Assessment of nutraceutical attributes of selected wild edible fruit plants used by Vhavenda people of the Thulamela Local Municipality

by

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Dissertation

submitted in fulfilment of the requirements

for the degree of

Magister Scientiae (MSc)

in

Biological Sciences

in the

Faculty of Science, Engineering and Agriculture

at the

University of Venda

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DECLARATION

I, Nephawe Rinae Lucy hereby declare that “the dissertation for the Master of Science in Botany at the University of Venda, hereby submitted by me has not previously been submitted for a degree at this or any other University, and that is my work in design and execution and that all reference material contained therein has been duly acknowledged”.

.... 30 April 2024 ....

Nephawe Rinae Lucy **Date**

DEDICATION

This project is dedicated to my mother Nephawe Nkhuliseni Patience who always guided and inspired me to be a diligent person. You are remarkably close to my heart.

ACKNOWLEDGEMENTS

Primarily, I would like to thank Almighty for the wonderful gift of a healthy life he has accorded me throughout my study, giving me strength and determination up until completion. I would like to express my sincere gratitude to the following individuals for their assistance and support towards the study: I would like to thank my parents Nephawe Maanda Mick and Nephawe Nkhuliseni Patience for your encouragement and love. I thank you for your unconditional assistance throughout the data collection period.

To my supervisor Dr Mokgaetji Georginah Mokganya and my co-supervisor Prof Ntakadzeni Edwin Madala, I deeply appreciate the opportunity to gain experience under your great guidance. Thank you for all the academic insights and motivation to always work hard. I appreciate that you never gave up on me but guided me with great love and patience.

To my field work assistants, Munyai Vhutshilo, Ndwamai Mulalo, Ndou Tshihumbudzo and Ramaswiela Adivhaho your great endurance during data collecting is highly acknowledged.

I would also like to extend my appreciation to my aunt Nephawe Ndiafhi Precious, thank you for the constant support and academic assistance. Your guidance and motivation always reminded me that I could make it.

I would also like to extend my gratitude to the University of Venda and the Departments of Biological Sciences, Biochemistry, and Microbiology for their endless support.

ABSTRACT

Wild edible fruit plants are highly valued in many remote rural areas, where they play a significant role in fulfilling the sociocultural and livelihood needs of people. Many of these plants are harvested by local communities to augment and diversify their diets as well as to promote local food security. The decline in the use of wild edible fruit plants may be due to a lack of knowledge of their nutritional value, their potential for income generation, and medicinal and cultural uses. This study focused on the documentation of utilisation, conservation, and nutraceutical composition of the wild edible fruit plants of the Thulamela Local Municipality. Information on wild edible fruit plants was gathered through semistructured interviews, fieldwork, and a literature review. A total of 39 wild edible fruit plants of 22 botanical families recorded during the study were used as food, beverage, and additionally as medicine. The number of wild edible fruit plants with medicinal properties was 39 whereas those used to make beverages were 9. Wild edible fruit plants of Thulamela municipality were experiencing challenges due to human activities resulting in declining, endangered, or even extinct. Results of this study also showed the use of wild edible fruit plants as firewood because 10 out of 39 were mentioned for this use. In this study the UHPLC-qTOF-MS/MS-based in source collision induced dissociation method was utilized to generate fragmentation data to assist in the differentiation of closely related isomers.

Wild edible fruit plants have been used for centuries in traditional medicine and for nutritional purposes. *Strychnos* species has not yet been fully decoded, and due to the inherent complexity of plant metabolomes, the characterization of *Strychnos* photochemistry remains challenging.

Thus, in this study, we propose the use of molecular networking to unravel the families using the metabolome analysis of two *Strychnos* species (*Strychnos pungens* and *Strychnos spinosa*) and highlight the relevance of molecular networking in exploring the chemotaxonomy of plants. This allows visualization of chemical classes and the variety of substructures within the molecular families.

Keywords: wild edible fruit plants, conservation, nutraceutical, lc-ms and molecular networking.

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LIST OF ACRONYMS

SANBI	South African National Biodiversity Institute
VDM	Vhembe District Municipality
UV	Ultraviolet
WEPs	Wild edible plants
RFC	Relative Frequency of Citation
IKS	Indigenous Knowledge System
ISCID In	Source collision-induced dissociation
ESI	Electrospray ionisation
MS	Mass spectrometry
MSI	Metabolomics Standard Initiative
CGAs	Chlorogenic acids
FAO	Food and Agriculture Organization
HIV	Human Immunodeficiency Virus
H	Hydrogen
UHPLC	Ultra-high performance liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry

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CHAPTER 1: BACKGROUND INFORMATION

1.1. INTRODUCTION

1.1.1 Definition of wild edible fruit plants

Wild edible fruit plants are a term given to species that are neither cultivated from its natural habitat, domesticated but easily accessible to serve as source of food (Molla *et al*., 2011). The term is used interchangeably with other similar terms such as traditional and indigenous plants throughout the ethnobotanical literature (Van der Hoeven *et al*., 2013; Tardio & Pardo-de-Santayana, 2008). The local Tshivenda translation of the wild edible fruit plants is 'miri ya daka' simply means "plants that grow in the wild" (Mokganya *et al*., 2017)

1.1.2 Attributes of wild edible fruit plants

Rural communities are still gathering and consuming many edible wild plants. They are still using them as a supplement to their essential need for food. Their significance is evidenced by relaxed accessibility and nutritional benefits, especially vitamin and micronutrient provisions (Mukhtar, 2019). Local people have obtained knowledge of wild edible fruit plants from fieldwork. Knowledge of wild edible fruit plants can be used to combat food insecurity and malnutrition challenges and is transferred from one generation to the other (Lulekal *et al*.,2011).

Wild edible food plants including wild edible fruit plants have been an essential element for the human diet since from time immemorial (Mukhtar, 2019). Remarkably, almost 75000 plant species that grow in the wild are known to be edible and only two hundred species are successfully being domesticated with only 30% contributing as food supply to a massive 95% of the world's plant food consumption (Mukhtar, 2019).

Inhabitants of the Thulamela Municipality and Vhembe District Municipality at large are not excluded from the 95% of the world's plant food consumption. The unambiguous evidence is provided from the findings of the study conducted by Mabogo (1990) wherein indication of wild food plants consumption was apparent. Additionally, Rampedi & Olivier, (2013) documented a total of 41 wild food plant species and mentioned that some can be utilized in production of different beverages.

It is of paramount importance to consider that wild edible fruit plants can be utilized in many various important ways. Worth noting, several studies had demonstrated the nutritional importance of certain wild edible food plants species in terms of provision of protein, minerals such as iron and calcium as well vitamin content (Rampedi & Olivier,2013, Mokganya *et al*., 2021). This notion highlights the pivotal role that these plants play in enriching the diets of vulnerable rural populations (Rampedi & Olivier, 2013). Furthermore, wild edible fruit plants always occupied a vital role in the lives of people by satisfying their spiritual and material needs (Rampedi & Olivier,2013). This is true especially in rural areas, where indigenous plants contribute significantly as source of food, medicine, firewood, and shelter constructions (Rampedi, 2010).

On the other hand, Kitula (2007) reinforced the "nutritional values of wild edible fruit plants by reporting availability of minerals like sodium, potassium, magnesium, iron, calcium, and phosphorus" from the studied plant species. In addition, wild edible fruit plants can be utilised medicinally to treat and cure various diseases (Mokganya *et al*, 2017). In other parts of the world, they are often used in different formulations called 'Ayurveda' an Indian Folk- medicine for the provision of immune enhancers to fight many various diseases (Frison *et al*., 2006).

1.2. PROBLEM STATEMENT

The use of wild edible fruit plants is declining in all areas of South Africa and the agronomic knowledge of their production is scarce. The decline in the use of the wild edible fruits plants may be due to lack of knowledge on their nutritional value, their potential for income generation, and medicinal and cultural uses. Lack of knowledge about nutritional composition and ways of preservation have also been suggested as reasons for the low use of indigenous plants. Wild edible fruit have the potential to provide a valuable source of nutrition in areas with hot and dry climates that are normally characterised by elevated levels of food insecurity, as is the case in most rural communities. Wild edible fruit plants are threatened by deforestation whereby popular but slow growing and or naturally rare plant species are often under pressure. Currently deforestation is one of the most widespread environmental threats contributing to the depletion of natural resources in the Vhembe region. Wild edible fruit plants have played a very vital part in supplementing the diet of the people. The dependence on these fruits has gradually decline as more exotic fruits have been introduced. However, many people in Vhembe areas still use them as a supplement of their basic need of food. Some of them are preserved for use in dry period or sold in rural market.

Most importantly, it is apparent that the popularity of these wild forms has recently decreased. Apart from their traditional use of food, potentially they have many advantages (Bhatia *et al.* 2018).

1.3 MOTIVATION OF THE STUDY

Wild edible fruit plants play pivotal role in the provision of essential nutrients that prevent the problem of malnutrition in the human's body. Moreover, they have potential towards households' income generation and family nutrition improvements. Additionally, some wild edible fruit plants possess medicinal properties which are useful to the well-being of the local communities. This study will contribute to adding knowledge on wild edible fruit plants used and found in the study area by sensitizing and creating awareness to the communities and organisations alike on sound harvesting methods, prioritization of species for domestication and designing appropriate processing and application methods.

1.4 AIM

To document utilisation, conservation and assess nutraceutical composition of the wild edible fruit plants of the Thulamela Local Municipality.

1.5 OBJECTIVES

- To identify and document popular native wild edible fruit plant species.
- To study the indigenous conservation methods for the selected fruit plant species
- To evaluate the nutraceutical composition of the selected popular wild edible fruit plant species.

1.6 RESEARCH QUESTIONS

1.6.1 Which species of wild edible fruit plants are used by the residents of Thulamela Local Municipality?

1.6.2 Which indigenous methods are used for the conservation of the documented wild edible fruit plant species?

1.6.3 What is the nutraceutical composition of the selected wild edible fruit plant species of the Thulamela Local Municipality.

CHAPTER 2: LITERATURE REVIEW

2.1 DESCRIPTION OF UTILISATION, CULTURAL VALUES AND CONSERVATION OF WILD EDIBLE FRUIT PLANTS

2.1.1 Description of wild edible fruits plants

‘Wild edible plants’ (WEPs) are regarded as species that are neither cultivated nor domesticated but grow naturally in the wild. Wild edible fruit plants are gathered from diverse habitats such as forests and disturbed areas that resulted from agricultural activities (Beluhan & Ranogajec, 2010). Different wild edible fruit plants have played a significant role in all geographical regions of the world throughout human history (Sekeroglu *et al*., 2006). Poor communities through the world are dependent on these wild plants in numerous ways including provision of food, nutrition, and improvement of rural livelihoods. Even nutritional superiority of some of the wild edibles was also reported over the cultivated food plants (Burlingame, 2000).

In recent time, the traditional consumption of wild edible plants is still supplemented with staple crop plants (Lulekal *et al*., 2011). The role of wild edible fruits plants in ensuring food and nutritional security to the rural or Indigenous communities is now widely recognized. Unfortunately, data available on their identification, composition, or nutritional properties, use and management or user’s preferences is scanty or less documented (Frison *et al*., 2006).

Wild plants are a crucial source of food, healthcare, and material subsistence in many of the developing world and carry a strong association with human livelihood. Wild edible fruit plants are the most important food source for the human population and continue to be significant contributors to the global food basket (Sundriyal & Sundriyal, 2004).

The word wild in this context refers to species that are not intentionally grown and managed by humans, including those minimally managed to prevent overgrowth or overharvest. This includes both native and alien plants, regardless of the preservation level of the habitats. Many earlier ethnobotanical works focused on lists of useful plants and had a strong tendency to focus on the scouting of new drug sources and new non-wood forest products, both of which can be economically lucrative (Frison *et al*., 2006). However, in recent years, there has been a growing interest in exploring the traditions of using wild plants beyond material and medicinal purposes and focus on wild edibles, as their roles become better understood in terms of local nutrition, dietary diversity, income generation, healthcare, reduction of micronutrient deficiency, and food security through diversification (Sekeroglu *et al.,* 2006).

There is now a consensus that information on wild edibles, including various modes of utilisation and preparation, constitutes an important part of ethnobotanical knowledge and therefore that elucidating region-specific patterns of their habitat management and consumption assists policymaking in the areas of natural conservation, human nutrition, and healthcare. This is particularly the case as lack of extensive data is one of the major barriers that prevent optimal decision making tailored to local conditions (Lulekal *et al*., 2011).

2.1.2 Utilisation of wild edible fruit plants

Since time immemorial the wild edible fruit' plants have been a source of hidden harvest which had supplemented the community with food and income. The traditional knowledge system and economic demand of a community influences the exploitation of wild edible fruit plants in a particular area. In terms of wild edible fruits, its consumption indicates user's evaluation of the fruits in terms of its availability, tastes, and preference along with duration of settlement near to a forest from where the community is procuring these fruit (Jasmine *et al*., 2007). World's poorest of the poor are those who are deprived of a dignified life and are most vulnerable dominates South Asia, Latin American, Northern Africa, Sub-Saharan Africa, and the Caribbean (Nazarudeen, 2010).

There is prevalence of undernourishment in these regions due to production of insufficient food grains and at times food supplies are not easily available. Foods obtained from wild edible fruit plants serve as buffer food rescuing lives during food shortages and famines. The vulnerability towards hunger and malfunctioned ecosystem services leads these people rely for food and other products more on intensive agriculture (Ericksen *et al*., 2009). Studies strongly indicate that the WEPs can significantly substantiate the global food basket in today's era of climate instability" (Maghembe, 1995).

These vulnerabilities have developed a greater understanding among the scientists, policy makers, national governments, and international institutions of a strong linkage between nutritional security and biodiversity for formulating policy support to promote utilization, value addition and conservation of WEPs (Bharucha & Pretty, 2010).

Wild edible fruit plant improves nutrition and increase food security particularly for a rural poor without which families would go hungry or become malnourished (Kitula, 2007). Scarpa & Arenas, (2007) enumerated 57 wild plant species traditionally used as sources of food by Chorote people of Salta Province, Argentina. In China Kang *et al*. (2014) recorded 81 wild vascular plant species utilized by the Tibetans as food. Several research provided detailed knowledge of edible wild plants in specific locations in Africa (Becker, 1986), all of which showed that wild plants are essential components of many African' diets during periods of seasonal food shortage especially immediately after planting and before harvesting.

2.1.3 Traditional knowledge and cultural value

Traditional or folk knowledge on plants is relationship between a society and its environment developed by the community variedly from region to region based on their real-world experience and empirical testing (Aumeeruddy, 1996). Wild edible fruit plants have been fundamentally associated with needs, tradition, and culture of Indigenous people (Addis *et al*., 2013). The Indigenous people are the custodian of these plant resources and the traditional knowledge associated with it (Demel & Abeje, 2004). Documenting traditional knowledge based on ethnobotany of wild edible fruit plants will help in identifying species for domestication and is necessary to create rich and complex production systems for its sustainable development and utilisation through commercialisation and conservation as well (Bell, 1995).

The past studies like Reddy *et al.* (2000) showed that different tribes have intensively used several similar wild fruits with different uses. This proves the diversification of knowledge among the Indigenous people in region to region and nation to nation. The intensity of use and knowledge of the wild edible fruit plants was reported to be function of characteristic of the used plants, habitat of the plants where it is found, frequency of food shortages and people's way of life in terms of their social, cultural, religious, and economical domains (Suresh *et al.,* 2014).

This knowledge differs among the communities and within a community in terms of age and gender where senior women folks were reported to be more knowledgeable in describing these plants as compared to their male counterparts (Koizumi, 2005; Shrestha & Dhillon, 2006). This is because communities and individuals may have different objectives, interest's, perceptions, beliefs and access to information and resources. The use of wild edible fruit plants in the life of rural and Indigenous people is not only in terms of food, income, or farm inputs but also in terms of social, cultural, and religious purposes as in sacred groves (Arnold, 1995; Clarke *et al*., 1996; Arora, 1998; Ohiokpehai & Ramosweu, 1999; Ohiokpehai, 2003; Pala *et al*., 2013). Unfortunately, this traditional culture and knowledge is losing importance by the encroaching traditions of modern outside community along with spread of agriculture and homogenisation of agricultural landscapes (Pretty, 2003).

The use of these wild plants for food and other uses by the rural and Indigenous communities can be continued by developing harmonious correlation among farming and wild biodiversity (Pretty, 2003). To deliver the economic benefits of the development programs meant for the rural and Indigenous communities, it is imperative to renew, document and utilise the traditional knowledge systems of the target communities where the intangible, edible, economic and environmental roles of the wild edible fruits plants can be the key (Pretty, 2003).

2.1.4 Forests degradation, biodiversity loss, and depletion of wild edible fruit plants.

Forest and woodland depletion and degradation have been progressing at an alarming rate in South Africa despite protective attempts by the colonial governments and later by the independent state government (Abdallah & Monela, 2007). Wild food and medicinal resources are suffering because of land clearing and harvesting for charcoal and timber rather than for food and medicine (Marshall, 1998). According to Marshall (1998), the number of wild food and medicinal plants is significantly declining. According to Abdallah and Monela (2007), using wild fruit products is ineffective and not sustainable.

