Title: Biological evaluation of ethnobotanical selected medicinal plants used in the management of male sexual health

by

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Submitted in partial fulfilment of the requirements of the degree

Doctor of Philosophy: Life Sciences

Department of Life and Consumer Sciences

College of Agriculture and Environmental Sciences

University of South Africa

November 2023

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DECLARATION

I, **Tsumbedzo Nemandalali** (Student number:**43323928**) declare that the thesis/dissertation, which I hereby submit for the degree Doctor of Philosophy (Life Sciences) at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: 

Date: 15 November 2023

ACKNOWLEDGEMENTS

- I would like to express my sincere gratitude and appreciation to my supervisor, Professor Thilivhali Emmanuel Tshikalange, for allowing me to pursue my PhD studies under his guidance and for his invaluable assistance, direction, and support in bringing this project to a successful conclusion. I appreciate your perseverance and wise advice.
- To my co-supervisor, Dr. Monde Nyila, I am grateful for your comments and ideas, which helped us to create the finest possible version of this dissertation.
- The author cordially thanks the people of the Thengwe area for inhabiting the study area and sharing their traditional knowledge.
- I also want to express my sincere gratitude to the Thengwe Tribal Council for letting me carry out the study there. We appreciate the financial support provided by the National Research Foundation and the University of South Africa.
- I would also like to thank the Staff member and Student of the Department of Plant Sciences, University of Pretoria who have contributed in one way or the other towards the success of this study. I am truly indebted for your effort, time and assistance that have in no small way contributed to the success of my research.
- Finally, I am truly grateful to my family (my wife Ndiadifuna, my sons Phimo and Ndalo and my siblings) for their unconditional love and support in those challenging times. I wish to thank my mother Sylvia Tshamano Nemandalali for being a pillar of my strength, thank you for those endless prayers and your

guidance, unconditional love. Most importantly, to the almighty GOD, thank you for the gift of life and for making the completion of this study a reality.

SUMMARY

Backround: Ethnobatanical knowledge is mostly confined among the locals, hence proper enquiry, and documentation of medicinal plants in a specific area can aid the constantly evolving scientific community in protecting different species and developing long-term cure to the menace of various ailments. In many African societies, men still rely on the long cultural history of utilizing medicinal plants to treat and manage their sexual health. There are many lifestyle risks factors (alcoholism, diabetes, sexually transmitted diseases and psychological factors) that affect men sexual and reproductive health. The current renewed global interest in traditional medicine and natural products to sustain health dictates the investigation of ethnobotanical important medicinal plants into their efficacy and safety against male sexual disorders such as impotence and erectile dysfunction. The current study aims to investigate the importance of selected ethnobotanical plants used by local men in villages under the Thengwe tribal council and also to determine their biological activities associated with men sexual health.

Methodology: The study was conducted in two phases after obtaining ethical approval from the University of South Africa ethics committee and permission to conduct the study from the Thengwe Tribal Council, Vhembe district in Limpopo province (South Africa). During phase 1, Twenty-one plant species were selected based on their ethnobotanical uses in the treatment and management of sexual health in men. A total of 23 participants (males) of different age groups ranging between 18 to 80 years were identified and selected from various villages to participate in the interviews. Among them, were from ages 18-30 (27%), 31-40 (22%), 41 to 50 (17%), 51 to 60 (16%) and those above 60 years (17%). All the participants gave their assent

for the use of their ethnobotanical knowledge, in accordance with the principle of educated prior informed consent. A quantitative matrix method as developed by De Beer & Van Wyk (2011) was used to determine Species Popularity Index (popularity or importance of each species) and the Ethnobotanical Knowledge Index (participant knowledge of plant local use). The Relative Frequency of Citation (RFC) indices was also utilized to further get insight into the importance of plant species in villages under The Thengwe tribal authority.

During phase 2 of the study, ten medicinal plants were selected for biological activity evaluation based on the availability of plant material, the species popularity and Relative Frequency of Citation indices. Acetone extracts were investigated for their antioxidant activity, anti-inflammatory activity against 15-lipoxygenase, inhibition of nitric oxide production on RAW 264.7 macrophages and antimicrobial activity against both *Neisseria gonorrhoeae* and *Candida albicans*. Preliminary phytochemical analysis was also done by determination of total flavonoids and total phenolic content. Cytotoxicity of the extracts was assessed using the the XTT reduction assay against Vero monkey kidney cells.

Results: The most popular species according to their species popularity index (SPI) and the relative frequency citation (RFC) were *B. mollis* (4.52; 0.95), *G. livingstonei* (4.29; 0.83), *R. caffra* (4.54; 0.91), *S. longepedunculata* (4.03; 0.78), *S. panduriforme* (3.80; 0.78), S. *prunioides* (4.67; 0.95) and *O. tenax* (4.42; 0.91). The highest ethnobotanical knowledge index (EKI) value on avarage was found in age group 61 and above (0.81), followed by age group 51-60 (0.74) and 41 – 50 (0.75). High usage of traditional medicine poses considerable risk of loss of biodiversity in the area, and an agent intervention to prioritise plant resources for conservation and sustainable use

is required. *Diospyros mespiliformis* extract was less toxic and showed significant antioxidant (IC_{50} = 8.34 µg/ml), anti-inflammatory (IC_{50} = 63 µg/ml) and antigonococcal (MIC= 0.39 mg/ml) activities. Extracts from *Garcinia livingstonei* and *Rhoicissus tridentata* also exhibited promising bioactivity. While these three extracts seem to inhibit the production of nitric oxide, the rest of the plant extracts seem to promote its production. Medications such as sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra) work by enhancing the effects of nitric oxide in the body to promote better blood flow to the penis, which can help with erectile function. Therefore, maintaining healthy nitric oxide levels is important for preventing erectile dysfunction, and reducing nitric oxide can have the opposite effect.

Conclusion: Medicinal plants have been used for ages and contain chemicals that can assist men to treat, maintain and manage their sexual health. Ethnobotanical studies have revealed many important medicinal plants used traditionally in the treatment and management of men sexual health, but only few of them have been validated scientifically. The current study demonstrated for the first time the importance and potential of the selected medicianal plants used traditionally in the treatment and management of men sexual health. These plants have potential therapeutic effects and can be used as leads in the development of new pharmaceuticals to manage men sexual disorders.

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LIST OF ABREVIATIONS

WHO - World Health Organization

ED - Erectile Dysfunction

STD - Sexually Transmitted diseases

STI - Sexually Transmitted Infection

UNAIDS - United Nation Programme on HIV/Aids

SPI - Species Popularity Index

ISC - International Information System Security Certification Consortium

FAO - Food and Agricultural Organization of the United Nation

IVF - *In Vitro* Fertilisation

ICSI - Intracytoplasmic Sperm Injection

DNA - Deoxyribonucleic Acid

MDA - Malondialdehyde

ROS - Reactive Oxygen Species

OS - Oxidative Stress

BMI- Body Mass Index

HPGaxis - Hypothalamic-Pituitary- Gonadal

EKI - Ethnobotanical Knowledge Index

TFC - Total Flavonoid Content

TPC - Total Phenolic Content

DPPH - 2,2-diphynyl-1-picrylhydrazyl

DMEM - Dulbecco's Modified Eagle's Medium

PSF - Penicillium Streptomycin Fungizone

MIC - Minimum Inhibitory Concentration

IIEF - International Index of Erectile Function

ANOVA - Analysis of Variance

SD - Standard Deviation

TS - Tryptone Soya

DMSO - Dimethyl Sulfoxide

NO - Nitric Oxide

RTI - Reproductive Track Infection

RFC - Relative frequency citation

STRUCTURE OF THE THESIS

The thesis is summarized into six (6) chapters.

CHAPTER 1: This chapter contains a general overview and context of the study. It presents a literature review on the importance of plants and the role that they play in human life. It also addresses the challenges faced by plants through over-utilization and deforestation that leads to plants being Red-Listed as endangered species in South Africa. The statement of research problem, the research justification, hypothesis, aim, and study objectives were highlighted.

CHAPTER 2: This chapter includes a review of the pertinent literature on the usage of medicinal plants in South Africa for the treatment and management of men sexual health. It provides a list of various men sexual illnesses that include premature ejaculation, erectile dysfunction, diabetes, and sexual transmitted diseases.

CHAPTER 3: Describes the selected plants used in the study. The taxonomy of the plants and their conservation status is explained.

CHAPTER 4: Deals with the ethnobotanical survey and description of study areas; and gives the initial set of research outcomes, with a focus on the region's traditional knowledge systems of medicinal plants employed in the treatment and management of men sexual problems in selected villages under Thengwe Tribal Council.

CHAPTER 5: The second set of research findings on the bioactivity of drugs typically used to treat and control male sexual health are presented. A

discussion of the biological activities and an experimental conclusion are also included.

CHAPTER 6: Comprises the general conclusion and the summary of answers to the research questions and recommendations for future studies.

CHAPTER 1: INTRODUCTION

1.1 Background

Throughout history, plants played a central role in satisfying people's spiritual, and material needs. Indigenous plants are especially important in rural areas, where they supply valuable resources, such as shelter, clothes, firewood, medicine, and food (Chadare et al., 2010; Omotayo et al., 2020, Grunewald, 2009, Nyanga et al., 2008). Plants have been part of the universe for so many centuries surviving in harsh weather conditions and defending themselves against regular enemies such as bacteria, fungi and viruses through the secretion of chemical compounds used as defence mechanisms. Scientists, botanists, and conservationists have traditionally given little attention to indigenous plants (Schwartz, 1956; Peters and Maguire, 1981; Moshe, 2004; Shackleton, 2004, Pei et al., 2020). However, South African research interest on indigenous plant species resurged in the late 1980s. Despite synthesising complex molecules with specific stereochemistry, plants can exhibit biological activities with novel modes of action due to the presence of phytochemicals including phenols, flavonoids, and terpenoids (Houghton, 1996; Jafri et al., 2023). Most African communities still depend on medicinal plants to treat a variety of illnesses. About three quarters of the world's population relies on plants to treat many illnesses, according to the World Health Organization, 2015 (WHO). Ademoelja (2000), reported that 25 % of modern medicines that are consumed globally contain herbal ingredients derived from plants.

Since the dawn of the human race, plants and plant-based products have been used for treating various men health problems, including stimulating sexual desire, improving performance and enjoyment. The undaunted and continuous search for substances that can enhance men sexual performance dates back to ancient China,

Egypt, Hinduism, and Roman cultures (Shah, 2002). Indians and the Chinese have traditionally believed that eating an animal's sexual organ, such as goat testicles, can improve a men's sexual ability, the same as the Romans (Shah, 2002). Ancient Egyptians used aphrodisiac medications, which involved applying oiled baby crocodile hearts to the penis or ingesting a mixture of 37 ingredients, including pine, salt, and watermelon (Smith et al., 1974; Nunn, 1996). A variety of medicinal plants, including *Securidaca longepedunculata* are used by some local tribes in Limpopo to boost sexual performance (Mongalo et al., 2015). Locally, the Tsonga tribe is known to use medicinal plants to increase the size of their genitals whereas Vhavenda are known to use medicinal plants to increase their sexual desire and performance. Indigenous medicinal plants have been investigated for their potential to be developed to treat a variety of men sexual problems. Different plant parts (root, stem bark, leaves) and preparation methods are traditionally used to treat and manage men sexual health. This present study investigates ethnobotanical selected medicinal plants used traditionally by men to treat and manage sexual health in Thengwe, Limpopo Province.

1.2 The literature contextualisation of the study and research motivation

Although men sexual health has been a serious issue for decades, better solutions to the problem are still being investigated. Sexual health and function play a key role in determining the quality of life. Sexual inactiveness has been a major health problem for many centuries as it can cause considerable distress and unhappiness in a relationship between both men and women (Singh et al., 2012). Sexual dysfunction affects millions of men, both young and old, for a variety of causes, including diseases, hormonal imbalance, injury, nutritional imbalance, and an extremely low testosterone

level. According to Gregoire, (1999) men with sexual dysfunction believe that their relationship quality is less important to their sexual problems than their partners or women with such problems. Physical activities or physiological issues might lead to male sexual dysfunction. Men sexual health problems increase from generation to generation, and this plays a major role in decreasing the quality of sexual relationships between men and women. The kind of human lifestyle and food consumed are reported to contribute to increasing the statistics of men's sexual problems worldwide. A lack of exercise, an abundance of fat-rich foods and high cholesterol are known to result in diseases such as strokes and erectile dysfunction in about 322 million men worldwide (Aytac et al., 1999). In South Africa, about 6 million men above the age of 40, and 50% of them suffer from weak erections (Men's Clinic, 2015). However, statistics do not include men who cannot perform sexual intercourse through other problems such as mental illnesses and injuries, heart diseases, stress, etc.

The inability of men to perform sexual activities due to various illnesses has been reported to reduce self-esteem, compromise well-being and limit interpersonal relationships (Rendell et al., 1999). Due to the loss of physical intimacy, sexual intercourse and feelings, many married couples end up divorcing (Wagner et al., 2000).

Many illnesses that result in erectile dysfunction in men have been researched using medicinal plants. Many medicinal plants have been studied to treat erectile dysfunction and the Bapedi speaking people in South Africa are currently using 21 plant species belonging to 20 genera from 15 families to treat erectile dysfunction (Semenya and Potgieter, 2013). Based on a study conducted by Semenya and Potgieter (2013), the most common plant types in homes were in the families Hyacinthaceae, Fabaceae

Celastraceae, and Asteraceae. Although the studied species' active chemical constituents and their method of action are unknown, it is conceivable that these plant families contain bioactive secondary metabolites that fight ED or other related illnesses (Semenya and Potgieter 2013). Other research results demonstrated that species from the Apocynaceae (Sharma, 1960; Principe, 1989), Fabaceae (New,1984) Hypoxidaceae (Bose et al., 2008), Asteraceae (Zepeda et al., 2009), contain the aphrodisiac potential to assist in the management of ED.

1.3 Problem statement

Men's sexual health has been a serious problem affecting males for many decades and a better solution to the problem is still being investigated. Sexual health, sexual satisfaction, and function are significant determinants of quality of life. Sexually inactivity has been a major health problem for many centuries as it can cause considerable distress and unhappiness in human relationships (Singh et al., 2012). Millions of men, both young and old, experience sexual dysfunction today for a variety of causes, including disease, hormonal imbalance, injury, nutritional imbalance, diet, and incredibly low testosterone levels. Men with sexual dysfunction are less likely to perceive the quality of their general relationship more relevant to their sexual problems than their partners or women with sexual problems (Gregoire, 1999). Physical or physiological issues may be the root course of men's sexual dysfunction.

Men's sexual health problems have been increasing from generation to generation and this is playing a major role in decreasing the quality of life for both men and women. Lifestyle and food are reported to contribute to increasing the statistics of male sexual problems worldwide. Lack of exercise and food with a lot of fat have been

reported to result in illnesses such as strokes, and high cholesterol which also results in the inability to perform sexual intercourse.

1.4 Relevance of the research

By 2025, it is anticipated that 322 million men will experience erectile dysfunction globally, with the highest projection rises estimated in Asia, South America, and Africa, (Aytac et al., 1999). South Africa has about 6 million men above the age of 40, and 50% of them suffer from weak erections (Men's Clinic, 2015). However, this statistic does not include men who cannot perform sexual intercourse due other problems such as mental illnesses and injuries, heart diseases, stress etc.

The inability to perform sexual activities through various illnesses has been reported to reduce self-esteem, compromising well-being, and limiting social relationships (Rendell et al., 1999). The results subsequently lead to loss of emotional attachment between romantic partners and physical intimacy and at sometimes to divorce (Wagner et al., 2000).

Many illnesses that result in erectile dysfunction in men have been researched using medicinal plants. In addition to the several medications that have been researched, Bapedi-speaking people in South Africa currently use 21 plant species from 20 genera and 15 families to cure erectile dysfunction. According to Semenya and Potgieter's (2013) study, 9.5 % of plant species in Bapedi households are representatives of the Celastraceae, Asteraceae, Fabaceae, Apocynaceae, Hyacinthaceae, and Hypoxidaceae.

Although the active chemical components and mode of action of the examined species are unknown, the identified plant families may contain bioactive secondary metabolites that are effective against ED or related illnesses, (Semenya and Potgieter, 2013). Other research shows that species from the Apocynaceae (Sharma, 1960; Principe, 1989), Asteraceae (Zepeda et al., 2009), Fabaceae (New 1989) and Hypoxidaceae (Bose et al., 2008) contains aphrodisiac potential that assists in the management of ED.

1.5 Research questions

The current study seeks to answer the following research questions:

- Are the selected plants from previous studies well-known in the current study area?
- What is the level of ethnobotanical knowledge of the selected plants among the research participants of different ages?
- Do the selected medicinal plants possess the biological activities associated with men's sexual health?

1.6 Aims and Objectives

The aims and objectives of this study are as follows:

- To determine the Species Popularity Index (SPI) of the selected medicinal plants used to manage men's sexual health in villages under the Thengwe Tribal Council.

- To quantify the ethnobotanical knowledge of the selected medicinal plants among the research participants.
- To evaluate the biological activities of ethnobotanical selected medicinal plants associated with men's sexual health.

1.7 Hypotheses

This study aims to test the following hypotheses linked to the research questions:

- The selected medicinal plants used in the management of men's sexual health are well-known among the research participants.
- The selected medicinal plants possess biological activities associated with the management of men's sexual health.

1.8 Ethical consideration

Before the commencement of the research, the ethics application was submitted and approved by the University of South Africa CAES HREC committee (2016 September). Verbal consent was obtained from all the research participants.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The sexual health of individuals, couples, and families is essential to their overall health and happiness, as well as to the social and economic development of communities and countries. WHO (2015) reported that, sexual health-related issues may cause negative consequences and, or conditions such as infections with sexually transmitted infections (STIs), reproductive tract infections (RTIs) and human immunodeficiency virus (HIV), their adverse outcomes (such as cancer and infertility), and sexual dysfunction. Sexual activities in human life are recognized as an important component of a normal and healthy lifestyle and general well-being (Singh et al., 2013). For centuries, people have investigated methods on how to achieve sexual desire, sexual techniques or skills, and sexual health living (Gundidza et al., 2009). Many compounds known as aphrodisiacs have been developed and used to achieve the necessary excitation.

Herbal medicine has been in existence long before Western medicines were developed in Europe (Singh et al., 2013). The World Health Organisation (2015) described traditional medicine as the sum of all knowledge and practices, whether explicable or not, employed in diagnosing, preventing, and eliminating bodily, mental, or societal imbalances. Plants are exposed to different weather conditions and other enemies such as bacteria, viruses, fungi, etc. Unfortunately, plants are unable to escape the danger posed by these enemies simply by moving away and tend to defend themselves through a chemical defence system (Van Wyk and Gericke, 2000). Many valuable medications have been produced from medicinal plants that were used in traditional medicine to treat a wide range of ailments. Different kinds of plants have

been investigated on their role stimulating and improving sexual performance and virility. Chauhan et al., (2014) reported that effective therapies are still in demand even though many synthetic medications are accessible and/or utilized to treat sexual issues. These drugs' disadvantages include their high cost and potential for major side effects.

Various illnesses associated with men sexual problems have been investigated and drugs have been developed to treat them. Illnesses such as erectile dysfunction, malfunction of hormones, diabetes, sexually transmitted diseases, and permanent injuries can result in the inability to perform sexual intercourse. Several herbal plants such as *Securidaca longepedunculata* (Frasen) (Polygalaceae) *Wrightia natelensis* (Stapf) *(*Apocynaceae) and *Rhoicissus tridentana* L.F (Wild and Drumm) (Vitaceae) common in Venda (Limpopo) are utilized to treat erectile dysfunction (Rakuambo et al., 2006). Gilani and Atta ur-Rahman (2005) reported that aspirin (antipyretic), digoxin, morphine (pain killer), quinine (hypertension) and tubocuraríne are a few examples of drugs, which were discovered through ethnobotany studies.

Chauhan et al., 2014 discussed the Indian traditional medicine used for many years in India. Vajikarana herbs have been utilized by old men who want to have sexual pleasure or secure the affections of women, as well as those suffering from senile decay or sexual incapacity, and people who have been debilitated by sexual excesses (Chauhan et al., 2014). According to Rasendra Sara Sangrah in ayurvedic text Vajikarana remedy makes a man sexually strong and allows him to thoroughly satisfy the heat and amorous ardours of young maidens (Sharma, 1988; Puri, 2002).

Numerous researchers have discussed the factors that contribute to men sexual dysfunction, including issues in romantic relationships with sexual partners (Singh et

al., 2012, Chauhan et al., 2014, Masuku et al., 2020). Men who suffer from hypogonadism (low production levels of testosterone) can lead to low libido or ED. Even various drugs, such as antidepressants and blood pressure medications, are known to cause sexual problems in men.

2.2 Premature ejaculation

Premature ejaculation is when men are unable to control and hold ejaculation sufficient to permit both partners to enjoy sexual intercourse. Premature ejaculation affects most men, and it has been reported that in South Africa 6 million men above the age of 40 have experienced some sort of ED or early ejaculation problems (Men's Clinic, 2015). This can often cause ejaculation quickly after or, in severe situations, before penetration. Premature ejaculation affects about 20% of males, and in most cases, there is no physical explanation (Gregoire, 1999). It is more common in younger men, and there may be a learning process associated with improved sexual performance. Undoubtedly, anxiety plays a significant part in some men's ejaculation speed. Psychological techniques like the "pause and squeeze" technique are used to improve ejaculation control and lessen performance anxiety. Reported success rates are conflicting, and long-term follow-up suggest that benefits are not maintained (Rosen and Leiblum, 1995).

