

Ethnopharmacology of a selection of plants used for sexually transmitted infections in Thaba ‘Nchu, Free State

Brian Ngobeni

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Promoter: Dr I.T. Manduna (DSc Botany)
Co-promoter: Prof. NJ Malebo (PhD Microbiology)
Co-promoter: Prof. SS Mashele (PhD)

BLOEMFONTEIN
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DECLARATION OF INDEPENDENT WORK

DECLARATION WITH REGARD TO INDEPENDENT WORK

I, NGOBENI BRIAN, identity number ##### and student number #####, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Doctor of Health Sciences Biomedical Technology, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

SIGNATURE OF STUDENT

DATE

This work is dedicated to my late father Herbert Ngobeni and late grandmother Tryphinah Mochike for supporting my vision and education when it was not fashionable to do so. May their souls rest in peace.



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A variety of medicinal plants are used by traditional healers in Thaba 'Nchu, Free State province, South Africa to treat sexually transmitted infections (STIs). This study evaluated the pharmacological properties of some medicinal plants identified through an ethnobotanical survey conducted through semi-structured interviews with the local traditional healers. A selection of the plants was investigated for antimicrobial, anti-inflammatory and antioxidant activities to confirm their pharmacological properties and safety. Plant extracts were prepared through liquid-solid extraction method using methanol, acetone, and distilled water. Phytochemical compounds that contribute to the pharmacological properties of plants were assessed qualitatively through standard methods and quantitatively using the Folin-Ciocalteu, the aluminium colorimetric method and Liquid Chromatography Mass Spectrometry (LCMS). Antimicrobial activities of the plant extracts were determined using the Broth microdilution method, while toxicity of the extracts was evaluated using Hoechst 33342 nuclear dye and the propidium iodide exclusion method against African green monkey kidney cell line or Vero cells, while 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used against the RAW 264.7 cell line. Nitric oxide (NO) assay against RAW 264.7 cell line and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method were employed to indicate the anti-inflammatory and antioxidant activities of the plant extracts, respectively. A total of 35 medicinal plant species were recorded for the treatment of STIs in the study area. Most of the medicinal plants were from Asteraceae (21%), Fabaceae (17%), Solanaceae (13%) and Hyacinthaceae (13%). *Bulbine narcissifolia*, *Pentanisia prunelloides*, *Hypoxis hemerocallidea*, *Scabiosa columbaria*, *Xysmalobium undulatum*, *Rumex lanceolatus*, *Eucomis autumnalis*, *Dicoma anomala* and *Salvia runcinata* were the most favoured plants. The most preferred method for preparation of remedies were decoctions made from the roots. *Hermannia depressa*, *Berkheya*

onopordifolia, *S. runcinata* and *S. runcinata* were selected for further studies because of high frequency of mention and availability in the study area. Phenols, flavonoids, tannins, saponins, alkaloids, steroids and glycosides were detected qualitatively across all the plant extracts. *H. depressa* acetone and *S. columbaria* aqueous extracts had the highest Total Phenolic Content at, 8.45 ± 0.006 and 7.65 ± 0.004 mg GAE/g respectively. Methanol and acetone extracts of *S. columbaria* were found to have the highest Total Flavonoid Content at 3.56 ± 0.01 and 2.29 ± 0.03 mg quercetin equivalents QE/g respectively. Pharmacologically important compounds such as Skimmin, 14-Methylmonocrotaline-like, 3',4'-Dimethoxyluteolin, Isosakuranetin, Carsonic acid, Jaceosidin, Waltherione C, Methoxyestradiol, and many other compounds were detected in some extracts. The compounds have antibacterial, anti-inflammatory, antioxidant, hepatoprotective and many other properties which may explain their use in the treatment of STIs. The aqueous extracts of the plants used in the study and the methanol extract of *S. columbaria* were not toxic. However, the acetone and methanol extracts of all four plants were toxic. *S. runcinata* methanol and acetone extracts showed toxicity by affecting 50% of Vero cells at a lower CTC₅₀ of 3.93 µg/ml and 3.96 µg/ml. Acetone extracts of *S. columbaria* had a lower CTC₅₀ of 6.09 µg/ml, while *H. depressa* methanol and acetone extracts affected Vero cells at a lower CTC₅₀ of 4.97 µg/ml and 4.65 µg/ml respectively. The cytotoxicity could be attributed to the presence of toxic compounds such as alkaloids, tannins, and glycosides (Canadine, Xanthoxylin, Rosmarinic acid Matairesinol, Salvigenin). All methanol and acetone extracts showed significant antimicrobial activity against almost all microbial species used against in the study at concentrations ranging from 0.1 to 1.25 mg/ml. The antimicrobial activity of *S. runcinata* acetone extracts against colistin antibiotic resistant *Escherichia coli* at MIC 1.25 mg/ml is important to highlight. *H. depressa* acetone, *S. columbaria* methanol and *S. runcinata* methanol extracts showed strong

anti-inflammatory activity by inhibiting the release of Nitric oxide by 60%. *H. depressa* aqueous ($IC_{50}=9.081 \mu\text{g/ml}$), methanol ($5.197 \mu\text{g/ml}$) and acetone ($3.576 \mu\text{g/ml}$) and *B. onopordifolia* acetone ($9.834 \mu\text{g/ml}$) extracts showed the strongest antioxidant activity when compared to Ascorbic acid ($5.69 \mu\text{g/ml}$) and Trolox ($6.166 \mu\text{g/ml}$). The antimicrobial, anti-inflammatory and antioxidant activity of the medicinal plants selected for this study validates their use for the treatment of STIs and related oxidative stress and inflammatory conditions. These plants may serve as a source for development of novel antimicrobial, anti-inflammatory and antioxidant agents.

LIST OF ABBREVIATIONS

Abbreviation	Meaning
2ME2	Methoxyestradiol
μl	Microlitre
μg/ml	Microgram per millilitre
AG	Aminoguanidine
AIDS	Acquired Immune Deficiency Syndrome
AlCl ₃	Aluminium Chloride
ATCC	American Cell Culture Collection
BV	Bacterial Vaginosis
CAF	Central Analytical Facilities
CAFSaB	Centre of Applied and Food Sustainability and Biotechnology
CFU	Colony Forming Units
CGA	Chlorogenic Acid
CO ₂	Carbon Dioxide
COX-2	Cyclooxygenase-2
CTC ₅₀	Half maximal cytotoxic concentration
CUPRAC	Cupric-Reducing Antioxidant Capacity
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
ESI	Electrospray Ionization
GAE/g	Gallic Acid Equivalent Per Gram
GCMS	Gas Chromatography Mass Spectrometry

FBS	Fetal Bovine Serum
FC	Frequency of citation
FRAP	Ferric Reducing Antioxidant Power
HIV	Human Immune Virus
HPV	Human Papilloma Virus
ICF	Informant Consensus Factor
IC ₅₀	Half maximal inhibitory concentration
IL-6	Interleukin 6
iNOS	Inducible nitric oxide synthase
IQE	Quercetin Equivalents
kV	Kilovolt
LCMS	Liquid Chromatography Mass Spectrometry
LPS	Lipopolysaccharide
MCF-7	Michigan Cancer Foundation-7
MIC	Minimum Inhibitory Concentration
MSD	Mass Selective Detector
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaNO ₃	Sodium Nitrate
NO	Nitric oxide
NMR	Nuclear magnetic resonance
PAs	Pyrrolizidine alkaloids
PC	Positive control
PGE2	Prostaglandin E2
PI	Propidium Iodide

psi	Pound per square inch
QE/g	Quercetin Equivalent Per Gram
RA	Rosmarinic acid
ROS	Reactive oxygen species
RPM	Revolutions per minute
RT	Retention time
SANBI	South African National Biodiversity Institute
SE	Standard Error
SI	Selectivity Index
STIs	Sexually Transmitted Infections
TB	Tuberculosis
TFC	Total Flavonoid Content
THM	Traditional Herbal Medicine
TOF-MS	Time-Of-Flight Mass Spectrometer
TPC	Total Phenolic Content
URIC	University Research and Innovation Committee
UT	Untreated Control
UV	Use Value; Ultraviolet
XCT	X-ray Computed Tomography
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

Sexually transmitted infections (STIs) are primarily transmitted through vaginal, anal, and oral sex; however, some infections can also be transmitted non-sexually, such as from mother to child during pregnancy or childbirth (National Institute for Communicable Diseases, 2022). STIs such as gonorrhoea, chlamydia, candida infections, syphilis, or trichomoniasis typically result in significant morbidity, mortality, and stigma, while more than a million people worldwide receive a diagnosis of one or more of these conditions every day (Wagenlehner *et al.*, 2016). STIs are one of the major health issues that developing nations such as South Africa usually deal with. Poverty, commercial sex lifestyles, a lack of knowledge about STIs and their symptoms, and access to modern medical facilities all contribute to the prevalence of these infections. STIs also affect child-bearing young women and can be passed on during childbirth (Sharifi-Rad *et al.*, 2021). Complications and devastating effects such as infertility, cancer, and pregnancy complications also have adverse effects on patients' psychosocial health and quality of life. STIs such as human papilloma virus can also increase HIV's infectiousness and susceptibility (Unemo *et al.*, 2017). Therefore, the management of STIs is a major priority for the health sector, globally.

STIs are currently still a significant public health issue despite increased awareness, prevention measures, and technological advancements (Carmona-Gutierrez *et al.*, 2016). Regular testing allows for early infection detection because no vaccines are available to prevent these STIs. Early detection will allow for effective treatment to stop further transmission and reduce infection-related morbidity (Unemo *et al.*, 2017). However, no single treatment is 100 % successful due to a variety of factors, such as

patient compliance to treatment, post-treatment nausea or diarrhoea, immune response, and microorganism factors such as antimicrobial resistance (Kong *et al.*, 2019). Given the above-mentioned factors it is pertinent to look for new, efficient, and affordable antimicrobial drug from natural products derived from natural sources such as plants, animals, soil, and microorganisms.

The term 'medicinal plant' refers to a variety of plants that have therapeutic properties. Over 50 000 plant species, or more than a tenth of all plant species in the world, are used in pharmaceutical and cosmetic products (Ugbogu *et al.*, 2009; Shakya, 2016). Many diseases that are difficult to treat using conventional medicine can be treated using medicinal plants. Medicinal plants have several benefits such as fewer side effects, improved patient tolerance, relative affordability, and acceptance due to a long history of use, which all contribute to their rising popularity (Tumpa *et al.*, 2015). Plants are a rich source of active compounds with direct or indirect therapeutic effects (Jamshidi-Kia *et al.*, 2018). Research to study these compounds for the development of new and potent agents for the treatment of STIs and other microbial diseases is therefore imperative.

Bioactive compounds are substances that are produced and stored in the bodies of plants that have pharmacological effects on living organisms (Shakya, 2016; Mandal *et al.*, 2021). Plant metabolites fall into two categories: primary metabolites and secondary metabolites. Primary metabolites are essential for the growth and development of the human body. These include glucose, starch, polysaccharides, protein, lipids, and nucleic acid. Alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, and volatile oils are secondary metabolites (Shakya, 2016). Antimicrobial, anti-inflammatory, antioxidant, and other pharmacological properties

are among the activities of these bioactive compounds (Kandar, 2021). Therefore, the bioactive compounds properties validate the use of plants for the treatment of STIs.

In South Africa, as with other nations, traditional healers are usually the custodians of traditional medicine. They hold a vast amount of indigenous knowledge regarding STIs, including their treatment using medicinal plants (Maema *et al.*, 2019). Such information was then passed down orally from generation to generation, which helped develop the traditional medical system that is still used today in many nations around the world (Qureshi *et al.*, 2016). Studies in ethnobotany document plant resources that are used in traditional medicine and that can be used to target novel compounds, resulting in the development of new drugs for the treatment of particularly complex and minor diseases (Qureshi *et al.*, 2016). Thaba 'Nchu is a settlement in the Free State province of South Africa where traditional healers predominate, meeting the healthcare needs of the local population. The study area is located in South Africa's grassland biome, which is abundant in flora with a wide range of therapeutic benefits used to treat STIs and many other ailments. The current study investigated the ethnopharmacology of a selection of plants used to treat STIs in Thaba 'Nchu.

1.1. RESEARCH OBJECTIVES

1.1.1. Problem statement

Sexually transmitted infections (STIs) continue to be a significant public health concern in South Africa. The country has one of the highest rates of HIV/AIDS infections in the world, with an estimated 7.7 million people living with the virus in 2021 (Van Schalkwyk *et al.*, 2021). In addition to HIV, there are many other types of STIs that are prevalent in the country, including gonorrhoea, chlamydia, syphilis, herpes, and human

papillomavirus (HPV). One of the main challenges in addressing the STIs epidemic in South Africa is the lack of awareness and knowledge about these infections (Francis *et al.*, 2018). Many people are unaware of the risks associated with unprotected sex and the importance of regular testing and treatment. This is compounded by social and cultural barriers such as stigma, discrimination, and gender inequality, which prevent many people from seeking help. In addition, the use of modern drugs comes with many challenges such as high prices, side effects and the emergence of drug resistant strains which make the treatment of STIs, and other microbial infections complicated. New sources of drugs such as medicinal plants are required to counter these shortcomings. Continued research on medicinal plants can contribute to the discovery of novel compounds or drug leads. Even though several drugs have already been developed from medicinal plants using indigenous knowledge or ethnobotany, more and more plant traditional uses still need to be documented to preserve the information for future use by generations to come. Therefore, the current study will document and evaluate the pharmacological properties of medicinal plants used for the treatment of STIs in Thaba 'Nchu.

1.1.2. Aim of the study

The aim of the study is to evaluate the pharmacological properties of the plants used to treat STIs in Thaba 'Nchu, Free State, South Africa.

1.1.3. Objectives of the study

1. To document traditional knowledge of plants used in the treatment of sexually transmitted infections by traditional healers of Thaba 'Nchu, Free State.
2. To detect and identify bioactive compounds from a selection of plants used to treat STIs.

3. To evaluate the antimicrobial, anti-inflammatory and antioxidant activities of the plants as well as the toxicity.

1.2. STUDY LAYOUT

This study was conducted in a sequence of steps described in Figure 1.1. An ethnobotanical survey was carried out to document medicinal plants used for the treatment of STIs in Thaba 'Nchu, Free State province, South Africa. The ethnobotanical survey data were analysed to identify the most used plants used for the treatment of STIs in the study area. *Berkheya onopordifolia*, *Hermannia depressa*, *Scambiosa columbaria* and *Salvia runcinata* extracts were investigated for their bioactive compounds, toxicity, antimicrobial, anti-inflammatory and antioxidant activities.

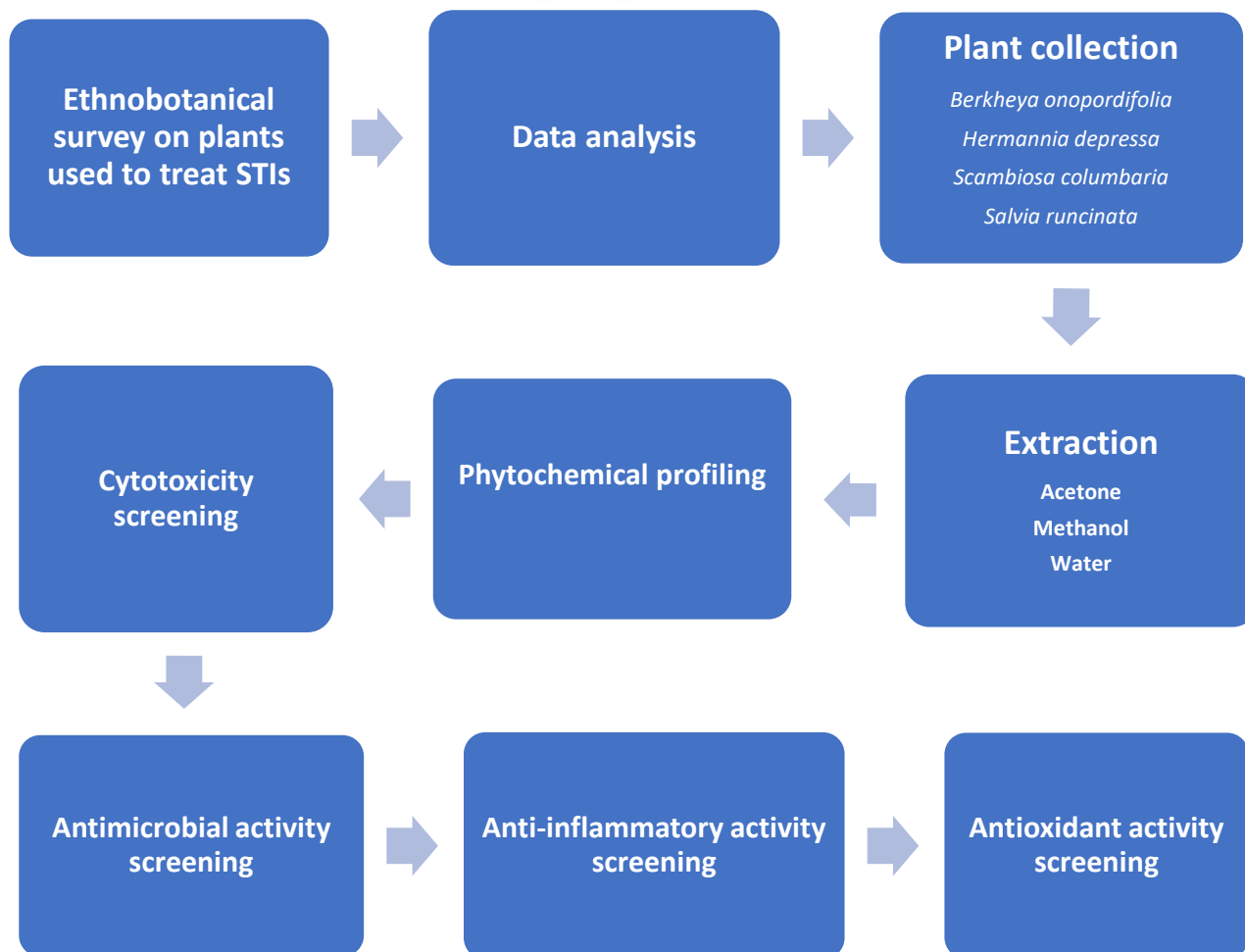


Figure 1.1: The summary of steps taken to achieve the objectives of this study.

1.3. OVERVIEW OF THE STUDY

This study achieved four objectives. Through an ethnobotanical survey, **Chapter 3** documented plants used for the treatment of STIs in Thaba 'Nchu, a village located in the Free State province of South Africa. The analysis of the ethnobotanical survey results gave birth to a selection of four commonly used plants (*Berkheya onopordifolia*, *Hermannia depressa*, *Scambiosa columbaria* and *Salvia runcinata*) used for the treatment STIs by traditional healers in the study area. **Chapter 4** is a review of the selected medicinal plants studied and reported in this thesis. This chapter focuses on the description, habitat, traditional uses, bioactivities and the phytochemistry of the selected plant species used to treat STIs. The gaps that currently exist in the literature of interest for the selected plants were utilised to substantiate the need to conduct

research on the plants in this study. The descriptions, habitats, traditional uses, pharmacological properties, and phytochemistry of the selected plants were covered. **Chapter 5** investigated the phytochemicals of the selected plant extracts qualitatively and quantitatively using various methods. Chapters **6-8** report on the evaluation of the bioactivities of the selected plants such as cytotoxicity, antimicrobial, anti-inflammatory and antioxidant activities. **Chapter 9** provides an overall concluding discussion based on the findings from all the chapters and future recommendations.

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CHAPTER 2

LITERATURE REVIEW

2.1. THE BURDEN OF SEXUALLY TRANSMITTED INFECTIONS

Sexual transmitted infections are the world's major public health concern. There are more than 25 STIs globally, some of which result in detrimental health problems if left untreated, while some contribute to the spread of HIV/AIDS infections (Semenya *et al.*, 2013). According to World Health Organization (WHO), more than one million infections are acquired every day (WHO, 2016). There are an estimated 357 million new infections with the following four STIs annually: chlamydia (131 million), gonorrhoea (78 million), syphilis (5.6 million) and trichomoniasis (143 million) (Newman *et al.*, 2015; WHO, 2016). More than 500 million people are living with genital HSV (herpes) infection, and more than 290 million women have a human papillomavirus virus (HPV) infection, which is one of the most common STIs (WHO, 2015; WHO, 2020). STIs such as herpes and syphilis can increase the risk of HIV acquisition in three-fold or more. Mother-to-child transmission of STIs can result in stillbirth, neonatal death, low-birthweight and prematurity, sepsis, pneumonia, neonatal conjunctivitis, and congenital deformities (WHO, 2015).

