An inventory, nutritional analysis and biological evaluation of underutilized indigenous vegetables used to manage non-communicable diseases in Thulamela local municipality, Vhembe District Municipality, South Africa

By

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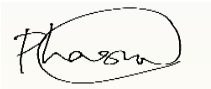
Declaration

I declare that this research is my own work and the work of others has been correctly acknowledged. This work has not been submitted to any other higher education institution.

Signed (student)  Date: 06 July 2022

Dedication

This work is dedicated to myself.

Acknowledgement

First, I would like to thank God for strength, wisdom, knowledge and good health throughout my study. Proverbs 3:5-6

I would like to thank my supervisor Dr N.A Masevhe and my co-supervisors Dr R.B Mulaudzi and Prof S.O. Amoo for their prompt feedback, continuous support, guidance, patience, motivation, enthusiasm and immense knowledge. Your insightful feedback pushed me to sharpen my thinking and brought my work to high level. I could not have completed this work without your unwavering supervision, I am realy grateful.

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Conference contribution

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Abstract

Non-communicable diseases have emerged as a serious public health concern around the world, with a high death rate. The study aim was to document and evaluate the biological activities of underutilized indigenous vegetables used by local people in Thulamela Local Municipality to manage non-communicable diseases. An open structured interview was used to conduct an ethnobotanical survey, and 25 underutilized indigenous vegetables belonging to 13 families were documented. Cucurbitaceae was the dominant family, constituting 21% of the documented species, followed by Urticaceae with 13% of the species. Six of the documented species, which are *Citrullus colocynthis, Cleome gynandra, Cucumis africanus, Oxygonum dregeanum, Pouzolzia mixta*, and *Sonchus oleraceus*, were selected and evaluated using standard laboratory procedures for quantifying nutritional, phytochemical constituents, antioxidant, and anti-diabetic activities.

The above-mentioned six vegetables contained a considerable amount of important nutrients. *Cucumis africanus* has the highest levels of ascorbic acid, Ca, Fe, and Mg. In comparison to other vegetables studied, *Citrullus colocynthis* had the highest levels of total phenolics, total flavonoids, and condensed tannins. The free radical-scavenging activity of all the plant extracts was moderate compared to the positive control (ascorbic acid). On the other hand, dichloromethane and acetone extracts exhibited high antioxidant activity in the beta-carotene-linoleic acid antioxidant system. Most dichloromethane, acetone and distilled water extracts showed low alpha-glucosidase inhibition compared to the positive control (acarbose). However, the dichloromethane extract of *Citrullus colocynthis* and acetone extract of *Cucumis africanus* exhibited significantly high alpha-glucosidase inhibitory activity than acarbose.

According to the information gathered, people in Thulamela Local Municipality rely on indigenous vegetables for medicine to treat non-communicable diseases. The quantified nutritional and phytochemical contents and the antioxidant and alpha-glucosidase inhibitory activities exhibited by the selected underutilized indigenous vegetables confirm their traditional uses as food and medicine to manage diabetes and other non-communicable diseases. To obtain precise data that can be used to verify these findings, in vivo methods should be used.

Keywords: Non-communicable disease, Underutilized indigenous vegetables, Nutritional value, Antioxidant activity, and Antidiabetic activity.

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List of abbreviations

α-amylase	Alpha-amylase
α-glucosidase	Alpha-glucosidase
BHT	Butylated hydroxytoluene
Ca	Calcium
CE	Catechin equivalent
Cu	Copper
DMSO	Dimethyl sulfoxide
DPPH	1,1-Diphenyl-2-picrylhydrazyl radical
EC_{50}	Half maximal effective concentration
Fe	Iron
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
H_2SO_4	Sulfuric acid
IC_{50}	Half-maximal inhibitory concentration
K	Potassium
LE	Leucocyanidin equivalent
Mg	Magnesium
Mn	Manganese
NaOH	Sodium hydroxide
NCDs	Non-communicable diseases
P	Phosphorus
pNP	*p*-nitrophenol
RDA	Recommended dietary allowance
SSA	Sub-Saharan Africa
UIVs	Underutilized indigenous vegetables
WHO	World Health Organisation
Zn	Zinc

Chapter 1

Introduction

1.1. Background

For an extended period, underutilized indigenous vegetables have proven to be a valuable natural resource for sustaining the human diet and meeting primary health care needs. Ancient people learned about the therapeutic use of these vegetables through trials and errors (Qureshi *et al.*, 2016). Different indigenous vegetables are used worldwide by various cultural groups as food sources and traditional medicine. Recently, they have intrigued the attention of researchers such as botanists, agriculturalists, economists, biologists and nutritionists (Kwinana-Mandindi, 2014). This has mainly been activated by the increasing recognition of these species contributions to people's lives economically, nutritionally and medicinally.

Underutilized indigenous vegetables are essential because they improve the economic status of people who sell them, thus contributing positively to household income and alleviating poverty (Mazumder *et al.*, 2019). Furthermore, they are vital nutritionally because of essential nutrients such as vitamins, minerals and amino acids. Underutilized indigenous vegetables also contain organic compounds that are beneficial for healthy living and ensure proper mental and physical functioning (Nyadanu and Lowor, 2015). Underutilized indigenous vegetables are important medicinally because of the bioactive compounds such as tannins, flavonoids, phenolics and alkaloids. Beneficial biological activities of these bioactive compounds include antioxidant, antidiabetic, antimicrobial and anti-inflammatory effects for the prevention, management and treatment of various human diseases (Ayinde *et al.*, 2016; Ijeomah *et al.*, 2012; Kwenin *et al.*, 2011).

Mahlangu *et al.* (2014) reported more than 45000 indigenous plant species in Sub-Saharan Africa, of which 1000 species can be consumed as indigenous vegetables. The Khoisan people and Bantu-speaking tribes have lived in Southern Africa for the past 120 000 years. They relied heavily on gathering indigenous vegetables and plants from the wild in order to survive (Van Rensburg *et al.*, 2007). Therefore, indigenous vegetables have been an essential part of the South African culture for thousands of years (Van Rensburg *et al.*, 2007). While the use of indigenous vegetables is practised in all the provinces in South Africa. The Limpopo Province has been described as the leading province in consuming and using indigenous vegetables (Bvenura and Afolayan, 2015). The prevalence of non-communicable diseases is increasing rapidly in South Africa (Ndinda, 2018). Therefore, there is a need to develop new or cost-effective ways that can be used to combat these diseases. Epidemiological studies have shown that increased utilization of underutilized indigenous vegetables is linked to decreased risks and incidences of nutrient deficiency disorder and non-communicable diseases (Mazumder *et al.*, 2019).

1.2. Non-communicable diseases

Non-communicable diseases (NCDs) are not contagious and cannot be passed from one person to another. These diseases take a long time to develop and usually have no symptoms initially. They are long-term diseases that usually advance slowly enough to cause chronic symptoms (Puoane *et al.*, 2012). Cardiovascular diseases, diabetes, cancer, hypertension, and chronic respiratory illnesses are among the diseases that fall into this category (Kufe *et al*., 2016; Jeet *et al.*, 2017). They have a greater impact on people during their productive years of life (Onagbiye *et al.,* 2019; Maimela *et al.*, 2016).

Non-communicable diseases are caused by a complex interaction of genetic, physiological, environmental, and behavioural factors. There are two types of non-communicable disease risk factors, namely modifiable and non-modifiable risk factors. Unhealthy diets, lack of physical activity, tobacco use, and excessive alcohol intake are modifiable risk factors, while age, sex, and family history are non-modifiable risk factors (Ndinda *et al.*, 2018; Bawah *et al.*, 2018). The number of deaths from NCDs has been rising worldwide, and they are now among the top causes of death (Nojilana *et al.*, 2016). However, by 2030, NCDs are expected to account for 46% of all deaths in Sub-Saharan Africa (SSA) (Bolarinwa *et al.*, 2020). NCDs have been more prevalent in South Africa over the last 15 years, accounting for an estimated 37% mortality and 16% disability-adjusted life years (Maimela *et al.*, 2016). According to Statistics South Africa (2018), diabetes, cerebrovascular disorders, hypertensive diseases, chronic lower respiratory diseases, and other forms of cardiac disease are amongst the diseases that cause death in each province. The risk factors differ in these provinces, but the effects remain the same (Maimela *et al.*, 2016).

The development of NCDs can be prevented, managed, controlled, and treated by reducing risk factors and using conventional and herbal medicine (Bollyky, 2013). Furthermore, detecting and addressing NCDs in their infancy can help to avoid complications and death (Ezzati and Riboli, 2012). These therapeutic options work to slow down the advancement of the disease and improve the body's functionality. Diabetic patients, for example, can decrease the risk for diabetes by changing their lifestyle and taking oral glucose-lowering medications and herbal medicines. However, available oral medications such as sulfonylureas, biguanides, thiazolidinediones, meglitinide, and alpha-glucosidase inhibitors have side effects. These include cardiac problems, fracture risk, severe risk of liver disease, higher risk of bladder cancer, upper respiratory infections, and nausea (Ghani, 2015; Ezuruike and Prieto, 2014; Prabhakar *et al.*, 2014). Thus, an increasing number have resorted to using natural products, including underutilised indigenous vegetables, as an alternative.

The use of underutilized indigenous vegetables has recently gained popularity globally. Indigenous vegetables are dependable, cheap, accessible and readily available (Ülger *et al.*, 2018; Kisangau, 2017). Since ancient times, these vegetables are often used as a source of therapeutic agents since they contain nutrients and pharmacologically active compounds (Bosede *et al.*, 2017). They are used as natural remedies to prevent and cure various diseases. Therefore, their inclusion in our diet serves dual purposes (Prasad *et al.*, 2017). Epidemiological studies indicate that increased consumption of leafy vegetables is responsible for decreased risk of nutrient deficiency disorders and NCDs (Matenge *et al.*, 2017). Underutilized indigenous vegetables also contain antioxidants necessary in neutralizing free radicals, which are human chemical hazards. The antioxidants present in green leafy vegetables can be helpful in the management of oxidative stress and age-related human ailments (Darkwa and Danka, 2013; Oseni and Olawoye, 2015).

The leaves of *Momordica charantia* (Hoque *et al.*, 2017), *Momordica foetida, Momordica balsamina* (De Wet *et al*., 2016), *Amaranthus hybridus* (Chintamunnee and Mahomoodally, 2012) as well as the seeds and fruits of *Citrullus colocynthis* (Rahimi *et al.*, 2012) have been documented to have anti-diabetic properties. Vegetables such as *Abelmoschus esculentus* have been used to treat diabetes and other illness such as cholesterol, syphilis and nutritional diseases (Jain *et al*., 2012). The leaves and the roots of *Cleome gynandra* have been used for diabetes, to lower high blood pressure, prevent kidney stones, and treat urinary tract infections, bladder stones and chronic kidney infections (Mishra *et al*., 2011). Vegetables such as *Bidens pilosa* have pharmacological properties such as anticancer, anti-hypertensive and antioxidant effects (Bairwa *et al.*, 2010).

1.3. Problem statement

Indigenous vegetables are an essential part of the diets of many rural African households. The consumption of indigenous vegetables plays a crucial nutritive role, especially for people living in rural areas (Moyo *et al.*, 2013). Despite their nutritional content and health benefits, they remain vastly underutilized, neglected, and underexploited (Njume *et al.*, 2014). In some areas, they are regarded as weeds due to ignorance. Most of the studies that have been conducted on underutilized indigenous vegetables have focused more on indigenous vegetables' sustainable use and management, yield improvement, conservation status, preservation methods, nutritional content, nutrient intake and consumption of indigenous foods, seed production and medicinal use (Mokganya and Tshisikhawe, 2018; Senyolo *et al.*, 2018; Mungofa *et al*., 2018; Mabala, 2018; Rankoana, 2016; Nyembe, 2015; Mahlangu *et al.*, 2014; Mbhenyane *et al.*, 2013; Mbhatsani *et al.*, 2011; Maanda and Bhat, 2010; Mbhenyane *et al.*, 2005; Nesamvuni *et al.*, 2001).

However, the medicinal values of these vegetables have not been thoroughly investigated, although rural communities who consume them daily have a relatively long life span free from diseases. Apart from the most consumed indigenous vegetables, some are still undocumented but pivotal in rural communities. The knowledge about indigenous vegetables remains mainly with older people. Therefore, this knowledge will soon be lost without proper documentation and preservation of this indigenous knowledge, especially about their preparation, administration, dosage, medicinal values, and nutritional content (Oladele, 2011). It is critical that indigenous vegetables be accurately documented and preserved for the sake of the present and future generations.

1.4. Rationale of the study

The indigenous knowledge orally transferred from generation to generation is fast disappearing because of the technology and cultural changes in ethnic groups. For the same reason, proper inventories and restoration of indigenous vegetables are vital. This study has the potential to highlight important species that can improve the human diet and provide leads for the discovery of new drugs. Those who sell these vegetables in the streets will continue with their excellent work because they will become more aware of what their products contribute. Furthermore, people who cannot afford modern medicine can use these vegetables to treat or manage non-communicable diseases. Young people will learn about the critical value of indigenous vegetables, which they have grown to neglect and dislike. Therefore, it is essential to document and conduct some assays on these vegetables to provide valid information that will benefit local people positively.

1.5. Hypothesis

There are many undocumented indigenous vegetables rich in nutritional and medicinal values used by rural people to sustain their livelihood.

1.6. Aims

This study aimed to document the available indigenous knowledge on underutilized indigenous vegetables used to manage non-communicable diseases in Thulamela Local Municipality in Limpopo Province and evaluate the antioxidant and α-glucosidase inhibitory activities of some selected underutilized indigenous vegetables.

1.7. Objectives

The objectives of this study were:

- To identify and document indigenous knowledge on underutilized indigenous vegetables used to manage non-communicable diseases;
- To assess the nutritional value of some selected underutilized indigenous vegetables;
- To determine the phytochemical content of some selected species;
- To evaluate the antioxidant and α-glucosidase inhibitory activities of some selected species.

1.8. Research questions

Which underutilized indigenous vegetables are used to manage non-communicable diseases? Which plant parts of the vegetables are used? What is the life form of these vegetables, and in what season(s) are these vegetables available? How are they administered? Are there other materials added to enhance their healing properties? How are these vegetables preserved? How are they prepared to manage non-communicable diseases?

1.9. Outline of the study

Chapter 1: This chapter briefly introduces underutilized indigenous vegetables used for nutritional and medicinal purposes (including management of non-communicable diseases). It also highlights the problem statement, rationale, hypothesis, aims, objectives, and research questions of the study.

Chapter 2: It provides an inventory of underutilized indigenous vegetables used to manage non-communicable diseases in Thulamela Local Municipality, as documented in this study.

Chapter 3: This chapter indicates the nutritional values of selected vegetables from this study. Their mineral (calcium, copper, iron, phosphorus, manganese, magnesium, potassium, and zinc), ascorbic acid and β-carotene contents were quantified and discussed concerning their nutritional values.

Chapter 4: This chapter details the phytochemical screening using qualitative and quantitative methods and the evaluation of biological properties (antioxidant and antidiabetic activities) of the selected plant species.

Chapter 5: This chapter summarises the overall conclusion and recommendations of this study.

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Chapter 2

An inventory of underutilized indigenous vegetables used to manage non-communicable diseases in Thulamela Local Municipality, South Africa.