2.3 Erectile dysfunction

Erectile dysfunction (ED) is "Defined as an inability to achieve and maintain an erection sufficient for satisfactory sexual intercourse". Around 50% of men worldwide between

the ages of 40 and 70 suffer from ED, which is fairly common. It is estimated that over 152 million men are suffering from ED worldwide and by 2025 the number is expected to be around 322 million (Malvia et al., 2011; Aydin and Senel, 2020; Masuku et al., 2020). The three main processes involved in penile erection are smooth muscle relaxation, arterial dilation, and venous constriction. The smooth muscles of the penis, known as the corpora cavernosa, which contain penile blood vessels, relax when a man gets aroused, allowing the arteries inside to open. The penis becomes erect when blood flows into and fills it. The pelvic floor muscles, commonly known as kegels, then squeeze the veins leading to the penis, trapping the blood.

Erectile dysfunction is a multifactorial disorder that is associated with ageing, a range of organic and psychogenic conditions, including hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular disease, depression, and behaviours such as alcoholism, drug abuse and smoking (Burnett, 2006). Penile erection (Figure 2.1) is a complex process involving psychogenic and hormonal input, and a neurovascular nonadrenergic, noncholinergic mechanism. Nitric oxide (NO) is believed to be the main vasoactive nonadrenergic, non-cholinergic neurotransmitter and chemical mediator of penile erection.

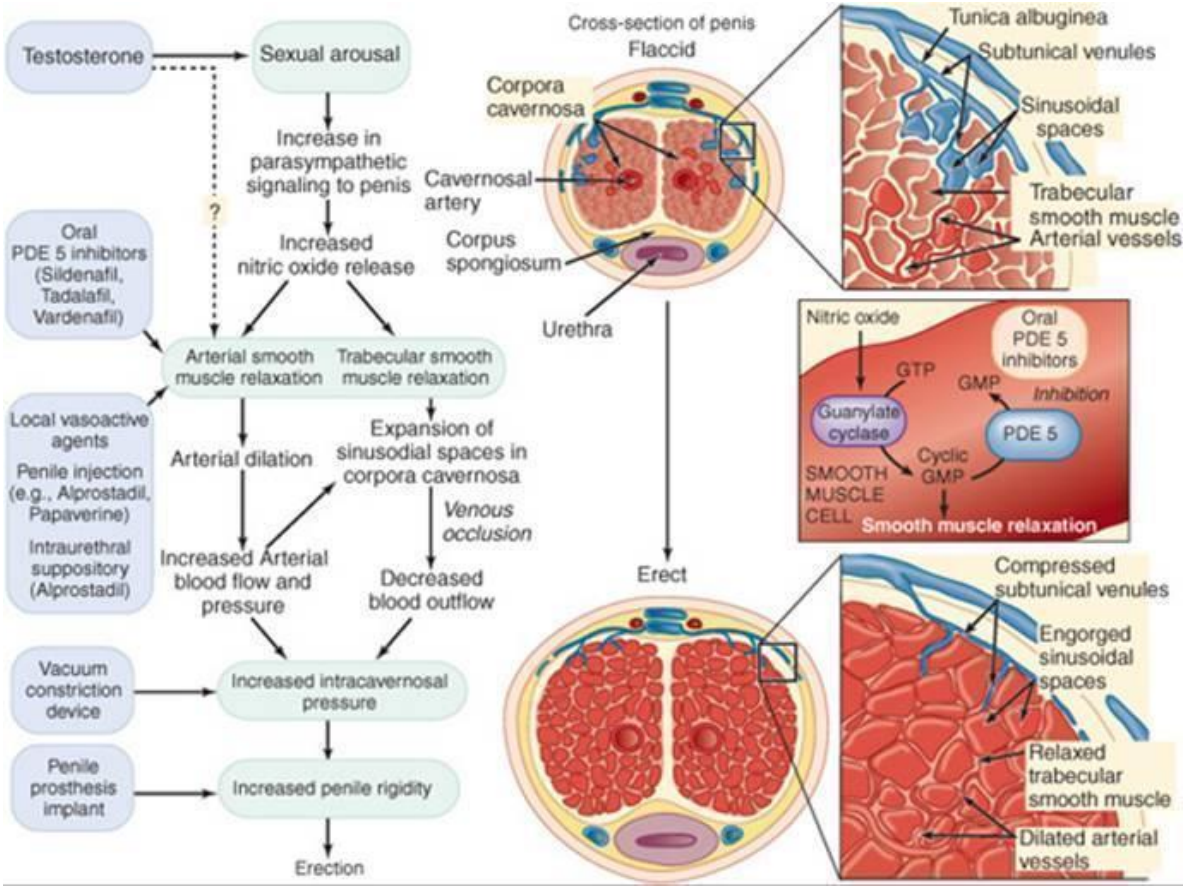

Figure 2.1: Mechanism of erection and sites of action of various treatment modalities for erectile dysfunction (ED). (https://www.mitchmedical.us/pharmacotherapy-principles/pathophysiology)

Erectile dysfunction (ED), sometimes called male impotence, is a common medical condition that affects the sexual life of millions of men (Ontorsi et al., 2003; Habsigh, et al., 2003). According to Singh et al, (2013) erectile dysfunction is defined as men's persistent inability to obtain and maintain an erection sufficient for naturally satisfactory intercourse. The National Institute of Health (1993) defined ED as the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse. On the other hand, Roper (2001) defines ED as the complete inability to achieve full erection, and the inconsistent ability to do so, or to sustain brief erections (premature ejaculation). It is one of the distresses that men can experience in their life.

Most men describe ED as one of the most embarrassing illnesses that a man can experience and this has been affecting men's lives as some see ED as an untreatable disease. Many incidents have been reported when men took their lives due to the problems of ED that affect men's sexual life and social lives.

Erectile dysfunction is considered one of the most important public health problems, since it affects a higher percentage of men. Erectile dysfunction is a serious medical and social symptom that occurs in 10% - 52% of men (Singh et al., 2013). It has been discovered that men between 17 and 96 years could suffer from ED as a result of physiological or physical health problems. Several scholars have reported various factors causing ED and they can be both mental and physical. Factors such as stress, anxiety, heart disease, diabetes, hypertension, hypercholesterolemia, obesity, diabetes, tobacco use, alcoholism, and sleep disorders affect ED (Ajiboye et al., 2018). ED is an inevitable process of ageing and prevalent in over 50% of men between 50 and 70 years of age (Singh et al., 2012). A study of ageing males showed that 52% of respondents have some degree of erectile dysfunction, with 35% of men aged 40-70 reporting moderate or complete impotence (Morgentelar, 1999). It is projected that about 322 million men worldwide are likely to develop ED by the year 2025. The projection shows that high increases are expected in Africa, South America and Asia (Aytac et al., 1999). A study conducted in Ghana found that early ED affects 74.4% of interviewed patients (Amidu et al., 2010). Locally, De Klerk and De Villiers (2003) showed that in a sample of 730 black and mixed-race males from the Western Cape Province of South Africa, 76.4% of black and about 77.7% of mixed-race men suffered from ED.

2.3.1 Risk factors for men erectile dysfunction

A variety of variables, including anomalies in the physical, psychological, physiological, and biochemical systems, come together to cause erectile dysfunction. Because ED typically develops as a result of an underlying ailment like diabetes and/or heart disease, the precise aetiology of ED is frequently difficult to pinpoint. Psychological problems like stress, depression, and anxiety can also contribute to ED. As a complicated neurovascular phenomenon, the physiological basis of erection depends on neuronal, vascular, hormonal, and psychological components (Aboua et al., 2014).

2.3.1.1 Diabetes

Diabetes refers to diseases characterised by chronically elevated blood glucose concentration above the range due to insufficient insulin or body tissue resistance to insulin hormone (Ogurtsova et al., 2017). There are two types of diabetes commonly known as type 1 diabetes and type 2 diabetes. Type 1 diabetes is also called insulin-dependent diabetes. It used to be called juvenile-onset diabetes, because it often begins in childhood. This type of diabetes may be caused by a genetic predisposition. It could also be the result of faulty beta cells in the pancreas that normally produce insulin. Several of medical risks are associated with type 1 diabetes. Many of them stem from damage to the tiny blood vessels in the eyes (called diabetic retinopathy), nerves (diabetic neuropathy), and kidneys (diabetic nephropathy). Even more serious is the increased risk of heart disease and stroke.

Type 2 diabetes used to be called adult-onset diabetes, but with the epidemic of obese and overweight kids, more teenagers are now developing type 2 diabetes. Type 2

diabetes is also called non-insulin-dependent diabetes. Type 2 diabetes is often a milder form of diabetes than type 1. Nevertheless, type 2 diabetes can still cause major health complications, particularly in the smallest blood vessels in the body that nourish the kidneys, nerves, and eyes. Type 2 diabetes also increases the risk of heart disease and stroke. Type 2 diabetes is by far, the most common form of diabetes accounting for 95% of diabetes cases in adults (Giugliano et al., 2010, Giugliano et al., 2019).

Diabetes may begin with erections that are less firm than before, and then progress to those which are shorter duration and less firm. Diabetes is an established risk factor for sexual dysfunction in men; a threefold increased risk of erectile dysfunction (ED) was documented in diabetic compared with nondiabetic men (Feldman et al., 1994, Giugliano et al., 2019, Giugliano et al., 2010).

The men will continue to have an interest in sex and be able to have orgasms. It is thought that this process occurs in about half of all diabetic men and is caused by diabetic neuropathy. Report from experts on men's sexual health reported that it can be slowed down and treated, but that actual tissue damage cannot be reversed (Chauhan et al., 2014). It is therefore very important to report symptoms as early as possible in order to get effective treatment. Sometimes impotence is a flag of undiagnosed diabetes, and then the impotence has a rapid onset. It comes with the other classic signs of diabetes. In these cases, it can usually be resolved with good control of glucose levels. Men with diabetes can develop sexual problems because of damage to nerves and small blood vessels.

Diabetes can result in men’s sexual problems for diseases such as erectile dysfunction and Retrograde Ejaculation**.** These illnesses affect other parts of the body that form part of the system which requires it to play a role before certain reactions occur. For

example, when a person wants to lift an arm or take a step, the brain sends nerve signals to the appropriate muscles. In 2012, more than 371 million people had diabetes, and this is expected to rise to 552 million by 2030, rendering previous estimates very conservative (Maiorino et al., 2014).

2.3.1.2 Sexually transmitted diseases

Sexually transmitted diseases (STD) have been a problem for both men and women for many centuries and still are even in the current world. STD's come with a wide variety of agents ranging from bacteria, viruses, etc. and they continue to damage other parts of the body if not treated. There are over 30 bacterial, viral and parasitic pathogens that have been identified to date that can be transmitted sexually. STDs colonize the genital region and often cause only mild symptoms (Ochsendorf, 2008). Oschsendorf (2008) also reported that infectious agents have different modes of impairing infertility (in men: organ damage, cell damage via mediators of inflammation, obstruction, binding to spermatozoa; in women: pelvic inflammatory disease and tubal obstruction). Bacterial sexually transmitted diseases such as gonorrhoea may impair male infertility. In a study conducted in Europe, gonorrhoeic urethritis was reported to be associated with urethral strictures (Dohle, 2003). Chronic infections (gonorrhoea) can cause urethral strictures and epididymal-orchitis. Although gonorrhoea is no longer a threat due to medical treatment improvement of gonorrhoea, some other chronic diseases such as human immunodeficiency virus (HIV) are currently a concern to STD's.

South Africa has the biggest and highest profile HIV epidemic in the world, with an estimated 8.3 million (20% of HIV globally) people living with HIV in 2021 (Ugwu and Ncayiyana, 2022). Through this study people will be able to have an idea of which

option they can take to resolve male sexual health problems which will help to address the issue of divorce that results from sexual problems in relationships. Men who suffer from ED will be able to attend to their sexual problems. The research will give hope to the male who has low faith in herbal medicine to resolve their sexual inactiveness. Herbal medicine will also address the challenge of high prices of modern medicine to treat male sexual health problems. Men will be able to use herbal medicine to enhance their sexual performance to satisfy their sexual partners.

2.3.1.3 Stress and depression

Many men do not realise that there are different types of erections – three, to be exact. A reflexive erection is due to physical stimulation while a psychogenic erection is triggered by visual or mental images. A nocturnal erection occurs during sleep. Once some of these erections are not occurring normally then it is a sign of erectile dysfunction.

Stress is regarded as one of the important factors of sexual dysfunction and infertility. It has been reported that stress can induce men sexual disturbances expressed as erectile dysfunction, ejaculatory disorders, loss of libido, a decrease in the frequency of intercourse (Lenzi et al., 2003), and a reduction of spermatogenesis (Nargund, 2015). The effects occur partly via the suppression of the hypothalamic-pituitary-gonadal (HPG-axis) axis (Nargund, 2015). In addition, a recent study also demonstrated that the dopaminergic system also contributed an important role in the regulation of men's sexual function (Hull et al., 2004). Therefore, the men sexual dysfunction appears to be a complex, multifactorial condition. Various aspects of sexual functions are affected in men with stress including sexual interest and poor

sexual performance (such as decreased libido, potency or satisfaction of erection or orgasm and premature ejaculation. In many cases, stress is the underlying factor, but it causes anxiety which then triggers more stress in vicious cycle.

Depression acts much like an anchor, weighing men down in body and mind, affecting all aspects of life physically and mentally. It has been reported that men who suffer from depression tend to think of it as something akin to sadness. While depression may cause feelings of sadness, it goes much deeper, and it tends to linger well beyond any specific cause or trigger. Some of the most common symptoms of depression include the following: feelings of sadness, emptiness, or hopelessness, angry outbursts, irritability or frustration, loss of interest in most or all normal activities, tiredness or lack of energy, anxiety, agitation, or restlessness, feelings of worthlessness or guilt, difficulty thinking or concentrating. These symptoms can make it difficult to take pleasure in much of anything, let alone sex (Baldwin, 2001).

2.3.1.4 Physical Injuries

Penile injury or fracture is one of the rare urological illnesses that occur because of an abrupt end to an erect penis (Nason et al., 2013). An injury to the penis can happen through an accident and during sexual intercourse. In Western society, the most common causes of fracture are during sexual intercourse (Kamdar et al., 2008).

The male reproductive system is very sensitive, it is easily affected by multiple risk factors, such as chronic diseases, environmental contaminants, drug toxicity and unhealthy lifestyle (Chen et al., 2019). The male reproductive system is a network of internal and external organs that interact from one part to another until the final reaction is achieved. For men to reach erection many activities need to happen before

erection, starting from the pituitary gland. Penis erection consists of a series of neurovascular activities caused by a net increase in the inflow of blood to the penis (Mariya and Oleg, 2013). The extent of erection depends on the balance between the amounts of blood perfusion flowing from veins (Jackson, 2013). The penis is in a state of relaxation when the arterial perfusion is low and the blood flow is balanced with the venous output (Muniz et al., 2013). When the arterial infusion of blood increases and the venous outflow volume decreases, the cavernous sinus of the penis becomes swollen and erects because of blood flow. Thus, penis erection is inseparable from artery perfusion, venous blood flow, and fine adjustments between them. Any physiological and pathological factors that cause injury or disturbance to these two aspects can lead to hemodynamic changes in the corpus cavernosum and can directly induce erectile dysfunction (Chen et al., 2019).

2.3.1.5 Hormonal imbalances

Hormonal imbalance refers to the malfunctioning of the hormone in such a way that it affects the reaction of human body parts and that ultimately tampers with human life. Hormonal imbalance occurs when body's glands secrete too little or too many hormones required to induce the reaction expected in the human body at a required time. One of the hormonal imbalances that some men suffer from is called hypogonadism, which affects their sexual lives. Hypogonadism refers to the decreased production of testosterone in males. According to the Cleveland clinic, this can result from the pituitary gland or the master gland in the brain for hormone production not stimulating the testicles to make testosterone or the failure of the testicles to produce adequate testosterone. Low testosterone levels in men lead to experience of low libido

(sex drive), erectile dysfunction, decreased energy, and decreased muscle mass and thinning of the bones which also affect male sexual health (Cleveland Clinic, 2022). Testosterone is one of the most popular hormones in both male and females due to its importance in the human body. The popularity of the male sex hormone derives from the fact that it acts in all organs and systems and has a significant influence on important aspects of life such as physical appearance, behaviour, mentality, abilities, sexuality and social status (Zitzmann and Nieschlag, 2001). According to Monti et al., (1977), testosterone levels in males have no significant association with different kinds of sexual activity, except for the frequency of masturbation. Similar results were reported in couples; in men there was no correlation of testosterone levels to sexual activity of any kind but in women sexual gratification, as well as frequency of intercourse, were positively related to testosterone levels (Persky, 1978). Therefore, it is assumed that in men testosterone levels are positively linked to libido, but that sexual activity in the partnership is moderated by the relationship itself (Zitzmann and Nieschlag, 2001).

2.3.1.6 Obesity

Obesity has been viewed as an interplay within a pathological loop between chronic inflammation and chronic oxidative stress disorder (Colquitt et al., 2014). The WHO defined obesity as “abnormal or excessive fat accumulation that present expressed as an abnormal and excessive body mass index of above 30 kg/ m^3. To determine a person's weight status, Body mass index (BMI) is calculated by dividing the weight in kilograms by the squared height of the individual, in meters (kg/m^2) (WHO, 2015). Esposito et al., (2008), showed that obesity plays a key role in reducing the

ability to perform sexual intercourse due to excessive amounts of fat that slows down the body. According to Han (2011), men had a higher waist circumference, even if they had a normal BMI. In addition, there is a strong belief that diet also plays a role in obesity and the occurrence of ED. Ramírez et al., (2016) reported poorer dietary intake of green vegetables and nuts as well as adverse lifestyle factors like tobacco smoking, alcohol consumption and inactivity in male patients with ED compared to those without the disease. In 2014, obesity was estimated to affect 1.9 billion adults worldwide (WHO, 2015; Chukwuonye, et al., 2015; Younossi, 2018).

2.3.2 The role of Nitric oxide in erectile dysfunction

Increased blood flow to the penis and an erection are the results of NO-activated relaxation of the corporal cavernosal smooth muscle tissue. The parasympathetic and sympathetic branches of the autonomic nervous system regulate the start of penile erection. Nerve stimulation triggers the nNOS to release NO. This subsequently starts a chain reaction that stimulates the synthesis of NO in endothelial cells via eNOS and iNOS (Davies, 2015).

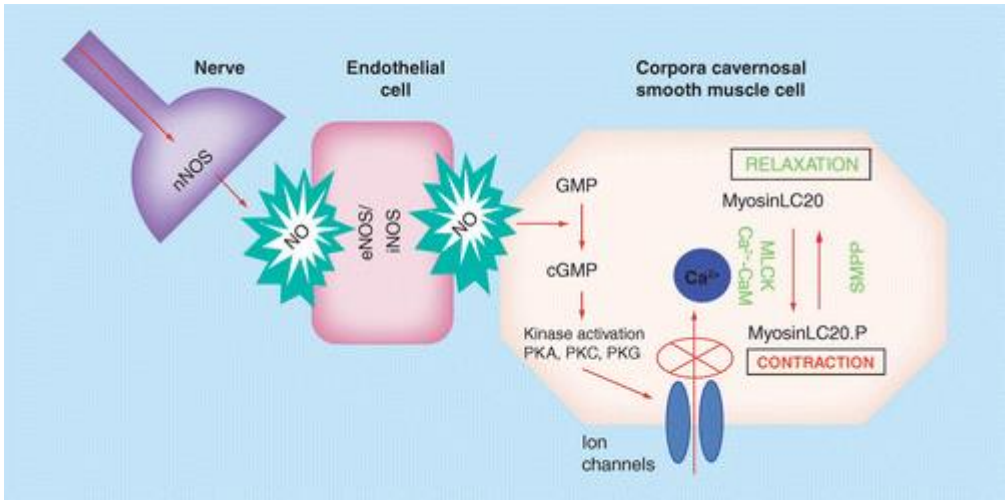

Figure 2.2: Nitric oxide pathways involved in erectile physiology (Davies, 2015).

Because it is involved in the relaxation of the smooth muscle, nitric oxide is a key factor in vasodilation. The corpora cavernosa is compressed and unable to receive blood if the smooth muscle is held taut. Since vascular illnesses and nitric oxide deficit have both been implicated as probable causes of ED in some patients, this finding suggests that 40% of men over 40 who experience ED symptoms also have cardiovascular disease (Burnett, 2006).

Increased blood flow to the penis and an erection are the results of NO-activated relaxation of the corporal cavernosal smooth muscle tissue. The erectile response caused by NO is currently amplified by pharmacological therapies for erectile dysfunction, such as phosphodiesterase-5 inhibitors (Davies, 2015).

2.4 Men Infertility

Fang et al., (2022) defined infertility as the inability to conceive naturally despite engaging in regular sexual activity without the use of contraceptives for 12 months. Infertility affects more than 15% of couples globally today. Among them, male infertility affects no less than 50% of infertile couples. Male infertility (MI) is a multifactorial condition that affects about 50% of male partners in infertile marriages and results in the disorder of male reproductive dysfunctions. After cancer and cardiovascular disease, male infertility has risen to become the third most challenging disease in the world. Although traditional therapies including medicine, surgery, and cutting-edge methods have assisted many infertile men in getting their female partners pregnant, effectiveness is not sufficient and is linked to negative effects (Fang et al., 2022).