Over 900 000 pregnant women infected with syphilis result in approximately 350 000 adverse birth outcomes including stillbirth in 2012 (Newman *et al.*, 2015; Korenromp *et al.*, 2018). HPV infections cause 528 000 cases of cervical cancer and is responsible for 266 000 cervical cancer deaths annually. STIs such as gonorrhoea and chlamydia are major causes of pelvic inflammatory disease (PID) and infertility in women (WHO, 2020). Acquired Immune Deficiency Syndrome (AIDS) is one of the biggest challenges to the public health care and the most infectious disease of the 20th and 21st century

globally (Doda *et al.*, 2018). It is rated as the fourth leading cause of death in the world especially in poor countries. In 2014, 39.9 million people were diagnosed with HIV, two million new cases were also recorded, and 2.1 million deaths were recorded due to AIDS (Pendse *et al.*, 2016; Elbadawi and Mirghani., 2017; Doda *et al.*, 2018). In 2016, 36.7 million people were further infected with HIV, 1.8 million new cases were recorded, and one million deaths of which more than two thirds of the statistics were attached to Africa (Korenromp *et al.*, 2019). Meanwhile, in 2021, South Africa was reported to have 7.5 to 8.2 million HIV positive adults and children (UNAIDS, 2021). This large burden of disease is an indicator of the necessity for appropriate strategies to aid in the management of STIs.

2.2. THE AETIOLOGY OF COMMON STIS

2.2.1. Chlamydia

Chlamydia is a common STI caused by *Chlamydia trachomatis* bacterial infection, and it usually cause inflammatory pelvic diseases that may result in infertility or ectopic pregnancy (Dukers-Muijrs *et al.*, 2022). According to the WHO, approximately 130.9 million people globally were newly infected with chlamydia, and it has also been declared as the leading cause of bacterial sexually transmitted infection globally (O'Connell and Ferone., 2016; WHO, 2016). Compared to other bacterial STIs, prevalence is relatively high because asymptotically infected individuals may not seek treatment, and after single dose therapy, repeat infection is common (Dukers-Muijrs *et al.*, 2022). Infection is more frequently identified in young women than in young men primarily because of single marital status, new sex relationships or marriages, smoking and related socio-economic status (Jorgensen *et al.*, 2015; O'Connell and Ferone, 2016). Symptoms of genital *C. trachomatis* infection in women include changes in vaginal discharge, intermittent, intermenstrual and/or post-coital

bleeding and may also present urinary tract infection symptoms such as dysuria (O'Connel and Ferone., 2016). *C. trachomatis* is the most common cause of urethritis of nongonococcal origin in men, and infections in men are mostly asymptomatic as compared to women. Men also present symptoms such as a small amount of watery urethral discharge and dysuria (Beeton et al., 2019).

2.2.2. Gonorrhoea

Gonorrhoea is caused by the bacterium *Neisseria gonorrhoeae*, which is one of the most frequently reported STIs, with 106 million new cases reported worldwide each year, according to World Health Organization (WHO) estimates (Rice *et al.*, 2017). Gonorrhoea is a bacterial infection transmitted almost solely by sexual contact and it affects mainly the mucous membranes of the urethra and cervix and, less commonly, those of the rectum, oropharynx, conjunctivae, and other internal organs, such as the liver, the heart, and the lining of the brain (Ndowa *et al.*, 2012). If not treated early, the rising genital infection in women leads to pelvic inflammatory disease (PID), which is the major complication of female infertility and one of the most common causes of infertility in men and women, including neonatal blindness (Rice *et al.*, 2017). Asymptomatic men make up approximately two-thirds of all infected men at any time and are the main cause of infection spread. More than half of infected women may be asymptomatic, but sometimes after direct (speculum) assessment of the cervix they show physical symptoms of inflammation (Rice *et al.*, 2017). Gonorrhoea is on the rise around the world and many antibiotic-resistant strains are emerging. Appropriate treatments and preventive measures are urgently needed, including effective vaccines.

2.2.3. Syphilis

Syphilis is a bacterial infection caused by the bacterium *Treponema pallidum* which can be transmitted sexually and from mother to child during pregnancy. Untreated primary or secondary syphilis infections in the second or third trimesters of pregnancy can cause fatal foetal outcomes. Adverse pregnancy outcomes associated with syphilis (i.e., congenital syphilis) include early foetal death, stillbirth, premature birth, low birth weight, neonatal mortality, and congenital infection in children (Gomez *et al.*, 2013; Wijesooriya *et al.*, 2016). The World Health Organization (WHO) estimated 36.4 million people worldwide to have been diagnosed with syphilis. There is an estimation of more than 12 million new syphilis infections in the world each year, and 90% of the cases are found in resource-limited countries. The WHO also estimated that the annual new syphilis cases among adults aged 15 to 49 in the African region were 3.4 million (Mutagoma *et al.*, 2016). Syphilis infection continues to be an important STI due to its prevalence, infectiousness, and disease in both infected individuals and health systems. Its importance is highlighted by the fact that its ulcerative lesions facilitate the acquisition of HIV; its potential for vertical transmission is another important aspect of syphilis (Mutagoma *et al.*, 2016).

2.2.4. Herpes simplex virus (HSV)

Herpes is a lifelong STIs infection caused by herpes simplex virus (HSV) type 1 (HSV-1) and type 2 (HSV-2). HSV-1 is marked by recurrent reactivations at the infection site. HSV type 1 is typically spread through oral-to-oral and oral-to-genital contact (Looker *et al.*, 2020). Orolabial herpes, commonly known as cold sores, are most frequently caused by HSV type 1 infection, which can also lead to encephalitis, keratitis, and ocular sequela (Whitley and Baines, 2018). It was estimated that in 2016, 192 million people aged 15 to 49 years had HSV-2 infection. HSV type 2 is primarily transmitted

through sexual contact causing genital herpes. Genital ulcer disease, also known as vesicles, ulcers, fissures, or other painful lesions on or around the genital skin and mucosa is the most common way to identify genital herpes (Looker *et al.*, 2020). Infants are susceptible to contracting HSV from a genitally infected mother as well as via oral contact with postpartum caretakers (Naser *et al.*, 2022). Moreover, evidence indicates that HSV type 2 infection raises the chance of contracting HIV. It was estimated that 491 million people were infected by HSV-2 infection. With both HSV type 1 and type 2, virus shedding can be symptomatic or asymptomatic (Looker *et al.*, 2017). Hence, the high global frequency of HSV infection is caused by the fact that infected people might be asymptomatic but infectious, allowing these viruses to be spread unintentionally (James *et al.*, 2020).

2.2.5. Human papilloma virus

Human papilloma virus (HPV) is a prevalent sexually transmitted DNA virus that infects the reproductive system's squamous epithelium, the anal transformation zone, and the mucosal epithelium of the larynx, tonsils, and oropharynx (Zacharis *et al.*, 2018). HPV is spread by sexual intercourse at all places of contact, and regardless of socio-economic position, its occurrence is generally high in both genders (Bosch *et al.*, 2013). Almost all cases of cervical cancer and a sizable part of anogenital (vulvar, vaginal, penile, and anal) and oropharyngeal cancers are linked to HPV infection (De Sanjose *et al.*, 2018; Zacharis *et al.*, 2018). Warts and benign papillomas are two additional skin and mucosal lesions that are linked to HPV infection (Doorbar *et al.*, 2015). Both men and women may get HPV, however, due to the increased vulnerability of cervical cells to HPV infection, the burden of infection is significantly greater in women (De Sanjose *et al.*, 2018).

2.2.6. Bacterial vaginosis

Bacterial vaginosis (BV) is the most common cause of excessive vaginal discharge caused by vaginal dysbiosis among women of reproductive age (Muzny *et al.*, 2019; Coudray and Madhivanan, 2020). BV is a vaginal disorder characterised by a change from Lactobacilli dominance to a mixture of anaerobic bacteria (Abou Chacra *et al.*, 2022). The vagina is made of a community of bacteria characterised by abundant Lactobacilli that evolve during the life of a woman, depending on age, hormonal oestrogen levels, sexual conduct, and environment (Muzny *et al.*, 2019); Abou Chacra *et al.*, 2022). The vaginal microbial community is crucial for women's health; yet, when it is impaired, it is linked with a variety of negative symptoms including vaginal malodour, discharge, itching, and elevated vaginal pH. BV can increase the risk of contracting many STIs such as HIV, *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and HSV-2 (Redelinghuys *et al.*, 2020).

2.3. THE RELATIONSHIP BETWEEN STIS, INFLAMMATION AND OXIDATIVE STRESS

STIs commonly result in persistent inflammation in the affected area and oxidative stress in the body. When there is an infection, injury, or irritation, inflammation is a natural response of the body, and it triggers the release of different inflammatory mediators such as cytokine and chemokines to fight pathogens (Dutta *et al.*, 2021). Chronic inflammation can be detrimental and has also been linked to various diseases, including cancer and cardiovascular diseases (Ferrucci and Fabbri, 2018). STIs can also cause oxidative stress which occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralise them (Das *et al.*, 2022). ROS are produced naturally during normal cellular metabolism, but excessive ROS can damage cellular structures and lead to degenerative diseases (He

et al., 2017). It has been shown that STIs including chlamydia, gonorrhoea, and HIV increase oxidative stress levels and lead to chronic inflammation, which can result in long-term health issues (Ona *et al.*, 2016; Bryan *et al.*, 2020). While it is important to develop effective treatment agents for STIs, it is equally important to search for drugs that will assist with the management of inflammatory and oxidative stress disorders caused by STIs.

2.4. THE CHALLENGES ASSOCIATED WITH THE TREATMENT OF SEXUALLY TRANSMITTED INFECTIONS

STIs pose a significant public health challenge, with millions of new cases diagnosed each year worldwide. While effective treatments exist for many STIs, their successful implementation can be challenging due to a variety of factors (Eisinger *et al.*, 2020). One key challenge is the emergence of antimicrobial-resistant strains of STIs such as *Neisseria gonorrhoeae* and *Mycoplasma genitalium*, which can make it difficult to treat infections with traditional antibiotics (Lin *et al.*, 2021; Sethi *et al.*, 2017). Stigma and discrimination associated with STIs can also hinder diagnosis and treatment efforts, as affected individuals may be reluctant to seek care or disclose their infection status to sexual partners. Inadequate access to healthcare, including screening and treatment services, can also pose a barrier to effective STI management (Gottlieb *et al.*, 2014). Asymptomatic infections and limited awareness of STIs among the general population can further complicate diagnosis and treatment efforts (WHO, 2020). STIs are effectively treated with various prescribed synthetic drugs; however, patients frequently have unpleasant side effects making STIs' treatment even more difficult (Table 2.1). Addressing these challenges requires a comprehensive approach that includes increased awareness and education, improved access to care, and the

development of new treatment strategies such as the use of medicinal plants to treat STIs.

Table 2.1 The common STIs, treatment drugs and associated side effects

STIs	Treatment	Common side effects	References
Chlamydia	Azithromycin, oxytetracycline, tetracycline, ofloxacin, levofloxacin or doxycycline	Nausea, vomiting, diarrhoea, headache, dizziness, rash, fatigue, abdominal pain	CDC. (2021). Chlamydia - CDC fact sheet (detailed). WHO, 2016 Páez-Canro <i>et al.</i> , 2019 Silverberg <i>et al.</i> , 2021
Gonorrhoea	Ceftriaxone and azithromycin or doxycycline	Injection site pain, diarrhoea, liver damage, vomiting, nausea, allergic reaction, abdominal pain, diarrhoea	CDC (2021). Gonorrhea - CDC fact sheet (detailed). Workowski and Bolan, 2015
Syphilis	Benzathine penicillin G, doxycycline, tetracycline, azithromycin, ceftriaxone	Injection site pain, fever, rash, allergic reaction, chills, muscle aches, joint pain, gastrointestinal upset, liver problems	CDC (2021). Syphilis - CDC fact sheet (detailed). WHO, 2016
Herpes	Antiviral medication (acyclovir, valacyclovir, famciclovir, penciclovir, docosanol)	Nausea, headache, diarrhoea, dizziness, abdominal pain, skin burn, itching and redness on the application site.	Hazra <i>et al.</i> , 2022 WHO, 2020
Human papilloma virus (HPV)	No cure, but vaccines available	Mild fever, pain at injection site	CDC (2022). Human Papillomavirus (HPV) - CDC fact sheet (detailed). Ardekani <i>et al.</i> , 2022
Trichomoniasis	Metronidazole or tinidazole	Nausea, headache, metallic taste	CDC (2020). Trichomoniasis; CDC fact sheet (detailed). Silverberg <i>et al.</i> , 2022

HIV	Antiretroviral therapy (ART)	Nausea, diarrhoea, fatigue	Esser <i>et al.</i> , 2007
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2.5. THE IMPORTANCE OF MEDICINAL PLANTS IN TREATMENT OF DISEASES

Human reliance on plants dates to the dawn of humans and are common sources of medicine. The interest in the use of herbal medicine has increased in both traditional and modern systems of medicine throughout the years (Jamshidi-Kia *et al.*, 2018). Credible evidence can be cited for the use of herbs for the treatment of diseases and for the regeneration and strengthening of body systems in ancient medical systems such as Ayurvedic, Unani and traditional Chinese medicine (Aslam and Ahmad, 2016). This global interest in the use of medicinal plants has contributed to the increase in the demand for medicinal plants resulting from the collection of native plants (Zahra *et al.*, 2020). It is estimated that approximately 70 to 80% of the population in developing countries is dependent on plant sourced remedies due to high prices associated with pharmaceutical drugs, their side effects and accessibility (Aslam and Ahmad, 2016; Mustafa *et al.*, 2017).

Over 50 000 species are used in pharmaceutical and cosmetic products. The term 'medicinal plant' refers to a plethora of plants with medicinal properties. Such plants are a rich source of compounds that can be used to synthesise the drugs (Rasool Hassan, 2012). Scientific growth and commercial interests on medicinal plants have been observed in developing countries in recent decades, primarily because of the economic potential and widespread acceptance of plant products (Kord *et al.*, 2019). The use of plant resources, especially medicinal plants, has led to beneficial results, including self-sufficiency and economic opportunities for residents. In the year 2006, the market value of medicinal and aromatic plants was estimated at \$60 billion

annually which is expected to increase drastically by 2050 due to the current global interest in herbal drugs (Kord *et al.*, 2019).

2.6. THE IMPORTANCE OF ETHNOBOTANICAL KNOWLEDGE AS A LEAD TO TREATMENT OF DISEASES

'Ethnobotany' in the year 1896 was defined and published by *Hershberger* as a field that addresses cultural statuses of tribes who use plants for food, protection, hunting, agricultural purposes, timber, fencing, firewood, building materials and religious/cultural purposes (Rahman *et al.*, 2019). Researchers have defined ethnobotany differently as a study of direct interaction of humans and plants in their culture (Martin, 2004; Nasab and Khosravi, 2014). Medicine and botany always had a strong and close connection and many of the modern medicines have plant origins (Rahman *et al.*, 2019). Traditionally, the use of medicinal plants has been a long-standing practice among the human population, with information being transferred from generation to generation within communities (Offiah *et al.*, 2011).

Information on the use of medicinal plants is slowly being lost because it is transferred from generation to generation verbally rather than by means of documentation (Offiah *et al.*, 2011; Vitalini *et al.*, 2013). Most cultures have extensive unrecorded indigenous knowledge about the use of medicinal plants for disease treatment, therefore recording and preserving traditional knowledge about the use of medicinal plants to discover new medicines is much more important (Kord *et al.*, 2019). Ethno-pharmacological surveys have gained interest among researchers in documenting and highlighting important herbal medicine practices and plants used in the health care systems (Watkins *et al.*, 2011; Ahmad *et al.*, 2018). Ethno-pharmacological survey is a critical stage that can be used in the development of new raw plant remedies and new drugs

that can be used to treat diseases that cannot be treated using standard healthcare systems, because plants are known to produce unrivalled chemical diversity and natural products either as pure compounds or as homogeneous plant extracts (Ahmad *et al.*, 2018; Mustafa *et al.*, 2017).

While the western use of ethnobotanical information has attracted interest and is recognised by most academic and industrial researchers, ethnobotanical surveys are important in highlighting the important plant species that are commonly used to treat diseases in various health care systems and can also assist in finding new crude plant mixtures that are used to treat diseases that are not curable using the modern standard health care systems (Mustafa *et al.*, 2017; Hani *et al.*, 2022). Therefore, there is a need for scientific investigations on medicinal plants using indigenous knowledge (Hosseinzadeh *et al.*, 2015).

Plants will continue to contribute directly to the development of drugs as well as providing a wide variety of compounds that can serve as the starting point to produce new drugs with better pharmacological properties (Rhaman *et al.*, 2019). Seventy-five per cent of new natural chemical components are derived from medicinal plants and are already being used as potential alternatives to combat infectious diseases (Lukhande *et al.*, 2007; Mustafa *et al.*, 2017). In current pharmacopeia approximately 25 % of drugs and a significant number of synthetic analogues are prepared on prototype compounds derived from plants (Mandal *et al.*, 2017; Mustafa *et al.*, 2017). Morphine, quinine, taxol, vincristine, serpentine, aspirin, and many other drugs are examples of drugs that were developed or discovered from plants (Mustafa *et al.*, 2017). The latter drugs have made significant contributions to resolving the health

issues brought on by cancer, blood, and inflammatory disorders, and have opened the door for the development of other drugs.

2.7. BIOACTIVE COMPOUNDS PRESENT IN MEDICINAL PLANTS

Plant compounds are categorised into primary or secondary metabolites. Primary metabolites are known to be distributed in nature and plants are also known to produce secondary metabolites synthesised by primary metabolites. For years, medicinal plants and secondary metabolites have been playing a role in reducing the burden of infectious diseases (Anand *et al.*, 2019). There are over 400 000 plant species on earth with scientifically unexplored reservoirs of bioactive compounds (Mushtaq *et al.*, 2018). Investigations on bioactive active compounds from medicinal plants using screening techniques showed that these compounds possessed various therapeutic properties (Ghorbanpour *et al.*, 2017). Plant-derived compounds have improved hard-to-treat diseases such as cancer, tuberculosis, STIs and many other diseases (Srinivasahan and Durairaj, 2014; Jamshidi-Kia *et al.*, 2018). While drugs of synthetic origin are currently being used to treat diseases, their use has been found to have side effects or cause damage to the body; hence the use of medicinal plants and natural products is currently being recognised (Jamshidi-Kia *et al.*, 2018).

The essential advantages of the use of medicinal plants for treatment of diseases is their safety, being economical, effective and their availability (Pandey and Tripathi, 2014). It is estimated that there are approximately 200 000 secondary metabolites in the plant domain which can be classified based on biosynthetic origin in three major categories, namely phenylpropanoids, alkaloids and terpenoids (Yang *et al.*, 2016). Plants have rich biological compounds that are mostly secondary metabolites such as alkaloids, flavonoids, tannins, phenols, and steroids which possess and produce

physiological actions to the human body (Zhao *et al.*, 2015). Most bioactive compounds are known to possess properties such as anticancer, antimicrobial, antioxidant, anti-diarrheal, wound healing, and many other activities (Table 2.2) (Ingle *et al.*, 2017). Bioactive compounds can be derived from any part of the plants such as bark, leaves, flowers, seeds, roots, etc. Because various plant parts of the same plant typically have varied concentrations of bioactive compounds, it is crucial to understand their phytochemical profiles to decide which plant parts to employ for a certain activity or synergistic activity.

Table 2.2 A summary of pharmacologically important phytochemicals and their properties

Phytochemicals	Biological activity	Examples
Alkaloids	Hepatoprotective, immunomodulatory, antispasmodic, antimalarial, analgesic, diuretic, anti-inflammatory and cardioprotective.	Morphine, caffeine, Berberin, codeine (Aslam <i>et al.</i> , 2016 Debnath <i>et al.</i> , 2018)
Glycosides	Anti-cancer, antifungal and antibacterial.	Amygdalin, gentiopicrin, and rographolide, polygalin, Cinnamyl acetate (Johnson <i>et al.</i> , 2021)
Polyphenols (Flavonoids, phenolics tannins)	Antifungal, antibacterial, anti-mycobacteria, anticlotting, and anti-inflammatory.	Quercetin, resveratrol, kaempferol and quercitrin, caffeic acid, flavones, rutin, naringin, hesperidin and chlorogenic, tannic acid, gallic acid and ellagic acid.

