; Kemboi et al., 2021; Gundidza and Sibanda, 1991; Machaba et al., 2024; Mashile et al., 2019b; Shai et al., 2013, 2020)
<i>Berchemia zeyheri</i> (Sond.) Grubov Family: Rhamnaceae Habit: Tree	Moneyi (PI); pink ivory (E), rooihout (A); umNeyi (W); xiniyani (XT); moye (T); muniane (V); umNini (X); umNini (Z)	Least concern	Eastern Cape, Limpopo, and Mpumalanga	Edible fruit	Root-headache	Drupe	Bark- antibacterial, toxicity, anthelmintic	Heartwood- Flavanone, isoflavanone-benzofuranoid	Leaves- ash, protein, fibre	(Bekker et al., 2000; Mashile et al., 2019a; McGaw et al., 2007; Mudau et al., 2021; Shai et al., 2020)
<i>Ziziphus mucronata</i> Willd. Family: Rhamnaceae Habit: Tree	Mothalo (PI); Ncecenyi, Mphasamhala (XT); buffalo thorn (E); blinkblaar-wag-'n-	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North-West,	Edible fruit	Leaves, roots mixed with lotion-skin problems	Drupe	Bark- antioxidant, antibacterial, antifungal Fruit- hepatoprotective, antioxidant Leaves-antioxidant, antibacterial, antidiabetic,	Root- Flavanols, flavonoids, phenols	Seeds- ash, fibre, protein, dry matter, P, Ca, K, Mg, Mn, Fe, Cu, Zn Leaves- ash, protein, fibre	(Abolaji et al., 2021; Adewusi and Steenkamp, 2011; Aganga and Mosase, 2001; Foyet et al., 2019; Ibrahim et al., 2012; Kwape et al., 2013; Mashile et al.,

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
	bietjie (A); isilahla (Z); umphafa (X); umlahlabantu (W); mokgalo (T); mutshetshete (V)		and Northern Cape.				anxiolytic, antidepressant, cytotoxicity, anticholinesterase Root- antioxidant			2019a, b; Motlhanka et al., 2008; Mthi et al., 2016; Nemudzivhadi and Masoko, 2015; Olajuyigbe and Afolayan, 2011, 2012; Tshikalange et al., 2016; Wado et al., 2020)
<i>Ziziphus zeyheriana</i> Sond. Family: Rhamnaceae Habit: Shrub	Mothalo fasane (PI); dwarf buffalo-thorn (E); haakbessie (A)	Least concern	Free State, Gauteng, Limpopo, Mpumalanga, and North-West.	Edible fruit	Not specified	Drupe	Not available	Not available	Not available	(Mashile, et al., 2019b)
<i>Agathisanthemum bojeri</i> Klotzsch Family: Rubiaceae Habit: Herb	Mavunge (XT)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West	Not specified	Root decoction-swollen testicles	Capsule	Aerial parts, roots-antifungal Leaves, stem-antibacterial, antioxidant, toxicity, antityrosinase Whole plant-antiplasmodial	Not available	Shoots, roots-P, C, N	(Lall et al., 2019; Muthaura et al., 2015; Runyoro et al., 2006; Treydte et al., 2006; Tshikalange et al., 2016)
<i>Canthium inerme</i> (L.f) Kuntze Family: Rubiaceae Habit: Tree	Turkey-berry (E); gewone bokdrol (A); umvuthwamini (X, Z); mvutwamira (W); muvhivvela-shadani (V)	Least concern	Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West, and Western Cape	Edible fruit	Not specified	Berry	Leaves-antibacterial, anthelmintic, anti-amoebic	Leaves- tannins, phenolics	Fruit- sugar, protein, fat, moisture Leaves- protein, fibre	(Bhatta et al., 2013; McGaw et al., 2000; Shai et al., 2020; Wilson and Downs, 2012)

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
<i>Pavetta gracilifolia</i> Bremek. Family: Rubiaceae Habit: Tree	Kleinbruids bos (A); small bride's bush (E)	Least concern	Eastern Cape, KwaZulu-Natal, Limpopo, and Mpumalanga	Not specified	Root decoction-painful feet	Drupe	Roots-antibacterial, antifungal	Not available	Not available	(Mophuting, 2015; Tshikalange et al., 2016)
<i>Vangueria infausta</i> Burch. Family: Rubiaceae Habit: Tree	Mmilo (PI); mpfilwa (XT); ild medlar (E); wilde mispel (A); muzwilu (V); umtulwa (Z); umvilo (X), umviyo (Nd), mmilo (T)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North-West, and Northern-Cape	Edible fruit-alcoholic beverage.	Bark, roots decoction-sexually transmitted infections/diseases, toothache Roots-snake bites	Drupe	Fruit- antioxidant, antibacterial, antifungal Leaves-hypolipidemic, antioxidant, antibacterial Root bark-antiplasmodial Roots- antibacterial, antifungal, antiviral, cytotoxicity	Root- Alkaloids, anthranoids, flavonoids, glycosides, polyphenols, saponins, tannins	Fruit- protein, moisture, dry matter, fat, carbohydrate, energy, N, P, K, Ca, Mn, Fe, Cu, Na, Zn, Mg	(Abosi et al., 2006; Amusan et al., 2007; De Boer et al., 2005; Gwatidzo et al., 2018; Kebonye et al., 2021; Kiteme et al., 2023; Maluleke et al., 2024; Mashile et al., 2019a, b; Mause et al., 2021b; Mothapo, 2014; Motlhanka et al., 2008; Mthethwa et al., 2014; Sanad, 2020; Shackleton et al., 2000; Shai et al., 2020; Tshikalange et al., 2016)
<i>Vangueria pygmaea</i> Schltr. Syn: <i>Pachystigma pygmaeum</i> (Schltr.) Robyns Family: Rubiaceae Habit: Subshrub	Mmilofasane (PI); Dwarf Crowned-medlar (E); Goubos A); Umkukuzela (Nd)	Least concern	Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North-West.	Edible fruit-alcoholic beverage.	Bark, leaves-sexually transmitted diseases or infections.	Drupe	Leaves- anticancer, toxicity (syn)	Leaves-pavettamine (polyamine) (syn)	Not available	(Bode et al., 2010; Ellis et al., 2010; Fourie et al., 1995; Mashile et al., 2019b; Saeed et al., 2016; Shai et al., 2020)

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
<i>Flacourtia indica</i> (Burm.f.) Merr. Family: Salicaceae Habit: Tree	Governor's Plum (E); muvhamba-ngoma (V); umqokolo (Nd)	Least concern	Eastern Cape, KwaZulu-Natal, Limpopo, Mpumalanga, and North West	Edible fruit	Not specified	Drupe	Fruit- antioxidant Stem bark- antioxidant Whole plant- antinociceptive, anti-inflammatory, diuretic, analgesic, antipyretic Leaves- antiasthmatic, antioxidant Roots- antifungal	Stem bark, leaves- phenols, flavonols Roots- Heneicosane, squalene Fruit- Rutin, salicylic acid glycoside, caffeoylshikimic acid	Fruit and seed- moisture, fat, protein, sugar, vitamin B ₁ , vitamin, C	(Alakolanga et al., 2014; Chun and Kundu, 2013; Eramma and Gayathri, 2021; Eramma and Patil, 2023; Islam et al., 2023; Misra and Misra, 2016; Selim et al., 2021; Shackleton et al., 2000; Swati et al., 2009; Tiwari, 2017; Tyagi et al., 2011)
<i>Pappea capensis</i> Eckl. & Zeyh. Family: Sapindaceae Habit: Tree	Bushveld cherry (E); doppruim (A); indaba (Z); ilitye (X); mongatane (S); liletsa (W); xikwakwax u, gulaswimbi (XT)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West, Northern Cape, and Western Cape	Not specified	Bark decoction- Penis enlargement, reduction of breasts in men	Berry	Leaves- antibacterial, antifungal, anticonoccal, antioxidant, cytotoxicity, antiplasmodial Twigs- antiplasmodial, cytotoxicity Whole plant- anticancer	Leaves- Epicatechin, isoquercitrin, quercitrin, juglanin, guaijaverin Stem bark- phenolics, flavonoids, terpenoids, fatty acids and derivatives	Fruit- ash, moisture, fat, fibre, carbohydrates Leaves, stem bark- fibre, protein, Se, Fe, Zn, Cr, Cu, Ni, Mn, Co, V, Mo, Al, Hg, Pb, vitamin C, vitamin A, vitamin E, vitamin B ₁ and B ₂	(Karau et al., 2012; Mabuza et al., 2022; Muruthi et al., 2023; Osuga et al., 2006; Paksio and Samson, 2021; Pendota et al., 2017; Potts et al., 2013; Sibiyi et al., 2021; Tajuddeen et al., 2021; Tshikalange et al., 2016)
<i>Solanum tomentosum</i> L. Family: Solanaceae Habit:	Slangappel bos (A); Nthomane (XT)	Least concern	Mpumalanga, Eastern Cape, Free State, Northern Cape, Western Cape	Not specified	Root infusion- eyes	Berry	Leaves- antifungal, antibacterial Stem- anticancer Roots- antifungal, anti-HIV, anti-inflammatory	Fruit- phenolics	Fruit- dry matter	(Aliero and Afolayan, 2006; Fouché et al., 2008; Kaushik et al., 2017; Mamba et al., 2016; Tshikalange et al., 2016)

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
<i>Ximenia americana</i> L. Family: Olacaceae Habit: Tree	Ditsadi (PI); ntsengele (XT); small blue sourplum (E); kleinblousu urpruim (A); umkholots hwana (Z); umtfundvuka (W); morotologana (T); mutanzwa (V)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North-West.	Edible fruit	Bark, roots-wounds, constipation	Drupe	Leaves- antioxidant, antibacterial, antidiabetic, anti-inflammatory, analgesic, antiproliferative, antitumor, toxicity Root- antitumor, antioxidant, toxicity Root bark- anti-inflammatory, toxicity Stem bark- antioxidant, antidepressant,	Leaves- Epicatechin, catechin, rutin, isoquercetin, avicularin	Fruit- moisture, ash, fibre, protein, fat, vitamin C, Na, K, Ca, Mg, P Leaves- moisture, fibre, protein, ash, N, Na, K, Ca, P, Mg Seeds- fat	(Abel et al., 2023; Bakrim et al., 2022; Feyssa et al., 2012; Hemamalini et al., 2011; Mashile et al., 2019a; Muhammad et al., 2019; Okhale et al., 2017; Olabissi et al., 2011; Pare et al., 2019; Shai et al., 2020; Shettar et al., 2015, 2017; Tanko et al., 2017; Togbossi et al., 2020)
<i>Ximenia caffra</i> Sond. Family: Olacaceae Habit: Tree	Motjhidi (P); large sourplum (E); grootsuurpruim (A); umThunduluka-obmvu (Z)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North-West.	Edible fruit-jam	Leaves- diarrhoea, fever, infertility	Drupe	Not available	Fruit juice- Flavonoids, phenols, organic acids	Fruit- Na, Zn, K, P, Ca, Fe, moisture, fibre, protein, fat, carbohydrate	(Goosen et al., 2018; Mapunda and Mligo, 2019; Mashile et al., 2019a, b; Rankoana et al., 2015; Shai et al., 2020)
<i>Englerophytum magalismontanum</i> (Sond.) T.D. Penn Family: Sapotaceae Habit: Tree	Ditlhatjwa tsa tlhaga (P); Transvaal milkplum (E); stamvrug (A); mothatswa (T); munombelo (V);	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North-West.	Edible fruit-jam, syrup, wine	Not specified	Berry	Leaves, bark- antibacterial, antifungal, anti-inflammatory, antioxidant, toxicity, anticholinesterase Fruits- antioxidant	Leaves- Naringenin	Fruits- moisture, ash, protein, fibre, carbohydrate, energy, fat, vitamin C, vitamin B ₁ , vitamin B ₂ , vitamin B ₃ , Al, Ca, Fe, K, Mg, Mn, P, Pb, Se, Zn, Cu, Na, B	(Akhawaya et al., 2018; Dzoyem and Eloff, 2015; Mashile et al., 2019a, b; Netshituni et al., 2022; Nkosi et al., 2022; Olaokun et al., 2022; Shai et al., 2020; Sibiya et al., 2020, 2021; Swenson et al.,

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
	amanumbela (Z); umnumbela (T)								Leaves- Ca, Fe, K, Mg, Mn, P, Zn, Cu	2023; Wehmeyer, 1966)
<i>Mimusops zeyheri</i> Sond. Family: Sapotaceae Habit: Tree	Nhlantswa (XT); Transvaal red milkwood (E); moepel (A); umpushane (Z); mubululu (V)	Least concern	Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and North-West.	Edible fruit	Not specified	Berry	Leaves- antibacterial Seed oil- antiproliferative	Leaves- Phenols, alkaloids, flavonoids, terpenoids, tannins, quinones, saponins, glycosides	Fruit- dry matter, organic matter, protein, ash, carbohydrates Seeds- fat, fibre, protein, organic matter, dry matter, vitamin E, Ca, Mg, P Leaves- N, P, K, Ca, Mg, Zn, Cu, Fe, Al, Mn	(Chivandi et al., 2012; Fakudze et al., 2023; Mabadahanye et al., 2022; Mashile et al., 2019b; Mngadi, 2017; Omotayo et al., 2020; Shai et al., 2020)
<i>Lantana rugosa</i> Thunb. Family: Verbenaceae Habit: Shrub	Bird's brandy (E); wildesalie (A); sekwebetane (N); utywala bentaka (X); impema, utshwala benyoni (Z); molutoane (S)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West, and Western Cape	Edible fruit	Leaves, roots- fever, diarrhoea	Berry	Leaves- anthelmintic, antibacterial, antifungal, toxicity Stem- antibacterial, antifungal,	Leaves- lantanin	Not available	(Mashile et al., 2019b; McGaw et al., 2005; Shai et al., 2020; Sibiya et al., 2021; Suliman, 2010)
<i>Lippia javanica</i> (Burm.f.) Spreng. Family: Verbenaceae Habit: Shrub	Lemon bush (E); koorsbossie (A); umsutane (W); inzinziniba	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo,	Not specified	Decoction- respiratory problems	Nut	Leaves- antioxidant, antibacterial, antifungal	Leaves- Tannin, flavonoids, terpenoids, alkaloids, phenols, α -thujene, α -pinene, camphene,	Aerial parts- Ash, moisture, fat, Fibre, protein, carbohydrate, Ca, N, Mg, K, P, Na, Zn, Cu, Mn, Fe	(Afolayan et al., 2021; Endris et al., 2016; Osunsanmi et al., 2019; Tshikalange et al., 2016)

Plant species	Common name (s)	Conservation status in South Africa	Province of availability in South Africa	Nutritional use (s)	Medicinal uses and methods of preparation	Fruit type	Pharmacological studies	Phytochemicals detected/isolated from plants	Nutritional composition	References
	(X); umswazi (Z)		Mpumalanga, North West					sabinene, myrcene, α -Phellandrene, α -terpinene		
<i>Rhoicissus tridentata</i> (L.f.) Wild & R.B.Drumm. Family: Vitaceae Habit: Liana	Wild grape (E); wildedruif (A); ulatile, (X); umthwazi (Z); lumbu (XT); morara-oathaba (S); murumbula-mbudzana (V)	Least concern	Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West, Northern Cape	Not specified	Root decoction-sexually transmitted infections	Berry	Root bark, leaves, tubers, stem bark-antioxidant Leaves- antidiabetic Roots- uterotonic	Root bark, leaves, tubers, stem bark-catechin, epicatechin, gallic acid, epigallocatechin-gallate Roots- 3-O- α -L-rhamnopyranoside, trans-resveratrol 3-O- β -glucopyranoside, asiatic arjunolic acids, quercetin 3-O-rhamnopyranoside, catechin, β -sitosterol, linoleic acid	Not available	(Mshengu et al., 2023; Mukundi et al., 2015; Naidoo et al., 2006; Tshikalange et al., 2016)

Common names: Afrikaans (A), English (E), Ndebele (Nd), Setswana (T), Sepulana (PI), Sesotho (S), Swati (W), Xhosa (X), Xitsonga (XT), Zulu (Z)

Minerals: Phosphorus (Ph), Calcium (Ca), Copper (Cu), Sodium (Na), Potassium (K), Nickel (Ni), Chromium (Cr), Cadmium (Cd), Nitrogen (N), Manganese (Mn), Zinc (Zn), Iron (Fe), Magnesium (Mg),

Table 2.3 An inventory of wild fruit plants in different regions of the Mpumalanga Province, South Africa.

Authors	District	Local Municipality	Ethnic group	Interviewee recruitment method	No. of participants	No of wild fruit plants	No. of families of wild fruit plants	Voucher specimens	Dominant plant families	Study focus	Economic aspect
(Shackleton et al., 2000)	Ehlanzeni	Bushbuckridge	Tsonga Pedi	Random sampling, structured questionnaire	180 households	54	Unspecified	Unspecified	Ebenaceae Anacardiaceae	Edible	Yes
(Tshikalange et al., 2016)	Ehlanzeni	Bushbuckridge	Tsonga	Purposive sampling, Semi-structured questionnaire	15	55	42	Yes	Fabaceae	Medicinal	Unspecified
(Mashile et al., 2019a)	Ehlanzeni	Bushbuckridge Mbombela Thaba Chweu	Sepulana	Snowball, semi-structured questionnaire	68	21	19	Unspecified	Phyllanthaceae	Edible and woody	Unspecified
(Mashile et al., 2019b)	Ehlanzeni	Bushbuckridge Mbombela Thaba Chweu	Sepulana	Snowball, semi-structured questionnaire	82	33	26	Yes	Anacardiaceae Rhamnaceae	Edible	Unspecified
(Shai et al., 2020)	Ehlanzeni	Bushbuckridge	Sepulana	Questionnaire, semi-structured interviews	41	31	17	Yes	Anacardiaceae Rubiaceae	Edible and medicinal	Unspecified

2.10.2.3 Growth form of wild fruit species

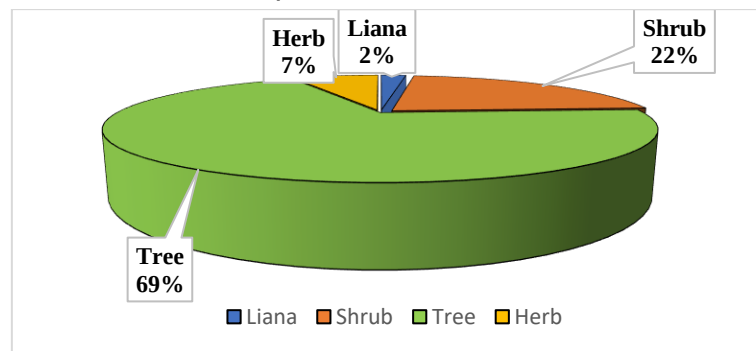


Figure 2.7 Growth form of wild fruit plants used in Mpumalanga Province, South Africa.

The analysis of the growth form of documented wild fruits showed that trees (69%) were dominant, followed by shrubs (22%), herbs (7%), and lianas (2%) (Figure 2.7). Similarly, Ramachandran (2007) observed that trees were the most dominant growth form of wild fruit species, followed by shrubs, and the least being lianas. The high proportion of trees and shrubs could be linked to their ability to endure extended dry seasons, accounting for their copiousness and ease of access throughout the year (Tolossa et al., 2013). Additionally, the Mpumalanga Province is dominated by savanna woodlands (Brown et al., 2022; Ifegbesan, 2009; Prinsloo, 2015) consisting of sparsely distributed tree species (Campos et al., 2011; Pickering and Bunn, 2007), which could be a factor influencing the abundance of trees.

2.10.2.4 Nutritional uses and types of fruit produced by wild fruit species



Figure 2.8 Marula fruit processed into local (jam and wine) and international products (cream liqueur) (Hyslop, 2012; Mutuwa, 2021).

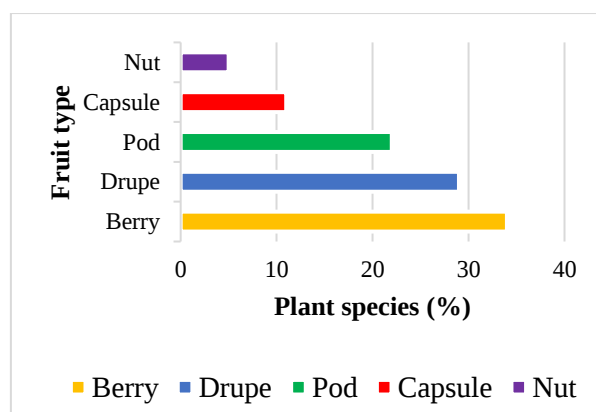


Figure 2.9 Percentages of different fruit types among wild fruit plants

The study revealed that 51% of wild fruit plant species are utilised for their nutritional value, primarily through their fruit, while the edibility of the remaining 49% was not specified. The study further revealed that the different types of wild fruits are processed into beverages, jams, juice, wine, syrup, and soft porridge by the local people (Table 2.2, Figure 2.8). The fruit types observed include berries, drupes, pods, capsules, and nuts (Figure 2.9). The most dominant fruit types were berries (34%) and drupes (29%). Similar to the findings of the current study, berries and drupes were the most common fruit types of wild fruit species in a study conducted by Mahapatra et al. (2012). Drupes, also known as stone fruits are single-seeded fleshy fruits (Bobrov and Romanov, 2019; Datta and Rawat, 2003), while berries are single or multi-seeded fruits with watery pulp (Datta and Rawat, 2003). These edible fruits are a great source of nourishment for local people since they are vitamin and mineral rich (Nazar et al., 2022). The consumption of wild fruits such as wild berries and drupes dates back to prehistoric times and still forms an integral part of the biocultural identity of local communities (Aguilera and Toledo, 2022). Surprisingly, in addition to the edible fruit, *E. crispa* leaves are used to prepare herbal tea (Table 2.2). The current results are in line with a prior study by Marwat et al. (2011) which demonstrated that the fruit and leaves of some wild fruit plant species such as *Grewia tenax* are edible and provide nutritional value to rural communities. This further indicates that wild fruit species can potentially possess nutritional benefits in other parts other than the fruit.

2.10.2.5 Ethnobotanical uses of wild fruits

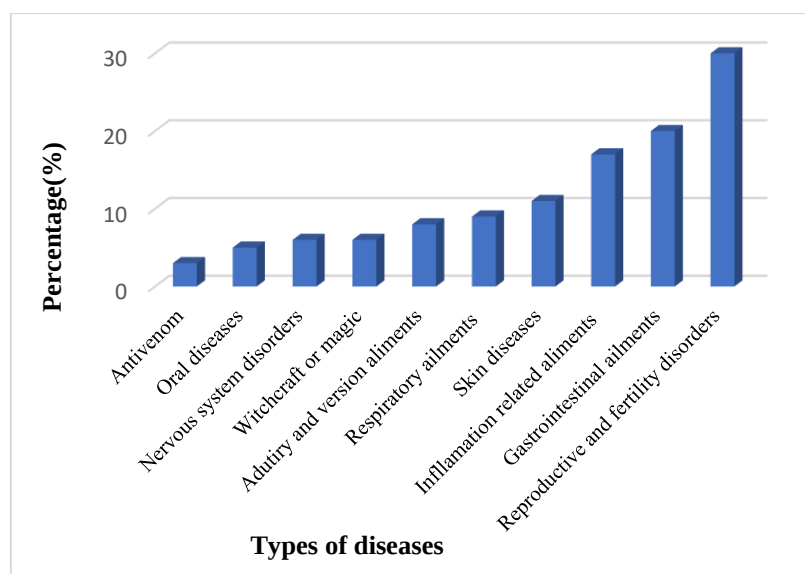


Figure 2.10 Percentage of wild fruit plants used to treat various categories of ailments in the Mpumalanga Province, South Africa.

In the current study, 76% of the wild fruit plant species were employed in addressing a range of ailments and diseases. The study revealed the traditional medicinal use of these plants for treating various diseases, including reproductive, respiratory, gastrointestinal, skin, urinary, renal, wounds, eye problems, toothache, and as antivenom (Table 2.2 and Figure 2.10). Fabaceae plant species, including *Macrotyloma maranguense* and *Senna italica* subsp. *arachoides*, are commonly used for treating reproductive disorders and diseases, particularly sexually transmitted infections, as noted by Mathibela et al. (2019) in the Blouberg area of South Africa.

2.10.2.6 Medicinal plant parts

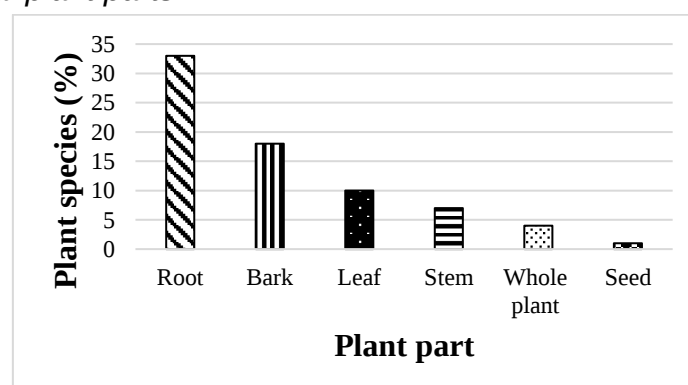


Figure 2.11 Percentage of plant parts used for medicinal purposes in Mpumalanga Province, South Africa.

The present findings showed that most plant parts are used for medicinal purposes (Figure 2.11); roots (33%), bark (18%), and leaves (8%) are among the most dominant medicinal parts. Unsurprisingly, these results resonate with previous studies that indicated that roots were the most commonly used plant part, followed by leaves and bark for medicinal purposes (Ndhlovu et al., 2023; Pascaline et al., 2011). Novotna et al. (2020) highlighted that local traditional health practitioners refer to roots as "real medicine." The frequent use of roots may be attributed to the fact that, even during extended dry seasons, they remain in the soil and are easily accessible (Tolossa et al., 2013). However, in other studies, the leaves are the predominantly used plant part because they are the most easily accessible part of the plant (Agisho et al., 2014; Chaachouay et al., 2022; Megersa and Woldetsadik, 2022). Furthermore, Alamgeer et al. (2018), indicated that preference of leaves over other plant parts may be due to the fact that they are the photosynthetic organs containing the photosynthates which might be responsible for their medicinal values.

A major challenge in conserving medicinal plants is the unregulated and unsustainable over-harvesting driven by the growing global demand for these plants (Raju and Das, 2024). To ensure their long-term availability, it is essential to raise awareness about sustainable harvesting practices and the responsible use of medicinal plants. Such efforts not only protect biodiversity but also foster socioeconomic development (Alemu et al., 2024).

Local communities use various harvesting techniques depending on the part of the plant required, often utilising a single plant in multiple ways, such as cutting the bark, plucking leaves, tapping sap, or extracting roots. Educating these communities on sustainable harvesting methods is vital to prevent over-harvesting and ensure the continued availability of medicinal plants. Best practices include carefully extracting roots without damaging the taproot, selectively removing stem bark to avoid harming inner layers, and plucking leaves without breaking shoots. These approaches help preserve plant populations, support biodiversity conservation, and promote the sustainable use of medicinal resources (Bukuluki et al., 2014).