It is now well-accepted that oxidative stress (OS) is the primary cause of male infertility. Although sperm physiology requires only small amounts of reactive oxygen species (ROS), their elevated level disrupts sperm function and causes men infertility through mechanisms like lipid peroxidation (MDA) and DNA damage (Sheweita et al., 2020). Many biological and environmental factors including infections, and diabetes can impact male fertility.

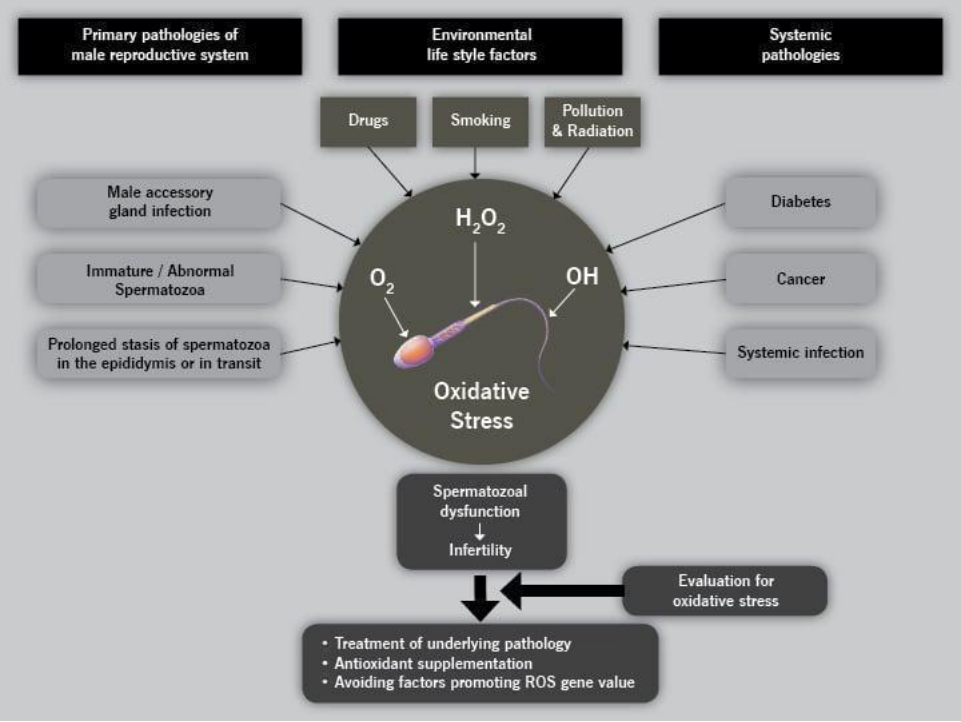

Figure 2.3: Biological and environmental factors that impact fertility (https://consultqd.clevelandclinic.org/what-the-internist-needs-to-know-about-infertility).

Male infertility treatments can be classified as either noninvasive which mainly contain antioxidant therapy, hormone stimulation therapy and lifestyle modifications. Invasive treatments include *in vitro* fertilisation (IVF), intracytoplasmic sperm injection (ICSI) and intrauterine insemination. However, both treatments are expensive and have many side effects such as the increased risk of developmental malformation and childhood cancers. Therefore, natural treatment in the form of medicinal plants or

traditional medicine remains an alternative source for most male patients to improve their fertility (Fang et al., 2022).

Recently there has been an increased interest in complementary therapies for infertility, and several antioxidants, dietary regimens, and medicinal plants have been suggested for the treatment of reproductive issues in infertile and sub-fertile couples. In many cultures across the world, medicinal plants with antifertility or fertility-boosting properties have been utilised to either reduce or improve male fertility. These plants' fertility-related qualities have also attracted the attention of contemporary scientific studies (Roozbeh et al., 2021).

2.5 Medicinal plants in the treatment and management of men sexual problems.

Nearly as old as humanity itself is the usage of plants or items derived from plants to arouse sexual desire and to improve performance and enjoyment. The active natural components of medicinal plants are effective in treating sexual diseases, can enhance sexual behaviour and performance, and aid in spermatogenesis and reproduction (Chauhan et al., 2014).

To find novel chemical compounds effective in treating sexual and erectile dysfunction, a number of herbal medicines have been evaluated for their impact on sexual behaviour and fertility.

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CHAPTER 3: SELECTED PLANTS USED IN THE STUDY

3.1 Plant selection

A list of 21 traditional medicinal plant species was selected based on the previous surveys done by Mophuting and Tshikalange (2015) and Tlakula, (2016) (Table 3.1). These studies indicated that the chosen medications were used to treat and manage issues related to male sexual health.

Table 3.1: List of medicinal plants, their vernacular name in Tshivenda, growth type, part used, main uses, major phytochemicals, and biological activities.

Botanical name	Vernacular name (in Tshivenda)	Growth habit	Part used	Main uses	Major Phytochemicals	Biological activities	References
Apiaceae *Heteromorpha arborescens* var abyssinica	Muthathavhanna	Shrub	Roots	Abdominal pains, Diarrhoea, Aphrodisiac	alkaloids saponins phenol	Antifungal α-glucosidase inhibitor	Adamu et al., 2016 Asfaw et al., 2023 Ajao et al ., 2019 Abifarin et al., 2020
Apocynaceae *Rauvolfia caffra* Sond.	Munadzi	Tree	Latex, bark, leaves	Toothache, Wounds, High blood pressure, Epilepsy	Alkaloids	Anti-seizure Anti-microbial	Chipiti et al., 2021 Bitombo et al., 2021
Apocynaceae *Tabernaemontana elegans* Stapf	Muhatu	Shrub	Bark	STIs	Alkaloids phenols terpenes steroids	Anti-mycobacterial, Antiplasmodial	Lawal and Tshikalange, 2020 Naidoo et al., 2021
Apocynaceae *Wrightia natalensis* Stapf	Musunzi	Shrub/small tree	Roots	Aphrodisiac	Tyrosinase	Antibacterial	Lawal and Tshikalange, 2020
Celastraceae *Elaeodendron transvaalense* (Burtt Davy) R.H. Archer	Mukuvhazwivhi	Shrub/small tree	Roots Bark Leaves	STIs	Triterpenes	Antimicrobial	Maroyi and Semenya 2019,
Clusiaceae *Garcinia livingstonei* T. Anderson**.**	Mupimbi	Tree	Roots Bark Leaves	STIs Aphrodisiac	Xanthones Flavonoid	anti-bacterial, anti-malarial, anti-atherosclerosis, anti-cancer, anti-hypertension	Khamthong and Hutadillok towatana, 2017
Combretaceae *Terminalia prunioides* M.A. Lawson	Mutwari	Tree	Bark	STIs	proanthocyanidins	anti-inflammatory, Antimicrobial activity anti-fungal	Masoko, 2007 Lawal et al., 2019
Fabaceae *Cassia abbreviata* Oliv.	Mulumanamana	Shrub/small tree	Bark Leaves	STIs	Tannins, phenolics, flavonoids, glycosides, alkaloids, anthraquinone	Anti-HIV	Leteane at al., 2012. Chauke et al., 2015 Kabuka et al., 2022
Loganiaceae *Strychonos madagascariensis* Poir	Mukwakwa	Tree	Fruit Bark	Aphrodisiac	Alkaloids	Antibacterial, Antifungal	Adebowale, A., 2014., van Rayne et al.,, 2022

Botanical name	Vernacular name (in Tshivenda)	Growth habit	Part used	Main uses	Major Phytochemicals	Biological activities	References
Loganiaceae *Strychnos spinosa Lam.*	Muramba	Shrub/small tree	Fruit	STIs, Aphrodisiac	Alkaloids	Antibacterial, Antifungal	Asuzu and Nwosu, 2017., Omotayo and Aremu, 2021
Moraceae *Ficus abutilifolia* Miq.	Tshikululu	Tree	Leaves Bark	STIs	Phenolics, saponins, flavonoids, tannins	Anti-bacterial	Danmalam et al., 2012 Madeleine et al., 2020 Taiwo et al., 2016
Phyllanthaceae *Bridelia mollis Hutch.*	Mukumba-kumba	Tree	Bark Roots	STIs	Gallocatechin, isoflavone, bridelonine Proanthocyanidins	Anti-inflammatory Anti-gungal Anti-microbial Anti-plasmodial,	Ngueyem et al., 2009 Lawal et al., 2019
Phyllanthaceae *Pseudolachnostylis maprouneifolia* Pax	Mutondowa	Tree	Stem bark roots leaves	chronic diseases cardiovascular infection, neural degeneration Diabetes, obesity	Phenolics	Anti-flammatory Antioxidants Anti-microbials	Moura et al., 2018 Kasali et al., 2021 Augustino et al 2011 Matowa et al., 2020
Picrodendraceae *Androstachys johnsonii* Prain	Musimbiri	Tree	Roots Bark Leaves	Aphrodisiac	Flavone Defensin	Anti-bacterial Anti-fungal	Molotja et al., 2012
Polygalaceae *Securidaca longepedunculata* Fresen	Mpesu	Tree	Roots	Aphrodisiac	Phenols Terpenoids	Anticonvulsant Antiparasitic, Pesticidal	Mongalo et al., 2015
Proteaceae *Faurea saligna* Harv.	Mutango	Tree	Leaves Roots	STIs	Proanthocyanidins	Anti Inflammatory	Lawal et al., 2019
Rutaceae *Zanthoxylym capanse* (Thumb) Harv.	Mununguu	Shrub	Leaves Bark	STIs	Alkaloids Terpenoids	Antibacterial Antioxidant Anticancer	Bodede et al., 2017
Solanaceae *Solanum panduriforme* Hochst	Mututulwa	Herb	Fruit	STIs	Alkaloids Phenols Terpenoids	Antibacterial	Karanja et al., 2021
Urticaceae *Obetia tenax* (N.E.Br.) Friis	Muvhazwi	Shrub/small tree	Leaves	STIs	Terpenoids	Antioxidant Antidiabetic	Mahlangeni et al., 2020
Vitaceae *Rhoicissus tridentata* (L.f.) Wild & R.B.	Murumbula mbudzana	Creeper	Rootstock	STIs Impotence Infertility	Terpenoids	Antimicrobial Anticancer	Dube et al., 2021
Diospyros mespiliformis Hochst. Ex A. DC	Musuma	Tree	Bark Leaves fruits	Syphilis Leprosy Pneaumonia	Alkaloids Flavonoids gycosides	Antioxidant Antiinflamatary Anticancer Antimicrobial	Burkil, 1995

3.1.1 *Cassia abbreviata* Oliv.

Description

Cassia abbreviata (family: Fabaceae, common names: wild senna, long-tail cassia, sjambok pod, (Muvhonelathangu) in Tshivenda. *C. abbreviata* subsp. beareana (Figure 3.1) is a deciduous tree of small to medium size with a height of 7 m. The bark of this tree, which ranges in colour from dark brown to grey and black, covers its slender trunk. Older trees' frequently have deep ridges in the bark. The leaves, which tend to drop slightly, are composed of multiple leaflets, green when young and a darker green as they age. It has yellow, sweet-smelling flowers that are large, loose, and become brown-vented with age, and its fruit is cylindrical and dark brown, hanging (Venter and Venter, 2009).

Distribution

Natural habitats of this species include arid lowland bushveld, open woodland, and sometimes riverbanks. They are popular in East Africa, in Southern Africa (Limpopo Province, Mpumalanga), Swaziland, and even Somalia (Brown, 2013).

Ethnobotanical Usage

In Southern Africa, traditional medicine, parts of *C. abbreviata* are utilised to treat various illnesses and the root is well known to treat toothache. The smoke from smouldering twigs can be inhaled to relieve headache. In South Africa and Botswana, the roots of *C. abbreviata* are ground into fine powder, soaked in water and used to cleanse dirty blood after a woman has miscarried (Setshogo and Mbereki, 2011).

Figure 3.1: The leaves and flowers of *Cassia abbreviata* (http://pza.sanbi.org, 2020).

Conservation Status

Cassia abbreviata is endangered and is reported to rank number 3, scoring 401 with a frequency of 33 of the top 10 priority, medicinal trees in the Shinyama region, Zambia (Dery et al., 1999).

3.1.2 *Faurea saligna* Harv.

Description

Faurea saligna (family: Proteaceae, common names: African beech also known as (willow beechwood), and Mutango in Tshivenda. In KwaZulu-Natal*, F. saligna* (Figure 3.2) grows to a height of up to 20 m in the mature stage, but is usually a small, willowy tree. Its long, narrow, hairless leaves measure 65-125 mm in length, waxy, slightly

sickle-shaped, lanceolate-elliptic, and drooping, (Mbambezeli, 2008). The margins of the leaves are entirely, green during summer and red in autumn season. Leaf stalks are about 20 mm in length. During fruiting, these flowers turn silvery hairy and are slender, pale yellow.

Distribution

The north-eastern region of southern Africa, including Zimbabwe, Mozambique, North-West province, Gauteng, Limpopo province, Mpumalanga, Swaziland, and KwaZulu-Natal, is known to be favourable for *F. saligna.* Faurea is the only genus in the Proteaceae family that is not endemic in the south-western Cape (Mbambezeli, 2008).

Ethnobotanical Usage

The *Faurea saligna* tree makes woods and is suitable for furniture. The tree is also processed to make poles. Its woods are known to be resistant to termites. It also makes good firewood. Through soaking the *Faurea saligna* stem bark in the water, it makes the red dye.

Figure 3.2: The leaves and flowers of *Faurea saligna* (http://pza.sanbi.org, 2020).

Conservation status

Faurea saligna is not a threatened species as it is well-distributed and common and grows in large populations in Southern Africa.

3.1.3 *Diospyros mespiliformis* Hochst. Ex A. DC.

Description

Diospyros mespiliformis (family name: Ebenaceae, common names: African ebony also known as jackal-berry, it's called Musuma in Tshivenda. *D. mespiliformis* (Figure 3.3) is a tall, upright tree with a dense evergreen canopy that can grow to a height of 25 m (Etkin, 1997). The bark is blackish-grey and is rough in texture. The colour of the bark's new inner skin is reddish. It has simple, alternating, leathery, and dark green leaves.

Distribution

Diospyros mespiliformis is common in the savannah and woodland areas, often on termite hills. It is distributed along the eastern part of the African continent, all the way from Ethiopia to the southern part of Swaziland. It grows vegetatively in areas with good rainfall with little or no frost areas. The tree is also common in West African regions such as Guinean and Sudanian woodlands and is generally widespread in such localities across Africa except in the Congo Basin (Kasimu et al., 2017).

Ethnobotanical Usage

The children of indigenous people relish *Diospyros mespiliformis* ripe fruits. Fruits can be consumed when fresh or are dried and placed in storage for later consumption.

Sometimes people use the young twigs as toothbrushes. The wood is strong and used for artistic purposes to make spoons and canoes. Infusion of the leaves is used to treat fever, pneumonia, syphilis, leprosy, and yaws (Von Maydell, 1990). Diseases such as infections of the skin, arthritis, and headaches are also treated with the leaves. Fruits and leaves are chewed or applied as an infusion for treating gingivitis, toothache, and for wound dressing to prevent infection (Burkill, 1995). Root decoctions are consumed to eliminate internal parasites like worms. The trees provide shade and make an incredible screen or windbreak.

Figure 3.3: The leaves and fruits of *Diospyros mespiliformis* (http://pza.sanbi.org, 2020)

Conservation status

The tree is not specifically endangered or protected in South Africa, but because of its importance to the ecology and the food chain in which it is embedded, it should be given some protection.

3.1.4 *Ficus abutilifolia* (Miq.) Miq.

Description

Ficus abutilifolia (family: Moraceae, common names: large-leaved rock fig, rock wild fig), Tshikululu in Tshivenda. *F. abutilifolia* (Figure 3.4) is a small to medium-sized, deciduous to semi-deciduous tree that grows up to 5 m in height and is generally encountered along streams (Palgrave, 2002). It has a distinctive bark that is immediately noticeable. It is colourless to yellowish-white and smooth, powdered, or slightly flaky. The branchlets are thick with no hairs and marked with leaf and stipule scars. The stem is typically twisted or distorted.

Distribution

Ficus abutilifolia grows on sandstones as well as granite and is frequently found on or near rock outcrops (Burrows and Burrows, 2003), basalts, and ironstone. The tree is widely distributed on the African continent (Burrows and Burrows, 2003; Palgrave, 2002). It is distributed along KwaZulu-Natal, Mpumalanga, Gauteng, and the northern parts of Mozambique, Zimbabwe, Botswana, Namibia, Malawi, Zambia, Ethiopia, Somalia, and west of Guinea.

Ethnobotanical Usage

Ficus abutilifolia wood is light and soft but strong (Burrows and Burrows 2003); it dries to a yellow-brown colour and works and polishes easily but is not very useful to people in general. The plant's milky latex is used to treat skin warts, while decoctions made from the leaves are used to encourage human fertility and good crop yields. (Taiwo et al., 2016; Singh, and Sharma, 2023). In addition, men use bark decoctions as a strengthening tonic (Hutchings, 1996).

A **B**

Figure 3.4: The leaves, fruits (A) and widespread roots of *Ficus abutilifolia* (http://pza.sanbi.org, 2020).

Conservation Status

Ficus abutilifolia is categorized under species that are not threatened.

3.1.5 *Garcinia livingstonei* T. Anderson

Description

Garcinia livingstonei (family: Clusiaceae, common names: African mangosteen). Is a small tree that grows to a height between 10-16 m and is pyramidal when young. However, later it spreads, and develops thick, woody branches with yellowish-red resin. The leaves are often three in a whorl, varied in shape but typically egg- or lance-shaped, typically 60-110 mm 30-55 mm, blue-green with whitish veins, and waxy. Joseph et al., (2017) describe the fruit as orange-yellow, orange to reddish-orange in

colour, with a soft juiciness and slightly acidic taste. Fruits (Figure 3.5) contain moderate amounts of minerals and have a high carbohydrate content (FAO, 1988).

Distribution

The plant is widespread in warmer parts of Africa such as Somalia, Guinea and Southern Africa.

Ethnobotanical Usage

In traditional medicine, the root of the tree is used as an aphrodisiac, especially when powdered. According to Palgrave (2002), the fruits can be fermented to create a delicious alcoholic beverage. Borers are attracted to the wood, but it has been utilised for general purposes.

Figure 3.5: The fruits of *Garcinia Livingstone* (http://pza.sanbi.org, 2020).

Conservation status

Garcinia livingstonei is not listed on under threat plant species.

3.1.6 *Obetia tenax* (N.E.Br.) Friis

Description

Obetia tenax (family name: Urticaceae: common name: giant nettle, stinging nettle tree, mountain nettle, rock tree-nettle, tree nettle,) Muvhazwi in Tshivenda. *O. tenax* (Figure 3.6) is usually a small, deciduous, succulent tree or shrub, which grows between 2 to 7 m tall in the Northern part of Limpopo province. The main stem is smooth, the bark is dark to reddish bronze in colour and has noticeable lenticels, and when peeled, it reveals clear sap. Flowers are often produced in late winter to early spring, in branching clusters, and range in colour from greenish yellow to white (Shackleton et al., 1998)

Distribution

Obetia tenax grows naturally in the Eastern Cape, Gauteng, KwaZulu-Natal Limpopo, Mpumalanga, and the Northwest province of South Africa. It can be found in Swaziland, Botswana, Zimbabwe, and Mozambique but is not native to South Africa. It thrives in forest, thorn-veld, and valley bushveld, as well as on dry, rocky hillsides, among loose stones, and occasionally next to streams (Neuwinger, 2000).

Ethnobotanical Usage

Due to its strong fibre from the bark of *O. tenax* is traditionally used to construct cable, rope, thatching, and sleeping mats. The leaves are cooked and eaten with porridge, and it is also used in traditional medicine. Root pulp is applied and used as a remedy for snakebites (Neuwinger, 2000; Shackleton et al., 1998). The hairs can lead to skin blisters and an excruciating burning discomfort. It can be a useful barrier plant in the garden.

A B

Figure 3.6: The leaves (A) and pruned (B) domesticated *Obetia tenax*.

Conservation status

Obetia tenax is assessed as the least concern and is not a threatened plant species.

3.1.7 *Pseudolachnostylis maprouneifolia* Pax

Description

Pseudolachnostylis maprouneifoli (family: Phyllanthaceae, common name: kudu berry) and Mutondowa in Tshivenda. *P. maprouneifolia* (Figure 3.7) is the only species in this genus and it exclusively occurs only on the African continent. *Pseudolachnostylis maprouneifolia* is roundly in shape with single-stemmed that grows to the height of 12 m in Mpumalanga and Limpopo where conditions are favourable. The bark is greyish to darkish brown. It is considered a slow growing tree, but juveniles tend to grow much quicker than established trees. It is a deciduous tree,

and it has an impressive display of crimson autumn colour, before losing its leaves in the winter. From July to November, the tree blooms, producing tiny, greenish-white flowers. On different trees, the sexes are separated (Maroyi, 2012).

Distribution

The kudu berry thrives in frost-free regions and can tolerate hot, dry conditions. It is naturally found in mixed deciduous vegetation, woodland, sandy, and on rocky ground. It is found throughout Zimbabwe and the northern provinces of Mpumalanga and Limpopo in southern Africa. It also exists in Botswana's and Namibia's northern regions (Palgrave, 2002).