The loss of biodiversity is a grave issue for the environment. Extinction, or the elimination of entire species, is currently happening at the greatest pace ever observed in human history, even without climate change (Myers & Spoolman, 2014). Because of human activity, "the biological diversity of the earth is quickly declining on both a direct and indirect level. Unknown but significant numbers of species have already gone extinct, and many others have threatened populations. To assure the survival of many species, humans must now step in (Frankham *et* *al*., 2004). The spread of wild edible fruit plants is impacted by climatic changes, including global warming. Genetic resources that were previously present in certain places have been lost because of the alterations” (Kayombo *et al*., 2013). Investigating the pressure on wild edible fruit trees within villages is significant since they are crucial for the villagers' health and culture (Stoffersen *et al*., 2011).

The rate of deforestation is significant in ecoregions under strain from human population growth, where floral variety is consequently relatively low (Chidumayo, 1996). According to Abdallah & Monela (2007) and Masanja (2013), the clearing of forests for agricultural purposes, overgrazing, the production of commercial and domestic fuel wood, mining, and forest fires for a variety of purposes (including the eradication of tsetse flies, shifting cultivation, hunting, and pasture management) are the apparent main causes of forest depletion and degradation. In South Africa, shifting cultivation may be responsible for more than 50% of the deforestation; charcoal is the second culprit. Additionally, there are reports of illegal mining and harvesting. Because some of the reasons of forest degradation and depletion are merely obvious or symptomatic, it is essential to have a comprehensive grasp of these causes (Misana *et al*., 1996). As a result, misinformation and negligence, poor agrarian policies, overcrowding, unrestricted access to forestlands, poverty, illiteracy, and corruption are the true causes of forest depletion and degradation (Misana *et al*., 1996). To effectively combat the depletion and degradation of forests, it is crucial to address these true drivers of deforestation.

According to Abdallah and Monela (2007), the destruction of forests has a few effects on both human livelihoods and the ecosystem". Acute shortages of timber, fuel wood, and other forest products and services; depletion of medicinal plant resources, which will inevitably lead to the loss of Indigenous knowledge (IK) that is frequently passed verbally from elders to the younger generation and is not documented in writing (Mhame, 2000); drying of water sources and shortage of water for various purposes; floods, sedimentation of rivers; and severe shortages of medicinal plants are just a few of the impacts (Mhame, 2000).

2.1.5. Nutraceutical and phytochemical content of wild edible fruit plants.

By promoting the intake of native fruits or their by-products, the development of dietary supplements using native plants with high nutritional content and phytochemical profiles can help fight non-communicable diseases. Based on the review of (Pfukwa *et al*., 2020), there is also little information available regarding the phytochemical makeup and bioactivity of these underutilised fruits, as well as their specific phenolic profiles and antioxidant qualities, except for Marula and Black monkey orange (Pfukwa *et al*., 2020).

The most recent data on a variety of native southern African fruits with nutraceutical qualities for food application is provided via metabolomic acid chemometrics. Except for xanthohumol, many phenolic metabolites in Sand apricot vine were present in low concentrations. The sour plum contained enormous amounts of procyanidin dimer B1 and procyanidin B5 (Nkosi *et al*., 2022).

The proximate composition of five Mozambican wild fruits was discussed in a prior study. In addition to being assessed for pH and titratable acidity, the dry matter, fat, protein, ash, soluble solids, and sugar content of *Adansonia digitata*, *Landolphia kirkii*, *Sclerocarya birrea*, and *Vangueria infausta* were also examined. The goal of the current study was to further analyse these fruits' nutritional value. The goal was to highlight the potential of the four wild fruits and chosen seeds as a potent remedy for addressing macro- and micronutrient deficiencies, particularly in children, by determining the amount of dietary fiber, organic acids, and minerals in each (Magaia *et al*., 2013).

The fruit of *Strychnos spinosa* is a healthy food source of both proteins and carbs. Additionally, it includes significant minerals like iron, zinc, copper, and manganese, indicating that eating *Strychnos spinosa* could help you get the zinc, iron, copper, and manganese your body needs. Micromineral deficiencies in people hinder growth and make them more susceptible to infections and mortalities, especially in youngsters. Phytochemicals are biologically active substances, such as flavonoids and phenolic acids, with health-promoting properties, such as anti-ageing and anti-inflammation, which were linked to their capacity to scavenge free radicals (Omotayo & Aremu, 2021).

The phytochemical studies conducted on the extracts of the twigs and stem wood of *Garcinia livingstonei* resulted in the isolation of two new benzophenone derivatives. So far, these benzophenone derivatives have not been reported from any other part of this plant. In the antioxidant activity studies, the benzophenone derivative was more active than the glycosylated derivative and the standard, vitamin C, used in these studies (Muriithi *et al*., 2016).

2.2 A REVIEW OF POPULAR WILD EDIBLE FRUIT PLANTS OF THE THULAMELA MUNICIPALITY

2.2.1. Description of *Englerophytum magalismontanum* (sond.) T.D.Penn.

The stem fruit is a small to medium (3-10 m) evergreen tree species, according to Palgrave (2002), and it can be found in a range of ecological environments, including rocky outcrops and slopes, wooded ravines, and along riverbanks. It is a member of the Sapotaceae family, sometimes known as the milkwood plant family, and there are roughly 22 native species in this genus (Van Wyk & Van Wyk, 1997). The geographic range of the species *Englerophytum magalismontanum* is wide, extending from Kwa-Zulu Natal in South Africa to Zimbabwe (Van Wyk & Gericke, 2003). One of the important species now used in local subsistence trade and domestication experiments is *Englerophytum magalismontanum*. In locations where it naturally exists, the species is highly sought after not only for food but also for medicinal purposes Du Preez (2007).

Figure 2.2.1: Photo of *Englerophytum magalismontanum* (sond.) T.D. Penn showing growth of fruits on the stem (**Source:** www.metafro.be/prelude/view_countrycc=ZA)

2.2.2 Description of the spiny-leaved monkey-orange (*Strychnos pungens*) Soler.

According to Van Wyk &Van Wyk (1997), the plant is a member of the Loganiaceae family. The common name "spiny-leaved-monkey orange" refers to the plant's creamy yellow bark, which is longitudinally ridged and covered in well-developed spines that frequently finish in a terminal spine. *Strychnos pungens* is “an evergreen shrub or tree that typically reaches heights of 0.6 to 8 meters”. Wild fruit that is “edible is collected for local consumption; in some locals, the fruit is of good quality and is highly prized as a food, while in other locals, the fruit has a variety of uses” (Palgrave, 2002). *Strychnos* species can be quite dangerous and are abundant in alkaloids, thus care must be used when using them (Palgrave, 2002).

Strychnos pungens is "a semi-deciduous shrub or small tree that grows in woodlands, mixed forests, and bushveld in southern and eastern Africa" (Akinnifesi *et al*., 2006). It has a rounded crown and broadly ovate oblong to round leaves. It often favors natural areas with rocky or sandy soil. Most species in the genus are used to treat fever and malaria in conventional medicine" (Ruffo *et al*., 2002). Both the roots and the leaves are "used to cure illnesses like dermatitis, gonorrhea, gastrointestinal problems, and STDs" (Ruffo *et al*., 2002). Additionally, *Strychnos pungens* fruit can be used with honey to alleviate coughs. Their ripening is indicated by fruits that turn from green to a yellowish-brown colour (Ruffo *et al*., 2002).

Figure 2.2.2: Photo showing fruits of spiny-leaved monkey-orange (*Strychnos pungens*) Soler**. (Source: www.zambiaflora.com)**

2.2.3. Description of spiny monkey orange (*Strychnos spinosa*) Lam

Strychnos spinosa is "one of the most important edible indigenous fruit trees in the wild." The fruit-bearing species of *Strychnos* belong to the family Loganiaceae. The tree has the capacity to stay edible in tropical heat, which is an important characteristic for food and nutrition security, as this will enhance availability and productivity (Ngadze *et a*l., 2017). In traditional medicine, *Strychnos spinosa* is often used in the treatment of venereal diseases, stomachrelated aches, and snake bite attack (Hedberg *et al*., 1983). *Strychnos spinosa* is known as a native or introduced species in many African nations. The plant has been reported across different African regions, including Southern Africa, East Africa, and West Africa. In South Africa, *Strychnos spinosa* grows well in four provinces (Eastern Cape, Limpopo, KwaZuluNatal, and Mpumalanga). Furthermore, the conservation status of *Strychnos spinosa* is categorized as “least concern” as its distribution and abundance possess a minimal risk of extinction (Aremu & moyo, 2022)

However, the plant has a recent record of declining occurrence in Benin and Burkina Faso (West Africa), which was attributed to factors such as agricultural activities, urbanisation, and animal breeding, rather than climate change and its impact. Although the distribution and availability of the *Strychnos spinosa* is uneven in Africa, its food-nutritional and economic potentials suggest the need for a more conscious and holistic conservation approach (Ngadze *et al*., 2017). *Strychnos spinosa* has several local uses, and it is known to be a rich source of nutrition and phytochemicals, thereby suggesting its potential health benefits. Given the increasing importance of *Strychnos spinosa* in food-nutritional sovereignty, as well as its ecological advantage, this review provides an appraisal on the potential for sustainable food– nutrition and economic prosperity of *Strychnos spinosa.* It is anticipated that "consolidated information on *Strychnos spinosa* is important to unfold its nutritional and economic potential (Omotayo & Aremu, 2021).

Figure 2.2.3: Photo showing fruits of *Strychnos spinosa* Lam (**Source:** www.africanplants)

2.2.4. Description of Mobola plum (*Parinari curatellifolia*) Planch.ex Benth.

"The Mobola plum belongs to the plant family Chrysobalanaceae (or the coco plum family). This family is comprised of three indigenous species in the Southern African region" (Van Wyk & Van Wyk, 1997). Related species such as *Chrysobalamus ciao* are also harvested for their fruits. In South Africa, *Parinari curatellifolia* occurs in areas classified as bushveld in the north-eastern areas of the Limpopo province. It usually thrives in deep, sandy soils on both gently sloping terrain and mountainous areas. The tree is evergreen and may grow up to ten metres high under favourable conditions. The leaves develop from sagging branches and are mostly dark greenish on the upper surface and grey to brown on the lower surface (Van Wyk & Van Wyk, 1997). The fruits are characteristically single-seeded, oval to round, brownish to yellow in colour and have distinctive greyish scales. The fruits are usually harvested between October and January and are known to have a pleasant taste and may also be used to make delicious syrups, jams, refreshing juices as well as various intoxicating traditional beverages. The research conducted on the mobola plum has focused on the nutrient content of the fruits (Du Preez *et al*., 2003). It has been established that the fruits have a high vitamin C content, ranging from 65-75 g/100g of fruit mass (Du Preez *et al*., 2003). In a study conducted in Malawi, the total carbohydrate content of these fruits was found to be approximately 88%. The kernels (nuts) have also been found to be rich in nutrients such as thiamine, magnesium, phosphorus, and zinc (Saka and Msonthi, 1994).

Figure 2.2.4: Photo showing fruits of mobola plum (*Parinari curatellifolia*) Planch.ex Benth.

(**Source:** https://silverhillseeds.co.za)

2.2.5. Description of sourplum (*Ximenia caffra*) Sond.

The sourplum is "a tiny tree or shrub that produces colorful fruit and has several traditional applications. To draw fruit-eating birds and numerous insects, it is best suited to growing in a bush clump or as part of a boundary screen in the garden (Palgrave, 2002). The Olocaceae family is small given that it has only five plant species (Van Wyk, 1997). *Ximenia caffra* is a deciduous tree that grows to a height of 6 m and has an untidy open crown. On younger branches, the bark is brown or green rather than dark grey and rough. Branchlets have spines at their tips. White sapwood and strong, reddish-brown heartwood can be found in trees. Nonaggressive root structure (Palgrave, 2002).

The leathery dark green leaves are often in clusters on short, spur branchlets. They are simple, 60 x 25 mm. They have been used in the treatment of conditions like inflamed eyes, dysentery, sexually transmitted diseases, and diarrhea because the chemical components in their roots and leaves have been reported to have anti-microbial, anti-inflammatory, and anti-schistosomic properties (Mathabe *et al*., 2006). Although their fruits are mostly consumed in rural areas; additionally, they are utilised in the production on non- acoholic and alcoholic beverages. The chemical profile of *Ximenia caffra* leaf was comprehensively analysed and led to the identification of ten polyphenol compounds, including phenolic acid and flavonoids (Rampedi & Olivier, 2013).

Figure 2.2.5: Photo of sourplum (*Ximenia caffra*) Sond. Showing growth of fruits on the stem

(**Source**: https://www.bio-innovation.org/work/wild-or-sour-plum)

2.2.6. Description of the Wild medlar (*Vangueria infausta*) Burch.subsp. *infausta*

Depending on the habitat, this deciduous shrub or small tree can grow to a height of 3 to 7 meters. It might have one stem or many, but commonly has more than one. The bark is smooth, ranging in colour from grey to yellowish brown, and it peels off in erratic, thin strips. Short, fuzzy hairs cover the branchlets, especially when they are young. Like other members of this family, the leaves are single and oriented in opposition. The leaf's full edge is present. "The leaf's form ranges from elliptic to ovate, and its net veining is clearly visible below. The leaves become twisted and harsh to the touch as they age" (Rampedi & Olivier, 2013).

The wild medlar is a member of the Rubiaceae family, which also includes about 160 other native species. *Vangueria infausta* grows in rocky ground typically found in bushveld, open forest, and grassland. "The use of the roots in the treatment of ailments like malaria and pneumonia is crucial in ethnomedicine" (Palgrave, 2002).

The plant produces 2.5–3.5 cm in diameter, spherical, edible fruits that turn yellow to brown when ripe. "The fruits contain a dry, sweet-sour flesh that may be considered an important dietary source of potassium (521 mg/100g fruit mass) and calcium (249 mg/100g fruit mass) minerals" (Van Wyk & Gericke, 2003).

Figure 2.2.6: Photo showing fruit of the Wild medlar (*Vangueria infausta*) Burch.subsp. in various stages of ripening**. (Source:** Own**).**

2.2.7. Description of the Brown ivory (*Berchemia discolor*) (Klotzsch) Hemsl.

Depending on the ecological setting, the brown ivory is a semi-deciduous tree that can reach heights of 20 m. It can be found frequently in rocky terrain and arid or semi-arid bushveld. A hefty, spherical crown is created by erect, widely spaced branches. "The bark and leaves of this indigenous species, like many others, are used in ethnomedicine to treat menorrhagia and infertility" (Van Wyk & Van Wyk, 1997). In terms of nutritional content, (Wehmeyer, 1986) recorded a potassium concentration of 270 mg/100g fruit mass and a vitamin C content of 50.3 mg/100g. These findings, along with the 6.1% protein level in the fruit pulp, demonstrate that this species has unrealised food potential in South Africa, especially in terms of meeting the nutritional needs of rural impoverished people" (Rampedi & Olivier, 2013).In some African countries, propagation trials are being carried out to facilitate their domestication, selection of superior germplasm for adoption and enhancement, as well as the commercialisation transition from informal to formal markets. Regarding traditional uses, "numerous foodstuffs such as pleasant, flavored porridge as well as non-alcoholic and alcoholic beverages might be produced from these fruits" (Du Preez, 2003).

Figure 2.2.7: Photo of showing fruits of the Brown ivory (*Berchemia discolor*) (Klotzsch) Hemsl. **(Source:** www.aluka.org)

2.2.8. Description of the Sand apricot vine (*Landolphia kirkii*) Dyer ex Hook. f.

The sand apricot vine is a "woody climber that grows throughout the northern KwaZulu-Natal and Limpopo provinces in thickets or thickets on rocky outcrops" (Schmidt et al., 2002). It occurs outside of South Africa in several nations, including Kenya, Mozambique, Tanzania, and Zimbabwe" (Cunningham & Shackleton, 2004). This species is one of forty native species in the Apocynaceae family, which is distinguished by opposing or whorled leaves and a milky or watery latex" (Van Wyk & Van Wyk, 1997).

The sand apricot "vine produces round, 80 mm-wide fruits that hang from a long, slender stalk. The fruit's skin is initially thin and greenish, but as it ripens, it thickens into a thick, woody shell that turns pale brown. Monkeys love the extremely sweet edible pulp surrounding the seeds" (Cunningham & Shackleton, 2004). When compared to other wild fruits, "the fruit flesh has a very low content of vitamin C (10.3 mg/100g), and has 193 mg/100g of potassium", according to Wehmeyer (1986). However, there is a dearth of information on further nutritional composition, phytochemical capabilities, ethnomedicinal features, and propagation potential (Rampedi & Olivier, 2013).

Figure 2.2.8: Photo showing fruits of the Sand apricot vine *Landolphia kirkii* -Dyer ex Hook.f. (**Source**: Own).

2.2.9 Description of Marula (*Sclerocarya birrea*) (A. Rich.) Hochst.subsp. *caffra*

Tropical Marula trees are found in Southern Africa, and because of their "great nutritional and economic worth, they make significant contributions to the local economies of their communities. The discovery of numerous more uses for the marula tree" (Mokganya *et al*., 2017) was not surprising. The tree is regarded as a versatile Southern African tree that is utilized by the locals for its fruit, cosmetic oil from the seed, and therapeutic compounds from the bark and leaves. Nine plant families out of the twenty-one discovered throughout the investigation have members with therapeutic qualities. Lastly, the fact that just one plant species' "barks are used, along with the fact that three of the Marula tree's parts are used the inner bark, the fruits, and the roots strengthens the tree's distinction" (Mokganya *et al*., 2017).