2.10.2.7 Mode of preparation

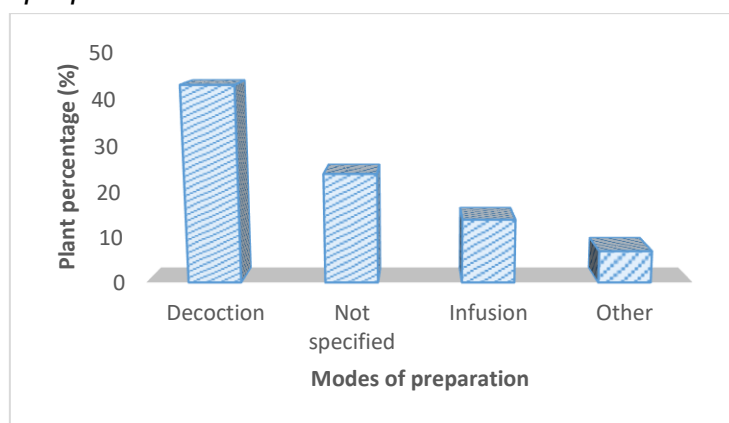


Figure 2.12 Mode of preparation of wild fruit plant part preparation for traditional medicinal use in the Mpumalanga Province.

The most frequent mode of traditional medicine preparation was decoction (43%), followed by infusion (14%; Figure 2.12). The current findings align with Ahmad et al. (2014), who also found that decoction was the commonly used mode of preparation. Decoction involves boiling plant parts, which makes the extraction of active principles more efficient and aids in preserving traditional medicine longer (Tugume and Nyakoojo, 2019). The second most common mode of preparation (infusion) involves soaking plant material in hot or warm water and allowing the mixture to cool (Tugume and Nyakoojo, 2019). However, the modes of preparation employed by the local people of Mpumalanga Province in their traditional medicines are not limited to decoctions and infusions. There are other methods employed such as chewing the plant material or adding the plant material to bathing water (Table 2.2). Lastly, 24% of mode of preparation of some plant species was not recorded. Existing inventories' quality is impacted by lack of information regarding plant parts, preparation methods, and uses. A comprehensive plant profile is crucial for ethnopharmacological research, as incomplete data could lead to the decontextualization of traditional knowledge and hinder scientific validation (Weckerle et al., 2018). As a result, all significant plant information must be recorded.

2.10.2.8 Pharmacological studies on wild fruit plant species

The findings of the current study indicate that pharmacological studies have been conducted on 93% of the plant species recorded in Table 2.2. The findings presented in Table 2.2 demonstrate that the antimicrobial properties, encompassing

antibacterial, antifungal, and antiviral activities, are extensively investigated among wild fruit plants. The results further indicate that the antimicrobial activities of 86% of these species have been studied. The increasing problem of antimicrobial drug resistance among pathogens linked to various diseases drives the primary interest in seeking antimicrobial agents from medicinal plants (Škovranová et al., 2024). Pharmacological studies play a vital role in novel drug discovery and in the maintenance of health by preventing and providing a cure for diseases such as cancer and acquired immunodeficiency syndrome (AIDS) (Süntar, 2020). Cabada-Aguirre et al. (2023) highlighted that 75% of healthcare professionals recommend the use of herbal medicines.

2.10.2.9 Phytochemical studies on wild fruit species

The study uncovered that 85% of wild fruit plant species have been studied for phytochemical composition with the leaves being the most studied part (Table 2.2). This indicates that most wild fruit plant species have been studied. Additionally, the current findings further revealed that phytochemical studies of the fruit or fruit components was conducted on 34% of the wild fruit plant species (Table 2.2). Contrast to the present study findings, other recent studies indicate that leaves are the most used medicinal plant part, prompting researchers to study their phytochemistry (Alemu et al., 2024; Gillani et al., 2024; Pandey et al., 2024). Additionally, a study of Ardalani et al. 2021 shows a growing interest in the study of the phytochemistry of the roots of medicinal plants and that they offer while highlighting that the phytochemistry of the roots needs to be explored more. Therefore, comprehensive research is needed on fruit phytochemistry, as it is an edible component of wild fruit species and often used for medicinal purposes.

Phytochemical studies vary in nature, with some studies conducting preliminary screening and others focusing on compound identification and isolation. About 70% of preliminary screening was conducted on wild edible fruits, while 30% compounds were identified or isolated from these plants. Epicatechin, a compound found in various plant species, was observed in *Sclerocarya birrea* Hochst., *Ficus sur* Forssk., *Pappea capensis* Eckl. & Zeyh, and *Ximenia americana* L., while quercetin and its derivatives were found in *Schotia brachypetala* Sond., *Morus alba* L., *X. americana* L., and *Vitis vinifera* L (Table 2.2). Epicatechin, a polyphenol found in raspberries and leaves, is known for its antioxidant and anti-allergic properties (German et al., 2024). Quercetin,

another plant polyphenol, also exhibits antioxidant and anti-inflammatory properties (Frez et al., 2024). These findings suggest that wild fruit species are rich in beneficial phytochemicals for human health. Further research is needed on fruit phytochemistry, as fruits are an edible component of wild fruit species.

2.10.2.10 Nutritional composition of wild fruit plant species

The nutritional compositions of various components of wild fruit plants from 62 plant species including 27 wild fruits are listed in Table 2.2. It is important to determine the nutritional contents of wild fruits since they serve as supplementary foods and essential nutrients for local people (Tesfay et al., 2024). However, the results indicate a predominant focus on the nutritional content of leaves, accounting for 60% of the studied plant species. The interest in assessing the nutritional profiles of stems and leaves arises from the fact that some of these plant species are browsed by animals. For example, *Diospyros mespiliformis* Hochst. ex A.DC., *Parinari curatellifolia* Planch. ex Benth., and *Syzgium cordatum* Hochst. Ex Krauss bear edible fruits, yet the plants are utilized as browse species for livestock in the Bushbuckridge local municipality (Chepape et al., 2014).

2.10.2.11 Conservation status

In the current study, all the plant species excluding *Elaeodendron transvaalense* (Burt Davy) R.H. Archer are classified as of "Least concern". Currently, *E. transvaalense* is listed as "Near threatened" in the South African National Red List Data for plants. *E. transvaalense* is mainly exploited for its bark, which is commonly sought after and traded in traditional markets for medicinal purposes (Khumalo et al., 2019). Factors such as over-harvesting, destructive bark harvesting, and the clearing of land for agricultural and urban development are responsible for the decline in the wild population of *E. transvaalense* in South Africa (Maroyi and Semanya, 2019). As noted by Williams et al. (2013), plant species can be classified as "Least concern", "Critically Endangered", "Endangered", "Vulnerable", "Near Threatened", "Data deficient", "Extinct", or "Extinct in the wild". Assessment of the conservation status of indigenous plants is paramount because a threat to these species may negatively impact the natural ecosystem (Moraswi et al., 2019). The primary threats to biodiversity, leading to an increased risk of extinction for numerous plant taxa in South Africa, stem from the over-utilisation of plant resources and anthropogenic pressures (Moraswi et al., 2019). It is also crucial to use plants of "least concern" sparingly to prevent extinction

in the future. It is further essential to assess the conservation status of non-evaluated plants and non-listed plants because habitat loss, harvesting, and trade may threaten their existence (Williams et al., 2013). Indigenous communities have their own methods of preserving valuable plants. For instance, Mashabela and Otang Mbeng (2021) and Seile et al. (2022) confirmed that locals in Ehlanzeni District of Mpumalanga Province conserve plants through seed storage and cultivation in their home gardens, and harvest only parts they need, demonstrating their concern for protecting their plant resources.

2.10.2.12 Comparison of ethnobotanical studies reporting on wild fruits

Table 2.3 presents various interview recruitment methods, including snowball (40%), purposive (20%), and random (20%) sampling. Purposive sampling is an efficient method in ethnobotany (Tongco, 2007), while random sampling helps draw general conclusions on large populations (Vogl et al., 2004). However, Alemu et al. (2024) explored random, purposive, and snowball techniques in their study. Semi-structured questionnaires were used in 80% of the studies. Fakchich and Elachouri (2023) argue that structured questionnaires result in low novel discoveries since the scope is restricted to the interests of the researcher. Additionally, Silva et al. (2014) recommends inventory-based type of interviews since they ensure the complete record of species in an area.

Shackleton et al. (2000) covered a significantly higher number of participants (180 households) in their study than the rest of the studies (Table 2.3). Paniagua-Zambrana et al. (2018) highlighted having a higher number of participants as an excellent method for acquiring a lot of information in ethnobotanical studies. In a recent study, Alemu et al. (2024) interviewed 388 participants, a number that is significantly higher than the studies in Table 2.3 which had less than 100 participants (Mashile et al., 2019a, b; Shai et al., 2020; Tshikalange et al., 2016). Additionally, Tshikalange et al. (2016) interviewed the least number of participants but recorded the highest (82) number of wild fruit species compared to other studies. Table 2.3 indicates that ethnobotanical studies often concentrate on the researcher's interests, rather than utilizing all available data on a species. For instance, Mashile, S. et al. (2019b) and Shackleton et al. (2000) focused only on edible wild fruit species, whereas Shai et al. (2020) focused on both medicinal and edible wild fruit species.

Table 2.4 Edible wild fruit plant species cited in all five ethnobotanical studies.

Plant species	No. of citations	Ethnobotanical studies
<i>C. spinarum</i>	5	(Mashile et al., 2019a, b; Shackleton et al., 2000; Shai et al., 2020; Tshikalange et al., 2016))
<i>D. mespiliformis</i>	5	(Mashile et al., 2019a, b; Shackleton et al., 2000; Shai et al., 2020; Tshikalange et al., 2016)
<i>S. madagascariensis</i>	5	(Mashile et al., 2019a, b; Shackleton et al., 2000; Shai et al., 2020; Tshikalange et al., 2016)
<i>V. infausta</i>	5	(Mashile et al., 2019a, b; Shackleton et al., 2000; Shai et al., 2020; Tshikalange et al., 2016)

Different numbers of plant species were recorded in the five different studies as highlighted in Table 2.3. However, some wild fruit plant species such as *V. infausta* and *S. madagascariensis* (Table 2.4) were cited in all the five-ethnobotanical studies reviewed in this paper which indicates their popularity and importance in the Bushbuckridge local Municipality.

The current study indicates that 20% of the previous investigations have focused on the economic aspects of wild fruit species. The study highlights the economic use and domestication of wild fruit species by indigenous communities in Bushbuckridge, highlighting the need to encourage the cultivation and processing of these plants for specific products (Shackleton et al., 2000), whereas, Nkosi et al. (2020) highlighted the commercial and nutritional value of indigenous wild fruit species in KwaZulu-Natal Province, South Africa, ranking them based on local markets and potential international market production. Hence, there is dire need to document and explore the cultivation/domestication as well as commercialisation of wild fruit species in the Mpumalanga Province as this could potentially benefit the community.

2.10.2.13 Economic value of wild fruit species

The trade of indigenous fruits and their products serves as a source of supplementary or main income for households (Mugari et al., 2024). The rise in demand for fruit in urban areas and limited economic opportunities in rural areas are driving the

increasing commercialisation of edible wild plant fruits (Seyoum et al., 2015). The production, sale, and consumption of wild fruits contributes to economic status of local people (Jabeen et al., 2024). Furthermore, several wild fruits are sold in local, national, and international markets (Termote et al., 2012). Some South African indigenous wild plant species whose fruit/ product has reached both local and international markets include *S. birrea* (Sardeshpande and Shackleton, 2019), *Adansonia digitata*, *Cucumis metulifer*, *Strychnos spinosa* Lam. (Hlangwani et al., 2023). The Marula tree is an important commercial plant from which products such as clay face mask, lip balm, soap, purifying face mask, serum, cream, body lotion, body wash gel, face wash, toner, body scrub, shampoo and conditioner are developed and sold nationwide by companies such as Portia M, Africa organics, The Victorian Garden, Lulu and Marula, and Iwori Beauty of Africa among others (Gebashe et al., 2022). The study of Shackleton et al. (2000) shows that the people of Bushbuckridge Municipality have long practiced indigenous fruit trade, selling *S. madagascariensis*, *C. spinarum*, and *V. infausta* fruits (Figure 2.13).

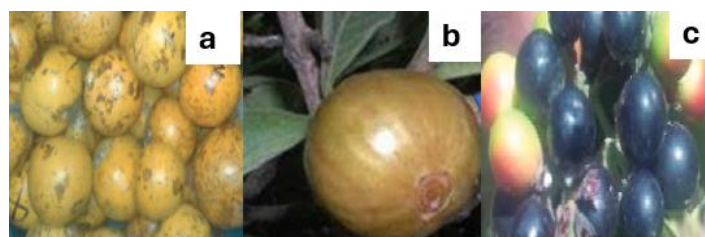


Figure 2.13 Wild fruit plants of commercial significance in the Mpumalanga Province, (a) *S. madagascariensis* (Akweni et al., 2022), (b) *V. infausta* (Mdungazi et al., 2024), and (c) *C. spinarum* (Siyum and Meresa, 2021).

2.10.2.14 Research gaps identified on studies on wild fruit species in Mpumalanga Province

Tshikalange et al. (2016) argue that the Mpumalanga Province consists of different ethnic groups, including Tsonga, Zulu, Swati, Pedi, and Ndebele. However, the results (Table 2.2) indicate that only the Sepulana- (50%), Tsonga- (33%), and Pedi- (17%) speaking ethnic groups are often consulted about the wild edible fruits. Therefore, there is a need to investigate the differences in the uses of these plants amongst different ethnic groups, given that the Mpumalanga Province is culturally diverse. Maroyi and Rasethe (2015) highlighted the differences and similarities in the use of wild plants by the Bapedi people of South Africa and the Chikaranga-speaking people

of Zimbabwe. Joshi et al. (2020) studied the utilisation of wild plants between three ethnic groups in Nepal, Asia and highlighted how these kinds of studies help reveal cultural differences in rural or urban settings.

Despite the rich diversity of wild fruit species in South Africa (Table 2.2), there has been limited success in their commercialisation and recognition in international markets due to the lack of innovation when it comes to value addition (Mabhaudhi et al., 2017; Sileshi et al., 2023). Notably, "amarula" from *S. birrea* stands out as one of the few South African products that have successfully penetrated the international market (Sileshi et al., 2023). This underscores the neglect and underutilisation of numerous other wild plant species (Issa-Zacharia et al., 2024; Sahoo et al., 2021; Shembe et al., 2023). Currently, there is a predominant emphasis on cultivating and commercialising conventional crops in South Africa, posing challenges exacerbated by climate change (Ntshidi et al., 2022). There is also a bias extending towards the cultivation of conventional and exotic crops rather than focusing on wild plants (Mabhaudhi et al., 2017; Nxusani et al., 2023). However, incorporating alternative crops such as wild fruit species is crucial for sustaining agriculture in the country, contributing significantly to health, food and economic security (Aga and Gagabo, 2024; Sulaiman et al., 2023).

As indicated in Table 2.2, wild fruits offer versatile consumption options, either fresh or processed into various products. Recent studies continue to prove both the nutritional and medicinal value of wild plants (Chaudhary et al., 2024; Ningthoujam et al., 2024). Medicinal and nutritional applications of wild fruit species can serve as a basis for their commercialisation (Seleteng-Kose et al., 2023). According to Shackleton et al. (2000), 15% of participants were involved in the local trade of wild fruits in Bushbuckridge, especially that of *S. madagascariensis* and *V. infausta*. This suggests that participation in the wild fruit trade was limited in the community. The remaining investigations in Table 2.3 have not delved into or pinpointed the economic utilisation or advantages of these plant species within the communities where the wild fruit species were documented. Consequently, there is a necessity to assess the current status of wild fruit species trade in these communities or to promote and support such trade. Furthermore, it is imperative to expand the trade of wild fruit species products beyond local markets. Thus, domesticating wild fruit species can facilitate large-scale production and commercialisation, thereby contributing to the local economy (Sileshi

et al., 2023). Integrating wild fruits into farming practices is also essential for safeguarding them from extinction, especially in the face of urbanisation and conventional farming methods (Borelli et al., 2020; Sileshi et al., 2023). Hence, a comprehensive research effort is needed to explore and encourage the domestication and innovative efforts to boost the commercialisation of wild fruit species in the Mpumalanga Province.

2.11 Plant species selected for further studies

The following six wild fruit plant species were selected based on their frequent citation in existing literature, traditional uses, research gaps in bioassays, and the availability and ease of access for collection.

2.11.1 Botanical description of *Carissa spinarum* L.



Figure 2.14 *Carissa spinarum* L. (a) whole plant and (b) leaves

Carissa spinarum L. (Figure 2.14) is a spiny evergreen shrub (Sanwal and Chaudhary, 2011). The woody plant has forked branches (Berhanu et al., 2020). Leaves are ovate, leathery, and exude white latex (Fatima et al., 2013). The plant produces a cluster of fragrant white flowers with a tint of purple or pink. The edible fruits are green and turn red to purplish black when ripe (Smyth and Sheridan, 2022).

2.11.2 Botanical description of *Diospyros mespiliformis* Hochst. ex A.DC

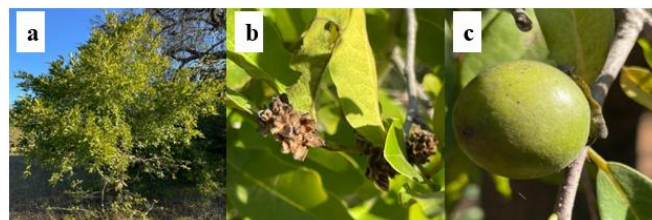


Figure 2.15 *Diospyros mespiliformis* Hochst. ex A.DC. whole plant (a), flowers (b), and fruit (c)

Diospyros mespiliformis Hochst. ex A.DC. (Figure 2.15) is a tree that can grow up to 25 m with an evergreen dense canopy (Ahmed and Mahmud, 2017). The tree produces fruit that are almost spherical and about 25 mm in diameter. When ripe, they turn into a fleshy yellow to purple colour (Nyambe et al., 2019b).

2.11.3 Botanical description of *Euclea crispa* (Thunb.) Gürke



Figure 2.16 *Euclea crispa* (Thunb.) Gürke whole plant (a) and leaves (b)

Euclea crispa (Thunb.) Gürke (Figure 2.16) is an evergreen and hardy shrub or tree (Palanisamy et al., 2018) growing up to 6 m in height (Alayande et al., 2020). The plant consists of a dense canopy of leaves that are simple, leathery, rigid, and dull green in colour. The bark ranges from grey to brown in colour. Furthermore, the plant bears yellow to greenish-white coloured small, waxy, and pendulous flowers (Palanisamy et al., 2022).

2.11.4 Botanical description of *Ficus thonningii* Blume

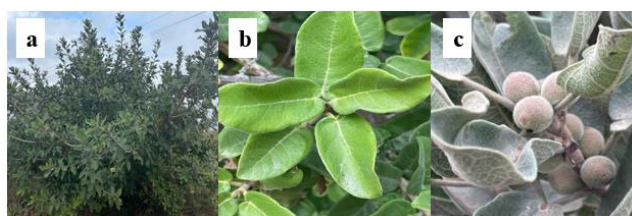


Figure 2.17 *Ficus thonningii* Blume whole plant (a), leaves (b), and fruit (c)

Ficus thonningii Blume (Figure 2.17) is a tree that grows from 6 to 21 m high, with a round, spreading crown that is densely packed (Maiha et al., 2013). All parts of the plant exude excessive milky latex. It has simple, glossy, dark green, thin leaves. A milky juice often distinguishes the fruits, which can come single or in pairs (Coker et al., 2015). Juvenile branches have hairy bark with a stipular cap covering the growth tip, whereas older branches and stems have smooth, grey bark. The fruit of the fig tree is hairy and usually borne in the leaf axils, sessile or on peduncles to 10 mm in length (Berg and Corner, 2005).

2.11.5 Botanical description of *Strychnos madagascariensis* Poir.

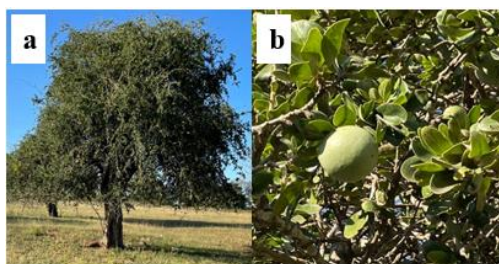


Figure 2.18 *Strychnos madagascariensis* Poir. whole plant (a), fruit and leaves (b)

Strychnos madagascariensis Poir (Figure 2.18) grows to a height of 15 m, often multi-stemmed, with flat and densely branched, twiggy and spreading crown. The bark of *S. madagascariensis* is pale grey in colour, smooth or grooved, with no spines but with often hard, short, and rigid lateral side shoots which give the impression of thorns. The leaves of *S. madagascariensis* are simple, opposite, or clustered on lateral twigs, thin, elliptic to oval in shape, shiny, leathery, dark green above and pale green beneath. The flowers are trumpet-shaped, small on short stalks, in axils of leaves and yellow green in colour with white hairs in the throat. The fruit of *S. madagascariensis* are large, globose or round berries, blue-green or green when young and ripening to orange-yellow (Maroyi, 2021). The fruit of this plant species contains edible pulp with a bitter-sweet taste. The fruit also contains large translucent seeds that are believed to be toxic (Van Rayne et al., 2020).

2.11.6 Botanical description of *Strychnos spinosa* Lam.



Figure 2.19 *Strychnos spinosa* Lam. whole plant (a), leaves (b), and fruit (c)

Strychnos spinosa Lam. (Figure 2.19) is a tree that can grow up to 7 m in height with a corky bark and spines that are curved or straight. Leaves are simple, oval, glabrous, and arranged oppositely with axillary spines. Fruits of this tree are edible and are bright green (unripe) with woody peels that are 3 to 4 mm thick. Meanwhile, as they ripen, the peel turns yellow-brown in colour. The fruit has a pleasantly sour taste and has a diameter of 10 to 15 cm with flat seeds that vary in size. It has hard-brown seeds and pale brown pulp that is edible, juicy, and sweet-sour (Abaci et al., 2017).

2.12 Concluding remarks

Skin diseases, whether infectious, inflammatory, or degenerative, pose significant global health and psychological challenges. Oxidative stress plays a critical role in skin damage, underscoring the importance of effective antioxidative treatments. Wild fruit species in Mpumalanga, with their potent antioxidant properties, offer promising solutions for managing skin conditions. Moreover, their antimicrobial effects could address multidrug-resistant pathogens like *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The Province of Mpumalanga boasts a rich diversity of wild fruits that serve dual roles as medicinal and nutritional resources, providing significant economic potential owing to their multifaceted uses. The research highlighted in this review predominantly centres on the Ehlanzeni district. Variations in traditional knowledge concerning wild fruit species among different cultures and ethnicities underscore the importance of exploring this knowledge across diverse regions and ethnic groups. These findings lay a crucial groundwork for future scientific inquiries aimed at comprehensive assessment and validation, while also preserving this significant cultural heritage. Thus, extensive documentation of wild fruit species in Mpumalanga Province and further investigation into their biological properties is recommended. Additionally, a thorough exploration of the commercial viability and domestication potential of these wild fruit species in Mpumalanga Province is recommended.

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Chapter 3.0: Antibacterial and antioxidant activities of the leaves of six wild fruit plant species

Summary

Antimicrobial drug resistance and side effects of conventional medicines necessitate research on the use of medicinal plants as alternatives. This study investigated the antibacterial and antioxidant properties of the leaves of wild fruit trees *Carissa spinarum*, *Diospyros mespiliformis*, *Euclea crispa*, *Ficus thonningii*, *Strychnos madagascariensis*, and *Strychnos spinosa*. The six wild fruit plant species were selected based on their frequent citation in existing literature, traditional uses, research gaps in bioassays, and the availability and ease of access for collection as shown in chapter 2. Hexane, acetone, and methanol leaf extracts were tested for antimicrobial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* since they are common pathogens of the skin associated with antimicrobial drug resistance. The hexane, acetone, and methanol extracts of the selected plants were tested for antibacterial activity using disc diffusion and microdilution assays, and for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing power (FRAP) assays. The methanol extract yielded the highest quantities of crude extract. The extracts of *D. mespiliformis*, *E. crispa*, and *F. thonningii* showed strong antimicrobial activity against *P. aeruginosa* with inhibition zones of up to 28 mm and minimum inhibitory concentrations (MICs) from 0.781–1.563 mg/mL. The acetone extract of *D. mespiliformis* also exhibited activity against *K. pneumoniae* (MIC: 3.125 mg/mL). The methanol extract of *E. crispa* displayed potent antioxidant activity, achieving a half-maximal inhibitory concentration (IC₅₀) of 1., which was comparable to ascorbic acid at concentrations of 62.5–250 µg/mL ($p > 0.05$). Acetone extracts of *S. spinosa* and methanol extracts of *C. spinarum* demonstrated good ferric-reducing power, however, all the plant extracts were significantly different ($p < 0.05$) from ascorbic acid and butylated hydroxytoluene (BHT) at 250 µg/mL. The acetone extract of *D. mespiliformis* had the most potent antibacterial activity while the methanol extracts of both *D. mespiliformis* and *E. crispa* had the strongest antioxidant activity, making them possible sources of novel compounds for combating skin infections and degenerative skin conditions.

3.1 Introduction

There is a significant burden on global healthcare systems and economic well-being due to the prevalence of both communicable and non-communicable diseases (Enfiyeci and Çavlin, 2023; Masanovic et al., 2023). Globally, these diseases have been linked to numerous mortalities (Shu and Jin, 2023), with non-communicable diseases accounting for 71 % of deaths per year (Ishak et al., 2023). Furthermore, Manyazewal et al. (2023) found that in Africa, the number of deaths caused by communicable and non-communicable diseases is on the rise. Oxidative stress generates free radicals which are associated with aging, inflammatory, and degenerative diseases (Mangrulkar et al., 2023). Degenerative diseases are non-communicable diseases (Basilicata et al., 2023). Inflammation, cancer, and skin irritations can all be attributed to high levels of free radicals oxidizing biomolecules, resulting in tissue damage and cell death (Bursal and Köksal, 2011). Antioxidants aid in the prevention of oxidation by scavenging free radicals in the human system (Adeshina et al., 2011; Rahaman et al., 2023).