Abstract

Non-communicable diseases are non-infectious diseases, causing a high mortality rate worldwide. The study aimed to document underutilized indigenous vegetables used to manage non-communicable diseases in Thulamela Local Municipality in Limpopo Province, South Africa. Ethnobotanical data were collected using open semi-structured interviews. A total of 80 respondents were interviewed, and 25 indigenous vegetables belonging to 13 families were reported. *Cucurbitaceae* was the dominant family with seven species, followed by *Urticaceae* with three species. The life forms of the recorded species were primarily herbs, followed by climbers, shrubs, creepers and trees. *Amaranthus dubius* (94%), *Bidens pilosa* (94%), *Momordica_foetida* (93%), *Momordica balsamina* (90%), *Momordica charantia* (90%), *Corchorus tridens* (89%) and *Pouzolzia parasitica* (89%) had the highest frequency indexes. Hypertension and diabetes were the most cited non-communicable diseases for which the indigenous vegetables were used. The information obtained showed that underutilized indigenous vegetables play an essential role in improving local communities' nutrition and health care.

Keywords: Non-communicable diseases, Underutilized indigenous vegetables, Ethnobotany, Herbs, Frequency index.

2.1. Introduction

Non-communicable diseases (NCDs) are non-infectious diseases, meaning that they cannot be transferred from one person to another. The causal factors of these diseases include lack of physical exercise, excessive alcohol consumption, tobacco usage, and poor diets (Ndinda *et al.*, 2018; Maimela *et al.*, 2016).

There is a burden of NCDs crisis in developed and developing countries. The World Health Organization predicted that the prevalence of non-communicable diseases would account for 73% of death and 60% of disease burdens from 2020 onwards (Anand *et al.*, 2007). Non-communicable diseases cause one in four deaths in sub-Saharan countries Bloomfield *et al.*, (2014) and contribute to the high death rate globally. The prevalence of these diseases is also rising rapidly in Africa (Nojilana *et al.*, 2016). Nojilana *et al.* (2016) reported that NCDs are among the top causes of death in South Africa. Hypertension and diabetes are the primary or common NCDs that affect most people (Cois, 2015).

Since the political transitioning, South Africa has experienced a rise in NCDs due to modernization, changes in diets, and sedentary lifestyles (Folb *et al.*, 2015; Mayosi *et al.*, 2009). An increasing number of people consume more processed foods than those grown in home gardens or growing in the wild. An unhealthy diet contains more energy-dense, processed foods, added sugar, salt, and fats (Cetthakrikul *et al.*, 2019; Spires *et al.*, 2016). However, this can be rectified by encouraging people to consume indigenous foods more often.

Underutilized indigenous vegetables grow naturally and abundantly in the wild and in cultivated areas. They are part of a significant biodiversity, and their potential was once recognized. Their recognition declined due to changes in time, the introduction of exotic vegetables, western food, modernization, and import of foods. These vegetables benefit rural people medicinally, nutritionally, and economically (Kumar *et al.*, 2014; Ijeomah *et al.*, 2012; Adebooye, 2011). Consumption of indigenous vegetables to combat NCDs is a trustworthy option as it has been used from the dawn of time and people survived free from many diet-related diseases (Cogill, 2015).

The objective of this study was to document indigenous knowledge on underutilized vegetables used to manage non-communicable diseases by local people in Thulamela Local Municipality, South Africa. Inventories of underutilized indigenous vegetables are essential because it's a way of documenting vegetables that indigenous people have been using to improve food security, health care, and standard of living since time immemorial. This also benefits indigenous knowledge by protecting traditional culture biodiversity and establishing an improved diet for the future generation (Negi *et al.*, 2018).

2.2 Materials and methods

2.2.1. Study site

The study was undertaken within four villages: Tshaulu, Duthuni, Lwamondo, and Thohoyandou in Thulamela Local Municipality in the far north of the Limpopo province, South Africa (Figure 2.1). The study area covered a total of 2 893.936 km² within the coordinates of 22° 56´ S and 30° 28´ E (Netshifhefhe *et al*., 2018). Various ethnic groups were found within this area; however, Vendas (63.64%) and Tsongas (32.85%) were more prevalent (Thulamela Local Municipality IDP, 2017/18). The area is characterized by gently to the very steep slope mountainous valleys (Semenya *et al.*, 2012). The average annual rainfall of approximately 500 mm per annum was recorded in the area between October and March (Mphephu *et al.*, 2017; Louw and Flandorp, 2017). The district falls within the greater Savanna Biome (Louw and Flandorp, 2017) and is rich in loam soil, bringing forth high agricultural activities. The area comprises sacred forests, namely Thathe holy forest, Tshatingwana forest, and conservancies' areas, e.g., Pafuri and Punda Maria Gates of Kruger National Park in Thulamela (Thulamela Local Municipality IDP 2018/19).

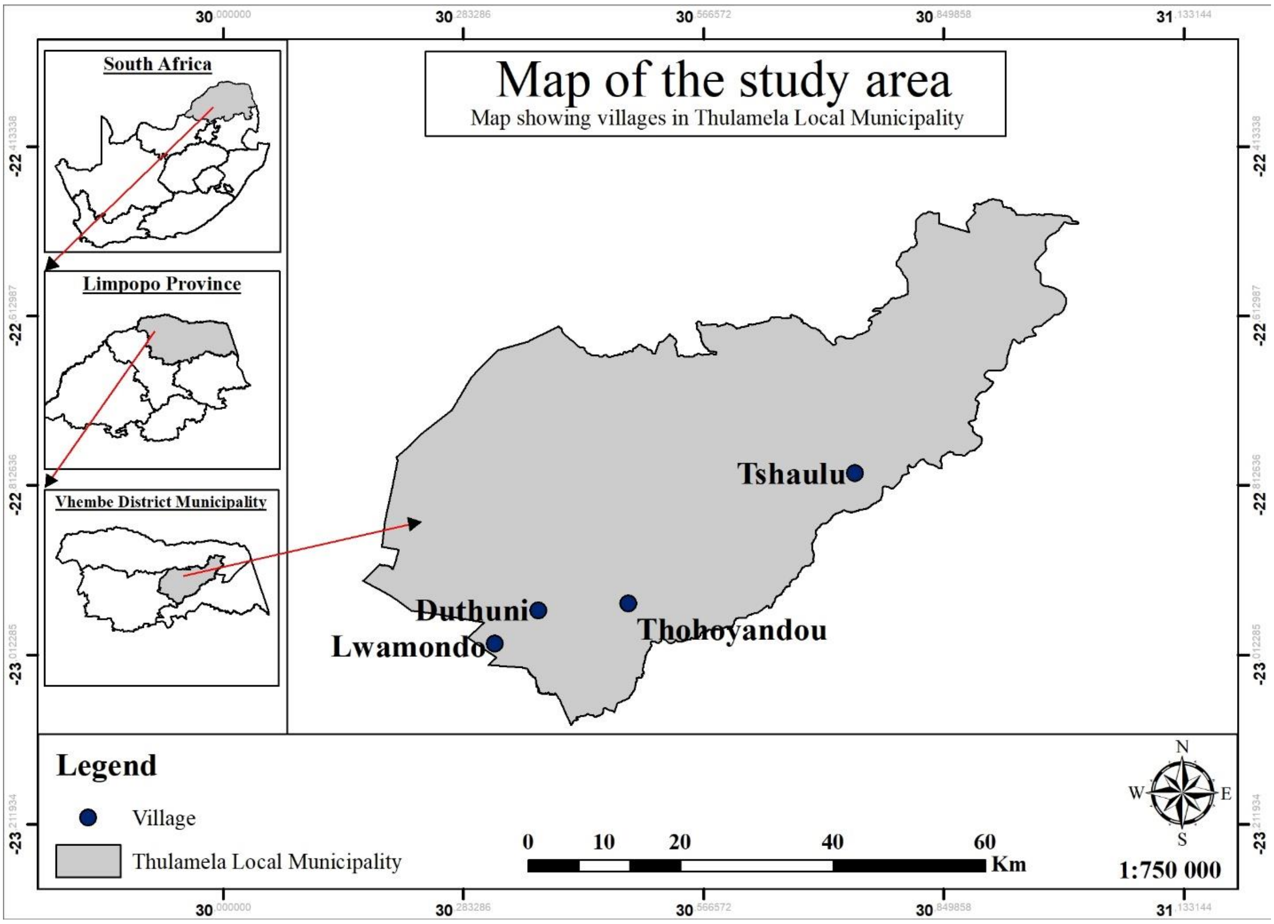

Figure 2.1. Map showing Thulamela Local Municipality in Vhembe District Municipality of Limpopo Province, South Africa (constructed by Mugari Dzivhuluwani).

2.2.2. Data collection

The traditional authorities granted permission for the collection of data. Ethnobotanical data were collected from August to November 2018 from local people. Interviews were conducted in Tshivenda (the native language of the respondents), which was translated to English during data analysis. Eighty participants from Tshaulu, Duthuni, Lwamondo, and Thohoyandou were interviewed through open semi-structured interviews (Appendix A). Twenty participants of no particular target group were selected from each village during the study. To get accurate information, interview responses from respondents were recorded using a phone (Samsung galaxy A20). Names of underutilized indigenous vegetables used to manage non-communicable diseases were obtained. In addition, information on plant parts used, vegetable life forms, how they are administered, things added to enhance the medicinal properties, and how are they preserved was also obtained. Some of the vegetables mentioned during the survey were identified and collected with the help of participants. Additionally, they were verified using online herbariums (http://apps.kew.org/herbcat; https://plants.jstor.org/compilation) and voucher specimens were deposited at the University of Venda, Botany Department, herbarium.

2.2.3. Data analysis

Data collected during the study were stored in Microsoft Excel 2013 database, which was also used to construct graphs. Data were analyzed using descriptive statistics to determine percentages and relative frequencies. The frequency index was calculated using relative frequency of citation (RFC), which signifies the local importance of each species.

The following formula calculates RFC:

$$RFC = \frac{FC}{N} \times 100$$

The index is determined by dividing the number of informants who mentioned the plant species (FC) by the total number of informants (N) in the survey (Atyosi *et al.*, 2019; Mokganya and Tshisikhawe, 2019).

2.3. Results and discussions

2.3.1. Demographic data

A total of 80 participants were interviewed, of which 40 were between the ages of 20-45, and the other 40 were between the ages of 46-65+. Female participants accounted for 75%, while 25% were males. This is not surprising because females are mostly the ones responsible for cooking and taking care of the family through domestic duties (Afolayan and Wintola, 2014). Women are the primary food producers worldwide (Vorster, 2007)**.** Indigenous leafy vegetables are called women crops in most African countries because they are grown and gathered by women for domestic uses and market sales (Ayanwale and Amusan, 2014). Van Rensburg *et al.* (2007) reported that males become involved only when the production of a particular indigenous vegetable is commercialized.

Of all the participants, 75% received formal education up to the tertiary level, and 25% did not receive any formal education. This shows that even though they have attained education, they did not abandon indigenous knowledge acquired through life experiences. About 55% of respondents had jobs such as teaching, cleaning in higher institutions, and street trading, whereas 45% are elderly people who live on the old-age pension and grant money from the government.

The results showed that elderly people were knowledgeable about underutilized indigenous vegetables than the young. Elderly people are more familiar because they are more involved in ploughing the field than younger people. This may be attributed to the fact that elderly people are the primary custodians of traditional knowledge and they are not profoundly or easily attracted to modernization (Mercy *et al.*, 2017; Dweba and Mearns, 2011). Lack of knowledge transfer from elderly people to the younger generation is due to the apathy the younger generation shows towards the indigenous knowledge system. The young generation has developed a reluctant attitude towards traditional knowledge and considers it knowledge of the past associated with poverty and low self-esteem (Dweba and Mearns, 2011; Afolayan and Jimoh, 2009).

2.3.2. Documented plant species and families

The current study documented 25 underutilized indigenous vegetables used by local communities to treat and manage non-communicable diseases (Table 2.1). Previous studies in this area focused on documenting indigenous vegetables used as the source of food, medicine, and nutrient content. For example, Mokganya and Tshisikhawe (2019) reported wild vegetables used medicinally in Vhembe District Municipality, Limpopo Province. Mokganya *et al.* (2019) documented edible vegetables focusing on their preparation and preservation methods in Vhembe District Municipality. Maanda and Bhat (2010) reported vegetables used as the food source in Venda. Nesamvuni *et al.* (2001) investigated the use and nutrient composition of edible wild plants commonly used in Venda. Similar to this study, indigenous vegetables such as *Sonchus oleraceus, Biden pilosa, Momordica balsamina, Momordica foetida, Cleome gynandra, Corchorus tridens, Amaranthus dubius*, and *Solanum retroflexum* were recorded in the studies mentioned above. This shows that these indigenous vegetables have multiple uses. Vegetables such as *Adenia digitata, Adenia gummifera, Cucumis anguria, Cucumis africanus, Datura ferox, Oxygonum dregeanum, Pouzolzia parasitic*, and *Pyrenacantha kaurabassana* were recorded for the first time in the present study. This may be attributed to the fact that they are not commonly found in areas where those previous studies were conducted.

Cucurbitaceae was the dominant family, accounting for 21% of the documented species, followed by *Urticaceae* with 13% (Figure 2.2). This coincides with the study by Mokganya and Tshisikhawe (2019) with Cucurbitaceae reported as a dominant family. Fruits from the species of the family Cucurbitaceae are rich in essential constituents vital for human and animal health (Shrivastava and Roy, 2013). They also contain excellent medicinal properties such as anti-HIV, antipyretic, anti-diarrhoeal, antioxidant, antidiabetic, antibacterial, anthelmintic, and hepatoprotective activities (Rajasree *et al.,* 2016; Ajuru and Nmom, 2017; Osuagwu and Edeoga, 2014; Saboo *et al.,* 2013). Species in Cucurbitaceae family are a good source of potassium, fibre, calories, and vitamins A, K, and C (Chunduri, 2013; Deyo and O'Malley, 2008). Species from the family Urticaceae contain various biological activities such as antihypertensive, anthelmintic, diuretic, antidiabetic, antiviral, and immune-modulatory properties (Ibrahim *et al.*, 2018).

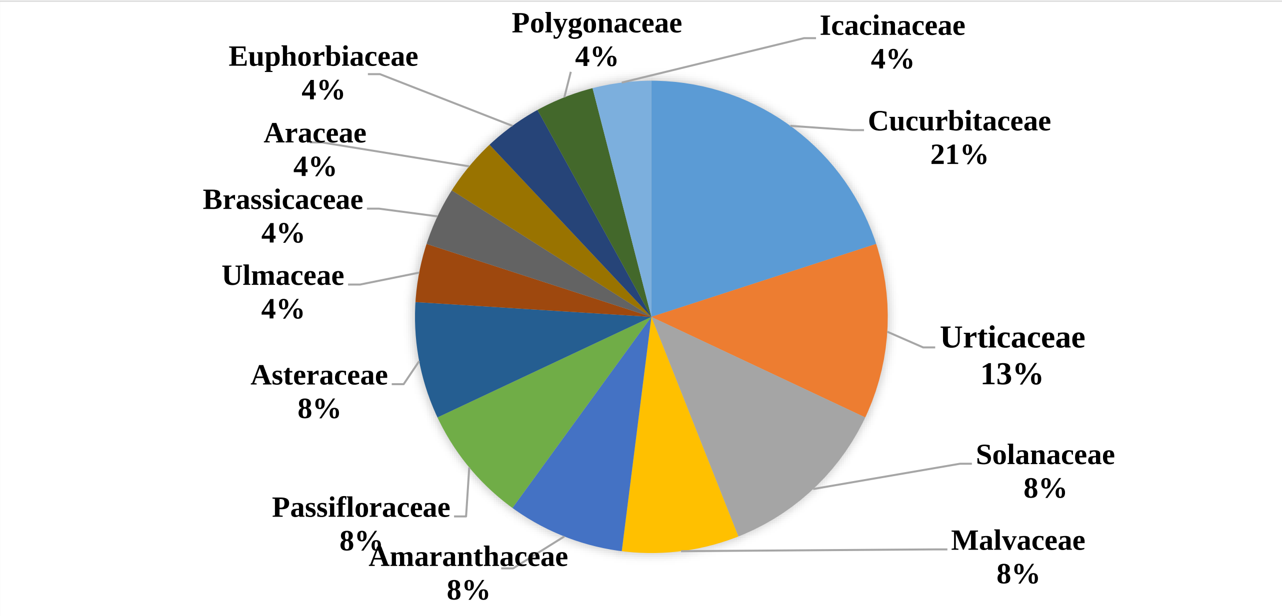

Figure 2.2. Families of the documented plant species.