Ethnobotanical Usage

Bark extracts from *Pseudolachnostylis maprouneifoli* are used to cure diarrhoea. Historically, it has also been utilized to treat pneumonia. As it grows naturally in frost-free regions like Limpopo and Mpumalanga, the tree provides lovely shade trees in parks and other public areas.

Figure 3.7: The young *Pseudolachnostylis maprouneifolia* (http://pza.sanbi.org, 2020).

Conservation status

Although *Pseudolachnostylis maprouneifolia* is not in danger, the ecology in which it occurs is because of several issues, including population increase, farming, improper game farming techniques, and perhaps global warming.

3.1.8 *Rauvolfia caffra* Sond.

Description

Rauvolfia caffra (family name: Apocynaceae, common name: Quinine tree) Munadzi in Tshivenda. *R. caffra* (Figure 3.8) is an evergreen tree with a rounded crown and a height of up to 30 m. It is commonly known as “quinine tree” and is utilised by natives in Sub-Saharan Africa for medicinal purposes (Nkunya, 1992). The stem is tall, naked, and straight. On younger branches, the bark is grey to brown, rough, and features noticeable leaf scars. As it ages, the bark becomes yellowish brown, thinly corky, and broken into little squares. The leaves are simple, in whorls of 3-6, clustered at the extremities of the short branches, slightly leathery, tapering to both ends, 120-280 mm long and 30-60 mm wide. With a high midrib and smooth margins, the leaf blade is glossy green above and lighter green below.

Distribution

South Africa is usually found in Limpopo, Mpumalanga, Kwa-Zulu Natal, Eastern Cape, Gauteng, North-West and Western Cape province, (Njau et al., 2014).

Ethnobotanical Usage

The wood is used to make for drumbeat, general timberwork in farming, and it is ideal for kitchen furniture and shelving, (Govender, 2017, Njau et al., 2014)

Figure 3.8: The *Rauvolfia caffra* (https://cjmgrowers.co.za/rauvolfia-caffra/).

Conservation status

Rauvolfia caffra is a protected tree in South Africa, there is no concern about its overuse for commercial purposes.

3.1.9 *Rhoicissus tridentata* (L.f.) Wild & R.B. Drumm.

Description

Rhoicissus tridentata (family name: Vitaceae: common names: wild grape, northern Bushman's grape, bitter grape,) Murumbula-mbudzana in Tshivenda. *R*. *tridentata* (Figure 3.9) is a deciduous, scrambling, shrubby creeper that occasionally develops

into a shrub or a small tree. It has tendrils and can reach heights of up to 3 meters. It features trifoliate leaves that emerge as a fresh green and develop into lustrous, dark, or bluish green, leathery leaves as they age. The plant's new branches typically have rust-coloured or silky greyish hairs covering them (Nqolo, 2008).

Distribution

Rhoicissus tridentata is widely distributed across all provinces of South Africa, and accures in tropical Africa and the Middle east.

Ethnobotanical Usage

The *Rhoicissus tridentata* is known for its medicinal purpose where parts of the plant such as tuberous rootstock are used to treat various illnesses. It contains a lot of flavonoids, which are a group of chemical substances made by plants that have positive health effects when consumed. Infertility, stomach, kidney, and bladder issues, as well as dysmenorrhea (a medical term for pain during menstruation), are all treated using the tuberous rootstock. (Dube et al., 2021).

Figure 3.9: The leaves and fruits of *Rhoicissus tridentata* (http://pza.sanbi.org, 2020).

Conservation status

The Rhoicissus tridentata species is not under threat with least concerned status.

3.1.10 *Solanum panduriforme* Eckl. & Zeyh.

Description

Solanum panduriforme is a perennial herb or herbaceous small shrub that is usually unarmed but may have a few prickles. The leaves are elliptic, whole, or slightly wavy on the margins, dark green on top and lighter and tomentose on the underside. Flowers are arranged in clusters along the branches, sometimes axillary or leaf-opposed; corolla is pale to deep blue, mauve or purple, infrequently white, 1.5 cm in diameter, and fragrant. When fruits (Figure 3.10) are ripe, they are spherical, green, and frequently striped or mottled with white, turning yellow to orange-brown (Baumann, 2005).

Distribution

According to Mey (1997), the distribution of *Solanum panduriforme* occurs in grassland along roadsides throughout South Africa's four provinces: the Free State, KwaZulu-Natal, the Eastern Cape, Swaziland, Lesotho, Namibia, and Botswana.

Ethnobotanical Usage

Solanum panduriforme is used for medicinal purposes to treat various illnesses. The roots are used to treat stomach-aches and tooth aches. Unripe green fruits are used to treat skin infections, toothache, and haemorrhoids as reported by (Pooley, 1998). *Solanum panduriforme* has been used in several studies to investigate its effectiveness to serve as botanical pesticides, an environmentally friendly alternative way of controlling pests in agricultural vegetable production.

A **B**

Figure 3.10: Ripen fruits (A) and a small *Solanum panduriforme* plant (B) (http://pza.sanbi.org, 2020).

Conservation status

Pelargonium panduriforme is very common in Southern Africa and is therefore not threatened.

3.1.11 *Strychnos madagascariensis* Poir

Description

Strychnos madagascariensis (family name: Loganiaceae, common name: black monkey orange) and Mukwakwa in Tshivenda. The black monkey orange tree has a single or more stems and a spreading, uneven, angular canopy. It is a densely branched tree that develops to the height of 5-8 m tall. The bark is light grey with white flecks that deepen over time. The simple leaves are oppositely oriented, green, hairy, and leathery, and have an entire border. The leaves are crowded on the tips of short thick branches and are not joined by an evident leaf stalk. It grows alone or in groups with other tree species and is commonly a lone tree (Van Rayne et al., 2020). *S. madagascariensis* fruits (Figure 3.11) edible.

Distribution

Strychnos madagascariensis is found throughout Sub-Saharan Africa, particularly in drought-prone locations and semi-arid Kalahari Sand-veld with 600 - 1500 mm rainfall; the tree is more common when demanding trees fail to thrive (SCUC, 2006). It is found in Botswana, Limpopo, North-West, Mpumalanga, Swaziland, and KwaZulu-Natal in Southern Africa. It can be found in the Sand Forest along the coast and is common in the bushveld savannah's woodland and thorn veld.

Ethnobotanical Usage

Many Loganiaceae genera are ethnobotanically helpful, with *Strychnos* being the most well-known for this due to the curarizing and other medicinal characteristics of its alkaloids (Bisset and Phillipson, 1971). The plant is frequently utilized in traditional medicine; the edible meat is pounded and dried. Despite being bitter, the dried seeds are regarded as a delicious treat for kids. The dried shells are used to make flutes and marimbas as musical instruments. The plant has been used medicinally as an emetic. Grounded roots are combined with hot water and consumed orally. A paste is made from the fruit for treating jigger fleas. Dried fruit shell is painted with different colours and used for house indoor decorations.

A **B**

Figure 3.11: Ripen (A) and unripe (B) fruits of *Strychnos madagascariensis* (http://pza.sanbi.org, 2020).

Conservation status

There is no recorded information about *Strychnos madagascariensis* conservation status.

3.1.12 *Strychnos spinosa* Lam.

Description

Strychnos spinosa (family name: Loganiaceae, common name: spiny monkey orange or green monkey orange) and Muramba in Tshivenda. The plant is deciduous shrub or small tree that grows up to the height of 10 m tall, with a trunk sometimes fluted, up to 25 cm in diameter, branching from low down. The tree is heavily branch, with flattish and irregular canopy (Mwaura et al., 2016). *S. spinosa* (Figure 3.12) blooms from September to February/Spring to Summer, producing tiny greenish-white flowers in dense heads at the extremities of branches. The huge, green, firm, silky fruits turn yellow as they ripen.

Distribution

It is widely distributed in tropical areas of African continent, from Senegal to South Africa. "Kwokwa or Kokiya" is one of the Hausa people of Nigeria's common names for it (Neuwinger, 1996).

Ethnobotanical Usage

The leaves, roots, and fruit (seeds) are crucial for therapeutic applications. The plant species used in traditional medicine to cure conditions like leprosy, diarrhoea, fever, ulcers, wounds, headaches, gastric and intestinal issues, and venereal infections (Neuwinger, 1996). Others use the unripe fruit and bark. Strychnine, along with other alkaloids present in the bark and unripe fruit, is thought to help humans resist the venom of some snakes (Hedberg et al., 1983, Mors et al., 2000). Strychnine, a potent stimulant of the central nervous system, may be able to counteract the respiratory depression brought on by these snakes' venom. It is employed as a purgative, to treat

uterine issues, and to soothe itchy eyes. In Central Africa, a decoction of the leaf or root is used as an analgesic.

Figure 3.12: The leaves of *Strychnos spinosa* (http://pza.sanbi.org, 2020).

Conservation status

Strychnos spinosa is very common in South Africa (Limpopo, Mpumalanga, and Kwa-Zulu natal and is therefore not threatened.

3.1.13 *Tabernaemontana elegans* Stapf

Description

Tebernaemontana elegans (Family name: Apocynaceae: common names: toad tree) is an unarmed shrub or tree mostly 1.5 to 5 m tall but occasionally reaching 12 m depending on the environmental conditions (Palgrave et al., 2003). The trunk can be between 50 to 300 mm in diameter with a corky, pale brown bark with longitudinal fissures. Twigs have prominent leaf scars that form transverse ridges. The tree is common in the far Northeast of Limpopo province (Thengwe area) where this study was conducted. The leaves are opposite, leathery, dark glossy green on top and lighter

below. The size of a leaf can range from 90 to 200 mm by 50 to 70 mm, and it is typically 2 to 4 times longer than it is wide. The fruit (Figure 3.13) is sub globose, paired, and has a green skin that is covered in light warts. Each fruit has two lateral ridges and one dorsal ridge and measures roughly 60 to 70 mm long by 40 to 50 mm wide. The fruit's wall is 5 to 15 mm thick and ranges in texture from leathery to woody. When fully grown, they split apart along one side, frequently while still attached to the tree, revealing the yellow pulp inside. Each fruit on the toad tree has a green, warty skin that resembles a toad, hence the common name.

Distribution

The Apocynaceae family, which includes over 100 species of *Tabernaemontana elegans*, is widely dispersed throughout the tropics (Mansoor et al., 2009). It is common in the Eastern Africa (Somalia) to south-eastern Africa, to South Africa and Swaziland.

Ethnobotanical Usage

Native South Africans use the medicinal plant *Tabernaemontana elegans* for a range of ailments, including tuberculosis, cancer, infertility, stomach aches, and several venereal diseases. In Tanzania, the Wabondei and Wadigo people in use the seeds, stem bark, and roots of *Tabernaemontana* elegans for medicinal purposes (Leeuwenberg, 1991). Infusions of the roots are consumed as an aphrodisiac and a treatment for lung conditions and stomach-aches, while the coagulated milky sap is used as a styptic. To treat tuberculosis, the roots are also macerated and taken twice daily. *Tabernaemontana elegans* stem barks are used for the treatment of malaria in Mozambique (Bandeira et al., 2001). Some venereal diseases are treated with a

potpourri of plant material that includes roots of *Tabernaemontana elegans*. A root decoction is used as a wash for wounds, a medicine for pulmonary conditions, and a treatment for chest pain. To combat tuberculosis, root ash maceration is consumed (Neuwinger, 2000). The endocarp, or inner layer of the fruit wall, is dried, ground up, and then submerged in water. After filtering, the water is used to cure cancer. Please be aware that certain plant parts used in traditional healers' concoctions have been identified as poisonous.

A **B**

Figure 3.13: The fruits (A) and (B) whole tree of *Tabernaemontana elegans*

Conservation status

Tabernaemontana elegans is not a threatened species and is commonly found in Limpopo, Mpumalanga, Kwa-Zulu Natal, and Swaziland.

3.1.14 *Terminalia prunioides* M.A. Lawson

Description

Terminalia prunioides (family name: Combretaceae, common name: Terminalia purple-pod) is a medium-sized tree or shrub endemic to southern and eastern Africa. *T. prunioides* (Figure 3.14) is a tiny deciduous tree that can grow to be multi-stemmed and 2.5 - 15 meters tall. The bole can be as large as 40 cm in diameter. The tree is harvested in the wild for usage as a source of food, medicine, and timber in the local community (Lawal and Tshikalange, 2020; Zhang et al., 2019*).*

Distribution

Terminalia prunioides is native to Southern Africa, and East African countries such as Zambia, Mozambique, Botswana, Kenya, Tanzania, Zimbabwe, and Lesotho. In South Africa, *T. prunioides* is commonly found in the northern provinces of South Africa, Limpopo, and Mpumalanga (Foden and Potter, 2005). The plant grows well in frost-free areas, sandy soils, coastal bushland, stony slopes, alluvial riverine thickets, or saline areas (Strohbach, 2000).

Ethnobotanical Usage

Postnatal abdominal pain is treated with a decoction of the plant (Adebayo et al., 2014). The bark of *T. prunioides* is chewed to treat illnesses such as coughs, sore throat, and stomach-aches, whereas the roots are chewed to treat colds. The rotten heartwood is sometimes pulverised and used as a fragrance for cosmetic purposes. It is used traditionally in the management of several human ailments including pain,

coughs, diarrhoea, and gastrointestinal discomfort (Urso et al., 2016). (Lawal and Tshikalange, 2020). Leaf extracts have been used to treat clinically significant pathogens such *Candida albicans*, *Staphylococcus aureus*, and *Enterococcus faecalis*, and antimicrobial properties have been documented.

Figure 3.14: The leaves and flowers of *Terminalia prunioides* (www.google.com, 2020).

Conservation of the plant

Terminalia prunioides is not a threatened species and is commonly found in both Eastern and Southern African countries.

3.1.15 *Zanthoxylum capense* (Thunb.) Harv.

Description

Zanthoxylum capense (Family: Rutaceae, common name: small knobwood) is a hardy, deciduous to semi-deciduous tree. The tree is small and multi-branched about 5 m in height, however, under favourable conditions it may reach 15 m (Nephawe, 2015). Young branches have a smooth bark with straight dark brown thorns, while older

branches and stems have light to dark grey bark with straight spines on a few dispersed cone-shaped knobs (20-30 mm), each of which is crowned with a small thorn (8-10 mm). The leaves (Figure 3.15) are made up of 4–8 pairs of oppositely spaced, 10–60 x 10–20 mm leaflets. The scalloped border of the glossy, dark green leaves has visible gland spots. They have an uneven compound with 4–8 pairs of leaflets plus a terminal one and are borne in clusters on short side stems. Leaves have a strong citrus smell when crushed. The blooms contain four sepals and four petals, are greenish-white in colour, and have a fragrant scent (Van Wyk and Gericke, 2000). The fruit is a round splitting capsule up to 5 mm in diameter, covered with glands, green, turning red when ripe, splitting later to reveal a single black, oil-rich seed per capsule. The wood is yellowish and hard. The seeds are shiny, dark brown to black, each containing a high number of volatile soils (Van Wyk et.al., 2009).

Distribution

The little knob wood tree is native to South Africa and can be found in the Southeastern parts of the continent, from the Western Cape region to the Zimbabwean granite and coastal Mozambique. (Schimidt et al., 2002; Luo et al., 2012). It has adapted to a variety of environments but is primarily found in dry to evergreen forest and on rocky hill slopes. Within the context of South Africa, the tree is widely distributed in eight provinces of SA, namely Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northwest, and Western Cape (Van Wyk et al., 2009).

Ethnobotanical Usage

The tree is well known for a wide range of ethnobotanical uses, nearly all parts of the tree are used from the fruits to the root. Early records reveal that little knob wood was

frequently utilized for traditional medicine, namely for flatulent colic, stomach aches, fever, snake bites, toothaches, and mouthwash. It is a traditional treatment for several diseases, including epilepsy (Bodede et al., 2017). Infusions or decoctions of the roots are used for snakebites, the root bark decoction to treat tuberculosis and paralysis, as a carminative remedy (used to treat flatulence) and to treat fever, the fruits or leaves are utilized. Mouthwashes are made from decoctions of roots or bark. (Bodede et al., 2017). Twigs and leaves are burnt, and the smoke is inhaled to ease dizziness. The bark is carefully removed from the twigs, flattened, and used as traditional toothbrushes (Adebayo et al., 2014). The Zulu-speaking people are known to use the leaves to heal sores.

Figure 3.15: The leaves and fruits of *Zanthoxylum capense* (http://pza.sanbi.org, 2020).

Conservation of the plant

Zanthoxylum capense is a protected tree in South Africa.

3.1.16 *Heteromorpha trifoliata* (Wendl.) Eckl. & Zeyh.

Description

Heteromorpha trifoliata (Family: Apiaceae, common name: parsley tree Muthatha-vhanna in Tshivenda) is a slender fast-growing tree that is drought resistant. The tree grows to a height of 3-7 m. An attractive feature of the tree is its glossy, waxy, coppery-coloured, peeling bark (Burrows et al., 2018). The leaves (Figure 3.16) can range from basic to differently complicated and vary in size and form. They can be yellow or red before falling in the fall and range in colour from pale green to greyish green. They have a parsley or parsnip-like aroma when crushed, hence the common name. Small heads of green or yellow flowers have a powerful scent, are unassuming, and are arranged in umbels, meaning that all the stalks grow from a single point. They start flowering in December-January. Phiri (2005), reported that tiny, winged fruits, which have two wings on one side and three on the other, are flattened, 5-7 m long, creamy brown, and begin to form in April.

Distribution

The parsley tree has its origin in South Africa, and it occurs in wooded grassland, bushveld and forest margins. Worldwide distribution is from Ethiopia, East tropical Africa to Swaziland, Lesotho and Zimbabwe, and it occurs further north in Africa. The Eastern Cape, Eastern Free State, Kwazulu-Natal, Gauteng, Mpumalanga, and Limpopo are the easternmost provinces of South Africa where it is prevalent (Maroyi, 2018).

Ethnobotanical Usage

The main use of *H. trifoliata* as reported (Palmer and Pitman 1972), is to treat intestinal worms in children, and abdominal pains and the infusion of leaves is used as an enema. It is also used to treat nervous and mental disorders. The roots and leaves are used in traditional medicine for a multitude of ailments. The bark is used as a vermifuge for horses. The smoke of the bark is inhaled for headaches. The roots are used for shortness of breath, coughs, colic, blood, stomach, and kidney purifiers as well as weakness in men. The volatile oils indicate that they are antibacterial and antifungal. The timber is very soft and decays quickly and is not good for firewood (Burrows et al., 2018).

Figure 3.16: The leaves and flowers of *Heteromorpha trifoliata* (https://www.gbif.org/occurrence/2625417327).

Conservation of the plant

The tree is listed under the Least Concern (LC) category in the Red List of South African Plants and is not a threatened species (Raimondo et al., 2009).

3.1.17 *Wrightia natalensis* Stapf

Description

Wrightia natalensis (Family: Apocynaceae: common name: Natal thick-fruit, Natal pachycarpus) is an evergreen shrub or tree that can grow from 1.6-1.8 metres in height. The bark of the plant is pale grey-brown smooth to finely fissured. Branches of the plant have pale lenticels, with milky sap present. The leaves are simple with opposite arrangements up to 10 x 2.5 cm long, they are light green. They often have follicles up to 32 x 1 cm which are dark green with pale lenticels. Its fragrant flowers (Figure 3.17) feature a creamy yellow corolla in the forks of branches. The fruits are dark green and carried in pods up to 32 centimetres in length. The seeds have an apical tuft of long silk-like hairs (Rao et al., 2018).

Distribution

Wrightia natalensis is native to Zimbabwe, Mozambique, Eswatini and South Africa. Within South Africa it is found in KwaZulu-Natal and Limpopo (Foden and Poter, 2005).

Ethnobotanical Usage

Wrightia natalensis's roots bark is used as an aphrodisiac to treat and enhance male sexual performance. The root bark powder and pieces are combined with traditional beer (Mabundu) and water, and the resulting concoction is either drunk right away or within two days. The powder is also used for the treatment of kidney stones. Bark and seeds are used to treat flatulence. Traditionally it is used in treating illnesses such as seizures, wounds, toothache, headache, eczema, scabies, dandruff, diarrhoea, and skin disorders (Rao et al., 2018).

Figure 3.17: The leaves and flowers of *Wrightia natalensis* (https://www.amazon.com, 2020).

Conservation of the plant

The tree is of least concern and is listed under the Least Concern (LC) category in the Red List of South African Plants (Raimondo et al., 2009).

3.1.18 Elaeodendron transvaalense (Burtt Davy) R.H. Archer

Description

Elaeodendron transvaalense (Family: common name: Celastraceae) refers to an irregular shrub, small to medium tree that fits perfectly in a small garden. It is also called the Bushveld saffron; the tree grows up to a height of about 6-8 m. In other places the tree may reach 18 m (Boon, 2010). It has a strikingly light grey, smooth bark that is occasionally finely fissured horizontally. Its dwarf spur-branchlets are characterised by a cluster of leaves (Figure 3.18) at the tips. On longer stems, the leaves are typically grouped in threes, but they can also alternate or be arranged

spirally. They range from light green to dismal grey-green, thin, and straight to narrowly oval. The leaves have an entire to finely serrated edge and are distinguished by prominent net veins on both sides. The summertime blooms (December to April) are tiny, greenish-white, and produced in groups of 20–30 heads on thin stalks. In autumn, flowers produce yellow, edible fruits that are up to 20 mm long, slightly elongated, and have a broad tapering to both ends (Burrows et al., 2018).