Degenerative diseases and disorders are often associated with or accompanied by microbial infections. For instance, atopic dermatitis is a skin condition linked to oxidative stress and microbial infections by bacteria such as *Staphylococcus aureus* in the cutaneous tissue (Alessandrello et al., 2024; Hulme, 2023). *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are some of the common bacterial pathogens affecting the human skin and associated with antimicrobial drug resistance (Rashid et al., 2020). Importantly, skin diseases cover a wide range of conditions that present major challenges in healthcare delivery and management, impacting people of all ages and demographics worldwide (Ryguła et al., 2024). Additionally, microbial pathogens can be triggers of oxidative stress due to the overproduction of free radicals during an infection (Canakci et al., 2005). Moreover, microbial infections are among the most common causes of death across the globe, regardless, the choice of antimicrobial treatment is limited owing to the occurrence of antimicrobial drug-resistant strains (Afrasiabi et al., 2020). This has caused researchers to resort to the utilisation of natural products as sources of novel antimicrobial drugs (Yang et al., 2023). Plant-based antioxidants are used to hinder and manage degenerative maladies (Akbari et al., 2022). Plant-based antioxidants include anthocyanins, beta-

carotene, flavonoids, lutein, polyphenols, organosulfur compounds, and vitamins A, C, and E among others (Rahmayanti and Ridwanto, 2023).

Plant secondary metabolites have been used to treat a wide variety of diseases since ancient times. These compounds have also been used as drug precursors, prototypes, and probes for pharmacology (Elshafie et al., 2023). Firoozbahr et al. (2023) also highlighted the importance of plants as a significant source of novel antimicrobials. Six plant species were selected based on the frequency of citation, traditional uses, and research gaps as highlighted in Chapter 2. The selected plant species include *Carissa spinarum* L. (Apocynaceae), *Diospyros mespiliformis* Hochst. Ex A. DC., *Euclea crispa* (Thunb.) Gürke (Ebenaceae), *Ficus thonningii* Blume (Moraceae), *Strychnos madagascariensis* Poir, and *Strychnos spinosa* Lam. (Loganiaceae). Chauke et al. (2024) also emphasized the importance of the six plant species as sources of medicine in the Mpumalanga Province. Hawas et al. (2022) reported that *D. mespiliformis* leaves exerted antioxidant and antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus*. *E. crispa* leaves possess antioxidant activity (Palanisamy et al., 2018) and antimicrobial activities against *Haemophilus influenzae* and *S. aureus* (Magama et al., 2003). Previous studies have shown that *F. thonningii* roots possess potent antioxidant activity and antimicrobial properties against *E. coli* and *Salmonella typhi* (Yahaya et al., 2021). *Diospyros mespiliformis*, *S. spinosa*, and *S. madagascariensis* fruit have good antioxidant activity (Mausse et al., 2021; Ndhlala et al., 2008; Oboh et al., 2020). Therefore, this study introduces new insights into the antioxidant and antimicrobial properties of selected plant species from Mpumalanga Province, highlighting the influence of environmental and climatic conditions on plant phytochemistry. Unlike most research, which often focuses on fruit, this study examines the antioxidant potential of leaves. The study evaluates the antimicrobial activity of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. spinosa*, and *S. madagascariensis*, determining the minimum concentrations at which they are effective. It also investigates their antioxidant properties, including their respective half-maximal inhibitory concentrations. The findings highlight the potential of indigenous plants from Mpumalanga Province as sources of antibacterial agents and antioxidants, offering promising applications in addressing antimicrobial resistance, combating oxidative stress, and managing dermatological conditions such as atopic dermatitis and skin aging.

3.2 Materials and methods

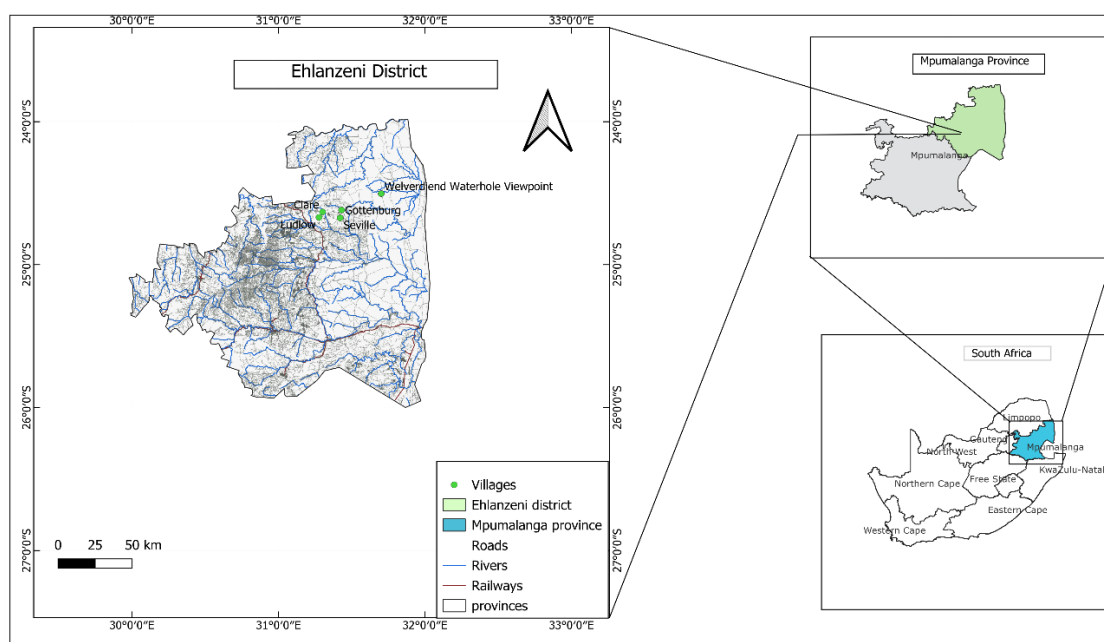


Figure 3.1 A map indicating the villages from which the selected plant species were collected. *C. spinarum* and *D. mespiliformis* (Gottenburg village), *E. crispa* (Clare), *F. thonningii* (Seville), and *S. spinosa* and *S. madagascariensis* (Welverdiend).

3.2.1 Plant collection

The leaves of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. madagascariensis*, and *S. spinosa* were collected from various villages in the Bushbuckridge local municipality, under Chief Mnisi's Tribal Council (Figure 3.1). Exploring leaves offers valuable insights into their medicinal properties, potentially revealing new therapeutic benefits and enhancing the overall understanding of the plant's healing potential. The plant species identities were confirmed at the University of Mpumalanga with the help of Dr L.J Ramarumo. Voucher specimens for each plant species were prepared and labelled with the following voucher numbers: *S. spinosa* (SC001), *C. spinarum* (SC002), *F. thonningii* (SC003), *D. mespiliformis* (SC004), *E. crispa* (SC005), and *S. madagascariensis* (SC006) and stored in the Indigenous Flora Research Laboratory at the University of Mpumalanga.

3.2.2 Plant extraction

Plant leaves were washed with distilled water, dried at room temperature, and ground into a fine powder with a heavy-duty blender (Zhongshan City Haitai Electrical Co. Ltd, China). Powdered plant material has an increased surface area that allows efficient

contact of the extraction solvent with the target phytochemicals (Azwanida, 2015). The ground plant material (100 g) was sequentially extracted with solvents in order of increasing polarity as described by Ahmed et al. (2017) with minor modifications. The ground plant material was macerated in 400 mL of acetone, methanol, and hexane. Extraction involved shaking the plant material for 48 hours at room temperature using an orbital shaker. The extract was filtered using Whatman's No. 1 filter paper and a rotary evaporator was used to remove the solvent. The resulting crude extract was weighed and kept at room temperature until further use.

3.2.3 Determination of antimicrobial activity

3.2.3.1 Bacterial strains and inoculum quantification

The plant extracts were screened against gram-positive American Type of Culture Collection (ATCC) of *Klebsiella pneumoniae* (ATCC-700603) and *Pseudomonas aeruginosa* (ATCC-27853) obtained from Davies Diagnostics (Pty) Limited, Gauteng. Standard isolates were used to ensure reproducibility and facilitate result comparison, given that susceptibility can vary significantly among different isolates of the same microbial species (Eloff, 2019). The microbial strains were resuscitated in a Nutrient agar medium and incubated overnight at 37°C before biological assays. For disc diffusion, the overnight bacterial cultures were spread into a Mueller-Hinton (MH) agar and for microdilution assay, the overnight cultures were transferred into the MH broth and quantified to a 0.5 McFarland standard. The inoculum was adjusted to roughly 5×10^5 CFU/mL for the microdilution assay. Furthermore, acetone served as the negative control, and amoxicillin trihydrate was the positive control. Amoxicillin is a broad-spectrum antibiotic used against various organisms including *K. pneumoniae* and *P. aeruginosa* (Abdulfatai et al., 2023).

3.2.3.2 Disc diffusion assay

The assay was conducted according to Sayeed et al. (2012) with some modifications. This technique has been applied as a preliminary step to measure the inhibition diameter produced around the disk. Petri dishes were loaded with 25 mL of Mueller-Hinton (MH) agar medium, allowed to solidify at room temperature in a biosafety cabinet, and then inoculated with overnight cultures of selected test bacteria using the spread plate method. Sterile Whatman filter paper discs (4 mm- diameter) were dipped into 10 µL of 100 mg/mL of each plant extract and allowed to stand for a few seconds to remove the excess extract. The discs were placed equidistant on the MH agar

inoculated with the pathogen and incubated overnight at 37 °C. The diameter of the inhibition zone, including the disc's diameter, was measured in millimetres (mm) around the disc.

3.2.3.3 Microdilution assay

The minimum inhibitory concentrations (MICs) of plant extracts were evaluated using the serial microplate method (Ramadwa et al., 2024). Plant extracts with a concentration of 100 mg/mL were serially diluted two-fold from well A to H of a 96-well microtiter plate. Each plant extract was tested in triplicates. A hundred microlitre (100 µL) of bacterial culture was added into the wells and incubated at 37°C overnight. After incubation, 40 µL of Iodonitrotetrazolium chloride (Sigma Aldrich, USA) dye at 0.2 mg/mL was added to the wells for visual assessment of microbial viability. The MIC was recorded as the lowest concentration of extract that inhibited bacterial growth.

3.2.4 Determination of antioxidant activity

3.2.4.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

The antioxidant potential of the plant extracts was determined in triplicate according to Mwinga et al. (2019) with slight modifications. A volume of 750 µL of 0.1 mM DPPH solution dissolved in methanol was added to plant extracts dissolved in their respective solvents at 250, 125, 62.5, 31.3, and 15.6 µg/mL. The total volume of the mixture of plant extract and DPPH solution was 5 mL. The control was prepared in a similar manner by replacing the plant extract with methanol. The reaction mixture was kept in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm using an E-SP1100-UV-P spectrophotometer (Biocom Biotech, Gauteng, South Africa). The decrease in absorbance indicated the presence of antioxidant activity (Masoko et al., 2010). L-ascorbic acid was used as a standard and a solvent in which each plant extract was dissolved as a blank. Equation ⁽¹⁾ was used to calculate the percentage of free radical scavenging activity (% RSA).

$$\% \text{ RSA} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \right] \times 100 \quad (1)$$

Where RSA is free radical scavenging activity.

The RSA was graphed against the concentration of the plant extract, and the half-maximal inhibitory concentration (IC₅₀) was calculated from the normalised logarithmic

regression curve. Plant extracts with the lowest IC₅₀ have the greatest radical scavenging effect (Asadujjaman et al., 2013).

3.2.4.2 Ferric-reducing antioxidant power (FRAP) assay

The reducing power of plant extracts was assessed following the method of Otang-Mbeng et al. (2012). Various concentrations of each plant extract (250, 125, 62.5, 31.3, and 15.6 µg/mL) in distilled water were combined with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1 % w/v). The mixture was incubated for 20 minutes at 50°C, after which 2.5 mL trichloroacetic acid (10 % w/v) was added. The mixture was then centrifuged for 10 minutes at 300 rpm. A 2.5 mL volume of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of Iron (III) chloride (0.1 % w/v), and the absorbance was measured at 700 nm. Butylated hydroxytoluene and L-ascorbic acid were used as positive controls.

3.2.5 Data analysis

The results of plant extraction were reported using descriptive statistics, expressed as percentages. The disc diffusion, microdilution, DPPH, and FRAP assays were performed in triplicates, with the outcomes expressed as the mean and standard deviation of these three replicates. Significant differences in the DPPH and FRAP results (inhibition percentages and absorbance values, respectively) were analysed using one-way ANOVA followed by Tukey's post hoc tests in SPSS version 27, with a significance threshold set at $p < 0.05$. The comparisons between positive controls and different solvent extracts (acetone, hexane, and methanol) were conducted independently for each plant species and for each assay to evaluate differences in antioxidant activity between the solvent extracts within the respective assay.

3.3 Results

3.3.1 Plant extraction

Table 3.1 Extraction yield of six wild edible fruit species leaves using various solvents.

Solvent	Yield (%)					
	<i>C. spinarum</i>	<i>D. mespiliformis</i>	<i>E. crispa</i>	<i>F. thonningii</i>	<i>S. madagascariensis</i>	<i>S. spinosa</i>
Hexane	2.12	0.9	1.22	1.38	4.19	2.04
Acetone	2.06	2.8	2.77	1.84	1.41	1.06
Methanol	5.79	10.39	10.09	1.53	2.99	1.96

Dried *C. spinarum*, *S. spinosa*, *E. crispa*, *F. thonningii*, *S. madagascariensis*, and *D. mespiliformis* leaves from the Bushbuckridge Local Municipality, Mpumalanga Province were serially extracted using, acetone, hexane, and methanol. The extraction efficiency of different solvents from the dried leaves is presented in Table 3.1. Methanol extracted the highest quantity of crude extracts from *D. mespiliformis* (10.39%), *E. crispa* (10.09%), and *C. spinosa* (5.79%). This suggests that methanol has a strong affinity for compounds in these plants compared to acetone and hexane. Acetone and hexane extracted extremely low crude extract quantities, with less than 5% obtained from all plant samples.

3.3.2 Disc diffusion assay

Table 3.2 Antibacterial activity of plant extracts against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* assessed through disc diffusion and broth microdilution assays.

Plant species	Extractants	<i>Klebsiella pneumoniae</i>		<i>Pseudomonas aeruginosa</i>	
		Disc diffusion Inhibition zone (mm) ^b	Broth microdilution Minimum inhibitory concentration (mg/mL)	Disc diffusion Inhibition zone (mm) ^b	Broth microdilution Minimum inhibitory concentration (mg/mL)
<i>C. spinarum</i>	Hexane	NA	50	22	6.25
	Acetone	NA	NA	25	3.125
	Methanol	NA	NA	15	6.25
<i>D. mespiliformis</i>	Hexane	NA	NA	28	6.25
	Acetone	NA	3.125	20	0.781
	Methanol	NA	6.25	19	1.563
<i>E. crispa</i>	Hexane	NA	NA	28	1.563
	Acetone	NA	12.5	22	1.563
	Methanol	NA	12.5	21	1.563
<i>F. thonningii</i>	Hexane	NA	NA	20	6.25
	Acetone	NA	25	21	1.563
	Methanol	NA	50	22	3.125
<i>S. madagascariensis</i>	Hexane	NA	100	25	12.5
	Acetone	NA	25	25	3.125
	Methanol	NA	100	25	12.5
<i>S. spinosa</i>	Hexane	NA	50	18	3.125
	Acetone	NA	25	19	3.125
	Methanol	NA	100	20	12.5
Amoxicillin		35	0.781	45	0.781

Results are expressed as means $\pm$ SD of three independent measurements; NA: no activity. ^b Diameter of inhibition zone including disc diameter of 4 mm (10 μ L of 100 mg/mL).

The disc diffusion assay method was employed to assess the antimicrobial properties of dried *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. madagascariensis*, and *S. spinosa* leaves against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. As detailed in Table 3.2, most of the plant extracts did not exhibit activity against *Klebsiella pneumoniae*, as indicated by “NA” (No activity). These results suggest that these plant extracts do not have antimicrobial activity against this strain. In contrast, *P. aeruginosa* was susceptible to all the plant extracts especially hexane extracts of *D. mespiliformis* and *E. crispa* with the highest inhibition zone of 28 mm, and *C.*

spinarum acetone extract and *S. madagascariensis* acetone, hexane, and methanol extracts with inhibition of 25 mm. Amoxicillin showed strong antimicrobial activity with inhibition zones of 35 mm and 45 mm for *K. pneumoniae* and *P. aeruginosa*, respectively.

3.3.3 Microdilution assay

The acetone and methanol extracts of *D. mespiliformis* showed high antibacterial activity against *K. pneumoniae*, with minimum inhibitory concentrations (MICs) of 3.125 mg/mL and 6.25 mg/mL, respectively (Table 3.2). In contrast, the hexane extract of *F. thonningii*, *E. crispa*, and *D. mespiliformis*, along with the acetone and methanol extracts of *C. spinarum*, showed no inhibitory effect on *K. pneumoniae*. However, all plant extracts displayed varying levels of inhibitory activity against *P. aeruginosa*. Particularly, the acetone extract of *D. mespiliformis* demonstrated excellent activity with an MIC of 0.781 mg/mL. Additionally, other extracts, including the methanol extract of *D. mespiliformis* and the methanol, hexane, and acetone extracts of *E. crispa* and *F. thonningii*, showed potent antibacterial activity with an MIC of 1.563 mg/mL. Amoxicillin, a positive control showed strong antimicrobial activity with low MIC values (0.781 and 0.781 mg/mL) for *K. pneumoniae* and *P. aeruginosa*, respectively.

3.3.4 DPPH assay

The dose-dependent radical scavenging activity of methanol, acetone, and hexane leaf extracts of six wild fruit plants is illustrated in Figure 3.2 and their relative half-maximal inhibitory concentration (IC₅₀) in Table 3.3. The IC₅₀ of the most potent extracts were in the order: L-ascorbic acid < *E. crispa* methanol extract ≤ *D. mespiliformis* methanol extract < *C. spinarum* methanol extract < *D. mespiliformis* acetone extract < *E. crispa* acetone extract < *S. madagascariensis* methanol extract < *S. spinosa* methanol extract (Table 3.3). None of the plant extracts demonstrated antioxidant activity higher than L-ascorbic acid, which showed 96.93% inhibition at a concentration of 250 µg/mL and the lowest IC₅₀ of 0.07 µg/mL. However, some plant extracts demonstrated antioxidant activities comparable to ascorbic acid. Notably, the methanol extracts of *C. spinarum* and *D. mespiliformis* exhibited a perfect similarity ($p = 1.00$), followed by the methanol extract of *E. crispa* ($p = 0.995$). The methanol extracts of *S. madagascariensis* ($p = 0.450$) and *S. spinosa* ($p = 0.0859$) also showed comparable antioxidant potential.

Table 3.3 Half-maximal inhibitory concentration (IC₅₀) of acetone, hexane, and methanol extracts of the leaves of six wild edible fruit species.

Plant species	Extractants	IC₅₀ (µg/mL)
L-ascorbic acid	Methanol	0.07
<i>C. spinarum</i>	Hexane	814231.50
	Acetone	228661.95
	Methanol	1.51
<i>D. mespiliformis</i>	Hexane	474 491.98
	Acetone	1.92
	Methanol	1.42
<i>E. crispa</i>	Hexane	82.27
	Acetone	3.19
	Methanol	1.42
<i>F. thonningii</i>	Hexane	49.40
	Acetone	20.49
	Methanol	11.14
<i>S. madagascariensis</i>	Hexane	34.81
	Acetone	13.07
	Methanol	3.29
<i>S. spinosa</i>	Hexane	134.29
	Acetone	10.38
	Methanol	3.60

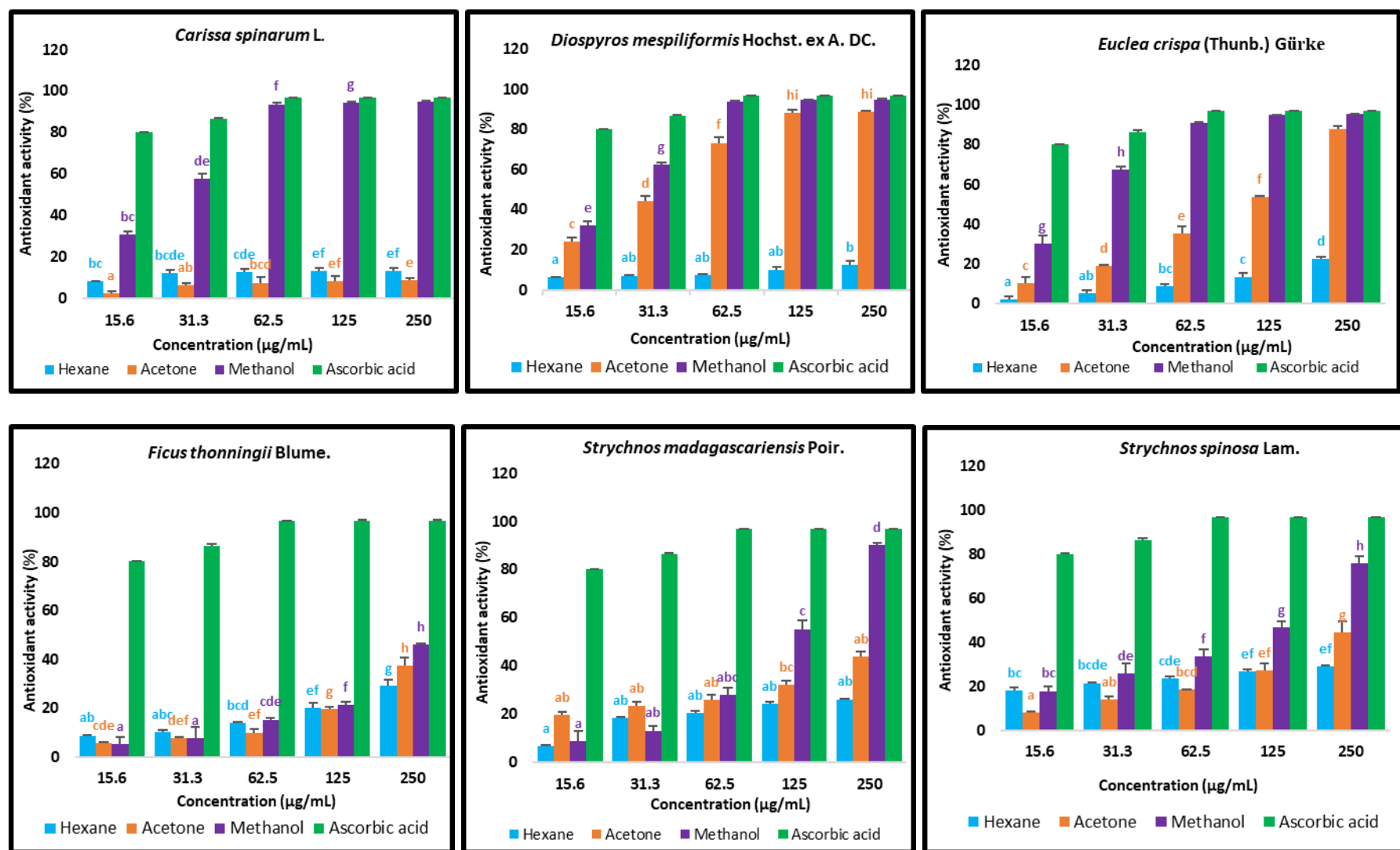


Figure 3.2 Antioxidant activity of hexane, acetone, and methanol leaf extracts from *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. spinosa*, and *S. madagascariensis*, evaluated against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Letter labels on the bar graphs indicate significant differences ($p < 0.05$) in the antioxidant activities of each plant extract compared to the positive control (ascorbic acid). Different letters indicate a significant difference ($p < 0.05$) between the antioxidant activities of plant extracts.

3.3.5 FRAP assay

The curves (Figure 3.3) show the dose-response reducing powers of methanol, hexane, and acetone extracts of selected plant species. The higher the absorbance of the reaction mixture at 700 nm the stronger the reducing power. The reducing power of the plant extracts and standards were as follows at 250 µg/mL: BHT> *S. madagascariensis* acetone extract> *S. spinosa* acetone extract> L-ascorbic acid> *C. spinarum* methanol extract> *E. crispa* methanol extract> *D. mespiliformis* methanol extract. The hexane, acetone, and methanol extracts of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, and *S. spinosa* showed statistically significant variations ($p < 0.001$) when evaluated independently within each plant species and compared to the positive controls, ascorbic acid and BHT. However, there was no significant difference between the hexane and acetone extracts of *S. madagascariensis* ($p = 0.058$).

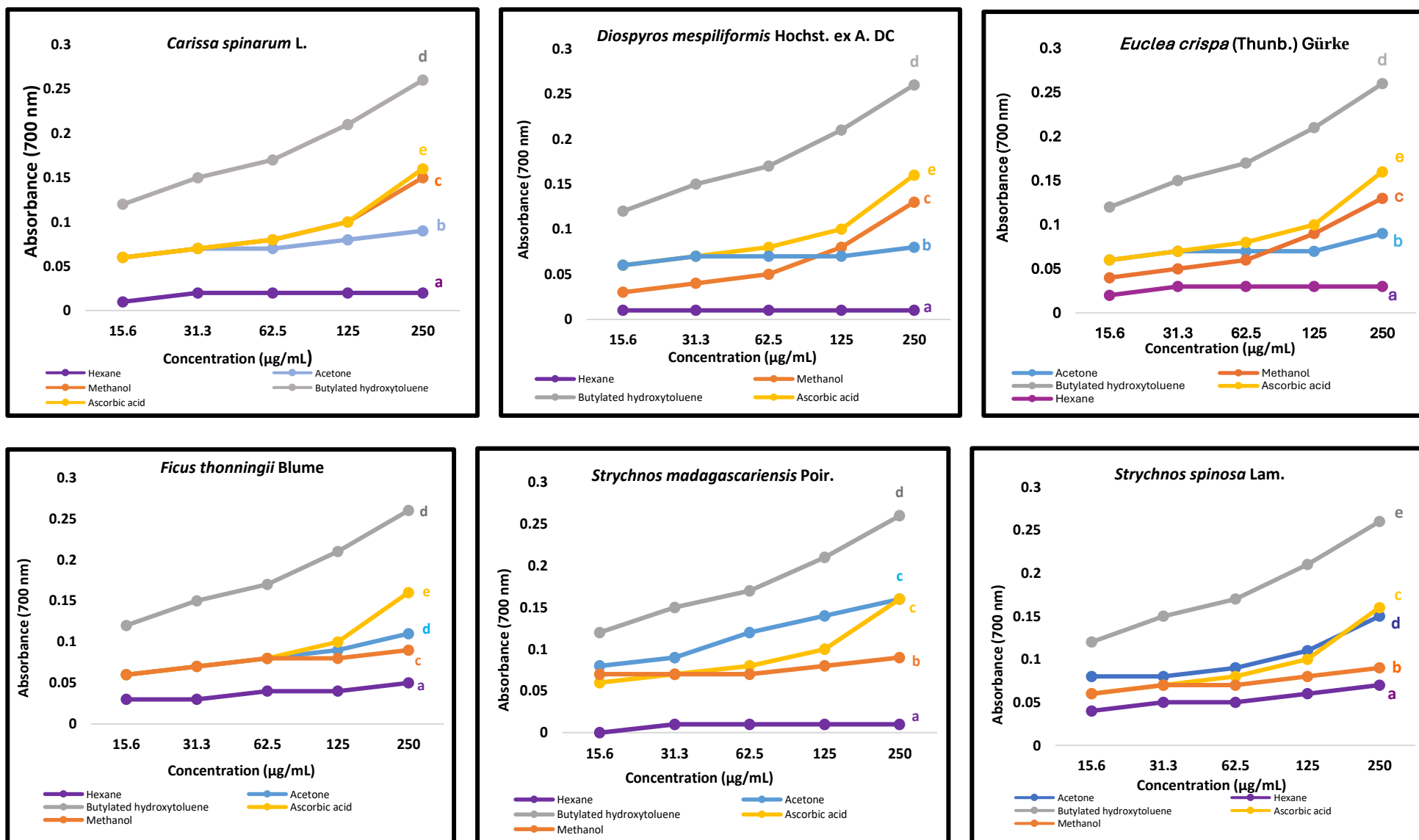


Figure 3.3 Ferric reducing power of hexane, acetone, and methanol leaf extracts from *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. spinosa*, and *S. madagascariensis*. Distinct letters on the line graphs indicate significant differences ($p < 0.05$) in the reducing power of each extract compared to the positive controls (ascorbic acid and butylated hydroxytoluene) at 250 µg/mL, whereas the same letter denotes no significant difference ($p > 0.05$).