		(Mushtaq and Wani, 2013 Cory <i>et al.</i> , 2018 Stavrou <i>et al.</i> , 2018 Rana <i>et al.</i> , 2022)
Saponins	Anti-inflammatory, antiviral, antioxidant, cholesterol reducing properties, plant defence, and haemolytic activities	Diosgenin and hecogenin (Nguyen <i>et al.</i> , 2020 Shi <i>et al.</i> , 2019; Kabera <i>et al.</i> , 2014; Zhao <i>et al.</i> , 2015)
Terpenes (carotenoids, steroids)	Antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory.	Artemisinin, α -carotene, β -carotene, lycopene, lutein, and zeaxanthin (Masyita <i>et al.</i> , 2022; Yang <i>et al.</i> , 2022; Tetali, 2019; Jahangeer <i>et al.</i> , 2021)
Anthraquinones	Anti-cancer, antimicrobial, anti-inflammatory	Rhein, salinos poramide and luteolin (Wu <i>et al.</i> , 2006; Choi <i>et al.</i> , 2013)

2.7.1. Alkaloids

Alkaloids are a group of compounds that contain nitrogen derived from amino acids. Alkaloid biosynthesis usually begins with the modification of amino acids through the process of decarboxylation or deamination, followed by further processes such as methylation, hydroxylation, and oxidation, and/or coupled with other compounds (Joosten and Van Veen, 2011; Yang *et al.*, 2016). There is an estimation of about 12 000 identified medicinal plants, and they are mostly found in plant families such as Manispermaceae, Fabaceae, Solanaceae, Papaveraceae and Berberidacea.

Alkaloids are most generally classified into five major classes, based on their biosynthetic amino acid: indolequinoline (tryptophane); tropan, pyrrolidine, and pyrrolizide alkaloids (ornithine); benzylisoquinoline (tyrosine); pyridine (pyridine); and quinolizidine and piperidine alkaloids (lysine) (Gu *et al.*, 2014). It is noteworthy that alkaloids are known to possess essential pharmacological properties and have also contributed to the discovery, isolation and development of drugs currently used to treat various diseases (Table 2.2).

2.7.2. Terpenoids

Terpenoids are one of the largest groups of plant secondary metabolites with most complex structures derived from a sequence of five carbon building blocks (C_5H_8) known as isoprene units. Single or combinations of two, three and four isoprene units are hemiterpenes, monoterpenes, sesquiterpenes and diterpenes, respectively (Gu *et al.*, 2014). Following the formation of basic carbon skeletons, successive modifications such as oxidation, reduction, isomerisation, and conjugation result in an enormous number of structures representing the most abundant class of plant-specific metabolites with more than 36,000 individual compounds (Gershenzon and Dudareva., 2007; Yang *et al.*, 2016). Carotenoids, steroids, and gibberellic acid are just some of the members of terpenoids. The important groups of terpenoids, from a pharmacological perspective, include sterols, essential oils, triterpenes, and saponins from which terpenoids of high medicinal and economic value were isolated, identified and discovered (Table 2.2). Terpenoids are known to possess vital pharmacological properties that are crucial for effectively treating a variety of diseases (Table 2.2). They are also known for inhibition of cholesterol synthesis and possess insecticidal properties; hence, useful for storing agricultural products (Zhao *et al.*, 2015).

2.7.3. Phenylpropanoids

Phenylpropanoids are commonly found in plants and are formulated from six carbon aromatic phenyl groups and three carbon propene tails which form a large group of metabolites that include monolignols, lignans, stilbenes, phenolic acids, and flavonoids (Yang *et al.*, 2016). They function as basic components of a variety of structural polymers, as well as floral pigments, fragrance compounds or signalling molecules for the mediation of bio-interactions, phytoalexins against herbivores and pathogens and defensive components against ultraviolet light radiation and other abiotic stresses (Vogt, 2010). Phenylpropanoids are also part of amongst other bioactive compounds in medicinal plants that belong to plant families such as Lamiaceae, Fabaceae (Leguminosae) and Asteraceae which have shown to possess biological properties such as free-radical scavengers, anti-inflammatory and anti-cancer activities (Korkina, 2007).

2.7.4. Flavonoids

Flavonoids is a low molecular weight, highly complex class of secondary metabolites consisting of over 9,000 structures. They are extremely significant phenolic compound subclasses and are widely distributed in plants such as fruits, herbs, spices, and tea. They are also widespread in terrestrial plants, including all vascular plants and some mosses plants (Tapas *et al.*, 2008; Panche *et al.*, 2016). Flavonoids are grouped based on aglycone core, and the groups include flavanones, flavones, flavonols, isoflavonoids, anthocyanins and proanthocyanidins. Flavonoids are essentially 1,3-diphenylpropan-1-one ($C_6-C_3-C_6$) derivatives, which are derived from condensing three malonylCoA molecules with one p-coumaroyl-CoA to form an intermediate chalcone. Chalcone isomerase converts chalcone into flavanones and transforms flavanones into different flavones, isoflavones, dihydroflavonols, flavonols and

anthoanidins (Ullah *et al.*, 2020). Flavonoids exhibit ample biological effects, *in vivo* as well as *in vitro*. Their antioxidant, antiasthmatic, anticlotting, anti-inflammatory, antimicrobial and anti-cancer activities are thus considered as health-promoting substances in the human diet (Karak, 2019). Many flavonoid compounds have been isolated and characterised from TMPs and have been verified for their exceptional pharmacological properties and multifarious structure. Furanoflavonoids were isolated from *Derris indica* (Lamk.) Bennet - a plant that is used in the treatment of bronchitis, whooping cough, and rheumatic joints (Rao *et al.*, 2009).

2.7.5. Saponins

Saponins are glycosides of both triterpenes and steroids distinguished by their bitter or astringent flavouring properties and haemolytic effects on red blood cells. Saponins are compounds whose active proportions are colloidal in water, produce lather when shaken and precipitate cholesterol (Kregiel *et al.*, 2017). They exist as glycosides, where the aglycone is terpenoid or steroidal. The combination of lipophilic sugars at the end of each saponin give them the ability to produce detergent like characteristics or soap like on membrane or skin (Kabera *et al.*, 2014). Saponins are commonly found in plants, and they possess physicochemical properties such as bitterness, foaming, sweetness, solubilisation, and emulsification. They are also known to hold biological properties that useful in the treatment of various diseases (Table 2.2).

2.7.6. Tannins

Tannins are polymeric phenolic compounds widely distributed in almost all plants and are composed of a very diverse group of oligomers and polymers. Starch, proteins, minerals, and cellulose may form the complex when combined with tannins. They are synthesised through the shikimic acid pathway, also known as the phenylpropanoid

pathway (Pizzi, 2021). Many phenolics such as isoflavones, coumarins, lignins, and aromatic amino acids are produced in the same route (Vuolo *et al.*, 2019). Tannins are water soluble molecules except for certain materials of high molecular weight. Naturally, they are subdivided into two groups: hydrolysable tannins which include gallotannins, ellagitannins, complex tannins and condensed tannins (Bule *et al.*, 2020). Plants that contain tannins are to be used for diarrhoea, diuretic against stomach, duodenal tumours and are also known to have important biological properties such as antifungal, antibacterial, anti-mycobacteria and anti-inflammatory activities (Compean and Ynalvez, 2014).

2.7.7. Glycosides

Glycosides are alcohol, phenol or sulphur compounds characterised by a sugar moiety connected by a bond to one or non-sugar portion. Naturally, most plants generate and store chemicals in the form of inactive glycosides that are activated by the hydrolysis of enzymes (Kytidou *et al.*, 2020). Glycosides are classified based on the nature of aglycone which includes any wide range of molecular types such as terpenes, phenols, steroids, and quinines. According to literature, their therapeutic actions include anti-cancer, antifungal, antibacterial, sedative, expectorant and digestive properties (Awuchi, 2019).

2.8. SAFETY OF MEDICINAL PLANTS

It has long been assumed that if a drug is effective, it will also have side effects. As a result, herbal medicines as drugs either have side effects or are ineffective. People seek therapy for diseases from medicinal plants or herbal medicine because they are generally considered safe, effective, and free of side effects (Nasri *et al.*, 2013). Poisoning from traditional medicine or plant products is not well documented in Africa,

owing to people's reluctance to admit poisoning and the misconception that herbal medicine has negative side effects (Ndhlala *et al.*, 2011). Poisoning usually occurs because of mislabelling of products, or products that are stored in wrong containers, confusing a toxic plant with something that is thought to be edible as well as taking incorrect doses. Nephrotoxicity and hepatotoxicity are the most common toxic effects caused by herbal medicines. Allergic reactions, neurotoxicity, cardiac toxicity, and even death are all possible side effects (Tariq *et al.*, 2018). As a result, it is also critical for manufacturers to clearly state the instructions for use and any potential side effects, as well as to ensure that the product's composition is standardised and monitored (Ndhlala *et al.*, 2011). It is also critical for researchers to investigate the toxicity of plants that are currently used to treat various diseases.

2.9. CONCLUSION

STIs continue to be a major health challenge that requires multidisciplinary and efficacious measures to address them. Even though numerous drugs have been developed over the years to combat STIs, factors such as the emergence of drug-resistant strains, high drug pricing, undesirable side effects, and the difficulty in accessing health facilities continue to make STIs a problematic global factor. Medicinal plants have proven over the years to be a qualified alternative in treating various diseases including STIs. The bioactive compounds found in plants contribute to the pharmacological properties of medicinal plants; hence, they are effective in treating diseases. South Africa is rich with flora that is currently being exploited by traditional healers to treat various diseases. Therefore, it is necessary to investigate efficacy of medicinal plants used by traditional healers to treat STIs.

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CHAPTER 3¹

Phytotherapy for sexually transmitted infections in Thaba 'Nchu, Free State province, South Africa

ABSTRACT

Medicinal plants have been prescribed by traditional healers for the treatment of STIs for years. This study documents therapeutic plants used against STIs in Thaba 'Nchu. Information was gathered through semi-structured interviews with traditional medical practitioners between March and December 2017. Information on medicinal plants, their local names, and their uses against STIs was gathered. The use value (UV) of the plant species, frequency of citation (FC) and the informant consensus factor (ICF) were calculated for the plants and STIs included in the study. Plants were used to treat infections such as Herpes zoster (ICF=0.4) as gonorrhoea (ICF=0.11), vaginosis (ICF=0.2), symptoms of HIV/AIDS (ICF=0.1) and pubic lice (ICF=0.1). A total of 35 plant species were identified for the treatment of STIs. Most medicinal plants in the study area came from the families Asteraceae (21%), Fabaceae (17%), Solanaceae (13%) and Hyacinthaceae (13%). *Bulbine narcissifolia*, *Pentanisia prunelloides*, *Hypoxis hemerocallidea*, *Scabiosa columbaria*, *Xysmalobium undulatum* *Rumex lanceolatus*, *Eucomis autumnalis*, *Dicoma anomala* and *Salvia runcinata* were

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Work from this chapter was also presented at the following national and international conferences:

- XVI Congress of the International Society of Ethnobiology and First World Socio Biodiversity Fair Conference held in Brazil from 4-11 August 2018.
- International conference on traditional knowledge and traditional medicine. Held at Vaal University of Technology from 3-5 April 2019.

frequently used. The preferred method for preparation of remedies were decoctions made from the roots (72%), whole plant (17%), leaves (7%), fruits (2%) and bark (2%) which were consumed orally. This study contributes to the documentation of plants used for STIs to conserve the information for future generations. A selection from the most frequently mentioned plants for treatment of STIs may be used for further phytopharmacological investigations and subsequently be used for the development of new antimicrobial agents for global use and for the socio-economic development of local communities.

3.1. INTRODUCTION

The World Health Organization (WHO) reported an estimated 357.9 million cases of STIs that occur annually worldwide (WHO, 2018). South Africa has high rates (7.9 million in 2017) of HIV infections and millions of residents are co-infected with other STIs (National Institute of Communicable Diseases, 2022). The burden of these STIs is a global cause for concern because of the negative health, economic and social implications (Chesson *et al.*, 2017). Addressing the burden of STIs requires concerted efforts from multiple angles including traditional knowledge and medicinal plants.

STIs are usually transferred from one person to the other through sexual contact. The most common pathogens responsible for STIs are *Treponema pallidum*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis* (WHO, 2007; Van Vurren *et al.*, 2010), which are treated with antimicrobial agents such as penicillin, azithromycin, amoxycillin, quinolone ciprofloxacin, metronidazole, famciclovir and many other drugs (Bowden *et al.*, 2002). However, most patients in developing countries - especially from rural areas - fail to seek therapy from official health care centres; therefore, they turn to traditional healers for help (Haque *et al.*, 2018).

Traditional healers are usually the first caregivers in rural areas because of traditional beliefs, poverty, and patient stigmatisation (Haque *et al.*, 2018; Leclerc-Madlala *et al.*, 2016). Additionally, limited accessibility to health care facilities, long waiting queues, lack of laboratories, shortages of drugs and poor attitudes of health workers make traditional medicine more appealing (Moteetee and Van Wyk, 2011; McGaw *et al.*, 2005; Cremers *et al.*, 2019).

Traditional medicine, especially medicinal plants, play an important role in the treatment and management of human diseases and ailments in South Africa (Thirumalai *et al.*, 2009). Plants are the greatest source of remedies for many health problems, as approximately 71 % of new drugs that have been approved since 1981 have directly or indirectly been derived from natural products (Anand *et al.*, 2019). The need for basic scientific investigations on medicinal plants using indigenous knowledge has become more relevant because they can lead to discovery of novel compounds that will contribute to the development of new drugs (Jamshidi-Kia *et al.*, 2017). However, some traditional communities have become less concerned about local resources and have adopted modern lifestyles, resulting in the loss of indigenous plant knowledge (Mathibela *et al.*, 2015). Urbanization, modernization of public services including education systems, and globalization of trade and belief systems, and the rapid loss of natural habitats also contribute to the loss of knowledge (Pilgrim *et al.*, 2007). Furthermore, ethnobotanical knowledge of plant uses is typically passed down across generations through oral tradition which can lead to distortion (Kose *et al.*, 2015; Hong *et al.*, 2015; Pizon *et al.*, 2016). As a result, documenting traditional medicinal plant applications has become essential and critical.

Information on medicinal plant use in the Free State Province of South Africa is readily available in literature (Meyer and Afolayan, 1995; Guillarnod, 1971; Watt and Breyer-Brandwijk, 1962; Shale *et al.*, 1999; Moteetee *et al.*, 2019, Moteetee and Kose, 2016; Mugomeri *et al.*, 2016; Kose, 2017; Moteetee and van Wyk, 2011); however, none is focused on the treatment of STIs in this region. Similar studies in South Africa have been carried out in other provinces. For example, ethnic groups such as Bapedi and Vhavenda in Limpopo (Sebua *et al.*, 2013; , Semenya *et al.*, 2013; Mulaudzi *et al.*, 2015; Mongalo *et al.*, 2017; Maema *et al.*, 2019), Zulu people in Kwa-Zulu Natal province (De Wet *et al.*, 2012; Naidoo *et al.*, 2013), Tsonga people in Mpumalanga province (Tshikalange *et al.*, 2016), Xhosa people in the Eastern and Western Cape province (Mulaudzi and Makhubela-Nkondo, 2006). Similarly, other studies have documented plants used by Basotho for treatment of various diseases in the Free State and Lesotho (Moteetee *et al.*, 2017; Kose, 2017; Semenya *et al.*, 2018; Kose *et al.*, 2021; Mokhesi and Modjadji, 2022). Therefore, the aim of this study is to document the medicinal plants used for treatment of STIs in Thaba 'Nchu located in the Free State province of South Africa. STIs were the most treated by traditional healers in this area.

3.2. Study area

Thaba 'Nchu is dominated by BaSotho and BaTswana residents who represent 80% of the overall population of the area. People in the area make a living through informal trade, agriculture, and some find employment in the nearest city, which is Bloemfontein (Moorosi, 1999). Thaba 'Nchu is one of the emerging towns of the Free State that has yet to be studied in terms of ethnobotany. Thaba 'Nchu is in the central part of the Free State province, covering an area of roughly 704 km², and is about 67 km east of Bloemfontein (29.2040° S and 26.8204° E) (Figure 3.1). The study area is in the

grassland biome, where the dominant plants are grasses, geophytes, and other small flowering plants. Thaba 'Nchu receives approximately 435 mm of rain annually, with most rainfall occurring during the summer. It receives the lowest rainfall (3mm) in June and the highest (73mm) in March. The average midday temperatures range from 15.4°C in June to 28.5°C in January. The region is the coldest during July when temperatures drop to 0.1°C on average during the night (Fourie *et al.*, 2019).

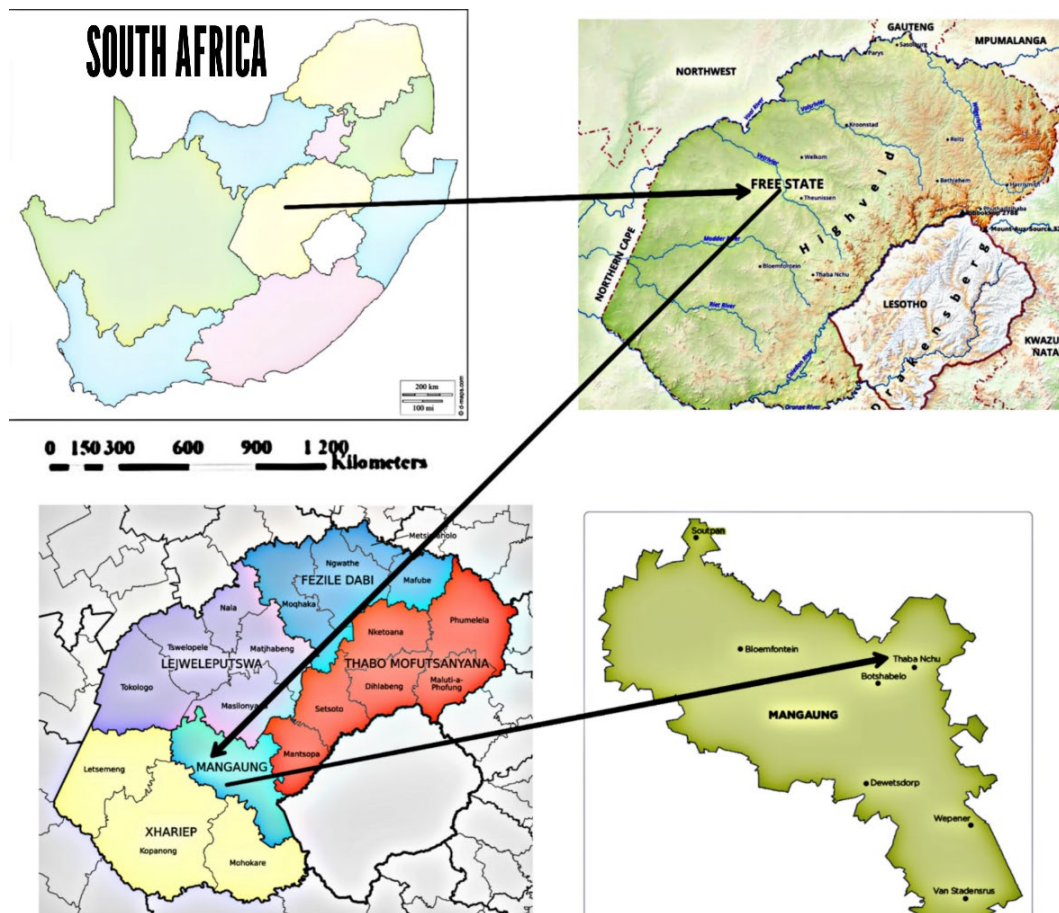


Figure 3.1: Geographical location of the study area

3.3. METHODS

3.3.1. Data collection

The protocol for this study was approved by the University Research and Innovation Committee (URIC) of the Central University of Technology, Free State, and all ethical

considerations associated with research in traditional/indigenous communities were observed. The traditional practitioners in the survey were identified and selected based on their willingness to participate using purposive sampling through a traditional healer's association found in the area. Before commencement, the study was explained by the researcher to the members of the association in the local Sesotho language. Thereafter, semi-structured interviews were conducted after obtaining informed consent from willing participants between March and December 2017. Prior to the main individual interviews, a focus group with traditional healers was held to test the reliability and validity of the questions. Twenty-four traditional healers were individually interviewed for information about medicinal plants used to treat STIs in the Thaba 'Nchu area. The data included the local names of the plants, the parts used, the mode of preparation, route of administration and the diseases treated. Plant specimens were collected with guidance from the traditional healers from the surrounding grasslands and from some home gardens. Plants were identified first by their vernacular names and later taken to SANBI (South African National Biodiversity Institute) in Bloemfontein for validation. Voucher specimens were deposited in the Centre of Applied and Food Sustainability and Biotechnology (CAFSaB) at the Central University of Technology, Free State.