Wild edible fruit plants can also be used for food, beverages, building materials, firewood, cosmetics, dye, and artifacts in addition to their medicinal capabilities. Of the twenty-seven recognised plant species, 34 percent are used as firewood. Food products accounted for 32% of all reported uses. Among the food products mentioned are "cooking oil made from Marula tree nuts, cooking soda made from Marula fruit skins, candy made from Marula fruit pulp, jam made from prickly pear fruits, and Marula kernels that can be eaten as a snack or used as spice" (Mokganya *et al*., 2017).

Marula trees are "one of the most adaptable indigenous species, claims (Palgrave, 2002). In fact, "the marula tree has received the greatest attention in terms of domestication and commercialisation out of all the trees that have been sold in South Africa" (Leakey *et al*., 2005). "The tree is dioecious (different sexes) and is known to yield thousands of fruits each season, totalling roughly 500 kg" (Shackleton, 2004). A few trees have, however, produced up to 1.5 tons of fruit mass year (Leakey, 1999). Light greenish fruits typically fall to the ground where they mature and turn yellow before being picked between January and March. The fruits and the tree have numerous traditional and sociocultural purposes. Marula fruit nutrients have been discovered to vary based on hereditary and environmental factors. However, most studies have found that "oranges have a greater vitamin C content of 194 mg/100g of fruit mass at 85% moisture, which is about 2-3 times more than the average 49 mg/100g found in most publications" (Du Preez *et al*., 2003).

Figure 2.2.9: Photo showing fruits of Marula (*Sclerocarya birrea*) (A. Rich.) Hochst.subsp. *caffra* (**Source**: www.pinterest.com).

2.2.10 Description of African mangostee (*Garcinia livingstonei).* T. Anderson.

A vast genus of trees or shrubs in the family Clusiaceae called Garcinia is polygamous, meaning that it has both male and female flowers on the same plant. It is found in tropical Asia, Africa, and Polynesia. According to Hutchings *et al*., (1996), “this family consists of the two genera *Hypericum* and *Garcinia*. Xanthones, flavonoids, benzophenones, lactones, and phenolic acids are only a few of the bioactive compounds that are abundant in garcinia” (Selvi *et al*., 2003). The last few years have seen the isolation of “xanthones and xanthone derivatives as well as other small and complex compounds from diverse species of *Garcinia*. In the rinds of *G. cambogia*, *G. indica*, and *G. cowa* fruit, hydroxy citric acid is present” (Jena *et al*., 2002).

The largest genus in the family Clusiaceae is “*Garcinia*, which has 400 species that are found throughout tropical Asia, Africa, New Caledonia, and Polynesia” (Waterman, 1986). According to Peres *et al*., (2000), “garcinia species are known to be abundant in oxygenated and prenylated phenol derivatives. Historically, lymphatitis, parotitis, struma, and other illness states have been treated with the leaves and seeds of other species, such as *Garcinia dulcin”* (Kasahara & Henmi, 1986). From *Garcinia livingstonei*, prenylated benzophenone, an HIV inhibitor, has been identified (Gustafson et al., 1992). “The African mangosteen, or *Garcinia livingstonei*, is a small to medium-sized tree that bears eatable fruits and develops at low elevations. Riverine edges and open woods are where you can find it, especially in South Africa. It has been noted that leaf and floral extracts have" (Diserens *et al.,* 1992).

Figure 2.2.10: Photo of African mangostee (*Garcinia livingstonei).* T. Anderson showing growth of fruits on the stem **(Source:**https://consultafrutas.blogspot.com/2013/07/imbegarcinia-livingstonei.html)

2.2.11. Description of Waterberry *(Syzygium cordatum)* (Hochst.)

The genus, "*Syzygium*," is derived from the Greek word "syzgios" which means "paired" (Grant *et al,* 1998). It is due to the leaves and twigs that grow at the same point. The specific name is the Latin name "*cordatus"* which means "heart shaped." It is in accordance with the heart-shaped base of the leaves. The genus "*Syzygium*" consists of more than 1100 species. The genus

Syzygium normally refers to trees of the Myrtaceae family. *Syzygium cordatum* is commonly known as Water-berry (English), Water-bessie (Afrikaans), uMdoni (Zulu), Mukute (Shona) and Montlho (North Sotho) (van Wyk *et al*., 2009). *Syzygium cordatum* is an evergreen and a medium to generous sized tree" (Drummond and Moll, 2002).

It grows to 8- 15 metres in height with a dense spreading rounded crown. "The stem is thick, dark brown fissured breaking up into irregular sections. "The leaves are simple, dark-green, broad, and circular, with a bluish green colour. The leaves have a distinctive arrangement in opposite pairs near the ends of the branches, each pair at right angles to the next. Its flowers are cream colour to pinkish with stems that are produced in clusters on the tips of the branches" (Young & Fox, 1982; van Wyk *et al.,* 2009*). Syzygium cordatum* trees grow rapidly in swamps, near fresh-water streams, in mountain grasslands, bushvelds and in places with high rainfall. *S. cordatum* trees are known as a sign of the availability of underground-water. *S. cordatum* are fire resistant and impervious to cold but not frost" (Grant *et al.,* 1998). *Syzygium cordatum* trees are native to RSA and are widely distributed in the eastern and northeastern parts of the country. They are found in the Eastern Cape, KwaZulu-Natal and across the northern part of South Africa" (Orwa *et al.,* 2009; van Wyk *et al.,* 2009). In Kwazulu-Natal, *S. cordatum* trees are signals of areas that are good for sugarcane plantations" (Grant *et al.,* 1998; Orwa *et al*., 2009).

"The fruiting season is usually from October to June in the RSA" (Drummond & Moll, 2002). The fruits of this family are used for both food and medicine in various parts of the world (van Wyk & van Wyk, 2013). *S. cordatum* trees produce sweet fruits. The fruits are found in clusters.

They are oval, glabrous, shiny, fleshy, up to 2 cm in length, red or red to purple black in colour and they are one seeded berry. The fruits are tipped with a calyx of 3-4 mm long" (Drummond & Moll, 2002; van Wyk & van Wyk, 2013). The seeds are about 2.8 cm thick. The ripened fruits are eaten raw. The fruits are eaten by people, birds, monkeys, bush pigs, and bush babies (Grant *et al.,* 1998). *Syzygium cordatum* fruits rank fourth (after *Sclerocarya birrea, Englerophytum magalismontanum* and *Strychnos pungens*) as a preferred delicious fruit among Indigenous South Africans" (De Lange *et al.,* 2005). *S. cordatum* fruits are often "fermented to produce potent intoxicating beverages" (Young & Fox, 1982). Jam and jelly are also manufactured from these fruits (Palmer & Pitman, 1972).

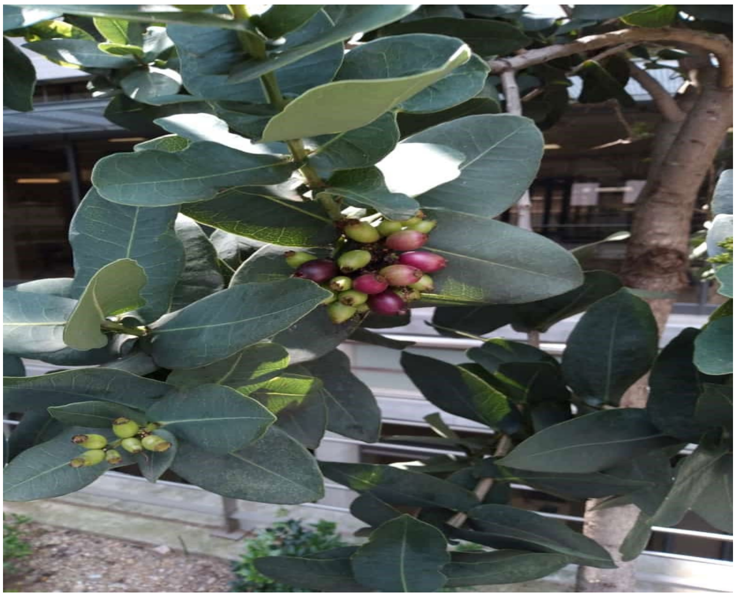

Figure 2.2.11: Photo showing fruits of Waterberry (*Syzygium cordatum)* (Hochst.) **(Source:** Own)

CHAPTER 3: STUDY AREA AND RESEARCH METHODS

3.1. STUDY AREA

3.1.1 DESCRIPTION OF STUDY SITE

Thulamela Municipality forms "part of the Vhembe district of Limpopo, and it forms part of the former Venda homeland. Thulamela municipality is located within the latitudes 22º 57º S 30º 29º E (Thulamela Local Municipality, 2015). The native residents of this area are Venda- and Tsonga-speaking people. Thulamela municipality was established in terms of the Local Government: Municipal Structures Act No. 117 of 1998 (South Africa, 1998a). "The Kruger National Park forms the boundary in the east, Mutale municipality forms the border in the north- east, and Makhado municipality in the south-west." "Forty-seven percent (47%) of the entire Vhembe district's population live in Thulamela municipality, and more than 85% of the people in this municipality live in tribal areas". "Thulamela municipality has a population size of 618 462, with a population growth of 0, 62% in 2011" (Statistics South Africa, 2016).

Figure 3.1.1. Thulamela Local Municipality map within the Vhembe District Municipality **(Source:** Thulamela Local Municipality, 2013/14**).**

3.1.2 Topography

The study area is surrounded by Thathe Vondo Mountains of the Thulamela Municipality (1439 metres above sea level) on the eastern side of Nzhelele that slopes gently into the Sibasa region. The eastern side of this mountains region also undulates into the hilly areas of Thohoyandou/Mphaphuli districts (Mabogo, 1990). The Maniini area has a gentle terrain, and is in a floodplain, making it vulnerable to flooding, compared with Tshilugwi village, which is characterized by gently rolling plains, as well as mountainous and hilly terrain (Musyoki, Thifhulufhelwi & Murungweni, 2016).

The area falls within the eastern part of the lowveld which forms part of the greater Limpopo basin and is characterized by 8% gently undulating slopes running in a north south direction (Mzezewa & Van Rensburg, 2011).

3.1.3 Geology and soil type

Precambrian basalts from "the Sibasa formation of the Soutpansberg group is found to the north of the area, and leucocratic biotite gneiss, leucocratic granite, and pegmatite, as well as grey biotite gneiss and migmatite, are found to the south in the Sand River gneiss of the central zone of the Limpopo belt. Precambrian igneous and metamorphic rocks dominate the region" (Hutten, 2015).

Hutton, Dresden, Tukulu, Sepane, and Vaalsrivier soils are "the most prevalent in the Lambani area" (Petja *et al.*, 2010:4). According to Musyoki *et al.*, (2016), "the geology and soil of Thulamela are composed of clay loam soil, sandy soil, and fertile clay soil".

3.1.4 Climate

Warm rainy summers (16–40 degrees Celsius) and mild dry winters (12–22 degrees Celsius) relate to the temperature and topography, respectively (Mabogo, 1990). According to Kabanda (2003), "annual rainfall ranges from 340mm in the west to 2000mm in the east. Venda rarely sees frost, and summer is when it rains the most" (Mabogo, 1990).

3.1.5 Vegetational composition

The least protected of South Africa's various forest kinds, the montane forest vegetation type is represented by this region. "A mist belt, montane forest, and woodland predominate in the Tshivhase region, which has a subtropical climate'" (Tshiguvho, 2008).

The district falls within the greater Savanna biome, also known as the bushveld, with some small pockets of grassland and forest biomes. These and other factors have produced a unique assortment of ecological niches, which in turn are occupied by a wide variety of plant and animal diversity. "The area's surprising biological diversity of flora and fauna is due to factors including its geographical location and varied topography" (Thulamela Local Municipality, 2017).

3.1.6 Selection of villages

To choose the communities, a simple random sample procedure was utilised. There was a list of every hamlet in the Thulamela Local Municipality of the Vhembe district. The four compass directions were used to group the communities. The names of the villages were written inside the little papers, which were then divided into four separate containers, one for each of the four compass directions, and used to separate all the grouped villages. Small pieces of paper were simply selected at random from the container using a straightforward random approach. The villages were chosen from the initial papers that were taken from several containers.

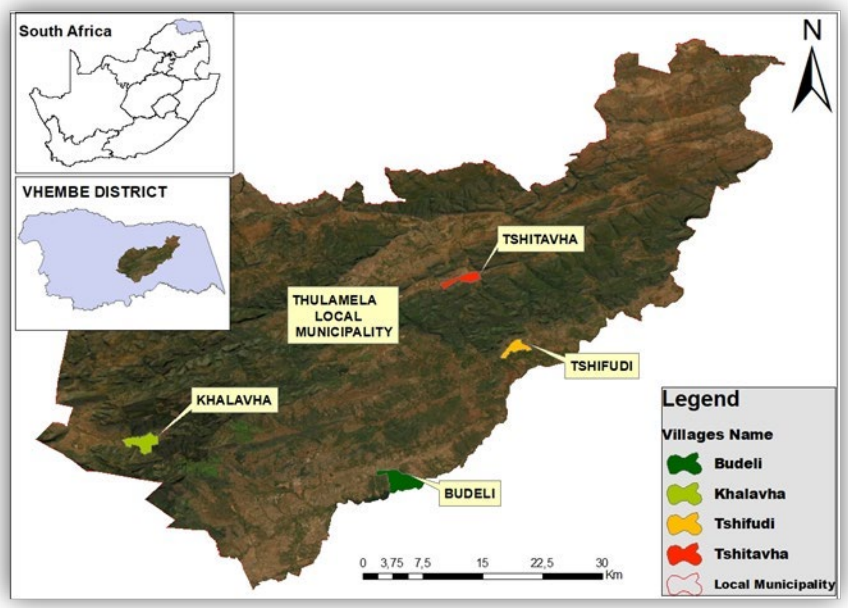

Figure 3.1.6 Picture showing villages that were visited during interview. (**Source**: Prepared by Ramagalela Takalani).

3.2. Ethical consideration

Before beginning the semi-structured interviews, the tribal leaders of the four chosen villages gave their verbal consent. Before completing interviews, formal written agreement was obtained from each participant in the study (Figure 3.2). The study's objectives were clearly explained to the respondents. The consent form said that the respondents' consented to the taking and use of pictures, digital recordings, and personal information.

Prior to the start of the interviews, respondents had the opportunity to clarify their understanding of the study's objectives by asking questions. As a result, in those circumstances where respondents declined to provide writing assent, verbal consent was gained. In certain cases, respondents refused to provide written consent because they were concerned that their signatures may be used for illegal activity.

Figure 3.2. Picture showing respondents signing consent forms. (**Source**: Own).

3.3. DATA COLLECTION METHODS

3.3.1 Methodology approaches of the study objectives

The findings of this research are attained by following different methodological approaches to entertain the three different objectives.

3.3.1.1 Selection of popular wild edible fruit plants of the Thulamela Municipality

To identify and document popular "wild edible fruit plant species of the study areas findings emerged from research" conducted by (Mokganya *et. al* 2017; Magwede; 2019 & Rampedi, 2013) were considered. "The identification of wild edible fruit plants was selected from these three papers based on their relative frequency of citation and use values of wild edible fruit plants."

3.3.1.2 Study of indigenous conservation method of wild edible fruit plants

To study the indigenous conservation methods for the identified popular wild edible fruit plants, knowledgeable people were interviewed using semi-structured questionnaires (Appendix A).

First, semi-structured interviews with the village chief were undertaken to identify informed locals who could take part in data gathering. "Respondents were sampled using the snowball method, in which further referrals of pertinent respondents were made until the needed sample size was reached" (Mashile, 2019). The initial respondents were identified by village leaders in this case. A total of 40 respondents participated in the study, with ten respondents coming from each of the four villages that were chosen (Figure 3.3.1.2).

"The administered questionnaire asked about knowledge of plant use, the socioeconomic characteristics, the conservation state of wild edible fruit plants, and other applications of these plants." It also asked about preservation techniques for future use.

Figure 3.3.1.2 Some of the respondents during administration of the questionnaire.

(**Source**: Own).

3.3.1.3 Collection of wild edible fruit plant for herbarium specimen and nutraceutical analyses

Special field trips were taken with some of the respondents of the four villages for voucher specimen collection. During this collection, "vernacular names of the identified popular wild edible fruit plants were registered." Voucher specimens were collected, mounted, and deposited to the University of Venda herbarium in the Department of Biological Sciences, Faculty of Science, Engineering and Agriculture. Field notes were recorded following methods applied by" Bhat *et al*., (1990). From all the inventoried wild edible fruit plants, species considered for nutraceutical analysis were selected by using the relative frequency of citation or quotation index (RFC) which is calculated by the following formula.

$$RFC = (FC/N) * 100,$$

Where FC is the number of informants who mentioned the wild edible fruit plants and N is the total number of informants" (Mokganya *et al*., 2018). A total of 13 species with RFC values ranging from 0.2-0.3 were selected and collected for further preparation prior to the nutraceutical analysis (Figure 3.3.1.3).

Figure 3.3.1.3: Collection of some of the selected wild fruits for nutraceutical analysis. (**Source**: Own).

3.3.1.3.1 Preparation of collected wild edible fruit plants for nutraceutical analysis.

Wild edible fruit plants were collected from mountains in different villages. Some of the selected species were collected in summer whereas some were collected in winter. Immediately, samples were stored in a cooler box and were then stored in a refrigerator, and in a cool dry place for further processing. Fruits were washed by running water to remove unwanted materials. The fruits were then peeled using a knife to separate seeds, pulp, and skin. Weights of fruit pulps were measured using the weighing balance and finally poured into separate vortex mixers. Fruit pulps were mixed with 20 ml of 80% of methanol in a vortex mixer for extraction. For maximum extraction, the homogenate was placed on the dragon shaker at room temperature (25^0c) for at least 24 hours. Following the extraction, the tissue debris was removed by centrifugation at 500 x g for 10 minutes. Prior to UHPLC-MS analyse, the pelleted extracted residues were re-constituted in 1m of 50% aqueous methanol and filtered through 0.22 µm nylon filters (Ramabulana *et.al*., 2021).