Distribution

Bushveld saffron is ubiquitous but not widely used. It grows in South Africa (KwaZulu-Natal, Mpumalanga, and throughout the northern regions of the country), Mozambique, Zimbabwe, and Zambia, as well as Botswana, Swaziland, Angola, and Namibia. It prefers lime-rich soils, grows in a variety of soils, and can be found in forests, bushveld, scrub, thornveld, and woodland, along streams, and frequently on termite mounds.

Ethnobotanical Usage

Elaeodendron transvaalense is regarded as one of the best traditional medicines for stomach problems and fever. A bark infusion is consumed as a general stomach conditioner. Additionally, the bark infusion is used as a drink in the treatment of several diseases, including kidney and bladder and complaints, skin diseases, swellings, haemorrhoids, and to improve appetite (Tshisikhawe and van Rooyen, 2013). The bark has a faint aromatic scent and is used to brew tea with a pleasant flavour. Chewing the leaves helps with throat issues, and drinking a leaf decoction can help prevent poisoning. The Zulu speaking people hold the opinion that adding a small piece of the bark to a gourd of curdled milk enhances the milk's quality. It is referred

to as mukuvhazwivhi by Venda traditional healers, which translates to "sin cleaner." The brittle, pale white wood of bushveld saffron has been utilized to manufacture tobacco pipes, headrests, spoons, ladles, and livestock troughs (Boon, 2010).

A B

Figure 3.18: The fruits (A) and leaves (B) of *Elaeodendron transvaalense* (http://pza.sanbi.org, 2020).

Conservation of the plant

Elaeodendron transvaalense is a near threatened species in South Africa. The plant is slow growing and is heavily harvested for local medicine and it cannot survive the repeated bark removal. (Raimondo et al., 2009).

3.1.19 *Androstachys johnsonii* Prain

Description

Androstachys johnsonii (family name: Picrodendraceae, common name: known as Lebombo-ironwood) is an evergreen tree that is usually found in hot savannas and grows well in rocky areas. It is renowned for its tough and durable wood. This medium-

sized, evergreen to deciduous tree can reach a height of 20 meters (Lemmens, 2011). It has deep green, spherical leaves that are white on the underside, the plant forms an attractive ornamental. The leaves (Figure 3.19) are circular and dark green, with a greyish-white underside. The flowers are golden and lack petals. It has 3-lobed yellowish to brown fruits (Schmidt et al., 2002).

Distribution

It is found in Mozambique, Swaziland, Zimbabwe, Malawi, and Madagascar, as well as South Africa, where it can be found in Limpopo province and the Lebombo Mountains in Mpumalanga (Schmidt et al., 2002).

Ethnobotanical Usage

Androstachys johnsonii is a common tree species found in South Africa's Vhembe region that is utilized for a variety of purposes. Traditional healers indicated using this species in traditional medicine, the bark is used together with the roots to make a decoction for treating sexual problems, and the roots of this species are used to treat infertility in women in Zimbabwe (Maroyi, 2018). *Androstachys Johnsonii* stems are used to make roofing for huts and thatched buildings. The leaves are fed either fresh or dry to cattle and goats (Murungweni et al., 2011).

Figure 3.19: The fruits of *Androstachys johnsonii* (http://pza.sanbi.org, 2020).

Conservation of the plant

Androstachys johnsonii has been largely exploited in the areas where it occurs and has been reported to have been widely used in Mozambique in 2005. Since it is slow growing, the recovery of the individuals that have been lost, has been slow. However, it is still widespread and not threatened, and is listed under the Least Concern (LC) category in the Red List of South African Plants (Raimondo et al., 2009).

3.1.20 *Securidaca longepedunculata* Fresen

Description

Securidaca longepedunculata (Family: Polygalaceae: common name: violet tree, also known as fibre tree), Mpesu in Tshivenda. The tree is small to medium-sized and can reach a height of 6 m and has distinctive pale greyish smooth bark. The leaves vary in size and shape, are alternating, and grow in clusters or crowded on dwarf spur branchlets that are often spine-tipped. They have incredibly fine hair while they are young, but they lose them as they grow older. Flowers (Figure 3.20) are up to 14 mm

long, pink to purple in short bunches, and produced in early summer (Van Wyk et al., 2009). They are around 10 mm long and have a long, slender stalk. Terminal and axillary sprays are 30-50 mm long and develop with the immature leaves. The fruit is spherical, up to 40 mm long, and has a characteristic membrane wing that is first purplish green before turning a light straw colour and it is visible from April to August.

Distribution

The species is mostly found in Angola, Benin, Botswana, Burundi, Cameroon, Chad, Côte d'Ivoire, Democratic Republic of the Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Kenya, Malawi, Mali, and Mozambique. It can be found in the provinces of Northwest and Limpopo in South Africa. Baloyi and Tshisikhawe (2009) found the tree in woodland and arid savanna soils.

Ethnobotanical Usage

The tree is the most widely used traditional medicinal plant in South Africa, being used to treat practically every possible condition. The roots are exceedingly deadly, smell like wintergreen oil, and contain methyl salicylate, which could explain why they are used as arrow poison in several parts of Africa, especially West Africa. The roots and bark are used to cure chest pain, headaches, inflammation, TB, infertility issues, venereal infections, and constipation. It is also used in abortion and ritual suicide. Chewing the roots can also help relieve toothache (Van Wyk et al., 2009).

To treat gonorrhoea, the roots of the violet tree and the dwarf custard apple are used. Powdered roots or wood scrapings are applied to the forehead to alleviate headaches, while root infusions are used to wash tropical ulcers. The Vhavenda people of Limpopo use the roots to treat mental problems and to protect children from disease during

breastfeeding. Many Africans are also said to utilize powdered violet tree roots as a sexual stimulant for men. When a man is sexually weak, he is given the powdered root and *mageu*, a beverage made from maize or sorghum, by the Vhavenda people. The bark is used to manufacture soap, fishing nets, baskets, strong threads for sewing bark cloth, and fibre for baskets (Van Wyk and Gericke, 2000).

Figure 3.20: The flowers of *Securidaca longepedunculata* (http://pza.sanbi.org, 2020).

Conservation Status

The roots of *S. longepedunculata*, a vulnerable and protected species, are the main target of those who use this plant, making it challenging for the plant to endure continuous harvesting.

3.1.21 *Bridelia mollis* Hutch.

Description

Bridellia mollis (family name: Phyllanthaceae, common name: velvet sweet berry) Mukumba-kumba in Tshivenda. It is an ornamental tree that may grow up to 9 meters tall and has a thick, leafy, spherical crown. The twigs and shoots have thick velvety coverings. The rough greyish-brown bark may have longitudinal striations at some time. Young branches are reddish to pale brown and densely velvety. The tree's leaves (Figure 3.21) are huge, alternating, soft, velvety, and plain, and they droop. Although the size can vary even on the same branch, they are oval and can measure up to 13 x 7 cm. The colour of the leaves is bright green above and a little lighter below. The tiny, unassuming reddish-yellow or green blossoms can reach a width of 4 mm. The edible fruit, which resembles a drupe, is a fleshy, nearly spherical berry. According to Maroyi (2018) and Palgrave (2002), it can be up to 1 cm wide and around the size of a pea.

Distribution

The trees are indigenous to South Africa, it is found in Mpumalanga, Limpopo and North-West province. Botswana, Zimbabwe, Mozambique (central, north, and extreme south), Zambia, Malawi, and northern Namibia (Schmidt et al., 2002). *Bridellia mollis* is found near water and grows at medium to low elevations. They can also be found in sand-veld (dry sandy soil) and granite (an igneous rock that forms underground when silica-rich molten rock cools) as well as other rocky outcrops (Setshogo and Venter, 2003).

Ethnobotanical Usage

In South Africa, a leaf infusion is used topically to treat burning, itching, and sores. In Venda, Limpopo province, South Africa, a leaf root infusion of *B*. *mollis* is taken orally as an emetic and treatment for dysentery and piles (Mbayo et al., 2016). In South Africa, root decoction is used orally as a treatment for parasitic worms, malaria, and fever. In Botswana, the leaves are cooked, and the solution is applied to the body as a relaxing lotion. The root decoction is taken orally as a treatment for diarrhoea. In Zimbabwe, leaf infusion is utilized topically for black blisters or skin blisters, while root decoction is taken orally for cough and stomach aches.

Figure 3.21: The leaves of *Bridellia mollis* (https://treesa.org, 2020).

Conservation Status

Bridellia mollis is characterised as least concern (LC) and is not threatened.

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CHAPTER 4: ETHNOBOTANICAL SURVEY

4.1 Introduction

Ethnobotanical studies are essential to determine the knowledge of the community about the surrounding plants in their area (Mechaala et al., 2021). Ethnobotany is defined as the study of interactions between people and plants, and it is largely centered on the use of traditional medicines. Ethnobotany research is critical for understanding the dynamic links between biological variety and social and cultural systems (Ahmad et al., 2014).

Vhembe district in Limpopo province of South Africa is one of the areas in Southern Africa known to have many medicinal plants used to treat and manage men sexual health. The area consists of various and diverse medicinal plants of importance and traditionally known to treat various male sexual illnesses such as erectile dysfunctions and aphrodisiac activities. Important species such as *Securidaca longepedunculata* used to treat multiple illnesses have been conserved and regulated for access in the area due to overuse (Moeng, 2010).

However, other species, namely, *Tabernaemontana elegans*, *Terminalia pruniodes*, *Strychonos spinosa*, *Bridelia mollis,* and *Garcinia livingstonei,* are facing the threat of over-use in the area indigenous knowledge of using medicinal plants to treat human ailments is on the verge of extinction because it is passed down orally from generation to generation without the use of a writing system, and many traditional healers do not keep written records (Kaido et al., 1997). Documentation of the traditional knowledge of medicinal plants through ethnobotanical surveys is critical to ensure the rightful owners of the knowledge are credited and to prevent overuse of the plants that could lead to extinction. The survey of medicinal plants in an area is vital to conserve that

landscape's traditionally important plant species (Sivasankari et al., 2013). Based on ethnobotanical surveys, several active natural products have been discovered from medicinal plants (Boudjela et al., 2013). According to Internationa Society of Ethnobiology (ISE) code of ethics, 2006) about three-quarters of the world's population rely on plants for treatments for many illnesses. It has been reported that 25% of modern medicines consumed globally contain herbal ingredients (Ademoelja, 2000). In Africa, 70% of the population seeks medical advice from traditional healers (Kamanzy et al., 2002). It has also been reported that the African continent is known for its wealth with biodiversity and is considered the cradle of humanity. Africa has an enormous plant resource of about 40.000-45.000 plant species, of which 5000 are medicinal species (Mahomoodally, 2013; Fajinmi et al., 2017).

Medicinal plants growing in the surrounding area under Vhembe district, plays an vital role not only as primary health care needs, but are now being collected by villagers for sale purposes (Semenya and Potgieter, 2014). The indigenous knowledge and experience gathered by local people on plants they grew up using for medicinal purposes drove them to sell the medicinal plants to generate income. Around 231 medicinal plants were reportedly traded at the muthi stores and by street vendors that were investigated, with roots being the most popular product (Moeng, 2010). Traditional medicine is highly used around Thengwe villages by adult males who seek to treat and improve their sexual health.

In recent years, traditional medicine has received massive attention in urban areas, especially to treat men's sexual problems. In most cases, the roots, bark, and leaves collected from medicinal plants are left in the shade for 4-5 days to dry. Drying in the shade is important to avoid losing most compounds through direct sunlight. The dried

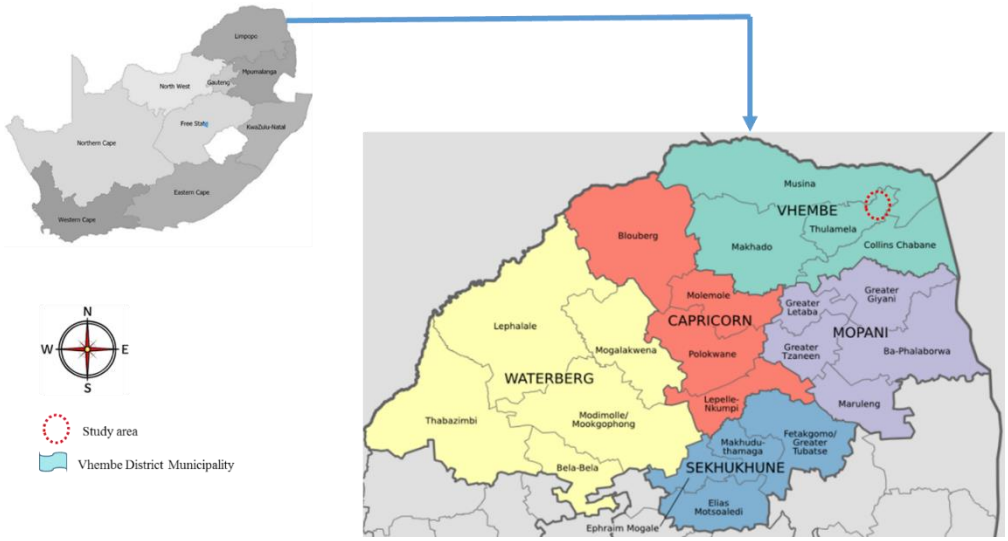
parts of medicinal plants are grinded to form a powder mixed and consumed with the traditional beer (Kamatenesi-Mugisha, 2005).

Many valuable drugs are developed from medicinal plants used traditionally to treat various illnesses. Different kinds of plants have been investigated in their role in stimulating and improving sexual performance and virility. Chauhan et al., 2014 reported that many synthetic drugs available and used to treat sexual problems have drawbacks, including being expensive and their ability to provoke severe adverse effects. Therefore, effective treatments with fewer side effects are still in demand.

4.2 Materials and Methods

4.2.1 Study area

Thengwe Tribal Authority jurisdiction is located 35 km from Thohoyandou, 205 km northeast of the province capital Polokwane and under the leadership of the headman, his Majesty Vho-Thovhele Nethengwe. Thengwe Tribal Authority falls under Thulamela municipality in the Vhembe District Municipality (Figure 4.1C), situated far north of Limpopo Province of South Africa which lies between 22.7696° S and 29.9741° E, and covers an area of 25 597 km² (Figure 4.1D).



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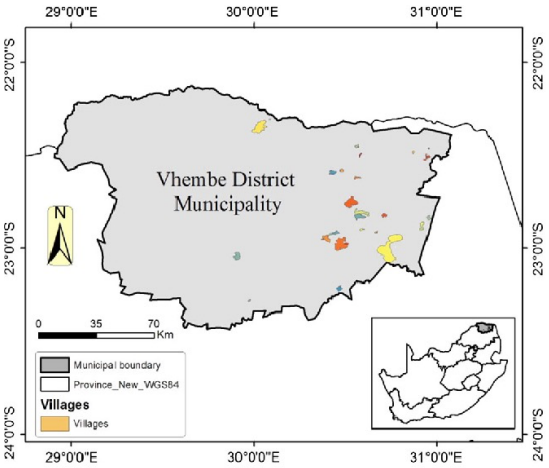

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Figure 4.1. South Africa Map showing Limpopo province (A); District municipalities in Limpopo province including Vhembe District Municipality (B); The geographical position of the study area of Vhembe District Municipality (C) and the four local municipalities falling under the Vhembe District Municipality (D) in Limpopo Province, South Africa. Source: Google, 2000.

Formerly, Thengwe was found in the Mutale Local Municipality, now part of the Thulamela and Musina Local Municipalities (Figure 2B). The soils of the villages are mainly reddish brown, sandy loam that was derived from sandstone (Cowling et al.,

1997). The general texture of the soil is loamy clay to sandy, and the soil pH is primarily neutral. The annual mean rainfall is roughly 350 mm; however, it varies, with the annual mean maximum and minimum temperatures being 26.5°C and 16°C, respectively (Mzezewa et al., 2010). Majority of people speak Tshivenda Language.

The Vhavenda-speaking people are situated in the northern part of South Africa in Limpopo province. According to (Acocks, 1988), this area is in the bushveld semiarid northern-eastern part of South Africa. More than 90% of residents have facilities to access sanitary toilets and safe drinking water, and 90% have access to electricity. The area is favourable for farming, rich in fertile soils, and most tropical and subtropical crops are grown. The main crops produced are maize, tomatoes, vegetables, citrus, and tropical and subtropical fruits. Wild plant resources are of great importance for local people who still rely on the forest for food, herbs, firewood, etc. Some villagers believe in medicinal help to heal their different sicknesses. The herbalist generates income by collecting medicinal plants from the wild, preparing, packaging, and selling them in local towns (Moeng, 2010).

4.2.2 Ethnobotanical data collection

The most recent (2008) revision of the International Society of Ethnobiology's (2006) code of ethics was strictly adhered to when ethnobotanical data was collected. According to the concept of informed consent, all participants gave their approval for the use of their ethnobotanical knowledge. The study aims, anticipated outcome, and envisaged publication were thoroughly explained to each participant. Ethnobotanical information was collected using questionnaires by semi-structured interviews with participants whose informed consent was obtained before the interviews. The

interviews were conducted in Tshivenda, the local language. A total of 23 participants (men) different age groups ranging from 18 to 80 years were identified and selected from various villages to participate in the interviews. Among them, were from ages 18-30 (27%), 31-40 (22%), 41 to 50 (17%), 51 to 60 (16%) and those above 60 years (17%). All the participants were selected based on their interest in the local medicinal plants.

Several field trips were undertaken between December 2017 to May 2019 in order to identify willing research participants and to collect ethnobotanical data based on both informal and formal interviews. Tshivenda language was used to conduct all the interviews since it is the participants and researcher's mother tongue. During formal interviews the matrix method as described by De beer and Van Wyk, 2011 was followed. The flip-file of 21 Plant images was the primary research tool, which was used in accordance with the matrix approach of recording and quantification developed by De Beer and Van Wyk (2011). Due to this, we were able to compute the values for the Species Popularity Index (SPI) and the Ethnobotanical Knowledge Index (EKI), both of which were suggested and utilised for the first time by De Beer and Van Wyk (2011). To further get insight into the importance of plant species in villages under the Thengwe tribal council, the Relative Frequency of Citation (RFC) indices was also utilized in addition to the matrix method.

The participants were shown sets of 21 "images" made up of herbarium specimens but enhanced with colour photographs, which often display various aspects of each plant that are not visible on specimens, such as the flower's habit and colour. Plant names and uses were documented according to 23 participants' knowledge through interaction between the researcher, participant, and each "image". Because most of

the data had already been collected during phase 1, this procedure ensured the community's active engagement in summarizing and validating the data's accuracy. The following questions were asked using a questionnaire: (1) Does the person know the plant? (2) Can you remember the name of a plant? (3) Can anyone recollect any uses for the plant (such as food, medicine, or other practical uses)? Each participant's biological details, as well as the origin/source of their knowledge, were also documented. The use of Tshivenda language allowed the researcher to record and accurately record subtle nuances that would usually be lost during interpretation and translation. These formal interviews were complemented with informal discussions while doing fieldwork.

Figure: 4.2 The pictures of selected plants used during interview of participants.

The following step was to enter the data into the Matrix of participants against species. Matrix population models have grown in popularity as effective methods for studying the dynamics of age-structured populations (Caswell, 2001; Oli, 2003). Each cell has four digits: (1) species known or unknown; (2) name known or unknown; and (3) uses known or unknown, to which numerical values of 1, 2, and 3 were assigned, with 0 signifying no knowledge of the plant and its uses. The final digit is the sum of three values (with a maximum value of six). The sum of each participant's scores yields a quantifiable indication of his knowledge of valuable plants. The researcher expressed each participant's ethnobotanical knowledge as a fraction (ratio) of the maximum possible score of 384, obtained by multiplying the total number of 21 plants by the maximum score of each species. The value of this “Ethnobotanical Knowledge Index” (EKI) thus varies between 0 and 1. In this study, for example, the highest EKI was 4 (as expected for the most knowledgeable participants). The Matrix also reflects the importance or popularity of each species based on the number of participants who are familiar with the plants and their different uses. The “Species Popularity Index” (SPI) is a fraction (ration) of the maximum possible score obtained by multiplying the number of participants with the maximum score for each plant.

4.2.3 Collection of plants and preservation

All the plant specimens were collected in both flowering and fruiting conditions. Samples of medicinal plants were collected for scientific identification and herbarium preparation following standard procedure (Jain and Rao, 1977). Voucher specimens of the collected plant species were made and submitted to the H.G.W.J. Schweickerdt Hebarium (University of Pretoria) for scientific identification. Photographs of the plant

species were also taken. About 98% of the plants were collected from the wild area, and 2% were from the backyards of the household in villages. Specimen number, local name, location, and identification points were remarked on each herbarium sheet and field notebook. Digital photo of medicinal plants was attentively taken. Data on each plant were recorded by using the data capture form.

4.2.4 Data analysis

The obtained data was entered into the Matrix of participants against species. Matrix population models have become popular and powerful tools for investigating the dynamics of age or stage-structured populations. Each cell includes four digits, namely (1) species known or not; (2) Name known or not; (3) uses known or not, to which numerical values of 1, 2, and 3 were respectively allocated, with 0 indicating no knowledge of the plant and its uses. The last digit represents the sum of three values (with a maximum value of 6). By adding the scores for each participant, a quantitative measure of his knowledge of useful plants is obtained. The researcher expressed each participant's ethnobotanical knowledge as a fraction (ratio) of the maximum possible score of 384, obtained by multiplying the total number of 21 plants by the maximum score of each species. The value of this “Ethnobotanical Knowledge Index” (EKI) thus varies between 0 and 1. For example, the highest EKI in this study was 4 (as expected for the most knowledgeable participants). The Matrix also indicates the importance or popularity of each species as measured by the number of participants who know the plants and their uses. The “Species Popularity Index” (SPI) is a fraction (ration) of the maximum possible score obtained by multiplying the number of participants with the maximum score for each plant.