Figure 3.2: Semi-structured interviews conducted with the traditional healers of Thaba 'Nchu

3.3.2. Data analysis

Following the interviews, the local importance of each plant species reported by traditional healers in the study area for treating STIs was calculated using the Informant Consensus Factor (ICF) and the use value (UV).

3.3.2.1. Informant consensus factor

The consensus of informants within a community and between cultural groups discloses which plants are important and regularly used. This is relevant for conservation purposes and aids in plant selection for pharmacological and

phytochemical research (Khan *et al.*, 2014). ICF was used to identify plants with cultural significance as well as consensus on how to use plants. The informant consensus factor was calculated using the formula:

$$ICF = \frac{Nur - Nt}{Nur - 1}$$

Where:

Nur = the number of used citations in each disease category.

Nt = the number of species used in each disease category.

The ICF is used to ensure that the information obtained is homogeneous. A high value (near #1) indicates that a specific plant species is used by the majority of informants interviewed for treating a disease category, whereas a low value (0 or close to 0) indicates that each informant used a different plant species, and that disagreement was expressed among informants regarding the treatment of that disease category by a specific plant species (Zaid *et al.*, 2017).

3.3.2.2. Frequency of citation (FC)

FC was used to evaluate the most preferred plants or more used plant species for treatment of STIs. The FC of the species of plants being utilised was evaluated using the following formula [37]:

For diseases treated:

$$FC = \frac{\text{Number of times a particular disease was mentioned}}{\text{Total number of times that all the diseases were mentioned}} \times 100$$

For plant species used:

$$FC = \frac{\text{Number of times a particular species was mentioned}}{\text{Total number of times that all species were mentioned}} \times 100$$

3.3.2.3. Use value (UV)

The use value is a quantitative method that describes the importance of a plant based on its frequency of mention by respondents, and it was calculated according to the following formula (Polat and Satil, 2012):

$$UV = U/N$$

Where:

UV = is the use value of a species.

U = the number of citations per species.

N = the number of informants.

A high UV score for a plant indicates that there are many usage reports for that plant, which may also be considered an important plant in the community, whereas a low score indicates that informants reported less usage reports for that plant.

3.4. RESULTS AND DISCUSSION

3.4.1. The participants

An ethnobotanical survey was conducted to document plants used for STIs by traditional healers in Thaba 'Nchu. The results of the study showed that the age of the participants ranged between 20 and 80 years. More females (79.2%) than males (20.8%) participated in the study (Table 3.1). This kind of female dominance is contrary to findings in Lesotho (Moteetee and van Wyk, 2011) where most of the traditional healers were men because of the belief that women should manage home duties such as taking care of the families, including children and the elderly. In this study, there were more women, which conforms to the general setup of a community dominated by female-headed households in the Free State province (Marais, 2002). They practice traditional healing to earn an income and provide for their families. Additionally, men from the community usually find work in places that are far away

from Thaba 'Nchu, therefore they no longer participate in traditional healing in the area. The trends that were observed in this study also adhere to the Age, Gender, and Dynamics of Knowledge Hypotheses (Gaoue *et al.*, 2017; Torres-Avilez *et al.*, 2016) which states that women and older people tend to have more knowledge of local medicinal plants. Most traditional healers who participated in the study were between the ages of 40 to 59, accounting for 45% of all participants, with the remainder being either young or old (Table 3.1). Most traditional healers had a traditional calling to practice traditional healing, while some used it as their primary source of income to support their families due to unemployment.

Table 3.1: Demographic characteristics of individual participants (n=24).

Age (years)	Female	Male	Number	Percentage (%)
20-29	2	1	3	12.5
30-39	3	1	4	16.7
40-49	4	1	5	20.8
50-59	6	0	6	25.0
60-69	1	1	2	8.3
70+	3	1	4	16.7
Total	19	5	24	100

3.4.2. STIs commonly treated using medicinal plants in the study area

The participants in Thaba 'Nchu commonly treated STIs such as gonorrhoea, herpes zoster, HIV, pubic lice, and vaginosis. However, some traditional healers did not differentiate STIs and generally used the term "*Mafu a thobalano*" to refer to any diseases that affect the reproductive organs obtained from having sex. According to the frequency of mention (Figure 3.2) and frequency of citation (Table 3.2), herpes

zoster, followed by gonorrhoea and vaginosis were the most treated STIs in the study area. The ICF ranged from 0.4 to 0 for all the disease categories treated in the community. This showed weak consensus (heterogeneity) across all the reported diseases and indicates that multiple plant resources are used to treat the same STI.

Table 3.2: FC and ICF values of the STIs treated using medicinal plants in Thaba 'Nchu

STIs	Local name	FC (%)	ICF
Vaginosis	'Seso'	19.23	0.2
HIV/AIDS	'Phamokate'	7.7	0.1
Herpes zoster	'Lebanta'	30.8	0.4
Pubic lice	'Dinta tsa senana'	7.7	0.1
Gonorrhoea	'Lerothodi'	22.1	0.11
Unspecified STIs	'Mafu a thobalano'	11.5	0

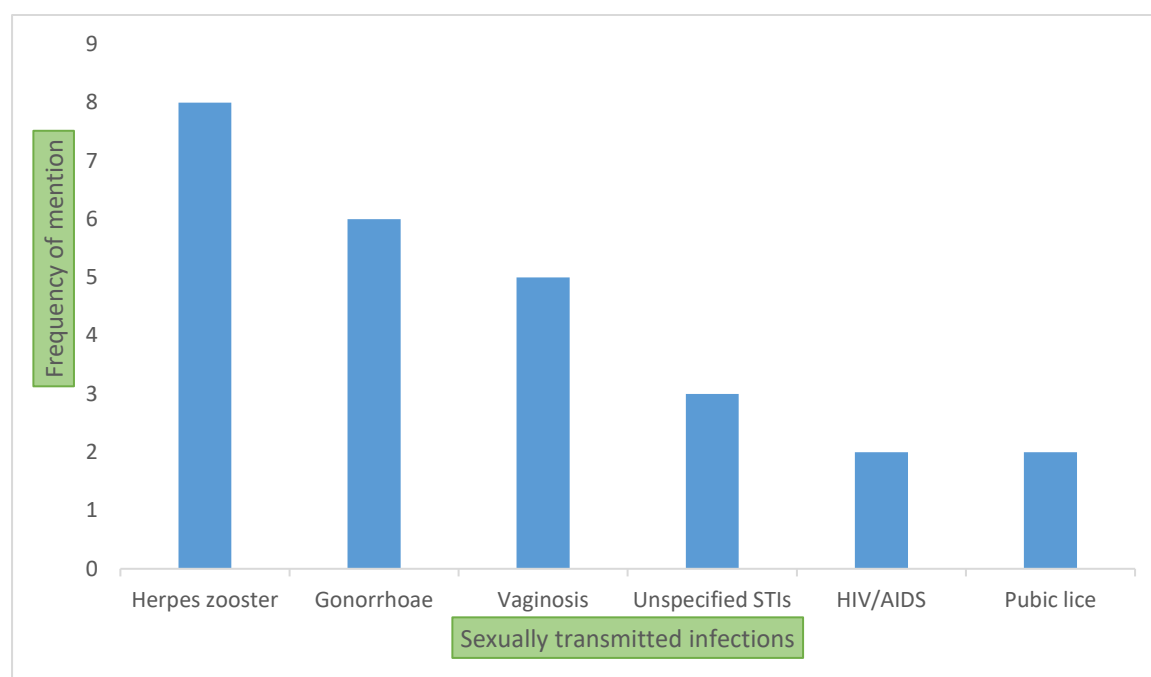


Figure 3.3: Frequency of mention of STIs treated with medicinal plants

3.4.3. Plants used to treat sexually transmitted infections

Information on plants species used to treat STIs, local names (in Sesotho), families, scientific names, use value as well as frequency of citation were reported by traditional healers during ethnobotanical interviews, as presented in Table 3.3. The ethnobotanical survey revealed that 35 plant species that belong to 24 families are used for various STIs in the study area. The family with the largest number of useful species was from Asteraceae, which amounted to 21% of all the mentioned species, followed by Fabaceae at 17% (Fig 3.3). Asteraceae was also found to be one of the dominant families in an ethnobotanical study conducted in the Limpopo province on plants used for STIs by Bapedi people (Semenya *et al.*, 2013). In contrast to the findings of this study, Fabaceae was found to have the greatest number of plant species utilised to treat various diseases in earlier ethnobotanical studies (Kose *et al.*, 2015, Moteetee and Van Wyk, 2011; Afolayan *et al.*, 2014; Steenkamp, 2003).

Plants of the Asteraceae family have been used as astringents, antipyretics, anti-inflammatories, hepatoprotective, diaphoretics in fevers, smooth muscle relaxants, nerve tonics, laxatives, and for the treatment of wounds, bleedings, headaches, pains, and spasmodic illnesses, flatulence, dyspepsia dysentery, lumbago, leucorrhoea, haemorrhoids, ulcers, and disorders causing cachexia (Achika *et al.*, 2014). Additionally, other species such as *Achillea millefolium*, *Artemisia absinthium*, *Matricaria recutita*, *Parthenium hysterophorus*, and *Inula crithmoides* from the family were found to contain bioactive compounds such as flavonoids (apigenin), phenolics (myricetin, ferulic acid, quercetin), sesquiterpenic compounds, terpenoids, alkaloids and cardiac glycosides known to have various biological activities such as antioxidant, antimicrobial, anti-inflammatory and many other properties (Bessada *et al.*, 2015),

which explains the dominance of plants from the Asteraceae family in the current study.



Figure 3.4: The most cited plant families in the area of study in percentages

The 10 most mentioned plants were *Bulbine narcissifolia*, *Pentanisia prunelloides*, *Dicoma anomala*, *Scambiosa columbaria*, *Xysmalobium undulatum*, *Rumex lanceolatus*, *Eucomis autumnalis*, *Salvia runcinata* and *Hypoxis hemerocallidea* (Figure 3.4). Most of the species mentioned by the traditional healers in Thaba 'Nchu were also mentioned in previous studies for the treatment of STIs (Kose *et al.*, 2015; Shale *et al.*, 1999; Moteetee and Van Wyk, 2011; Jäger *et al.*, 1996; Moffett, 2010; Moteetee, 2017). Plants such as *E. autumnalis*, *B. onopordifolia*, *P. prunelloides* and *S. runcinata* have antimicrobial, anti-inflammatory and antioxidant properties (Kamatou *et al.*, 2005; Madikizela *et al.*, 2013; Muleya *et al.*, 2015; Bisi-Johnson *et al.*, 2017; Bodede and Prinsloo, 2020). Therefore, the selection of these plants for preparation of remedies used to treat STIs by the informants may be attributed to the above-mentioned biological activities and indicates that the therapeutic properties of

the plants are reliable and could thus possibly lead to the discovery of new bioactive compounds and products (Sop *et al.*, 2012).

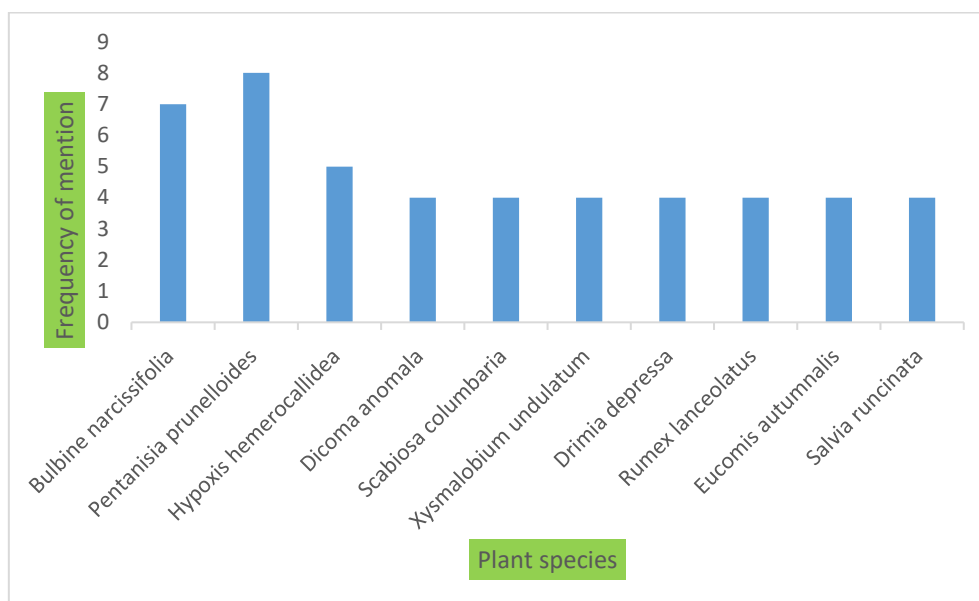


Figure 3.5: The most cited plants by informants in the study area

3.4.4. Use value

The use value (UV) across all the mentioned plants ranged between 0.33 and 0.04. The UV for *P. prunelloides* (0.33), *B. narcissifolia* (0.29), *H. hemerocallidea* (0.25), *S. runcinata* (0.17), *E. autumnalis* (0.17), *D. anomala* (0.17), *R. lanceolatus* (0.17), *S. Columbaria* (0.17), *X. undulatum* (0.17), *D. depressa* (0.17) were the highest values (Table 3.3). Plants that were mentioned in most disease categories by many informants often have a high use value, whereas species that are only used by a few informants usually have a low use value (Sop *et al.*, 2012). This implies that the above-mentioned plant species are frequently used to treat a variety of STIs in the study area. The high use value of these plants indicates their extensive usage in the study area by traditional healers. These may also be due to their wide distribution in the area. High use values are also useful in indicating the importance of plants in treating STIs in the study area and can be selected in determining their safety and pharmacological

effects. This may also influence the development of plant-derived products that can be used to treat STIs in the study area.

Table 3.3: Medicinal plants used to treat STIs in Thaba 'Ncnu

Scientific name	Local name	Family	Voucher specimen number	Plant parts	STIs treated	Companion plants	Method of preparation and route of administration	UV	FC (%)
<i>Bulbine narcissifolia</i> Salm-Dyck	Khom-ea-balisa	Asphodelaceae	B001	Leaves	Gonorrhoea		Decoction (Orally)	0.29	8.3
				Flowers	Vaginosis	<i>Rumex lanceolatus</i> , <i>Leucosidea sericea</i> , <i>Metalsia muricata</i> , <i>Gunnera perpensa</i> , <i>Eucomis autumnalis</i>			
<i>Euclea coriacea</i> A.DC	Monna-mots'o	Ebenaceae	B002	Roots	Gonorrhoea	<i>Hypoxis hemerocallidea</i>	Decoction (Orally)	0.04	1.19
<i>Pentanisia prunelloides</i> (Klotzsch ex	Setima-mollo	Rubiaceae	B003	Whole plant	Herpes zoster	<i>Pentanisia prunelloides</i> <i>Xysmalobium</i>	Decoction (Orally)	0.33	9.52



Eckl. & Zeyh.) Walp.						<i>undulatum, Dicoma anomala, Turbina oblongata, Metalasia muricata, Salvia runcinata and Nasturtium officinale</i>			
					Gonorrhoea	<i>Bulbine narcissifolia</i>	Plant powder (Topically)		
						<i>Bulbine narcissifolia, Solanum aculeatissimum, Berkheya onopordifolia and Hypoxis hemerocallidea</i>	Decoction		



						<i>Bulbine narcissifolia, Hypoxis hemerocallidea</i>	Bath		
<i>Dicoma anomala Sond. subsp. cirsioides (Harv.)</i>	Hloenya	Asteraceae	B004	Roots	HIV/AIDS	<i>Xysmalobium undulatum, Nasturtium officinale, Cussonia paniculata</i>	Decoction (Orally)	0.17	4.76
<i>Scabiosa columbaria L.</i>	Selomi	Dipsacaceae	B005	Whole plant	Gonorrhoea	<i>Xysmalobium undulatum and Hermannia depressa</i>	Decoction (Orally)	0.17	4.76
				Roots	Unspecified STIs	<i>Scabiosa columbaria, Hermannia depressa,</i>	Decoction (Orally)		



						<i>Helichrysum caespititium</i>			
<i>Asparagus microraphis</i> (Kunth) Baker	Lereratau	Asparagaceae	B006	Roots	HIV	<i>Scabiosa columbaria</i> and <i>Agave Americana</i>	Decoction (Orally)	0.04	1.19
<i>Hermannia depressa</i> N.E.Br.	Seletjane	Malvaceae	B007	Roots	Gonorrhoea		Decoction (Orally)	0.08	2.4
					Unspecified STIs	<i>Scabiosa columbaria</i> and <i>Salvia runcinata</i>	Decoction (Orally)		
<i>Xysmalobium undulatum</i> (L)	Poho-Ts'ehla	Apocynaceae	B008	Roots	HIV	<i>Dicoma anomala</i> and <i>Nasturtium officinale</i>	Decoction (Orally)	0.17	4.76
<i>Helichrysum caespititium</i> (DC)	Phate-ea-ngaka	Asteraceae	B009	Whole plant	Unspecified STIs	<i>Scabiosa columbaria</i> and <i>Hermania depressa</i>	Decoction	0.04	1.19
<i>Drimia depressa</i> (Baker) Jessop	Moretele	Hyacinthaceae	B0010	Roots	Herpes zoster	Vimbela and coarse salt	Ashes (Topically)	0.17	4.76



<i>Withania somnifera</i> (L) Dunal	Mofera-ngope	Solanaceae	B0011	Roots	Gonorrhoea	<i>Xysmalobium undulatum</i> f	Decoction (Orally)	0.08	1.19
<i>Rumex lanceolatus</i> Thunb	Khamane	Polygonaceae	B0012	Roots	Gonorrhoea	<i>Eucalyptus spp</i>	Decoction (Orally)	0.17	4.76
					Vaginosis	<i>Lessertia depressa, Dicoma anomala, Eucomis autumnalis and Euphorbia clavarioides</i>			
<i>Leucosidea sericea</i> Eckl. & Zeyh.	Cheche	Rosaceae	B0013	Leaves	Vaginosis	<i>Bulbine narcissifolia, Rumex lanceolatus, Metalasia muricata, Gunnera perpensa, Eucomis autumnalis</i>	Decoction (Orally)	0.04	1.19



<i>Euphorbia clavarioides</i> Boiss	Sehlooko	Euphorbiaceae	B0014	Roots	vaginosi	<i>Lessertia depressa, Dicoma anomala, Eucomis autumnalis</i>	Decoction (Orally)	0.04	1.19
<i>Eucomis autumnalis</i> (Mill.)	Khapumpu/Mathethebale	Hyacinthaceae	B0015	Roots	Vaginosi	<i>Lessertia depressa, Dicoma anomala, Euphorbia clavarioides</i>	Decoction (Orally)	0.17	4.76
<i>Metalasia muricata</i> R.Br.	Tee-ae-baroa	Asteraceae	B0016	Whole plant	Vaginosi	<i>Bulbine narcissifolia, Rumex lanceolatus, Leucosidea sericea, Gunnera perpensa, Eucomis autumnalis</i>	Decoction (Orally)	0.04	1.19



Salvia runcinata L.f	Mosisili	Lamiaceae	B0017	Roots	Pubic lice	<i>Rumex lanceolatus,</i> <i>Hypoxis hemerocallidea,</i> <i>Eucomis autumnalis,</i> <i>Selaginella caffrorum, Bulbine narcissifolia,</i> <i>Drimia depressa</i>	Decoction (Orally)	0.17	4.76
						<i>Drimia depressa</i> and <i>Aloe ferox</i>	Bath (Topically)		
Gunnera perpensa L.	Qobo	Gunneraceae	B0018	Whole plant	Vaginitis	<i>Bulbine narcissifolia,</i> <i>Rumex lanceolatus,</i> <i>Leucosidea sericea, Eucomis autumnalis</i> f	Decoction (Orally)	0.04	1.19