4.4 Discussion

4.4.1 Plant extraction

Extraction yield is affected by the type of solvent, the solvation power, and the affinity (Sagili et al., 2023). Polar compounds are most commonly extracted using a polar solvent such as methanol (Samaraweera et al., 2023). Similar to the results of the present study, previous studies have proven methanol as the best solvent for high plant extract yield (Egra et al., 2023).

4.4.2 Disc diffusion

Contrary to the current findings, Tiruneh et al. (2022) found that the methanol extract of *C. spinarum* leaves exhibited antimicrobial activity against *K. pneumoniae* with an inhibition zone of 11.0 ± 0.23 mm at 100 mg/mL. The butanol fraction of *E. crista* leaves at 10 mg/mL had an inhibition zone of 18 mm against *K. pneumoniae* and 16 mm against *P. aeruginosa* (Alayande et al., 2017) which is comparable to the 21 mm inhibition zone obtained from the methanol and acetone extracts of *E. crista* (Table 3.3). A study conducted by (Ijoma and Ajiwe, 2023) revealed that the hexane and methanol leaf extracts of *F. thonningii* had an inhibition zone of 19.2 and 20 mm respectively against *K. pneumoniae* which is contrary to the findings (Table 3.2) wherein the plant extracts showed no activity against *K. pneumoniae*. The variations in the antimicrobial activities in the current study as compared to the literature may be resulting from differences in the geographical location, seasonality, and climatic conditions from which the plant species were collected since geographical and climatic conditions affect the phytochemistry of plants and thereby their biological activity (Adeosun et al., 2022). Furthermore, different types of cultures of the same bacterial strain may have different susceptibilities to plant extracts. For instance, Alayande et al. (2017) found differences in the antibacterial activity of *E. crista* leaves against *K. pneumoniae* (ATCC 13047) and *K. pneumoniae*.

3.4.3 Microdilution assay

Hexane leaf extracts of *D. mespiliformis* demonstrated strong antibacterial activity against *P. aeruginosa* with MICs ranging from 156.25 to 312.5 µg/mL (Ebbo et al., 2019) like the hexane, acetone, and methanol leaf extracts (Table 3.2). The current study found that the methanol and acetone extracts of *E. crista* had much higher MICs of 12.5 mg/mL against *K. pneumoniae*. Interestingly, the methanol, hexane, and acetone extracts of *E. crista* (Table 3.2) each had an MIC of 1.563 mg/mL against *P.*

aeruginosa which may indicate that all the plant extracts contain antimicrobial phytochemicals whose mechanism of action needs to be studied. Ultimately, *E. crispa* and *D. mespiliformis* exhibited very similar activities with MIC of 1.563 mg/mL against *P. aeruginosa* which may result from the fact that both species belong to the Ebenaceae family and may contain similar compounds. For instance, Pretorius et al. (2003) identified a flavonoid with antibacterial activity known as rutin from the leaves of *E. crispa*. Hawas et al. (2022) also isolated rutin from the leaves of *D. mespiliformis*. The current research indicates that the methanol and acetone extracts of *F. thonningii* had poor antimicrobial activities with MICs of 25 and 50 mg/mL against *K. pneumoniae*. The findings of the current study also displayed that the acetone and methanol extracts of *S. madagascariensis* and *S. spinosa* had similar activity against *P. aeruginosa* (Table 3.2), due to shared phytoconstituents responsible for antimicrobial activity among plants of the same family and genus (Islam et al., 2024).

3.4.4 Comparison between disc diffusion and microdilution assay

The disc diffusion assay was used for preliminary screening to obtain qualitative results in the form of inhibition zones while the microdilution assay was used to determine the quantitative results in the form of the MIC of plant extracts (Palladini et al., 2023). The current findings proved the microdilution assay to be the most sensitive method of determining the antibacterial activities of plant extracts. Similarly, Scorzoni et al. (2007) found that plant extracts did not show antifungal activity in disc diffusion but showed activity in microdilution assay. Scorzoni et al. (2007) further elaborated that the antimicrobial effectiveness of different samples may not always be accurately detected due to variances in physical properties like solubility, volatility, and diffusion in agar. Moreover, factors such as agar volume, microbial strains, disk adsorption, disk size, quantity of compound applied, type and strength of agar, and pH, can all impact the size of inhibition zones. The disc diffusion method is a simple, cost-effective technique that allows multiple antimicrobial agents to be tested on a single plate, with results easily interpreted through visible bacterial growth since bacterial growth can be directly observed on the plate (Salam et al., 2023).

3.4.5 DPPH assay

Diospyros mespiliformis and *Euclea crispa* are members of the Ebenaceae family with excellent antioxidant activities. Previous studies show that Ebenaceae plant species have good antioxidant activities including *Diospyros ebenum* (Baravalia et al., 2009).

The results (Figure 3.3) indicate that methanol extracts of *E. crispa*, *C. spinarum*, *D. mespiliformis*, and *S. madagascariensis* had excellent antioxidant activities at >90% which is comparable to ascorbic acid which was the most potent with 96.93% radical scavenging ability. Furthermore, the results (Figure 3.3) show that the acetone extracts of *D. mespiliformis* and *E. crispa* exhibited considerable antioxidant activities with 88.51% and 87.9%, respectively. Hexane extracts had the least antioxidative effect in each plant species with the highest antioxidative effect with IC₅₀ of 34.81 µg/mL observed in *S. madagascariensis*. The current findings (Table 3.3) indicate that *D. mespiliformis* methanol and acetone extracts of leaves possess good antioxidant activity with an IC₅₀ of 1.42 and 1.92 µg/mL respectively. Hegazy et al. (2019) also found the fruit of *D. mespiliformis* to exhibit good antioxidant activity with percentage inhibition of up to 87.36% comparable to the 88.51% inhibition by the leaves obtained in the current study (Figure 3.2). *E. crispa* also showed great antioxidant activities with IC₅₀ of 1.42 µg/mL (Table 3.3). However, Palanisamy et al. (2018) found a much higher IC₅₀ value of 135.4 ± 0.7 µg/mL from the ethanolic extract of *E. crispa* leaves. *C. spinarum* methanol extract also showed notable activities against DPPH with an IC₅₀ of 1.51 µg/mL. Liu et al. (2021) isolated ten phytochemicals from the ethanol fraction of the root bark of *C. spinarum* that displayed antioxidant activities against DPPH, especially one unidentified compound with an IC₅₀ value of 16.5 ± 1.2 µM. The methanol extracts of two plant species belonging to the Loganiaceae family, *S. spinosa* and *S. madagascariensis* had IC₅₀ of 3.29 and 3.60 µg/mL, respectively. The IC₅₀ values are almost equal which may be because they belong to the same family and genus (*Strychnos*). Isa et al. (2014) evaluated the antioxidant potential of the leaf of *S. spinosa* using DPPH radical assay and the results showed free radical scavenging activities of acetone, methanol, and dichloromethane/methanol extracts with IC₅₀ values ranging from 33.66–230.15 µg/mL. From their study, methanol extract had the most noteworthy activity with IC₅₀ of 36.56 µg/mL which is much lower compared to the current findings. *F. thonningii* had the least antioxidant activity with the highest scavenging activity of 46.19% exhibited by methanol extract at 250 µg/mL. However, Fongang et al. (2015) reported that the methanolic stem roots extract has good free radical scavenging activity (68.30, 75.20, and 81.26%) at 10, 50, and 100 µg/10 µL, respectively.

3.4.6 FRAP assay

Ferric-reducing power (FRAP) assays measure the ability of plant extracts to reduce ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) due to their reductive properties. When iron (III) chloride (FeCl_3) is added to a solution containing the ferrous (Fe^{2+}) form, a chemical reaction whereby the Fe^{3+} ions from FeCl_3 react with the Fe^{2+} ions to form Prussian, blue-coloured complex forms. Thus, the extent of reduction can be assessed by measuring the formation of Perl's Prussian blue at 700 nm. Greater absorbance signifies a stronger ferric-reducing power (Bursal and Köksal, 2011). In the current study (Figure 3.3), hexane extracts showed the least ferric-reducing power in all the selected plant species. However, the positive control (BHT) had the most potent reducing power incomparable to the rest of the plant extracts. Interestingly, the reducing power of *S. madagascariensis* was comparable to ascorbic acid. The study also showed a dose-dependent relationship between the plant extract concentration and the ferric-reducing power. The increase in concentration results in an increase in the reducing power. The current study showed that the ferric-reducing power of plant extracts was good at 125 and 250 $\mu\text{g/mL}$. The present findings indicate that the methanol extract of *E. crispa* exhibits strong ferric-reducing power, consistent with the findings of Palanisamy et al. (2018), who reported similar activity in the ethanolic extract of *E. crispa* leaves. Additionally, previous studies by Liu et al. (2021) have shown that the root bark of *C. spinarum* possesses significant ferric-reducing power, which aligns with the current results observed for the methanol extract of *C. spinarum* (Figure 3.3). The ferric-reducing abilities of *S. madagascariensis* and *S. spinosa*, and *D. mespiliformis* and *E. crispa* were closely related since they belong to the Loganiaceae and Ebenaceae families respectively. The findings of the present study are further supported by Achika et al. (2023), who reported that the stem bark of *Strychnos innocua* Del., a member of the Loganiaceae family, exhibited strong ferric-reducing power that increased with concentration.

3.4.7 Comparison between DPPH and FRAP assays

The current study revealed that some plant extracts exhibited a high scavenging effect against DPPH but low reducing power. For example, methanol extracts of both *S. madagascariensis* and *S. spinosa* showed high DPPH radical scavenging activity but weaker ferric-reducing power than acetone extracts. Conversely, methanol extracts of *D. mespiliformis*, *E. crispa*, and *C. spinarum* demonstrated strong DPPH radical scavenging activity and ferric-reducing power. Prakash et al. (2011) found that some

plant extracts exhibit good DPPH radical scavenging activity and good ferric-reducing power. Additionally, high antioxidant activity is linked to high total phenolic content (El Kamari et al., 2024). Methanol extracts showed the best activity in both assays compared to acetone and hexane (Figures 3.2 and 3.3), indicating that methanol is an excellent solvent for extracting antioxidant compounds. Hexane extracts, however, showed less activity in both assays. Hossain and Shah (2015) reported that hexane extracts exhibited the lowest antioxidant activity when compared to more polar solvents such as ethanol, butanol, and chloroform. They further emphasized that the higher antioxidant activity observed in polar solvent extracts was linked to their phenolic content. Similarly, Johari and Kong (2019) found that hexane extracts had the lowest phenolic content, which corresponded to the weakest antioxidant activity, particularly in comparison to methanol extracts.

3.5 Concluding remarks

The study demonstrates that the leaves of selected wild fruit plant species exhibit significant antimicrobial and antioxidant properties, influenced by the choice of extraction solvent. These findings scientifically support the traditional use of these plants in managing infectious skin conditions and oxidative stress-related skin disorders. The leaves of *C. spinarum*, *D. mespiliformis*, *E. crispa*, *F. thonningii*, *S. madagascariensis*, and *S. spinosa* exhibited strong antimicrobial activity, highlighting their potential in treating bacterial skin infections. Additionally, the leaves of selected plant species especially *C. spinarum*, *D. mespiliformis*, *E. crispa*, *S. spinosa*, and *S. madagascariensis* showed notable antioxidant activity, suggesting their role in combating oxidative damage linked to skin aging and inflammation. These results underscore the therapeutic potential of wild fruit plant species from Bushbuckridge Local Municipality, Mpumalanga Province, as valuable sources of bioactive compounds. Further research should focus on isolating and characterising the active compounds responsible for these antimicrobial and antioxidant effects. Additionally, *in vivo* studies are necessary to validate the efficacy of the leaves of the studied wild fruit plant species observed *in vitro*, and future investigations should expand antimicrobial testing to a wider range of bacterial strains for a more comprehensive assessment. Cytotoxicity evaluations are also crucial to ensure the safe application of these plant extracts in healthcare, dermatology, and skin care product development. The study

indicates that the leaves of wild plant species possess antimicrobial and antioxidant properties, which are influenced by the solvents used for extraction. These findings provide scientific support for the traditional use of these plants in treating infectious skin conditions and oxidative stress.

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Chapter 4.0: The nutritional and mineral composition of wild fruit plants harvested from Mpumalanga Province

Summary

In both developed and developing countries, food insecurity is an increasing concern often accompanied by various health conditions including skin conditions, which are highly susceptible to malnutrition. The study evaluated the nutritional composition of the fruits of *Diospyros mespiliformis*, *Ficus thonningii*, and *Strychnos spinosa*. Additionally, the study examined the mineral content of both the leaves and fruits of *D. mespiliformis*, *F. thonningii*, and *S. spinosa*. The goal was to investigate their potential applications in combating food insecurity linked to skin health while supporting skin maintenance through dietary use. Nutritional analysis revealed that *F. thonningii* had the highest moisture (82.07%) and crude fat (3.92%) content, while *S. spinosa* seeds had the highest crude protein (31.20%). Fibre content was highest in the peels of all species, with *S. spinosa* peel containing 44.21%. Elemental analysis indicated high levels of beneficial minerals, such as calcium, iron, and magnesium, with *S. spinosa* leaves having notably high manganese (Mn) content (1 728 mg/Kg). *D. mespiliformis* and *F. thonningii* leaves efficiently bioaccumulated zinc (Zn) with a bioaccumulation factor (BAF) of 1.25 and 1.58 respectively, while *S. spinosa* exhibited a high BAF of 25.08 for Mn. The fruits of all three species contained arsenic (As), cadmium (Cd), and chromium (Cr), with elevated carcinogenic risk factors beyond 0.001 mg/Kg/day for As and Cd, and 0.012 mg/Kg/day for Cr. *D. mespiliformis* fruit had the highest carcinogenic risk factor of 0.0063 for Cr. While these fruits present valuable nutritional benefits, their potential toxicity warrants further investigation. Strategies for mitigating metal contamination could enhance their safe use in food security initiatives and health-related applications.

4.1 Introduction

Global food security is declining as developing countries fail to meet the demands for safe, nutritious, and affordable food in their communities (Mkhize et al., 2023). Nearly half of the world's population cannot afford a healthy diet because the world's food systems are in disarray (Von Braun et al., 2023). This can be attributed to climate change, increased population growth (Mirón et al., 2023; Mutengwa et al., 2023), and the recent outbreak of pandemics such as the COVID-19 virus among others (Alabi

and Ngwenyama, 2023). Conflicts between Gaza and Israel, Russia and Ukraine, have had negative socioeconomic impacts on global food and nutrition security (Hassoun et al., 2024; Lu, 2024; Raposo et al., 2023). Southern Africa is highly prone to food and nutrition insecurity due to climate change (Mutengwa et al., 2023). The concept of food security is defined as “everyone having access, at all times, to enough food to sustain a healthy and active lifestyle”, while nutrition security focuses on the impact of nutrition on health, i.e. food insecurity and diet-related diseases (Seligman et al., 2023). Furthermore, a healthy diet is important for good health and nutrition, and the prevention of chronic diseases (Gelaye, 2023).

Food insecurity is broadly associated with malnutrition (Spoede et al., 2021) especially undernutrition, which is caused by an inadequate intake of food. But overnutrition is caused by excessive food intake, or specific nutrient deficiencies (Serra et al., 2018). Nutrition deficits involve varying degrees of deficiencies in energy, protein, vitamins, and trace elements (Dupont et al., 2018). Previous studies have shown that food insecurity leads to undernutrition (Hasan et al., 2023; Lye et al., 2023). Furthermore, many health conditions in South Africa are attributed to malnutrition (Mkhize et al., 2023). Notably, both undernutrition and overnutrition can alter the physiology of the skin (Piccardi and Manissier, 2009). Hence, nutrition plays a critical role in maintaining the health and condition of the skin. For instance, extreme calorie/protein deficiency (Kwashiorkor) causes biochemical changes in the skin (Piccardi and Manissier, 2009). In addition to impaired wound healing, malnutrition increases the likelihood of developing pressure ulcers (Stotts and Moulder, 2022). Other skin defects such as acanthosis nigricans, acrochordons, and keratosis pilaris often result from overnutrition (Yosipovitch et al., 2007). Furthermore, obese individuals experience complications in wound healing because their skin folds, and creates a moist environment that promotes bacterial growth (Munoz et al., 2020). Diets rich in fruits are recommended to prevent chronic inflammatory skin conditions such as psoriasis (Duarte et al., 2012). Importantly, fruits are considered essential components of a high-quality diet (Wallace et al., 2020).

Since the skin is the largest human organ with tissue that has high proliferative potential, it is essential to consume sufficient quantities of proteins, carbohydrates, and fats (Michalak et al., 2021). Indigenous fruits are rich sources of nutrients, vitamins, and minerals necessary for human health, supporting skin health and

offering potential benefits for preventing diseases and combating food insecurity (Aguilera and Toledo, 2024). Mytton et al. (2014) highlights that fruit consumption protects against various diseases and recommends strategies to increase fruit consumption. Therefore, this study aims to determine the proximate and elemental composition of three wild fruit plant species harvested from the Mpumalanga Province and to deduce their potential to curb food and economic insecurity while promoting good health.

4.2 Materials and methods

4.2.1 Sample collection and preparation

The leaves and fruit samples of *Diospyros mespiliformis* Hochst. ex A.DC., *Strychnos spinosa* Lam., and *Ficus thonningii* Blume and their corresponding soil samples were collected from various villages (Table 4.1) under the Jurisdiction of the Mnisi Tribal Council in Bushbuckridge Local Municipality, Mpumalanga Province (refer to figure 3.1 in chapter 3). The fruit peel, seeds, and pulp of *D. mespiliformis* and *S. spinosa* were mechanically separated. *F. thonningii* fruits were used whole due to the small size of the fruit. The leaves were rinsed with distilled water, air-dried, and ground into a fine powder. The soil samples were passed through a 2 mm sieve to remove debris and particles.

Table 4.1 Dates of collection of the samples that were collected and analysed for nutritional and elemental content.

Plant species	Sample	Month of collection	Season	Name of village
<i>Diospyros mespiliformis</i> Hochst. ex A.DC.	Soil	August	Winter	Gottenburg
	Leaves			
	Fruit	May	Autumn	
<i>Strychnos spinosa</i> Lam.	Soil	August	Winter	Wolverdiend
	Leaves			
	Fruit	May	Autumn	
<i>Ficus thonningii</i> Blume.	Soil	August	Winter	Seville
	Leaves			
	Fruit	May	Autumn	

4.2.2 Proximate analysis

4.2.2.1 Moisture content

A mass of 5 g of fruit samples in triplicates was weighed and placed in pre-weighed crucibles. The samples were dried in an oven set at 60°C to a constant weight. The crucibles with dried samples were transferred to the desiccator and allowed to cool at room temperature and weighed (Jacob et al., 2016). The percentage moisture content was calculated using equation (1) below.

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

Where W1-mass of a dried empty crucible (add the rest of the masses)

4.2.2.2 Ash content

A mass of 5 g of fruit samples was weighed and placed in the dried and pre-weighed crucibles and reweighed (W2). The samples were dried in a muffle furnace at 500°C for 3 hours until a grey residue was obtained. The crucibles were cooled in a desiccator and weighed (W3) (Jacob et al., 2016). The percentage ash content was calculated using equation (2) below.

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (2)$$

4.2.2.3 Crude fat

Round bottom flasks (250 ml) were oven-dried at 105°C for 30 minutes, cooled in a desiccator and weighed. A mass of 2 g (W1) of the samples was weighed into labelled extraction thimbles and lightly plugged with cotton wool. The round bottom flasks were filled with 300 ml of hexane. The thimbles were placed inside the Soxhlet extraction apparatus and allowed to reflux for 8 hours. The heating temperature was adjusted to 50–55°C. The thimbles were removed, and the hexane was recycled. When the flasks were almost free of the hexane, they were removed and dried for 1 hour at 105°C until the solvent was completely dry. The flasks were cooled in desiccators and weighed (W2) (Jacob et al., 2016). The percentage fat content was calculated using equation (3) below.

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

4.2.2.4 Crude fibre

A mass of 2 g (W_s) of defatted samples was weighed into a 250 ml conical flask, and 200 ml of 1.25% sulphuric acid was added. The sample was heated for 30 minutes and filtered using poplin cloth in a Buchner funnel and washed with hot water until no traces of acid were observed using the pH litmus paper. The residue was transferred back to the 250 ml conical flask and 200 ml of 1.25% sodium hydroxide was added. The sample was heated for 30 minutes and filtered using poplin cloth and washed with hot water until no base was detected. The sample was transferred into a crucible and dried in an oven, cooled, and weighed (W_{cd}). The crucible was placed in a muffle furnace and ashed for 6 hours, cooled, and weighed (W_{ca}) (Jacob et al., 2016). Equation (4) below was used to calculate the percentage fibre.

$$\% \text{ Fibre} = \frac{W_{cd} - W_{ca}}{W_s} \times 100 \quad (4)$$

4.2.2.5 Protein

The nitrogen content was estimated using the Kjeldahl model. The crude protein was calculated by multiplying the evaluated nitrogen with 6.25. A mass of 1 g of sample was weighed and transferred into a digestion flask followed by the addition of two selenium tablets as a catalyst. A volume of 12 ml of sulphuric acid was added, and the tubes were heated until clear solutions were obtained. The clear solutions were transferred to a 50 mL volumetric flask made to the mark. 10 ml of digest followed by 10 ml of 40% sodium hydroxide (NaOH) solution were pipetted into a Kjeldahl distiller. A conical flask containing 5 ml of 2% boric acid and 3 drops of mixed indicator were placed under the condenser outlet. At the end of distillation, the ammonium sulphate solution was converted to ammonia. The ammonia gas produced condensed and was collected as a liquid into the conical flask containing boric acid and mixed indicator. The nitrogen in the distillates was determined by titrating with 0.01 M of HCL. Colour changes from green to pink marked the endpoint (Jacob et al., 2016). Equation (5) below was used to estimate the % of nitrogen and equation (6) to estimate the % crude protein.

$$\% N = \frac{(S-B) \times N_{acid} \times 0.014 \times D}{\text{Weight of sample} \times V} \times 100 \quad (5)$$

$$\% \text{Crude protein} = 6.25 \times \%N (* \text{Correction factor}) \quad (6)$$

S= sample titration reading; B=blank titration reading; N= normality of HCL; D= Dilution of sample after digestion; V= volume taken for distillation; 0.014= milliequivalent weight of nitrogen.

4.2.2.6 Carbohydrate

Carbohydrate estimate was calculated using equation (7) below (Jacob et al., 2016).

$$\%Carbohydrate = 100 - (\%Ash + \%Protein + \%Fat + \%Fibre) \quad (7)$$

4.2.2.7 Energy value (Ev)

The energy/calorific value of fruit samples expressed in kilocalories was calculated by multiplying the values of protein, fat, and available carbohydrate by 4.00, 9.00, and 4.00 respectively, and adding the resulting products (Jacob et al., 2016).

4.2.3 Elemental analysis

The 0.5 g samples were digested using the microwave-assisted closed-vessel method with 10 ml of 70% nitric acid (HNO₃) in ceramic vessels. After a 1 hour pre-digestion and sealing, the samples were subjected to microwave digestion at 500 W for 30 minutes, followed by 650 W for 15 minutes. Forced ventilation was used to cool the bombs for 15 minutes. The sample digests were filtered into 50 ml volumetric flasks, using 0.45 µm filters. The volumetric flasks were filled with double distilled water and transferred to polyethylene bottles for analysis of the elements. The samples were analysed for Arsenic (As), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Calcium (Ca), Copper (Cu), Manganese (Mn), Magnesium (Mg), Iron (Fe), Lead (Pb), Selenium (Se), and Zinc (Zn) by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The analytical wavelengths were selected based on minimum spectral interferences and maximum analytical performance. Initially, the 3 most sensitive lines were selected. From these lines, the lines with no interfering elements were then selected (Shelembe, 2014).

4.2.3.1 Quality assurance

Table 4.2 Certified and found values of certified reference material (strawberry leaves).

Elements	Wavelength (λ)	Measured	Certified
Cr	283.563	1.944553	2.15$\pm$0.34
Cu	324.752	10.732277	10
Fe	259.939	198.0613	818$\pm$48
Mn	257.61	64.68946	171$\pm$10
Zn	213.857	39.07021	24$\pm$5

The validity of an analytical technique was assessed by examining the certified reference material (CRM, strawberry leaves, LGC-7162, Community Reference Bureau of the Commission of the European Communities, Brussels, Belgium). The wavelength that yielded the most satisfactory CRM outcomes, characterized by high intensity and absence of interfering elements, was chosen. Reagent blanks and calibration standards for each element in double-distilled water (within the estimated ranges) were prepared from 1000 mg/L stock standard solutions (Fluka Analytical, Sigma, Switzerland) to generate five-point calibration curves representing concentration strength (refer to appendix 4, figures A4.1 and A4.2). The most suitable linear fit of the curves was selected. All samples, including calibration standards and blanks, underwent analysis in 70% nitric acid to eliminate matrix effects and minimize spectral interferences. The CRMs were analysed to confirm the accuracy and precision of the calibration curve and were also utilized to accept or reject it. Method accuracy was assessed by comparing the mean experimental values from three replicates to the certified values for each analyte using the CRMs (Table 4.2). A two-sample t-test (assuming equal variances) verified accuracy by showing no statistically significant differences between the means ($p > 0.05$). Method precision, indicating the degree of measurement consistency upon repeated analysis of an analyte, was evaluated by comparing the %RSD for the CRM, which should ideally be within 20% of the true value. The experimental values within the 95% confidence interval fell within the appropriate range specified for the CRM. Hence, the analytical method was deemed acceptable (Shelembe, 2014).