The following formula was used to determine the Relative Frequency of Citation (RFC) of the plant species:

RCF = FC/N (0 < RFC < 1),

Where **FC** is the number of respondents who mentioned the use, and N is the total number of respondents in the research.

4.3 Results and Discussion

4.3.1 Diversity of valuable plants in the Thengwe area

Since ancient times, people from many different cultures have used plants as valuable and secure natural sources of medicine as well as agents of therapeutic, industrial, and environmental benefits. Plants that could be utilized to treat and manage men sexual health have been documented by medical historians. Inability to perform sexual activity and sexual dissatisfaction reduces the well-being, quality of life, and decreases work productivity in both male and females (Elterman et al., 2021). Nowadays, several factors like obesity, anxiety, stress, various disease conditions, and excessive use of synthetic medicines have increased the risk of erectile dysfunction. Information on 21 beneficial plant species encountered during the survey is recorded and briefly discussed in Table 4.1. For each species, the various uses as given by participants are listed. In most cases, all participants agreed about the use(s) of the plant, as indicated in Table 4.1. Apocynaceae, Loganiaceae, and Phyllanthaceae were the most represented among the selected plants from the study area. About 57% of the selected plants' growth type was trees, 33% were shrub/small trees, 5% were herbs, and 5% were creepers.

Table: 4.1. Indicates the medicinal plants of South Africa and their significant importance to human health.

Botanical name	Vernacular name (in Tshivenda)	Anecdotes from interviews conducted from villages under Thengwe Tribal Authority
Apiaceae *Heteromorpha arborescens* var abyssinica	Muthathavhanna	The roots are used men as an aphrodisiac.
Apocynaceae *Rauvolfia caffra* Sond.	Munadzi	Bark used to treat wounds for sexually transmitted diseases
Apocynaceae *Tabernaemontana elegans* Stapf	Muhatu	Roots used to treat wounds for sexually transmitted diseases, edible fruits,
Apocynaceae *Wrightia natalensis* Stapf	Musunzi	Roots are used as an aphrodisiac
Celastraceae *Elaeodendron transvaalense* (Burtt Davy) *R.H. Archer*	Mukuvhazwivhi	Bark use to treat various diseases including STD's
Clusiaceae *Garcinia livingstonei* T. Anderson.	Mupimbi	Roots are used as an aphrodisiac, Edible fruit
Combretaceae *Terminalia prunioides* M.A. Lawson	Mutwari	Fruits are boiled to make tea
Ebenaceae *Diospyros mespiliformis* Hochst. Ex A. DC	Musuma	Roots used to treat various diseases, edible fruits
Fabaceae *Cassia abbreviate* Oliv.	Mulumanamana	Roots are used as an aphrodisiac, STD's.
Loganiaceae *Strychnos madagascariensis* Poir	Mukwakwa	Males use the roots are used as an aphrodisiac, food.
Loganiaceae *Strychnos spinosa* Lam.	Muramba	Males use the roots are used as an aphrodisiac, food
Moraceae *Ficus abutilifolia* (Miq.) Miq	Tshikululu	Bark used to treat STD's.
Phyllanthaceae *Pseudolachnostylis maprouneifolia* Pax	Mutondowa	Bark used to treat STD's, Stomach problems
Phyllanthaceae *Bridelia mollis* Hutch.	Mukumba-kumba	Edible fruits, wood used for general timber
Picrodenndraceae *Androstachys Jinsonii* Prain	Musimbiri	Wood used to make fence
Polygalaceae *Securidaca longepedunculata* **Fresen**	Mpesu	Males use the roots are used as an aphrodisiac
Proteaceae *Faurea saligna* **Bharv.**	Mutango	STD's
Rutaceae *Zanthoxylum capense* (Thunb.) Harv.	Munungu	Roots are used as an aphrodisiac, STD's.
Solanaceae *Solanum panduriforme* E. Mey	Mututulwa	STD's
Urticaceae *Obetia tenax*	Muvhazwi	Leaves used as vegetable, medicinally
Vitaceae *Rhoicissus tridentata* (L.f.) Wild & R.B. Drumm.	Mutumbulambudzana	Aphrodisiac, edible fruits

A wide diversity of plants in the Thengwe area is still a source of medicine and food or used for various other purposes. There are several intriguing new records of plants that are locally significant and frequently used in the research area but have never been documented. There were several new vernacular names and uses that were noted. For the first time, the following three species are identified as having ethnobotanical value for improving male sexual performance and treating male sexual issues: *Wrightia natalensis, Heteromorpha trifoliata, Garcinia livingstonei*. In addition, there are three others often used well-known medicinal plants and food plants documented (Mabogo, 1990). For example, *Eleadendron transvaalense* treats gonorrhoea and other sexually transmitted diseases. Literature reference to the use of *Solanum panduriforme* includes using the roots to treat toothache and stomach-ache and its fruits to treat wounds. *Securidaca longepedunculata* is one of the dominant plants in Thengwe and is well known locally by Vhavenda for its use as a booster for male sexual performance.

The Species Popularity Index (SPI) was obtained by adding the total score each participant got from six participants in the specific age group (for example, 18-30) on each plant species (Table 2). The total score was divided by the possible highest score available (36). The Ethnobotanical Knowledge Index (EKI) was calculated by adding the total score of each participant in all plant species and divided by the total number of participants per age group (6), multiplied by the number of plant species (21) which gave (126). The total score of all six participants was divided by the total number of participants to get an average of 0.65 for the specific age group (for example, 18-30).

Table 4.2. The Matrix of 21 plant species at Thengwe with scores reflecting the knowledge of five different age groups of participants.

Scientific Names	Age Group 18-30						Age Group 31-40					Age Group 41-50				Age Group 51-60				Age Group 61 & Above			
	P#1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23
C. abbreviata	1203	0000	0000	1236	0000	1034	1236	1236	1236	1236	1236	1203	1236	1236	0000	1236	1236	1034	1203	1236	1236	0202	1236
F. saligna	1236	1034	1034	0000	0000	1034	1236	0000	1236	1236	1236	1236	1034	1001	0000	1236	0000	1236	1203	1236	1001	0202	0000
D. natalensis	1236	0000	0000	0000	0000	0000	1236	0000	0000	1034	1236	1236	1034	1236	0000	1236	0000	0235	0000	0000	1034	0000	1236
F. abutilifolia	1236	1034	1034	0000	0000	0235	1203	1200	0000	0000	0000	1236	0000	0000	0000	1236	1236	1203	1236	1236	1001	1236	1236
G. livingstonei .	1236	1236	1236	1034	1236	1236	1203	1203	1236	0000	1236	1236	1236	1236	1236	1236	0000	1236	1236	1236	1236	1236	1236
O. tenax	1236	0000	1236	1236	1236	1236	1236	1236	1236	1236	1236	1034	1236	1236	1236	1236	1236	1034	0000	1236	1236	1236	1236
P. maprouneifolia	1034	0000	0000	1236	0000	0235	1236	0000	1236	0000	0000	1236	0000	0000	0000	1236	0000	1236	1236	1236	1236	1236	0000
R. caffra.	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1034	1236	0000	1203	1236	1236
R. tridentate	1236	1203	1203	1236	0000	1236	1203	1236	0000	0000	0000	1236	1236	1236	1236	1236	1236	1236	0000	1236	1236	1236	1236
S. panduriforme	1236	1236	1236	1236	0000	1236	1236	1236	1236	1236	0000	1034	1236	0000	0000	1236	1236	1236	1236	0000	1236	1236	1236
S. madagascariensis	1203	0000	0000	1034	1236	1034	0000	1236	1236	0000	0000	1236	1236	1236	0000	1236	0000	1236	1236	1236	1034	1236	1001
S. spinose	1404	1236	1236	1236	1236	1236	1236	0235	1236	1236	1236	1034	1236	1236	1236	1236	1236	0001	1236	1236	1236	1236	1236
T. elegans	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1034	1236	1236	1236	1236	1236	1236	1236	1236	1236	1034	1236
T. prunioides	1203	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236	0000	1236	1236
Z. capense	1203	1236	1236	1236	0000	1236	1236	0000	0000	1236	1236	0000	1034	1236	1236	1236	1236	1236	1236	1236	1236	1236	1236
H. arborescens	1236	0000	1236	0000	1034	0000	1001	1236	1034	0000	1236	1236	1236	1236	0000	1236	1236	0000	0000	1236	1236	1236	1236
W. natalensis	0000	0000	0000	1230	0000	1236	1236	0000	0000	1236	0000	1236	0000	1236	1034	1236	1236	0000	0000	1236	0000	1236	1236
E. transvaalense	1236	0000	1236	1236	0000	0000	1236	0000	0000	1236	0000	1236	1236	1236	0000	1236	0000	1034	1236	1236	0000	1236	1236
A. johnsonii	1236	0000	0000	0000	0000	0000	1236	0000	0000	0000	0000	1236	1236	1236	0000	1236	0000	1034	1203	1236	1236	1236	0203
S. longepedunculata	1236	1203	1203	1236	1236	1236	1236	1236	1034	1236	0000	1236	1236	1236	0000	1236	1236	1034	1236	1236	1001	1236	1236
B. mollis	1236	1236	1236	1236	1236	1236	1236	1236	0000	1236	1236	1236	1236	1236	1236	1236	1236	1034	1236	1236	1236	1236	1236
EKI	**0.82**	**0.49**	**0.63**	**0.68**	**0.46**	**0.71**	**0.87**	**0.54**	**0.59**	**0.65**	**0.57**	**0.86**	**0.86**	**0.82**	**0.46**	**1**	**0.66**	**0.72**	**0.69**	**0.90**	**0.61**	**0.87**	**0.79**

#P is short for participant.

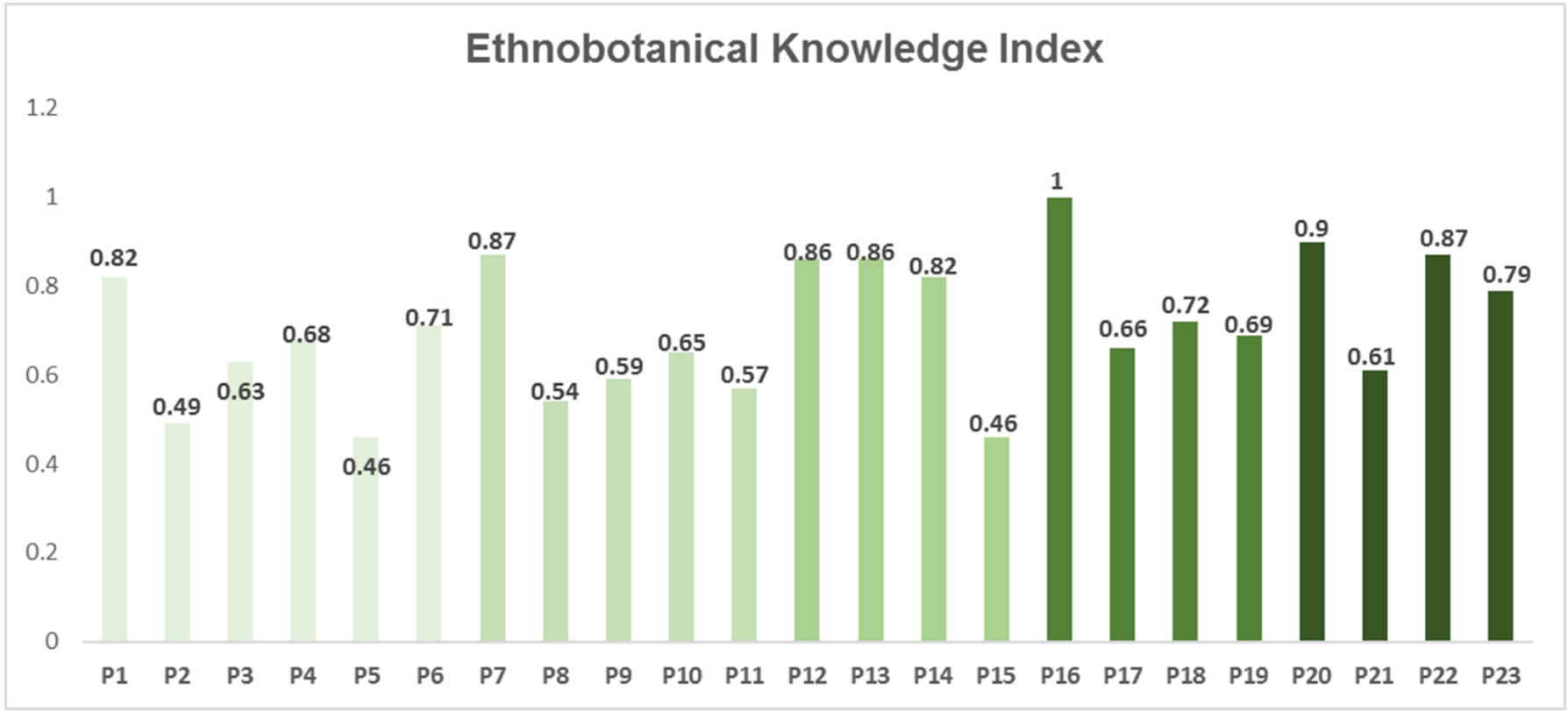

Figure: 4.3 The graph illustrates the Ethnobotanical Knowledge Index of different ages in the participants from ages 18-30, 31-40, 41-50, 51-60 and above 60 years.

The quantification of Thengwe area data is presented as a matrix in Table 2. All 21 species are listed with their Species Popularity Index (SPI), and all 23 participants with their Ethnobotanical Knowledge Indices. The most popular and widely known plant species in the Thengwe area (arranged by their ISP values) are *Tebernaemontana elegans* (4.83), *Terminalia pruniodes* (4.67), *Strychonos spinosa* and *Strychonos spinosa* (4.54), *Bridelia mollis* (4.52), and *Garcinia livingstonei* (4.29), and so on. The EKI of the age groups in all 21 plant species was higher in age 60 and above (0.81). The EKI seemed to grow gradually with age but dropped slightly in the age group 31-40.

The relative frequency citation (RFC) for medicinal plants ranged from 0.43 to 1 for 23 participants and 21 plant species. This confirms the importance of these medicinal plants in the villages under the Thengwe tribal council. *B. mollis* (0.95), *G. livingstonei* (0.83), *R. caffra* (0.91), *S. longepedunculata* (0.78), *S. panduriforme* (0.78), *S. prunioides* (0.95) and *O. tenax* (0.91) had the highest RFC index values while the rest of plants had moderate RFC values.

Table 4.3. The species popularity index of 21 plant species at Thengwe with scores reflecting the knowledge of five different age groups of participants.

Plant Species Scientific Names	Species Popularity Index (SPI)						Relative Frequency Citation (RFC)
	Group ages						
	18-30	31-40	41-50	51-60	61& above	Total SPI per age group	
Cassia abbreviate	0.36	1.00	0.63	0.79	0.83	**3.61**	**0.65**
Faurea saligna	0.50	0.80	0.46	0.63	0.38	**2.76**	**0.56**
Diospyros mespiliformis	0.17	0.53	0.67	0.46	0.42	**2.24**	**0.47**
Ficus abutilifolia	0.53	0.20	0.25	0.88	0.79	**2.64**	**0.47**
Garcinia livingstonei	0.94	0.60	1.00	0.75	1.00	**4.29**	**0.83**
Obetia tenax	0.83	1.00	0.92	0.67	1.00	**4.42**	**0.91**
Pseudolachnostylis maprouneifolia	0.42	0.40	0.25	0.50	0.75	**2.32**	**0.52**
Rauvolfia caffra	1.00	1.00	1.00	0.92	0.63	**4.54**	**0.91**
Rhoicissus tridentata	0.67	0.30	1.00	0.75	1.00	**3.72**	**0.65**
Solanum panduriforme	0.83	0.80	0.42	1.00	0.75	**3.80**	**0.78**
Strychonos madagascariensis	0.47	0.40	0.75	0.50	0.71	**2.83**	**0.60**
Strychnos spinosa	1.00	0.83	0.92	0.79	1.00	**4.54**	**0.91**
Tabernaemontana elegans	1.00	1.00	0.92	1.00	0.92	**4.83**	**1**
Terminalia prunioides	0.92	1.00	1.00	1.00	0.75	**4.67**	**0.95**
Zanthoxylum capense	0.75	0.60	0.67	1.00	1.00	**4.02**	**0.78**
Heteromorpha arborescens	0.44	0.57	0.75	0.50	1.00	**3.26**	**0.65**
Wrightia natalensis Stapf	0.33	0.40	0.67	0.50	0.75	**2.65**	**0.52**
Elaeodendron transvaalense	0.50	0.20	0.75	0.67	0.75	**2.87**	**0.60**
Androstachys johnsonii	0.17	0.20	0.75	0.54	0.83	**2.49**	**0.43**
Securidaca longepedunculata	0.83	0.73	0.75	0.92	0.79	**4.03**	**0.78**
Bridelia mollis	1.00	0.60	1.00	0.92	1.00	**4.52**	**0.95**
Ethnobotanical Knowledge Index (EKI)	**0.63**	**0.64**	**0.75**	**0.76**	**0.80**		

4.3.2 The Matrix methods

This new Matrix method's main advantages and unique features are highlighted. Recently, the matrix method is commonly used in ethnobotanical research (De Caluwé et al., 2009; Grace et al., 2009; Tardio and Santayana 2008; Taklehaymanot and Giday 2010; Taklehaymanot, 2009). It provides comparative quantitative data of medicinal plant use (across all categories of utilisation), allowing for future

comparisons within and between different participants (e.g., average EKI-values) and between the plants in different areas (e.g., average SPI-values). Using images in combination with specimens makes the study independent of the flowering season and the need to study the plants *in situ*. The most knowledgeable participants are often old and frail and cannot walk/travel long distances. The fact that all plants are shown to all participants excludes the possibility of false negative results. In all or most surveys done thus far, the data were based on the information given by participants. It is very likely that important plants were left out – not because they are not well known, but because they were momentarily forgotten. The significance of this new methodology is perhaps not immediately apparent. For example, the first question, "Do you know this plant?" is trivial because it establishes whether the person is familiar with the plant, even if he does not have a name for it. Young participants typically knew many plants for which they did not have names. They often recognise the plants by their flowers, colour, or leaves and can point them out in the veld.

The second question (the name of the plant) allows for an accurate recording of the full variation of vernacular names of the plant. In spelling and pronunciation, vernacular names often vary subtly from person to person or village to village. The study's results indicate that the acquisition of knowledge of the plants follows a predictable progression from young to older people. Young males first learn to recognise the edible plants and can communicate by learning their names. Those particularly curious and interested in plants will typically be observant and come to know many other plants, e.g., medicinal plants, without necessarily bothering to learn their names.

In most areas, younger participants recognised a more significant number of plants used for food even though they didn't know the names. Adults know the names of a

broader range of plants, including those used for non-medicinal purposes, e.g., crafts. The elderly people fall into three categories: (1) those reasonably ignorant of plants and their uses; (2) people with knowledge about those plants that are in daily use in the community, and (3) local experts with a profound knowledge of the plants, their identities, and their sensory and morphological properties.

4.4 Conclusion

The present study reveals that the men around the Thengwe area where the study was conducted have sound knowledge of different traditional medicine used in the villages to treat and manage sexual health. The knowledge of the use of traditional medicine appears to be more profound in old-aged males, and it can be concluded that the experience plays a significant role. Older men acquired knowledge of medicinal plants used to treat their sexual ailments from their ancestors and long practice. There is low interest from the younger generation in acquiring more knowledge about the use of medicinal plants. Medicinal plants such as *Securidaca longepedunculata*, *Eleoadendron transvaalense*, *Heteromorpha trilfoliata*, *Bridelia mollis*, *Androstrachys jonsonii*, *Strychonos madagascariensis* were more popular for the treatment and management of male sexual ailments. On the other hand, *Solanum panduriforme*, *Diospyros mespiliformis*, *Zanthoxylum capense*, *Terminalia pruniodes* and *Rauvolfia caffra* showed to be more popular for other ailments such as stomach ulcers, toothache, stomach cleansing and woods.

There is an urgent need to educate the younger generation about the conservation of medicinal plants for future use and to avoid the extinction of the plants. There is also a necessity to explore the traditional ethnomedicinal wealth and knowledge with

pharmacological aspects. With the high demand for medicinal plants, especially by males who relocated to urban areas due to work and searched for better opportunities, the control and conservation of plants should be stricter.

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CHAPTER 5: BIOACTIVITY OF MEDICINAL PLANTS USED TRADITIONALLY FOR THE TREATMENT AND MANAGEMENT OF MEN SEXUAL HEALTH

5.1 Introduction

In many African communities, the use of ethnobotanical indigenous knowledge is of vital importance in the management of men sexual health. Because of the long history of use in various cultures and the present resurgence of interest in using natural products to maintain health globally, the use of medicinal plants in managing men sexual disorders is beneficial. More investigation into the effectiveness of herbal treatments for men sexual disorders is important as a method to acknowledge the benefits and functions of traditional medical knowledge in the delivery of healthcare (Kamatenesi-Mugisha and Oryem-Origa, 2005). Medicinal plants are preferred over synthetic pharmaceutical drugs because of their availability, abundance, low cost and cause fewer or no side effects.