<i>Malva parviflora</i> L.var	Tikamotse	Solanaceae	B0019	Leaves	Herpes zoster		Decoction (Orally)	0.08	2.4
<i>Berkheya</i> <i>onopordifolia</i> (dc.) o.hoffm. ex burt davy	Mohatollo	Asteraceae	B0020	Roots	Gonorrhoea	<i>Solanum</i> <i>aculeatissimum</i> , <i>Berkheya</i> <i>onopordifolia</i> , <i>Hypoxis</i> <i>hemerocallidea</i> , <i>Bulbine</i> <i>narcissifolia</i>	Decoction (Orally)	0.04	1.19
<i>Achyranthes</i> <i>aspera</i> Linn.	Lemanamana	Fabaceae	B0021	Roots	Pubic lice	<i>Achyranthes</i> <i>aspera</i> , 'Madubula' antiseptic	Bath (Topically)	0.04	1.19
<i>Solanum</i> <i>aculeatissimum</i> Jacq	Thola	Solanaceae	B0022	Roots	Gonorrhoea	<i>Solanum</i> <i>aculeatissimum</i> , <i>Berkheya</i> <i>onopordifolia</i> , <i>Hypoxis</i> <i>hemerocallidea</i> ,	Decoction (Orally)	0.08	2.4



						<i>Bulbine narcissifolia</i>			
<i>Agave americana</i> L. subsp. americana	Lekhala	Agavaceae	B0023	Whole plant	Vaginitis	<i>Drimys depressa</i> , <i>Eriosephalus punctatus</i> , <i>Salvia runcinata</i> , <i>Hypoxis hemerocallidea</i> , <i>Eucomis autumnalis</i>	Decoction (Orally)	0.04	2.4
<i>Eucalyptus</i> spp	Boloukomo	Myrtaceae	B0024	Bark	Gonorrhoea	<i>Rumex lanceolatus</i>	Decoction (Orally)	0.04	1.19
<i>Lessertia depressa</i> Harv.	Mmusapelo	Fabaceae	B0025	Roots	Vaginitis	<i>Dicoma anomala</i> and <i>Eucomis autumnalis</i> , <i>Euphorbia clavarioides</i>	Decoction (Orally)	0.04	1.19
<i>Schizocarpus gerrardii</i> (Baker) Van der Merwe	Letjoetlane	Hyacinthaceae	B0026	Roots	Vaginitis	<i>Bulbine narcissifolia</i>	Decoction (Orally)	0.04	1.19



<i>Turbina oblongata</i> A. Meeuse	Mothukho	Convolvulaceae	B0027	vine plant	Herpes zoster	<i>Xysmalobium undulatum</i> and <i>Dicoma anomala</i> , <i>Pentanisia prunelloides</i> , <i>Metalasia muricata</i> , <i>Salvia runcinata</i> and <i>Nasturtium officinale</i>	Decoction (Orally)	0.04	1.19
<i>Selaginella cafferorum</i> (Milde)	Moriri-oa-matlapa	Selaginellaceae	B0028	Roots	Pubic lice	<i>Rumex lanceolatus</i> , <i>Hypoxis Hemerocallidea</i> , <i>Eucomis autumnalis</i> , <i>Salvia runcinata</i> , <i>Bulbine narcissifolia</i> , <i>Drimia depressa</i>	Bath (topically)	0.04	1.19



<i>Elephantorrhiza elephantine</i> (Burch) Skeels	Mositsane	Fabaceae	B0029	Roots	vaginitis	<i>Agave americana</i> , <i>Drimys depressa</i> , <i>Eriosephalus punctatus</i> , <i>Salvia runcinata</i> , <i>Hypoxis hemerocallidea</i> , <i>Eucomis autumnalis</i>	Decoction (Orally)	0.08	2.4
<i>Eriosephalus punctatus</i> DC	Sehalahala-sa-matlaka	Asteraceae	B0030	Roots	Vaginitis	<i>Agave americana</i> , <i>Drimys depressa</i> , <i>Salvia runcinata</i> , <i>Hypoxis hemerocallidea</i> , <i>Eucomis autumnalis</i> , <i>Elephantorrhiza elephantine</i>	Decoction (Orally)	0.04	1.19



<i>Melolobium microphyllum</i> (L.F.)	Mofahlatoeba	Fabaceae	B0031	Roots	Unspecified STIs		Decoction (Orally)	0.04	1.19
<i>Cussonia paniculata</i> Eckl. & Zeyh. Subsp. Sinuate	Mots'ets'e	Araliaceae	B0032	Roots	HIV	<i>Heteromorpha trifoliata</i>	Decoction (Orally)	0.08	2.4
<i>Heteromorpha trifoliata</i> (H. L. Wendl.) Eckl. & Zeyh.	Monkhoane	Apiaceae	B0033	Roots	HIV	<i>Cussonia paniculate</i>	Decoction (Orally)	0.04	1.19
<i>Hypoxis hemerocallidea</i> syn.	Moli	Hypoxidaceae	B0034	Roots	Gonorrhoea	<i>Solanum aculeatissimum</i> , <i>Berkheya onopordifolia</i> , <i>Bulbine narcissifolia</i>	Decoction (Orally)	0.25	7.14



					vaginitis	<i>Agave americana</i> , <i>Drimys depressa</i> , <i>Eriosephalus</i> <i>punctatus</i> , <i>Salvia</i> <i>runcinata</i> , <i>Eucomis</i> <i>autumnalis</i> and <i>Elephantorrhiza</i> <i>elephantina</i>			
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3.4.5. The plant parts used for remedy preparation

The plant parts that are usually used for the preparation of remedies used to treat STIs are shown in Figure 3.5. Roots were reported to be the most used plant part, followed by whole plant and leaves. This corroborates the Optimal Defence Theory (Gaoue *et al.*, 2017; Coley *et al.*, 2003), which states that humans select medicinal plants to optimise secondary chemistry and are likely to select plant organs that are less likely to be attacked by herbivores. However, the excessive use of roots and whole plants has a negative impact on the growth of plant populations, whereas the use of aerial plant parts would be more sustainable (Giday *et al.*, 2003; Ghimire *et al.*, 2008). For example, El Houssine Bouiamrine *et al.* (2017), Swemmer *et al.* (2019) and many other studies showed the importance of harvesting plants sustainably and further recommended the establishment programmes of conservation and sensitisation of rural populations on the protection of biodiversity. In addition, future research and interventions may also advocate for sustainable use of medicinal plants in the Thaba 'Nchu area through the development of medicinal plant gardens and applying the measures recommended in the above-mentioned studies to reduce misuse and avert extinction.

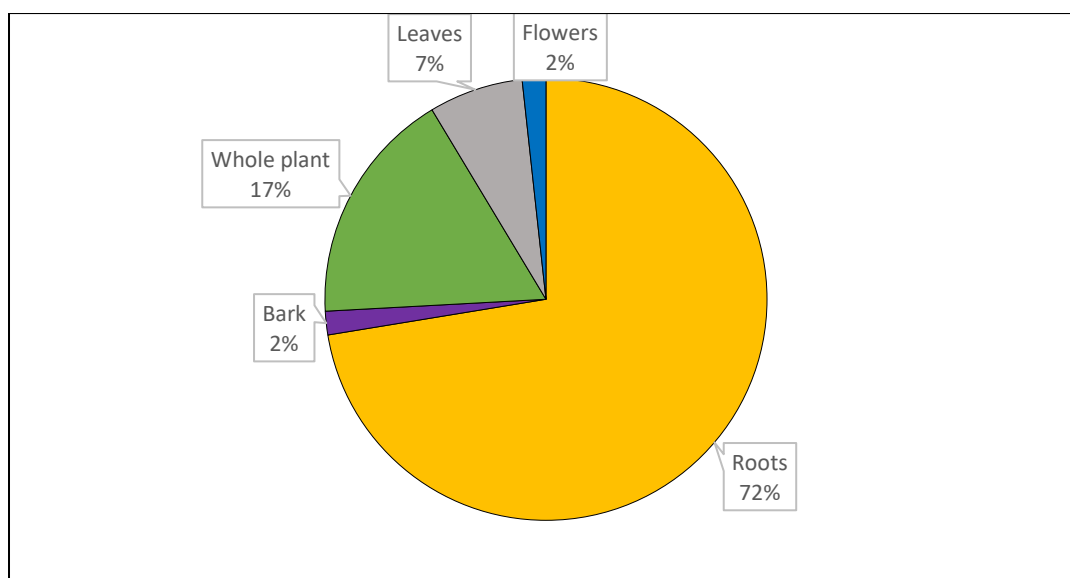


Figure 3.6: Plant parts used for preparation of remedies

3.4.6. Plant collection and method of preparation

The informants mentioned that they personally collect most of the plants they use to treat their patients and only buy and cultivate those that are difficult to access. For storage purposes, the plants are usually dried out and ground into fine powder. The informants mentioned that plants can be stored up to five years when ground into fine powder. Grinding the plants allows the solvent to have better contact with the plant material, thus allowing better compound extraction and therefore increase the efficacy in treatment of diseases (Azwanida, 2015). The informants utilised most of the plants used for STI treatment in the research region individually or in combination. The use of a combination of plants species against a particular type of ailment may imply that the ailment is prevalent in that area of study (Hossan *et al.*, 2010). Decoctions are the most often utilised remedy preparation method in the research area, possibly due to their simplicity of preparation and administration (Table 3.3). Herbal administration was mostly oral, and some plants were topically applied especially when treating skin infections (Table 3.3). This is consistent with other studies that previously documented

that most remedies used for treatment of STIs were prescribed orally (Semenya *et al.*, 2013; Chigora *et al.*, 2007; Kambiz and Afolayan, 2001).

3.5. CONCLUSION

The study documented the indigenous knowledge of medicinal plants used to treat symptoms of STIs in Thaba 'Nchu, Free State province, South Africa. The results showed that an overall of 35 medicinal plants from 24 families are used for treatment of various STIs such as herpes zoster, vaginosis, pubic lice, HIV/AIDS, and gonorrhoea. The majority of plants used by traditional healers for treatment of STIs are from Asteraceae, Fabaceae, Solanaceae and Hyacinthaceae families. *Bulbine narcissifolia*, *Pentanisia prunelloides*, *Hypoxis hemerocallidea*, *Scabiosa columbaria*, *Xysmalobium undulatum* *Rumex lanceolatus*, *Eucomis autumnalis*, *Dicoma anomala* and *Salvia runcinata* were frequently used for treatment of STIs in the study area. Roots were the most used plant parts for preparation of different remedies and decoction is the common method of remedy preparation. Medicinal plants in the area might be endangered or go extinct in a long run due to the collection method and the use of roots for remedy preparation. Sustainable harvesting and use of medicinal plants to avoid further loss of plants needs to be encouraged. Many of these plants have previously been reported for antimicrobial activities which may justify their roles as natural remedies for sexually transmitted infections in this study. Further studies are needed to determine the dosages, minimum inhibitory concentrations, biological activities, and toxicities, and characterise the active phytochemicals. The plants recorded in this study can serve as a basis for further research of novel drugs that can be used to treat STIs and other microbial infections.

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CHAPTER 4

A literature review of a selection of plants used for STIs in Thaba 'Nchu

ABSTRACT

Ethnobotany has facilitated the development of many modern medicines from medicinal plants, which are regarded as rich sources of traditional medicines. After conducting the ethnobotanical survey, four plants utilized for the treatment of STIs were selected for further investigation. Traditional uses, therapeutic benefits, and phytochemical composition of *Berkheya onopordifolia*, *Hermannia depressa*, *Scambiosa columbaria*, and *Salvia runcinata* are all covered in this chapter. These plants have a track record of being used traditionally to treat a wide range of diseases in many different communities throughout Southern Africa and many other nations. Literature on *Salvia runcinata* and *Scambiosa columbaria* biological activities, safety, active compounds, and isolated compounds is available. The plants have been reported to exhibit biological activities such as antioxidant, antimicrobial, anti-inflammatory, cytotoxic, antituberculosis and many other activities. The biological activities, active ingredients, and isolated compounds information on *Hermannia depressa* and *Berkheya onopordifolia* is extremely limited. Even though there are reports on the traditional use of *Hermannia depressa* and *Berkheya onopordifolia* and *Scambiosa columbaria* for the treatment of various diseases, extensive studies based on investigation of their biological activities, active compounds and isolated compounds and safety is required. It is evident that all the plants mentioned have the potential to be developed into novel herbal drugs and other health-related products.

4.1. INTRODUCTION

STIs continue to be a significant public health issue around the world. They affect millions of people every year and can cause severe health complications if left untreated (Van Gerwen *et al.*, 2022). STIs are often accompanied by stigma and discrimination, which can hinder individuals from seeking testing and treatment (Unemo *et al.*, 2019). The challenges associated with STIs include limited access to healthcare, inadequate education on prevention methods, and the emergence of antibiotic-resistant strains (Grad *et al.*, 2016). To address the challenges posed by STIs, it is important to focus on prevention, diagnosis, and explore alternative treatment options (Unemo *et al.*, 2017). One potential alternative could be the use of medicinal plants. Prioritising research in this area may lead to the identification of new and effective treatments for STIs that could alleviate the burden on healthcare systems and reduce the spread of these infections.

To date, a significant amount of people in developing countries still use plants for their primary health care needs. Many countries around the world, including those in the African continent have traditional medicine systems in place and continue to use them (Nyamukuru *et al.*, 2017). Between 50 000 and 70 000 plant species are used worldwide in conventional medicine (Kilic *et al.*, 2020). South Africa has over 30 000 species of higher plants because of its rich floral biodiversity, and 3 000 of these plants have been found to be used in traditional medicine (Thring and Weitz, 2006). Traditional medicine is important in the management of diseases, either for preventative, curative, or palliative purposes (Mothibe and Sibanda, 2019). Hence, there is a greater interest and need to search for plants with medicinal benefits.

Eighty per cent of people in the world use traditional medicine to cater for their primary health care needs (Ahn, 2017). Due to its natural makeup and less side effects, traditional medicine has been used for thousands of years in both developed and developing countries (Jamshidi-Kia *et al.*, 2018). Traditional medicine has also continued to be the most accessible and reasonably priced treatment option in the primary healthcare system of resource-poor nations (Maroyi, 2013). Traditional knowledge on the use of medicinal plants to treat diseases has been passed down from generation to generation through trial and error. As a result, documentation of indigenous ethnobotanical knowledge is critical for the preservation and utilisation of biological resources, especially as the use of medicinal plants declines due to modernisation and urbanisation (Yuan *et al.*, 2016).

Plant medicinal properties have been studied in recent scientific developments around the world due to their potent therapeutic potential, few side effects, and economic viability (Ssenku *et al.*, 2022). Medicinal plants are used as raw materials in the development of drugs that provide people with effective and affordable health care (Jain *et al.*, 2019). All plants produce phytochemicals known to be beneficial to health because they cannot be produced by the human body (Shakya, 2016). Many plants have been found to have pharmacological effects due to the presence of metabolites that are divided into primary and secondary metabolites (Hussein and El-Anssary, 2019). Primary metabolites are organic compounds such as glucose, starch, polysaccharide, protein, lipids, and nucleic acid that are essential for human growth and development (Wink, 2015). Secondary metabolites produced by plants include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, etc. (Anulika *et al.*, 2016). Therapeutic potential of plants in treating diseases is due to

the presence of secondary metabolites (Jain *et al.*, 2019). Given the above information, there is a need to investigate plants to develop new affordable and effective drugs against various diseases using the plant secondary metabolites. This chapter will review the description, traditional uses, phytochemical, and pharmacological aspects of four plants used for STIs and other diseases in Thaba 'Nchu, Free State Province of South Africa. *Hermannia depressa* (Sterculiaceae), *Scambiosa columbaria* (Dipsacaceae), *Salvia runcinata* (Lamiaceae) and *Berkheya onopordifolia* (Asteraceae) were selected from ethnobotanical information obtained from traditional healers who practice in the study area. The criteria utilised to choose plants for more research were frequency of citation (FC) (Figure 4.1), availability of the plants in the study region, and the depth of the plant research.

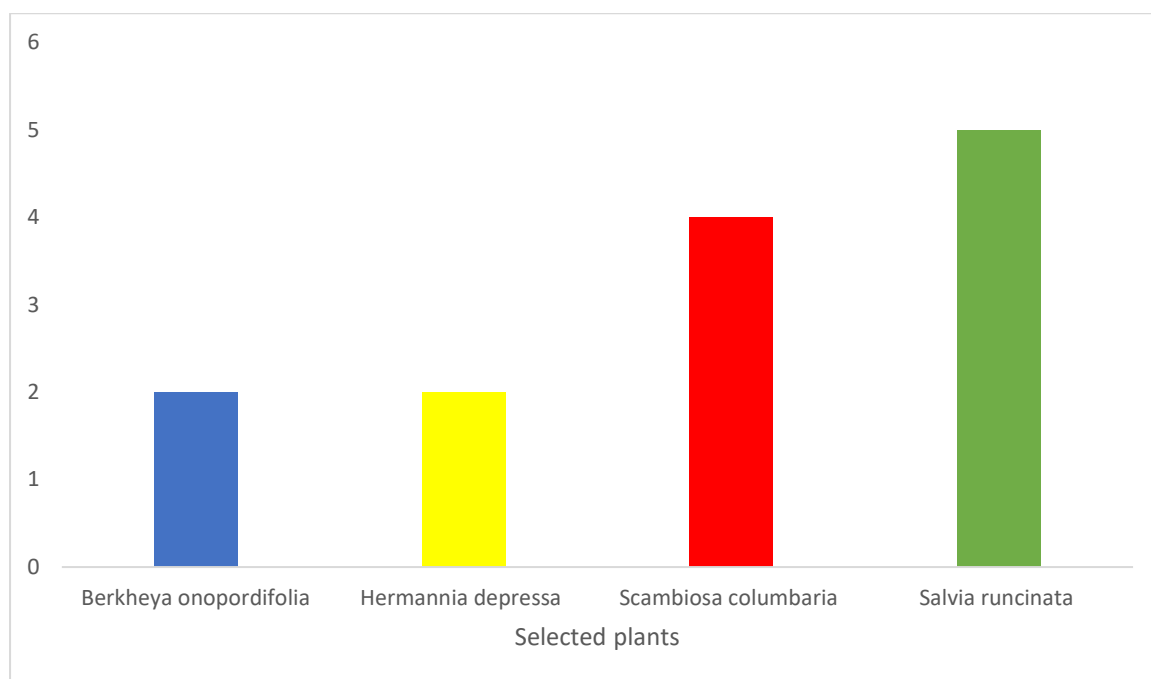


Figure 4.1: The frequency of citation (FIC) of the selected medicinal plants

4.2. METHODS

4.2.1. Search criteria

The information in this review was obtained from articles and research papers indexed in data bases such as Science Direct, Google Scholar, Elsevier, Medline, SCOPUS, Sabinet African Journals/ SA e-publications and Pubmed on the plants of interest and their traditional medicinal uses, phytochemistry and their pharmacological effects were studied, and related articles and papers were considered. The scientific articles were retrieved using the following search terms: the selected plant name and description, traditional uses, pharmacological properties, phytochemical screening, toxicity, and the isolated compounds. For example, for review of “*Berkheya onopordifolia traditional uses*”, a total of 100 papers were reviewed. After the preliminary review, 40 articles were found to be suitable for the aim of this chapter and the overall study, and therefore the rest of the articles were excluded.

4.3. RESULTS AND DISCUSSION

4.3.1. *Scabiosa columbaria* L.

4.3.1.1. Vernacular names

English: Scabios', **Afrikaans:** Bitterbos; **Sesotho:** Selomi; **Isixhosa:** silawu; **Isizulu:** Ibheka.

4.3.1.2. Synonyms

Scabiosa austro-africana Heine

4.3.1.3. Scientific classification

Kingdom: plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Dipsacales;
Family: Caprifoliaceae; Genus: Scambiosa L.; Species: *Scabiosa columbaria* L.

4.3.1.4. Distribution, habitat, and description

South African provinces, Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northwest, Northern Cape, Western Cape, Lesotho, and Swaziland all contain significant populations of *S. columbaria*, which is primarily found in grasslands, rocky slopes, and bushveld habitats. It is a perennial evergreen herb with branches which grow from a woody rootstock. The basal leaves form a rosette with serrated or toothed margins. The upper surface is light green and slightly hairy. The inflorescences are carried on multi-stemmed stalks that can reach 750 mm in height, with flowerheads measuring 25-35 mm in diameter. Flowers are white or pink and appear in the spring and summer (Figure 4.1). *S. columbaria* grows in grassland, bush veld, and rocky slope areas (Matthews, 1972).



Figure 4.2: *Scambiosa columbaria* L. Leaves and flowers

4.3.1.5. Traditional uses

According to literature, *S. columbaria*'s aerial parts, roots, leaves and the whole plant are used throughout South Africa, Lesotho, Swaziland, Italy, Turkey, and Iberian Peninsula to treat more than 25 ailments including STIs. Leaves, roots and whole plant parts of *S. columbaria* are used traditionally to treat wound-healing, baby powder, colic, sterility, heartburn, and period pain, STIs, abdominal pains, promotion of labour, constipation, diuretic, eye problems, female fertility, heartburn, high blood pressure, pregnancy complications, womb cleansing, reducing mother-child HIV transmission, respiratory problems, diphtheria, flu, skin infections, uterine disorders, and wounds (Van Wyk, 2011; Maroyi, 2019; Sagbo *et al.*, 2020; Ngoben *et al.*, 2023).