3.3.1.3.2 Nutraceutical analysis of selected wild edible fruit plant species -high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-qTOF-MS).

A UHPLC high-definition quadrupole time-of-flight MS instrument (UHPLC-qTOF SYNAPT G1 HDMS system, Waters Corporation, Manchester, UK) fitted with an Acquity HSS T3 C8 column (150 mm × 2.1 mm with a particle size of 1.7 µm) (Waters, Milford, MA, USA) were used to chromatographically analyse the extracts". "A sample volume of 3µL was injected and the column was housed in a column oven thermo-stated at 60^0C. A binary solvent system was used consisting of solvent A: 0.1% formic acid in Milli-Q water and solvent B: acetonitrile (UHPLC grade) with 0.1% formic acid". "A binary solvent gradient (with solvent A and B) with a flow rate of 0.4 mL/min was used to separate analytes over 30 min". "The separation conditions were: 2% B over 0.0-2.0 min, 2-60% B over 2.0-24 min, 60-95% B over 24–25 min, from 25-27 min the conditions were maintained at 95% B and the column was washed 95-2% B over 27–28 min. The column was then allowed to re-equilibrate 2% B over a 2 min isocratic wash (Ramabulana *et.al*., 2021).

The chromatographic effluent was further analysed utilising the SYNAPT G1 high-definition mass spectrometer operating in both positive and negative electrospray ionisation (ESI) modes.
"The MS conditions were set as follows: capillary voltage of 2.5 kV, sampling cone voltage of 30 V, extraction cone of 4.0 V, source temperature of 120°C, cone gas flow of 50.0 L/h, desolations gas flow of 550 L/h, m/z range of 100-1000, scan time of 0.2 s, interscan delay of

0.02 s, mode: centroid and lock mass: leucine-enkephalin (556.3 Da). "For downstream structural elucidation, the MS analyses were set to result in both unfragmented and fragmented analytes by ramping the collision energy from 15 to 60 eV in a series of fragmentation experiments" (MS^E)" (Madala *et al.,* 2016).

CHAPTER 4: RESULTS AND DISCUSSION

4. 1. Demographics

A random sample of four villages within Thulamela Local Municipality was taken, namely Tshitavha (Sambandou), Habudeli, Khalavha, and Tshifudi. Each village was visited, and twenty homesteads were surveyed. Most of the participants (n=48/80) were females, while the remaining (n=32/80) were males. Pitso and Lebese (2014) noted that "women were overrepresented in the sample as they are regarded by community leaders as the primary knowledge holders and guardians of the main community activities".

Table 4.1.1 Characteristics of informants(n=80)

Personal information	N	Percentage%
Gender		
Female	**45**	**56.25%**
Male	**35**	**43.75%**
Education level		
No education	**51**	**57.50%**
Primary education	**15**	**12.50%**
Secondary education	**21**	**20%**
Tertiary education	**13**	**10%**

4.1.2. Age group of respondents

The age distribution of "all participants interviewed were above 35 years old (Figure 4.1). Fifteen percent 15% of interviewees were between the ages of 35-45 years, 22% were between the ages of 46-55 years, 30% were between the ages of 56 and 65 years, and 33% were at the age of 66 years and above".

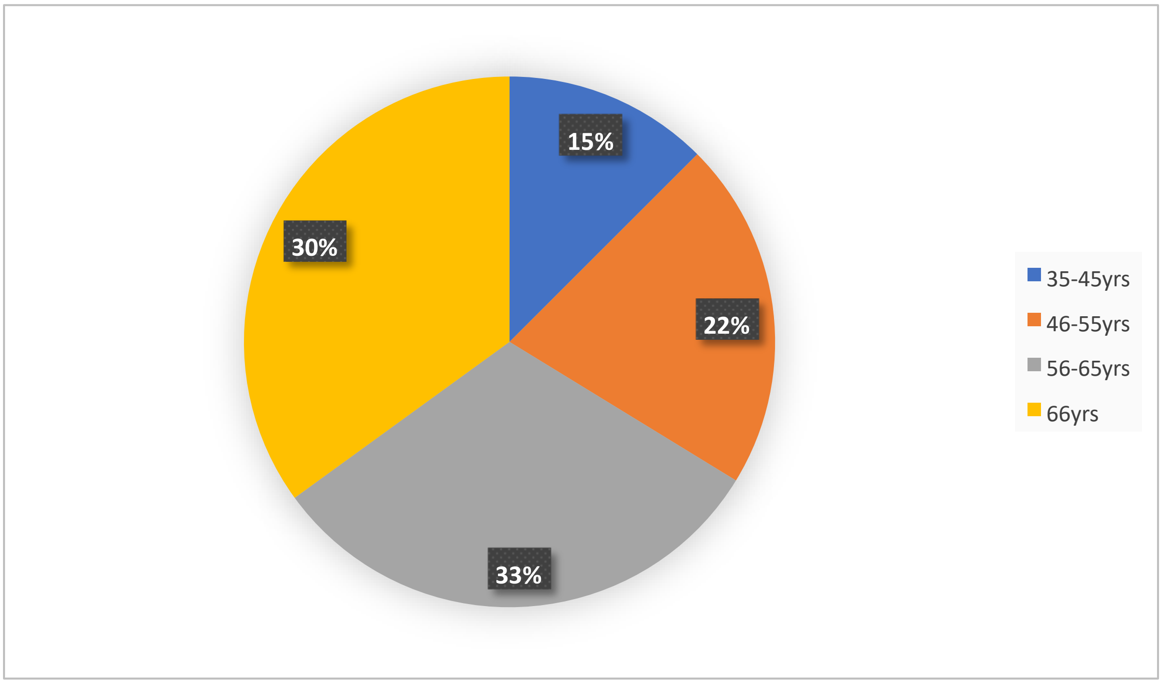

Figure 4.1: Age distribution of respondents per village.

4.1.3. Residence

All participants were staying in rural areas, Tshitavha Sambandou, Habudeli, Khalavha, and Tshifudi. Almost 70% of interviewees were Tshivenda speaking, and 30% were Xitsonga speaking.

4.1.4. Level of education

Forty-six percent 51% of respondents interviewed had no formal education, 15% percent had primary education, 21% had secondary education, and approximately 13% had tertiary education (Figure 4.2).

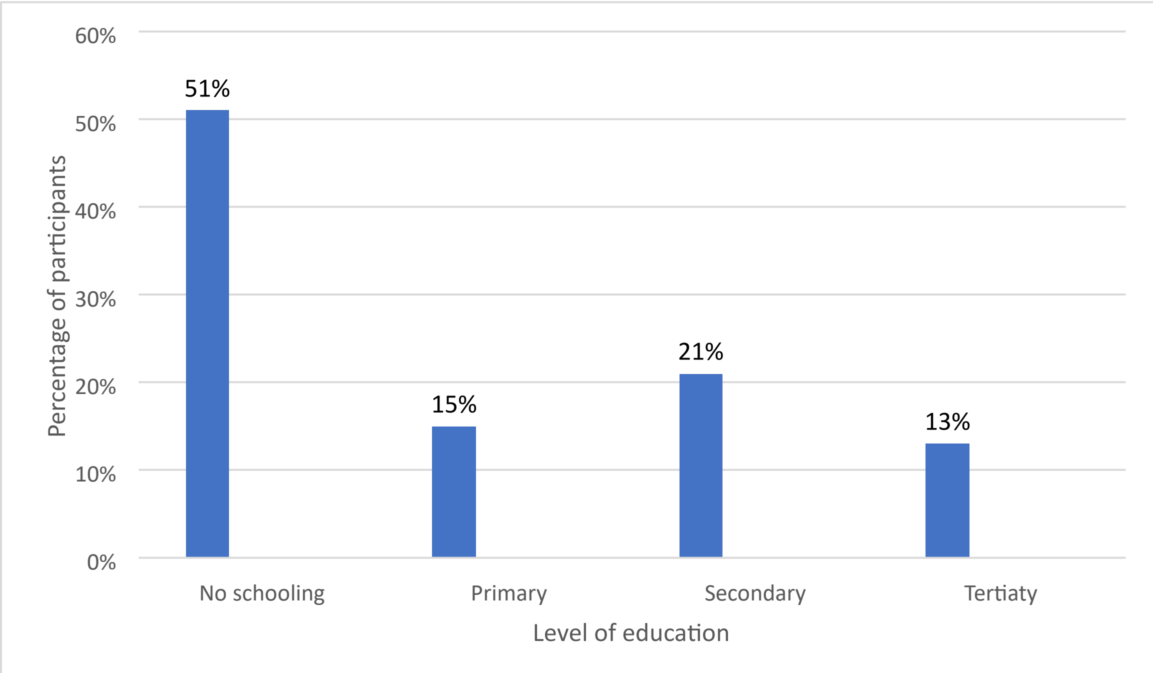

Figure 4.2 Level of education of respondents among the villages.

4.1.5. Taxonomic diversity

A total of thirty-nine wild edible fruits plant species, which belong to 22 families that are used as food, beverages and their respective medicinal purpose were recorded (Table 4.1.3). The species comprise different forms, such as trees, shrubs, climbers, bulbs, and herbs that are used to treat different ailments.

Table 4.1.2 List of wild edible fruit plant species used by participants.

Family	**Common names(V)= Venda (E)= English**	**Scientific name and Herbarium number**	**Relative Frequency of citation**	**Medicinal uses (Diseases)**	**Plant parts used**	**Methods of preparation**
Anacardiacae	Mufula (V) Marula (E)	*Sclerochyra birrea* (A. Rich) Hochst.subsp (LRN01)	0.17	Vaginal infection	Roots; bark	Decoction; burning
Anacardiaceae	Muthasiri (V) Roger's currant (E)	*Searsia rogersii* (Schonland) Moffett (LRN02)	0.17	Diarrhea (children)	Roots	Decoction
Annonaccae	Muembe (V) Wild custard apple (E)	*Anonna senengalensis* Pers. Subsp. (LRN03)	0.16	Treating bilharzia	Tuber	Decoction
Annonaceae	Muhuhuma (V)Baboons breakfast(E)	*Hexalobus monopetaus* (A. Rich.) Engl.) (LRN04)	0.18	For treating asthma	Leaves& fruits	Infusion
Annonaceae	Mudzidzi (V)Red hook berry(E)	*Artabotrys brachypetalus* Benth. (LRN05)	0.16	Infusion is taken orally for infertility in men.	Leaves& fruits	Infusion

Apocynaceae	Mavhungo(V) (E)Ruber vine	*Landolphia kirkii* Dyer ex Hook.f. (LRN06)	0.30	Infusion is taken to expel worms.	Roots	Decoction
Apocynaceae	Murungulu(V)Num – num(E)	*Carissa bispinosa* (L.) Desf. Ex Brenan (LRN07)	0.22	Stomach cleansing for newborn	Leaves	Infusion
Apocynaceae	Muthowa (V) Rhodesian rubber tree(E)	*Diplorhynchus condylocarpon* (Mull.Arg.) Pichon (LRN08)	0.10	Cleaning vigina after menstrution	Barks	Decoction (barks are dried & grinded to make powder)
Apocynaceae	Muhatu (V)Toad tree (E)	*Tabernaemontana Elegans* (Stapf.) (LRN09)	0.10	Treatment of painful menstruation	Leaves	Infusion
Cactaceae	Mudoro (V)weet prickly pear(E)	*Opuntia ficus-indica* (LRN10)	0.21	It applied on affected tooth	Leaves	Infusion
Chrysobalanaceae	Muvhula (V)Mobola plum(E)	*Ficus sycomourus* L. subsp. sycomorus (LRN11)	0.12	Used for stomach pain.	Bark's sap (phula in Tshivenda)	Liniments
Chrysobalanaceae	Muvhula (V) Mobola plum(E)	*Parinari curatellifolia* Planch. Ex Benth. (LRN12)	0.15	Blood puriffication	Roots	Decoction (barks are dried & grinded to make powder)
Cluciaceae	Mupimbi (African mangostee)	*Garcinia livingstonei* T. Anderson (LRN13)	0.25	Decoction is used as contraceptive	Roots	Chewing

Ebenaceae	Musuma (V)Jackal berry(E)	*Diospyros mespiliformis* Hochst. Ex A.DC (LRN14)	0.18	For treating headache(roots)	Roots and leaf	Infusion & Decoction
Ebenaceae	Muthaladzi (V) Blue bush (E)	*Mimusops zeyheri* Sond. (LRN15)	0.19	For constipation	Leaves	Decotion
Ebenaceae	muṱangulenyele(V) blue guarri(E)	*Euclea crispa* (Thunb) Gurke subsp. *crispa* (LRN16)	0.10	Flue	Leaves	Inhalation
Ebenaceae	Muthala (V)Transvaal blue bush(E)	*Diospyros lycioides* Desf (LRN17)	0.10	Applied to wounds to facilitate healing.	Leaves	Burning
Ebenaceae	Mutangule (V)Blue guarri(E)	*Euclea divinorum* Hiern (LRN18)	0.10	Treating snake venom	Bark and roots	Decoction
Euphorbiaceae	Munzere(V) Mitseeri (E)	*Bridelia micrantha* (Hochst.) Baill. (LRN19)	0.10	Stomach wounds	Bark and roots	Decoction
Fabaceae	Mutondo(V) Transvaal teak(E)	*Pterocarpas angolensis* DC. LRN20	0.28	Eye disoder	Roots	Soaking (Decoction)
Fabaceae	Munembenembe(V) Monkey pod(E)	*Senna petersiana* (Bolle) Lock LRN21	0.15	Decotion of roots used for mouthwash	roots	burning (mix with tshinyai)

Fabaceae	Mukolokote (V)Camel's foot(E)	*Pillostigma thonningii* Schumach.) MilneRedh. LRN22	0.10	Used for digestion.	Leaf and roots	Infusion & Decoction
Malvacaee	Muvhuyu (V)Boabab(E)	*Adansonia digitata* A L. LRN23	0.18	Mosquito repellent	Barks & Roots	Decoction
Malvaceae	Mupfuka(V)Sand raisin(E)	*Grewia microthyrsa* K. Schum.ex Burret LRN24	0.11	Diabetes	Leaves	Infunsion
Meliaceae	Mutshikili(V) Forest mahogany(E)	*Trichilia dregeana* Sond LRN25	0.11	Diabetes. Cancer, fever	barks	Decoction
Moraccae	Muhuyu (V)Wild fig(E)	*Ficus sycomorus* L. subsp. *sycomorus* LRN26	0.25	Diarrhoea treatment	Roots	Decoction
Moraceae	Muumo(V) Common wild fig(E)	*Ficus ingens* (Miq) Miq. LRN27	0.10	Treating snake venom	Leaves	Infusions
Moraceae	Mutamvu(V) Knobbly fig(E)	*Ficus Sansibarica* Warb. LRN28	0.10	Applied as a lotion to get rid of body rash.	Leaves	Infusions
Myrtaceae	Mutu (V)Waterberr(E)	*Syzygium cordatum* Hochst.ex.C. Krauss LRN29	0.23	Decoction is taken for body pains.	Leaves	Infusions

Myrtaceae	Thawi (V)	*Syzygium gerradi*i (Harv.ex Hook.f) LRN30	0.10	Infunsion for stomach pain.	Leaves	Infusions
Ochnaceae	Murombe (V)Wild pear(E)	*Ochna pulchra* Hook.f. LRN31	0.16	Blood puriffication	Leaves	Decotion boil or soak
Olacaceae	Mutanzwa (V) Sour plum(E)	*Ximenia caffra* Sond. LRN32	0.21	Womb & Kidney cleansing	Leaf, bark, and roots	Decoction, Boil, or soak
Proteaceae	Mutebvu (V)macadamia nut(E)	*Macadamia integrfolia* LRN33	0.18	Intestinal worms	Roots	Decotion
Rhamnaceae	Munii (V) Birdplum(E)	*Berchemia zeyheri* (Sond) Grubov LRN34	0.12	Wounds, Stomachache, diarrhea,	Roots	Decoction
Rubiaceae	Muzwilu (V)wild meldar(E)	*Vangueria infausta* Burch. Subsp. *infausta* LRN35	0.26	For treating diarrhea (Barks)	Barks	Decoction
Rubiaceae	Muthombothi (V)	*Canthium mundianum* Cham & Schltdl. LRN36	0.03	Diarrhoea treatment	Barks	Decoction

Sapotaceae	Munombelo (V)Transvaal milkplum(E)	*Englerophytum magalismontanum* (Sond) T.D.Penn. LRN37	0.26	For treating wound	Roots	Decoction
Strychnaceae	Muramba (V)Spiny monkey-orange(E)	*Strychnos spinosa* Lam. LRN38	0.23	To treat cancer	Barks (Latex)	Decoction
Strychnaceae	Mukwakwa (V)Black monkey orange(E)	*Strychnos pungens* sole*r* LRN39	0.29	Treating Headache	Bark and root	Decoction