4.2.3.2 Bioaccumulation factor (BAF)

The BAF quantifies the capacity of plants to accumulate and retain heavy metals from the soil. It is determined by comparing the concentration of metals in the plant to the concentration of metals in the soil it grows in as defined in equation (8) below.

$$BAF = \frac{[metal]_{\frac{leaves}{fruit}}}{[Metal]_{soil}} \quad (8)$$

A BAF value of 1.00 is an indication that the plant can only take up elements but cannot accumulate in the tissues. A BAF value >1.00 implies that the plant may have the ability to absorb and accumulate elements in its tissues (Islam et al., 2022).

4.2.3.3 Human health risk assessment

4.2.3.3.1 Estimated daily intake (EDI) indices

The EDI of elements through the consumption of wild fruits was calculated using the element concentrations in mg/Kg (Kavcar et al., 2009), the recommended daily intake (RDI) of fruits (0.4 Kg/day) (Mehri et al., 2024), as well as the average body weight (BW) in kg 70 Kg for an adult (Zergui et al., 2024) as shown in equation 10 below.

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

4.2.3.3.2 Target Hazard Quotient (THQ)

Table 4.3 The oral toxicity reference dose (RfD) of toxic minerals (Adeagbo et al., 2024).

Mineral	RfD (mg/Kg/day)
As	0.0003
Co	0.0003
Cd	0.00005
Cr	0.003
Cu	0.04
Fe	0.7
Mn	0.14
Zn	0.3

The health risk associated with consuming wild fruits was evaluated as a non-carcinogenic hazard through the THQ using equation 11 below.

$$THQ = \frac{EDI}{R_f D} \quad (11)$$

The exposed population is assumed to be safe when the $THQ < 1$, but if the value of $THQ > 1$, there is an unacceptable risk of adverse non-carcinogenic effects on human health (Adeagbo et al., 2024).

4.2.3.3.3 Target Carcinogenic Risk (TCR)

The potential cancerous health risk via the consumption of wild fruits was estimated using the toxicity index known as slope factor (SF) to compute the potential cancerous health risk of an individual developing cancer over a lifetime as a result of exposure to possible carcinogens using equation 12 below.

$$TCR = SF \times CDI \quad (12)$$

Where the oral carcinogenic SF from USEPA (2015) was; 1.7, 0.38, 0.5, and 0.009 (mg/Kg/day)⁻¹ for As, Cd, and Cr respectively converts the chronic daily intake (CDI) to the incremental risk of individual developing cancer. The target carcinogenic risk factors of Co, Cu, Fe, Mn, and Zn were not calculated because their carcinogenic slope factor was not established since the metals have no carcinogenic effect (Kumar, 2021). Slope factor As=1.5 (Mshengu et al., 2023); Cd= 0.38 , Cr= 0.5 (Adeagbo et al., 2024). The USEPA recommendation for carcinogenic limit is 1.0×10^{-4} (Adeagbo et al., 2024).

4.2.4 Data analysis

Data were presented as the mean of three independent replicates $\pm$ standard deviation (SD). The coefficients were obtained using Statistical Package for the Social Sciences (SPSS) version 30.0.0. The correlation ranges between + 1 and -1, where + 0.8 correlation indicates a strong positive relationship, while - 0.8 correlation indicates a negative relationship. The significant difference was determined using Post Hoc's Tukey and LSD tests in SPSS, with the significant difference determined at $p < 0.05$.

4.3.1 Proximate analysis

4.3 Results

Table 4.4 Nutritional composition of wild fruits harvested from the Mpumalanga Province.

Plant name	Proximate analyses (%)							
	Fruit part	Moisture	Ash	Crude fat	Crude fibre	Protein	Carbohydrates	Energy value (Kcal/100 g)
<i>D. mespiliformis</i>	Peel	38.13±1.03 ^d	2.93±0.07^{bc}	1.73±0.03 ^{bc}	31.23±0.58^b	2.37±1.02 ^f	61.78±2.09 ^c	272.13±2.83^c
	Pulp	59.13±0.59^b	2.21±0.13 ^{bc}	2.5±0.25^{ab}	2.95±0.20 ^f	6.20±0.29^d	86.02±0.41^a	391.38±2.25^a
	Seeds	37.73±1.13 ^d	3.41±0.46 ^{ab}	1.13±0.12 ^{bc}	11.40±0.05 ^{de}	5.37±0.63 ^d	78.70±1.18^b	346.39±2.27^b
<i>S. spinosa</i>	Peel	39.93±2.74 ^d	2.98±0.13 ^{bc}	1.47±0.03 ^{bc}	44.21±0.33^a	8.83±0 ^e	42.95±1.17 ^e	218.61±1.97^d
	Pulp	76.33±0.18^a	5.87±0.93^a	0.25±0.75 ^c	10.30±0.15 ^e	23.62±1.04 ^b	59.55±0.51 ^c	342.55±0.55 ^c
	Seeds	51.27±1.87 ^c	3.55±0.06 ^{ab}	1.63±0.37 ^{bc}	13.13±0.43 ^d	31.20±0.29^a	51.85±1.9 ^d	341.45±0.05^b
<i>F. thonningii</i>	Whole fruit	82.07±1.75^a	1.2±0.2 ^c	3.92±0.42^a	17.55±0.30 ^c	20.60±1.47 ^c	55.16±1.02 ^{cd}	346.68±2.18 ^b

The results are expressed as the mean of three replicates ± standard error (SE). Statistical analyses were performed separately for each nutrient. Identical superscript letters within the same column indicate no significant difference ($p > 0.05$). The analyses were conducted using Post Hoc's Tukey and LSD tests, with the significant difference determined at $p < 0.05$.

The analysis of the three fruit species revealed diverse nutritional components (Table 4.4). Moisture content was highest in the whole fruit of *F. thonningii* (82.07 %), followed by the pulp of *S. spinosa* (76.33%) and *D. mespiliformis* (59.13%). *S. spinosa* pulp contained the largest ash content (5.87%), while crude fat was most abundant in the whole fruit of *F. thonningii*, with *D. mespiliformis* pulp ranking second (2.50%). Crude fibre was found to be significantly higher in fruit peels than in pulp or seeds, with the highest amounts in *S. spinosa* peel (44.21%) and *D. mespiliformis* peel (31.23%). The lowest crude fibre content was in the pulp of *D. mespiliformis* (2.95%). Crude protein was most abundant in *S. spinosa* seeds (31.20%), followed by *S. spinosa* pulp (23.62%) and *F. thonningii* whole fruit (20.60%). Carbohydrate content was highest in the pulp, seeds, and peel of *D. mespiliformis*. Regarding the energy values, the pulp of *D. mespiliformis* had the highest energy content, while *S. spinosa* peel had the lowest.

Table 4.5 Elemental analysis of the fruit and leaf samples of selected plant species and the soil samples from their respective sites.

Elements	Wavelength (λ)	Concentration (mg/Kg)								
		<i>D. mespiliformis</i>			<i>F. thonningii</i>			<i>S. spinosa</i>		
		Soil (Site A)	Leaves	Fruit	Soil (Site B)	Leaves	Fruit	Soil (Site C)	Leaves	Fruit
As	228.81	0.4 ± 0.01 ^f	0.2 ± 0.01 ^d	0.1 ± 0.00 ^b	0.3 ± 0.01 ^e	0.2 ± 0.01 ^d	0.2 ± 0.02 ^c	0.2 ± 0.01 ^d	BDL	BDL
Cd	228.80	0.3 ± 0.01 ^b	0.3 ± 0.02 ^b	0.2 ± 0.01 ^a	0.3 ± 0.01 ^b	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.02 ^a
Ca	315.89	1 502 ± 2.08 ^e	2 793 ± 0.58 ^f	395.9 ± 2.09 ^c	753 ± 0.50 ^d	12 190 ± 0.44 ^h	4 933 ± 3.00 ^g	247.9 ± 0.46 ^a	17 240 ± 2.00 ⁱ	388.2 ± 0.09 ^b
Cu	224.7	9.7 ± 0.09 ^g	9.3 ± 0.02 ^f	3 ± 0.03 ^a	6.6 ± 0.03 ^d	6.1 ± 0.08 ^c	7 ± 0.01 ^e	4.8 ± 0.02 ^b	6 ± 0.01 ^c	3.1 ± 0.00 ^a
Co	228.62	2.6 ± 0.05 ^f	BDL	BDL	1.5 ± 0.01 ^e	0.1 ± 0.00 ^a	BDL	1 ± 0.00	0.4 ± 0.00 ^c	0.2 ± 0.01 ^b
Cr	205.56	26.6 ± 0.02 ^h	3 ± 0.03 ^c	2.2 ± 0.01 ^a	6.5 ± 0.09 ^f	4 ± 0.27 ^d	2.8 ± 0.00 ^{bc}	8.1 ± 0.07 ^g	4.6 ± 0.03 ^e	2.7 ± 0.00 ^b
Fe	239.56	1 948 ± 0.57 ⁱ	91.5 ± 0.27 ^d	21.1 ± 0.50 ^a	1 647 ± 2.00 ^h	330.5 ± 0.05 ^f	157.6 ± 0.06 ^e	1 195 ± 1.00 ^g	31.9 ± 0.08 ^c	28.6 ± 0.00 ^b
Pb	217.00	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mg	285.21	1 026 ± 0.29 ^d	1 149 ± 0.11 ^e	483.1 ± 0.56 ^c	445.5 ± 0.69 ^b	1 896 ± 0.56 ^h	1 555 ± 5.03 ^g	213.5 ± 0.27 ^a	1 508 ± 0.55 ^f	455.3 ± 0.44 ^b
Mn	260.57	72 ± 0.20 ^g	9.4 ± 0.10 ^b	2.1 ± 0.10 ^a	73.6 ± 0.10 ^g	43.5 ± 0.20 ^e	16.9 ± 0.10 ^c	68.9 ± 0.10 ^f	1 728 ± 3.00 ^h	21.3 ± 0.10 ^d
Se	196.03	2.2 ± 0.10 ^b	4.9 ± 0.06 ^e	4.5 ± 0.08 ^d	3.2 ± 0.01 ^c	6.4 ± 0.07 ^h	6 ± 0.03 ^g	1.8 ± 0.04 ^a	5.7 ± 0.00 ^f	1.8 ± 0.01 ^a
Zn	213.86	45 ± 0.00 ^e	56.3 ± 0.10 ^h	27.8 ± 0.01 ^b	20.7 ± 0.01 ^a	32.7 ± 0.00 ^c	53.3 ± 0.62 ^f	44.9 ± 0.03 ^e	40.8 ± 0.00 ^d	55 ± 0.06 ^g

BDL= Beyond detection limit. The results are presented as the mean of three independent replicates (n) ± standard deviation (SD). Significant differences among the samples were assessed using the Tukey test at a significance level of $p < 0.05$. Identical superscript letters within a row denote no significant difference ($p > 0.05$) between the samples while differing letters indicate a significant difference.

4.3.2 Elemental analysis

4.3.2.1 Mineral composition

The elemental analysis (Table 4.5) highlighted varying mineral concentrations. Arsenic (As) levels were higher in soils than in plant tissues, though *F. thonningii* fruit contained as much arsenic as its leaves (0.2 mg/Kg). Cadmium (Cd) was equally present in the soils of sites A and B, with *D. mespiliformis* leaves reflecting their soil concentration (0.3 mg/Kg). The calcium (Ca) content in *S. spinosa* leaves (17,240 mg/Kg) was notably high, surpassing even the calcium-rich soils at Site A (1,502 mg/Kg). Copper (Cu) concentrations were highest in *D. mespiliformis* leaves from Site A and *F. thonningii* fruit. Cobalt (Co) content was undetectable in *D. mespiliformis* but highest in *S. spinosa* leaves and fruit. Chromium (Cr) levels were elevated in *S. spinosa* leaves, and all fruit samples exhibited similar chromium concentrations (2.2-2.7 mg/Kg).

Iron (Fe) was the most abundant mineral in all soil and plant samples, with *F. thonningii* leaves and fruit having the highest concentrations. Lead (Pb) was undetectable across all samples. Magnesium (Mg) was abundant in *F. thonningii* leaves (1,896 mg/Kg) and dominant in the fruit of *F. thonningii* and *S. spinosa*. Manganese (Mn) was present in large quantities in *S. spinosa* (1,728 mg/Kg), and its fruit contained the highest manganese levels among the species.

Selenium (Se) content was highest in *S. spinosa* leaves, while zinc (Zn) concentrations varied, with the highest levels in *D. mespiliformis* leaves and *S. spinosa* fruit. Overall, these results highlight the nutritional and elemental richness of these species, with each fruit part offering unique contributions.

Table 4.6 Correlation coefficients (r) and significance (p) levels between elemental composition, proximate components, and soil samples of *Diospyros mespiliformis* fruit.

		Correlations																											
		AsF	AsS	CaS	CaF	CdF	CdS	CoS	CrF	CrS	CuF	CuS	FeF	MgF	MgS	MnF	MnS	SeF	SeS	ZnF	ZnS	Ash	Protein	Moisture	CrudeFat	Fibre	Carbohydrate	Energy value	
AsF	Pearson Correlation	1	1.000**	-0.327	-0.980	0.619	-0.500	-0.982	0.500	-0.995	-0.933	-0.945	-0.939	-0.927	-0.449	-0.091	0.778	0.655	-0.778	0.655	-0.029	0.189	-0.327	-0.156	-1.000**	0.277	-0.345	-0.327	0.303
	Sig. (2-tailed)		0.000	0.788	0.129	0.575	0.667	0.121	0.667	0.061	0.234	0.212	0.224	0.245	0.703	0.942	0.433	0.546	0.433	0.546	0.981	0.879	0.788	0.901		0.821	0.776	0.788	0.804
AsS	Pearson Correlation	1.000**	1	-0.327	-0.980	0.619	-0.500	-0.982	0.500	-0.995	-0.933	-0.945	-0.939	-0.927	-0.449	-0.091	0.778	0.655	-0.778	0.655	-0.029	0.189	-0.327	-0.156	-1.000**	0.277	-0.345	-0.327	0.303
	Sig. (2-tailed)		0.000	0.788	0.129	0.575	0.667	0.121	0.667	0.061	0.234	0.212	0.224	0.245	0.703	0.942	0.433	0.546	0.433	0.546	0.981	0.879	0.788	0.901		0.821	0.776	0.788	0.804
CaS	Pearson Correlation	-0.327	-0.327	1	0.511	0.575	-0.655	0.143	-0.967	0.416	-0.034	0.000	0.633	0.268	-0.697	-0.911	-0.849	-0.929	-0.339	-0.929	-0.935	-0.900	1.000**	0.984	1.000**	-0.999*	-0.774	-0.786	-1.000**
	Sig. (2-tailed)	0.788	0.788		0.659	0.637	0.546	0.909	0.121	0.727	0.978	1.000	0.564	0.543	0.509	0.270	0.355	0.242	0.780	0.242	0.231	0.091	0.100	0.113		0.033	0.436	0.425	0.016
CaF	Pearson Correlation	-0.980	-0.980	0.511	1	-0.448	0.315	0.924	-0.664	0.994	0.842	0.860	0.989	0.984	0.260	-0.112	-0.888	-0.793	0.635	-0.793	-0.172	-0.383	0.511	0.351	1.000**	-0.465	0.149	0.130	-0.488
	Sig. (2-tailed)	0.129	0.129	0.659		0.704	0.796	0.250	0.538	0.068	0.363	0.341	0.095	0.115	0.832	0.929	0.304	0.417	0.562	0.417	0.890	0.750	0.659	0.772		0.692	0.905	0.917	0.675
CdF	Pearson Correlation	0.619	0.619	0.540	-0.448	1	-0.990	-0.756	-0.371	-0.540	-0.860	-0.842	-0.310	-0.279	-0.980	-0.839	-0.913	-0.189	-0.975	-0.189	-0.804	-0.655	0.540	0.680	-1.000**	-0.583	-0.951	-0.945	-0.562
	Sig. (2-tailed)	0.575	0.575	0.637	0.704		0.091	0.454	0.758	0.636	0.342	0.363	0.800	0.820	0.128	0.367	0.992	0.879	0.143	0.879	0.406	0.546	0.637	0.524		0.604	0.200	0.212	0.620
CdS	Pearson Correlation	-0.500	-0.500	-0.655	0.315	-0.990	1	0.655	0.500	0.415	0.778	0.756	0.171	0.139	.998*	0.908	0.156	0.327	0.933	0.327	0.880	0.756	-0.655	-0.778	1.000**	0.693	0.985	0.982	0.674
	Sig. (2-tailed)	0.667	0.667	0.546	0.796	0.091		0.546	0.667	0.728	0.433	0.454	0.891	0.911	0.037	0.275	0.901	0.788	0.234	0.788	0.315	0.454	0.546	0.433		0.512	0.109	0.121	0.529
CoF	Pearson Correlation																												
	Sig. (2-tailed)																												
CoS	Pearson Correlation	-0.982	-0.982	0.143	0.924	-0.756	0.655	1	-0.327	0.959	0.984	0.990	0.857	0.840	0.610	0.277	-0.645	-0.500	0.882	-0.500	0.218	0.000	0.143	-0.034	1.000**	-0.091	0.516	0.500	-0.117
	Sig. (2-tailed)	0.121	0.121	0.909	0.250	0.454	0.546		0.788	0.182	0.113	0.091	0.345	0.366	0.582	0.821	0.554	0.667	0.312	0.667	0.860	1.000	0.909	0.978		0.942	0.655	0.667	0.925
CrF	Pearson Correlation	0.500	0.500	-0.982	-0.664	-0.371	0.500	-0.327	1	-0.581	-0.156	-0.189	-0.768	-0.788	0.549	0.817	0.933	0.962	0.156	0.962	0.851	0.945	-0.982	-0.933	-1.000**	0.971	0.640	0.655	0.977
	Sig. (2-tailed)	0.667	0.667	0.121	0.538	0.758	0.667	0.788		0.606	0.901	0.879	0.443	0.422	0.630	0.391	0.234	0.121	0.901	0.121	0.352	0.212	0.121	0.234		0.154	0.557	0.546	0.137
CrS	Pearson Correlation	-0.995	-0.995	0.416	0.994	-0.540	0.415	0.959	-0.581	1	0.895	0.909	0.967	0.959	0.362	-0.005	-0.834	-0.724	0.714	-0.724	-0.067	-0.282	0.416	0.249	1.000**	-0.368	0.253	0.235	-0.393
	Sig. (2-tailed)	0.061	0.061	0.727	0.068	0.636	0.728	0.182	0.606		0.295	0.273	0.163	0.183	0.764	0.997	0.372	0.485	0.494	0.485	0.958	0.818	0.727	0.840		0.760	0.837	0.849	0.743
CuF	Pearson Correlation	-0.933	-0.933	-0.034	0.842	-0.860	0.778	0.984	-0.156	0.895	1	.999*	0.752	0.731	0.740	0.442	-0.500	-0.339	0.952	-0.339	0.386	0.176	-0.034	-0.210	1.000**	0.086	0.659	0.645	0.060
	Sig. (2-tailed)	0.234	0.234	0.978	0.363	0.342	0.433	0.113	0.901	0.295		0.022	0.458	0.478	0.469	0.708	0.667	0.780	0.199	0.780	0.747	0.887	0.978	0.866		0.945	0.542	0.554	0.962
CuS	Pearson Correlation	-0.945	-0.945	0.000	0.860	-0.842	0.756	0.990	-0.189	0.909	.999*	1	0.778	0.753	0.717	0.412	-0.529	-0.371	0.941	-0.371	0.355	0.143	0.000	-0.176	1.000**	0.052	0.633	0.619	0.026
	Sig. (2-tailed)	0.212	0.212	1.000	0.341	0.363	0.454	0.091	0.879	0.273	0.022		0.436	0.457	0.491	0.730	0.645	0.758	0.220	0.758	0.769	0.909	1.000	0.887		0.967	0.564	0.575	0.984
FeF	Pearson Correlation	-0.939	-0.939	0.633	0.989	-0.310	0.171	-0.857	-0.768	0.967	0.752	0.774	1	.999*	0.114	-0.258	-0.947	-0.875	0.513	-0.875	-0.317	-0.516	0.633	0.487	1.000**	-0.592	0.000	-0.019	-0.613
	Sig. (2-tailed)	0.224	0.224	0.564	0.095	0.800	0.891	0.345	0.443	0.163	0.458	0.436		0.020	0.928	0.834	0.209	0.321	0.657	0.321	0.795	0.655	0.564	0.676		0.597	1.000	0.988	0.580
FeS	Pearson Correlation	-0.927	-0.927	0.658	0.984	-0.279	0.139	0.840	-0.788	0.959	0.731	0.753	.999*	1	0.082	-0.289	-0.957	-0.890	0.486	-0.890	-0.347	-0.543	0.658	0.514	1.000**	-0.617	-0.032	-0.051	-0.638
	Sig. (2-tailed)	0.245	0.245	0.543	0.115	0.820	0.911	0.366	0.422	0.183	0.478	0.457	0.020		0.948	0.813	0.188	0.301	0.677	0.301	0.774	0.634	0.543	0.656		0.577	0.980	0.968	0.560
MgF	Pearson Correlation	-0.449	-0.449	-0.697	0.260	-0.980	.998*	0.610	0.549	0.362	0.740	0.717	0.114	0.082	1	0.931	0.212	0.381	0.911	0.381	0.906	0.792	-0.697	-0.813	1.000**	0.734	0.994	0.991	0.715
	Sig. (2-tailed)	0.703	0.703	0.509	0.832	0.128	0.037	0.582	0.630	0.764	0.469	0.491	0.928	0.948		0.239	0.864	0.751	0.271	0.751	0.278	0.418	0.509	0.396		0.476	0.072	0.084	0.492
MgS	Pearson Correlation	-0.091	-0.091	-0.911	-0.112	-0.839	0.908	0.277	0.817	-0.005	0.442	0.412	-0.258	-0.289	0.931	1	0.555	0.693	0.697	0.693	.998*	0.961	-0.911	-0.970	1.000**	0.932	0.966	0.971	0.922
	Sig. (2-tailed)	0.942	0.942	0.270	0.929	0.367	0.275	0.821	0.391	0.997	0.708	0.730	0.834	0.813	0.239		0.625	0.512	0.509	0.512	0.039	0.179	0.270	0.157		0.237	0.166	0.154	0.254
MnF	Pearson Correlation	0.778	0.778	-0.849	-0.888	-0.013	0.156	-0.645	0.933	-0.834	-0.500	-0.529	-0.947	-0.957	0.212	0.555	1	0.984	-0.210	0.984	0.606	0.764	-0.489	-0.742	-1.000**	0.820	0.322	0.339	0.835
	Sig. (2-tailed)	0.433	0.433	0.355	0.304	0.992	0.901	0.554	0.234	0.372	0.667	0.645	0.209	0.188	0.864	0.625		0.113	0.866	0.113	0.586	0.446	0.355	0.468		0.388	0.791	0.780	0.371
MnS	Pearson Correlation	0.655	0.655	-0.929	-0.793	-0.189	0.327	-0.500	0.982	-0.724	-0.339	-0.371	-0.875	-0.890	0.381	0.693	0.984	1	-0.034	1.000*	0.736	0.866	-0.929	-0.849	-1.000**	0.908	0.484	0.500	0.919
	Sig. (2-tailed)	0.546	0.546	0.242	0.417	0.879	0.788	0.667	0.121	0.485	0.780	0.758	0.321	0.301	0.751	0.512	0.113		0.978	0.000	0.473	0.333	0.242	0.355		0.275	0.679	0.667	0.763
SeF	Pearson Correlation	-0.778	-0.778	-0.339	0.635	-0.975	0.933	0.882	0.156	0.714	0.952	0.941	0.513	0.486	0.911	0.697	-0.210	-0.034	1	-0.034	0.651	0.470	-0.339	-0.500	1.000**	0.388	0.858	0.849	0.364
	Sig. (2-tailed)	0.433	0.433	0.780	0.562	0.143	0.234	0.312	0.901	0.494	0.199	0.220	0.657	0.677	0.271	0.509	0.866	0.978		0.978	0.549	0.688	0.780	0.667		0.746	0.343	0.355	0.763
SeS	Pearson Correlation	0.655	0.655	-0.929	-0.793	-0.189	0.327	-0.500	0.982	-0.724	-0.339	-0.371	-0.875	-0.890	0.381	0.693	0.984	1.000*	-0.034	1	0.736	0.866	-0.929	-0.849	-1.000**	0.908	0.484	0.500	0.919
	Sig. (2-tailed)	0.546	0.546	0.242	0.417	0.879	0.788	0.667	0.121	0.485	0.780	0.758	0.321	0.301	0.751	0.512	0.113	0.978		0.978	0.549	0.688	0.780	0.667		0.746	0.343	0.355	0.763
ZnF	Pearson Correlation	-0.029	-0.029	0.935	-0.172	-0.800	0.880	0.218	0.851	0.067	0.386	0.355	-0.317	-0.347	0.000	0.998*	0.606	0.736	0.651	0.736	1	0.976	0.935	-0.982	1.000**	0.985	0.962	0.954	0.944
	Sig. (2-tailed)	0.981	0.981	0.231	0.890	0.406	0.315	0.860	0.352	0.958	0.747	0.769	0.795	0.774	0.278	0.000	0.586	0.473	0.549	0.736	1	0.140	0.231	0.118		0.198	0.205	0.194	0.215
ZnS	Pearson Correlation	0.189	0.189	-0.990	-0.383	-0.655	0.756	0.000	0.945	-0.282	0.176	0.143	-0.516	-0.543	0.792	0.961	0.764	0.866	0.470	0.866	0.976	1	-0.990						

Sample types: Fruit= F; Soil= S. Elements: Arsenic= As; Calcium=Ca; Cadmium= Cd; Chromium=Cr; Copper= Cu; Iron=Fe; Magnesium= Mg; Manganese= Mn; Selenium= Se; Zinc= Zn.
Correlation coefficient (*r*): Strong correlation $r > 0.7$ or $r < -0.7$; Moderate correlation $0.3 < r < 0.7$ or $-0.3 < r < -0.7$; Weak correlation $r < 0.3$ or $r > -0.3$. **Significance level (*p*):** Significant

The correlation analysis (Table 4.6) of proximate compositions and elemental content in *D. mespiliformis* revealed a range of relationships, with some statistically significant and others not. Proximate-to-proximate correlations within the fruit showed notable trends. For example, fat exhibited a perfect positive correlation with energy ($r = 1$, $p = 0.017$), indicating that fat strongly contributes to the energy content of the fruit. Conversely, fat had a strong negative correlation with ash ($r = -0.999$, $p = 0.033$), suggesting that higher ash content is associated with lower fat levels. While fibre showed a perfect positive correlation with moisture ($r = 1$), the relationship was not statistically significant ($p > 0.05$). Similarly, protein displayed a strong negative correlation with fibre ($r = -0.874$, $p = 0.324$) and energy ($r = -0.989$, $p = 0.096$), though these relationships were also not significant. Moisture exhibited a perfect positive correlation with carbohydrates ($r = 1$), while its relationship with energy was negative ($r = -1$), both of which lacked statistical significance.