2.3.3. Plant life forms

Most of the documented vegetables were herbs (20), followed by climbers (2) (Figure 2.3 and Table 2.1). Most indigenous vegetables studies reported herbs as the most dominant plant life form (Mercy *et al.*, 2017; Bvenura and Afolayan, 2014; Abubakar *et al.*, 2012; Mokganya and Tshisikhawe, 2019; Dansi *et al.*, 2008). Herbs have gained popularity due to their culinary and therapeutic uses (Singab and Eldahshan, 2015).

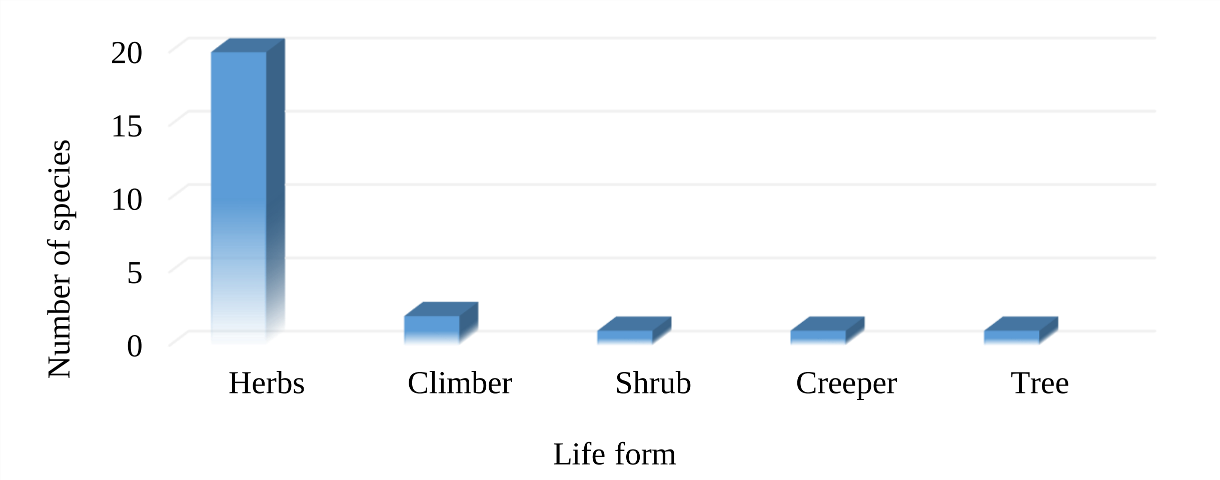

Figure 2.3. Life forms of vegetables documented in Thulamela Local Municipality.

2.3.4. Plant part used

The present study revealed that leaves were the used plant part of these vegetables (Table 2.1). This study concurred with previous studies conducted by various researchers (Shosan *et al*., 2014; Joseph *et al*., 2020; Amjad *et al*., 2015; Asowata-Ayodele *et al*., 2016). Leaves are the cheapest and most abundant part of the vegetables (Fasuyi, 2006). Leaves are the site for synthesising organic substances that have potency in treating diseases and produce many specific nutrients (Al-Yousef *et al*., 2017; Olivier *et al*., 2016; Afolayan and Wintora, 2014). The use of leaves more than other plant parts is beneficial for the conservation of the plant as the abundant removal of leaves does not affect the plant's survival (Lee *et al*., 2019).

2.3.5. Cited non-communicable diseases

The most cited NCDs were hypertension and diabetes (Figure 2.4), each having at least ten vegetables managing them (Table 2.1). These are the dominant NCDs that affect most people around the world and burdens the health system (Oni *et al*., 2015). According to the WHO (2013), "NCDs can be prevented and treated if correct and effective precautions are followed" (p. 7). They can be treated using modern drugs or herbs that have been part of humankind diet from the dawn of time.

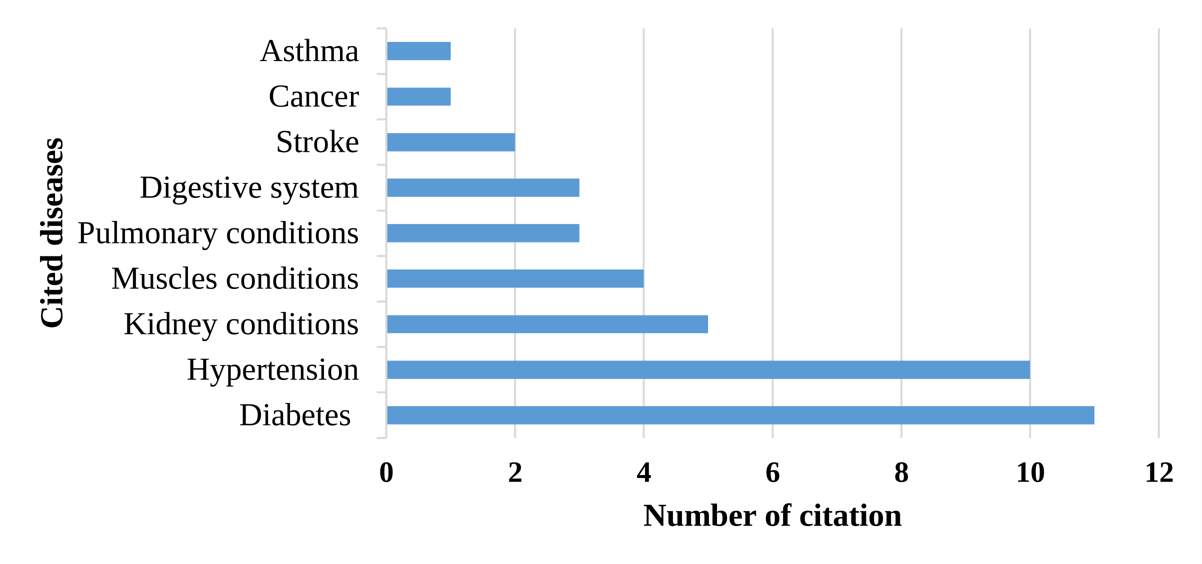

Figure 2.4. Diseases cited during the survey.

2.3.6. Method of preparation and traditional medicinal uses of documented species

The documented vegetables are administered in cooked form and eaten as potherbs. The decoction from leaves is taken orally. These vegetables are prepared on their own, and some are cooked combined. For example, *Amaranthus dubius, Cleome gynandra, Adenia digitata, Adenia gummifera, Solanum retroflexum* and *Celtis africana* are prepared individually. In contrast, *Momordica foetida* and *Corchorus tridens, Momordica balsamina* and *Datura forex, Citrullus colocynthis* and *Corchorus tridens* are prepared in combination. These preparation methods are standard when preparing indigenous vegetables. Kwinana-Mandindi (2014) noted that cooking vegetables in combination contribute to several health benefits as vegetables can synergistically complement each other nutritionally and medicinally. Leaves of *Amaranthus dubius* are cooked and eaten as potherbs to treat fever and kidney stones, and stomach aches (Adebayo and Amoo, 2019; Alegbejo, 2013).

The decoction from the leaves of *Biden pilosa*, *Momordica foetida* and *Momordica balsamina* is taken orally to stabilize hypertension and diabetes (Table 2.1). In the current study, *B. pilosa* was used to treat diabetes and hypertension. Other researchers have reported the use of *B. pilosa* to treat influenza, diabetes, gastroenteritis, hypertension, symptoms of malaria, and fever (Afolayan and Sunmonu, 2010; Ashafa and Afolayan, 2009). The use of *M. foetida* and *M. balsamina* documented in the present study concur with the data obtained by Mokganya and Tshisikhawe (2019) that they are used to stabilize blood and sugar levels. Several authors reported that *M. foetida* and *M. balsamina* are used to treat diabetes and diseases such as rheumatoid arthritis, neurodegeneration, coughing, stomachache, and hypertension (Oloyede and Aluko, 2012; Semenya *et al.*, 2012; Waako *et al.*, 2005).

The leaves of *Cleome gynandra* are cooked as potherbs and used to treat diabetes. This aligns with the study conducted by Mishra *et al.* (2011), who indicated that *C. gynandra* protects the body from diabetes and various diseases such as carcinogenesis, cardiovascular, renal disorders, diabetes, and ulcers. Furthermore, it is rich in biological activities such as antimalaria, antimicrobial (Onyango *et al.*, 2013), anti-inflammatory, anticancer (Moyo *et al.*, 2018), and antioxidant properties (Sowunmi and Afolayan, 2015).

The leaves of *Corchorus tridens* and *Abelmoschus esculentus* are cooked as potherbs (Table 2.1) to treat muscle joint pain and skeletal muscles, while *Abelmoschus esculentus* is additionally used to treat diabetes. These results agree with the information reported by Amin (2011) that *Abelmoschus esculentus* is used to manage diabetes. Mucilage from this vegetable effectively cleans away toxic substances and bad cholesterol (Jain *et al.*, 2012). It is also an effective remedy for ulcers and joint healthiness (Gemede *et al.*, 2015; Jain *et al.*, 2012).

The decoction from *Solanum retroflexum* leaves is taken orally, and the leaves are also cooked as potherbs. The current study revealed that this vegetable is used to treat hypertension. Särkinen *et al.* (2018) and Umair *et al.* (2017) reported that *S. retroflexum* is used to treat cuts and wounds, breast cancer, chickenpox, fever, diarrhoea, ulcer, piles, cardiac pain, diabetes, and sore eyes. Mampholo *et al*. (2016) stated that it is used for stomach pains, duodenal ulcers, swollen glands, and tooth problems. Leaves are also used to treat earache in children (Mokganya and Tshisikhawe, 2019, Umair *et al.*, 2017).

Table 2.1: Inventory of underutilized indigenous vegetables used to prevent and/or treat non-communicable diseases in Thulamela Local Municipality in Limpopo Province, South Africa.

Scientific names and Families	Local names and common names	Voucher specimen # and life form	Frequency index (%)	Non-communicable diseases	Plant part used and administration method	Previously reported medicinal uses	Previously reported biological activities
***Abelmoschus esculentus* (L.) Moench. Malvaceae**	Delele Mandande (V), Lady's finger (E)	MC12 Herb	75%	Muscles joints pain, diabetes and kidney stones	Leaves. They are cooked and eaten as potherbs.	Reduce chronic diseases, diarrhoea, diuretic effect, treat dysentery, diabetes ulcer and joints (Chowdhury *et al.*, 2019; Durazoo *et al.*, 2019; Sindhu and Puri, 2016; Gemede et al., 2014; Roy *et al.*, 2014).	Antioxidant, antidiabetic, antispasmodic, anticancer, antibacterial, antifatigue, anti-inflammatory and antifungal (Chowdhury *et al.*, 2019; Durazoo *et al.*, 2018; Ayushi *et al.*, 2016; Sindhu and Puri, 2016; Caluete *et al.*, 2015; Roy *et al.*, 2014 Gemede *et al.*, 2014).
***Achyranthes aspera* L. Amaranthaceae**	Mukuluvhali (V), Chaff flower(E)	MC04 Herb	63%	Digestive system and hypertension.	Leaves. They are cooked and eaten as potherbs.	Treat pneumonia, mild astringent in bowel complaints, diarrhoea and dysentery (Vijayaraj and Vidhya, 2016) Treat chest pain, stomach aches and cough (Adebayo and Amoo, 2019).	Antiviral, antidiabetic, anti-inflammatory, antimicrobial, anti-parasitic, anti-allergic and antioxidant (Vijayaraj and Vidhya, 2016).
***Adenia digitata* Burtt Davy Passifloraceae**	Dundu (V), Wild granadilla(E)	MC06 Climber	38%	Cancer.	Leaves. They are cooked and eaten as potherbs.	No information	No information

***Adenia gummifera* Harms Passifloraceae**	Belea (V), Monkey rope (E)	MC15 Climber	38%	Hypertension.	Leaves. They are cooked and eaten as potherbs.	No information	No information
***Amaranthus dubius* Mart. Ex Thell Amaranthaceae**	Vowa (V), Spleen amaranth (E)	MC17 Herb	94%	Kidney stones.	Leaves. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Fever and kidney problems (Adebayo and Amoo, 2019). Used for children and lactating mothers, treat haemorrhage, anaemia and stomach ache (Alegbejo, 2013).	No information
***Bidens pilosa.* Asteraceae**	Mushinzhi (V), Blackjack (E)	MC01 Herb	94%	Hypertension and diabetes.	Leaves. Decoction of leaves is taken orally to manage diabetes and hypertension. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Treat inflammation and tumours (Borges *et al.*, 2013). Glandular sclerosis, wounds, colds and flu, chronic hepatitis and urinary tract infections (Arthur *et al.*, 2012; Sundararajan *et al.*, 2006).	Antimicrobial, diuretic, hypotensive activities, anticancer and antipyretic (Alikwe *et al.*, 2014; Sundararajan *et al.*, 2006). Antitumor, anti-inflammatory antidiabetic and anti-hyperglycemic, antioxidant, immunomodulator y, antibacterial, antimalarial, antifungal and antihypertensive (Bartolome *et al.*, 2013)
***Celtis africana* Burm.f. Ulmaceae**	Luvhambo (V), White stinkwood (E)	MC08 Tree	78%	Unblock coronary arteries	Leaves. They are cooked and eaten as potherbs.	Treatment of cancer indigestion and edema (Perveen *et al.*, 2011).	Antioxidant activity (Adedapo *et al.*, 2009; Perveen *et al.*, 2011). Anti-inflammatory

							(Borquaye *et al*., 2020).
***Citrullus colocynthis (L.) Schrad.* Cucurbitaceae**	Mutshatsha (V), bitter apple, bitter cucumber and bitter gourd (E)	MC21 Herb	84%	Hypertension, diabetes, stroke and kidney stones	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Diabetes, leprosy, common cold, cough, asthma, bronchitis, jaundice, joint pain, cancer, toothache, wounds, Hypertension, dermatological problem, gynaecological, urinary and pulmonary infections. (Gupta *et al.*, 2018; Teixeira da Silva and Hussain, 2017; Uma and Sekar 2014; Benariba *et al.*, 2013). Carcinoma, endothelioma, leukaemia, purgative, rheumatism and snakebite (Najafi *et al.*, 2010).	Anti-inflammatory; Anti-candidal and antibacterial; anticancer, anti-lipidemic, antifertility, antimicrobial, cytotoxic, insecticidal antidiabetic and anti-tumour (Pravin *et al.*, 2013; Faisal *et al*., 2018; Teixeira da Silva and Hussain, 2017)
***Cleome gynandra* L. Brassicaceae**	Murudi (V), Stinkweed (E)	MC03 Herb	88%	Diabetes.	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Rheumatism, piles, malaria, headache, fever, prevent sepis on wounds (Sowunmi and Afolayan, 2015). Prevent blood loss and treat anaemia. (Mokganya and Tshisikhawe, 2019).	Antidiabetic and antioxidant activity (Mishra *et al*., 2011). Anticancer, antibacterial, anti-inflammatory, analgesic, antidiarrheal and antimalarial (Abdullah *et al*., 2016).