Table 4.1.3 List of wild edible fruits plants species with additional uses

Family	**Common names**(V)= Venda (E)= English	**Scientific name**	**Relative Frequency of citation**	**Additional uses**
Anarcadiaceae	Mufula (V) Marula(E)	*Sclerochyra birrea* (A. Rich) Hochst.subsp	0.17	For making beverages, Jam, and cooking soda
Anarcadiaceae	Muthasiri (V) Roger's curran t (E)	*Searsia rogersii* Schonland) Moffett	0.17	Used to make energy drink
Anonnaceae	Muembe (V) Wild custard apple (E)	*Anonna senengalensis* Pers. Subsp.	0.16	For making juice
Annonaceae	Muhuhuma (V) Baboon's breakfast(E)	*Hexalobus monopetaus* (A. Rich.) Engl.)	0.18	For firewood
Annonaceae	Mudzidzi (V)	*Artabotrys brachypetalus* Benth.	0.16	For making jam

	Red hook berry(E)			

Apocynaceae	Mavhungo(V) Ruber vine (E)	*Landolphia kirkii* Dyer ex Hook.f.	0.30	For firewood and roofing
Apocynaceae	Murungulu (V) Num – num (E)	*Carissa bispinosa* (Mull.Arg.) Pichon	0.22	For making juices
Apocynaceae	Muthowa (V) Rhodesian rubber tree (E)	*Diplorhynchus condylocarpon* (Stapf.)	0.10	For making firewood
Apocynaceae	Muhatu (V) Toad tree(E)	*Tabernaemontana Elegans* L. subsp. Sycomorus	0.10	For making soda
Cactaceae	Mudoro (V) Weet prickly pear(E)	*Opuntia ficus-indica*	0.18	For making beer and jam
Myrtaceae	Mugwavha (V) Yellow guava(E)	*Psidium guajava* Planch. Ex Benth.	0.12	For making juice, beer

Chrysobalanaceae	Muvhula(V) Mobola plum(E)	*Parinari curatellifolia* Planch. Ex Benth.	0.15	Making soft porridge and beer
Cluciaceae	Mupimbi (V) African mangosteen (E)	*Garcinia livingstonei* T. Anderson	0.21	For making juice
Ebenaceae	Musuma (V)		0.18	For firewood
	Jackal berry (E)	*Diospyros mespiliformis* Hochst. Ex A. DC		
Ebenaceae	Muthaladzi (V) Blue bush (E)	*Mimusops zeyheri* Sond.	0.19	For firewood
Ebenaceae	Mutangule (V) (Blue guarri) (E)	*Euclea divinorum* Hiern	0.1	For firewood
Ebenaceae	muṱangulenyele (V) Blue guarri(E)	*Euclea crispa* (Thunb) Gurke subsp. Crispa	0.10	Timber

Ebenaceae	Muthala (V) Transvaal blue bush (E)	*Diospyros lycioides* Desf	0.10	For construction
Euphorbiaceae	Munzere (V) Mitseeri(E)	*Bridelia micrantha* (Hochst.) Baill	0.10	Mosquito repellent
Fabaceae	Mutondo (V) Transvaal teak(E)	*Pterocarpas angolensis* DC.	0.28	Fence and ornamental
Fabaceae	Munembenembe (V) Monkey pod(E)	*Senna petersiana* (Bolle) Lock	0.15	Cosmetic oil and cordage
Fabaceae	Mukolokote (V) Camel's foot (E)	*Pillostigma thonningii* (Schumach) Milner edh	0.10	Used for digestion.
Malvacaee	Muvhuyu(V) Baobabs(E)	*Adansonia digitata* A L.	0.28	For making yogurt
Malvaceae	Mupfuka (V) sand raisin(E)	*Grewia microthyrsa* K. Schum.ex Burret	0.11	For firewood, porridge cooking

Meliaceae	Mutshikili (V) Forest mahogany(E)	*Trichilia dregeana* Sond.	0.11	For making milk
Moraccae	Muhuyu (V) Wild fig (E)	*Ficus sycomorus* L. subsp. Sycomorus	0.25	For firewood, Lotion
Moraceae	Muumo(V) Common wild fig (E)	*Ficus ingens* (Miq) Miq	0.10	Treating snake venom
Moraceae	Mutamvu(V) Knobbly fig(E)	*Ficus Sansibarica* Warb.	0.10	For making Juice
Myrtaceae	Mutu(V) Waterberry (E)	*Syzygium cordatum* Hochst.ex.C. Krauss	0.23	For making beverage
Myrtaceae	Thawi	*Syzygium gerradi*i (Harv.ex Hook.f)	0.10	For making juice
Ochnaceae	Murombe (V) Wild pear (E)	*Ochna pulchra* Sond. Hook.f.	0.16	For making juice
Olacaceae	Mutanzwa (V)	*Ximenia caffra* Sond.	0.21	For making Beverage

	Sour plum (E)			
Proteaceae	Mutebvu (V) Macadamia nut (E)	*Macadamia integrfolia*	0.18	For making butternuts
Rhamnaceae	Munii (V) Birdplum (E)	*Berchemia zeyheri* (Sond) Grubov	0.12	For firewood
Rubiaceae	Muzwilu (V) wild meldar(E)	*Vangueria infausta* Burch. Subsp. Infausta	0.26	For firewood
Rubiaceae	Muthombothi	*Canthium mundianum* Cham & Schltdl	0.03	For making beverage
Sapotaceae	Munombelo (V) Transvaal milkplum(E)	*Englerophytum magalismontanum* (Sond) T.D.Penn.	0.26	For making beverage
Strychnaceae	Muramba (V) Spiny monkey-orange (E)	*Strychnos spinosa* Lam.	0.23	For making beverage, Firewood

Strychnaceae	Mukwakwa (V) Black monkey orange(E)	*Strychnos pungens* Soler	0.29	For making juice, beer, and artifact

4.2 Sources and season of availability of wild edible fruit plants

4.2.1. Sources of wild edible fruit plants

During the study, it was found that all the respondents who were interviewed gather wild edible fruits from communal land. However, they also purchase some of the fruits from street vendors. "The participants mentioned that there are several limitations to the number of fruits they can harvest, including competition with wildlife" (Rampedi & Olivier, 2013). The wild fruits are also consumed by birds and monkeys before humans can harvest them, resulting in damage to the fruits. To overcome these limitations, "some fruits like *Landolphia kirkki* and *Strychnos spinosa* are collected before they are fully ripe, buried in the sand to hasten the ripening process, and then consumed."

4.2.2. The seasonal availability of plant species

"The seasonal availability of fruit species presented in Table 4.1 was determined through information gathered from respondents and confirmed by existing literature, as shown in Table 4.1.4." (Van Wyk, 1997; Palgrave, 2002; Van Wyk & Gericke, 2003). "The spring season months (August, September, and October) are usually dry, but this is also the period during the year when atmospheric temperature begins to increase." "The spring period is characterised by the development of plant leaves as well as flowers." In addition, "it is the time when certain fruits begin to ripen and thus marks the beginning of the harvesting period. About 75% of the “fruit species depicted in Table 4.2 begin to ripen in spring and can be harvested during August October period”. “When the rainfall period intensifies during the summer months (November, December, and January), it heralds the ripening of more than 70% of the fruit types listed in Table 4.1.4”. The proportion of fruits that ripen during the autumn months (February, March, and April) is extremely low, comprising only 13% of the plants in Table 4.14.

Table 4.1.4. Wild edible fruit plants and their season of availability in Thulamela Limpopo

Table 4.1.4. Wild edible fruit plants and their season of availability in Thulamela

Local name Venda	Scientific name	summer	Spring	Autum	Winter
Mufula (Marula)	*Sclerochyra birrea*	■			
Muthasiri (Roger's currant)	*Searsia rogersii*		■		
Muembe (Wild custard apple	*Anonna senengalensis*	■	■		
Muhuhuma (Baboons breakfast)	*Hexalobus monopetaus*	■	■		
Mudzidzi (Red hook berry)	*Artabotrys brachypetalus*	■			
Mavhungo (Ruber vine)	*Landolphia kirkii*	■			
Murungulu (Num - num)	*Carissa bispinosa*	■			
Muthowa (Rhodesian rubber tree)	*Diplorhynchus condylocarpon*			■	
Muhatu (Toad tree)	*Tabernaemontana Elegans*	■	■		
Mudoro (weet prickly pear)	*Opuntia ficus-indica*	■	■		
Muvhula (Mobola plum)	*Ficus sycomurus*	■			
Muvhula (Mobola plum)	*Parinari curatellifolia*		■		
Mupimbi (African mangosteen)	*Garcinia livingstonei*	■			
Musuma (Jackal berry)	*Diospyros mespiliformis*	■	■		
Muthaladzi (Blue bush)	*Mimusops zeyheri*	■			
muṱangulenyele (blue guarri)	*Euclea crispa*	■	■		
Muthala (Transvaal blue bush)	*Diospyros lycioides*		■		
Mutangule (Blue guarri)	*Euclea divinorum*				■
Munzere (Mitseeri)	*Bridelia micrantha*	■			
Mutondo (Transvaal teak)	*Pterocarpas angolensis*	■			
Munembenembe (Monkey pod)	*Senna petersiana*	■			

Mukolokote (Camel's foot)	*Pillostigma thonningii*				
Muvhuyu (Boabab)	*Adansonia digitata*				
Mupfuka (sand raisin)	*Grewia microthyrsa*				
Mutshikili (Forest mahogany)	*Trichilia dregeana*				
Muhuyu (Wild fig)	*Ficus sycomorus*				
Muumo (Common wild fig)	*Ficus ingens*				
Mutamvu (Knobbly fig)	*Ficus Sansibarica Warb.*				
Mutu (Waterberry)	*Syzygium cordatum*				
Thawi	*Syzygium gerradii*				
Murombe (Wild pear)	*Ochna pulchra*				
Mutanzwa (Sour plum)	*Ximenia caffra*				
Mutebvu (macadamia nut)	*Macadamia integrfolia*				
Munii (Birdplum)	*Berchemia zeyheri*				
Muzwilu (wild meldar)	*Vangueria infausta*				
Muthombothi	*Canthium mundianum*				
Munombelo (Transvaal milkplum)	*Englephytum magalismontanum*				
Muramba (Spiny monkey-orange)	*Strychnos spinosa*				
Mukwakwa (Black monkey orange	*Strychnos pungenssoler*				

4.3. UHPLC-QTOF-MS identification and characterisation of the bioactive metabolites

Table 4.1.5 displays the identification and characterisation of bioactive metabolites in various fruit species, including “*Syzygium cordatum*, *Pterocarpus angolensis*, *Ficus sycomorus*, *Strychnos spinosa*, *Landolphia kirkii*, *Carissa bispinosa*, *Ximenia caffra*, *Garcinia Livingstonei*, *Englerophytum magalismontanum*, *Opuritia ficus* and *Strychnos pungenssoler* using UHPLC-QTOF-MS.” These metabolites consist of hydroxycinnamic acids conjugated to different molecules, such as quercetin and sugar moieties. For example, chlorogenic acid is a conjugation of hydroxycinnamic acids with quinic acids, such as trans-5-caffeolylquinic acid and 3,4-caffeolylquinic acid. Additionally, flavonoids were identified as free molecules or conjugated with various sugar moieties, such as Kaempferol-3-O-glucoside, quercetin-3rhamosylhexose, and kaempferol rutinoside. “The phenolic metabolites identified in *Ficus sycomurus* and *Strychnos spinosa* contained higher levels of 3-Caffeoylquinic acid (3-CQA), 5-Caffeoylquinic acid (5-CQA), and 3,4-di-Caffeolyquinic acid” (Nkosi *et.al*, 2022).

According to (*Nkosi et.al,* 2022), “*Ximenia caffra* and *Landolphia kirkii* contained the highest levels of glucogallin”. “Flavonoids are important defensive compound which protect fruit against infestation by pathogens, acting either as pytoanticipins or phytoalexin” (Nkosi *et.al*, 2022). “Flavonoids such as Quercetin-3-O-rutinoside play a vital role in response to stress” (Sefater *et.al.,*2016). Rutin for an example, is a “bio flavonoid with strong antioxidant activity which has antimicrobial effect on pathogens, as evidenced by reduced conidial germination appressorium formation of pathogen” (Satari *et al*., 2021) Quercetin protected cells from oxidative injury to the cells during H_2O_2 treatment. Also, fruit extract rich in quercetin have been applied as an internsity recourse four functional food products and other food used to reduce the risk of age-related mascular degeneration. Amongst the identified flavonoids most were found to contain either quercetin or kaempferol aglycone moieties. "Phenolic compound with a catechol structure has been shown to possess high antioxidant activities" (Ramabulana *et al*., 2016). Thus, as "quercetin has a catechol structure on its B ring, this molecule is more potent and moreover, quercetin glycoside acylated with hydroxycinnamic acid has been shown to be overly sensitive against UV-B processing and has been explained by the presence of the catechol found in both molecules" (Ramabulana *et al*., 2016).

Table 4.1.5 Chlorogenic acid and flavonoids identified in twelve wild edible fruits using UHPLC-qTOF-MS.

No	Rt (min)	Mass (*m/z*)	Fragment ions	Molecular formular	Metabolite identity
1	13.9	353.08	191,179,135	$C_{16}H_{18}O_9$	3-Caffeoylquinic acid
2	15.0	353.08	173,135,179	$C_{16}H_{18}O_9$	4-Caffeoylquinic acid
3	16.2	353.08	191	$C_{16}H_{18}O_9$	5-Caffeoylquinic acid
4	30.0	515.14	353,179,191,173	$C_{25}H_{24}O_9$	3,4-di-Caffeolyquinic acid
5	35.1	515.16	353,191,179,135	$C_{25}H_{24}O_9$	3,5-di-Caffeoylquinic acid
6	35.9	515.17	353,191,179,173,	$C_{25}H_{24}O_9$	4,5-di-Caffeoylquinic acid
7	23.0	593.12	284,119	$C_{27}H_{30}O_{15}$	Kaempferol-3-*o*-rutinoside

8	3.3	315.09	271	$C_{16}H_{12}O_7$	Isorhamnetin
9	15.1	449.07	316,300,271	$C_{20}H_{18}O_{12}$	Kaempferol-3-O-glucioside
10	21.5	609.15	300	$C_{27}H_{30}O_{16}$	Quercetin-3- rhamonosylhexose
11	3.0	205.03	115	$C_7H_{10}O_7$	Methyl citric acid
12	9.9	461.06	285	$C_{21}H_{18}O_{12}$	kaempferol-3-O-glucuronide
13	19.8	447.09	285,175	$C_{27}H_{30}O_{15}$	Kaempferol-3-O-glucoside
14	30.0	447.08	285	$C_{27}H_{30}O_{15}$	Kaempferol-3-O-glucoside 1
15	35.0	447.09	285	$C_{27}H_{30}O_{15}$	Kaempferol-3-O-glucoside 2

16	1.2	191.01	155,111	$C_6H_8O_7$	Citric acid
17	11.7	337.09	191,163	$C_{16}H_{18}O_8$	3-O-p Coumaroylquinic acid
18	32.0	463.03	300	$C_{21}H_{20}O_{12}$	Quercetin-3-glycoside
19	30.0	565.02	303	$C_{27}H_{29}O_{16}$	Kaempferol-3-rhamnoside
20	5.9	331.06	211,169	$C_{13}H_{16}O_{10}$	Glucogallin
21	2.0	289.07	221,205	$C_{15}H_{14}O_6$	Catechin
22	18.5	433.07	301	$C_{20}H_{18}O_{11}$	Quercetin-3- O-pyranoside
23	11.1	577.12	407,289	$C_{30}H_{26}O_{12}$	Procyanidin dimer B1

24	11.6	577.133	289,407	$C_{30}H_{26}O_{12}$	Procyanidin B

4.3.1. Classification of *Ficus sycomurus* and *Strychnos spinosa* based on their chlorogenic acid.

Analyses of the crude aqueous methanol extracts prepared from the fruits of the "*Ficus Sycomorus* and *Strychnos spinosa* were conducted using an UHPLC-qTOF-MS operated in negative electrospray ionization ESI mode because most of the metabolites of interest in this study has been shown to ionize better in the negative mode" (Clifford *et al.,* 2003). "Visual inspection of these base peak intensity (BPI) chromatogram shows clear differences in peak intensities and presence/absence of some peaks" (Clifford *et al.,* 2003).

4.3.2. Characterisation of caffeoylquinic acids

Three peaks were identified with a precursor ion [M-H]- at m/z 353 and labelled as isomers of caffeoylquinic acids in Table 4.1.5 and figure 4.3.1. The metabolites were identified as 3Caffeoylquinic acid (trans-3-CQA), 4-Caffeoylquinic acid (trans-4-CQA), and 5Caffeoylquinic acid (trans-5-CQA), based on their elution order and fragmentation patterns. Additionally, a peak with product ions at m/z 191 and m/z 179 at 50% of the base peak was also identified as 3-CQA (Negovhela *et.al*, 2021). "A molecule with a product ion at m/z 179 and m/z 135 was identified as 4-CQA due to the presence of product ion m/z 173 dehydration product of quinic acid, which is only possible if acylation is on the 4-OH of the quinic acid" (Negovhela *et.al*, 2021).