4.3.1.6. Pharmacological properties and phytochemistry

According to literature, biological properties such as antibacterial, antifungal, antiprotozoal, antioxidant and anti-inflammatory activities have been evaluated for *S. columbaria*. Aqueous, a mixture of methanol and dichloromethane leaf and root extracts showed antibacterial activity against pathogens associated with STIs such as *Neisseria gonorrhoea*, *Gardnerella vaginalis*, *Oligella ureolytica*, and *Ureaplasma urealyticum* using microdilution method (Van Vuuren and Naidoo, 2010). The latter extracts further showed antifungal activity against *Candida albicans* using the same technique at MICs ranging from 2.0-8.0 mg/ml comparable to that of Amphotericin B (2.5 mg/ml) (Van Vuuren and Naidoo, 2010). Aqueous and a mixture of methanol and dichloromethane also showed antiprotozoal activities against *Trichomonas vaginalis*. The extracts showed antibacterial activity at minimum inhibitory concentration (MIC) ranging from 2.0-16.0 mg/ml, which is weaker than that of ciprofloxacin (0.01 mg/ml) (Van Vuuren and Naidoo, 2010). Antibacterial activities were also conducted using *S. columbaria* aqueous root extracts against various pathogens where the activity was

noted at MICs ranging from 1.3 and 8 mg/ml, which was higher than that of ciprofloxacin (0.01 mg/ml) (Seleteng-Kose, 2017).

To date, there is limited information on antimicrobial investigations conducted on *S. columbaria* (whole plant and extracts such as methanol, acetone, hexane, and many other extraction solvents). Leaves and flowers of *S. columbaria* methanol extracts showed high antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), Cupric reducing antioxidant capacity (CUPRAC) and ABTS⁺ radical scavenging capacity methods (Akar, 2021). *S. columbaria* (leaves) acetone and hexane extracts showed anti-inflammatory activity at 100µg/ml concentration using the nitric oxide assay against RAW 264.7 cell lines (Mhlongo *et al.*, 2022). According to literature, there is limited information on the antioxidant and anti-inflammatory activity investigations on *S. columbaria* whole plant extracts and isolated compounds. Therefore, there is a need to investigate the pharmacological properties of the plant using whole plant and isolated compounds.

Phytochemicals are responsible for pharmacological properties exhibited by plants. *S. columbaria* (Leaves) methanol extracts were found to contain compounds such as alkaloids, flavonoids, phenolics, tannins, steroids, terpenoids, and saponins (Sagbo *et al.*, 2020). These compounds are known to have biological properties such as antimicrobial, antioxidant, anti-inflammatory, cytotoxic and many other properties (Koche *et al.*, 2016). *S. columbaria* (leaves) methanol extracts were further found to contain compounds such as eucalptol, benzene, 1,4-dichloro-, phytol, pregn-5-ene-3, 20-dione, Batilol, aprabarbital and 3,5-Dihydroxy-6,7,8-trimethoxyflavone using gas

chromatography spectrometry (GCMS). These compounds are known to have anti-inflammatory, antioxidant, antibacterial, cytotoxic, anticancer, antinociceptive, antilarval, antifungal and anticonvulsant properties (Sagbo *et al.*, 2020). Gallic acid, catechin, chlorogenic acid, 4-OH benzoic, 4-OH benzaldehyde and caffeic are phenolic compounds detected using liquid chromatography mass spectrometry (LCMS) in flower and leaf parts of *S. columbaria* (Akar, 2021).

Scambiosin was isolated from the roots of *S. columbaria*, while compounds such as palmitic acid, phthalic acid, diisooctyl phthalate, bis-(ethylhexyl) phthalate and dibutyl phthalate were also isolated from the aerial parts (Watt and Brandwijk, 1927). Furthermore, loganin and sweroside were also identified from the roots of *S. columbaria*, and they are known to have antibacterial activities against various microorganisms such as *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and many other microbes at MICs ranging from 0.001-0.1 mg/ml. The various traditional uses and scientific evidence on the pharmacological effects and the phytochemistry is a clear indication that *S. columbaria* has the potential to be used as herbal medicine, and to be developed into new drugs to treat various diseases, including STIs, based on biologically active compounds that have been isolated from the plant. It is crucial to test the effectiveness of *S. columbaria* crude extracts and compounds against drug-resistant strains of STI pathogens to ensure that it has the potential to effectively combat the issues caused by drug-resistant pathogens, despite the plant already showing promising results against STI pathogens.

4.3.2. *Berkheya onopordifolia* (DC.) O.Hoffm. ex Burt Davy

4.3.2.1. Vernacular names

SeSotho: Mohato, Ntsoantsane, Pepetloane-e-meutla; **Zulu:** Ikhakhasi, Iphungula

4.3.2.2. Scientific classification

Kingdom: plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Asterales;

Family: Asteraceae; Genus: *Berkheya*; Species: *Berkheya onopordifolia* (DC.)

O.Hoffm. ex Burt Davy

4.3.2.3. Synonyms

Stobaea onopordifolia DC.

4.3.2.4. Distribution, habitat, and description

B. onopordifolia is distributed in the Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Mpumalanga, and Northwest provinces of South Africa (Raimondo *et al.*, 2009).

Based on current evidence, *Berkheya* consists of approximately 75 species mainly distributed in South Africa. Most of the species have yellow ray flowers, some have white ray flowers, and the dominant coverage of sharp spines makes the *Berkheya* species difficult and painful to collect, and the genus is not well known and under collected, despite being a prominent component of many plant communities (Figure 4.2). *Berkheya* species are mostly perennial herbs and less commonly shrubs, and they occur in several bioregions but are concentrated where they are most strongly associated with the winter-rainfall desert (succulent Karoo) vegetation (Bergh and Helme, 2014).



Figure 4.3: *Berkheya onopordifolia* (DC.) O.Hoffm. ex Burt Davy leaves and flowers.

4.3.2.5. Traditional uses

Some species in the genus are used traditionally to treat coughs, gonorrhoea, rheumatism, and abdominal disorders (Odeleye *et al.*, 2011). *Berkheya* genus plants, such as *Berkheya setifera*, have also traditionally been used to treat fever, malaria, skin diseases, sexually transmitted infections, gastro-intestinal disorders, inflammatory, respiratory, and reproductive issues (Maroyi, 2019).

4.3.2.6. Pharmacological properties and phytochemistry

Although there is information on the traditional use of *B. onopordifolia* in treating ailments, there is only limited information available on the pharmacological properties and phytochemistry of the plant. Sesquiterpenoids, polyphenols and derivatives of thiophene (isocomene, β -isocomene and modhepene) were isolated from *Berkheya* species (*Berkheya setifera*) (Hutchings *et al.*, 1996; Maroyi, 2019). *B. setifera* and other *Berkheya* species are known to possess biological activities such as antibacterial, antifungal, anti-inflammatory, antimalarial, antioxidant, haemolysis, and cytotoxic activities (Sibandze *et al.*, 2009; Odeleye *et al.*, 2011; Muleya, 2013; Muleya *et al.*, 2014; Seleteng-Kose *et al.*, 2019; Maroyi, 2019). There is a great need to investigate the pharmacological properties and phytochemistry of *B. onopordifolia* using various methods to affirm the traditional uses of the plant.

4.3.3. *Salvia runcinata* L.f.

4.3.3.1. Vernacular names

Afrikaans: Hardsalie, Wilde Salie; **SeSotho:** Mangoakoane, Mosisili

4.3.3.2. Scientific classification

Kingdom: Plantae; **Phylum:** Tracheophyta; **Class:** Magnoliopsida; **Order:** Asterales;
Family: Lamiaceae; **Genus:** *Salvia*.; **Species:** *Berkheya onopordifolia* (DC.) O.Hoffm.
ex Burtt Davy

4.3.3.3. Synonyms

Salvia monticola Benth, *Salvia monticola* Benth. var. *monticola*, *Salvia runcinata* L.f.
var. *grandiflora* Skan, *Salvia runcinata* L.f. var. *nana* Skan, *Salvia sisymbriifolia* Skan

4.3.3.4. Distribution and habitat and description

South African provinces, Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, Northwest, Western Cape, Lesotho, and Botswana all have significant populations of *S. runcinata*, which thrives in grassy areas, heavy, damp soils, or disturbed areas (Kamatou *et al.*, 2008). The genus name '*Salvia*' is derived from a Latin name '*Salvare*' which means to heal or to be safe and unharmed which refers to its medicinal properties of some of its species (Blumenthal *et al.*, 2000; Kamatou *et al.*, 2008). About 30 *Salvia* species have been identified in Southern Africa and are easily recognised their square stems and rugose, entire, toothed, and lobed leaves (Arnold and De Wet, 1993). Flowers are clustered in racemes, spike-like racemes, spikes, and panicles are usually large and brightly coloured, depending on the species (Figure 4.3) (Kamatou *et al.*, 2008).



Figure 4.4: *Salvia runcinata* L.f. leaves, stems, and flowers

4.3.3.5. Traditional uses

The whole plant of *S. runcinata*, including its leaves and stems, has been traditionally used to treat a total of various ailments. Remedies involve preparing a decoction, paste, or using burned plant material *S. runcinata* is used traditionally to treat urticaria, purgative for infants, blood purifier, appetite booster, fever, headache, digestive disorders, sores, throat inflammation, STIs and female related ailments (Watt and Breyer-Brandwijk, 1962; Kamatou *et al.*, 2008; Moteetee and Van Wyk, 2011; Kamatou *et al.*, 2005; Kamatou and Viljoen, 2010, Ngobeni *et al.*, 2023).

4.3.3.6. Pharmacological properties and phytochemistry

S. runcinata has been found to have various pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, cytotoxic, and antituberculosis activities (Kamatou *et al.*, 2008). When tested using the agar diffusion method, *S. runcinata* methanol extracts displayed weak antimicrobial activity against gram-negative bacteria, yeast, and moulds. However, the same extracts were found to exhibit antimicrobial activity against these gram-negative bacterial species at a minimum inhibitory concentration (MIC) greater than 1.6 mg/ml (Gono-Bwalya, 2003). A mixture of methanol and chloroform extracts of the aerial parts of *S. runcinata* demonstrated antioxidant activity using ABTS and DPPH assays, with an IC₅₀ value of 19.4 and 19.3 µg/ml, respectively. In addition, methanol extracts showed good antioxidant activity, with IC₅₀ values below 16 µg/ml. *S. runcinata* essential oils showed anti-inflammatory activity by inhibiting cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2), which are inflammatory mediators in the inflammation process (Gono-Bwalya, 2003). *S. runcinata* has also showed toxicity against three human cancer cell lines, including the breast adenocarcinoma (MCF-7), the colon adenocarcinoma (HT-29) and the

glioblastoma (SF-268) cell lines, using the sulforhodamine B assay. However, the results may be considered positive for anticancer properties (Kamatou *et al.*, 2008).

S. runcinata contains significant amounts of volatile compounds such as α -bisabolol and (E)-nerolidol, making it a potential natural source of α -bisabolol. As α -bisabolol is an essential ingredient in many cosmetic products, this makes *S. runcinata* a valuable plant for the cosmetic industry (Kamatou and Viljoen, 2010). α -Bisabolol has been used extensively in dermatological and cosmetic formulations, including aftershave creams, hand and body lotions, deodorants, lipsticks, sun care and after-sun products, baby care items, and sport creams (Kamatou and Viljoen, 2010). The plant was also found to contain non-volatile phenolic compounds such as rosmarinic acid (RA), carnosic acid, carnosol and ursolic acid (Kamatou *et al.*, 2008). The therapeutic effects reported on RA are antioxidant, anti-inflammatory, antibacterial, anti-angiogenic, anti-mutagenic, antidepressant and anti-allergenic effects (Nunes *et al.*, 2017). Therefore, *S. runcinata* can be considered as a natural source of RA. Carnosic acid, carnosol and ursolic acid are known to possess biological activities such as antioxidant, antimicrobial, anticancer, anti-inflammatory, anti-angiogenic, hepatoprotective, neuroprotective, antidiabetic, and cardioprotective activities (Johnson, 2011; Birtic *et al.*, 2015; O'Neill *et al.*, 2020). In studies by Gono-Bwalya (2003), Fisher (2005), Kamatou *et al.* (2008), Kamatou *et al.* (2010), and Kamatou *et al.*, (2005), *S. runcinata* extracts demonstrated antimicrobial activities (antibacterial, anti-plasmodial, antimalarial, and antituberculosis). The above-mentioned compounds may be the cause of these effects. *Salvia* species are known to have anti-inflammatory properties, and their traditional use as medicinal herbs includes wound healing (Kamatou, 2006). Sesquiterpenic alcohol levels in *S. runcinata* were found to be high, allowing the plant

to inhibit the 5-lipoxygenase and COX-2 enzyme, thus having anti-inflammatory activity (Gono-Byalwa, 2003; Kamatou *et al.*, 2005; Kamatou, 2006). Alkaloids and phenolics were also identified as some of the compounds found in *S. runcinata* (Kamatou, 2006). Many alkaloids are poisonous to both animals and humans. Hence, the plant exhibited the highest toxicity profile against Graham cells using MTT toxicity assay in a study conducted by Kamatou *et al.*, (2005). *Salvia runcinata* has been traditionally used to treat different illnesses, indicating its potential as a traditional herbal medicine. Additionally, the plant displays pharmacological properties and has numerous bioactive compounds that have been identified and isolated. It is necessary to test the plant's antimicrobial activity against drug-resistant microbes related to sexually transmitted infections. Further studies are needed to examine the isolated compounds for pharmacological effects such as anti-inflammatory, antioxidant, cytotoxicity and may other properties to increase the chances of developing novel drugs from the plant.

4.3.4. *Hermannia depressa* N.E.Br.

4.3.4.1. Vernacular names

English: Doll's rose, **Sesotho:** Seletjane or Phate-ya-ngaka, **Afrikaans:** Poprosie, Moederkappie, Rooiopsag **Zulu:** Umphuphuthelo or Itsshesizwe.

4.3.4.2. Synonyms

Mahernia erodioides Burch. ex DC. var. *latifolia* Harv.

4.3.4.3. Scientific classification

Kingdom: plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Malvales; Family: Malvaceae; Genus: *Hermannia*; Species: *Hermannia depressa* N.E.Br.

4.3.4.4. Distribution, habitat, and description

Hermannia depressa is widely distributed in the Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, Northwest, and Western Cape provinces of South Africa. It typically grows in terrestrial habitat types. There are approximately 141 species of *Hermannia* that are endemic to South Africa. *H. depressa* is a prostrate herb that grows from a woody perennial taproot with spreading stems and flat, ground-level leaves. The leaves are large and typically carried on short stalks with a purplish to reddish brown tinge. The flowers are short, erect stalks that droop and come in a variety of colours, including orange, pinkish, mauve, and yellow cream. It is sparsely hairy and slightly glandular, occurring in grasslands and marshes (Figure 4.4) (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996).



Figure 4.5: Leaves of *Hermannia depressa* N.E.Br.

4.3.4.5. Traditional uses

The plant is used traditionally to treat abdominal pains during pregnancy, nausea, diarrhoea, heartburn, stomach-ache, emetic, cough and boost appetite and pregnant women (Reid *et al.*, 2005; Kose *et al.*, 2015).

4.3.4.6. Pharmacological properties and phytochemistry

Although *H. depressa* has a history of being used traditionally to treat different ailments, there is inadequate scientific knowledge about the plant's pharmacological properties and the compounds that have been isolated from it. The aerial acetone extracts of *H. depressa* have demonstrated activity against breast (MCF-7) and cervical (HeLa) cancer cell lines. This suggests that the extracts may have anti-cancer properties (Molefe, 2013). *H. depressa* also showed antibacterial properties against *Bacillus pumilus* and *Pseudomonas aeruginosa* at MIC values 0.098 and 0.36 mg/ml, respectively (Xaba and Buwa, 2016). *H. depressa* showed low antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (Reid, 2002). Moreover, acetone extracts obtained from the aerial parts of *H. depressa* exhibited anthelmintic properties against parasitic gastrointestinal nematodes that affect livestock (Anofi and O, 2013). Tannins are amongst one of the phytochemicals detected through standard methods from leaf, stem, and root extracts of *H. depressa* (Reid *et al.*, 2005). *H. depressa* methanol, ethanol, acetone, and aqueous extracts were found to contain tannins, saponins, flavonoids, and terpenoids (Xaba and Buwa, 2016). As per our extensive search through available literature, there is limited information on phytochemical analysis using LCMS and GCMS that have been performed on *H. depressa*, and no attempts have been made to isolate any bioactive compounds from it. While there is scientific proof of the antibacterial properties of *H. depressa*, it is important to assess the efficacy of this plant against

STI-related pathogens and drug-resistant strains. This is necessary to determine its potential in the treatment of STIs and the development of novel therapeutic drugs.

4.5. CONCLUSION

There is limited information regarding the isolated compounds, phytochemicals, and biological activities of *B. onopordifolia*. To validate the *B. onopordifolia* traditional use for treating diseases, extensive research on the biological activities, toxicity, active compounds, and isolation must be conducted. *S. columbaria* has been studied for its anti-inflammatory and antioxidant properties using different methods only on leaves and flowers. Preliminary screening of *S. columbaria* crude extracts was conducted using standard methods, and characterisation of active compounds was also conducted using LCMS on leaves and flowers. Further investigations on antimicrobial activities and other biological activities such as anticancer, cytotoxicity etc. as well as isolation of biologically active compounds is recommended to validate their traditional uses. Antimicrobial and anticancer activities of the root extracts of *H. depressa* have been studied. The root extracts of *H. depressa* have only undergone preliminary phytochemical screening; no active compounds have been isolated or characterised. Further investigations still need to be done to explore more biological properties and active compounds to authenticate *H. depressa* ethnobotanical uses. Biological properties and active compounds of *Salvia runcinata* have all been extensively studied. However, *S. runcinata* has not been tested for its antimicrobial activity against the microbes responsible for STIs. It is crucial to conduct research on such antimicrobial activity, as STIs are drug-resistant, and they have a negative health impact on the community of Thaba 'Nchu. Therefore, this study will focus on validating

the ethnobotanical uses of these four plants to treat STIs through the evaluation of their antimicrobial, anti-inflammatory, antioxidant, and toxic properties.

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CHAPTER 5²

Phytochemical profiling of the crude extracts of *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata* and *Scambiosa columbaria*

ABSTRACT

The bioactive compounds that contribute to the pharmacological value of extracts from *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata* and *Scambiosa columbaria* were evaluated in this chapter. The qualitative phytochemical screening was conducted using standard calorimetric methods and Liquid Chromatography Mass Spectrometry (LCMS). Folin-Ciocalteu and aluminium colorimetric methods were used to quantitatively evaluate phenols and flavonoids, respectively. Phenols, flavonoids, saponins, terpenoids, alkaloids and tannins were qualitatively detected from *H. depressa*, *S. columbaria*. *H. depressa* acetone and *S. columbaria* extracts. The latter extracts had the highest phenolic content $8,45 \pm 0,006$ & $7,65 \pm 0,004$ mg GAE/g respectively. The highest presence of flavonoids was observed from the methanol and acetone extracts of *S. columbaria*, $3,56 \pm 0,01$ and $2,29 \pm 0,03$ mg quercetin equivalents QE/g respectively. All the extracts examined in this study had a variety of bioactive compounds that were identified using LCMS. Most bioactive compounds found in the plants are known to have a wide range of biological capabilities, including antibacterial, anti-inflammatory, antioxidant, hepatoprotective, and many other properties. The selected plants' medicinal properties and their ability to traditionally treat diseases such as STIs may be related to the presence of bioactive compounds. More research must be done on the bioactive compounds to enhance the

²Some parts of this chapter were presented at African Traditional and Natural Product Medicine Conference hosted by the University of Limpopo at the Protea Hotel Ranch Resort from 19-21 October 2022.

likelihood for the development of new STIs drugs from plants and the discovery of novel compounds.

5.1. INTRODUCTION

Secondary metabolism of plants is nearly an inexhaustible source of phytochemicals displaying limitless biological activities (Carvalho *et al.*, 2019). Phytochemical analysis of plants proved that medicinal plants contain compounds such as alkaloids, flavonoids, steroids, phenols, coumarins, and glycosides (Kumar *et al.*, 2019). Phytochemicals are secondary metabolites that have various biological properties such as antioxidant, antimicrobial, anticancer and anti-inflammatory activities (Kumar *et al.*, 2019). Therefore, pharmaceutical companies regard plants as a great option to provide many leads that are important for the development of new drugs, and current scientific investigations are propelled towards naturally occurring bioactive compounds from plants (Takaidza *et al.*, 2018).