***Colocasia esculenta* (L.) Schott. Araceae**	Mufhongwe	MC13 Herb	50%	Pulmonary congestion and hypertension.	Leaves. They are cooked and eaten as potherbs.	It is used to treat liver ailments, stomatitis, haemorrhoids and constipation (Azubike *et al.*, 2017). Promote menstruation, relieve stomach problems, and treat cysts (Agyare *et al.*, 2016). High blood pressure, ulcer and pulmonary congestion (Vasant *et al.*, 2012).	Anti-inflammatory, anti-h hypertensive, anti-hepatotoxic, antifungal, antibacterial antidiabetic, hypoglycemic, hypolipidemic, antioxidant and anti-cancer activities (Chinonyelum *et al.*, 2017; Vasant *et al.*, 2012).
***Corchorus tridens* L. Malvaceae**	Delele (V), Wild jute (E)	MC19 Herb	89%	Muscle joints and skeletal muscles.	Leaves. They are cooked and eaten as potherbs.	Medicine for fever, anemia, piles, asthma,stomach disorder, (Nyadanu *et al.*, 2017).Treatment of heart disease, gonorrhea, toothache fever, diabetes (Ngomuo *et al.*, 2017)	Antioxidant, antibacterial, antidiabetic (Barku *et al.*, 2014).
***Cucumis africanus* L.f. Cucurbitaceae**	Tshinyagu (V), African wild cucumber (E)	MC11 Herb	84%	Hypertension and diabetes.	Leaves. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Manage obesity and wound healing (Abifarin *et al.*, 2019).	Antioxidant activity (Abifarin *et al.*, 2019). anti-inflammatory (Abifarin et al., 2020)
***Cucumis anguria* L. var. longaculeatus J.H.Kirkbr. Cucurbitaceae**	Muthangavhavhe (V), Gooseberry gourd gherkin (E)	MC25 Herb	63%	Kidney stones	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Stomach ache, jaundice, hemorrhoids, and prevent stone formation in the kidney (Thiruvengadam and Chung, 2012).	Antioxidant and antimicrobial activity (Thiruvengadam and Chung, 2015; Yoon *et al.*, 2015). Anticancer (Chung *et al.*, 2018).

***Datura ferox* Nees Solanaceae**	Zavhazavha (V), Jimson weed, Prickly apple (E)	MC18 Herb	69%	Asthma.	Leaves. They are cooked and eaten as potherbs.	Malaria, asthma, hepatitis, dermatitis, diuretic and rheumatism (Çakir *et al.*, 2014).	Antioxidant, antibacterial and antifungal activities (Çakir *et al.*, 2014).
***Manihot esculenta.* Subsp. *Peruviana* Euphorbiaceae**	Mutumbula	MC22 Herb	71%	Diabetes.	Leaves. They are cooked and eaten as potherbs.	Ringworms, tumour, sores, fever, headache, diarrhoea, snakebite, diabetes (Nwose *et al.*, 2017; Bahekar and kale, 2013)	Anti-hemorrhoid, anti-inflammatory, antimicrobial (Bahekar and kale, 2013).
***Momordica balsamina* Wall Cucurbitaceae**	Tshibavhe (V), African cucumber (E)	MC14 Herb	90%	Diabetes.	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Treat wounds, stomachic, fevers, yaws, skin diseases and relieve period pain (Thakur *et al.*, 2009).	Anti-HIV, antioxidant, analgesic, anti-diabetic, anti-bacterial and anti-viral (Thakur *et al.*, 2009).
***Momordica charantia* Cucurbitaceae**	Nngutshiphaswi, lugulusekene (V) balsam pear bitter melon, bitter cucumber and African cucumber (E)	MC25 Herb	90%	Hypertension and diabetes.	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Treat cancer, diabetes (Daniel *et al.*, 2014; Ahmad *et al.*, 2016). Treat dysentery, rheumatism and gout (Upadhyay *et al.*, 2015).	Anti-fungal, anti-viral and anti-tumorous (Ahmad *et al.*, 2016). Antidiabetic activity, anti-cancer activity and antidepressant activity (Kumar *et al.*, 2011; Joseph and Jini, 2013).
***Momordica foetida* Schumach. Cucurbitaceae**	Nngu khulwane/Mukake (V), African wild cucumber (E)	MC16 Herb	93%	Hypertension and diabetes.	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Malaria, hypertension, peptic ulcer, diabetes mellitus and fever (Acquaviva *et al.*, 2013; Froelich *et al.*, 2007).	Antioxidant (Acquaviva *et al.*, 2013; Molehin and Adefegha, 2013).

Oxygonum dregeanum **Meisn. Polygonaceae**	Mutanye/Tanye	MC23 Herb	79%	Hypertension and diabetes.	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Malaria and fever (Nafuka, 2014).	Antiplasmodial activity (Nafuka, 2014).
Pouzolzia mixta **Solms var shirensis Urticaceae**	Mutanzwa (V), Soap nettle (E)	MC07 Shrub	88%	Digestive system and kidney diseases.	Leaves. They are cooked and eaten as potherbs.	Treat painful uterus, the expulsion of retained placenta, infertility, venereal disease, and dilate birth canal (Sewani-rusiki, 2013).	
Pouzolzia parasitica **(Forssk.) Schweinf. Urticaceae**	Makhuluadzaluma/ Gwikwitimba (V), Small stinging nettle (E)	MC10 Herb	89%	Muscles joint pain.	Leaves. They are cooked and eaten as potherbs	Treat lymphadenitis (Giday *et al.*, 2009).	No information
Pyrenacantha kaurabassana **Baill. Icacinaceae**	Galange	MC09 Creeper	54%	Stroke.	Leaves. They are cooked and eaten as potherbs.	Treat genital warts De Wet *et al.*, 2012.	No information
Solanum retroflexum **Dunal Solanaceae**	Muxe (V), Wonderberry (E)	MC20 Herb	88%	Hypertension. *Arachis hypogaea*	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia emetic* are added as condiments.	Stomach pains, duodenal ulcers, and swollen glands and for treatment of teeth problems (Mampholo *et al.*, 2016).	Antioxidant activity Managa *et al.*, 2020.
Sonchus oleraceus **L. (L.) Asteraceae**	Shashe (V), Common sow thistle (E)	MC02 Herb	88%	Diabetes and digestive system. *Arachis hypogaea*	Leaves. Decoction of leaves is taken orally. They are cooked and eaten as potherbs. Roasted seeds of *Cucurbita pepo*, kernels of *Sclerocarya birrea*, *Arachis hypogaea*, and *Trichilia*	Wounds and burns, gastrointestinal infection, inflammation, diabetes and cardiac dysfunction, kidney and liver disorders (Mallik *et al.*, 2014; Khan *et al.*, 2010).	Antioxidant, anticancer and anti-inflammatory (Khan *et al.*, 2010).

					emetic are added as condiments.		
Urtica dioica **L.** **Urticaceae**	Dzaluma (V), Stinging nettle (E)	MC03 Herb	76%	Pulmonary activator and muscle joint pains.	Leaves. They are cooked and eaten as potherbs.	Blood purifier, diuretic, nasal and menstrual rheumatism, eczema, anaemia, nephritis, haematuria, jaundice, menorrhagia and vaginal discharge (Joshi *et al.*, 2014; Kukrić *et al.*, 2012)	Antibacterial, antioxidant, analgesic, anti-inflammatory, antiviral, immunomodulatory, hepatoprotective, anti-colitis and anticancer effects (Joshi *et al.*, 2014).

2.3.7. Condiments

When underutilized indigenous vegetables are prepared as potherbs, some condiments are added to enhance their taste and medicinal properties. Condiments may be made from the fruit and roasted seeds of *Cucurbita pepo,* kernels of *Sclerocarya birrea, Arachis hypogaea, Trichilia emetic*, to mention a few. Condiments are added to vegetables such as *Amaranthus dubius, Biden pilosa, Citrullus colocynthis, Cleome gynandra, Cucumis africanus, Cucumis anguria, Momordica foetida, Momordica balsamina, Momordica charantia, Oxygonum dregeanum, Solanum retroflexum* and *Sonchus oleraceus,* (Table 2.1). These condiments contain medicinal properties that activate the vegetables to become more effective in combating diseases. The fruits of *Cucurbita pepo* relieve intestinal inflammation, increase appetite, cure leprosy, and purify the blood (Saboo *et al.*, 2013), and the seeds are used to treat hypertension and prevent the formation of kidney stones (Adnan *et al.*, 2017; Bardaa *et al.*, 2016; Gutierrez, 2016). The seeds of *Arachis hypogaea* are used to prevent cardiovascular disease, maintain cholesterol and sugar levels, improve brain power and memory, clear skin, and have anti-ageing benefits (Sigh and Kunwar, 2018; Settalurin *et al.*, 2012). *Sclerocarya birrea* nuts add a piquant flavour to food dishes. The kernels are rich in oil that contain amino acids, fatty acids, antioxidants, and protein (Mariod *et al.*, 2012). The seeds of *Trichilia emetica* are rich in fats that add flavour to vegetable dishes (Adinew, 2014; Komane *et al.*, 2011).

2.3.8. Preservation

The results revealed that these vegetables grow in abundance during summer; therefore, there are ways to preserve them to be available in lean times and other seasons. These vegetables are preserved by drying in the sun and shade on empty sacks, flat stones, bamboo branches, and straight wood from trees used for fire. The drying methods vary as some are dried cooked, e.g., *Amaranthus dubius* is cooked with *Biden pilosa*, *Cleome gynandra* and *Solanum retroflexum*. They are moulded into round shapes, aligned on flattened empty sacks, flat stones, bamboo branches or straight wood and dried in the sun. They are dried for three to five days, depending on the weather. Some vegetables are dried raw in the shade, e.g., *Corchorus tridens, Momordica foetida, Momordica balsamina,* and *Momordica charantia*. They are shade-dried to prevent some nutrients from 'fading' and preserve the green colour. Sun-drying is the oldest method that has been used since ancient times, and it is affordable (Afolabi, 2014; Masarirambi *et al.*, 2010).

2.4. Botanical description of selected underutilized indigenous vegetables

Six underutilized indigenous vegetables were selected based on their frequency index, availability, and traditional use in managing non-communicable diseases. The selected species were evaluated for their nutritional and biological activities. The selected vegetables are mainly herbs, i.e., *Citrullus colocynthis, Cleome gynandra, Cucumis africanus, Oxygonum dregeanum, Pouzolzia mixta, and Sonchus oleraceus*. Below is a short description of each vegetable.

Citrullus colocynthis* (*L.*) *Schrad.* Bitter cucumber (English), Mutshatsha (Venda), *Cucurbitaceae

Citrullus colocynthis (Figure 2.5) is a trailing perennial herb. It is found in sandy soil and mountainous areas in Limpopo and North West provinces in South Africa. Leaves have a deltoid margin, the pale green colour above, ashy colour beneath and scabrid on both surfaces. Furthermore, leaves have a rough, hairy texture. Fruits are rounded, green in colour and white glabrous when ripe. Fruits are filled with dry, spongy, bitter pulp (Hussain *et al.*, 2014; Pravin, 2013).

Figure 2.5. Leaves of *C. colocynthis* (Picture taken at Duthuni, January 2020

Cleome gynandra* L. Stinkweed (English), Murudi (Venda), *Brassicaceae

Cleome gynandra (Figure 2.6) is an erect herb that grows 250-600 mm tall. It is more branched, and becomes woody with age. Leaves are palmately compound with 3-5 leaflets. Leaflets taste bitter and radiate from the tip of the leaf stalk. The fruits are long-stalked, dry, dehiscent siliques. The stem is sticky with glandular hairs and marked with parallel lines. It is usually found in open areas, uncultivated land, wasteland and arable land. It grows well in dry or moist soil in a humid climate and full sunlight (Sango *et al.*, 2016). This vegetable occurs throughout the tropics and subtropics of Africa. In South Africa, it is found in Limpopo, North West, Gauteng, Mpumalanga, KwaZulu-Natal, Free State and Northern Cape provinces (Foden and Potter, 2005; Essou *et al.*, 2017).

Figure 2.6. Leaves of *C. gynandra* (Picture taken at Thohoyandou, January 2020).

Cucumis africanus **L.f. Wild cucumber (English*)*, Tshinyagu (Venda),** ***Cucurbitaceae***

Cucumis africanus (Figure 2.7) is a perennial herb with rootstock thick and woody. This vegetable is found in the woodlands of Angola, Zimbabwe, Namibia and South Africa (Abifarin *et al.*, 2019). In South Africa, it is found in Limpopo, North West, Gauteng, Mpumalanga, KwaZulu-Natal, Northern, Western and Eastern Cape provinces. It grows on deep and well-drained soils such as white, red and brown sand, grey or red loam and gravely or stony ground. It is also found on saline soils with underlying limestone formations and blackish clay soil (Foden and Potter, 2005).

Figure 2.7. Leaves of *C. africanus* (picture taken at Duthuni, January 2020).

Oxygonum dregeanum* Meisn. Mutanye/tanye (Venda), *Polygonaceae

Oxygonum dregeanum (Figure 2.8) is an erect perennial herb that grows up to 50 cm. leaves are slightly succulent, long and narrow. In Africa, this vegetable is found in Angola, Mozambique, Zambia, Botswana and South Africa. In South Africa, it is found in KwaZulu-Natal, Limpopo and Mpumalanga provinces. It grows in open grassland, rocky mountain grassland and wooded grassland (von Staden, 2015; Hyde *et al.*, 2021).

Figure 2.8. Leaves of *O. dregeanum* (Picture taken at Tshaulu, March 2020).

Pouzolzia mixta* Solms var. mixta, Soap nettle (English), Mutanzwa (Venda), *Urticaceae

Pouzolzia mixta (Figure 2.9) is a multi-stemmed shrub with conspicuously bicoloured leaves spirally arranged with smooth margins. The bark is dark reddish-brown and smooth, and branchlets have a velvety surface. The leaves are simple, spirally arranged and ovate with a tapering apex and lobed base. The leaves have contrasting upper and lower textures and tend to stick together and to clothing. The upper surface is dark green, while the lower is silvery white-felted. It is found in Swaziland, Botswana, Zimbabwe, Malawi and South Africa. In South Africa, it is found in Mpumalanga, KwaZulu-Natal, Gauteng, North West and Limpopo provinces (Sewani-Rusike, 2013). It grows on a rocky hillside, in open woodland, wooded grassland and along riparian thickets in bushveld (Foden and Potter, 2005).

Figure 2.9. Leaves of *P. mixta* (picture taken at Duthuni, March 2020).

Sonchus oleraceus* var asper, Common sow thistle (English), Shashe (Venda), *Asteraceae

Sonchus oleraceus (Figure 2.10) is an annual herb and a noxious winter weed that grows up to 1.2 m. The leaves are lanceolate to oblong in shape, hairless and no spikes, and dark green with pale white to purple veins. Young leaves are short-stalked, partially lobed, while old leaves are long-stalked and deeply lobed. The stem is green with a reddish-purple touch, hollow, and releases milky sap when cut. It grows annually in various habitats such as orchards, farmland, roadsides, pastures, gardens and fields. It grows in different soil conditions and prefers full sun (Alrekabi and Hamad, 2018). It is native in Europe and is widely spread worldwide in North America, South America, Africa, Asia, Australia and New Zealand (Gomaa and Hassan, 2014; Puri *et al.*, 2018).

Figure 2.10. Leaves of *S. oleraceus* (Picture taken at Thohoyandou, February 2020).

2.5. Conclusion

The results from this study reported underutilized indigenous vegetables that are primarily used as source of food and manage some non-communicable diseases. Out of the 25 documented underutilized indigenous vegetables, 17 species were reported by other authors for treating diseases other than non-communicable diseases. However, *Adenia digitata, Adenia gummifera, Cucumis anguria, Cucumis africanus, Datura ferox, Oxygonum dregeanum, Pouzolzia parasitic,* and *Pyrenacantha kaurabassana* were reported for the first time for treatment of non-communicable diseases. Therefore, this study has added valuable information about the medicinal values of underutilized indigenous vegetables. Additionally, the documentation of the use of these species has the potential for preserving indigenous knowledge on underutilized wild vegetables that are highly consumed for management of non-communicable diseases in the Thulamela Local Municipality. The documented vegetables should be investigated further for their nutritional content and biological effects, such as antioxidant and anti-diabetic activities, for their valorisation. This may serve as a lead in developing new ways to encourage the younger generation and residents from other communities to include these vegetables in their dishes to combat non-communicable diseases.