Erectile dysfunction is a major health issue forming a major part of sexual function disorders. According to Ajoa et al (2018) sexual dysfunction is “characterised by the inability to achieve, keep and maintain penile erection enough for stimulating coitus”.

The drugs (e.g., sildenafil) that are currently used for the treatment of erectile dysfunction may cause serious side effects such as headaches, dizziness, indigestion, heartburn, and stuffy nose. Medicinal plants are important natural sources of alternative treatment options for the management of male sexual health. These plants can be used in the discovery and development of drugs containing natural bioactive compounds with fewer or no side effects (Masuku et al., 2020).

The optimal male sexual health includes the desire for sex, the ability to get and sustain erection. However, many risk factors such as sexually transmitted infections (STI's), diabetes, surgery, smoking, alcoholism, high blood pressure, drugs, etc cause sexual dysfunction in males of all ages. Other endocrine disorders such as adrenal insufficiency, atherosclerosis, hypogonadism, and hypothyroidism are also responsible for erectile dysfunction (Goel and Maurya, 2020).

The risk factors such as diabetes, hypertension and smoking adversely affect the activity of Nitric oxide in penile tissue which can lead to erectile dysfunction. Sexual transmitted diseases such as chlamydia infects the prostate gland, leading to prostatitis which can cause erectile dysfunction. Nitric Oxide (NO) is thought to play a very critical role in the penile erection physiology.

5.2 Materials and methods

The methodology of this chapter was followed as illustrated in figure 5.1.

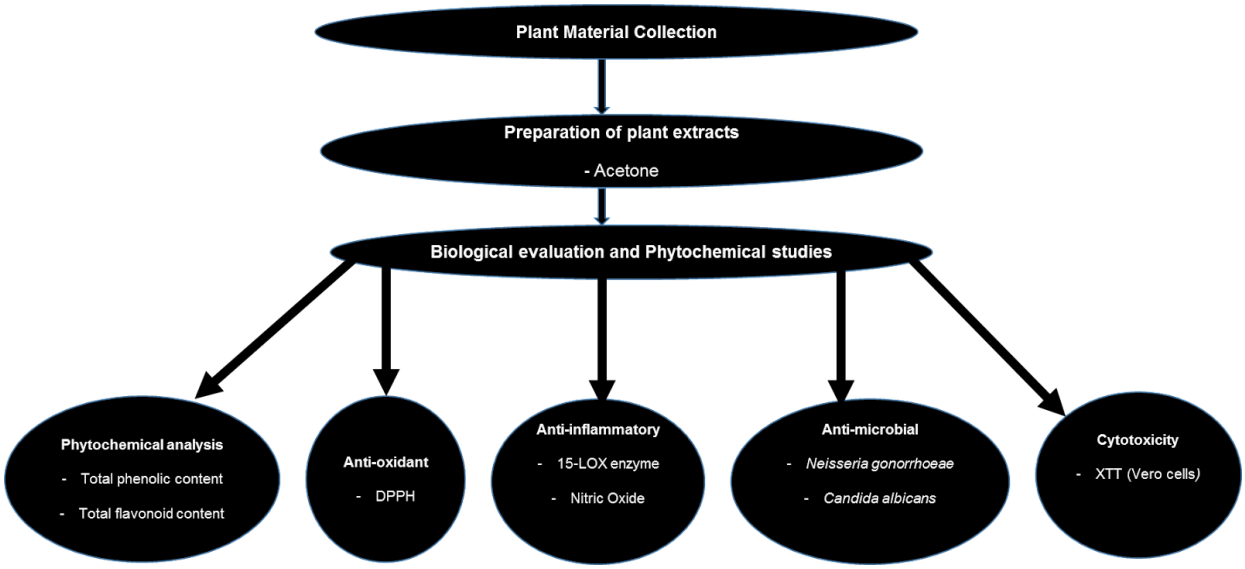

Figure 5.1: Study flow diagram.

5.2.1 Plant Material collection

The plants material (leaves, bark, and roots) was collected from villages in Thengwe. The voucher specimens were placed in the HGWJ Schweickerdt Herbarium at University of Pretoria, where the identity of the plant species was confirmed. The plant material was collected and dried at room temperature in a well-ventilated chamber before being reduced to a fine powder with a laboratory mechanical grinder.

5.2.2 Preparation of extract

Each plant material was extracted from five grams of fine powder using 100 acetone of acetone for 48 hours at room temperature. The extracts were filtered using a Buchner funnel using Whatman No. 1 filter paper. Using a rotary evaporator, the collected filtrate was concentrated to dryness (under vacuum). The dried extracts were weighed, determined their percentage yield, and stored in glass vials at 4 ^{0}C until use.

5.2.3 Phytochemical analysis

5.2.3.1 Determination of Total flavonoids content (TFC)

To determine total flavonoid content, a volume of 300 ul of ethanol was added to 100 ul of extract solution of 1 mg/ml concentration. Thereafter 20 ul of 10% aluminium chloride solution was added. Distilled water was then added to make up the solution up to 1 mL and incubated at room temperature. After 30 min of incubation, the absorbance was measured using a spectrophotometer at a wavelength of 450 nm. The total flavonoid content of the extracts was measured using a standard curve made up of various amounts of quercetin. The results were expressed as mg/g equivalent to quercetin.

5. 2.3.2 Determination of total phenolic content (TPC)

The Folin-Ciocalteu method, as described by Adebayo et al (2015), was used to determine total phenolic content. A volume of 250 ul of Folin-Ciocalteu reagent was added to 25 ul of extract solution of 1 mg/ml concentration. The mixture was incubated at room temperature for 5 min and thereafter 750 ul of 20% anhydrous sodium carbonate was added to terminate the reaction. Distilled water was added to make the volume up to 5 ml and the solution was incubated at room temperature (in the dark) for 2 h before the absorbance was then read at a wavelength of 760 nm. The total phenolic content of the extracts was calculated using a standard curve made with various doses of Gallic acid. The results were represented in milligrams per gram of gallic acid equivalent (Mayur et al. 2010).

5. 2.4 Antioxidant assay

5.2.4.1 DPPH (2,2-diphenyl-l-picrylhydrazyl) assay

The DPPH radical scavenging potential was measured as described by Lawal et al (2020) and Ogidi et al (2020). The extracts and Ascorbic acid (Positive control) stock solutions were at concentrations of 10 and 2 mg/ml respectively. Thereafter 20 ul of these samples were added to the first wells of a 96-well plate containing 200 ul of distilled water before serially dilution was done to obtain a final concentration ranging from 3.9-500 ug/m for the extracts and 0.78 – 100 ug/ml for the positive control. Methanol was also prepared in the same manner as the extracts in order to serve as a vehicle control. In each well 90 ul of DPPH solution in methanol (0.04 M) was added, however in those wells that serve as blanks (colour controls) methanol was added instead. The 96-well was then left in a dark room for 30 min before the

absorbance were read using a microplate reader at 517 nm. The radical scavenging percentage was calculated using the following equation:

(Absorbance vehicle control –Absorbance sample)/Absorbance vehicle control x 100

The GraphPad Prism 4 software was used to calculate IC_{50} values from percentage radical scavenging values.

5.2.5 Inhibition of Nitric Oxide production and viability of LPS-activated RAW 264.7 macrophages.

5.2.5.1 Cell culture

The Murine RAW 264.7 macrophage cells obtained from the American Type Culture Collection (Rockville, MD, USA) were cultured in plastic culture flasks comprising Dulbecco's improved Eagle's medium (DMEM) with L-glutamine, supplemented with 10% foetal calf serum (FCS) and 1 % penicillium/streptomycin/fungizone (PSF) solution. The cells were maintained at 37 ^{0}C under 5% CO_2 and split twice a week. The cells were seeded in a 96 well plate (40 000/well density) and incubated in a medium containing 5 ug/mL concentration of LPS. Thereafter various concentrations of plant extracts dissolved in 0.2 % DMSO and diluted with DMEM. Quercetin was used as a positive control while the untreated cells served as a negative control.

5.2.5.2 Determination of Nitrate production

The Nitric Oxide produced by treated macrophages was assessed by determining the nitrate concentration in the culture supernatant with the use of Griess reagent. The 96 well plates were covered and incubated in the dark for 15 minutes. After incubation

for 24 hours, 100 ul of supernatant from each well was transferred into a new 96 well plate and Griess reagent of equal volume was added. The absorbance of resultant solutions in the plates was determined using a BIOTek Synergy Multi-Detention microplate reader after 10 min at 550 nm. The percentage inhibition by the extracts was calculated based on its ability to inhibit Nitric Oxide production by the cells. The cells in the media without the extracts (containing triggering agents and DMSO) were considered as 0 % inhibition. The following formula was used to calculate the percentage inhibition of Nitric Oxide by the plant extracts:

100 – (Absorbance $_{extract}$ – Absorbance $_{blank}$) / (Absorbance $_{negative\ control}$ – Absorbance $_{blank}$)

5.2.6 Antimicrobial assay

5.2.6.1 Preparation of inoculums

The antimicrobial activity of the plant extracts was determined against a bacterial and a fungal pathogen associated with sexually transmitted diseases (STD's). To ensure sterility both pathogens were cultured in a fume hood and standard protocols were adhered to. The standard American Type culture collection strains (ThermoFisher, South Africa) of *Neisseria gonorrhoeae* (ATCC14018) and *Candida albicans* (ATCC 10231) were used. To make primary cultures, *N. gonorrhoeae* on GC chocolate Agar for 48 hours at 37 ^{0}C in 5% CO_2. Tryptone Soya agar was used to culture *C. albicans* which was incubated for 24 hours at 37 ^{0}C. For each pathogen, 24 h inoculum suspensions are prepared using Mueller-Hinton (MH) broth for *N. gonorrhoeae* and Tryptone Soya (TS) broth for *C. albicans*. To standardise the final concentration of the inoculums, a spectrophotometer at wavelength of 600 was used. Both pathogens were

inoculated in a sterile broth and prepared to a density of 1.5 x 10^5 colony forming units per ml (CFU/ml) which correspond to 0.5 McFarland Standard.

5.2.6.2 Minimum inhibitory concentration assay

To determine the antimicrobial activity of the extracts, the microdilution method as described by Eloff, 1998 was used to get the minimal inhibition concentrations of extracts against *Neisseria gonorrhoea* and *Candida albicans*. Briefly, 25 mg of each plant extracts was dissolved in 100 µl of 10% DMSO before adding 900 µl of broth to make a final concentration of 25 mg/ml (stock solution). The extracts and controls (samples) were tested in triplicate using a 96-well plate. Ciprofloxacin was used as the positive control while the negative controls included broth with microorganisms and 10% DMSO in order to ascertain if it does not affect the growth of the pathogen. In all the wells, 100 µl of a broth used to prepare inoculum suspension was added. A 100 µl of extract stock solution was added to the first row of the plate, where after two-fold serial dilutions were prepared. In each well, 100 µl of inoculum was then added. The plate was covered and incubated at 37 ^{0}C for 24 hours. The final concentrations of the extracts ranged from 0.004 to 6.25 mg/ml, while the final concentration of the positive controls ranged from 0.005-0.625 mg/ml. Microbial growth was determined, a pink colour change after addition of 15 µl of PrestoBlue (viability reagent) to microplate wells and 15 minutes' incubation period. A blue colour indicated microbial inhibition (antimicrobial activity). The MIC values were determined visually and recorded for each extract and positive controls.

5.2.7 Cytotoxicity assay

The extracts were assessed for their cytotoxicity using the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxyanilide salt) reduction assay against Vero monkey kidney cells as previously described by Lawal and Tshikalange et al., 2019. In 96-well microtitre plates, cells were seeded at a density of 1 × 105 cells/mL (100 µL) and incubated at 37 °C and 5% CO_2 in a humidified environment for 24 hours. After incubation, extracts (100 µL) at varying final concentrations (3.13 to 400 µg/mL.) were added to the wells containing cells and the plates were further re-incubated for 72 h. Actinomycin-D (m 0.05 – 3.91 x 10-4 µg/mL) was used as a Positive control, DMSO as a negative control and a suitable blank control with equivalent volume of acetone was also included. Then, 100 µL of XTT was added to the test and blank wells before the plates the plates were re-incubated for further 2 hours. Thereafter, the absorbance was measured using a multiple-well plate reader at 490 nm at a reference wavelength of 690 nm. The percentage of cell growth inhibition was calculated based on a comparison with untreated cell and the IC_{50} (50 % inhibitory concentration) was determined (Adebayo et al., 2015).

5.2.8 Statistical analysis

The experiments were conducted in triplicate and the results were expressed as mean ± standard deviation (SD). A GraphPad Prism 4 software was used to subject all data to one-way analysis of variance (ANOVA). Tukey's multiple comparison test was used to separate the means where a significant difference was observed ($P < 0.05$).

5.3 Results and discussion

5.3.1 Crude extract yields

The highest and the lowest percentage yield was observed with *E. transvaalense* and *R. caffra* with 26% and 2.2% percent respectively. The rest of the plant extracts yields ranged between 2.7 and 23%.

5.3.2 Phytochemical analysis

All the plant extracts investigated were found to be rich in flavonoids compounds with values ranging from 16 to 170 mg/g QE. Similarly, the total phenolic content of all the plant extracts were significantly high with values ranging from 420 to 93 mg/ml GAE as shown in Table 1.

5.3.3 Antioxidant activity

The antioxidant potential of the plant extracts was evaluated using the DPPH method. Extracts *G. livingstonei* and *P. maprouneifolia* exhibited the highest antioxidant activity with IC_{50} values of 6.86± 0.85 µg/ml, 5.48± 2.17 µg/ml respectively. Moderate antioxidant activity was observed in extracts from *D. mespiliformis* (8.34± 2.18 µg/ml) *R. caffra* (14.39 ± 1.02 µg/ml) and *S. madagascariensis* (14.39±0.68 µg/ml) whereas the remaining plant extracts had low activity comparatively (varying from 42.9±2.16 to 131.33 ±1.07 µg/ml). The study conducted by Muriithi et al (2016), reported a better than vitamin C antioxidant activity of benzophenone isolated from the twigs and stem wood of *Garcinia livingstonei*. Oxidative stress and inflammation, which disrupt nitric oxide (NO) production directly or by causing resistance to insulin, are central determinants of vascular diseases including ED. Most of cases of erectile dysfunction (ED) are linked to risk factors for oxidative stress including diabetes, smoking,

hypercholesterolemia, and hypertension (Zhang et al., 2011; Meldrum et al., 2012). The use of antioxidant supplements is common in clinical settings and is considered a viable treatment for ED. According to a study by Su et al (2022), the effect of antioxidant therapy alone on ED may be modest. However, the use of antioxidant compounds and the combination of PDE5 inhibitors and antioxidants was linked to better erectile dysfunction and can be viewed as an adjunctive therapeutic approach for ED. The combination of PDE5 inhibitors and antioxidants significantly improved the International Index of Erectile Function (IIEF) score (SMD = 1.1; 95% CI: 0.51, 1.68; P .001) and the sexual satisfaction score (SMD = 1.28; 95% CI: 0.06, 2.51; P = 04) when compared to the PDE5 inhibitors alone. Therefore, antioxidants may be helpful in treating disorders related to infertility in both men and women, including erectile dysfunction, according to a growing body of research.

Antioxidant-rich plants may benefit men's sexual health, while the association is not always clear-cut and may change based on personal circumstances. Antioxidants are substances that assist in shielding the body's cells from harm resulting from free radicals, which are unstable molecules that can cause a number of health problems, including problems related to sexual health. By lowering oxidative stress and protecting blood vessels, antioxidants can aid in enhancing blood flow. An erection can only be achieved and maintained with proper blood flow. Antioxidants shield the endocrine system, which helps maintain hormonal equilibrium. Hormone balance is essential for preserving sexual function and libido. By shielding sperm from oxidative damage, antioxidants may increase the fertility and quality of sperm. Antioxidants are beneficial to general health and well-being, both of which have a positive effect on sexual health.

Table: 5.1 Plant extract yield, phytochemical analysis, anti-oxidant and anti-inflammatory activity

Plant extracts	Voucher specimen	Plant part	Yield (%)	TFC (mg/g QE)	TPC (mg/g GAE)	DPPH assay IC_{50} (µg/ml)	LOX assay IC_{50} (µg/ml)	NO assay IC_{50} (µg/ml)
Diospyros mespiliformis	PKT0094	Leaves	7.7	170	93.0	8.34 ± 2.18	63 ± 2.17	148.5
Elaeodendron transvaalense	PKT0071	Bark	26.0	116	197	106 ± 0.61	>83	163.0
Garcinia livingstonei	MPT0021	Bark	23.0	60	420	6.86 ± 0.85	>83	77.94
Pseudolachnostylis maprouneifolia	TE0218	Leaves	4.8	107	260	5.48 ± 2.17	67 ±4.34	422.9
Rauvolfia caffra	MPT0080	Bark	2.2	25	170	14.39± 1.02	>83	>500
Rhoicissus tridentate	MPT0029	Roots	4.8	16	130	42.9±2.16	>83	450
Securidaca longepedunculata	PKT0152	Roots	14.0	17	142	117 ± 0.19	>83	>500
Strychnos madagascariensis	TE0222	Fruits	2.7	56	230	14.2± 0.68	>83	>500
Tabernaemontana elegans	MPT0079	Bark	2.8	90	374	131.3 ±1.07	>83	>500
Wrightia natalensis	MPT0041	Roots	16.0	14	82	69.96 ±1.07	>83	>500
Ascorbic acid			-	-	-	9.96 ±2.04	-	30.0
Quercetin			-	-	-	-	29 ±1.21	-

Table: 5.2 Antimicrobial activity and cytotoxity of plant extracts

Plant extracts	Antimicrobial activity, MIC (mg/ml) *N. gonorrhoeae*	*C. albicans*	Cytotoxicity Assay (IC_{50} µg/ml)
D. mespiliformis	0.39	6.25	Nd
E. transvaalense	>6.25	3.12	88±1.04
G. livingstonei	0.39	6.25	>100
P. maprouneifolia	>6.25	6.25	> 100
R. caffra	>6.25	>6.25	Nd
R. tridentata	0.39	6.25	> 100
S. longepedunculata	>6.25	>6.25	Nd
S. madagascariensis	>6.25	>6.25	Nd
T. elegans	6.25	>6.25	21.5± 14.80
W. natalensis	6.25	>6.25	> 100

5.3.4 Anti-inflammatory activity

15-lipoxygenase inhibition activity

Inflammation is a natural response of the body to injury or infection. It is characterised by redness, swelling, and pain. When inflammation occurs in the body, it can cause a variety of health problems, including erectile dysfunction. This is because inflammation can damage the blood vessels that supply the penis with blood, leading to reduced blood flow and difficulty achieving or maintaining an erection. Additionally, inflammation can cause hormonal imbalances that can lead to erectile dysfunction.

In this study, the plant extracts were evaluated for their inhibition activity against 15-Lipoxygenase enzyme and their results are expressed in table 1 as IC_{50} values. Extracts of *E. transvaalense*, *G. livingstonei*, *R. caffra*, *R. tridentata*, *S. longepedunculata*, *S. madagascariensis*, *T. elegans* and *W. natalensis* did not inhibit or showed significant inhibition against 15-LOX as their IC_{50} values were greater than 83 µg/ml. Only *D. mesipiliformis* (63± 2.17 µg/ml) and *P. maprouneifolia* (67±4.34 µg/ml) showed good inhibition against 15-LOX in comparison with positive control quercetin which had IC_{50} value of 29±1.21 µg/ml.

Plants with anti-inflammatory properties may have a positive impact on sexual health in men, indirectly, by addressing certain underlying health issues that can affect sexual function. Inflammation in the body can contribute to a variety of health problems, including those related to sexual health.

Nitric Oxide Production inhibitory activity

LPS-activated RAW 264.7 macrophages were used to evaluate the Nitric Oxide production inhibitory activity of the plant extracts. As presented in table, five of the plant extracts tested (*S. longepedunculata*, *S. madagascariensis*, *T. elegans*, *W. natalensis* and *R. caffra*) did not show NO inhibition capacity as their IC_{50} values were greater than 500 µg/ml. Extracts from *D. mespiliformis* (148.5 µg/ml), *E. transvaalense* (163 µg/ml), *G. livingstonei* (77.94 µg/ml), *P. maprouneifolia* (422 µg/ml) and *R. tridentate* (450 µg/ml) showed moderate to poor suppression of NO production when compared with positive control quercetin with IC_{50} value of 30.01± 0.7 µg/ml.

In men, nitric oxide reduction may exacerbate erectile dysfunction. A naturally occurring substance in the body, nitric oxide (NO) is essential to the physiological

mechanisms that lead to and sustain an erection. It relaxes the blood vessels in the penis, increasing blood flow, by acting as a vasodilator. The erection is dependent upon this increased blood flow (Davies 2015). Low nitric oxide levels can cause the penis' blood vessels to dilate improperly, which would limit blood supply to the penile tissues. One common sign of erectile dysfunction is difficulty getting or keeping an erection, which can be caused by this decreased blood flow (Burnett, 2006).

5.3.5 Antimicrobial activity

Determination of Minimum inhibitory concentration of the extracts against selected pathogens.