Secondary metabolites play an important role in shielding plants from environmental stress such as herbivore, bacterial invasions inter-plant competition and their survival (Bednarek and Schulze-Lefert, 2009; Zaynab *et al.*, 2018). Secondary metabolites are also attractive to pollinators and seed-dispersing animals and protect plants against ultraviolet (UV) radiation (Gomez-Caracava *et al.*, 2014). Secondary metabolites are responsible for colour, odour, and taste of plants, and most of them are commercially important and have been used in important sectors such as pharmacology, chemistry, agriculture, and drug development (Cannes do Nascimento and Fett-Neto, 2010). Analysis of secondary plant products has progressed considerably over the last 20 to 30 years. The use of modern analytical techniques such as chromatography,

electrophoresis, isotope techniques and enzymology have succeeded in the elucidation of exact structural formulas and the most important biosynthetic pathways of secondary metabolites or bioactive compound (Kabera *et al.*, 2014). Some of the useful drugs developed from plant bioactive compounds include vinblastine, vincristine, taxol, podophyllotoxin, codeine, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, aspirin, atropine, pilocarpine, capsaicin, allicin, curcumin, artemisinin, and ephedrine (Devanesan *et al.*, 2018).

Secondary metabolites from plants are subdivided into three major classes such as terpenoids, alkaloids and phenols. Terpenoids are responsible for attracting beneficial mites for the ingestion of herbivorous insects in plants and have numerous medicinal properties such as anti-inflammatory, anti-cancer, anti-malarial, cholesterol synthesis inhibition, antiviral, and antibacterial activities (Kappers *et al.*, 2005). Alkaloids are nitrogen-containing secondary metabolites derived from amino acids such as tryptophan, tyrosine, phenylalanine, and lysine, as well as ornithine (Patra *et al.*, 2013). Alkaloids are also known to protect plants from herbivorous attacks and UV radiation. They possess pharmacological activities such as anti-inflammatory, anti-diabetic, antimalarial and antimicrobial activities (Kabera *et al.*, 2014). Phenolic compounds are one of the largest groups of secondary metabolites produced by plants and are subject to a great number of chemical, biological, agricultural, and medical analysis. They are classified by their ability to exhibit properties such as antioxidant, anti-inflammatory, anti-carcinogenic and other biological properties (Park *et al.*, 2001; Kabera *et al.*, 2014). Some simple phenols also possess bactericidal, antiseptic and antihelmintic properties (Pengelly, 2004). Given the pharmacological properties of compounds found in plants, it shows that plants may be effective in treating STIs.

Plants are an important source of pharmacologically active compounds with many drugs developed directly or indirectly from plants. These bioactive compounds have made a significant contribution to disease control (Velu *et al.*, 2018). Therefore, it is of utmost importance to analyse bioactive compounds from plants to validate their traditional use in the treatment of diseases, and documentation of such information is useful in the discovery of new drugs. In this chapter, phytochemical evaluation of acetone and aqueous extracts of *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata* and *Scambiosa columbaria* was conducted.

5.2. METHODS

5.2.1. Extraction process and storage

B. onopordifolia (roots), *H. depressa* (roots), *S. runcinata* (whole plant) and *S. columbaria* (whole plant) plant material was collected in Thaba 'Nchu for the investigation of their phytochemical profile. The plant parts were collected, washed, oven dried at 40°C for 72 hours, and ground to powder. The dry, powdered material (50g) of the plants was weighed, pulverised, and soaked in 100% methanol, acetone, or water for 48 hours on a rotary shaker. After filtering the extracts, further extraction with a new solvent was carried out until the solvent was clear. The solvent of extraction was evaporated at 40°C, under a vacuum, using a Buchi Rotavapor. All extracts were stored in the fridge at 4°C until required. Aqueous, methanol and acetone extracts of the selected plants were used throughout the experiments.

5.2.2. Phytochemical analysis of the plant extracts

Biochemical tests we conducted to screen for the presence of various phytochemicals such as alkaloids, flavonoids, saponins, steroids, glycosides, phenols, terpenes, phlabotannins and tannins using standard methods by Dhawan *et al.* (2017). These types of phytochemicals were selected for screening because they are known to have pharmacological effects such as antimicrobial, anti-inflammatory, antioxidant and many more properties useful in the treatment of STIs (Nyamai *et al.*, 2016).

5.2.2.1. Test for alkaloids

Ten milligrams of each extract were dissolved in 2 mL of reagent Wagner. The appearance of reddish-brown precipitates after the extracts are dissolved indicates the presence of alkaloids in the plant extract.

5.2.2.2. Test for flavonoids

In 10 mg of each extract, a few drops of diluted NaOH were added. The appearance of a yellow colour that disappears or becomes colourless after a few drops of diluted H₂SO₄ confirms the presence of flavonoids in the extracts of plants.

5.2.2.3. Test for saponins

For 20 mL of distilled water, ten milligrams of each extract were diluted. The test tubes were then shaken by hand for 15 minutes, where the formation of foam on top of the test tube indicated the presence of saponins in the extract from the plant.

5.2.2.4. Test for steroids

Ten milligrams of each extract were taken and 1 mL of concentrated H₂SO₄ was slowly added into a test tube of each extract. The presence of a dark reddish green colour indicated steroid content in the plant extract.

5.2.2.5. Test for tannins

In 45 per cent of ethanol, 10 mg of each plant extract was dissolved. The test tubes were then boiled for five minutes, and 1 ml of 15 per cent ferric chloride solution was added to each. The appearance of a greenish to black colour indicated tannin content in the plant extract.

5.2.2.6. Test for glycosides

The glycoside test was conducted using the Molisch test, where 2 mg of extracts was mixed with 10 ml water, filtered and the filtrate concentrated. Drops of Molisch's reagent were added and mixed, followed by the addition of 2ml of concentrated sulphuric acid through the side of the test tube. The appearance of a reddish violet ring suggested the presence of glycosides (Kodangala *et al.*, 2010).

5.2.2.7. Test for phlobatannins

A few drops of 1% HCl were added and boiled to 1 ml of the extract. A red precipitation meant that phlobatannins were present.

5.2.2.8. Test for terpenoids

0.5 ml of acetic anhydride and 1 ml of sample extract and a few drops of concentrated sulphuric acid (H_2SO_4) were mixed. A bluish, green precipitate indicated that terpenes were present (Anyasor *et al.*, 2010).

5.2.3. Determination of total phenolic compounds content

Total phenolic content of the selected plant extracts was performed using the method of Singleton *et al.* (1999) with modifications as introduced by Tahrani *et al.* (2019). Hundred microliters of each extract solution at a concentration of 1 mg/ml, 100 μ l of 50% Folin-Ciocalteu reagent and 2ml of 7.5 % sodium carbonate were prepared in triplicates. The mixture was incubated for 30 minutes at room temperature, and the

absorbance was measured at a wavelength of 720nm. Gallic acid was used to formulate a standard curve (0 to 350 µg/ml; $Y = 0.0029X - 0.0121$; $R^2 = 0.995$), and the total phenolic content of gallic acid equivalent (GAE) extracts was calculated using the following formula:

$$C = c \times V/m$$

Where:

C= is the total amount of phenolic compounds in mg/g of plant extract in GAE;

c= is the concentration of gallic acid from the calibration curve (mg/ml);

V= is the extract volume (ml); and

m= refers to the weight of pure organic plant extract (g).

5.2.4. Determination of total flavonoid compounds content

Total flavonoid content was determined using aluminium colorimetric method by Pothitirat *et al.* (2009) with modifications by Vongsak *et al.* (2013). 50µl of crude extracts (1 mg/ml ethanol) were made up to 1 ml with methanol, mixed with 4 ml of distilled water and then 0.3 ml of 5% NaNO_2 solution; 0.3 ml of 10% AlCl_3 solution was added after 5 minutes of incubation, and the mixture was incubated for 10 minutes. Then, 2 ml of 1 mol/l NaOH solution were added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The absorbance of the mixture was measured at 415 nm against a blank sample without aluminium chloride using UV–VIS spectrophotometer. The content of total flavonoids was calculated as mean $\pm$ SD (n=3) and expressed as grams of quercetin equivalents (IQE) in 100 g of the extract and dried powder.

5.2.5. Identification of bioactive active compounds using LCMS

LC-MS/MS analysis to identify active compounds from crude extracts of *B. onopordifolia*, *H. depressa*, *S. runcinata*, and *S. columbaria* extracts was carried out at Central Analytical Facilities (CAF), Stellenbosch, South Africa using the Waters Synapt G2 spectrometry system with ESI positive/negative. An Agilent mass selective detector (MSD) Ion trap x-ray computed tomography (XCT) mass spectrometer with an Electrospray Ionization (ESI) ion source was connected to the column outlet. Chromatographic separation was achieved on a C18, 2.1 x 150 mm analytical column. For 0.50 minute, the column was held at 100% solvent A (0.1% formic acid in water) and 0% solvent B (0.1% formic acid in acetonitrile), followed by a 12:00 minute step gradient from 0% A to 100% B, followed by 3 minutes at 100% A at a flow rate of 0.4 ml/min, while the injection volume was set at 0.01 ml. Data collection and analysis were performed on a computer using an Acquity binary solvent manager instrument system. The following working conditions were used throughout all MS experiments: for electrospray ionisation with negative ion polarity the capillary voltage was set to 3 kV, the drying temperature to 350°C and cone voltage of 15V, the maximum nebuliser pressure to 15000 psi and the seal wash was 5 min. The total run time was 15 minutes, and the scan speed was 26 000 m/z/s (ultra-scan mode).

5.3. RESULTS AND DISCUSSION

5.3.1. Qualitative phytochemical screening using standard methods

The phytochemical screening results of aqueous, methanol and acetone extracts of *H. depressa*, *S. runcinata*, *S. columbaria* and *B. onopordifolia* using standard methods are displayed in Table 5.1. The presence of the phytochemical was denoted by a positive (+) and absence by a negative (-) sign, while (-) denoted a lack of

phytochemicals and no change in colour. All *S. runcinata* and *S. columbaria* extracts, as well as *H. depressa* methanol extracts, contained alkaloids. Alkaloids were also detected in *B. onopordifolia* methanol and acetone extracts. The presence of alkaloids in *H. depressa*, *S. runcinata*, *S. columbaria* and *B. onopordifolia* suggests that the plants may be used for the development of anticancer, anticholinergic, antimalarial, anti-bacterial, antifungal, anthelmintic, cardiogenic, anticonvulsant, anti-inflammatory and analgesic drugs (Kaur and Arora, 2015; Kainsa *et al.*, 2012, Marella *et al.*, 2013; Nassiri, 2013; Machado *et al.*, 2021). Phlobatannins were not detected in all the plant extracts tested in this chapter.

All *H. depressa* and *S. columbaria* extracts were found to contain terpenoids. Terpenoids were identified in *S. runcinata* and *B. onopordifolia* aqueous extracts, as well as in *S. runcinata* acetone extracts. Various studies have reported that terpenoids such as monoterpenes, sesquiterpenes, diterpenes and sesterterpenes have biological activities such as anticancer, anti-inflammatory, antiviral, and antibacterial activities (Duetz *et al.*, 2001; Smolinski and Pestka, 2003; Wu *et al.*, 2004; Ludwiczuk *et al.*, 2017). The presence of terpenoids in all the plants studied in this chapter might lead to the development of medicines that may be used to treat viral and bacterial infections, inflammatory diseases, and cancer. A test for steroids was positive for *B. onopordifolia* methanol and acetone extracts, suggesting its potential effects against cancer, inflammatory diseases, cardiovascular diseases, arthritis, temporal arthritis, dermatitis, allergic reactions, asthma, hepatitis, systemic lupus erythematosus, etc. (Sultan and Raza, 2015). Traces of tannins were detected in *H. depressa* (aqueous, methanol and acetone), *S. runcinata* (aqueous and acetone) and *S. columbaria* (aqueous and acetone) extracts. Tannins are known to possess biological properties

such as antioxidant, radical scavenging, antimicrobial, antiviral, antimutagenic, anti-inflammatory, anti-cancer, and antidiabetic (Sieniawska, 2015). Due to their tannin content, *H. depressa*, *S. runcinata* and *S. columbaria* may be useful in the development of drugs with the latter biological properties. Glycosides were also detected in *S. runcinata*, *S. columbaria* (Methanol and acetone) and *B. onopordifolia* (aqueous and methanol) extracts. Saponins were detected from *H. depressa*, *S. runcinata* and *S. columbaria*, while flavonoids were only detected in *S. runcinata* acetone extracts. The results of Sagbo *et al.* (2020) contradict those of this study, where *S. columbaria* tested positive for flavonoids. Ethanol was used as an extraction solvent by Sagbo *et al.* (2020), this may have influenced the negative results for flavonoids. According to literature, flavonoids are known to possess biological properties such as anticancer, antiviral, antioxidant, antidepressant, hepatoprotective, haemolytic, anti-inflammatory, and antimicrobial properties (Kaunda and Zhang, 2019, Sparg *et al.*, 2004; Fang *et al.*, 2020).

Table 5.1: Qualitative phytochemical analysis of aqueous, methanol, and acetone crude extracts of *B. onopordifolia*, *H. depressa*, *S. columbaria*, and *S. runcinata*

	<i>H. depressa</i>			<i>S. runcinata</i>			<i>S. columbaria</i>			<i>B. onopordifolia</i>		
	Aq	Methanol	Ace	Aq	Meth	Ace	Aq	Meth	Ace	Aq	Meth	Ace
Alkaloids	-	+	-	+	+	+	+	+	+	-	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	-	+	+	+	+	+	-	-
Tannins	+	+	+	+	-	+	+	-	+	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	+	+
Phenols	-	+	+	+	-	-	+	-	-	-	-	-
Glycosides	-	-	-	-	+	+	-	+	+	+	+	-
Saponins	+	+	+	+	-	-	+	-	-	-	-	-
Flavonoids	-	-	-	-	-	+	-	-	-	-	-	-

Ace= Acetone; Meth= Methanol; Aq= Aqueous; (+) =presence of a phytochemical; (-) =absence of a phytochemical; each experiment was conducted in triplicates.

5.3.2. Total flavonoid content (TFC)

The TFC of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* aqueous, methanol and acetone extracts are displayed in Table 5.2. The equation of calibration curve of quercetin standard is displayed in Figure 5.1. Amongst all 12 crude extracts, roots of *B. onopordifolia* methanol (11.77 ± 0.081 mgQE/g), whole plant *S. runcinata* methanol (5.15 ± 0.01 mgQE/g), and whole plant *S. columbaria* methanol (3.56 ± 0.01 mgQE/g) extracts contained the highest flavonoid compounds content. The methods and solvents used in extraction are responsible for the dissolution of the plant compounds. Additionally, plant compounds can have either a polar or non-polar nature (Siddhuraju and Becker, 2003). Methanol extracts displayed the highest total flavonoid content in this chapter. This may be attributed to the solubility of polyphenols in more polar solvents such as methanol. It is also apparent that the recovery of phenolic compounds is dependent on the solvents used and their polarity (Tepal, 2016). Flavonoids are a class of polyphenolic compounds with a wide range of properties and chemical structures. They have antibacterial, antiviral, anti-allergic, vasodilatory, and anti-inflammatory properties, as well as antioxidant, antimutagenic, and anti-carcinogenic properties (Ribarova *et al.*, 2005; Tepal, 2016). Therefore, *B. onopordifolia*, *S. runcinata* and *S. columbaria* plants can be used to develop drugs with the above-mentioned properties to treat various diseases.

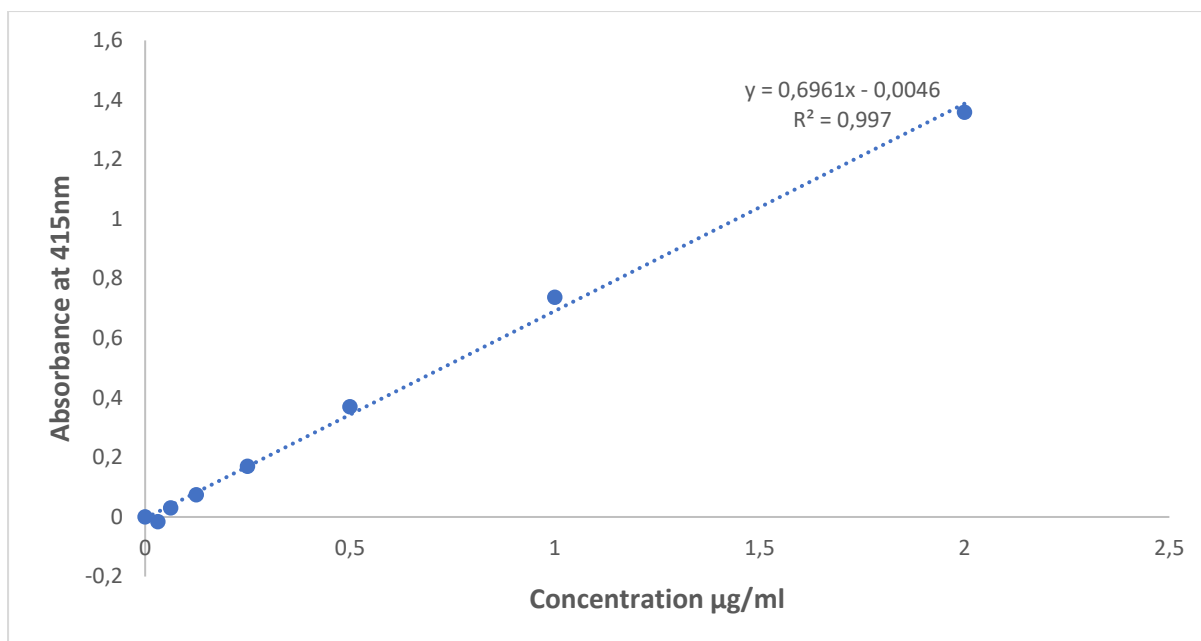


Figure 5.1: Standard curve of quercetin

Table 5.2: Total phenolic content and flavonoid content of *B. onopordifolia*, *H. depressa*, *S. columbaria* and *S. runcinata* extracts.

Plant name	Extracts	Total phenolic content (mg GAE/g of extract)	Total flavonoid content (mg QE/g of extract)
<i>B. onopordifolia</i>	Acetone	0.49±0.008	24.30±0.008
	Methanol	2.50± 0.003	11.77±0.081
	Aqueous	0.49±0.007	0.176±0.008
<i>S. runcinata</i>	Acetone	2.93±0.009	2.29±0.030
	Methanol	3.97± 0.007	5.15±0.010
	Aqueous	3.06±0.005	2.28±0.011
<i>S. columbaria</i>	Acetone	2.93±0.009	2.29±0.030
	Methanol	3.13±0.010	3.56±0.010
	Aqueous	3.28±0.010	1.79±0.020
<i>H. depressa</i>	Acetone	8.45±0.006	0.58±0.020
	Methanol	7.65±0.004	0.53±0.001
	Aqueous	5.66±0.002	0.97±0.010

All experiments were conducted in triplicates; All the data was expressed as means ± standard deviation.

5.3.3. Total phenol content (TPC)

The TPC of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* aqueous, methanol and acetone extracts are displayed in Table 5.2. The calibration curve from gallic acid showed maximum absorbances at 765 nm wavelength (Figure 5.2). Amongst all the 12 crude extracts, *H. depressa* acetone (8.45 ± 0.006 mgGAE/g), methanol (7.65 ± 0.004 mgGAE/g) and aqueous (5.66 ± 0.002 mgGAE/g) extracts contained the highest number of phenolic compounds. The TPC results correlate with the preliminary phytochemical screening results in this chapter where both acetone and methanol *H. depressa* were found to contain phenols. This study is the first to report on the total phenolic content of *H. depressa*. The high TFC in *H. depressa* extracts demonstrated in this study could explain the antimicrobial, antibacterial and anti-inflammatory activities previously reported (Reid *et al.*, 2005; Essop *et al.*, 2008).