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Chapter 3

Nutritional analysis of six selected underutilized indigenous vegetables documented in Thulamela Local Municipality, South Africa.

Abstract

Evaluation of nutrient content of underutilized indigenous vegetables could be an effective tool to support their traditional use as sources of food and medicine. This study evaluated the nutritional content from leaves of *Cucumis colocynthis* (L.) Schrad., *Cleome gynandra* L., *Cucumis africanus* L.f., *Oxygonum dregeanum* Meisn., *Pouzolzia mixta* Solms var. mixta, and *Sonchus oleraceus* var asper, consumed to manage non-communicable diseases in Thulamela Local Municpality, South Africa. Various methods, including HPLC, were used to evaluate the content of ascorbic acid, β-carotene, calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), and zinc (Zn). The results indicated that *Cucumis africanus* had the highest ascorbic acid (38.02 mg/100 g dry weight), Ca (5766.66 mg/100 g), Fe (131.40 mg/100 g) and Mg (1939.33 mg/100 g) contents in comparison with the other vegetables. *Sonchus oleraceus* had the highest β-carotene, Cu and K contents. The highest Mn and Zn contents were found in *C. colocynthis.* The results demonstrated that these vegetables are excellent sources of vital nutrients. Therefore, their consumption could contribute to diet diversity for combating non-communicable diseases and nutrient deficiency-related ailments.

Keywords: Underutilized indigenous vegetables, Nutritional content, Non-communicable diseases, Nutrients deficiency diseases.

3.1. Introduction

The prevalence of non-communicable diseases such as diabetes, cardiovascular diseases, hypertension and cancer presents a global health crisis (Gowshall and Taylor-Robinson, 2018). In Africa, the prevalence of these diseases in Africa is increasing rapidly and may account for 46% death by 2030 (Bolarinwa *et al.*, 2020; Mayosi *et al.*, 2009). One of the causal factors of non-communicable diseases is a change in diet. This is the transition from consuming traditional foods to consuming processed foods, more foods of animal origin, with more added sugar, salt and fat and more energy-dense (Spires *et al.*, 2016). This behaviour has resulted in the prevalence of non-communicable diseases worldwide. This issue can be mended by transitioning back to traditional foods such as underutilized indigenous vegetables rich in nutrients needed by the body to function correctly (Van der Hoeven *et al.*, 2016).

Underutilized indigenous vegetables are plants whose leaves or aerial parts have been incorporated into the local food culture over a large span, and are consumed raw or cooked (Ayanwale *et al.*, 2016). These plant species grow in the wild, semi-cultivated and cultivated areas. They are included in plant groups sought as part of the diet and for therapeutic purposes (Kisangau, 2017). Furthermore, they are the cheapest source of valuable nutrient composition due to their easy availability (Maseko *et al.*, 2018; Kisangau, 2017). The rich nutritional content of the vegetables helps maintain good health and prevent diseases (Kamga *et al.*, 2013). The World Health Organisation (WHO) has led the way in developing a solution for non-communicable diseases. The consumption of indigenous vegetables was one of the methods suggested to decrease the growing prevalence of non-communicable diseases (Branca *et al.*, 2019).

People from Limpopo Province (South Africa) have discovered various underutilized indigenous vegetables to supplement their diet from time immemorial (Mbhenyane *et al.*, 2013). These vegetables are eaten as main dishes, relish to starchy staple foods, and supporting foods. In addition, they contribute to food security by providing alternative options to common vegetables and help balance the diet by adding diversity to the menu to make it healthier and enjoyable. It is vital to consume a variety of vegetables in one dish because a single vegetable does not usually have all the nutrients required by the body (Kamga *et al.*, 2013; Usunobun and Okolie, 2015).

This study aimed to assess the nutritional properties of six selected underutilized indigenous vegetables consumed to manage non-communicable diseases. Finding solutions by consumption of indigenous vegetables is a powerful tool in maintaining and enhancing health and nutritional status (Essack *et al.*, 2017; Van der Hoeven *et al.*, 2016).

3.2. Materials and methods

3.2.1. Selection, collection and grinding

Six underutilized indigenous vegetables were selected from an ethnobotanical survey (described in Chapter 2) to document indigenous vegetables consumed in the Thulamela Local Municipality, South Africa. Their selection was based on their frequency index and traditional use. The leaves of *Citrullus colocynthis* (L.) Schrad, *Cleome gynandra* L, *Cucumis africanus* L.f., *Oxygonum dregeanum* Meisn, *Pouzolzia mixta* Solms, and *Sonchus oleraceus* L. (L.) were collected from their natural habitats in Duthuni, Thohoyandou, and Tshaulu villages in

Thulamela Local Municipality, Limpopo Province, South Africa. The leaves sampling period was from December 2019 to March 2020. Voucher specimens were prepared and deposited at the University of Venda herbarium. Samples were taken to the lab and washed with water to remove dirt. The vegetables were air-dried in the shade at room temperature. Dried leaves were ground into a fine powder using an electric blender, stored in airtight envelopes and locked in a dark locker until analysed.

3.2.2. Determination of nutrient content

3.2.2.1. Determination of ascorbic acid content

Ascorbic acid was determined using the method described by Odriozola-serrano *et al.* (2007) with minor modifications. The standard solution was prepared by dissolving 10 mg of ascorbic acid in 10 ml of water. After that, 2.5 ml was transferred into a 10 ml volumetric flask to make a working solution. An amount of 0.2 g sample was weighed into the test tube. Ten millilitres metaphosphoric acid, prepared by dissolving 4.5 g of metaphosphoric acid in 100 ml of distilled water was then added to the test tube with sample. The mixture was sonicated for 30 min and centrifuged for 2 min at 2000 rpm. The mixture was filtered into a 10 ml volumetric flask. The extract was transferred into the vial for High-Performance Liquid Chromatography (HPLC) analysis with acetonitrile: water: formic acid (99: 0.9: 0.1) as the mobile phase. The separation was performed using a C_{18} column at 35 ℃, with Pump mode set as isocratic. The injection volume was 20 µl, and the flow rate was set at 1ml/ min. Ascorbic acid was detected at a 245 nm wavelength. The results were expressed as milligrams of ascorbic acid per 100 g sample dry weight (mg/100 g DW). Tests were carried out in triplicates.

3.2.2.2. Determination of beta-carotene content

Beta-carotene was determined using the method described by Biehler *et al.* (2010) with minor modifications. Methanol (5 ml) was added to 0.2 g of the sample. The tubes were vortexed for 5 sec, then 15 ml of 1:1 acetone-hexane was added. Sample mixtures were sonicated for 15 min. Saturated sodium chloride (5 ml) was added to the sample mixtures, followed by vortexing for 5 sec. The tubes were then centrifuged for 2 min at 2000 rpm. The upper hexane layer was transferred into the vial for further analysis using HPLC with these conditions: mobile phase: acetonitrile: DCM: MeOH (7:2:1). The separation was performed using a C_{18} column at 35℃. Pump mode was isocratic. The injection volume was 20 µl, and the flow rate was set at 1ml/min. The wavelength was 450 nm. Tests were carried out in triplicates.

3.2.2.3. Determination of mineral element content

The mineral elements were quantified using an inductively coupled plasma – optical emission spectrometry (ICP-OES) (ICPE-9820, Shimadzu Corporation, Kyoto, Japan) as described by Ang and Lee (2005), with slight modification (Uddin *et al.*, 2016). During the digestion, 0.5 g of the sample was weighed and transferred into the flat bottom flask, and 10 ml of freshly prepared aqua regia (nitric-hydrochloric acid) was added. The mixture was boiled gently over a hot plate for 4–5 h until the aqua regia had evaporated completely. After that, 10 ml of deionized water was added to the flask with the sample, and the mixture was filtered with Whatman 42 (2.5-µm particle retention) filter paper. A sufficient amount of deionized water was added to the filtered supernatant to make a 50 ml final volume.

All analyses were run in triplicates and data subjected to one-way analysis of variance (ANOVA) using Statsoft (Statistica 8) software. Results were reported as mean ± standard error of triplicate analyses. The mean values were compared using Duncan's Multiple Range Test, and the significant difference was P = 0.05.

3.3. Results and discussion

Underutilized indigenous vegetables are an essential part of a balanced diet, and their daily consumption in the right amount helps prevent non-communicable ailments (Imathiu, 2021). Due to this, rural people have resorted to using underutilized indigenous vegetables to treat, cure, manage and prevent non-communicable diseases. Additionally, these vegetables are considered protective foods because they maintain good health and prevent various diseases due to their high nutrients concentrations (Kumar *et al.*, 2020). Nutrients in indigenous vegetables vary due to factors such as maturity, post-harvest handling, soil type and climate. Therefore, dietary diversification is critical to improving food, nutritional security and combating or alleviating nutrient-related diseases (Kumar *et al.*, 2020).

This study evaluated different nutrients content, namely ascorbic acid, β-carotene, Ca, Cu, Fe, Mg, K, Mn, P, and Zn in *Citrullus colocynthis, Cleome gynandra, Cucumis africanus, Oxygonum dregeanum, Pouzolzia mixta,* and *Sonchus oleraceus* (Table 3.1). *Cucumis africanus* exhibited the highest ascorbic acid, Ca, Fe, and Mg content compared to other analysed vegetables. However, S*onchus oleraceus* contained the highest β-carotene, Cu and K content. The concentration of Zn and Mn were significantly higher in *Citrullus colocynthis*. *Cleome gynandra* had the highest phosphorus content than other analysed vegetables (Table 3.1). This indicates that nutrient content varies in these vegetables. Therefore, a diet incorporating different vegetables is vital to provide enough nutrients.

The level of Ca (5766.66 mg/100 g) and Mg (1939.33 mg/100 g) exhibited by *Cucumis africanus* in this study was two times higher than Ca (2974 mg/100g) and Mg (1022 mg/100g) reported in *C. metuliferus* by Odhav *et al.* (2007). Furthermore, the content of Fe (131.40 mg/100g) in *C. africanus* was six times higher as compared to Fe (20 mg/100g) level in *C. metuliferus* reported by Odhav *et al.* (2007).

The nutrients mentioned above play an essential role in the proper functioning of the human body. For example, calcium is vital for the coagulation of blood, the proper functioning of the heart and nervous system and the normal contraction of muscles (Usunomena and Paulinus, 2015). Furthermore, it plays a vital role in forming bones and teeth (Ntuli, 2019; Satter *et al.*, 2016). Iron is an essential component in the formation of haemoglobin. It maintains the normal functioning of the central nervous system and the oxidation of carbohydrates, protein, and fats (Satter *et al.*, 2016). Ascorbic acid is an antioxidant that strengthens the immune system by scavenging free radicals in the body (Jiménez-Aguilar and Grusak, 2017; Hudiyanti *et al.*, 2018; Usunomena and Paulinus, 2015). Mibei *et al.* (2011) stated that a small daily intake of vitamin C boosts the immune system. Therefore, *Cucumis africanus* consumption daily helps balance vitamin C required in the body.

In the present study, S*onchus oleraceus* contains the highest level of β-carotene (4.46 mg/100 g), Cu (5.02 mg/100 g) and K (4026.66 mg/100 g) in comparison to other analysed vegetables (Table 3.1). Similar to our findings, Juhaimi *et al.* (2017) reported *Sonchus oleraceus* as a good source of Cu and K. In this study, β-carotene and Cu contents in *Sonchus oleraceus* are higher than those reported by Oliveira *et al.* (2019) in *Sonchus arvensis* (1.8 $mg.100g^{-1}$ and 1.0 $mg.100g^{-1}$, respectively). The Cu content (5.02 mg/100 g) of *S. oleraceus* recorded in the current study is lower than the content of Cu reported by Jimoh *et al*. (2011) in *S. oleraceus* and *S. asper* found in the same genus.

Beta-carotene is an antioxidant that protects lipids and other biological systems by restricting the activation of oxygen, quenching singlet oxygen and sensitizers, and scavenging peroxyl radicals at low oxygen pressures (Agiang *et al.*, 2017). Potassium is a common electrolyte required for keeping the heart, brain, kidney, muscle tissues, and other vital organs of the human body in good condition (Umerah and Nnam, 2019; Usunobun and Okolie, 2015). Ekmekcioglu *et al.* (2016) indicated that lower potassium intake is associated with a higher risk of being affected by type 2 diabetes. Therefore, the consumption of *Sonchus oleraceus* can help prevent the development of diabetes.

Data obtained in the present study indicate that the leaves of *Citrullus colocynthis* contain the highest content of Zn (8.96 mg/100 g) and Mn (24.53 mg/100 g) compared to other analysed vegetables (Table 3.1). Similarly, the presence of Fe, Zn and P in *Citrullus colocynthis* was reported in a study reviewing the nutritional and medical importance of *Citrullus colocynthis* (Bhasin *et al.*, 2020). Zinc is an integral part of many enzymes in the human body and plays catalytic, structural and regulatory roles. In addition, it promotes wound healing, regulates immune function, and is necessary for protein synthesis (George *et al.*, 2013).

Pregnant and lactation women and children are more vulnerable to zinc deficiency. Zinc deficiency during pregnancy results in increased infections, preeclampsia, miscarriages, low birth weight, neurological malformations and neurological impairment (Narváez-Caicedo *et al.*, 2018, Ackland and Michalczyk, 2016).

The current study revealed that *Cleome gynandra* had the highest P (1467.33 mg/100 g) content (Table 3.1). The high phosphorus content in *Cleome gynandra* was also recently reported by Moyo *et al*. (2018). Phosphorus (P) is a vital mineral found in every body cell as it facilitates metabolic processes and it is an essential component of bone minerals (Asaolu *et al*., 2012). Additionally, it serves as a phosphate buffer and contributes to forming high-energy compounds such as adenosine triphosphate (ATP) (Soetan *et al*., 2010).

The nutritional content of *Pouzolzia mixta* and *Oxygonum dregeanum* were analysed for the first time in this study. Therefore, this study has added valuable information about the nutrient content of these vegetables and their consumption is encouraged. However, *Pouzolzia mixta* and *Oxygonum dregeanum* did not reveal the highest values of minerals evaluated, but they showed a good amount of Ca, Cu. Fe, K, Mg, Mn, P, and Zn nutrients (Table 3.1).

Table 3.1: Nutrient properties (mg/100 g sample dry weight) of analysed vegetables.

Sample name	Ascorbic acid	β-carotene	Ca	Cu	Fe	K	Mg	Mn	P	Zn
Citrullus colocynthis	13.16±0.18[c]	3.30±0.09[bc]	4046.66±40.55[b]	4.22±0.017[c]	70.26±0.86[c]	1081.33±10.72[f]	650.00±7.02[d]	**24.53±0.29[a]**	435.33±4.05[e]	**8.96±0.19[a]**
Cleome gynandra	25.45±0.68[b]	4.24±0.03[a]	3153.33±46.66[d]	4.95±0.28[a]	97.53±0.89[b]	2580.00±30.55[c]	794.66±10.34[b]	5.37±0.05[e]	**1467.33±16.38[a]**	7.41±0.40[b]
Cucumis africanus	**38.02±0.36[a]**	3.62±0.11[b]	**5766.66±48.07[a]**	4.66±0.11[ab]	**131.40±1.33[a]**	1393.33±30.20[d]	**1939.33±19.19[a]**	7.68±0.08[d]	469.33±4.37[d]	6.68±0.04[b]
Oxygonum dregeanum	12.08±0.31[de]	3.27±0.01[c]	1636.66±16.82[f]	4.02±0.13[c]	50.86±0.43[d]	3880.00±75.71[b]	340.66±2.40[e]	3.14±0.041[f]	698.00±6.00[b]	4.86±0.05[c]
Pouzolzia mixta	11.61±0.38[e]	3.52±0.07[bc]	3560.00±34.64[c]	3.95±0.25[c]	15.28±1.27[e]	1312.00±94.11[e]	708.66±53.53[c]	11.61±1.01[b]	477.33±31.67[d]	2.77±0.25[d]
Sonchus oleraceus	9.14±0.06[f]	**4.46±0.19[a]**	2166.66±63.59[e]	**5.02±0.03[a]**	52.53±1.59[d]	**4026.66±107.28[a]**	337.33±7.68[e]	10.18±0.31[c]	628.00±17.08[c]	7.91±0.21[a]

Values are expressed as the mean ± standard error of triplicate analyses. Mean values carrying different superscripts within a column are significantly different at P = 0.05.