Element-to-element correlations between fruit and soil were assessed to understand the influence of soil composition on fruit nutrient content. A perfect positive correlation was observed between Cu in fruit and soil ($r = 0.999$, $p = 0.022$) and between Fe in fruit and soil ($r = 0.999$, $p = 0.020$), both of which were statistically significant. These findings highlight a strong dependency of fruit copper and iron content on soil levels. Conversely, Cd in fruit was strongly negatively correlated with Cd in soil ($r = -0.990$, $p = 0.091$), although the relationship was not statistically significant. Moisture in fruit also showed a perfect positive correlation with its soil counterpart ($r = 1$), though this relationship was not tested for significance. Conversely, cadmium in fruit was strongly negatively correlated with cadmium in soil ($r = -0.990$, $p = 0.091$), and chromium in fruit was strongly negatively correlated with chromium in soil ($r = -0.581$, $p = 0.606$), though neither relationship was statistically significant.

Relationships between elemental and proximate compositions within the fruit showed varying degrees of association. Zn displayed a strong positive correlation with protein ($r = 0.983$, $p = 0.118$), and magnesium showed a very strong positive correlation with fibre ($r = 0.994$, $p = 0.072$), though neither relationship reached statistical significance. Fat had a moderate negative correlation with cadmium ($r = -0.583$, $p = 0.604$), and fibre was strongly positively correlated with selenium ($r = 0.858$, $p = 0.343$), though these were also not significant. Relationships between elements within the soil showed weaker trends. For instance, iron and zinc exhibited a moderate negative correlation

($r = -0.543$, $p = 0.634$), which was not statistically significant. For additional information on the correlation coefficients of *F. thonningii* and *S. spinosa*, refer to appendix 4, tables A4.1 and A4.2.

4.3.2.2 Bioaccumulation factor

Table 4.7 Bioaccumulation factors of toxic and microminerals in the leaves and fruit of wild fruits.

Plant species	Element	Concentration (mg/Kg)			Bioaccumulation factor	
		Soil	leaves	Fruit	Leaves	Fruit
<i>D. mespiliformis</i>	As	0.4	0.2	0.1	0.5	0.25
	Cd	0.3	0.3	0.2	1	0.67
	Cr	26.6	3	2.2	0.11	0.08
	Co	2.6	BDL	BDL	BDL	BDL
	Cu	9.7	9.3	3	0.96	0.31
	Fe	1 948	91.5	21.1	0.05	0.01
	Mn	72	9.4	2.1	0.13	0.03
	Zn	45	56.3	27.8	1.25	0.62
<i>F. thonningii</i>	As	0.3	0.2	0.2	0.67	0.67
	Cd	0.3	0.2	0.2	0.67	0.67
	Cr	6.5	4	2.8	0.62	0.43
	Co	1.5	0.1	BDL	0.07	BDL
	Cu	6.6	6.1	7	0.92	1.06
	Fe	1 647	330.5	157.6	0.20	0.10
	Mn	73.6	43.5	16.9	0.59	0.23
	Zn	20.7	32.7	53.3	1.58	2.57
<i>S. spinosa</i>	As	0.2	BDL	BDL	BDL	BDL
	Cd	0.2	0.2	0.2	1	1
	Cr	8.1	4.6	2.7	0.57	0.33
	Co	1	0.4	0.2	0.4	0.2
	Cu	4.8	6	3.1	1.25	0.65
	Fe	1 195	31.9	28.6	0.03	0.02
	Mn	68.9	1 728	21.3	25.08	0.31
	Zn	44.9	40.8	55	0.91	1.22

BDL: Beyond detection limit

The metal bioaccumulation (Table 4.7) results indicate that *D. mespiliformis* leaves can absorb and accumulate Zn, whereas the fruit was not effective in bioaccumulating any of the metals studied, as all metals had a bioaccumulation factor below one. *Ficus thonningii* leaves efficiently bioaccumulated Zn, while the fruit effectively accumulated both Cu and Zn. *S. spinosa* leaves exhibited a notably high BAF for Mn, at 25.08, and the fruit showed the ability to bioaccumulate Zn with a BAF of 1.22.

4.3.2.3 Health risk assessment

4.3.2.3.1 Target Hazard Quotient/non-carcinogenic risk and Carcinogenic risk

Table 4.8 Health risk assessment of toxic elements detected in the edible parts (Pulp) of wild fruit plants.

Plant species	Elements	Fruit (mg/Kg)	Estimated daily intake	Target hazard quotient	Carcinogenic risk
<i>D. mespiliformis</i>	As	0.1	0.001	1.9	0.0009
	Cd	0.2	0.001	22.86	0.0004
	Cr	2.2	0.012	4.19	0.0063
	Co	BDL	BDL	BDL	
	Cu	3	0.017	0.43	
	Fe	21.1	0.121	0.17	
	Mn	2.1	0.012	0.09	
	Zn	27.8	0.159	0.53	
<i>F. thonningii</i>	As	0.2	0.001	3.81	0.0017
	Cd	0.2	0.001	22.86	0.0004
	Cr	2.8	0.016	5.33	0.008
	Co	BDL	BDL	BDL	
	Cu	7	0.040	1	
	Fe	157.6	0.900	1.29	
	Mn	16.9	0.100	0.69	
	Zn	53.3	0.305	1.02	
<i>S. spinosa</i>	As	BDL	BDL	BDL	BDL
	Cd	0.2	0.001	22.86	0.0004
	Cr	0.2	0.001	0.38	0.0006
	Co	2.7	0.015	51.43	
	Cu	3.1	0.018	0.44	
	Fe	28.6	0.163	0.23	
	Mn	21.3	0.122	0.87	
	Zn	55	0.314	1.05	

BDL: Beyond detection limit. The carcinogenic risk of Co, Cu, Fe, Mn, and Zn was not calculated since they are not

The target hazard quotient/non-carcinogenic risk assessment (Table 4.8) revealed that the fruit of *D. mespiliformis* showed unacceptable levels of Cd (22.86), followed by Cr (4.19), and As (1.9). Similarly, the fruit of *F. thonningii* contained high levels of Cd (22.86), Cr (5.33), As (3.81), Fe (1.29), and Zn (1.02), while *S. spinosa* exhibited excessively high levels of cobalt Co (51.43), Cd (22.86), and Zn (1.05).

The carcinogenic risk assessment (Table 4.8) revealed that the fruit of *D. mespiliformis*, *F. thonningii*, and *S. spinosa* may pose an increased cancer risk to humans due to the presence of As, Cd, and Cr. The findings showed that in *D. mespiliformis* fruit, Cr presented the highest carcinogenic risk factor at 0.0063, followed by As at 0.0009 and Cd at 0.0004. In *F. thonningii* fruit, As had the highest

risk factor at 0.0017. For *S. spinosa* fruit, Cr had a carcinogenic risk factor of 0.0006, which was higher than Cd at 0.0004.

4.4 Discussion

4.4.1 Proximate analysis

Moisture content

The results of the study indicate that *F. thonningii* whole fruit had the highest water content (82.07%) followed by the pulp of *S. spinosa* fruit (76.33%) and *D. mespiliformis* pulp (59.13%). However, Jacob et al. (2016) found significantly lower percentages of moisture from the pulp of *D. mespiliformis* (6.89%) and *S. spinosa* (7.12%) compared to the current findings. The findings of another study found that the moisture content of the pulp of *S. spinosa* was 76.4% (Akweni et al., 2022) which is relatively close to the findings of the current study. Notably, fruits normally have a high water content ranging from 55–85 % (Rahman et al., 2024). Due to the high water content of fruits, some ethnobotanical studies show that wild fruits were used as thirst quenchers by local people (Das, 2018; Motlhanka and Makhabu, 2011; Muchuweti et al., 2005; Sathyavathi and Janardhanan, 2014). However, higher moisture content in fruits indicates that the fruit is highly perishable (Omolola et al., 2017). For instance, the papaya fruit is considered highly perishable due to its high moisture content leading up to 25% post-harvest losses (Mishra et al., 2015). Ramjan and Ansari (2018) concluded that amongst other factors, the moisture content controls the shelf life of fruits. Therefore, the currently studied fruit may have a low shelf life due to high moisture content. Previous research advises that fruits should be harvested before they ripen, reducing the carbon dioxide around them.

Ash content

Ash content refers to the inorganic material (Hitzl et al., 2015) found in the plant which contains the mineral content of the plant (Vermani et al., 2010). Therefore, the composition of ash depends on the type of plant material and the type of species (Babayemi et al., 2010). Furthermore, the ash content of different parts of the same plant may vary. Similarly, Zeng et al. (2014) also found varying quantities of ash from different parts of a Masson pine tree. Bakker and Elbersen (2005) noted that wetland species have higher ash content than C4 species further indicating that water-uptake

influences the ash content of a plant. This is justified by the perfect positive correlation between moisture and ash content in Table 4.6. High moisture content is associated with high ash content (Dagnew et al., 2021). Furthermore, the soil type and texture influence the ash content (Bakker and Elbersen, 2005).

Strychnos spinosa fruit pulp had the highest ash content of 5.87% while the fruit peel and seeds had 2.98% and 3.55% respectively. Mbhele et al. (2024) studied the ash content of various morphophytes of *S. spinosa* and one of them had an ash content of 5.83% comparable to the current findings (Table 5.2) of *S. spinosa* pulp which had an ash content of 5.87%. Furthermore, Mbhele et al. (2024) concluded that *S. spinosa* is a valuable fruit that can be used to address mineral deficiencies. Jacob et al. (2016) found that the ash content of *S. spinosa* pulp was 3.86 % and 4.66 % for *D. mespiliformis* pulp. *D. mespiliformis* showed varying quantities of ash between the pulp (2.21%), seeds (3.41%), and peel (2.93%). Magaji (2019) found that the pulp of *D. mespiliformis* had an ash content of 2.02 g/100g. Mbhele et al. (2024) highlighted that low ash content is associated with high moisture content which is justified by the results in Table 4.4 indicating that *F. thonningii* has the highest water content (82.07%) and the lowest ash content (1.2%). The results further revealed that different parts of the same fruit may contain varying quantities of ash (Table 4.4).

Crude fat

The results showed a strong negative correlation between the crude fat and ash content ($r = -0.999$) corresponding to the findings of (Ugese et al., 2008). Furthermore, the results show that the ash content influences the crude fat ($p = 0.033$) of a plant species. Fat plays three key roles as a nutrient: it provides a highly concentrated source of energy, acts as a carrier for fat-soluble vitamins, and supplies essential fatty acids that must be obtained through dietary fat. Additionally, fat helps transport certain bioactive compounds found in fruits, such as phytoestrogens and carotenoids, which are fat-soluble. Fatty acids are also needed to form cell structures and to act as precursors of prostaglandins (Sánchez-Moreno et al., 2006). *F. thonningii* had the highest oil content of 3.92 %. The results (Table 4.4) further indicate variances in the crude oil content between the fruit peel, pulp, and seeds of both *D. mespiliformis* and *S. spinosa*. The results (Table 5.3) show that the peel and pulp of *D. mespiliformis* had fat content of 1.73% and 2.5%. Muhammad et al. (2024) found that the fat content of

D. mespiliformis fruit peel and pulp was 1.76% and 2.89% respectively. Contrarily, Ebbo et al. (2019a) found that the peel and pulp of *D. mespiliformis* had a much lower crude fat content of 0.45% and 0.11% respectively. Figueiredo et al. (2008) elaborated on how various factors such as environmental conditions (climate, pests and diseases, pollution), geographic variations (soil type), physiological variations (seasonality, type of plant organ, development stage, secretory structure, and injuries) influence the production of essential oils. Hence, these factors may affect the production and bioaccumulation of essential oils leading to variances in the quality and quantity of these oils within the same species exposed to varying conditions (Zhang et al., 2023). However, it is important to note that previous studies have shown that plant essential oils have proven antimicrobial (Andoğan et al., 2002; Sahu et al., 2024), antioxidant (Moghaddam et al., 2015; Okoh et al., 2016), and anti-proliferative (Lampronti et al., 2006; Saengha et al., 2022) activities. A comparative study by Rustaie et al. (2016) to determine the biological activities of wild and cultivated fruits of *Bunium persicum* (Boiss.) B.Fedtsch., the results showed that the wild-grown plant had some variations in percentage compositions of the essential oils with the wild-grown plant exhibiting excellent biological activities than the cultivated plant. This therefore indicates that plants growing in the wild have a better potential of providing essential oils with potent beneficial properties. Furthermore, previous studies show that some wild fruits have edible oils that are rich in essential fatty acids, and important saturated and unsaturated fatty acids (Maikhuri et al., 2021). In general, the fat content of fruits is very low as reported in table 4.4 for all the studied fruit. This is supported by (Sánchez-Moreno et al., 2006) who also highlighted that the fat content of fruit is generally low. For instance, fatty acid deficiencies are associated with eczema, depigmentation, and impaired wound healing (Krutmann and Humbert, 2011).

Crude fibre

S. spinosa fruit peel, which is non-edible had the highest crude fibre of 43.85%. From the edible part of the three analysed fruits, *F. thonningii* (17.55%) had the highest crude fibre content compared to the pulps of *S. spinosa* and *D. mespiliformis*. An estimate of the crude fiber content in present foods is based on the amount of indigestible cellulose, pentosans, lignin, and other materials of this type in the food (Nembang, 2023). The RDA of fibre for adult men and women is 38 and 25 g/day (Satter et al., 2016). Crude fibre is also known as dietary fibre (Dai and Chau, 2017)

which consist of insoluble and soluble fibres (Mudgil, 2017). Insoluble fibres are indigestible but assist food in passing through the digestive system while soluble fibres absorb water and make it easy to expel waste (Stadler and Elledge, 2004). The benefits of dietary fibres include the lowering of cholesterol levels, regulating diabetes, and improvement of the digestive system serving as prebiotics (Mudgil, 2017). Hence, fibers play an important role in the relief of constipation (Dai and Chau, 2017). Dietary fibres can be used to relieve inflammation in psoriasis patients, hence, there is a correlation between the low intake of dietary fibre and psoriasis (Sawada et al., 2021).

Protein

The protein content of *D. mespiliformis* fruit pulp was 6.20% which is comparable to the 6.01 g/100 g found in the fruit pulp of *D. mespiliformis* by Magaji (2019). The protein content of *D. mespiliformis* seeds was 5.37% comparable to the findings of Ezeagu et al. (1996) who found that the protein content of the seeds was 5.47%. However, the *D. mespiliformis* fruit peel had the least protein content (2.37%) compared to the seeds and pulp. In contrast, (Muhammad et al., 2024) found that the protein content of *D. mespiliformis* fruit peel was 6.86%, and (Nyambe et al., 2019b) found that the fruit peel protein content was 1.6%.

The whole *F. thonningii* fruit had a considerable quantity of protein 20.60% (Table 4.4) which is higher than the protein content of *F. thonningii* seed reported in (Muhammad and Oluwaniyi, 2022). The protein content of *S. spinosa* pulp (23.62%) was higher compared to the findings of Lockett et al. (2000) who found that the fruit has a protein content of 11.70%; 3.3% (Amarteifio and Mosase, 2006); and 9.19% (Mbhele et al., 2024). However, the protein content of the *S. spinosa* seed (31.20%) is less than that of the commercial date palm (*Phoenix dactylifera* L.) seed with 36.40% (Bouaziz et al., 2008).

The RDA of protein intake for children, men, and women is 34 g, 56 g, and 46 g, respectively (Satter et al., 2016). In this study, the pulp of *S. spinosa* and the whole fruit of *F. thonningii* were found to contain significant protein levels (23.63% and 20.60%, respectively), which could contribute substantially to meeting protein requirements, particularly in children. Proteins are crucial in the diet because they provide amino acids, some are essential as the body cannot produce them independently. They are vital structural components of all cells and are necessary for

tissue building and repair, and the production of enzymes, hormones, and other substances. Proteins also play roles in immune function, blood clotting, and more, serving both regulatory and structural functions in the body. While fruits contain only small amounts of nitrogenous compounds (0.1–1.5%), making them generally poor protein sources, berries, cherimoya, and avocados have higher protein content than other fruits (Sánchez-Moreno et al., 2006). Cao et al. (2020) further elaborated that constructing, repairing, and mediating skin physiological functions are among the primary functions of proteins. Hence, they form an important part of body tissue and organs. Furthermore, deficiencies in any of these nutrients will have detrimental effects on the skin.

Carbohydrates

Carbohydrates showed strong positive relationship with moisture, fibre, fat, energy value and a weak negative inverse relationship with protein (Table 4.6). The highest carbohydrate content was observed from the pulp of *D. mespiliformis* (86.02%) > *D. mespiliformis* seeds (78.70%) > *D. mespiliformis* peel (61.78%). The carbohydrate content of the pulp (Table 2) is higher than the findings of (Magaji, 2019) 56.55 ± 0.72 g/ 100g., (Jacob et al., 2016) 60.47%, and (Aremu et al., 2019) 79.68 g/100 g on the pulp of *D. mespiliformis*. However, Muhammad and Oluwaniyi (2022) found that the pulp of *D. mespiliformis* fruit is 86.56%, comparable to the current study's findings. In addition, the current study findings on the carbohydrate content of *D. mespiliformis* seeds is comparable to the 77.21% found by (Ezeagu et al., 1996) in the seeds of *D. mespiliformis*.

The carbohydrate content of the *S. spinosa* pulp was 59.55 % which is less than the carbohydrate content of 62.47% of the pulp recorded by (Jacob et al., 2016). However, (Mbhele et al., 2024) found that the carbohydrate content of the fruit of thirty-two *S. spinosa* morphophytes ranged from 37.88–40.77 %. Furthermore, the carbohydrate content of *S. spinosa* pulp was higher than the carbohydrate content of *S. spinosa* seeds and peel (Table 4.4). The carbohydrate content of *F. thonningii* whole fruit was 55.16% which is higher than the carbohydrate content of the seed (40.02%) as discovered by Muhammad and Oluwaniyi (2022). The RDA of carbohydrates for adult males and females is 130 g/day (Satter et al., 2016). All the fruits in the current study can serve as good sources of carbohydrates, especially *D. mespiliformis* pulp.

Carbohydrates serve as the primary source of energy in the human diet. The energy generated from metabolizing carbohydrates can either be used immediately to meet the body's energy demands or stored as fat for later use (Sánchez-Moreno et al., 2006). Carbohydrates provide the body with energy (Posthauer et al., 2015).

Energy value

The current findings show that the energy value had a perfect inverse relationship with the moisture content as indicated by (Torrens Zaragoza, 2015). *D. mespiliformis* had the highest energy value of 391.38 Kcal/100 g which is higher than the findings of (Jacob et al., 2016) which was 348.86 Kcal/100 g. For *S. spinosa* fruit, the pulp had the highest energy value of 342.55 Kcal/100 g compared to its seeds and peel. However, Jacob et al. (2016) found that the pulp of *S. spinosa* was 303.38 kcal/100 g. However, the energy value of *F. thonningii* was 346.68 Kcal/100 g which was less than that of the seeds 382.93 Kcal/100 g (Muhammad and Oluwaniyi, 2022).

The energy value represents the amount of energy in a given weight of food and is a key determinant of energy intake because a greater intake of a low-energy-dense diet, such as fruits, makes excessive energy intake more difficult. Fruits add weight to meals without increasing calories, consequently decreasing energy density, and energy intake and reducing body weight. Additionally, low-energy-dense diets often are recommended for weight control (De Oliveira et al., 2008). Foods with higher energy levels such as refined grains and added sugars are associated with obesity while fruit has not been linked to obesity (Drewnowski and Darmon, 2005).

4.4.2 Elemental analysis

The presence of arsenic (As) in all soil samples aligns with findings by Rehman et al. (2021), who explained that Arsenic is a geogenic carcinogen commonly found in soils, with its levels varying due to the parent rock and anthropogenic activities. Although chronic arsenic exposure takes months or years to manifest, it can have detrimental effects on multiple organ systems, including the endocrine, nervous, and cardiovascular systems (Rokonuzzaman et al., 2022). Arsenic accumulation and chronic intake may have negative impacts on the endocrine, nervous, cardiovascular, hepatic, haematological, renal, dermal, and respiratory systems of the human body (Rehman et al., 2021).

All the soil samples in the current study contained Cadmium (Cd) and (Kubier et al., 2019) elaborates on how Cd levels in the soil are influenced by the weathering of rocks, sewage sludge, landfills, traffic, metal industry, mining, and incidents. Furthermore, Cd is known to compete with and displace zinc (Palmgren et al., 2008). The results show a weak negative correlation between Cd and Zn with $r = -0.804$. Cd levels were consistent across the fruit of *D. mespiliformis*, *F. thonningii*, and *S. spinosa*, each showing 0.2 mg/kg. However, the leaves of *D. mespiliformis* had a higher concentration of 0.3 mg/kg, matching the soil content. Cd, a highly toxic heavy metal, can cause severe health issues even at low concentrations, potentially leading to cancer, renal failure, cardiovascular dysfunction, hormonal imbalances, and death (Sharma et al., 2015).

Site A soil was rich in calcium (Ca) content (1 502 mg/kg). There was a moderate positive correlation between the soil and fruit with $r = 0.511$. Trees also influence the Ca content through biological weathering (Dijkstra et al., 2003), agricultural practices, and water movement (Beerling et al., 2018). The Ca content of *D. mespiliformis* fruit was 395.9 mg/kg, while other studies by Magaji (2019) reported a much higher content (69.44 g/100g). In *F. thonningii*, Ca concentrations were highest in the leaves (12 190 mg/kg) compared to the fruit (4 933 mg/kg). These values are consistent with research by Muhammad and Oluwaniyi (2022), who found 2 067.5 mg/100g in the seeds. Across all species, Ca was consistently higher in the leaves than in the fruit, with *S. spinosa* showing the highest concentrations (Table 4.5). Ca is essential for numerous physiological functions, including maintaining skin health, promoting cell differentiation, and supporting the epidermal barrier (Lee and Lee, 2018; Lin et al., 2024). A disturbance in Ca homeostasis can lead to skin conditions such as atopic dermatitis and psoriasis (Rinnerthaler et al., 2015).

The copper (Cu) content of *D. mespiliformis* was found to be 3 mg/Kg (Table 4.5), significantly lower than the 30.3 g/100g reported by Magaji (2019). In *F. thonningii*, Cu concentrations were 6.1 mg/Kg in the leaves and 7 mg/Kg in the fruit, while (Muhammad and Oluwaniyi, 2022) found 57.4 mg/100g in the seeds. The Cu content of the leaves and fruit of *S. spinosa* were 6 and 3.1 mg/Kg respectively, however, (Mbhele et al., 2024) found 2.70 mg/100 g from *S. spinosa* fruit. The Cu content of the soil and the fruit show a strong positive correlation ($r = 0.999$, $p = 0.022$). Cu plays a crucial role in skin health by supporting the synthesis of elastin, collagen, and melanin,

and deficiencies may lead to sagging skin conditions and hypopigmentation (Krutmann and Humbert, 2011). Among its many benefits, copper peptides have been shown to reduce photodamaged, speed wound healing, and calm irritated skin conditions (Polefka et al., 2012).

Cobalt (Co) was detectable in the leaves of *F. thonningii* (0.1 mg/Kg), however, (Muhammad and Oluwaniyi, 2022) found higher concentrations of Co (1.2 mg/100g) in the seeds of *F. thonningii*. *S. spinosa* leaves and fruits had cobalt content of 0.4 and 0.2 mg/Kg respectively. This corroborates that plant species take up 0.1 to 2.0 mg/Kg of Co in their tissues (Gál et al., 2008).

Chromium (Cr) was present in all soil and samples at varying concentrations (Table 4.5). Cr is considered a high environmental pollutant that is found in water, rocks and sediments, and minerals (Dey et al., 2023). It serves a vital role in insulin function and the metabolism of proteins, fats, and carbohydrates (Chowdhury et al., 2003). Trace elements like Cr are essential for skin health, and deficiencies can lead to skin modifications (Piccardi and Manissier, 2009). However, the Cr also places people at a risk of developing cancer (Table 4.8).

All the soil samples in the current study had high levels of iron (Fe). There was a significant ($p = 0.020$) directly proportional relationship between the Fe content of the soil and the fruit with $r = 0.999$. Bartholomeus et al. (2007) indicated that Fe content indicates the fertility and potential use of the soil for cultivation. The Fe content of *D. mespiliformis* was 21.1 mg/kg in the pulp, whereas Magaji (2019) reported a much higher value of 9.88 g/100g. In *F. thonningii*, Fe concentrations were higher in the leaves (330.5 mg/Kg) compared to the fruit (157.6 mg/Kg), with the seeds containing 434.1 mg/100g (Muhammad and Oluwaniyi, 2022). The highest Fe content observed in a study by (Mbhele et al., 2024) was 4.00 mg/100 g from the fruit of *S. spinosa* while the Fe levels in the current study were 31.9 and 28.6 mg/Kg for leaves and fruit respectively. Fe deficiencies are linked to pale, dry, and scaly skin, highlighting its importance in skin health (Krutmann and Humbert, 2011).

In this study, lead (Pb) levels were beyond the detection limit in all fruit, leaves, and soil samples, contrary to the findings of (Magaji, 2019) who found 5.06 g/100 g in *D. mespiliformis* pulp.

In the present study, site A soils were also rich in magnesium (Mg) than sites B and C. Mg is important for plant growth, but higher levels may be toxic to plants and may also have deleterious effects on the soil (Qadir et al., 2018). Mg concentrations in *D. mespiliformis* were 483.1 mg/kg in the pulp, while (Magaji, 2019) reported 24 g/100g. The leaves of *D. mespiliformis* had 1 896 mg/Kg, and the fruit had 1 555 mg/kg. Additionally, the findings of another study on the seed revealed 1184.10 mg/100 g magnesium content (Muhammad and Oluwaniyi, 2022). Mg plays a crucial role in many enzymatic reactions, and deficiencies can disrupt Ca and K levels, both vital for skin health (Al-Fartusie and Mohssan, 2017). *S. spinosa* fruit had Mg levels ranging from 9 to 69 mg/100 g (Mbhele et al., 2024) while the current study showed 455.3 mg/Kg in the fruit.

Ficus thonningii manganese (Mn) levels were 43.5 mg/Kg in the leaves and 16.9 mg/kg in the fruit, consistent with 39.3 mg/100g 72 seed concentrations. Mn is vital for normal body function, and its deficiency has been associated with skin lesions (Crossgrove and Zheng, 2004). Mbhele et al. (2024) found Mn levels ranging from 0.10 to 2.43 mg/100 g in *S. spinosa* fruit while the current study shows 21.3 mg/Kg in *S. spinosa* fruit.

Selenium was present in low concentrations across all soil, leaf, and fruit samples. According to (Brodowska et al., 2016) high levels of Se are associated with high Fe and organic matter content, while low levels are associated with acidic soils and magma-origin rocks. According to Pearson correlation coefficient, there was a positive moderate correlation ($r = 0.513$, $p = 0.657$) between Se and Fe in the fruit of *D. mespiliformis* and *F. thonningii* and a negative moderate correlation between Se and Fe in the soil samples ($r = -0.890$, $p = 0.301$). Se is critical for the development and function of skin keratinocytes and plays a role in skin antioxidant activity (Cao et al., 2020).