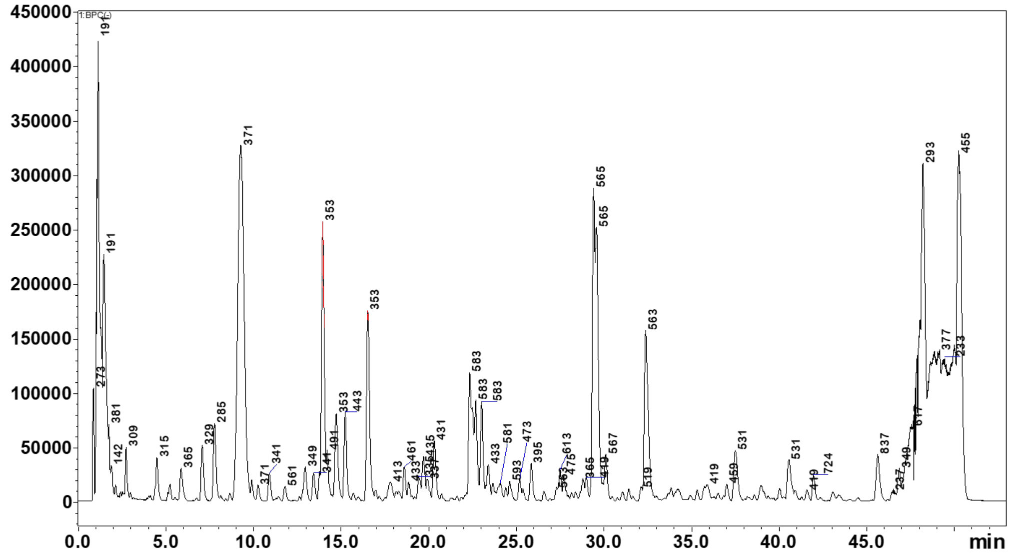

Figure 4.3.1. Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Ficus sycomurus.*

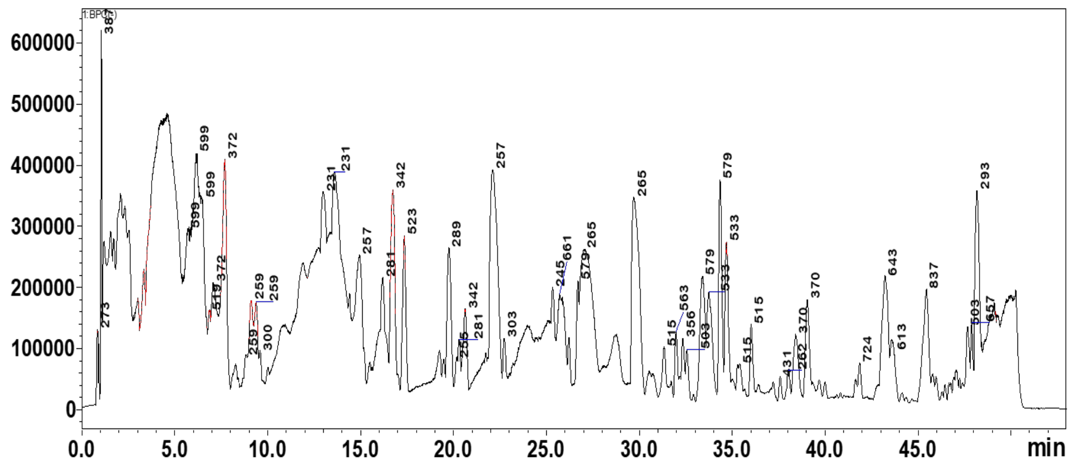

Figure 4.3.2 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Strychnos spinosa.*

4.3.3. Characteristic of di-caffeoylquinic acid (di-CQA)

Di-caffeoylquinic acid are known for having a molar mass (Mr) of 516. In this case, "three molecules were identified (Table 4.1.5) having a precursor ion $(M\text{-}H)^-$ at *m/z* 515" (Negovhela *et.al,* 2021). "Using the same approach as the one used for characterizing mono-acyl chlorogenic acid, the three molecules were identified as di-CQAs with acylation on position four (4) which suggest they could be geometrical isomers of either 3.4-diCQA or 4,5-diCQA. Both compounds showed MS2 base peaks at *m/z* 353, indicating loss of one caffeoyl residue, and the presence of secondary ions at *m/z* 173 and at *m/z* 191 in MS2 was enough to account for 4-acyl" (Negovhela *et.al,* 2021).

Chlorogenic acids (CGAs) are "the family of ester phytochemicals formed between cinnamic acid derivatives and quinic acids'' (Clifford *et al*., 2003). "These compounds are present in all plants and contribute a significant fraction of the total dietary intake of phenols in the daily human diet." Moreover, "they possess some notable bio-medical or pharmacological properties" Nobela *et.al*., 2021). CGAs are phenolic compounds produced through the shikimate- and phenylpropanoid pathways and have been identified in responses against both biotic and abiotic stressors (Ncube *et al.,*2014). "The most common naturally occurring cinnamic acid derivatives that have been reported to be utilised during the biosynthesis of these molecules is caffeic acid" (Ncube *et al.,*2014).

4.3.4. Characterisation of flavonoids

A molecule at retention time 9.9 min with precursor ion *m/z* 461.06 $[M-H]^-$ and MS2 fragment at *m/z* 285.03 obtained after loss of 176 amu (glucurone unit) was tentatively identified as kaempferol-3-O-glucuronide (Sefater *et.al.,*2016).

A molecule at retention time (Rt) 19.8 min was tentatively identified as kaempferol-3-Oglucoside (Figure 4.3.5) at *m/z* 447.0927 $[M-H]^-$ and fragment at *m/z* 285.03 obtained due to loss of a hexose moiety (162 amu) (Sefater *et.al.,*2016). "Same molecule at retention time of 30.0 and 35.0 min, respectively, also showed similar fragmentation patterns and as such, these three molecules were identified as geometrical isomers of caftaric acid hexose" (Sefater *et.al.,*2016).

A molecule at retention time at 21.5 min, respectively were annotated as quercetin-3-rhamonosylhexose (Rutin) (Figure 4.3.5) with a precursor ion at *m/z* 609.15 $[M-H]^-$ and a product ion at m/z 300.02 (Table 4.15), respectively, a quercetin aglycone following the loss of rutin. "Flavonoids are biologically active compounds that exist as either free aglycone or glycosides attached to various forms of sugar molecules. The glycosylation of these flavonoids can either be through O-glycosides or C-glycosides." Interestingly, "the attachment of these sugar molecules has been shown to have biological effect" (Nengovhela *et al.* 2020). However, "most phytochemical data have shown flavonoids glycosylation to be a complex biological phenomenon which is also prone to further chemical modification such as isomerization, acetylation, and acylation, thereby complicating the already complex metabolome." Elsewhere, attachment (through acylation) of other biologically active molecules such as cinnamic acids are responsible for the production of isomeric compounds" (Nengovhela *et al.* 2020).

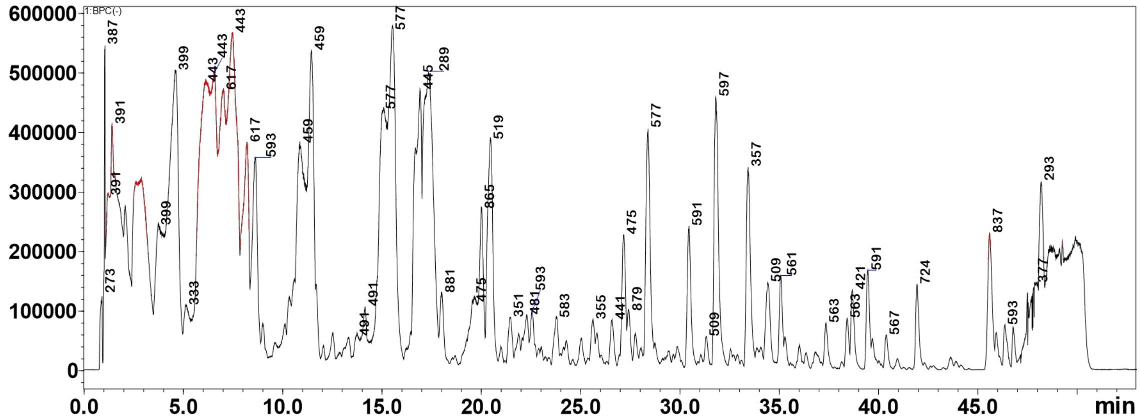

Figure 4.3.4 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Landolphia kirkii*

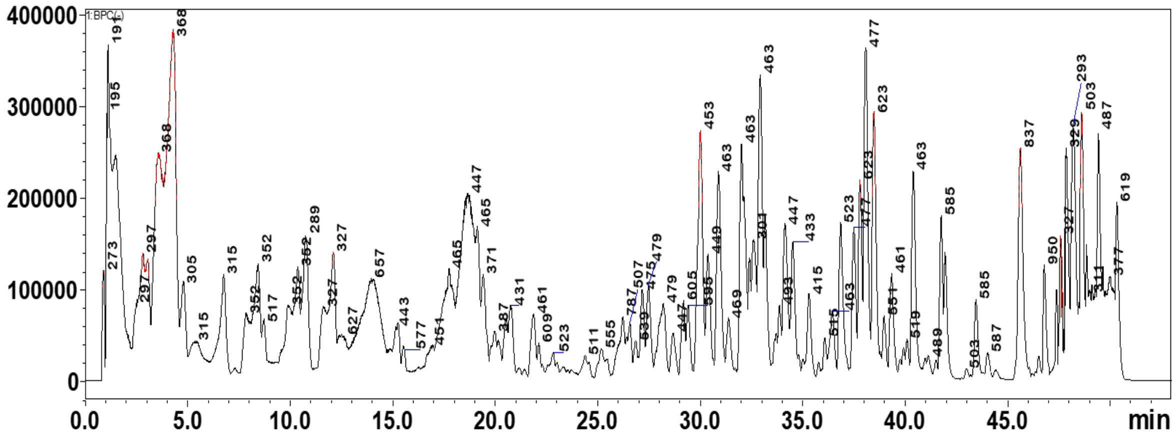

Figure 4.3.5 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Carissa bispinosa*.

4.3.6 Characterisation of organic acid

A molecule at retention time 3.05 min with a precursor ion $[M-H]^-$ at *m/z* 205.03 were detected in *Adansonia digitata* chromatograph (figure 4.3.6). And was annotated as methyl citric acid (table 4.1.5) these molecules were identified by (Barky *et.al*.,2021). Several studies have affirmed "the potential of dietary flavonoids in diabetes management through improving glucose metabolism and the lipids profile, thus protecting against diabetes and its aggravation" (Barky *et.al.,*2021).

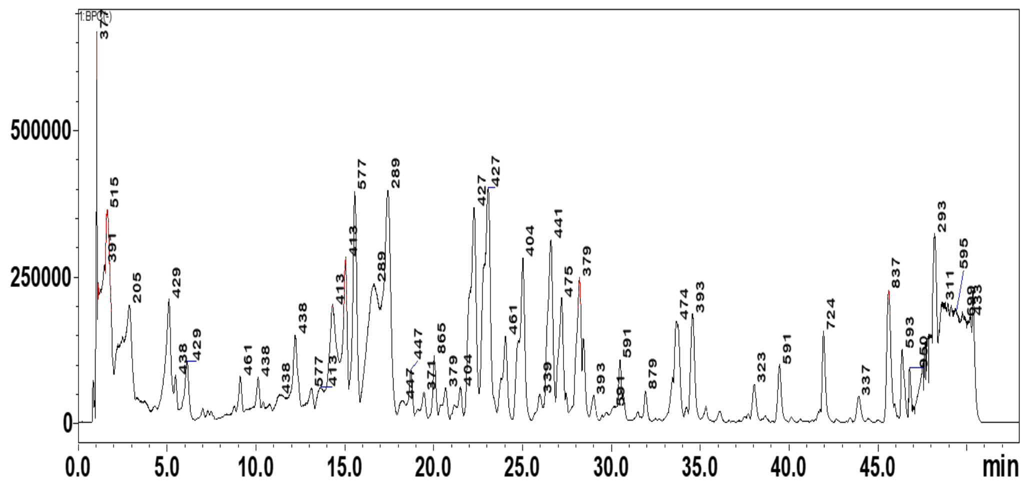

Figure 4.3.6 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Adansonia digitata*.

4.3.7 Classification of *Syzygium cordatum* and *Pterocarpus angolensis* based on their flavonoids and chlorogenic acid.

The flavonoid metabolites were characterised by analysing their MS fragmentation pattern and elution order from the UHPLC chromatogram. To confirm their identities, the KNAPSACK database and other literature reports were consulted" (Sefater *et.al.*,2016). Kaempferol has been reported as great significance in managing cancer-associated ailments as well as inhibiting oxidative stress (Wang *et.al.,* 2020). A molecule at retention time 39.5 min with a precursor ion at m/z 593.14 $[M-H]^-$ (figure 4.3.7) were identified as Kaempferol-3-o-rutinoside (Table 4.1.5) which are flavonoids" (Mabasa *et.al.,*2021).

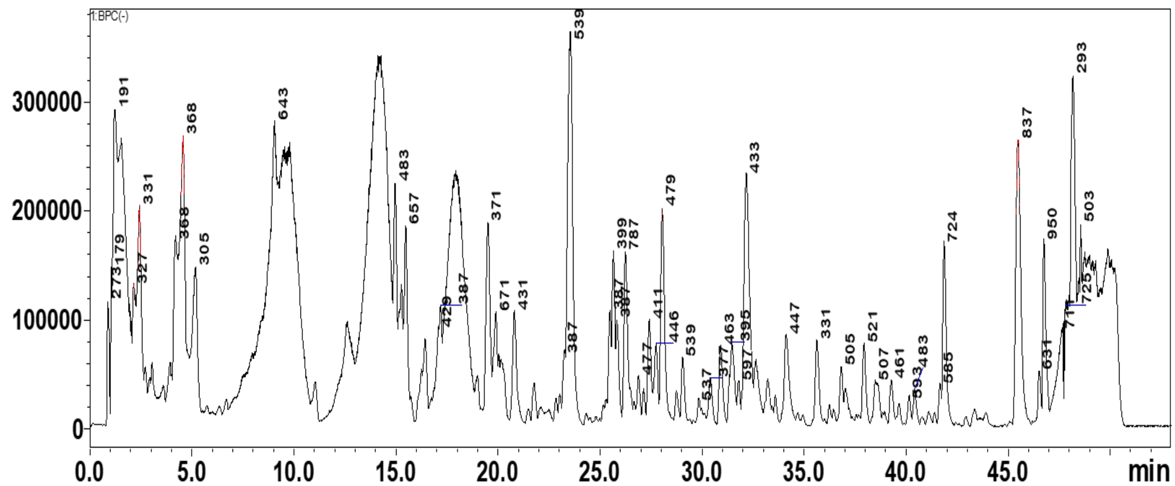

Figure 4.3.7 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Syzygium cordatum.*

A molecule at retention time 19.8 min was tentatively identified as kaempferol-3-O-glucoside with precursor ion at *m/z* 447.09 [M-H]$^-$ and fragments ion at *m/z* 285.03 obtained due to loss of a hexose moiety (162 amu) (Sefater *et.al.,*2016). “Molecules at retention time 14.59 min and 15.1 min respectively were annotated as caffeoylquinic acid (Table 4.1.5) by the loss of a Caffeolyl residue with a precursor ion of m/z 353. 09 (M-H)$^-$ and a product ion at *m/z* 191 figure 4.3.8” (Ncube *et al*., 2014).

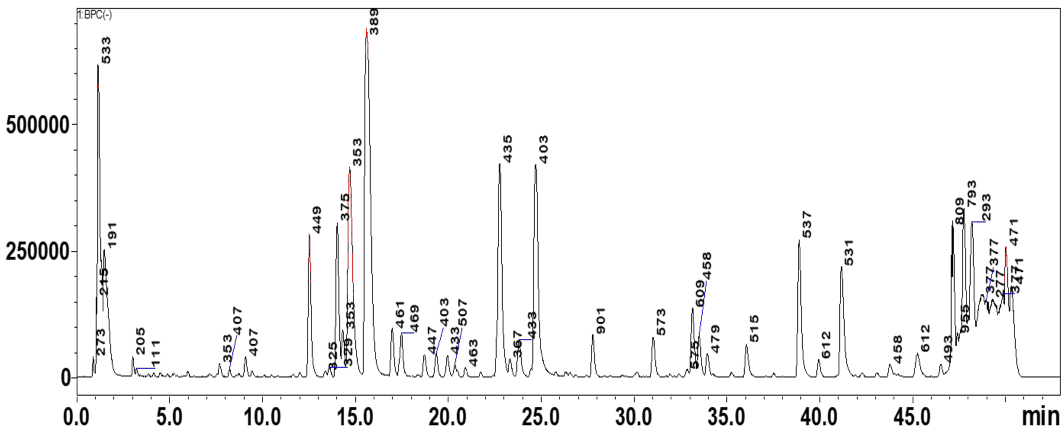

Figure 4.3.8 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Pterocarpus angolensis.*

4.3.9 Classification *of Ximenia caffra* and *Garcinia Livingstonei.*

Molecule at retention time 1.0 min with a precursor ion of [M-H] at m/z 191 were detected in

Ximenia caffra chromatogram (figure 4.3.9) these molecules were identified by (Ramabulana *et.al* 2020) as citric acid (Table 4.1.5). A “molecule with at retention time at 32.1 with a precursor ion of [M-H]- at m/z 577 with product ions at m/z 269 and 169 were detected in

Garcinia Livingstonei chromatogram (figure 4.3.10)". These molecules were identified by (Nobela *et.al*., 2021) asapigenin rutinoside.

From our findings of the twelve samples, there are fruits with small seed that has more metabolites, than those with small seed such as *Carissa bispinosa* that has more metabolites, than those with bigger seeds such as *Ximenia caffra* they have less metabolites. Now referring to the chromatogram, from figure 4.3.1 to figure 4.3.8 we can see that fruits with smaller seeds produces the best metabolites on the chromatogram peak. Referred from the below chromatogram, (figure 4.3.9 and figure 4.3.10) depicts fruits that contain big seeds have less metabolite, the chromatograph starts as a straight and gradually builds and reaches a peak.