Ca=Calcium, Cu=Copper, Fe =Iron, K=Potassium, Mg=Magnesium, Mn= Manganese, P=Phosphorus, Zn=Zinc.

3.4. Conclusion

The analysed underutilized indigenous vegetables showed a significant amount of ascorbic acid, β-carotene, Ca, Cu, Fe, K, Mg, Mn, P and Zn. These support their traditional use as sources of food and traditional medicine to manage non-communicable diseases. These vegetables can contribute valuable nutrients to the human diet. Therefore, their consumption should be promoted and included in the diet of people suffering from non-communicable diseases and nutrient deficiency-related ailments. There is a need to escalate the knowledge of nutrition and health benefits of underutilized indigenous vegetables to the municipality authorities, local health officers and rural communities.

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Chapter 4

Evaluation of antioxidant and antidiabetic potential of six selected underutilized indigenous vegetables used to manage non communicable diseases in Thulamela Local Municipality, South Africa.

Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by high blood sugar levels (hyperglycemia). The current study evaluates phytochemical content, antioxidant and antidiabetic activities of dichloromethane, acetone and water extracts in *Citrullus colocynthis, Cleome gynandra, Cucumis africanus, Oxygonum dregeanum, Pouzolzia mixta* and *Sonchus oleraceus* leaves. Phytochemicals were evaluated using standard qualitative and quantitative methods. The *in vitro* antioxidant activities were assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and beta-carotene/linoleic acid assays. The anti-diabetic activity was evaluated using the alpha-glucosidase enzyme, and acarbose was used as a positive control. Quantitative phytochemical analysis showed that *Citrulus colocynthis* had high total phenolics, flavonoids and condensed tannins compared with other indigenous vegetables tested. In the DPPH method, the acetone and water extracts showed moderate antioxidant activity compared to the positive control. In the beta-carotene-linoleic acid method, the dichloromethane and acetone extracts exhibited high antioxidant activity comparable to the butylated hydroxytoluene (BHT) positive control. Among the analysed vegetables, acetone extract of *Cucumis africanus* exhibited strong alpha-glucosidase inhibition activity with the IC_{50} value of 164.40 µg/ml. Dichloromethane extract of *Citrulus colocynthis* exhibited moderate inhibition activity, with the IC_{50} value of 309.97 µg/ml compared to the acarbose (positive control) wih IC_{50} values of 240.10 µg/ml. *Citrullus colocynthis*, *Sonchus oleraceus*, *Oxygonum dregeanum* antioxidant properties and *Cucumis africanus*, *Citrullus colocynthis*, *Pouzolzia mixta*, *Sonchus oleraceus* anti-diabetic activities were related to the presence of phytochemicals such as total phenolics, flavonoids, and condensed tannins. These results provide a modern scientific rationale, albeit *in vitro*, for their traditional use in managing diabetes and other non-communicable diseases.

Keywords: Underutilized indigenous vegetables, Diabetes mellitus, Antioxidant activity, Phytochemicals, Alpha-glucosidase.

4.1. Introduction

Diabetes is a metabolic disorder that affects the metabolism of carbohydrates, fats and protein. It is characterized by elevated glucose levels in the blood due to the pancreas' inadequate insulin production and usage (Hammeso *et al*., 2019; Asmat *et al.*, 2016). The effects of diabetes mellitus include long-term complications such as heart disease, stroke, dysfunction and failure of various organs and some secondary complications including nephropathy, retinopathy and neuropathy (Banerjee *et al*., 2017; Dewi and Maryani, 2015).

Diabetes is linked to a high risk of morbidity, mortality and burden to healthcare worldwide. The total number of people with diabetes worldwide is projected to rise to 366 million by 2030 (Shilubane *et al.*, 2016). The World Health Organisation (WHO) has predicted that the significant burden of diabetes mellitus will occur in developing countries. The number of adults with diabetes mellitus in sub-Saharan Africa (SSA) is projected to rise to 41.5 million by 2035 (Bailey *et al.*, 2016). Morris-Paxton *et al.* (2018) reported that 20 million people in South Africa are affected by diabetes, and 7% are adults.

There are two enzymes: α-glucosidase and α-amylase, amongst others, which are targeted in developing conventional treatment of type 2 diabetes. Alpha-amylase hydrolyzes complex starches to oligosaccharides, while alpha-glucosidase hydrolyzes oligosaccharides to glucose and other monosaccharides. Inhibition of these enzymes produces a postprandial anti-hyperglycemic effect by reducing the rate and extent of glucose absorption (Rege and Chowdhary, 2014). The decrease of postprandial hypoglycemia is necessary for diabetic patients because elevated glucose can cause diabetic complications. This can be achieved by retarding glucose absorption by inhibiting the carbohydrate hydrolysing enzymes in the digestive tract (Salahuddin *et al.*, 2020). However, synthetic enzyme inhibitors (acarbose, miglitol and voglibose) are associated with gastrointestinal side effects such as flatulence, abdominal cramp, bloating, and diarrhoea (Hamza *et al.*, 2015). This has led to the necessity of searching for alternative antidiabetic agents from plants that have lesser side effects.

Plants have always been a point of call for alternative medicine because they are rich in potent substances and have contributed positively to human health care since time immemorial (Jibril *et al.*, 2019). Shukri *et al*. (2011) reported that antioxidants from herbs can reduce oxidative damage and complications in diabetic patients. Therefore, antioxidants and α-glucosidase inhibitors from herbs are correlated and effective in treating diabetes mellitus. Thus, the current study evaluated the antioxidant and antidiabetic potential of selected underutilized indigenous vegetables used to manage diabetes.

4.2. Material and methods

4.2.1. Plant collection, drying and grinding

The leaves of *Citrullus colocynthis* (L.) Schrad, *Cleome gynandra* L, *Cucumis africanus* L.f., *Oxygonum dregeanum* Meisn, *Pouzolzia mixta* Solms, and *Sonchus oleraceus* L. (L.) were collected in their natural habitat in Duthuni, Thohoyandou, and Tshaulu villages in the Thulamela Local Municipality, Limpopo Province, South Africa, in December 2019 to March 2020. Voucher specimens (detailed in Table 2.1) were prepared and deposited at the University of Venda herbarium. Plant samples were taken to the lab and were washed with water to remove dirt before air-drying in the shade at room temperature. Dried leaves were ground into a fine powder using an electric blender, stored in airtight envelopes and locked in a dark locker until needed for use.

4.2.2. Extraction

Powdered leaves of *Citrullus colocynthis, Cleome gynandra, Cucumis africanus, Oxygonum dregeanum, Pouzolzia mixta,* and *Sonchus oleraceus* were extracted individually with three different solvents: dichloromethane, acetone, and distilled water. The resulting mixtures were decanted and filtered into labelled bottles using filter paper (Whatman No 24). The residues were further extracted two times. The dichloromethane and acetone plant extracts were condensed using an IKA RV Rotary evaporator. The plant extracts were poured into glass vials and placed in a fume hood to dry. Distilled water plant extracts were freeze-dried to give the crude dried extracts.

4.2.3. Phytochemical screening

4.2.3.1 Qualitative screening

Phytochemical screening is a way to detect bioactive compounds present in the plant extracts.

4.2.3.1.1 Test for alkaloids

Three ml of plant extract was stirred with 3 ml of 1% HCl, The mixture was warmed for 4 min then filtered. 3 ml of filtrate was treated with few drops of potassium mercuric iodide (Mayer's reagent). The formation of red precipitate shows the presence of alkaloids (Owoade *et al.*, 2018).

4.2.3.1.2 Test for tannins

Few drops of 5% ferric chloride solution were added into 1ml of extract. Blue or greenish to black color indicates the presence of tannins (Dhawan and Gupta, 2017).

4.2.3.1.3 Test for flavonoids

Two ml of extract was treated with few drops of 20% sodium hydroxide solution formation of intense yellow color, which becomes colorless on the addition of dilute hydrochloric acid, indicates the presence of flavonoids (Khamis and Aly, 2017).

4.2.3.1.4 Test for saponins

Five mg of each plant extract was dissolved in 6 ml of hot distilled water. Persistent foam shows the presence of saponins (Majouli *et al.*, 2017).

4.2.3.1.5 Test for steroids

Steroids were tested by adding 1 ml concentrated H_2SO_4 by the walls of the test tubes containing 5 milligrams of the plant extracts. The appearance of dark reddish-green colour confirmed the presence of steroids (Dhawan and Gupta, 2017).

4.2.3.1.6 Test for glycosides

Glycosides were tested by adding 2 ml of glacial acetic acid to 5 ml plant extracts, followed by 4-6 drops of ferric chloride solution and 1 ml of concentrated sulphuric acid. The formation of a brown ring at the interface confirmed the presence of glycosides (Khamis and Aly, 2017).

4.2.3.1.7 Test for terpenoids

Terpenoids were tested by adding 2 ml chloroform, then 3 ml of concentrated sulphuric acid to 5 ml plant extracts. The formation of the reddish-brown layer at the interface indicated the presence of terpenoids (Thangavel and Ramasamy, 2019).

4.2.3.2 Quantitative screening

4.2.3.2.1 Total phenolic content

Total phenolic content was determined according to the method described by Makkar (2003) with some modifications. The plant samples (in 10 ml of 50% methanol) were extracted through sonication for 20 min. A total of 450 µl distilled water was added to a test tube with

50 µl of the plant extract. After, 250 µl of 1 N Folin Ciocalteu reagent was added, followed by 1250 µl of sodium carbonate solution. The tubes were vortexed for 5 seconds and then incubated for 40 min at room temperature. The absorbance was measured at 725 nmwavelength. Gallic acid (0.1 mg/ml) was used as a standard solution. Total phenolic content was expressed as mg gallic acid equivalents per gram dry weight. The tests were carried out in triplicates.

4.2.3.2.2 Total flavonoids

The total flavonoids content was determined using the aluminium chloride method described by Marinova *et al.* (2005). The samples were extracted in 10 ml 50% methanol as previously outlined. An aliquot of 250 µl of plant extract was pipetted into a reaction tube containing 1 ml distilled water. To the working tube, 75 µl 5% $NaNO_3$ was added, and after a latent period of 5 min, 75 µl 10 % $AlCl_3$ and 0.5 ml of 1 M NaOH, and 0.6 mL of distilled water was added to the test tube sequentially. The mixture was vortexed, and absorbance was measured at 510 nm wavelength. A blank was prepared as previously outlined except that the plant extract was replaced with 50 % methanol. The total flavonoid content was expressed as mg per gram dry weight Catechin Equivalent (CE). Tests were analysed in triplicates.

4.2.3.2.3 Condensed tannins

Condensed tannins were determined using the method described by Porter *et al*. (1986). The sample was dissolved in 10 ml of 50% methanol through sonication for 20 minutes. A sample extract of 500 µl was added into a test tube. After that, 3000 µl of butanol-HCl reagent (prepared by mixing 950 ml butanol with 50% concentrated HCl 37%) and 100 µl of ferric reagent were added into the test tube. Ferric reagent was prepared by dissolving 2 g ferric ammonium sulphate in 100 ml 2N HCl, and it was stored in dark bottles.

The tubes were cabbed and incubated at 100℃ for 60 min and cooled at room temperature before absorbance was measured at 550 nm. The colour of heated and unheated blanks was checked. The unheated blank was prepared by adding 0.5 ml of the sample into the test tube, then adding 3ml of butanol-HCl reagent, followed by 0.1 ml of ferric reagent. The heated blank was prepared by adding a 0.5ml sample into the test tube, followed by 3ml of butanol (only) was added and 0.1 ml ferric reagent.

For calculations, the absorbance of the unheated mixture (considered a suitable blank) was subtracted from the absorbance of the heated mixture; the actual reading at 550 nm for the calculation of condensed tannins. Development of pink colour without heating the sample indicates the presence of flavan–4–ols. If this happened, one heated blank for each sample, comprising 0.5 mL of the extract, 3 mL of butanol, and 0.1 mL of the ferric reagent, was used. The determinations were carried out in triplicates. Condensed tannin content was calculated as leucocyanidin equivalent (LE) according to the following formula:

(A 550 nm x 78.26 x Dilution factor)/ (% dry matter).

4.2.4. Antioxidant assays

4.2.4.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging activity.

DPPH radical scavenging activity was determined using the method described by Sharma and Bhat (2009) with some modifications as described by Amoo *et al.* (2011). Briefly, dried dichloromethane, acetone, and water extracts were redissolved in 50% methanol at different concentrations. The concentration in this sequence was 30 mg/ml, 15 mg/ml, 7.5 mg/ml, 3.75 mg/ml etc. Thirty microlitres of the extracts was diluted with 50 % methanol to make the final volume of 750 µl. An equal volume of DPPH was added to make a total volume of 1500 µl. The tubes were vortexing for 5 seconds and incubated at room temperature for 30 min. After that, absorbance was read at 517 nm using a UV-visible spectrophotometer. Methanol was used as a negative control, while ascorbic acid was a positive control. Blank solution with methanol was included for each extract to correct any absorbance due to extract colour. The spectrophotometer was equillibrated with methanol, and tests were carried out in triplicates.

The radical scavenging activity was calculated using the following equation:

$$RSA\% = 1 - (A_{extract} - A_{blank})/A_{control} \times 100$$

Where $A_{extract}$ is the absorbance of the extracts, A_{blank} is the absorbance of a blank solution, and $A_{control}$ is the absorbance of the negative control. The EC_{50}, which is the concentration of the extract required to scavenge 50% of DPPH radical, was determined for each extract using GraphPad prism software.

4.2.4.2. Beta carotene/linoleic acid assay

The antioxidant activity using β-carotene/linoleic acid assay was determined using a method described by Amarowicz *et al*. (2003). A stock solution (emulsion) of carotene/linoleic acid (Sigma-Aldrich) was prepared as follows: first, 5 mg of beta carotene was dissolved in 1 ml of chloroform (this was done in the dark, and the bottle was covered with aluminium foil), then 100 µl of linoleic acid and 100 µl tween 20 were added; lastly, 1000 ml of deionized water was added and the mixture shaken vigorously. The dichloromethane, acetone, and distilled water dried extracts (10 mg) were dissolved in 1 ml 50% methanol. Butylated hydroxytoluene (BHT) was prepared at the same concentration range as the plant extract and used as a positive control, while methanol was the negative control. A volume of 2 ml emulsion was added to 100 µl of sample extracts. The same procedure was repeated on positive control (BHT) and negative control (methanol). The initial absorbance was recorded at 470 nm. The test tubes were then incubated in the water bath for an hour at 50 ℃; then, absorbance was re-recorded.

Antioxidant activity was calculated using the following formula:

$AAC = 1000x\ (A_{Sample\ (60\ min)} – A_{Control\ (60\ min)})/\ (A_{Control\ (0\ min)} – A_{Control\ (60min)})$ (A: absorbance).