The MIC values of the extracts *N. gonorrhoea* and *C. albicans* are shown in table 1. Acetone extracts of the ten medicinal plant samples were tested for their ability to inhibit *Neisseria gonorrhoeae* and *Candida albicans* using a microdilution method in order to determine their minimum inhibitory concentration. According to the results, extracts from *D. mespiliformis*, *G. livingstonei* and *R. tridentata* have the highest antigonnococal with MIC of 0.39 mg/l, followed by extracts from *T. elagans* and *W. natalensis* with MIC of 6.25 mg/ml respectively. The rest of the extracts exhibited an MIC greater than 6.25 mg/ml. When tested against *C. albicans*, all the plant extracts showed an MIC of greater or equal to 6.25 mg/ml except for *E. transvaalense* (3.12 mg/ml).

In a study by Sara et al (2018), Aqueous extracts of leaf and stembark of *D. mespiliformis* revealed high sensitivity against some pathogenic microorganisms

including *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Shigella spp*, *Staphylococcus aureus*, *Salmonella typhi* and *Streptococcus pyogenes*. Similarly, Adeniyi, et al 1996, reported broad spectrum antimicrobial activities of *D. mespiliformis* against Gram-positive, Gram-negative bacteria, and fungal strains. However, this study report for the first antigonococcal activity of *D. mespiliformis*. Although myriad factors contribute to the development of ED, recent research has indicated that STI's may play a significant role in the onset of this condition.

Antimicrobial plants may provide several health advantages, including an indirect benefit to men's sexual health. It's crucial to remember that there are a variety of intricate relationships between sexual health and antimicrobial plant qualities, and not all antimicrobial plants will directly enhance sexual health. The prevention and treatment of illnesses is aided by antimicrobial plants, which is beneficial to general health. Sexual health problems or pain can occasionally result from infections, such as urinary tract infections or sexually transmitted infections (STI's). These plants can help sexual health indirectly by controlling and avoiding such illnesses (Lawal et al., 2019). Additionally, several plants may include substances that affect circulation or hormone balance, which may have an impact on sexual health. For instance, ginseng and maca are two plants that are frequently linked to enhanced sexual performance and possible aphrodisiac properties. They may not have predominantly antibacterial modes of action, but they can nevertheless improve sexual and general health. It's critical to realize that a healthy lifestyle, stress reduction, regular exercise, and a balanced diet are all important components of sustaining excellent sexual health. Although certain plants possessing antimicrobial qualities can contribute to a more

comprehensive health strategy and indirectly enhance sexual health, they do not provide a direct or exclusive remedy for issues related to sexual health. It's best if you have particular queries or concerns about your sexual health.

5.3.6 Cytotoxicity assessment

The results indicated that extracts of *G. livingstonei*, *P. maprouneifolia*, *R. tridentata* and *W. natalensis* had the lowest cytotoxicity (IC_{50} > 100 µg/mL) on Vero Monkey kidney cell lines among those tested (Table 5.2). Extracts of *T. elegans* and *E. transvaalense* exhibited some toxicity with IC_{50} values of 21.5 and 88 µg/mL respectively. Although extracts were not tested, previous studies have shown that these plants have relatively low to moderate toxicity on Vero cells (Mohammed et al., 2016; Lawal et al., 2019; Thlapi et al., 2020; Canga et al., 2022).

5.3.7 Conclusion

For ages, traditional remedies have been used to treat issues with male sexual health, such as erectile dysfunction, early ejaculation, and poor libido. It has been discovered that medicinal herbs used historically to treat and maintain male sexual health also contain bioactive chemicals that can enhance male sexual health. To promote overall sexual health, increase desire, and enhance male sexual performance, these botanicals may be used alone or in conjunction with other treatments. Further research is needed to fully understand the mechanisms of action of these bioactive compounds and their potential benefits for male sexual health.

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CHAPTER 6: GENERAL CONCLUSION

This study has presented promising evidence of the potential (antioxidant, antimicrobial and anti-inflammatory) of medicinal plants used in the management of men sexual health. The findings of the study revealed that the selected medicinal plants possess bioactive phytochemicals against conditions associated with men sexual disorders. Therefore, the presented data validates and supports the traditional usage of medicinal plants by men in villages under Thengwe Tribal Authority.

6.1 Answers to research questions and hypotheses

The current study seeks to answer the following research questions:

- **Are the selected plants from previous studies well known in the current study area?**

  The data presented showed that the selected medicinal plants are well known in the study area, with an average Species Popularity Index of (SPI) and Relative Frequency citation (RFC) of 70 and 71% respectively.

- **What is the level of ethnobotanical knowledge of the selected plants among the research participants of different ages?**

  The study revealed an average Ethnobotanical Knowledge Indices (EKI) of the selected plants of 70% among the research participants.

- **Do the selected medicinal plants possess the biological activities associated with men sexual health?**

The selected medicinal plants investigated showed promising biological activities associated with the management of men sexual health. *Diospyros mespiliformis* extract exhibited the most significant bioactivity with less toxic.

The results of this study demonstrated that the following hypotheses can not be rejected:

- **The selected medicinal plants used in the management of men's sexual health are well-known among the research participants.**
- **The selected medicinal plants possess biological activities associated with the management of men's sexual health.**

6.2 Study contribution

This is the first study focusing on the importance and potential of medicinal plants used traditionally to treat and manage men sexual health. The current study will contribute to Indigenous Knowledge Sytems (IKS). The result of this study makes a valuable contribution to the Sustainable Development Goal (SDG) of good health and wellbeing by forming a basis for the development of pharmaceuticals to manage men sexual health.

6.3 Recommendations for future studies

The following further research studies are recommended based on the limitations of the current study.

- The current study should be replicated in other villages around Vhembe District municipality for future comparison.

- The extracts and bioactive compounds should be investigated against phosphodiesterase-5 (PDE5), a key enzyme involved in the physiological processes of smooth muscle contraction and relaxation. Inhibition of this enzyme is responsible for prolonging an erection by enhancing the effects of Nitric Oxide, thus increasing cGMP in the smooth muscle cells.
- Isolation, identification, and characterization of bioactive compounds responsible for the activities investigated.
- *In vivo* bioactivity and mechanism of activity studies of the extracts and bioactive compounds

APPENDIX 1: UNISA HEALTH RESEARCH ETHICS COMMITTEE APPROVAL LETTER

UNISA university of south africa

UNISA HEALTH RESEARCH ETHICS COMMITTEE

Date: 19/07/2018

NHREC Registration # : REC-170616-051
REC Reference # : 2017/CAES/078
Name : Mr T Nemandalali
Student #: 43323928

Dear Mr Nemandalali

Decision: Ethics Approval Renewal for period 01/07/2018 to 31/12/2018

Researcher(s): Mr T Nemandalali
TsumbedzoN@daff.gov.za

Supervisor (s): Dr TE Tshikalange
Emmanuel.Tshikalange@up.ac.za; 082-634-0680

Dr MA Nyila
nyilama@unisa.ac.za; 011-471-2294

Working title of research:

Biological evaluation of ethnobotanical selected medicinal plants used in the treatment and management of male sexual health

Qualification: PhD Life Science

Thank you for the submission of your progress report to the Unisa CAES Health Research Ethics Committee for the above mentioned research. As this is a high risk study, ethics approval is granted for a six-month period. After six months the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another six-month period.

Due date for progress report: 31 December 2018

*The **high risk application** was **reviewed** by the CAES Health Research Ethics Committee on 08 December 2017 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
2. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
5. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
6. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
7. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

The reference number ***2017/CAES/078*** *should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof EL Kempen
Chair of CAES Health REC
E-mail: kempeel@unisa.ac.za
Tel: (011) 471-2241

Prof MJ Linington
Executive Dean : CAES
E-mail: lininmj@unisa.ac.za
Tel: (011) 471-3806

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1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
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7. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:
*The reference number **2017/CAES/078** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof EL Kempen
Chair of CAES General Research ERC
E-mail: kempeel@unisa.ac.za
Tel: (011) 471-2241

DM Modise

Prof MJ Linington
Acting Executive Dean : CAES
E-mail: lininmj@unisa.ac.za
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APPENDIX 2: CONSET FORM

UNISA university of south africa

CONSENT FORM

TITLE OF RESEARCH PROJECT

Biological evaluation of ethnobotanical selected medicinal plants used in the treatments and management of men sexual health

Dear **Mr**/Mrs/Miss/Ms **T NEMANDALALI**..... Date:02/10/2016...

NATURE AND PURPOSE OF THE STUDY

RESEARCH PROCESS

1. The study requires your participation in a focus group interview to discuss the use of medicinal plant to treat and manage male sexual health.
2. The focus group will be led by a facilitator
3. The focus group environment offers you the opportunity to express your opinion on the subject of the use of medicinal plants used in the treatments and management of male sexual health.
4. There are no right or wrong answers, and all opinions will be valued.
5. You do not need to prepare anything in advance
6. All participants will be given the opportunity to express an opinion, or agree or disagree with the opinion of other focus group members. The group may debate the opinions of individual members of the group.

NOTIFICATION THAT PHOTOGRAPHIC MATERIAL, TAPE RECORDINGS, ETC WILL BE REQUIRED

Your attention is drawn to the fact that the focus group interview will be tape recorded to ensure that valuable information elicited during the interview is captured and the context of the information can be reviewed in detail. Following the focus group interview, the recorded material will be transcribed. You may peruse the transcription of the recording of the focus group interview in which you participated at any time.

CONFIDENTIALITY

The opinions of the focus group are viewed as strictly confidential and only members of the research team will have access to the information. No data published in dissertations and journals will contain any information thorugh which focus group members may be identified. Your anonymity is therefore ensured.

WITHDRAWAL CLAUSE

I understand that I may withdraw from the focus group at any time. I therefore participate voluntarily until such time as I request otherwise.

POTENTIAL BENEFITS OF THE STUDY

Due to the large number of males both young and adults sufering from various illnesses that results in inability to perform sexual intercourse which also affect their quality of life and the relationship with their partners should be researched using medicinal plants as an alternative to the challenge. This information will be used by different male age groups to use medicinal plants as an option to treat and manage whatever challenges they might be facing on their sexual health. This study may in future give hope to the use of medicinal plants in the treatment ana management of male sexual health, which might also open market of medicinal plants on trade. At the conclusion of the focus group interviews, participants will be compensated for their contribution.

INFORMATION (contact information of your supervisor)

If I have any questions concerning the study, I may contact the supervisor Dr T.E Tshikalange at Department of Plant Sciences and Soil Schience at University of Pretoria, Hatfield Campus, 012 430 2008. Cell: 082 634 0680

CONSENT

I, the undersigned, **Ndou Thivhalitshi Elson** (full name) have read the above information relating to the project and have also heard the verbal version and declare that I understand it. I have been afforded the opportunity to discuss relevant aspects of the project with the project leader, and hereby declare that I agree voluntarily to participate in the project.

I indemnify the university and any employee or student of the university against any liability that I may incur during the course of the project.

I further undertake to make no claim against the university in respect of damages to my person or reputation that may be incurred as a result of the project/trial or through the fault of other participants, unless resulting from negligence on the part of the university, its employees, or students.

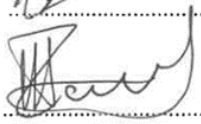
I have received a signed copy of this consent form.

Signature of participant: .....

Signed at ...Thengwe..... on .....02/10/2016.....

WITNESSES

1 

2 

APPENDIX 3: REASEARCH QUESTIONNAIRE

PhD Project Title: Biological evaluation of ethnobotanical selected medicinal plants used in the management of men sexual health.

Participant Name/ Number: _____ Age: _____ Place: _____

Plant species	**Assessments**		
	Do you know this plant? *Know the plant =1* *Do not rexcognise the plant = 0*	**Do you know the name of this plant?** *Know the name =2* *Do not know the name = 0*	**Do you know the use of this plant?** *Know the use =1* *Do not know the uset = 0*
1.			
2.			
3			
4			
5			

Plant species	**Assessments**		
	Do you know this plant? *Know the plant =1* *Do not rexcognise the plant = 0*	**Do you know the name of this plant?** *Know the name =2* *Do not know the name = 0*	**Do you know the use of this plant?** *Know the use =1* *Do not know the uset = 0*
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			

Plant species	**Assessments**		
	Do you know this plant? *Know the plant =1* *Do not rexcognise the plant = 0*	**Do you know the name of this plant?** *Know the name =2* *Do not know the name = 0*	**Do you know the use of this plant?** *Know the use =1* *Do not know the uset = 0*
14.			
15.			
16.			
17.			
18.			
19.			
20.			
21.			

APPENDIX 4: PERMISSION LETTERS FROM THE TRIBAL COUNCIL AND VILLAGE HEADMAN

THENGWE TRADITIONAL COUNCIL
LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF CO-OPERATIVE GOVERNANCE HUMAN SETTLEMTS & TRADITIONAL AFFAIRS

Ref: CH 11/8/4
Enq: Tovhowani N.G
Tel/Fax: 015 967 2922
Date: 07.09.2016

DEPARTMENT OF CO-OPERATIVE GOVERNANCE, HUMAN SETTLEMENTS & TRADITIONAL AFFAIRS
THENGWE TRADITIONAL COUNCIL
07 SEP 2016
BOX 39 MUTALE 0956
VHEMBE DISTRICT SUPPORT CENTRE

P.O Box 39
MUTALE
0956

To whom it may concern

1. This is to certify that Nemandalali Tsumbedzo of ID. No. 850426 5704 083 has been permitted to conduct research survey on medicinal use, treatment and management of male sexual health.

2. Research will be done at: Gundani village, Tshithuthuni village, Mufulwi village and Mafukani village.

3. Yours in service

KHOSI N.S. NETHENGWE
THENGWE TRADITIONAL COUNCIL
CELL: 082 5896 279, TEL / FAX: 015 967 2922

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University of South Africa

Department of life and consumer sciences

To whom it may concern

REQUEST FOR PERMISSION TO CONDUCT A RESEARCH SURVEY

A. Researcher

My name is Tsumbedzo Nemandalali (Student no: 43323928) PhD student at university of South Africa. The research I will be conducting involves ethnobotanical survey of medicinal used in the treatment and management of male sexual health.

I am hereby seeking your authorisation to conduct a research interviews in the community concerned. The researcher asks for permission to interview the community members on a one on one interview.

Signature: Nedalali Date: 06-September-2016

B. Community leader

I hereby Authorize the researcher to conduct the research survey in Tshithuthuni village.

Names: NDWAMBI MAFANEDZA HELSON

Signature: Ndwambi Date: 2016-09-06

VHAMUSANDA VHO-NDWAMBI MN
TSHITHUTHUNI VILLAGE (THENGWE)
06 SEP 2016
P.O BOX 1509 MUTALE 0956

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Reclaiming Africa's Intellectual Futures

UNISA

University of South Africa

Department of life and consumer sciences

To whom it may concern

REQUEST FOR PERMISSION TO CONDUCT A RESEARCH SURVEY

A. Researcher

My name is Tsumbedzo Nemandalali (Student no: 43323928) PhD student at university of South Africa. The research I will be conducting involves ethnobotanical survey of medicinal used in the treatment and management of male sexual health.

I am hereby seeking your authorisation to conduct a research interviews in the community concerned. The researcher asks for permission to interview the community members on a one on one interview.

Signature: ..... Date: 06-September-2016

B. Community leader

I hereby Authorize the researcher to conduct the research survey in MAFUKANI village.

Names: NEMAFUKANI NDILEHI SAMSON

Signature: ..... Date: 2016/09/06

VHA MUSANDA
VHO –NEMAFUKANI N.S
CELL NO: 073 067 1564
2016 -09- 06
MAFUKANI I & II MATAVHELA
BOX 70 MATAVHELA 0990
LIMPOPO PROVINCE

UNISA

University of South Africa

Department of life and consumer sciences

To whom it may concern

REQUEST FOR PERMISSION TO CONDUCT A RESEARCH SURVEY

A. Researcher

My name is Tsumbedzo Nemandalali (Student no: 43323928) PhD student at university of South Africa. The research I will be conducting involves ethnobotanical survey of medicinal used in the treatment and management of male sexual health.

I am hereby seeking your authorisation to conduct a research interviews in the community concerned. The researcher asks for permission to interview the community members on a one on one interview.

Signature: ..... Date: 06-September-2016

B. Community leader

I hereby Authorize the researcher to conduct the research survey in Gundani village.

Names: MARANDA M. Phineas

Signature: ..... Date: 06-09-2016

VHA MUSANDA VHO
MARANDA M.P.
PO Box 367 Mutale
Gundani Village
Cell 072 214 0258

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UNISA

University of South Africa

Department of life and consumer sciences

To whom it may concern

REQUEST FOR PERMISSION TO CONDUCT A RESEARCH SURVEY

A. Researcher

My name is Tsumbedzo Nemandalali (Student no: 43323928) PhD student at university of South Africa. The research I will be conducting involves ethnobotanical survey of medicinal used in the treatment and management of male sexual health.

I am hereby seeking your authorisation to conduct a research interviews in the community concerned. The researcher asks for permission to interview the community members on a one on one interview.

Signature: ..... Date: 06- September 2016

B. Community leader

I hereby Authorize the researcher to conduct the research survey in Mufulwi village.

Names: Nemufulwi Francis

Signature: Nemufulwi E.F Date: 06-September-2016

KHOSI VHO- NEMUFULWI E.F
MUFULWI VILLAGE
2016 -09- 06
P.O. BOX 02, MATAVIELA, 0990
CELL NO: 078 526 9857/ 073 840 9654
LIMPOPO PROVINCE

APPENDIX 5: SUBMITTED MANUSCRIPT TO THE INDIAN JOURNAL OF TRADITIONAL KNOWLEDGE

Ethnobotanical study of medicinal plants used to treat and manage men sexual health in villages under Thengwe Tribal council, Limpopo, South Africa

T Nemandalali, MA, Nyila & TE Tshikalange[b*]

[a]Department of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, University of South Africa, c/o Christiaan de Wet and de Wet and Pioneer Avenue, Florida 1719, Johannesburg, South Africa.

[b]Department of Plant and Soil Sciences, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

E-mail: *Ntsumbe85@gmail.com

The current research aims to identify the ethnobotanical plants collected by local men of the Thengwe area under the former Mutale local municipality. In the Thengwe area, the community members, especially men, prefer to use traditional medicines to treat and manage their sexual illnesses. Various age groups have different perceptions and understanding of traditional medicine, from 18 to above 70 years of age. During the study, participants of varying age groups were randomly selected, namely, 18-30, 31-40, 41-50, 51-60, and 61 and above. Semi-structured interviews with questionnaires were used for the collection of data. A voucher specimen of each plant species was used to identify plants. Plant species identified in the Herbarium specimen at the Department of plant sciences of the University of Pretoria, South Africa. The roots and barks of twenty-one plant species were used to treat and manage men sexual illnesses. These roots and barks were dried for five days at room temperature and ground to powder when completely dry. Male community members in the Thengwe area know local medicinal plants and commonly use them to treat and manage their sexual illnesses and enhance sexual performance. The dosage of the traditional medicines seems to be unknown, and the potential risk associated with toxicity. High usage of traditional medicine poses considerable risk of loss of biodiversity in the area, and an agent intervention to prioritise plant resources for conservation and sustainable use is required.

Keywords: Ethnobotany, Male sexual health, Medicinal plants, and Thengwe area.

Introduction

Ethnobotanical studies are essential to determine the knowledge of the community about the surrounding plants in their area. [1] Defined ethnobotany as the study of relationships between human beings and plants, and it is mainly based on traditional medicinal knowledge and popularity. The study of ethnobotany plays an important role in understanding the dynamic relationships between biological diversity and social and cultural systems [2]. Thengwe is one of the areas in Southern Africa known to have many medicinal plants used to treat and manage men sexual health. The area consists of various and diverse medicinal plants of importance and traditionally known to treat various men sexual illnesses such as erectile dysfunctions and aphrodisiac activities. Important species such as *Securidaca longepedunculata* used to treat multiple illnesses have been conserved and regulated for access in the area due to over-use. However, other species, namely, *Tabernaemontana elegans*, *Terminalia*

APPENDIX 6: FEEEDBACK FROM RESEARCH JOURNAL ON SUBMITTED MANUSCRIPT

Reviewer H comments on IJTK-6692

Dear colleague,
this is an interesting paper with some structural problems: REVISION REQUIRED

- the authors talk about PEOPLE, not animals, so instead of "males" they should use "MEN"!
- S/N no column should be removed from tables
- Tables 1, 2, 3: in scientific names AUTHORS MUST NOT be in italics! Only Genus and Species are supposed to be in italics
- VOUCHER NUMBERS ARE MISSING
- lots of spacing mistakes

While reporting from an interesting region, the manuscript in its current state lacks a series of important components to make it suitable for publication. As it stands, the ms is of very limited interest to international readers and cannot be considered without substantial revisions. As it stands it is not suitable for IJTK.

Detailed other problems:

GENERAL
- Introduction is very fragmentary and does not well introduce into the subject overall, nor the local conditions in particular. Thus the introduction needs to be expanded.
- the manuscript lacks any kind of hypothesis to be tested
- no statistical analysis whatsoever

METHODS
- no detailed information on identification (LITERATURE?), i.e. the identity of the species mentioned cannot be confirmed. No information on deposition of vouchers in a scientific collection, i.e. no information WHERE the specimens were deposited

RESULTS
- simplistic presentation of results
- no statistical analysis whatsoever. the authors should provide analysis of Informant Consensus, Use Value, Diversity of Uses, Cultural Value at least.

DISCUSSION
- very limited discussion that ignores a large part of the pertinent comparative literature