Site A and C soils had higher **Zn** content than site B. Zn is an essential toxic mineral when in excess and displaces other important minerals such as Mn and Fe (Palmgren et al., 2008). The correlation coefficient showed a moderate negative correlation between Zn and Fe ($r = -0.543$) indicating that their relationship is inversely proportional. Zn levels in *F. thonningii* were 32.7 mg/Kg in the leaves and 53.3 mg/Kg in the fruit. Muhammad and Oluwaniyi (2022) found that the seeds contained 63.6

mg/100g. Zn is a cofactor for wound healing enzymes, and its deficiency is linked to skin conditions like dermatitis (Krutmann and Humbert, 2011; Polefka et al., 2012). In the present study, *S. spinosa* had 55 mg/kg of Zn, however, (Mbhele et al., 2024) found Zn concentrations ranging from 0.10 to 0.80 mg/100 g from various morphotypes of *S. spinosa* fruit.

4.4.3 Bioaccumulation of metals across soil, leaves, and fruit

Fruits naturally accumulate metals from the soil during their growth and processing. These metals, which are not biodegradable, can persist (Mbhele et al., 2024) and move up the food chain. While some metals are essential micronutrients, they become toxic at elevated concentrations. Rising levels of environmental heavy metals can result in increased human intake, leading to serious health conditions (Randjelovic et al., 2014). The process of metal uptake in plants is influenced by various factors, such as soil metal concentrations, pH, cation exchange capacity, organic matter, plant type and variety, and even the plant's age (Jung, 2008).

The current study (Table 4.7) revealed intriguing patterns of metal bioaccumulation across different plant parts. In *D. mespiliformis*, for instance, the bioaccumulation factor (BAF) for all tested elements decreased from leaves to fruits. Similarly, in *F. thonningii*, the elements generally decreased from leaves to roots, except for Cd and As, which maintained a stable BAF (0.67) across leaves and fruits. Interestingly, Cu and Mn exhibited an increasing BAF from leaves to fruit in *F. thonningii*. For *S. spinosa*, the BAF of Cd was consistent (1) in both leaves and fruits, while Zn showed an increase from leaves to fruit.

Overall, most elements exhibited higher bioaccumulation in leaves than in fruits. Previous studies support this trend, suggesting that leaves typically contain higher concentrations of metals due to roots acting as barriers or leaves being the first receptors of contaminants from environmental sources (Islam et al., 2022; Zhao and Duo, 2015). Notably, *F. thonningii* leaves had an exceptionally high BAF of 25.08 for Mn, a trend corroborated by other studies (Assoumou et al., 2024; Kamal et al., 2016; Mulenga et al., 2022).

These findings suggest that *D. mespiliformis* is particularly effective at accumulating Zn in its leaves, while *F. thonningii* excels at accumulating Cu in its fruits and Zn in both leaves and fruits. Meanwhile, *S. spinosa* accumulates Mn in its leaves and Zn in

its fruits. Given their ability to bioaccumulate metals with BAF values greater than one, these plant species could serve as valuable candidates for phytoremediation of specific elements (Li et al., 2019).

4.4.4 Health risk assessment

4.4.4.1 Target Hazard Quotient/non-carcinogenic risk and Carcinogenic risk

Toxic elements are an increasing global environmental concern, leading to widespread contamination of water, air, and soil, which ultimately threatens the health of plants, animals, and humans. Plants can absorb these toxic substances, primarily through soil pollution, which can be caused by both natural processes and human activities when pollutant concentrations surpass safe limits (Sinha et al., 2023). Even trace amounts of hazardous toxic elements pose serious risks to public health (Rokonuzzaman et al., 2022).

In the current study (Table 4.8), results revealed alarming concentrations of harmful elements in fruit samples. The fruit of *D. mespiliformis* showed unacceptable levels of cadmium (Cd), followed by chromium (Cr), and arsenic (As). Similarly, the fruit of *F. thonningii* contained high levels of Cd, Cr, As, Fe, and Zn, while *S. spinosa* exhibited excessively high levels of Cd, Cd, and Zn. Notably, *D. mespiliformis* and *F. thonningii* lacked detectable levels of Co.

These findings raise concerns regarding the potential health risks to consumers of these fruits, as harmful elements such as Cd, As, Co, Cr, Fe, and Zn could accumulate in their bodies. Thus, it is imperative to enforce strict regulations on the consumption of these fruits (Adeagbo et al., 2024). Additionally, the toxic elements As, Cd, and Cr were found to exceed the target carcinogenic risk threshold of 1.0×10^{-4} , further underscoring the need for careful monitoring of their intake. Mathebula (2010) notes that the Barberton area of Mpumalanga Province is home to active gold mining operations. Additionally, the Mpumalanga province hosts the majority of South Africa's coal-fired power stations situated near the coal mines in the province. Unfortunately, coal mining activities in Mpumalanga have negative effects on both biodiversity and water resources. The open-cast and underground mining activities contribute to the contamination of surface and underground water, resulting in the elevated levels of toxic metals found in plant species from the area (Simpson et al., 2019).

The Bushbuckridge Local Municipality, with its mining history, adds another layer of concern. This region not only has a history of gold mining in the Hazyview area (Rowe, 2021) but is also involved in sand mining and stone crushing, as highlighted by Mathebula (2010). These activities further exacerbate land, air, and water pollution.

4.5 Concluding remarks

The nutritional and elemental analysis of *D. mespiliformis*, *F. thonningii*, and *S. spinosa* highlights their potential as valuable sources of nutrition and mineral content, making them promising candidates for addressing food insecurity and enhancing human health. Each species exhibited unique profiles, with notable levels of protein, fibre, and essential minerals such as calcium, iron, and zinc, which could be beneficial in skincare products and promoting healthy skin. Additionally, the high bioaccumulation of zinc and manganese in certain plant parts indicates the potential for developing nutraceuticals. Caution is warranted due to the presence of potentially toxic metals like arsenic, cadmium, and chromium in the fruits, as revealed by the bioaccumulation and carcinogenic risk assessments. While the leaves of *D. mespiliformis* and *F. thonningii* efficiently accumulated beneficial metals, their fruits, along with those of *S. spinosa*, showed elevated levels of metals with carcinogenic risks, particularly for Cr, As, and Cd due to the mining activities in the Bushbuckridge Local Municipality. The carcinogenic risk assessment underscores the need for careful consideration of the consumption of these fruits, especially in areas with heavy metal contamination, to mitigate potential long-term health risks. Overall, while these fruits offer significant nutritional and skincare potential, further research is necessary to evaluate and manage the risks associated with their consumption in polluted environments. Developing strategies for safe consumption could enhance their role in both food security and health applications.

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Chapter 5.0: General conclusion and implications for future research

Summary

This study aimed to explore the antimicrobial, antioxidant, and nutritional properties of several wild fruit plant species from the Bushbuckridge local municipality, Mpumalanga Province of South Africa. By identifying plants with therapeutic and nutritional value, the research sought to contribute to food and nutrition security and health improvement. The study's objectives and hypothesis were addressed through an integrated approach combining antimicrobial testing, antioxidant analysis, and nutritional evaluation.

5.1 Specific objectives and findings

A comprehensive literature review was conducted to identify wild fruit species with medicinal and nutritional potential in the Mpumalanga region. The review highlighted several wild plant species, particularly *Carissa spinarum*, *Diospyros mespiliformis*, *Euclea crispa*, *Ficus thonningii*, *Strychnos madagascariensis*, and *Strychnos spinosa*, which are the most used plant species for medicinal and nutritional purposes by the local people of Mpumalanga Province. These species were selected for further investigation based on their documented traditional uses and the availability of scientific data supporting their medicinal and nutritional value.

The antimicrobial properties of the leaves of the selected wild fruit species were evaluated against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, two bacterial pathogens associated with skin infections. The study revealed that *Diospyros mespiliformis*, *Euclea crispa*, and *Ficus thonningii*, exhibited significant antimicrobial activity, especially against *Pseudomonas aeruginosa*. Inhibition zones ranged up to 28 mm, and the minimum inhibitory concentrations (MICs) were as low as 0.781 mg/mL. These results support the hypothesis that wild fruit plant species possess medicinal properties, particularly as potential treatments for bacterial skin infections.

The antioxidant potential of the wild fruit species was assessed through DPPH and ferric-reducing power assays. The results demonstrated that *Euclea crispa* exhibited the highest antioxidant activity, comparable to ascorbic acid, followed by *Diospyros mespiliformis*. This finding reinforces the hypothesis that wild fruit plants have

medicinal properties, particularly in combating oxidative stress, which is associated with skin aging and related diseases. The high antioxidant potential of these species suggests their suitability for inclusion in therapeutic products aimed at reducing oxidative damage. Nutritional analysis was conducted on the fruits of the selected species, revealing that *F. thonningii*, *S. spinosa*, and *D. mespiliformis* are rich in essential minerals such as calcium, iron, magnesium, and zinc. The high moisture (82.07%) and protein (20.60%) content of *F. thonningii* and the protein content of *S. spinosa* pulp (33.62%) highlight the potential of these species to contribute to food security, particularly in regions facing nutritional deficiencies. These findings also affirm the hypothesis that wild fruits have nutritional value, supporting their role in addressing food insecurity. However, based on the findings of this study, the presence of toxic elements such as cadmium (Cd), arsenic (As), chromium (Cr), and zinc (Zn) in the fruit samples of *D. mespiliformis*, *F. thonningii*, and *S. spinosa* poses significant health risks. These elements, which exceed safe concentration levels, can accumulate in the human body upon consumption, potentially leading to serious health conditions. Furthermore, the concentrations of Cd, As, and Cr in the fruit exceeded the target carcinogenic risk threshold, indicating an elevated risk of cancer for consumers exposed to these fruits regularly.

5.3 Implications of the current findings in clinical practice and dermatology

- **Implications for dermatology and skin care:** The antimicrobial activity of certain wild fruit species, particularly against *P. aeruginosa*, suggests that these plants could serve as a natural remedy or complementary treatment for bacterial skin infections. These findings could support their integration into dermatological treatments, particularly in areas with limited access to antibiotics or where antibiotic resistance is a growing concern. The antioxidant properties observed in *D. mespiliformis*, *E. crista*, and the rest of the plant species may help combat oxidative stress, offering potential benefits in treating skin conditions such as premature aging, psoriasis, and eczema. The use of these plants as natural antioxidants could provide an alternative to synthetic compounds in skincare products.
- **Implications for clinical nutrition and food security:** The nutritional analysis showing that the fruits of these wild plants are rich in essential minerals,

highlights their potential role in combating micronutrient deficiencies, particularly in rural communities. The protein-rich pulp of *S. spinosa* and *F. thonningii* presents a valuable plant-based protein source, which could help alleviate food insecurity in underserved regions. Moreover, these fruits contain key nutrients such as calcium, iron, and zinc, which are crucial for immune function and overall health, reinforcing their potential as functional foods. However, despite these nutritional benefits, the study also identified significant health risks associated with the consumption of *D. mespiliformis*, *F. thonningii*, and *S. spinosa* due to elevated concentrations of toxic elements, particularly Cd, As, Cr. These heavy metals pose both non-carcinogenic and carcinogenic risks, which may lead to severe health complications, including organ toxicity and cancer, ultimately compromising food safety and nutrition security.

- **Implications for integrative and traditional medicine:** The antimicrobial and antioxidant properties identified in this study provide scientific validation of traditional practices that have long utilised these wild fruits for treating infections and skin diseases. These plants could be further developed into standardized formulations for use in both Western medicine and complementary therapies.
- **Regulatory and safety considerations:** The integration of these plants into clinical or nutritional practice will require careful regulatory oversight. Toxicity studies, clinical trials, and the establishment of safe usage guidelines are essential before these plants can be fully embraced in healthcare and nutrition.

5.4 Implications for future research

The findings from this study lay the groundwork for further research in the following areas:

- **Isolation and characterization of bioactive compounds:** Future studies should isolate the bioactive compounds responsible for the antimicrobial and antioxidant activities observed in these plants. This could lead to the development of new pharmaceutical and cosmetic products.
- **Sustainable harvesting and cultivation:** Research into sustainable harvesting methods and the development of cultivation practices that minimize heavy metal contamination is essential for ensuring the safe use of these plants in the future.

- **Formulation of therapeutic products:** There is potential for these wild plants to be developed into topical treatments, dietary supplements, or functional foods. Future research should explore the formulation and efficacy of such products.
- **Toxicity and Safety Studies:** It is essential to implement regular monitoring and risk assessment of wild fruit species in the Bushbuckridge Local Municipality and the Mpumalanga province to ensure they meet food safety standards. Additionally, phytoremediation and soil management strategies should be employed to reduce heavy metal contamination in the area. Promoting alternative safe food sources and raising awareness among local communities regarding the potential toxicity of these fruits can further enhance public health protection. Furthermore, reinforcing policy frameworks and regulatory measures will prevent exposure to hazardous elements and ensure sustainable utilisation of wild fruit plant species for food and medicinal purposes.

5.5 Concluding remarks

This study has successfully met its specific objectives, validating that wild fruit species from the Mpumalanga Province possess both medicinal and nutritional properties. The antimicrobial activity of the leaves against *P. aeruginosa* and the high antioxidant potential of *E. crista* further emphasize their potential for therapeutic applications in treating skin infections and related conditions. Moreover, the nutritional value of these species suggests they can contribute to food security by providing essential minerals and proteins, which are particularly important in areas with limited access to diverse food sources. However, the presence of toxic metals like arsenic, cadmium, and chromium in some species, particularly in the fruits, poses a significant concern regarding their safety for consumption. Future research should focus on developing methods to mitigate these risks, such as identifying and cultivating plants in less contaminated environments, or refining extraction and processing methods that reduce heavy metal uptake. In conclusion, the research highlights the **potential** of wild fruit species in addressing both health and food security challenges. Through continued exploration and responsible development, these plants could serve as valuable resources in the fields of medicine, nutrition, and sustainable agriculture.

Appendix A: Ethical approval



UNIVERSITY OF
MPUMALANGA

Creating Opportunities

B Maoneke (PhD)

School of Computing and Mathematical Sciences

Mbombela Campus.

Dear Sinorita Chauke

Protocol Reference Number: UMP/Chauke/230013937/MS/2024

Project Title: Nutraceutical properties of wild fruit plants in Mpumalanga

Approval Notification: In response to your application received on **15/09/2023**, The Research Ethics Committee Faculty Research Ethics Committee has considered the above mentioned application and the protocol has been granted **FULL APPROVAL**.

Any alteration/s to the approved research protocol i.e. Questionnaire/Interviews Schedule, Informed Consent form, Title of the project, Location of the study, Research Approach and methods must be reviewed and approved through the amendment/ modification prior to its implementation. In case you have further queries, please quote the above reference number.

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

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

Wishing you the best with your study.

Yours faithfully,

B Maoneke (Chair)

Cc: Faculty Research & Innovation Committee Chair:

M Bembe 06/03/2024

DECLARATION OF INVESTIGATOR(S)

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

06/03/2024

Signature

Date

PLEASE QUOTE THE PROTOCOL NUMBER ON ALL ENQUIRIES

Appendix B: Plant collection permit



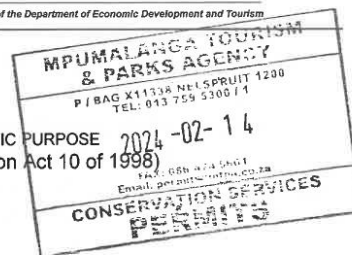
MPB. 1465

PERMIT

TO COLLECT/CONVEY PROTECTED PLANTS FOR SCIENTIFIC PURPOSE
(Issued in terms of the provisions of the Nature Conservation Act 10 of 1998)

Name and residential
Address of permit holder:

S. Chauke
Cnr R40 and D725, Nelspruit
Private Bag x 11283
Nelspruit 1200



Name and address of institution or department: **University of Mpumalanga**
NELSPRUIT

PARTICULARS OF PLANTS WHICH MAY BE COLLECTED

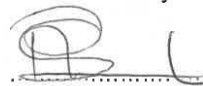
Number	Species
	Leaf & Fruit Samples
	Climbing num-num- <i>Carissa spinarum</i> L.
	Jackal berry- <i>Euclea crispa</i> (Thunb.) Gürke
	Blue guarri- <i>Diospyros mespiliformis</i> Hochst, ex, A. DC.
	Xirhomberhome- <i>Ficus thonningii</i> Blume
	Black monkey orange- <i>Strychnos madagascariensis</i> Poir.
	Green monkey orange- <i>Strychnos spinosa</i> Lam.

PLACE: MPUMALANGA

- No Specially Protected (*Encephalartos* spp, Mpumalanga Conservation Act) plants may be collected
- No herbarium specimen of Critically Endangered (SANBI Red List) plants may be collected
- Only species and quantities indicated on the original project application form may be collected
- Collecting on private land without written permission is a contravention of the permit conditions and shall render the permit invalid
- May only collect on public open areas (NOT on unfenced private and tribal land)
- MTPA must be supplied with a list of all locality information.
- Copies of scientific reports, thesis and/or scientific articles generated from the project must be sent to the MTPA Botanist at: Willem.vanstadén@mtpa.co.za

In terms of and subject to the provisions of the Nature Conservation Act (Act No. 10 Of 1998) and the regulations framed thereunder, the above-mentioned person is hereby authorised, subject to the conditions and requirements appearing on this permit, to collect and convey protected plants referred to above during the period of validity of this permit on behalf of the institution or department referred to above

Period of validity of permit: From date of issue to: **31 January 2025**


for CHIEF EXECUTIVE OFFICER


Signature of permit holder

Appendix C: Turnitin report

Nutraceutical properties of wild fruit plants in Mpumalanga

ORIGINALITY REPORT

11 %
SIMILARITY INDEX

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INTERNET SOURCES

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6	Callistus Bvenura, Learnmore Kambizi. "Food Security and Nutrition - Utilizing Undervalued Food Plants", CRC Press, 2024 Publication	<1 %
7	"Global Arsenic Hazard", Springer Science and Business Media LLC, 2023 Publication	<1 %
8	"Fruit Oils: Chemistry and Functionality", Springer Science and Business Media LLC,	<1 %

Appendix D: Supplementary data

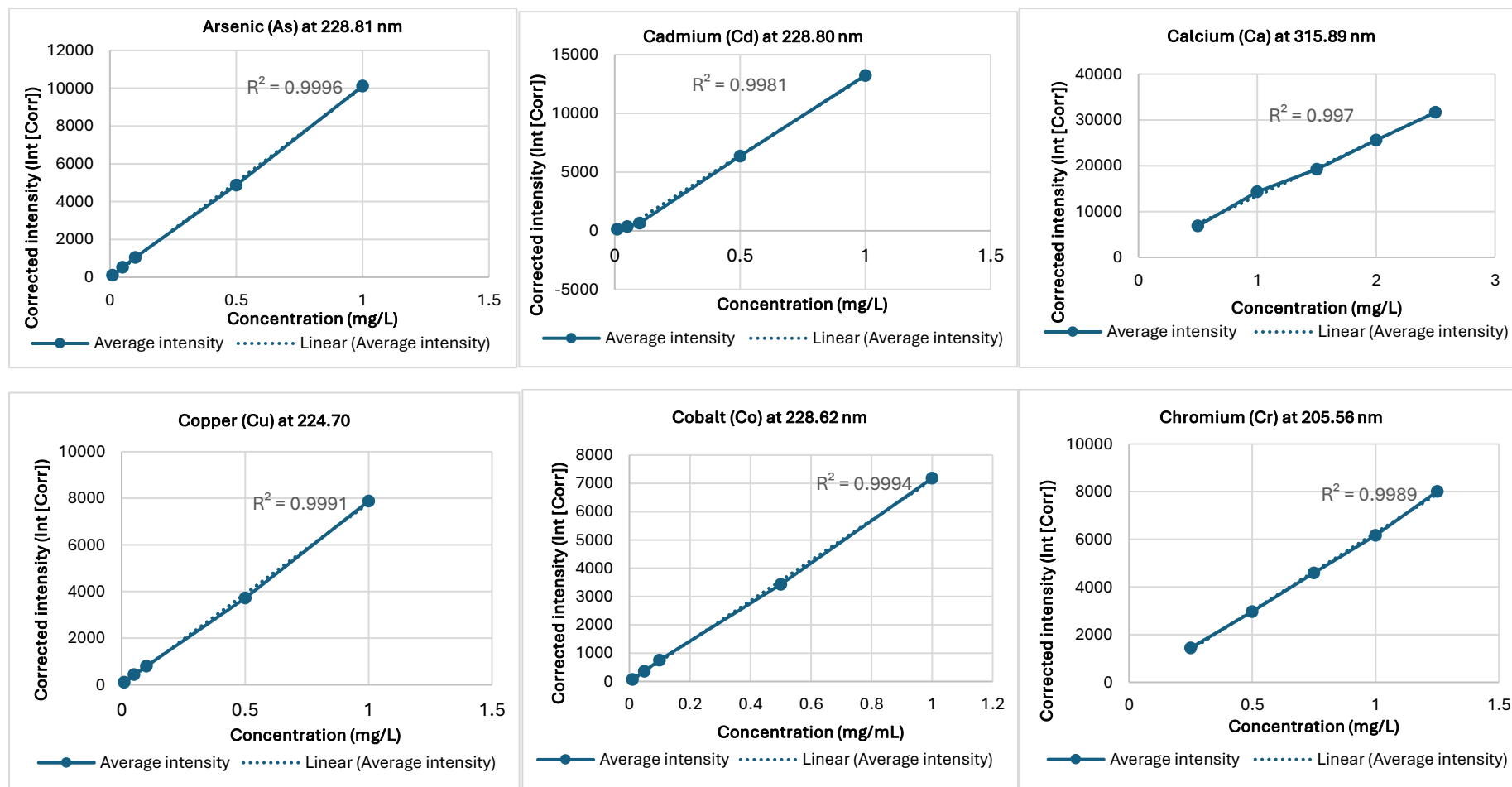


Figure D. 1 Calibration curves for arsenic (As) at 228.81 nm, Cadmium (Cd) at 228.80 nm, Calcium (Ca) at 315.89 nm, Copper (Cu) at 228.62 nm, Cobalt (Co) at 228.62 nm, and Chromium (Cr) at 205.56 nm, demonstrating the relationship between concentration (mg/L) and corrected intensity. The R^2 values, representing the goodness of fit and reliability of the calibration curves, were ≥ 0.99 for all elements.

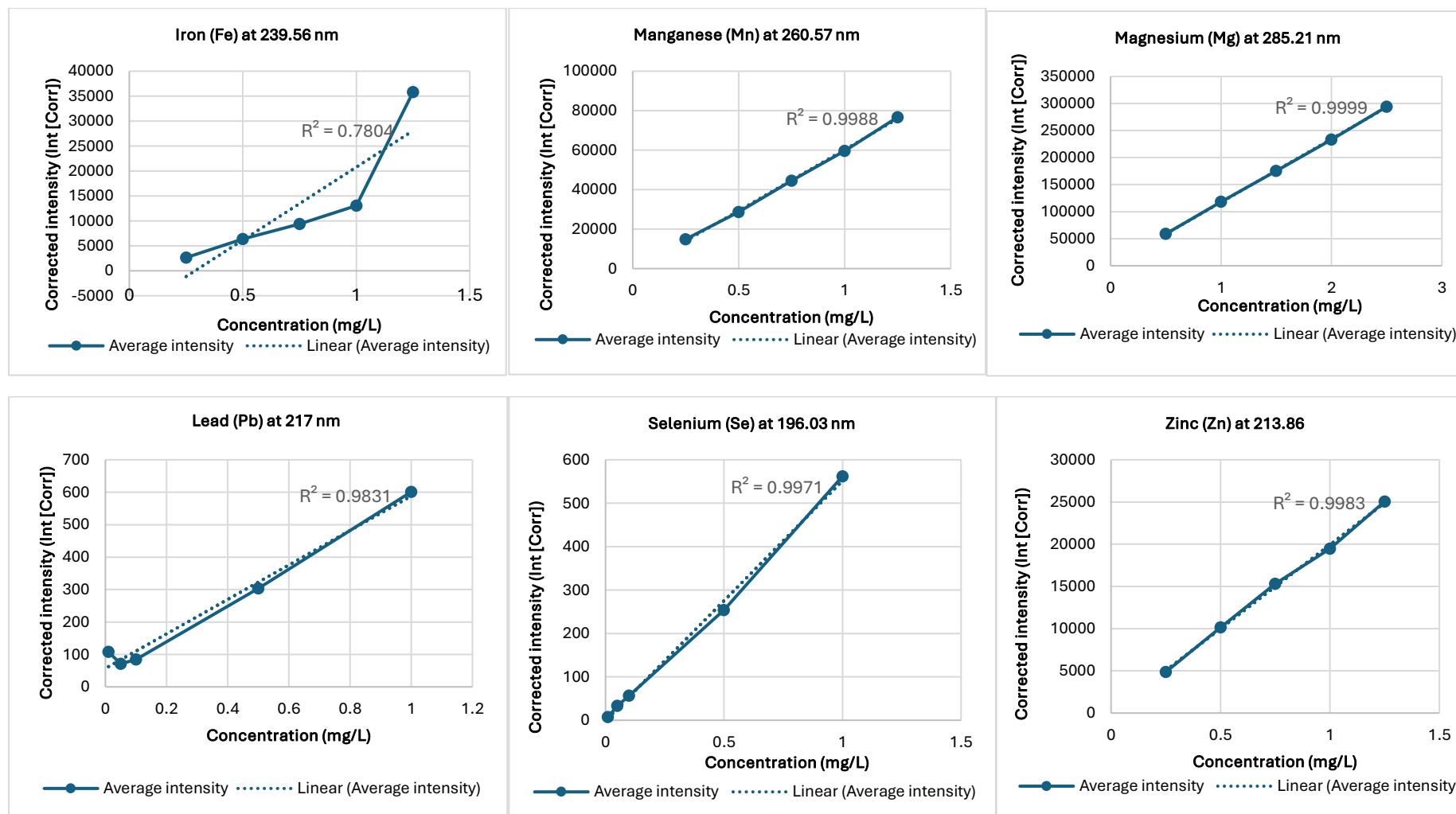


Figure D. 2 Calibration curves for Iron (Fe) at 239.56 nm, Manganese (Mn) at 260.57 nm, Magnesium (Mg) at 285.21 nm, Lead (Pb) at 217 nm, Selenium (Se) at 196.03 nm, and Zinc (Zn) at 213.86 nm, demonstrating the relationship between concentration (mg/L) and corrected intensity. The R^2 values, representing the goodness of fit and reliability of the calibration curves, were ≥ 0.99 for all elements except Iron.

Table D. 1 Correlation coefficients (r) and significance (p) levels between elemental composition, proximate components of *Ficus thonningii* fruit and respective soil samples from the collection site.