4.2.5. Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity was determined using a method described by Zhang *et al.* (2019) and Tao *et al.* (2013) with minor modifications. Briefly, a serial dilution technique using a 96 well microtiter plate was used. Different concentrations of the samples were prepared using dimethyl sulfoxide (DMSO). The enzyme solution was prepared by dissolving yeast alpha-glucosidase (0.5 Unit/ml) in 0.1 M potassium phosphate buffer (pH 6.8). About 20 µl of the extract and 20 µl of the enzyme solution were pipetted into the microtiter plate. After that, 40 µl of the substrate solution *p*-Nitrophenyl-*a*-D-glucopyranoside (5 mM) prepared in 0.1 M potassium phosphate buffer (pH 6.8) was added to start the reaction. The plates were incubated at 37℃ for 40 min. After incubation, 0.2 M sodium carbonate in 100 ml 0.1 M potassium phosphate buffer (pH 6.8) was added into each well to quench the reaction. Acarbose was used as a positive control. The control experiment contained the same reaction mixture, but the sample solution was replaced with phosphate buffer. The tests were carried out in triplicates.

The inhibition percentage was calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_{405}\text{ control - }A_{405}\text{ extract}}{A_{405}\text{ control}} \times 100\%$$

Where A_{405} control is the absorbance of the control and A_{405} extract is the absorbance of the extract.

4.2.6. Statistical analysis.

Analyses were run in triplicates and data subjected to one-way analysis of variance (ANOVA) using Statsoft (Statistica 8) software. Results were reported as mean ± standard error of triplicate analyses. The mean values were compared using Duncan's Multiple Range Test, and a significant difference was based on P = 0.05.

4.3. Results and discussion

4.3.1. Phytochemical screening

Phytochemicals are biologically active compounds in a plant. Phytochemical screening was done in *Citrullus colocynthis* (Mutshatsha), *Cleome gynandra* (Murudi), *Cucumis africanus* (Tshinyagu), *Oxygonum dregeanum* (Mutanye/Tanye), *Pouzolzia mixta* (Mutanzwa), and *Sonchus oleraceus* (Shashe). The results revealed the presence of alkaloids, steroids, tannins, glycosides, flavonoids, saponins, and terpenoids. These compounds serve as a lead of the medicinal potential certain vegetables have and how they can be used as a base to manufacture modern effective drugs (Sheikh *et al*., 2013).

Results showed that water extracted more compounds from the leaves of *Oxygonum dregeanum*, *Citrullus colocynthis*, and *Cleome gynandra* than acetone and dichloromethane. In *Oxygonum dregeanum* however, it extracted all the evaluated compounds. Water extract of *Citrulus colocynthis* and *C. gynandra* possessed alkaloids, steroids, tannins, glycosides, saponins and terpenoids. Dichloromethane extracted the least compounds from the leaves of *P. mixta* (Table 4.1).

Phytochemicals found in the aqueous extract of *Citrullus colocynthis* agree with Uma and Sekar (2014) observation. Owoade *et al.* (2018) and Ramakrishna *et al.* (2019) confirmed the presence of alkaloids, flavonoids, tannins and saponins in the leaves of *Citrullus colocynthis.* The results of the aqueous extract of *Cleome gynandra* aligned well with the study by Adhikari and Paul (2018), recording aqueous extract of *Cleome gynandra* to contain saponins, tannins, flavonoids, and glycosides. Additionally, Rajeselvam and Rose (2016) found that *Cleome* *gynandra* has saponins and tannins. This validates the use of these vegetables for medicinal purposes by local people. Consumption of these vegetables rich in phytochemicals helps promote good health and prevent diseases (Sango *et al.,* 2016). The results indicated that distilled water extracted more compounds than dichloromethane and acetone (Table 4.1). This was a good experimental observation because most traditional remedies are mainly prepared as water extracts (Mulaudzi *et al.,* 2019).

The curative ability of the selected vegetables is embedded in the presence of these phytochemical compounds. Their folklore usage to treat non-communicable diseases is validated by the presence of alkaloids, steroids, tannins, glycosides, flavonoids, saponins, and terpenoids. Alkaloids are known for their bioactivities such as antibacterial, anti-parasitic and anti-diabetic activities (Venkatesan *et al.,* 2019). Steroids are highly used in nutrition, herbal medicine, and cosmetics. Steroids act against cardiotonic activity (Sonan *et al.,* 2017; Yadav and Agarwala, 2011). Therapeutic activities of tannins are antimicrobial, antiviral, antimutagenic, antidiabetic, and anti-parasitic (Sieniawska, 2015; Ashok and Upadhyaya, 2012).

Glycosides can activate cardiac output, lower blood pressure, and lower heart diseases such as congestive heart failure and arrhythmia (Sonan *et al.,* 2017; Khan *et al.,* 2020). Flavonoids are known for their biological activities such as anti-inflammation, antioxidant, anti-allergic, antiviral, antidiabetic, and anticarcinogenic (Uma and Sekar, 2014; Umerah and Nnam, 2019). The saponins' foams qualifies them to be used in medicine and pharmaceutical industries to produce a frothy effect (El Aziz *et al.,* 2019; Venkatesa *et al.,* 2019). The presence of phytochemicals has given underutilized indigenous vegetables the potential to serve as protective food against non-communicable diseases (Gbadamosi and Kalejaye, 2017; Srivastav *et al.,* 2018).

Table 4.1: Phytochemical constituents in different plant extracts extracted by different solvents.

Extract	Phytochemicals							
	Plant species	Alkaloids	Steroids	Tannins	Glycosides	Flavonoids	Saponins	Terpenoids
Dichloromethane	*Citrulus colocynthis*	+	+	+	–	+	–	–
	Cleome gynandra	+	+	+	–	+	–	–
	Cucumis africanus	+	+	+	–	+	–	–
	Oxygonum dregeanum	+	+	+	+	–	–	–
	Pouzolzia mixta	+	–	–	+	–	–	–
	Sonchus oleraceus	+	+	+	+	–	–	–
Acetone	*Citrulus colocynthis*	+	+	–	+	–	+	+
	Cleome gynandra	–	+	+	–	–	–	–
	Cucumis africanus	+	+	+	–	–	–	+
	Oxygonum dregeanum	–	+	–	–	–	–	+
	Pouzolzia mixta	+	+	–	+	–	–	–
	Sonchus oleraceus	–	+	+	–	–	–	–
Distilled Water	*Citrulus colocynthis*	+	+	+	+	–	+	+
	Cleome gynandra	+	+	+	+	–	+	+
	Cucumis africanus	+	–	+	+	–	+	–
	Oxygonum dregeanum	+	+	+	+	+	+	+
	Pouzolzia mixta	+	–	+	+	–	–	–
	Sonchus oleraceus	+	–	–	–	–	–	–

Keys: (+) = present, (-) = not present.

4.3.2. The quantitative analysis

4.3.2.1. Total phenolic content

Total phenolic content was expressed as mg GAE/g and ranged from 3.34 mg GAE/g to 13.10 mg GAE/g. The highest value was obtained from *Citrulus colocynthis* (13.10 mg GAE/g), and the lowest value was obtained from *Cucumis africanus* (3.34 mg GAE/g). Total phenolics values were significantly different ($P < 0.05$) (Table 4.2). The value of *Cleome gynandra* (5.75 mg GAE/g) compares favourably to *Cleome gynandra* value (3.94 mg GAE/g) reported by (Moyo *et al.*, 2013). Additionally, the value of *Cleome gynandra* (5.75 mg GAE/g) in the current study is lower than the value (15.15 mg GAE/g DW) obtained by (Moyo *et al.*, 2018). Still, it compares well to the commonly consumed vegetable *Brassica oleracea* (5.84 mg GAE/g DW) (Moyo *et al.*, 2018). The results of *Citrulus colocynthis* (13.10 mg GAE/g) is lower than the data obtained by Owoade *et al.* (2018) in *Citrulus colocynthis* (58.76 mg in Gallic acid equivalent). Phenols are non-nutritive, secondary metabolites found abundantly in plants that function to promote significant health benefits and prevent various diseases (Farooq *et al.*, 2018). Saha *et al.* (2015) reported that phenolic compounds protect the human cell against oxidative damage and possess great anti-carcinogenic capabilities. Consumption of vegetables rich in phenols ameliorates and prevents the generation of chronic diseases (Alam *et al*., 2019).

4.3.2.2. The total flavonoid content

Total flavonoids values were significantly different ($P < 0.05$). *Citrulus colocynthis* (8.30 mg CE/g) had the highest flavonoids content, followed by *Sonchus oleraceus* (7.10 mg CE/g), *Oxygonum dregeanum* (3.9 mg CE/g) (Table 4.2). The flavonoid content recorded for *Cleome gynandra* (2.49 mg CE/g) in the present study is in agreement with the content (2.19 mg catechin/g) reported by Moyo *et al*. (2013) and was comparable to the level of *Cleome gynandra* (4.778 mg QE/g) obtained by Widodo and Pratiwi (2018) and (5.65 mg CE/g DW) obtained by Moyo *et al*. (2018). Furthermore, the value of *Citrulus colocynthis* (8.30 mg CE/g) was higher than the value (2.01mg/g of rutin equivalent) obtained by Owoade *et al.* (2018). Flavonoids are major plant constituents and have been known to possess several biological activities such as anti-inflammatory, antioxidant, anti-cancer, anti-allergic, and anti-carcinogenic activities (Obeng *et al.*, 2020; Rebaya *et al.*, 2014). Bhebhe *et al.* (2015) reported that flavonoids reduce the risk of hypertension and cardiovascular diseases.

4.3.2.3. Condensed tannin

Condensed tannins values varied from 0.01% in *S. oleraceus* to 0.05% in *Citrullus colocynthis*. The mean values of *Oxygonum dregeanum, Pouzolzia mixta*, and *Sonchus oleraceus* were not significantly different ($P > 0.05$) (Table 4.2). The content of condensed tannins in the present study was very low. However, Sowunmi and Afolayan (2015) reported that low tannins still significantly impact the body. Condensed tannins are natural compounds that occur naturally in various vegetables. Consuming plants rich in condensed tannins is highly beneficial for diabetic people because tannins reduce high glucose levels in the blood and scavenge free radicals (Bhebhe *et al.*, 2015).

Citrulus colocynthis had a high content of total phenols, flavonoids and condensed tannins compared with other analysed indigenous vegetables, followed by *Sonchus oleraceus* species, *Oxygonum dregeanum, Cleome gynandra, Pouzolzia mixta,* and *Cucumis africanus* (Table 4.2). The investigated vegetables contain a significant level of polyphenols meaning that they exhibit high antioxidant activity, contributing more to their medicinal properties (Pulipati *et al*., 2017). Jiménez-Aguilar and Grusak (2017) reported that polyphenols reduce the risk of cardiovascular and chronic diseases; therefore, high consumption of these vegetables could combat non-communicable diseases. Additionally, Pulipati *et al.* (2017) reported that polyphenols contain various chemicals and biological activities, including radical scavenging properties.

Table 4.2: Total phenolics, flavonoids and condensed tannins of selected vegetables.

Sample name	Total phenolics (mg GAE/g)	Flavonoids (mg CE/g)	Condensed tannins (% in dry matter) as leucocyandidn equivalent)
Citrulus colocynthis	13.10±0.25[a]	8.30±0.13[a]	0.05±0.00[a]
Cleome gynandra	5.75±0.13[c]	2.49±0.03[e]	0.03±0.00[c]
Cucumis africanus	3.34±0.15[d]	0.71±0.02[d]	0.04±0.00[b]
Oxygonum dregeanum	5.55±0.21[c]	3.94±0.06[c]	0.01±0.00[d]
Pouzolzia mixta	3.72±0.06[d]	2.36±0.15[e]	0.01±0.00[d]
Sonchus oleraceus	9.06±0.29[b]	7.10±0.09[b]	0.01±0.00[d]

Values are expressed as the mean ± standard error of triplicate analyses. Mean values carrying different superscripts are significantly different ($P \leq 0.05$).

4.3.3. Antioxidant activity

The antioxidant activity of the selected underutilized vegetables was determined using DPPH scavenging radical activity and beta-carotene/linoleic acid assays.

4.3.3.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

Free radicals play a significant role in the development of non-communicable diseases. These can be eliminated by antioxidants that inhibit oxidation and protect cells from damage. DPPH is one of the stable free radicals commercially available to evaluate the scavenging activity of plant extracts by decreasing the presence of free radical scavengers (Bouguerra *et al*., 2020; El Omari *et al*., 2019).

To determine the antioxidant activity of the selected underutilized indigenous vegetables, DPPH was used, and ascorbic acid used as a positive control. Table 4.3 shows the free radical scavenging activity of the dichloromethane, acetone, and water extracts of *Citrullus colocynthis, Cleome gynandra, Cucumis Africanus, Oxygonum dregeanum, Pouzolzia mixta* and *Sonchus oleraceus.* The results were interpreted as the lower the EC_{50} value the higher the antioxidant activity. The EC_{50} values obtained from analysed vegetables ranged from 7.57 to 187.83 (ug/ml), whereas the ascorbic acid had the lowest EC_{50} value of 2.08 (ug/ml). Acetone and water extract EC_{50} values were comparable to the positive control. The EC_{50} values exhibited by acetone and water extracts were not significantly different from ascorbic acid.

The highest scavenging activity was observed in *Citrulus colocynthis* water extract (7.57 ug/ml), which is approximately 3.5 fold lesser than the ascorbic acid positive control (2.08 ug/ml). Acetone extracts of *Citrulus colocynthis*, *Oxygonum dregeanum, Sonchus oleraceus,* and water extracts of *Citrulus colocynthis, Cleome gynandra, Cucumis africanus, Oxygonum dregeanum* and *Pouzolzia mixta* exhibited moderate radical scavenging activity, not far below the ascorbic acid (Table 4.3).

The results revealed acetone and water as good extractors because most of their EC_{50} values are lower and not significantly different compared with EC_{50} values revealed by dichloromethane extracts. This is interesting as it validates water use by local people in preparing and cooking vegetables. The solvent used for extraction plays a role in extracting antioxidants (Rebaya *et al.*, 2014). Acetone extract of *Cleome gynandra* and *Sonchus oleraceus* possessed tannins, and water extract of *Citrulus colocynthis, Cleome gynandra*, had tannins. Mishra *et al.* (2011) reported that phenolic compounds like tannins are responsible for the antioxidant activity in plant extracts. The studies by Mishra *et al.* (2011) and Meda *et al*. (2013) in *Cleome gynandra* showed that the leaves exhibited antioxidant activity. Bizuayehu *et al.* (2016) reported that the total number of polyphenols, flavonoids and tannins correlates with the antioxidant activities. Medini *et al.* (2014) reported that the antioxidant activity from plants is derived from phenols. In work done by Teugwa *et al.* (2013), *Sonchus oleraceus* possessed antioxidant activity due to higher content of total phenolic and flavonoid compounds. Free radical scavenging activity results from the existence of phenolic and flavonoids types compounds (Yin *et al.*, 2007). Dewi and Maryani (2015) reported a high correlation between total polyphenol content and radical scavenging antioxidant activity.

4.3.3.2. Beta-carotene/ linoleic acid assay

In the beta-carotene/linoleic acid assay, the substance's antioxidant activity level is determined by measuring oxidation products of linoleic acid that simultaneously attack highly unsaturated beta-carotene molecules, resulting in discolouration of its characteristic yellow colour (Hasibuan *et al*., 2013; Piaru *et al*., 2012; Shakeri *et al*., 2012). The presence of antioxidants can hinder the degradation of beta-carotene by reacting with the linoleate free radical (Mraihi *et al*., 2013; Piaru *et al*., 2012).