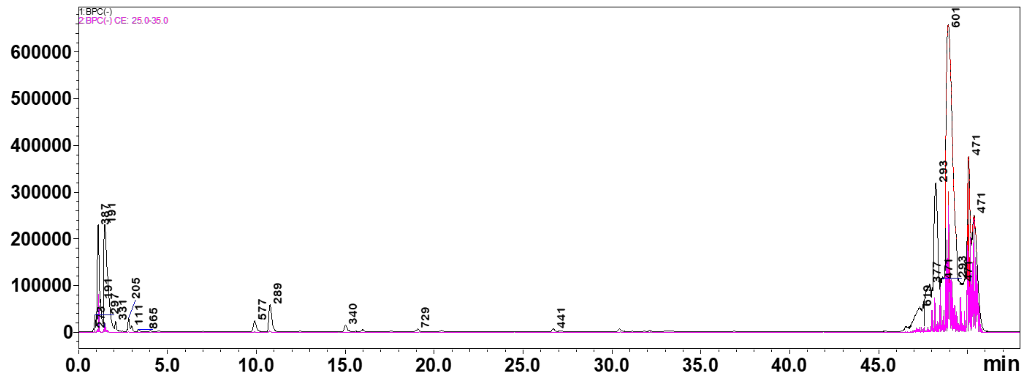

Figure 4.3.9 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Ximenia caffra*.

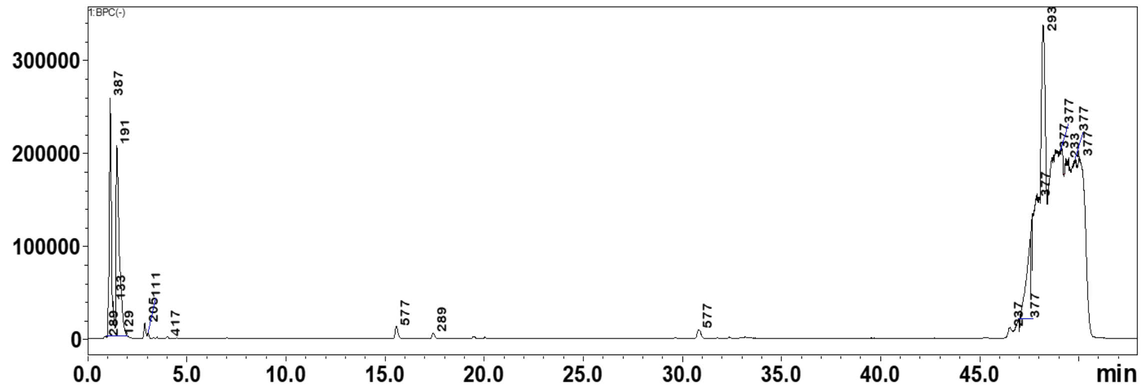

Figure 4.3.10 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Garcinia Livingstone.*

4.4. Molecular networking

Molecular network (MN) is a computational approach tool which classifies metabolites based on their structural similarities, thus molecules with similar structural moieties are grouped together to form a molecular family. During MN, tandem mass spectrometry (MS/MS) data comprising of both unfragmented and collision induced dissociation (CID) data is used to create a network of metabolites, whereby "metabolites with similar fragmentation patterns (especially due to common structural features) are connected to each other to form a molecular family" (Ramphinwa *et al.,* 2022).

4.4.1 Major Chemical Classes of *Syzygium cordatum*, *Garcinia livingstonei, Ximenia caffra* and *Englerophytum magalismontanum*.

Classical molecular networking was firstly applied in *Syzygium cordatum, Garcinia livingstonei, Ximenia caffra* and *Englerophytum magalismontanum.* The MS-Cluster algorithm was used to evaluate sample spectra similarities, grouping of ions within a predefined mass tolerance into consensus spectra, which are represented as nodes. Structurally related metabolites that share similar gas phase chemistries were grouped into molecular families based on their similarity scores. In the computed MN,1493 consensus spectra (nodes) were generated, with 1022 clustered into 165 independents spectral matching. Spectra not clustered into molecular families were represented as self-loop nodes at the bottom of the network. In the computed MN (Figure 4.4.1), fifty-seven of the nodes were putatively annotated through an automated library spectral matching, providing some insight into the chemical identities of the species but also alluding to the metabolome complexity of these plants and the lack of comprehensive spectral libraries. This analysis of chemical relationships between every MS/MS spectrum, visualizing the entire metabolome detected in a sample, revealed structurally related molecular families in *Syzygium cordaturm*, *Garcinia livingstonei, Ximenia caffra* and *Englerophytum magalismontanum***.**

Lipids such as glycerophospholipids for example were identified in *Syzygium cordatum*, *Garcinia livingstonei, Ximenia caffra* and *Englerophytum magalismontanum.*

"Glycerophospholipids are lipids with both phosphoric acid and fatty acid attachment and are classified as heterolipids" (Ramabulana *et.al.,* 2021). "These make up a vital component of the cell membrane and vital function of cellular physiology" (Ramabulana *et.al.,* 2021).

Other metabolites that are widely distributed in *Syzygium cordatum*, *Garcinia livingstonei*, *Ximenia caffra* and *Englerophytum magalismontanum* four species are flavonoids which cluster into a molecular family (Figure 4.4.1). Flavonoids are widely distributed in fruits and vegetables and are known exhibit health promoting effect such as antioxidant (Wang *et.al.,* 2020), cancer (Ramabulana *et.al.,* 2021) and anti-bacterial activities (Xie *et al*., 2015). These are used by "plants for growth and among other protection against oxidative stress and ultraviolet protection" (Falcone *et al*., 2012). "Flavonoids have structurally diverse aglycones as backbone namely chalcones, flavones, isoflavones and flavanols" (Safe *et al*., 2021). "Flavanone and anthocyanidin occur in various modified form through hydroxylation methylation and glycosylation by transferases. In figure 4.4.1 the flavonoids identified are isorhamnetin and Luteolin -O glucopyranoside aglycones that fall into four subclass which mostly were flavanols" (Ramabulana *et.al.,* 2021). In figure 4.4.1 the tannins identified are "Benzoic acid are phenolic compounds that interfere with iron absorption through a complex formation with iron when it is in the gastro-intestinal lumen which decreases the bioavailability of iron" (Safe *et al*., 2021).

The relationship of phenolic compounds with various hydroxylation systems varies significantly. The impact on iron absorption of several compounds (gallic acid, catechin, chlorogenic acid). "The main factor affecting whether phenolic compounds have an inhibitory effect on iron metabolism may be the presence of galloyl groups that bind iron" (Ramabulana *et.al.,*2021).

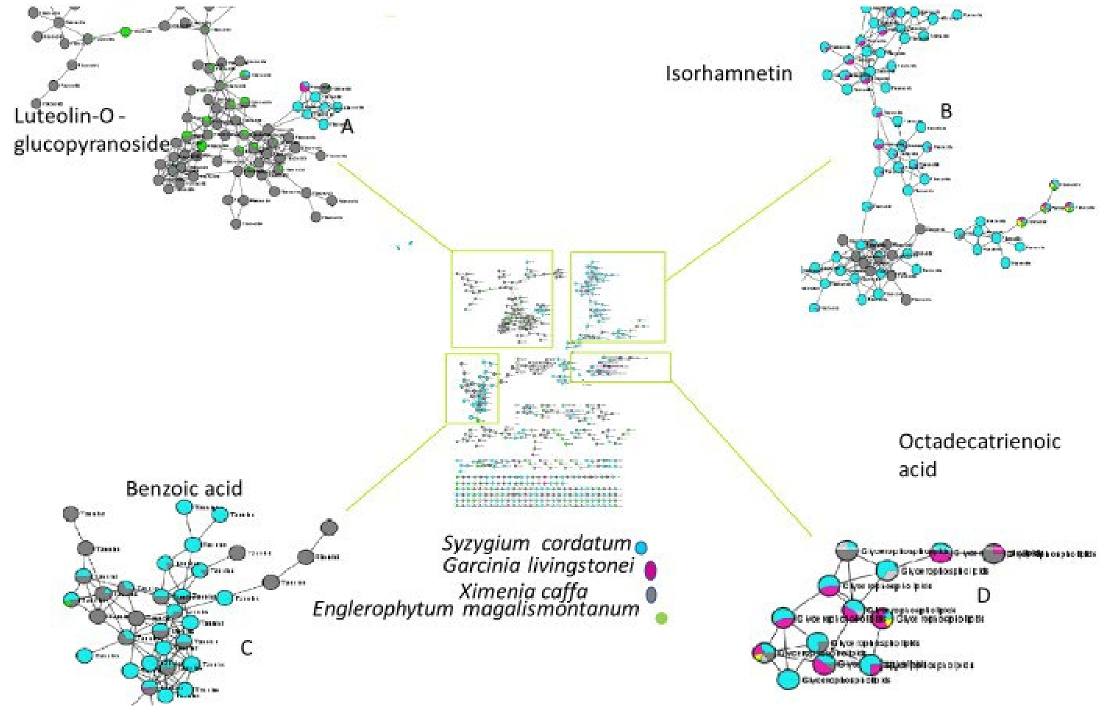

Figure 4.4.1. Molecular network of *syzygium cordaturm, Garcinia livingstonei, Ximenia caffra* and *Englerophytum magalismontanum* extract analysed liquid chromatograph- tandem- mass spectrometry using electrospray ionization in negative mode.

In figure 4.4.2. “Flavonoids identified are procyanidin B2 and Quercetin-3-O-alpha-L rhamopyranoside are specific two types of bioflavonoids and their potential benefits in treating musculoskeletal condition” (Wang *et.al.,* 2020). Flavonoids have been found to have potential benefits for connective tissue due to several reasons. These include their ability to limit inflammation and associated tissue degradation, enhance local circulation, and promote a strong collagen matrix (Wang *et.al.,* 2020). The tannins identified are “ellagic acid it may also prevent the growth of cancer cells and improve the safety and efficacy of some cancer drugs.” However, ellagic acid is poorly absorbed and is also eliminated quickly from the body” (Rios *et.al.,* 2018).

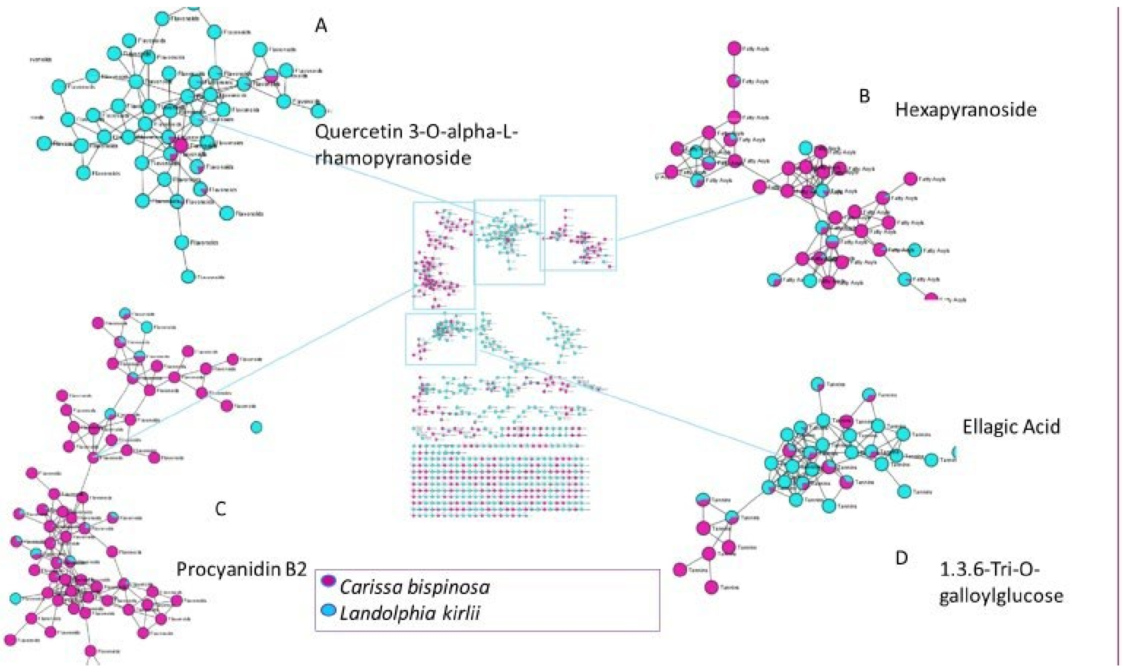

Figure 4.4.2. Molecular network of *Landolphia kirkii* and *Carrisa bispinosa* which belong to same family extract analysed liquid chromatograph- tandem- mass spectrometry using electrospray ionization in negative mode.

In figure 4.4.3 the chlorogenic acid identified is 3.4-di-o caffeoylquinic acid is one of the phenylpropanoids which have various bioactivates such as antioxidant (Ramabulana *et.al*.,2020), antibacterial, (Xie *et al*., 2015), anticancer (Ncube, 2014) and other biological effect. It is previously reported that 3.4-di-O-caffeolyquinic acid inhibited amyloid induced cellular toxicity on human neuroblastoma cell and increase the mRNA expression level of glycolytic enzymes and the intracellu lar ATP level. Putative annotation of metabolites that were matched and some unmatched by molecular networking was confirmed based on their accurate mass, which was used to generate molecular formulae that were searched across different outline databases. It is important to note that during MS/MS fragmentation, most flavonoids readily lose their sugar attachments, result in aglycones exposure for further fragmentation (Ramabulana *et.al*., 2021).

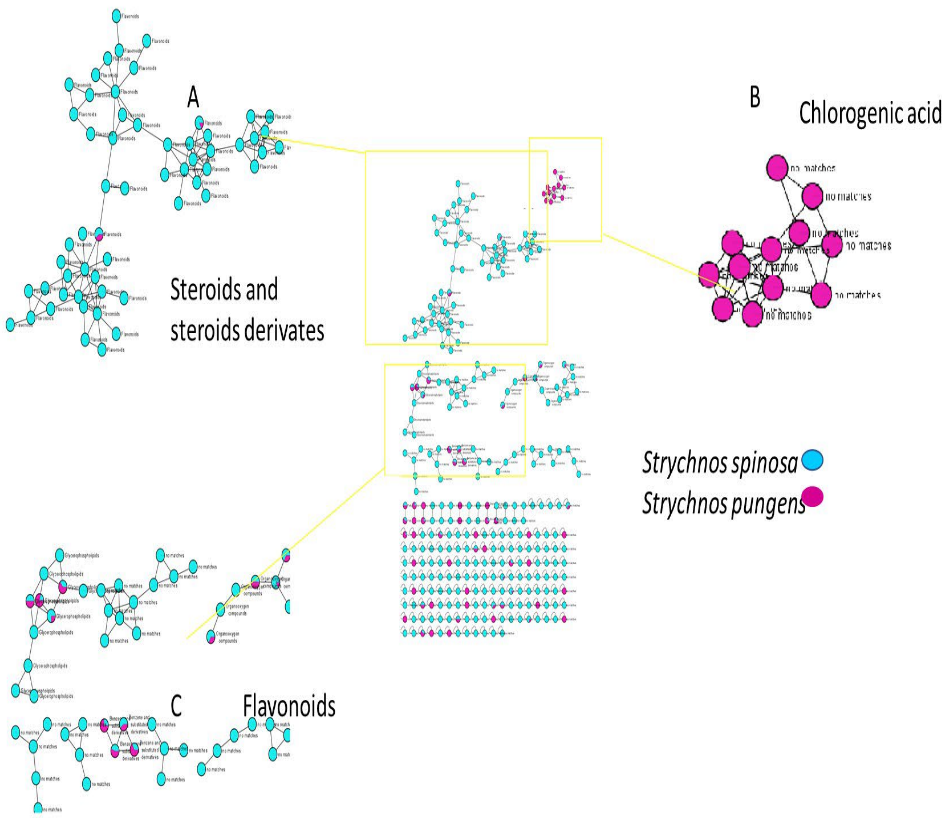

Figure 4.4.3 Molecular network of *Strychnos spinosa* and *strychnos pungens* extract analysed liquid chromatograph- tandem- mass spectrometry using electrospray ionization in negative mode.

4.4.4. The MolNetEnhancer

The MolnetEnhancer approach integrates results outputs from molecular mining tools (molecular network and MS2LDA) in *silico* annotation tool (network annotation propagation and dereplicator) and class annotation through class fire term to provide a comprehensive visualization of the chemical space within a metabolome.

Putative annotation of metabolites that were matched and some unmatched by molecular networking were confirmed based on their accurate mass, which was used to generate molecular formulae that were searched across databases (Ernst *et.al.,* 2019). The visualization of chemical space indicate that these species contain lipids and lipids like molecules, phenylpropanoids and polyketides, organic oxygen compounds, benzenoids, organic polymers, organ heterocyclic compounds and benzene and substituted derivates.