Correlations																																	
	AsF	AsS	CaS	CaF	CdF	CdS	CoF	CoS	CrF	CrS	CuF	CuS	FeF	FeS	MgF	MgS	MnF	MnS	SeF	SeS	ZnF	ZnS	Ash	Protein	Moisture	CrudeFat	Fibre	Carbohydrate	Energy value				
AsF	Pearson	1	1.000 ^{**}	-0.327		-0.980	0.619	-0.500			-0.982	0.500	-0.995	-0.933	0.945	0.224	0.224	0.245	-0.449	-0.091	0.778	0.655	-0.778	0.655	-0.029	0.189	-0.327	0.156	0.277	-0.345	-0.327	0.303	
	Sig. (2-tailed)		0.000	0.788	0.129	0.575	0.667				0.121	0.667	0.061	0.234	0.212	0.224	0.245	0.703	0.942	0.433	0.546	0.433	0.546	0.981	0.879	0.788	0.901	0.821	0.776	0.788	0.804		
AsS	Pearson	1.000 ^{**}	1	-0.327	-0.980	0.619	-0.500				-0.982	0.500	-0.995	-0.933	-0.945	-0.939	-0.927	-0.449	-0.091	0.778	0.655	-0.778	0.655	-0.029	0.189	-0.327	-0.156	-1.000 ^{**}	0.277	-0.345	-0.327	0.303	
	Sig. (2-tailed)		0.000	0.788	0.129	0.575	0.667				0.121	0.667	0.061	0.234	0.212	0.224	0.245	0.703	0.942	0.433	0.546	0.433	0.546	0.981	0.879	0.788	0.901	0.821	0.776	0.788	0.804		
CaS	Pearson	-0.327	-0.327	1	0.511	0.540	-0.655				0.143	-0.982	0.416	-0.034	0.000	0.633	0.658	-0.697	-0.911	-0.849	-0.929	-0.339	-0.929	-0.935	-0.990	1.000 ^{**}	0.984	1.000 ^{**}	-0.999 [*]	-0.774	-0.786	-1.000 ^{**}	
	Sig. (2-tailed)		0.788	0.788	0.659	0.637	0.546				0.909	0.121	0.727	0.978	1.000	0.564	0.543	0.509	0.270	0.355	0.242	0.780	0.242	0.231	0.091	0.000	0.113	0.033	0.436	0.425	0.016		
CaF	Pearson	-0.980	-0.980	0.511	1	-0.448	0.315				0.924	-0.664	0.994	0.842	0.860	0.989	0.984	0.260	-0.112	-0.886	-0.793	-0.635	-0.793	-0.172	-0.383	0.511	0.351	1.000 ^{**}	-0.465	0.149	0.130	-0.488	
	Sig. (2-tailed)		0.129	0.129	0.659	0.704	0.796				0.250	0.538	0.068	0.363	0.341	0.095	0.115	0.832	0.929	0.304	0.417	0.562	0.417	0.890	0.750	0.659	0.772	0.692	0.905	0.917	0.675		
CdF	Pearson	0.619	0.619	0.540	-0.448	1	-0.990				-0.756	-0.619	-0.540	-0.860	-0.842	-0.310	-0.279	-0.980	-0.839	-0.013	-0.189	-0.975	-0.189	-0.880	-0.655	0.540	0.680	-1.000 ^{**}	-0.583	-0.951	-0.945	-0.562	
	Sig. (2-tailed)		0.575	0.575	0.637	0.704	0.091				0.454	0.758	0.636	0.342	0.363	0.800	0.820	0.128	0.367	0.992	0.879	0.143	0.879	0.406	0.546	0.637	0.524	0.604	0.200	0.212	0.620		
CdS	Pearson	-0.500	-0.500	-0.655	0.315	-0.990	1				0.655	-0.500	0.415	0.778	0.756	0.171	0.139	.998 [*]	0.908	0.156	0.327	0.933	0.327	0.880	0.546	-0.655	-0.778	1.000 ^{**}	0.693	0.985	0.862	0.674	
	Sig. (2-tailed)		0.667	0.667	0.546	0.796	0.091				0.546	0.667	0.728	0.433	0.454	0.891	0.911	0.037	0.275	0.901	0.788	0.234	0.788	0.315	0.454	0.546	0.433	0.512	0.109	0.121	0.529		
CoF	Pearson																																
	Sig. (2-tailed)																																
CoS	Pearson	-0.982	-0.982	0.143	0.924	-0.756	0.655				1	-0.327	0.959	0.984	0.990	0.857	0.840	0.610	0.277		-0.645	-0.500	0.882	-0.500	0.218	0.000	0.143	-0.034	1.000 ^{**}	-0.091	0.516	0.500	-0.117
	Sig. (2-tailed)		0.121	0.121	0.909	0.250	0.454	0.546				0.788	0.182	0.113	0.091	0.345	0.366	0.582	0.821	0.554	0.667	0.312	0.667	0.860	1.000	0.909	0.978	0.942	0.655	0.667	0.925		
CrF	Pearson	0.500	0.500	-0.982	-0.664	-0.371	0.500				-0.327	1	-0.581	-0.156	-0.189	-0.768	-0.788	0.549	0.817	0.933	0.982	0.156	0.982	0.851	0.945	-0.982	-0.933	-1.000 ^{**}	0.971	0.640	0.655	0.977	
	Sig. (2-tailed)		0.667	0.667	0.121	0.538	0.758	0.667			0.788	0.182	0.113	0.091	0.345	0.366	0.582	0.821	0.554	0.667	0.312	0.667	0.860	1.000	0.909	0.978	0.942	0.655	0.667	0.925			
CrS	Pearson	-0.995	-0.995	0.416	0.994	-0.540	0.415				0.959	-0.581	1	0.895	0.909	0.967	0.959	0.362	-0.005	-0.234	-0.121	0.901	0.121	0.352	0.212	0.121	0.234	0.154	0.557	0.546	0.137		
	Sig. (2-tailed)		0.061	0.061	0.727	0.068	0.636	0.728			0.182	0.060	0.895	1	0.895	0.909	0.967	0.959	0.362	-0.005	-0.234	-0.121	0.901	0.121	0.352	0.212	0.121	0.234	0.154	0.557	0.546	0.137	
CuF	Pearson	-0.933	-0.933	-0.034	0.842	-0.860	0.778				0.984	-0.156	0.895	1	.999 [*]	0.752	0.731	0.740	0.442	-0.500	-0.339	0.952	-0.339	0.386	0.818	-0.724	-0.210	0.000	0.086	0.659	0.645	0.763	
	Sig. (2-tailed)		0.234	0.234	0.978	0.363	0.342	0.433			0.113	0.901	0.295	0.295	0.022	0.458	0.478	0.469	0.708	0.667	0.780	0.199	0.780	0.747	0.887	0.978	0.866	0.945	0.542	0.554	0.962		
CuS	Pearson	-0.945	-0.945	0.000	0.860	-0.842	0.756				0.990	-0.189	0.909	.999 [*]	1	0.774	0.753	0.717	0.412	-0.529	-0.371	0.941	-0.371	0.355	0.143	0.000	-0.176	1.000 ^{**}	0.052	0.633	0.619	0.026	
	Sig. (2-tailed)		0.212	0.212	1.000	0.341	0.363	0.454			0.091	0.879	0.273	0.022	0.436	0.457	0.491	0.730	0.645	0.758	0.220	0.758	0.769	0.909	1.000	0.887	0.967	0.564	0.575	0.984			
FeF	Pearson	-0.939	-0.939	0.633	0.989	-0.310	0.171				0.857	-0.768	0.967	0.752	0.774	1	.999 [*]	0.114	-0.258	-0.947	-0.875	0.513	-0.875	-0.317	-0.516	0.633	0.487	1.000 ^{**}	-0.592	0.000	-0.019	-0.613	
	Sig. (2-tailed)		0.224	0.224	0.564	0.095	0.800	0.891			0.345	0.443	0.163	0.458	0.436	0.202	0.928	0.834	0.209	0.321	0.657	0.321	0.795	0.655	0.564	0.676	0.564	0.676	0.564	0.988	0.580		
FeS	Pearson	-0.927	-0.927	0.658	0.984	-0.279	0.139				0.840	-0.788	0.959	0.731	0.753	.999 [*]	1	0.062	-0.289	-0.957	-0.890	0.486	-0.890	-0.347	-0.543	0.658	0.514	1.000 ^{**}	-0.617	-0.032	-0.051	-0.638	
	Sig. (2-tailed)		0.245	0.245	0.543	0.115	0.820	0.911			0.368	0.422	0.183	0.478	0.457	0.020	0.948	0.813	0.188	0.301	0.677	0.301	0.774	0.634	0.543	0.656	0.564	0.676	0.564	0.988	0.560		
MgF	Pearson	-0.449	-0.449	-0.697	0.260	-0.980	.998 [*]				0.610	0.549	0.362	0.740	0.717	0.114	0.082	1	0.931	0.212	0.381	0.911	0.381	0.906	0.792	-0.697	-0.813	1.000 ^{**}	0.734	0.984	0.991	0.715	
	Sig. (2-tailed)		0.703	0.703	0.509	0.832	0.128	0.037			0.582	0.630	0.764	0.469	0.491	0.928	0.948	0.239	0.864	0.751	0.271	0.751	0.278	0.418	0.509	0.396	0.476	0.072	0.084	0.492			
MgS	Pearson	-0.091	-0.091	-0.911	-0.112	-0.839	0.908				0.277	0.817	-0.005	0.442	0.412	-0.258	-0.289	0.931	1	0.555	0.693	0.697	0.693	.998 [*]	0.961	-0.911	-0.970	1.000 ^{**}	0.932	0.966	0.971	0.922	
	Sig. (2-tailed)		0.942	0.942	0.270	0.929	0.367	0.275			0.821	0.391	0.997	0.708	0.730	0.834	0.813	0.239	0.625	0.512	0.509	0.512	0.039	0.179	0.270	0.157	0.237	0.166	0.154	0.254			
MnF	Pearson	0.778	0.778	-0.849	-0.888	-0.013	0.156				-0.645	0.933	-0.834	-0.500	-0.529	-0.947	-0.957	0.212	0.555	1	0.984	-0.210	0.984	0.606	0.764	-0.849	-0.742	-1.000 ^{**}	0.820	0.322	0.339	0.835	
	Sig. (2-tailed)		0.433	0.433	0.355	0.304	0.992	0.901			0.554	0.234	0.372	0.667	0.645	0.209	0.188	0.864	0.625	0.984	0.866	0.113	0.586	0.466	0.355	0.468	0.388	0.791	0.780	0.371			
MnS	Pearson	0.655	0.655	-0.929	-0.793	-0.189	0.327				-0.500	0.982	-0.724	-0.339	-0.371	-0.875	-0.890	0.381	0.693	0.984	1	-0.034	1.000 ^{**}	0.736	0.866	-0.929	-0.849	-1.000 ^{**}	0.908	0.484	0.500	0.919	
	Sig. (2-tailed)		0.546	0.546	0.242	0.417	0.879	0.788			0.667	0.121	0.485	0.780	0.758	0.321	0.301	0.751	0.512	0.113	0.000	0.978	0.667	0.473	0.333	0.242	0.355	0.275	0.679	0.683	0.259		
SeF	Pearson	-0.778	-0.778	-0.339	0.635	-0.975	0.933				0.882	0.156	0.714	0.952	0.941	0.513	0.486	0.911	0.697	-0.210	-0.034	1	-0.034	0.651	0.470	-0.339	-0.500	1.000 ^{**}	0.388	0.858	0.849	0.364	
	Sig. (2-tailed)		0.433	0.433	0.780	0.562	0.143	0.234			0.312	0.901	0.494	0.199	0.220	0.657	0.677	0.271	0.509	0.866	0.978	0.736	0.549	0.688	0.780	0.667	0.746	0.343	0.355	0.355	0.763		
SeS	Pearson	0.655	0.655	-0.929	-0.793	-0.189	0.327				-0.500	0.982	-0.724	-0.339	-0.371	-0.875	-0.890	0.381	0.693	0.984	1.000 ^{**}	-0.034	1	0.736	0.866	-0.929	-0.849	-1.000 ^{**}	0.908	0.484	0.500	0.919	
	Sig. (2-tailed)		0.546	0.546	0.242	0.417	0.879	0.788			0.667	0.121	0.485	0.780	0.758	0.321	0.301	0.751	0.512	0.113	0.000	0.978	0.667	0.473	0.333	0.242	0.355	0.275	0.679	0.683	0.259		
ZnF	Pearson	-0.029	-0.029	-0.935	-0.172	-0.804	0.880				0.218	0.851	-0.067	0.386	0.355	-0.317	-0.347	0.906	.998 [*]	0.606	0.736	0.651	0.736	1	0.976	-0.935	-0.983	1.000 ^{**}	0.952	0.948	0.954		

Samples type: Fruit= F; Soil= S. Elements: Arsenic= As; Calcium=Ca; Cadmium= Cd; Chromium=Cr; Copper= Cu; Iron=Fe; Magnesium= Mg; Manganese= Mn; Selenium= Se; Zinc= Zn. **Correlation coefficient (r):** Strong correlation $r > 0.7$ or $r < -0.7$; Moderate correlation $0.3 < r < 0.7$ or $-0.3 < r < -0.7$; Weak correlation $r < 0.3$ or $r > -0.3$. **Significance level (p):** Significant $p < 0.05$ or not significant

Table D. 2 Correlation coefficients (r) and significance (p) levels between elemental composition and proximate components of *Strychnos spinosa* fruit and respective soil samples from the collection site.

		Correlations																											
		CaS	CaF	CdF	CdS	CoF	CoS	CrF	CrS	CuF	CuS	FeF	FeS	MgF	MgS	MnF	MnS	SeF	SeS	ZnF	ZnS	Ash	Protein	Moisture	CrudeFat	Fibre	Carbohydrate	Energy value	
CaS	Pearson Correlation	1	-0.982	-1.000	0.500	0.500	0.189	-0.327	0.082	0.826	-0.886	0.887	0.835	-0.850	0.666	0.664	-0.861	-0.982	1.000	0.877	1.000	0.918	0.619	-0.787	0.756	1.000	0.896	-0.690	
	Sig. (2-tailed)		0.121	0.000	0.667	0.667	0.879	0.788	0.948	0.381	0.306	0.305	0.371	0.353	0.536	0.538	0.339	0.121	0.000	0.320	0.000	0.260	0.575	0.423	0.454	0.000	0.293	0.515	
CaF	Pearson Correlation	-0.982	1	0.982	-0.655	-0.327	0.000	0.143	-0.269	-0.188	0.958	-0.784	-0.924	0.735	-0.513	-0.793	0.750	1.000	-0.982	-0.770	-0.982	-0.982	-0.976	-0.459	0.656	-0.866	-0.982	-0.964	0.814
	Sig. (2-tailed)	0.121		0.121	0.546	0.788	1.000	0.909	0.827	0.260	0.185	0.426	0.250	0.474	0.657	0.417	0.460	0.000	0.121	0.441	0.121	0.139	0.696	0.544	0.333	0.121	0.172	0.394	
CdF	Pearson Correlation	-1.000	0.982	1	-0.500	-0.500	-0.189	0.327	-0.082	-0.826	0.886	-0.887	-0.835	0.850	-0.666	-0.664	0.861	0.982	-1.000	-0.877	-1.000	-0.918	-0.619	0.787	-0.756	-1.000	-0.896	0.690	
	Sig. (2-tailed)	0.000	0.121		0.667	0.667	0.879	0.788	0.948	0.381	0.306	0.305	0.371	0.353	0.536	0.538	0.339	0.121	0.000	0.320	0.000	0.260	0.575	0.423	0.454	0.000	0.293	0.515	
CdS	Pearson Correlation	0.500	-0.655	-0.500	1	-0.500	-0.756	0.655	0.904	0.901	-0.844	0.044	0.894	0.931	-0.313	0.980	0.009	-0.655	0.500	0.021	0.500	0.803	-0.371	0.141	0.945	0.500	0.832	-0.972	
	Sig. (2-tailed)	0.667	0.546	0.667		0.667	0.454	0.546	0.281	0.285	0.360	0.972	0.295	0.080	0.797	0.129	0.994	0.546	0.667	0.986	0.667	0.407	0.758	0.910	0.212	0.667	0.374	0.151	
CoF	Pearson Correlation	0.500	-0.327	-0.500	-0.500	1	0.945	-0.982	-0.822	-0.075	-0.042	0.843	-0.060	-0.881	0.979	-0.316	-0.871	-0.327	0.500	0.855	0.500	0.915	0.990	-0.928	-0.189	0.900	0.064	0.282	
	Sig. (2-tailed)	0.667	0.788	0.667	0.667		0.212	0.121	0.386	0.952	0.973	0.362	0.962	0.313	0.130	0.795	0.327	0.788	0.667	0.347	0.667	0.127	0.091	0.244	0.879	0.667	0.959	0.818	
CoS	Pearson Correlation	0.189	0.000	-0.189	-0.756	0.945	1	-0.990	-0.963	-0.397	0.287	0.620	-0.383	-0.678	0.859	-0.609	-0.662	0.000	0.189	0.638	0.189	-0.217	0.888	-0.754	-0.500	0.189	-0.266	0.581	
	Sig. (2-tailed)	0.879	1.000	0.879	0.454	0.212		0.091	0.173	0.740	0.815	0.574	0.750	0.526	0.343	0.583	0.540	1.000	0.879	0.559	0.789	0.861	0.304	0.456	0.667	0.879	0.828	0.606	
CrF	Pearson Correlation	-0.327	0.143	0.327	0.655	-0.982	-0.990	1	0.915	0.262	-0.147	-0.726	0.247	0.776	-0.923	0.490	0.762	0.143	-0.327	-0.742	-0.327	0.075	-0.945	0.840	0.371	-0.327	0.125	-0.458	
	Sig. (2-tailed)	0.788	0.909	0.788	0.546	0.121	0.091		0.265	0.831	0.906	0.483	0.841	0.434	0.251	0.674	0.449	0.909	0.788	0.468	0.788	0.952	0.212	0.365	0.758	0.788	0.920	0.697	
CrS	Pearson Correlation	0.082	-0.269	-0.082	0.904	-0.822	-0.963	0.915	1	0.630	-0.534	-0.387	0.617	0.455	-0.689	0.800	0.435	-0.269	0.082	-0.408	0.082	0.471	-0.732	0.550	0.715	0.082	0.516	-0.778	
	Sig. (2-tailed)	0.948	0.827	0.948	0.281	0.386	0.173	0.265		0.566	0.641	0.747	0.576	0.699	0.516	0.410	0.713	0.827	0.948	0.733	0.948	0.687	0.477	0.629	0.493	0.948	0.655	0.432	
CuF	Pearson Correlation	0.826	-0.918	-0.826	0.901	-0.075	-0.397	0.262	0.630	1	-0.993	0.473	1.000	-0.405	0.129	0.970	-0.425	-0.918	0.826	0.453	0.826	0.982	0.068	-0.303	0.993	0.826	0.990	-0.978	
	Sig. (2-tailed)	0.381	0.260	0.381	0.285	0.952	0.740	0.831	0.566		0.075	0.686	0.010	0.735	0.917	0.157	0.720	0.260	0.381	0.701	0.381	0.121	0.957	0.804	0.073	0.381	0.089	0.134	
CuS	Pearson Correlation	-0.886	0.958	0.886	-0.844	-0.042	0.287	-0.147	-0.534	-0.993	1	-0.573	-0.995	0.509	-0.245	-0.935	0.528	0.958	-0.886	-0.554	-0.886	-0.997	-0.185	0.412	-0.973	-0.886	-1.000	0.947	
	Sig. (2-tailed)	0.306	0.185	0.306	0.360	0.973	0.815	0.906	0.641	0.075		0.612	0.065	0.660	0.843	0.232	0.646	0.185	0.306	0.626	0.306	0.046	0.882	0.730	0.148	0.306	0.014	0.209	
FeF	Pearson Correlation	0.887	-0.784	-0.887	0.044	0.843	0.620	-0.726	-0.387	0.473	-0.573	1	0.487	-997	0.935	0.244	-999	-0.784	0.887	1.000	0.887	0.631	0.911	-0.983	0.369	0.887	0.591	-0.278	
	Sig. (2-tailed)	0.305	0.426	0.305	0.972	0.362	0.574	0.483	0.747	0.686	0.612		0.676	0.048	0.231	0.843	0.034	0.426	0.305	0.015	0.305	0.565	0.270	0.118	0.759	0.305	0.588	0.821	
FeS	Pearson Correlation	0.835	-0.924	-0.835	0.894	-0.060	-0.383	0.247	0.617	1.000	-0.995	0.487	1	-0.419	0.145	0.966	-0.439	-0.924	0.835	0.466	0.835	0.985	0.084	-0.317	0.992	0.835	0.992	-0.974	
	Sig. (2-tailed)	0.371	0.250	0.371	0.295	0.962	0.750	0.841	0.576	0.010	0.065	0.676		0.725	0.908	0.167	0.710	0.250	0.371	0.691	0.371	0.111	0.947	0.794	0.083	0.371	0.079	0.144	
MgF	Pearson Correlation	-0.850	0.735	0.850	0.031	-0.881	-0.678	0.776	0.455	-0.405	0.509	-997	-0.419	1	-0.959	-0.170	1.000	0.735	-0.850	-999	-0.850	-0.570	-0.940	0.994	-0.297	-0.850	-0.528	0.205	
	Sig. (2-tailed)	0.353	0.474	0.353	0.980	0.313	0.526	0.434	0.699	0.735	0.660	0.048	0.725		0.183	0.891	0.014	0.474	0.353	0.034	0.353	0.614	0.222	0.070	0.808	0.353	0.646	0.869	
MgS	Pearson Correlation	0.666	-0.513	-0.666	-0.313	0.979	0.859	-0.923	-0.689	0.129	-0.245	0.935	0.145	-0.959	1	-0.116	-0.952	-0.513	0.666	0.943	0.666	0.314	998	-0.984	0.015	0.666	0.266	0.081	
	Sig. (2-tailed)	0.536	0.657	0.536	0.797	0.130	0.343	0.251	0.516	0.917	0.843	0.231	0.908	0.183		0.926	0.197	0.657	0.536	0.216	0.536	0.796	0.039	0.113	0.991	0.536	0.829	0.948	
MnF	Pearson Correlation	0.664	-0.793	-0.664	0.980	-0.316	-0.609	0.490	0.800	0.970	-0.935	0.244	0.966	-0.170	-0.116	1	-0.192	-0.793	0.664	0.222	0.664	0.906	-0.177	-0.061	0.991	0.664	0.927	-999	
	Sig. (2-tailed)	0.538	0.417	0.538	0.129	0.795	0.583	0.674	0.410	0.157	0.232	0.843	0.167	0.891	0.926		0.877	0.417	0.538	0.858	0.538	0.278	0.887	0.961	0.084	0.538	0.245	0.023	
MnS	Pearson Correlation	-0.861	0.750	0.861	0.009	-0.871	-0.662	0.762	0.435	-0.425	0.528	-999	-0.439	1.000	-0.952	-0.192	1	0.750	-0.861	-1.000	-0.861	-0.589	-0.932	0.991	-0.319	-0.861	-0.547	0.226	
	Sig. (2-tailed)	0.339	0.460	0.339	0.994	0.327	0.540	0.449	0.713	0.720	0.646	0.034	0.710	0.014	0.197	0.877		0.460	0.339	0.019	0.339	0.599	0.236	0.084	0.794	0.339	0.632	0.855	
SeF	Pearson Correlation	-0.982	1.000	-0.982	-0.655	-0.327	0.000	0.143	-0.269	-0.188	0.958	-0.784	-0.924	0.735	-0.513	-0.793	0.750	1	-0.982	-0.770	-0.982	-0.976	-0.459	0.656	-0.866	-0.982	-0.964	0.814	
	Sig. (2-tailed)	0.121	0.000	0.121	0.546	0.788	1.000	0.909	0.827	0.260	0.185	0.426	0.250	0.474	0.657	0.417	0.460		0.121	0.441	0.121	0.139	0.696	0.544	0.333	0.121	0.172	0.394	
SeS	Pearson Correlation	1.000	-0.982	-1.000	0.500	0.500	0.189	-0.327	0.082	0.826	-0.886	0.887	0.835	-0.850	0.666	0.664	-0.861	-0.982	1	0.877	1.000	0.918	0.619	-0.787	0.756	1.000	0.896	-0.690	
	Sig. (2-tailed)	0.000	0.121	0.000	0.667	0.667	0.879	0.788	0.948	0.381	0.306	0.305	0.371	0.353	0.536	0.538	0.339	0.121		0.320	0.000	0.260	0.575	0.423	0.454	0.000	0.293	0.515	
ZnF	Pearson Correlation	0.877	-0.770	-0.877	0.021	0.855	0.638	-0.742	-0.408	0.453	-0.554	1.000	0.466	-999	0.943	0.222	-1.000	-0.770	0.877	1	0.877	0.613	0.920	-0.987	0.347	0.877	0.572	-0.256	
	Sig. (2-tailed)	0.320	0.441	0.320	0.986	0.347	0.559	0.468	0.733	0.701	0.626	0.015	0.691	0.034	0.216	0.858	0.019	0.441	0.320		0.320	0.580	0.256	0.103	0.774	0.320	0.612	0.835	
ZnS	Pearson Correlation	1.000	-0.982	-1.000	0.500	0.500	0.189	-0.327	0.082	0.826	-0.886	0.887	0.835	-0.850	0.666	0.664	-0.861	-0.982	1.000	0.877	1	0.918	0.619	-0.787	0.756	1.000	0.896	-0.690	
	Sig. (2-tailed)	0.000	0.121	0.000	0.667	0.667	0.879	0.788	0.948	0.381	0.306	0.305	0.371	0.353	0.536	0.538	0.339	0.121	0.000	0.320		0.260	0.575	0.423	0.454	0.000	0.293	0.515	
Ash	Pearson Correlation	0.918	-0.976	-0.918	0.803	0.115	-0.217	0.075	0.471	0.982	-997	0.631	0.985	-0.570	0.314	0.906	-0.589	-0.976	0.918	0.613	0.918	1	0.255	-0.477	0.954	0.918	999	-0.921	
	Sig. (2-tailed)	0.260	0.139	0.260	0.407	0.927	0.861	0.952	0.687	0.121	0.046	0.565	0.111	0.614	0.796	0.278	0.599	0.139	0.260	0.580	0.260		0.836	0.683	0.194	0.260	0.032	0.255	
Protein	Pearson Correlation	0.619	-0.459	-0.619	-0.371	0.990	0.888	-0.945	-0.732	0.068	-0.185	0.911	0.084	-0.940	998	-0.177	-0.932	-0.459	0.619	0.920	0.619	0.255	1						

Samples type: Fruit= F; Soil= S. Elements: Arsenic= As; Calcium=Ca; Cadmium= Cd; Chromium=Cr; Copper= Cu; Iron=Fe; Magnesium= Mg; Manganese= Mn; Selenium= Se; Zinc= Zn. **Correlation coefficient (r):** Strong correlation $r > 0.7$ or $r < -0.7$; Moderate correlation $0.3 < r < 0.7$ or $-0.3 < r < -0.7$; Weak correlation $r < 0.3$ or $r > -0.3$. **Significance level (p):** Significant $p < 0.05$ or not significant $p \geq 0.05$.