The beta-carotene linoleic acid method was used to determine the antioxidant potential of the selected vegetable extracts, and butylated hydroxytoluene (BHT) was used as a positive control. This method was used to assess the extract's ability in protecting beta-carotene. In the present study, vegetable extracts' antioxidant activity was comparable to the BHT (positive control) (Table 4.3). Acetone extract of *Cucumis africanus* (81.49 ug/ml) and dichloromethane extract of *Citrullus colocynthis* (84.02 ug/ml) and *Cleome gynandra* (81.53 ug/ml) showed significant positive activity, which is comparable to the positive control (83.59 ug/ml) (Table 4.3). Similarly, antioxidant activity in the leaves of *Citrullus colocynthis* was reported by (Gupta *et al*., 2018). Saini and Yadav (2020) and Bamidele *et al.* (2017) have noted a good correlation between total phenolic content and antioxidant activity in plant species. Antioxidant activity in these vegetables may be due to the total phenols, flavonoids and tannins they exhibited (Table 4.2).

The solvent used for extraction plays a part in extracting antioxidant activity (Rebaya *et al.*, 2014). Therefore, acetone and dichloromethane was found to be the good antioxidant screening extractor than distilled water. There is epidemiological evidence correlating a higher intake of vegetables with antioxidant abilities to a lower incidence of non-communicable disease (Zhang *et al.*, 2015). This shows that the analysed vegetable can serve as a potential source of natural antioxidants.

4.3.4. Alpha-glucosidase inhibition

Table 4.3 shows alpha-glucosidase inhibition activity of *Citrullus colocynthis, Cleome gynandra, Cucumis africanus, Oxygonum dregeanum, Pouzolzia mixta* and *Sonchus oleraceus*. Alpha-glucosidase inhibition is used as a therapeutic way to control postprandial hyperglycemia (Dewi and Maryani, 2015). Alpha-glucosidase is a membrane-bound enzyme responsible for changing oligosaccharides, trisaccharides, and disaccharides into glucose and other monosaccharides in the intestinal lumen for easy glucose absorption in the bloodstream (Carpéné *et al.*, 2019; Vongsak *et al.*, 2015; Rengasamy *et al.*, 2013). Alpha-glucosidase inhibitors such as acarbose, miglitol and voglibose are used to inhibit the degradation of carbohydrates, thereby reducing glucose absorption and managing postprandial hyperglycaemia at the digestive level (Nimesh *et al.*, 2019; Dewi and Maryani, 2015). The benefits of alpha-glucosidase inhibition in treating diabetic patients have been reported by several researchers (Kalva *et al.*, 2018; Banerjee *et al.*, 2017; Zarei and Poursharifi, 2015).

In the current study, acarbose was used as an inhibitor of alpha-glucosidase. Several researchers have reported inhibition of alpha-glucosidase to effectively prevent and treat diabetic patients by reducing postprandial hyperglycemia (Aoki *et al*., 2019; Kalva *et al.*, 2018; Banerjee *et al.*, 2017; Kadouh *et al.*, 2016; Zarei and Poursharifi, 2015).

The lower the IC_{50} value the stronger the inhibition activity. Vegetables with IC_{50} values lower than the acarbose positive control have more robust inhibition activity, while vegetables with higher IC_{50} values have weaker inhibition activity. Therefore, in the present study, inhibitory activity among these dichloromethane, acetone, water extracts and positive control were compared based on their IC_{50} values. The IC_{50} values of the analysed vegetables were higher than the IC_{50} value of acarbose, except for the acetone extract of *Cucumis africanus*. This means that their inhibitory activity is lower than that of the positive control, except for the acetone extract of *Cucumis africanus* (Table 4.3).

Acetone extract of *Cucumis africanus* exhibited strong α-glucosidase inhibitory activity with an IC_{50} value of 169.27 ug/ml, compared to the acarbose with an IC_{50} value of 240.10 ug/ml. Moderate inhibitory activity was revealed by dichloromethane extract of *Citrullus colocynthis* with an IC_{50} value of 309.97 ug/ml compared to acarbose with an IC_{50} value of 240.10 ug/ml respectively (Table 4.3). The IC_{50} values obtained from the extracts, as mentioned above, were not significantly different when compared with the acarbose.

The antidiabetic activities of *Cucumis africanus*, *Oxygonum dregeanum* and *Pouzolzia mixta* were evaluated for the first time and *Cucumis africanus* revealed good inhibitory activity. Thus, leaves of *Citrullus colocynthis* and *Cucumis africanus* could be used as an alternative for diabetes mellitus treatment. This also supports why people at the Thulamela Local Municipality use these vegetables to manage diabetes. Inhibition found in the above-mentioned vegetables may be due to phenolic compounds such as flavonoids, tannins and alkaloids. Phenolic compounds have been reported to inhibit alpha-glucosidase (Koala *et al.*, 2021). Kadouh *et al.* (2016) reported that antioxidant compounds such as polyphenols and flavonoids exhibited alpha-glucosidase inhibitory activity *in vitro*.

Table 4.3: Free radical scavenging, antioxidant and alpha-glucosidase inhibitory activities of analysed vegetable extracts.

Solvent	Sample name	DPPH EC_{50} (µg/ml)	Beta-carotene linoleic acid (%) at 50 µg/ml	Alpha glucosidase inhibitory IC_{50} (µg/ml)
Dichloromethane	*Citrulus colocynthis*	187.83 ± 15.01^{a}	84.02 ± 2.33^{b}	309.97 ± 18.94^{hi}
	Cleome gynandra	87.17 ± 2.81^{c}	81.53 ± 0.11^{bcd}	759.77 ± 21.66^{cd}
	Cucumis africanus	56.62 ± 1.26^{d}	79.02 ± 0.31^{de}	601.57 ± 18.49^{ef}
	Oxygonum dregeanum	184.73 ± 5.70^{a}	78.07 ± 1.16^{de}	831.00 ± 62.03^{c}
	Pouzolzia mixta	108.83 ± 4.22^{b}	76.66 ± 0.95^{ef}	514.60 ± 1.13^{fg}
	Sonchus oleraceus	83.24 ± 8.15^{c}	79.77 ± 1.53^{cde}	574.63 ± 6.28^{ef}
Acetone	*Citrulus colocynthis*	14.99 ± 0.16^{efg}	79.58 ± 0.18^{cde}	656.10 ± 70.77^{def}
	Cleome gynandra	20.90 ± 0.12^{ef}	77.77 ± 0.55^{de}	627.33 ± 24.96d^{ef}
	Cucumis africanus	21.59 ± 0.38^{ef}	81.49 ± 1.29^{bcd}	169.27 ± 11.52^{j}
	Oxygonum dregeanum	9.25 ± 0.17^{fg}	79.19 ± 0.34^{de}	809.63 ± 12.96^{c}
	Pouzolzia mixta	26.35 ± 2.27^{e}	72.23 ± 0.77^{g}	409.80 ± 12.71^{gh}
	Sonchus oleraceus	12.60 ± 0.15^{efg}	73.43 ± 1.35^{fg}	666.90 ± 48.36^{de}
Distilled water	*Citrulus colocynthis*	7.57 ± 0.15^{fg}	72.52 ± 2.40^{g}	582.10 ± 106.67^{ef}
	Cleome gynandra	14.32 ± 0.55^{efg}	70.74 ± 0.94^{g}	nd*
	Cucumis africanus	14.07 ± 0.14^{efg}	71.00 ± 0.54^{g}	nd**
	Oxygonum dregeanum	11.76 ± 0.21^{fg}	73.74 ± 2.33^{fg}	1741.33 ± 79.48^{a}
	Pouzolzia mixta	13.73 ± 0.82^{efg}	72.32 ± 1.17^{g}	nd***
	Sonchus oleraceus	52.99 ± 0.88^{d}	94.95 ± 1.74^{a}	1261.00 ± 52.29^{b}
Acarbose				240.10 ± 9.52^{ij}
Ascorbic acid		2.08 ± 0.19^{g}		
BHT			83.59 ± 0.30^{bc}	

nd* = Not determined, 37.17% alpha-glucosidase inhibition at 3333.33 µg/ml (highest concentration evaluated); nd** = Not determined, 56. 92% alpha-glucosidase inhibition at 3333.33 µg/ml (highest concentration evaluated); nd*** = Not determined, 53.66% alpha-glucosidase inhibition at 3333.33 µg/ml (highest concentration evaluated). Values are expressed as the mean ± standard error of triplicate analyses. Mean values carrying different superscripts are significantly different $P < 0.05$. BHT=Butylated hydroxytoluene, DPPH=2,2-diphenyl-1-picrylhydrazy

4.4. Conclusion

Underutilized indigenous vegetables can combat various diseases. The results in the present study for phytochemical screening, antioxidant and antidiabetic activity from *Citrulus colocynthis, Cleome gynandra, Cucumis africanus, Oxygonum dregeanum, Pouzolzia mixta* and *Sonchus oleraceus* provides valuable information about the benefits of using these vegetables in the management of non-communicable diseases. *Citrullus colocynthis*, *Cucumis africanus, Sonchus oleraceus,* and *Oxygonum dregeanum* exhibited significant amounts of total phenolics and flavonoids, as well as antioxidant activity in both DPPH and beta-linoleic acid. Acetone extract of *Cucumis africanus* exhibited strong inhibitory activity while dichloromethane extracts of *Citrullus colocynthis* revealed moderate inhibitory activity compared to the acarbose. These concepts are related; therefore, it can be concluded that the presence of antioxidant and antidiabetic activity in these vegetables may be attributable to the presence of phenolic and flavonoid compounds. The result supports the traditional use of these vegetables in managing non-communicable diseases. However, these underutilized indigenous vegetables should be investigated further using *in vivo* methods to validate these results.

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Chapter 5

Overall summary, conclusion and recommendation

Non-communicable illnesses have become a global health burden, causing high morbidity and mortality. Indigenous vegetables have been used as source of food and medicinetime immemorial. As a result, local people use these vegetables to manage non-communicable diseases. Therefore, the study aimed to document indigenous knowledge on underutilized indigenous vegetables used to treat non-communicable diseases and assess their biological activities. The objectives that were identified to attain the aims are mentioned below.

5.1. Objectives

1. To identify and document indigenous knowledge on underutilized indigenous vegetables which are used to manage non-communicable diseases.
2. To assess nutritional value of underutilized indigenous vegetables.
3. To evaluate antioxidant and antidiabetic potential of underutilized indigenous vegetables used to manage non-communicable diseases.

5.1.1. Objective 1. To identify and document indigenous knowledge on underutilized indigenous vegetables which are used to manage non communicable diseases.

The importance of an ethnobotanical survey is revealing information about plants that are useful to local people. An ethnobotanical survey was conducted on underutilized indigenous vegetables used to manage non-communicable diseases. The study discovered that elderly females had a greater understanding of indigenous vegetables. A total of 25 underutilized indigenous vegetables belonging to 13 families were identified and documented. The documented vegetables were primarily herbs, with leaves being used more frequently than other plant parts. The use of leaves aids in plant conservation because removing many leaves does not harm the vegetables, unlike roots and stems.

These vegetables are administered as potherbs while cooked, and a decoction extracted from the leaves is taken orally. The vegetables are cooked individually, while some are combined to provide heightened nutritional and medicinal benefits. Condiments are also used to enhance their taste and medicinal properties.

Other researchers have reported *Sonchus oleraceus, Biden pilosa, Momordica balsamina, Momordica foetida, Cleome gynandra, Corchorus tridens, Amaranthus dubius,* and *Solanum retroflexum* for the treatment of non-communicable diseases and other ailments. This indicates that these vegetables serve a variety of functions in the lives of the local people. Vegetables such as *Adenia digitata, Adenia gummifera, Cucumis anguria, Cucumis africanus, Datura ferox, Oxygonum dregeanum, Pouzolzia parasitic,* and *Pyrenacantha kaurabassana* were recorded for the first time for the treatment of non-communicable diseases. This is an essential addition to the growing library of indigenous knowledge of vegetables that are helpful to local people's health and diet.

5.1.2. Objective 2. To assess nutritional value of underutilized indigenous vegetables.

Six underutilized indigenous vegetables were tested for their nutritional value. Although all of the vegetables studied contained significant amounts of the examined mineral elements and micronutrients, *Cucumis africanus* had the highest vitamin C, calcium, iron, and magnesium compared to the other vegetables. *Sonchus oleraceus* had the highest content of β-carotene, copper, and potassium. *Citrulus colocynthis* had the highest concentrations of Mn and Zn. This suggests that these vegetables could be helpful in dietary diversification, the management of non-communicable diseases, and the treatment of nutrient deficiency-related illnesses. This lends credence to their long-standing use as food sources.

5.1.3. Objective 3. To evaluate antioxidant and antidiabetic potential of underutilized indigenous vegetables used to manage non communicable diseases.

The phytochemical assay was conducted through qualitative and quantitative screening to evaluate bioactive compounds. Water was demonstrated as a better extractor than acetone and dichloromethane. This is fascinating since it is a universal solvent commonly used for the preparation of medicine and cooking by local people. Water extracted all the evaluated compounds from *Oxygonum dregeanum.* Few compounds were obtained from dichloromethane extract of *Pouzolzia mixta. Citrulus colocynthis* had high content of phenolics, flavonoids and condensed tannins, followed by *Sonchus oleraceus, Oxygonum dregeanum Cleome gynandra,* and other vegetables. This suggests that these vegetables contain bioactive substances that help repair and maintain the health of the local people.

The antioxidant activity of the selected vegetables was tested. This was done because antioxidant activities are linked to the therapeutic benefits of vegetables. DPPH and beta-carotene-linoleic acid assays were used to determine the vegetables' antioxidant activity. Compared to the ascorbic acid (positive control), the analysed vegetables' scavenging activity was moderate. Beta-carotene-linoleic acid method extracts exhibited positive activity comparable to a positive control (BHT). *Citrullus colocynthis, Oxygonum dregeanum, Pouzolzia mixta*, *Sonchus oleraceus* and *Cucumis africanus* showed positive antioxidant activity. This means they have antioxidant activity, boosting the body's immune system to combat non-communicable diseases. On the other hand, other methods must be utilised to compare outcomes and find reliable results.

Alpha-glucosidase was used to assess anti-diabetic activity from selected vegetables. The study found that IC_{50} values of the analysed vegetables were higher than that of the positive control (acarbose), except for the acetone extract of *Cucumis africanus*. However, dichloromethane extracts of *Citrullus colocynthis* revealed moderate inhibitory activity compared to the positive control. This means that their inhibitory activity is moderate compared to acarbose's inhibitory activity, except for the acetone extract of *Cucumis africanus*. This is based on the fact that the lower the IC_{50} value the stronger the inhibition activity and vice versa. This supports their use by local people in the management of diabetes and other non-communicable diseases. However, other assays used to evaluate anti-diabetic activity must be employed to validate these results.

5.2. Recommendation

More surveys are needed to document more ethnomedicine knowledge in the surrounding areas. Consumption of underutilised indigenous vegetables should be promoted based on their nutritional value and potential medicinal benefits, as demonstrated in this study. Their sustainable supply through cultivation and product development should also be promoted. To confirm antioxidants discovered in these vegetables, *in vivo* evaluation of the antioxidant activity and bioavailability must be utilized. The alpha-amylase enzyme and *in vivo* methods for the antidiabetic activity must be used to obtain precise results.

APPENDIX A

UNIVERSITY OF VENDA, BOTANY DEPARTMENT

A survey of underutilized indigenous vegetables consumed as a rich source of nutritional and medicinal values in Mutale Local Municipality, Vhembe District Municipality.

Questionnaire

Informant's personal details:

Name of the informant: ………………………………………………………..

Physical address and contact number………………………………………………………

Age……………………………………………………………………………….

Male: Female:

Education level:

Occupation:

Information about underutilized indigenous vegetables

Local names of underutilized indigenous vegetables that are used to manage non-communicable diseases

Plant life-form

Tree	Shrub	Herb	Bulb

Season of collection

Summer	Winter	Autumn	Spring

Vegetable part used

Roots	Leaves	Stem	Whole

Method of preparation:……………………………………………………………………….

Administration techniques:……………………………………………………………..

Other things that are added to enhance the healing properties:………………………………

Method of preservation………………………………………………………..