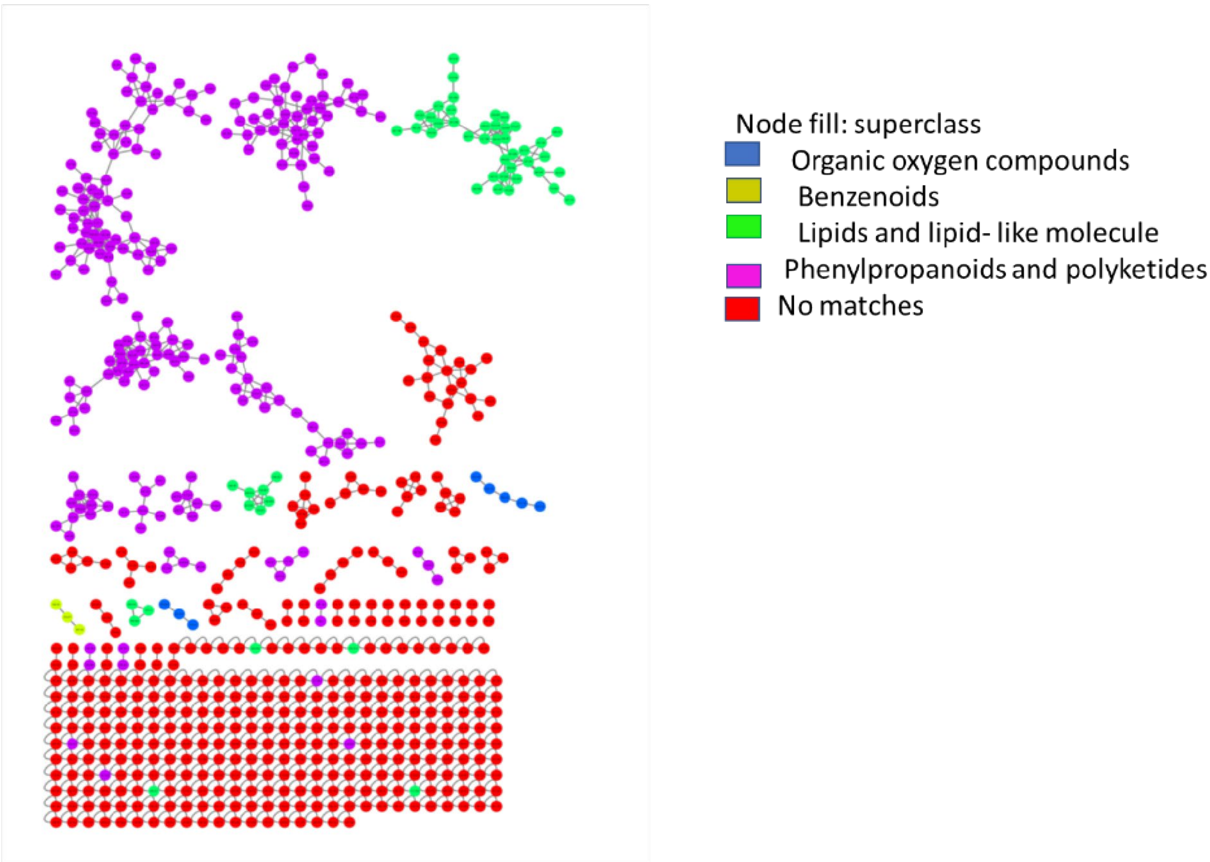

Figure 4.4.4. Chemical classification and structural elucidation of *strychnos spinosa* and *strychnos pungens*. An enhanced molecular network in which nodes are coloured based on their chemical superclass.

CHAPTER 5

5.1 CONCLUSION

In this study, the metabolomics tools and techniques were utilized to investigate and characterize the hydroxycinnamic acids and their ester derivatives found in wild edible fruits, which are important for both food and medicinal purposes. The distribution of chemically diverse esters of quinic and tartaric acid in these fruits was found to be variable. Using UHPLCqTOF-MS/MS-based metabolite profiling, twenty-four phytochemicals from various classes of natural products, such as hydroxycinnamic acids, flavonoids, and organic acids, were identified across twelve wild edible fruits. This approach is beneficial for the conservation of these plants by limiting the harvesting of the whole part. The study also revealed that *Ficus sycomorus* contained structurally complex chlorogenic acids (CGAs) as regio- and geometric isomers, as well as hetero-acyl CGAs. The application of MS fragmentation patterns allowed efficient discrimination of these isomers. The identified compounds possess known health-promoting benefits. The use of computational tools such as spectral libraries and molecular networking helped in the identification of known and structurally related metabolites in *Strychnos spinosa* and *Strychnos pungens*. The study showed the potential of computational tools in unraveling complex plant metabolomes and filling knowledge gaps of unexplored and partially explored metabolomes. Future perspectives include further mining of these metabolomes using more computational tools to discover novel compounds that could be potential natural products, and to describe approaches that will assist in a more automated exploration of complex metabolomes.

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APPENDICES

APPENDIX 1

NEPHAWE RINAE LUCY (14005307)

Master's in science

Supervisor: DR MOKGANYA M.G

RESEARCH QUESTIONNAIRE FOR THE PROJECT ENTITLED ' ASSESSMENT OF NUTRACEUTICAL ATTRIBUTES OF SELECTED WILD EDIBLE FRUIT PLANTS USED BY VHAVENDA PEOPLE OF THE THULAMELA LOCAL MUNICIPALITY.'

PERSONAL INFORMATION

Respondent name				
Gender	Male		Female	
Residence				

Age group	35-45		46-55		56-65		66+	
Level of education	No schooling				Primary education			
	Secondary education				Tertiary education			

B.

QUESTIONAIRRES	ANSWERS+
What is the preservation method of these fruits for future use?	
What is the conservation status regarding indigenous wild edibles fruits?	
What is the contribution of wild edible fruit plants in the socio - economic status?	
What are the different uses of selected wild edibles fruits?	

What is the plant population rate in this location?	
Do you experience difficulties in finding some wild edibles fruits plants?	
Who collect these wild edibles fruits plants among the members of your household?	
Do you sometimes cultivate any of the plants?	
Which resource management options do you think are most appropriate in achieving long term sustainable use of plants?	
Are there any forms of enforcement measures in place from Government to control use wild edibles fruits plants species?	

APPENDIX 2 CONSENT FORM FOR PARTICIPATION IN THE RESEARCH ENTITLED

"ASSESSMENT OF UTILIZATION, NUTRITIONAL AND CONSERVATION STATUES OF SELECTED WILD EDIBLE FRUIT PLANTS USED BY VHAVENDA PEOPLE OF THULAMELA LOCAL MUNICIPALITY."

You are invited to participate in a research study conducted by Nephawe Rinae Lucy. The purpose of this research is to document assessment of utilization, conservation, and the nutritional status of wild edible fruit plants in Thulamela Local Municipality. Your participation will involve the naming of the wild edible fruit plant that you know, and to show the researchers the locations of those plants.

This research may help us to know the nutritional values of some of the documented wild edible fruit plant. Participants are advised that we will do everything we can as researchers to protect their privacy. Your identity will not be revealed in any publication resulting from this study. Your participation in this research is voluntary. You may choose not to participate, and you may withdraw your consent to participate at any time.

If you have any questions, please contact Nephawe Lucy Rinae at the University of Venda at 015 962 8929

Consent

I have read this consent form and I give my consent to participate in this study.

Participant's signature: ____________________________ Date: ________________________

APPENDIX 3

Landolphia kirkii belong to family Apocynaceae and roots are also used for decoction of infusion is s taken to expel worms. The season availability of *Landolphia kirkii* are available in summer

Figure 3.4.1 Collection of *Landolphia kirkii*

APPENDIX 4

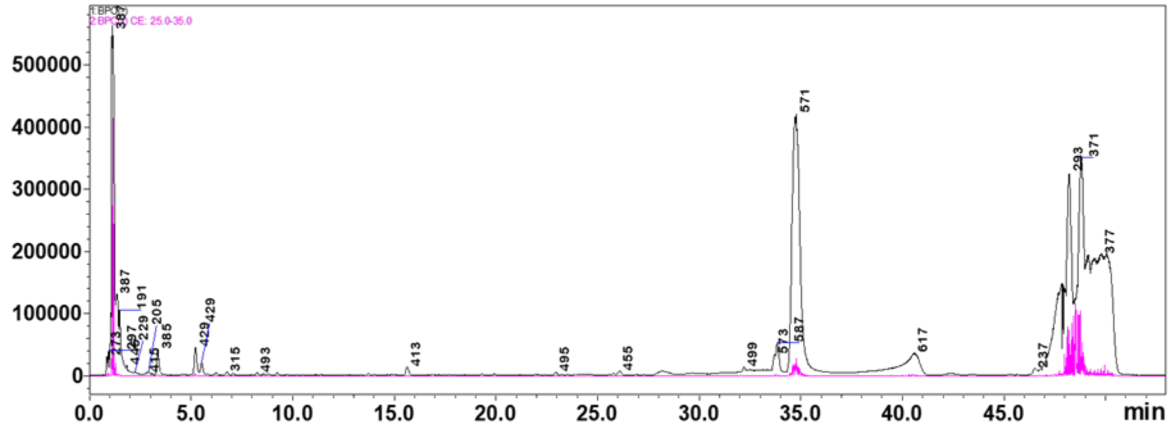

Figure 4.3.11 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Strychnos pungenssoler*.

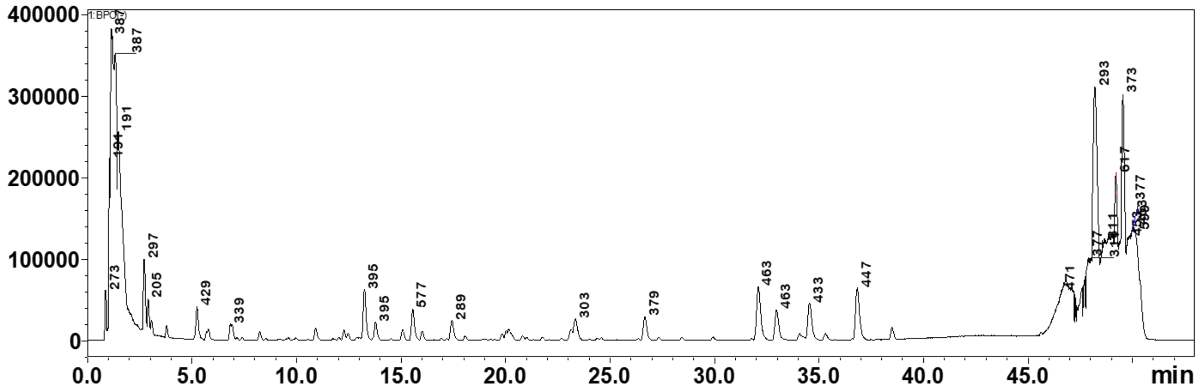

Figure 4.3.12 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Englerophytum magalismontanum*.

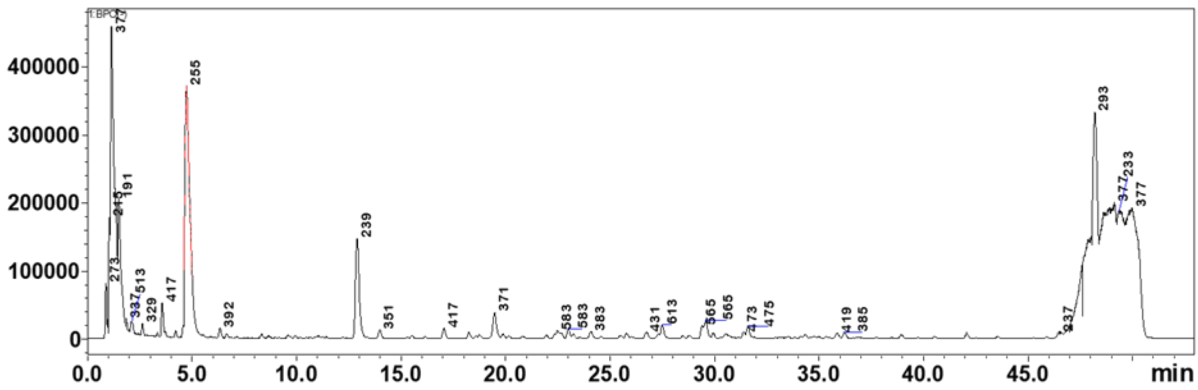

Figure 4.3.13 Representative UHPLC-qTOF-MS chromatogram showing metabolites present in the methanolic extract of *Opuntia ficus*.

APPENDIX 5

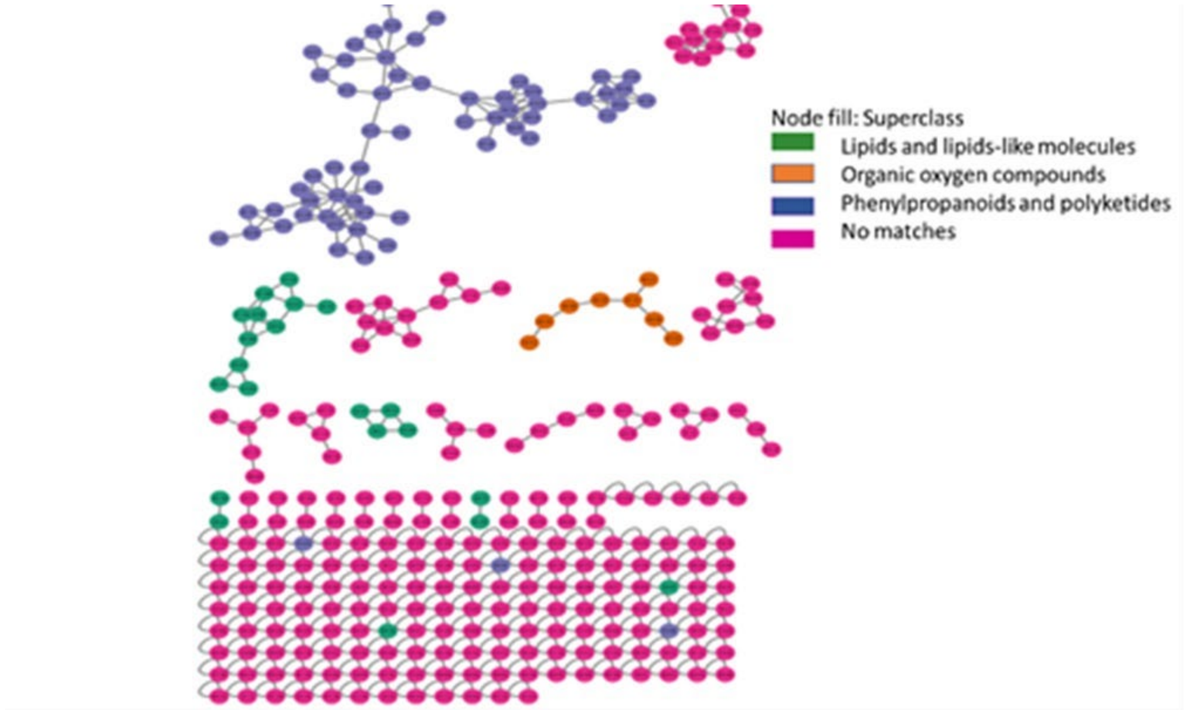

Figure 4.4.5 Chemical classification and structural elucidation of *Landolphia kirkii* and *Carissa bispinosa.* An enhanced molecular network in which nodes are colored based on their chemical superclass.

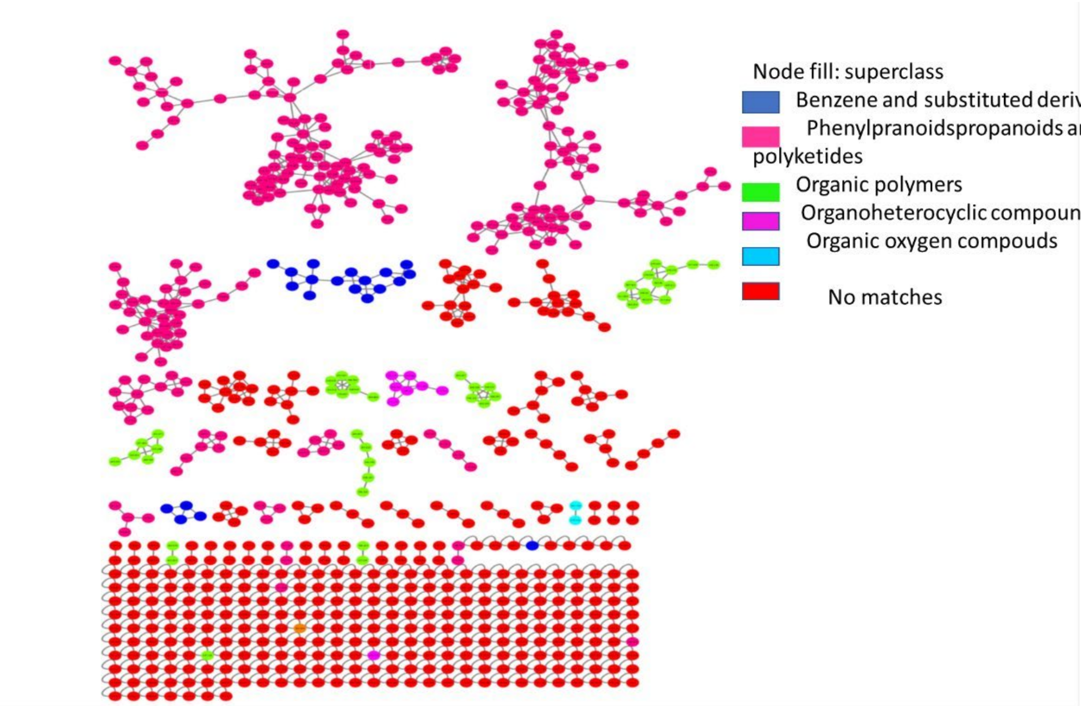

Figure 4.4.6 Chemical classification and structural elucidation of *Syzygium cordatum, Garcinia livingstonei, Ximenia caffra and Englerophytum magalismontanum.* An enhanced molecular network in which nodes are colored based on their chemical superclass.