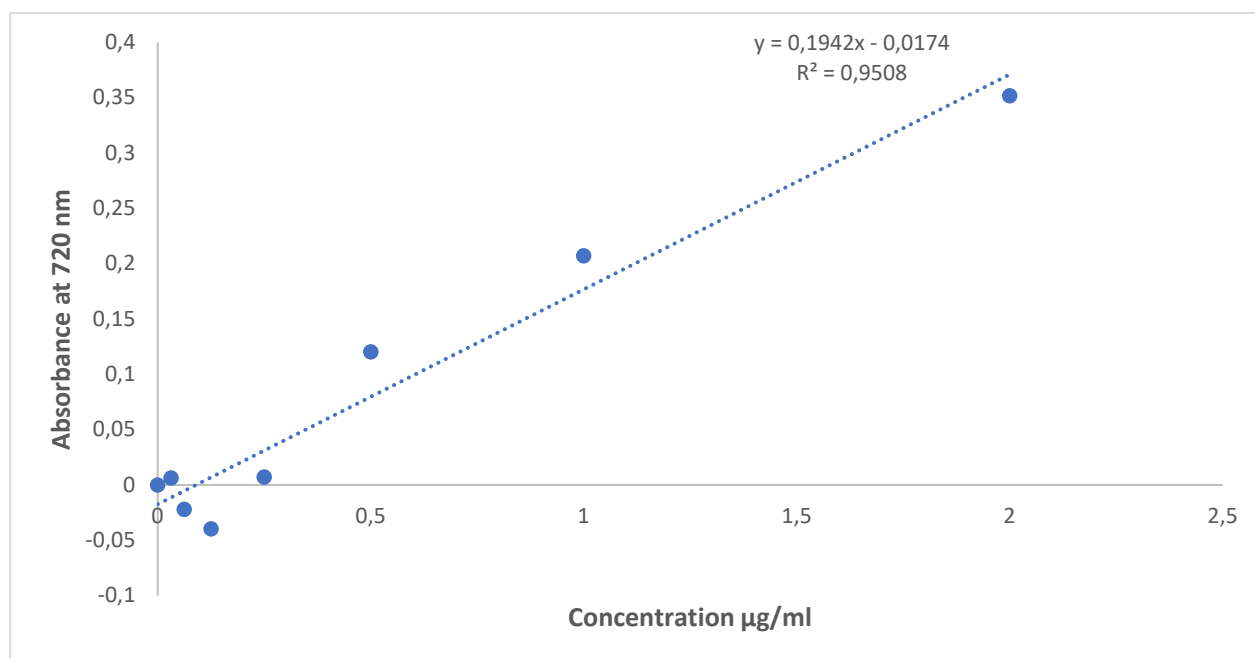


Figure 5.2: Standard curve of gallic acid

5.3.4. LCMS analysis of *B. onopordifolia* extracts in positive mode

The LCMS analysis chromatograms in positive mode for aqueous, acetone and methanol extracts of *B. onopordifolia* are displayed in the appendices section (appendix 1, 15, 23). Table 5.4 displayed the peak number, proposed compounds, retention times (RT), mode of analysis (+ or -), molecular weight and formula of *B. onopordifolia* extracts. The LCMS analysis of *B. onopordifolia* aqueous extracts in positive mode at RT 1.00 minutes, peak 1 matched a compound skimmin at m/z 325.116. Skimmin is one of the major pharmacologically active coumarin glycoside compounds with anti-inflammatory and reno-protective effects (Zhang *et al.*, 2013). Skimmin was further found to possess antidiabetic, antibilious, antiparasitic, antipyretic, aphrodisiac, aromatic and alterative properties (Zhang *et al.*, 2012; Lou *et al.*, 2020). Peak 2 showed a compound corresponding to 14-Methylmonocrotaline-like alkaloid at RT 11.84 minutes at m/z 362.155 which is a pyrrolizidine alkaloid belonging to the monocrotaline-like subgroup (Flores *et al.*, 2009). Isolated Pyrrolizidine alkaloids (PAs) are known to have antimicrobial, anti-inflammatory, anticancer and antiviral effects (Wei *et al.*, 2021). However, PAs are equally known to exert hepatotoxicity, genotoxicity and neurological damage effects on livestock, wildlife, and humans (Chen *et al.*, 2010; Jank and Rath, 2017; Ma *et al.*, 2018). PAs have been previously identified in plants from families such as Apocynaceae, Boraginaceae, Fabaceae, Orchidaceae, and Santalaceae (Wei *et al.*, 2021). Plants from the above-mentioned families are commonly used for boils, insect stings, sores, throat infections, ulcers, and snake bites. LCMS positive mode analysis of *B. onopordifolia* acetone extracts at RT 7.83 minutes revealed a compound corresponding to 3',4'-Dimethoxyluteolin at peak 2. The flavone 3',4'-Dimethoxyluteolin has previously been isolated from the flowers of *Antirrhinum majus* and *Leptadenia reticulata* (Al-Snafi, 2015). This compound has

shown moderate antimalarial activity against *plasmodium falciparum* k1 strain (Chaniad *et al.*, 2021). Moreover, flavones are known to exhibit useful biological activities such as anti-inflammatory, antioestrogenic, antimicrobial, anti-allergic, antioxidant, vascular, antitumor, and cytotoxic activities and are also used for cancer, cardiovascular and neuro-degenerative disorders (Verma and Pratap, 2010; Singh *et al.*, 2014; Hostetler *et al.*, 2017). *B. onopordifolia* methanol extracts positive mode analysis showed compound 4'-methoxynaringenin - also known as Isosakuranetin (Peak 7 and RT 9.53 minutes). Isosakuranetin is a flavonoid that was reported to possess hepatoprotective, antimicrobial and antioxidant properties (Filho *et al.*, 2008; Figueiredo-Rinhel *et al.*, 2013; Bae *et al.*, 2022).

Table 5.4: Most abundant phytochemical compounds present in *B. onoporaifolia* extracts by LCMS analysis in positive and negative mode

Extract	Peak	Proposed compound	m/z	RT (min)	Mode	Molecular formula	Ref.
Aqueous	1	Skimmin	325.116	1.00	+	C ₁₅ H ₁₆ O ₈	Perkowska <i>et al.</i> , 2021
	2	14-Methylmonocrotaline-like	362.155	11.84	+	C ₁₇ H ₂₅ NNaO ₆	Martins <i>et al.</i> , 2015
	1	Hexose/glucose	215.033	1.00	-	C ₆ H ₁₂ OCl	Sans <i>et al.</i> , 2017
	3	Formononetin	267.025	5.76	-	C ₁₆ H ₁₂ O ₄	Xiang <i>et al.</i> , 2009
	4	Isomer of formononetin	267.025	5.78	-	C ₁₆ H ₁₂ O ₄	Xiang <i>et al.</i> , 2009
	5	Isomer of formononetin	2670.25	5.85	-	C ₁₆ H ₁₂ O ₄	Xiang <i>et al.</i> , 2009
Acetone	2	3',4'-Dimethoxyluteolin	315.087	7.83	+	C ₁₇ H ₁₄ O ₆	Lusa <i>et al.</i> , 2016
	1	Azelaic acid	187.097	5.39	-	C ₉ H ₁₆ O ₄	Garelnabi <i>et al.</i> , 2010
	2	apigenin 2"-O-vanilloyl, C-hex, C-pen	7 13.172	6.71	-	C ₃₄ H ₃₄ O ₁₇	Geng <i>et al.</i> , 2016
	4	Demethoxysudachitin	329.174	9.40	-	C ₁₇ H ₁₄ O ₇	Güzel, 2020
	5	Vernolic acid	295.227	10.11	-	C ₁₈ H ₃₂ O ₃	Abdelhafez <i>et al.</i> , 2020
	6	Carsanoic acid	331.191	10.45	-	C ₂₀ H ₂₆ O ₄	Sharma <i>et al.</i> , 2020
	7	12-O-methyl carnosic acid	345.207	10.95	-	C ₂₁ H ₃₀ O ₄	Jesionek <i>et al.</i> , 2017
	8	Naringenin	271.226	11.13	-	C ₁₅ H ₁₂ O ₅	Fang <i>et al.</i> , 2006



Methanol	1	Waltherione D	364.271	3.86	+	C ₂₂ H ₂₂ NO ₄	Arroyo <i>et al.</i> , 2018
	2	Isomer of waltherione D	364.272	4.31	+	C ₂₂ H ₂₂ NO ₄	Cretton <i>et al.</i> , 2020
	6	2-Methoxyestradiol	303.233	10.17	+	C ₁₅ H ₁₀ O ₇	Yuan <i>et al.</i> , 2021
	7	4'-methoxynaringenin	287.203	10.84	+	C ₁₅ H ₁₀ O ₆	Yuan <i>et al.</i> , 2021
	1	Hexose/glucose	215.034	1.00	-	C ₆ H ₁₂ OCl	Sans <i>et al.</i> , 2017
	9	Jaceosidin	329.231	7.00	-	C ₁₇ H ₁₄ O ₇	Paje <i>et al.</i> , 2022
	10	Gramrione	329.232	7.04	-	C ₁₇ H ₁₄ O ₇	Rahman <i>et al.</i> , 2013
	14	Demethoxysudachitin	329.174	9.53	-	C ₁₇ H ₁₄ O ₇	Güzel, 2020
	15	Carsanoic acid	331.190	10.46	-	C ₂₀ H ₂₆ O ₄	Sharma <i>et al.</i> , 2020
	16	12-O-methyl carnosic acid	345.206	10.99	-	C ₂₁ H ₃₀ O ₄	Jesionek <i>et al.</i> , 2017

5.3.4. LCMS analysis of *B. onopordifolia* extracts in negative mode

The LCMS analysis chromatograms in negative mode for aqueous, acetone and methanol extracts of *B. onopordifolia* are displayed in the appendices section (appendices 2, 16, and 24). *B. onopordifolia* methanol, acetone, and aqueous extracts in LCMS analysis in negative mode results are displayed in Table 5.4, while the chromatogram is displayed in the appendices section. Formononetin was detected at Peak 3 and RT 5.76 minutes for *B. onopordifolia* aqueous extracts analysis. Formononetin is an isoflavone reported to have antioxidant, anticancer, antimicrobial, hepatoprotective, antihypertensive and anti-inflammatory activities (Jiang *et al.*, 2019; Dutra *et al.*, 2021). *B. onopordifolia* acetone extracts analysis revealed azelaic acid at peak 1 and RT 5.39 minutes. Unprocessed grains like rye, barley and wheat contain azelaic acid, a saturated dicarboxylic acid. Azelaic acid is well-known for its anti-keratinising, antibacterial, anti-inflammatory, cytotoxic and antioxidant properties, and most people with dermatological conditions tolerate it well (Pasca *et al.*, 2022).

Carsonic acid was detected at Peak 7 and RT 10.45 minutes when *B. onopordifolia* acetone extracts were analysed further. Carsonic acid is a major oxidised derivative of Corsonol, a diterpene found in many species of the Lamiaceae family such as salvia and Rosmarinus (Alonso *et al.*, 2019). Carsonic acid and other related diterpenes exhibit biological properties such as antibacterial, antioxidant, antidiabetic, antitumor, leishmanicidal, antiplasmodial, neuroprotective and inhibitory effects for Human Immunodeficiency Virus (HIV) (Bahri *et al.*, 2016; Alonso *et al.*, 2019). The above-mentioned therapeutic benefits of Carsonic acid may justify the traditional use *B. onopordifolia* in Thaba 'Nchu to treat STIs (Chapter 3). A compound corresponding to Naringenin was detected at peak 8 and RT 11.13 minutes. Naringenin is a flavonoid

found at various concentrations in tomatoes, grapes, lemons, and many other fruits. The compound is known to possess antioxidant, anti-inflammatory, hepatoprotective, anti-inflammatory, anticarcinogenic and many other effects (Patel *et al.*, 2018). At peak 1 and RT 7.00 minutes, *B. onopordifolia* methanol extracts analysis revealed a compound corresponding to a natural flavone Jaceosidin. The compound is known to exhibit a variety of pharmacological activities such as anti-inflammatory, antioxidant, anti-bacterial, antiallergic and anti-cancer activities (Nageen *et al.*, 2021).

5.3.5. LCMS analysis of *H. depressa* extracts in positive mode

The LCMS analysis chromatograms in positive mode for aqueous, acetone and methanol extracts of *H. depressa* are displayed in the appendices section (appendices 4, 9, and 17). The LCMS analysis of *H. depressa* aqueous, acetone, and methanol extracts results in positive mode are displayed in Table 5.5. Peak 3 post *H. depressa* aqueous extracts analysis revealed a compound corresponding to a naturally occurring quinolone alkaloid, Waltherione C at RT 4.85 min. Waltherione C has pharmacological properties such as cytotoxic, anti-trypanosomal, antimutagenic, antimicrobial, anti-HIV, antimalarial, antiviral, and anti-inflammatory activities (Heeb *et al.*, 2011; Tullius *et al.*, 2016; Chu *et al.*, 2019; Nirmala and Sridevi, 2021).

Methoxyestradiol (2ME2) was detected at peak 8 and RT 10.17 min when *H. depressa* acetone extracts were analysed. 2ME2 is an endogenous compound of oestrogen that has been reported to have both antiangiogenic, antitumor effects and has a potential to improve hypertension, glomerulosclerosis, hypercholesterolemia and may other diseases (Pribluda *et al.*, 2000; Mooberry, 2003; Verenich and Gerk, 2010). *H.*

depressa methanol extracts analysis revealed a compound corresponding to 4-O-caffeoylquinic acid at peak 2 and RT 4.11. 4-O-caffeoylquinic acid is a phenolic compound known to possess antioxidant, hyperlipidaemia, and many other activities (Benzarti et al., 2015; Ganzon *et al.*, 2018; Zdunić et al., 2020).

Table 5.5: Most abundant phytochemical compounds in aqueous extracts of *H. depressa* by LCMS analysis in positive and negative mode

Extracts	Peak	Proposed compound	m/z	Tf (min)	[M-H] ⁻	Molecular formula	Ref
Aqueous	1	Waltherione D	364.272	3.88	+	C ₂₂ H ₂₂ NO ₄	Arroyo <i>et al.</i> , 2018
	2	Isomer of Waltherione D	364.271	4.32	+	C ₂₂ H ₂₂ NO ₄	Cretton <i>et al.</i> , 2020
	3	Waltherione C	348.275	4.85	+	C ₂₂ H ₂₁ NO ₃	Silva <i>et al.</i> , 2022
	5	Buxifoliadine D	362.156	11.84	+	C ₂₃ H ₂₃ NO ₃	Wu and Chen, 2000
	7	Quercetin	301.093	5.06	-	C ₁₅ H ₁₀ O ₇	Vacek <i>et al.</i> , 2013
	9	Tricin	329.232	7.00	-	C ₁₇ H ₁₄ O ₇	Bao <i>et al.</i> , 2007
	10	Gramrione	329.231	7.73	-	C ₁₇ H ₁₄ O ₇	Rahman <i>et al.</i> , 2013
Acetone	1	Waltherione D	364.271	4.32	+	C ₂₂ H ₂₂ NO ₄	Cretton <i>et al.</i> , 2020
	4	8-Dihydroantidesmone	344.281	6.00	+	C ₁₉ H ₃₁ NO ₃ Na	Essono Mintsu <i>et al.</i> , 2022
	6	Hibtherin A	349.202	8.20	+	C ₂₂ H ₃₆ O ₃	Ma <i>et al.</i> , 2009
	7	2-Methoxy-5-octylaniline	236.166	9.93	+	C ₁₅ H ₂₆ NO	Zdorichenko <i>et al.</i> , 2019
	8	2-Methoxyestradiol	303.234	10.17	+	C ₁₅ H ₁₀ O ₇	Yuan <i>et al.</i> , 2021
	9	4'-Methoxynaringenin	287.203	10.84	+	C ₁₅ H ₁₀ O ₆	Yuan <i>et al.</i> , 2021
	1	Hexose/glucose	215.034	1.00	-	C ₆ H ₁₂ OCl	Sans <i>et al.</i> , 2017



	7	Jaceosidin	329.232	6.99	-	C ₁₇ H ₁₄ O ₇	Paje <i>et al.</i> , 2022
	8	Docosahexanoic acid	327.216	7.35	-	C ₂₂ H ₃₂ O ₂	Yuan <i>et al.</i> , 2022
	12	Vernolic acid	295.227	10.11	-	C ₁₈ H ₃₂ O ₃	Abdelhafez <i>et al.</i> , 2020
	13	6-Gingerol	293.211	10.48	-	C ₁₇ H ₂₆ O ₄	Yuan <i>et al.</i> , 2021
Methanol	2	4-O-Caffeoylquinic acid	355.104	3.84	+	C ₁₆ H ₁₈ O ₉	Yuan <i>et al.</i> , 2021
	2	Matairesinol	359.135	4.11	-	C ₁₆ H ₂₂ O ₉	Yuan <i>et al.</i> , 2022
	3	Pinoresinol	359.135	4.18	-	C ₁₆ H ₂₂ O ₉	Yuan <i>et al.</i> , 2022
	4	Medioresinol	389.147	4.44	-	C ₂₁ H ₂₄ O ₇	Irakli <i>et al.</i> , 2021
	5	7-Methoxytaxifolin-3-glucoside	403.162	5.10	-	C ₂₁ H ₂₂ O ₁₂	Jaiswal <i>et al.</i> , 2014
	7	Yunnaneic acid F	599.236	6.09	-	C ₂₉ H ₂₆ O ₁₄	Barros <i>et al.</i> , 2013
	8	Salvigenin	329.103	9.16	-	C ₂₀ H ₂₄ O ₄	Yuan <i>et al.</i> , 2022

5.3.6. LCMS analysis of *H. depressa* extracts in negative mode

The LCMS analysis chromatograms in negative mode for aqueous and acetone extracts of *H. depressa* are displayed in the appendices section (appendices 3 and 10). The LCMS analysis of *H. depressa* aqueous and acetone extract results in negative mode are displayed in Table 5.5. Peak 9 from *H. depressa* aqueous extracts analysis showed a compound corresponding to Tricin at RT 7.00. Tricin has bioactivities such as anti-allergy, anti-HIV, special genes inducer, anti-inflammatory, antioxidant, anti-ulcerogenic, anti-virus, potential diabetes suppression, pigmentation inhibition, herbicidal, antibacterial, antifungal, insecticidal, anti-plant-hoppers, and anti-weeds, which are like other flavonoids (Li *et al.*, 2016; Jiang *et al.*, 2020). Peak 7 showed a compound matching with a flavonoid Quercetin at RT 5.06. Quercetin is a widely distributed flavonoid found in various food sources such as fruits, vegetables, nuts, wines, and seeds (Kim and Park, 2018). Quercetin has various biological properties including antioxidant, anti-inflammatory, antibacterial, antiviral, radical-scavenging, gastroprotective, and immune-modulatory activities (Kim and Park, 2018; Massi *et al.*, 2017).

Peak 7 from *H. depressa* acetone extracts analysis revealed a compound corresponding to Jaceosidin. The compound is known for its anti-inflammatory, antioxidant, anti-bacterial, antiallergic and anti-cancer effects (Nageen *et al.*, 2021). 6-Gingerol was detected from *H. depressa* acetone extracts analysis at peak 3 and RT 10.48. 6-Gingerol is one of the major pharmacologically active phenolic compounds found in *Zingiber officinale* commonly known as ginger. 6-Gingerol has been reported to have pharmacological properties such as anticancer, antioxidant, anti-inflammation,

anti-platelet aggregation and antifungal properties (Young *et al.*, 2005; Wang *et al.*, 2014).

Peak 2 from *H. depressa* methanol extracts revealed a compound corresponding to Matairesinol, a lignan-type compound found in seeds, grains, vegetables, and fruits at RT 4.11 (Eklund *et al.*, 2003). The compound has been reported to exhibit toxic effects against T-cell lymphoma and colon cell lines (Su *et al.*, 2015; Yamauchi *et al.*, 2006). However, it has also been reported for its beneficial antioxidant, anti-inflammatory, IgE-suppressive, anti-cancer, and many other effects (Kawahara *et al.*, 2010; Lee *et al.*, 2012; Al-sayed *et al.*, 2020). Peak 3 revealed a compound corresponding to Pinoresinol at RT 4.18, which is a biphenolic compound isolated from a plant *Sambucus williamsii* used in traditional medicine in Europe, Asia, and North Africa to treat analgesic, antiviral, anti-inflammatory, homeostatic and diuretic-related ailments (Hwang *et al.*, 2010). Pinoresinols have been reported to possess antifungal, anti-inflammatory and hepatoprotective properties (Hwang *et al.*, 2010; Kim *et al.*, 2010; During *et al.*, 2012). Salvigenin was detected from *H. depressa* methanol extracts analysis at peak 8 and RT 9.16. Salvigenin is a flavonoid reported to be found in *Salvia officinalis* known to have anti-inflammatory, antitumor, immunomodulatory, cytotoxic, antinociceptive, antidepressant and anxiolytic activities (Noori *et al.*, 2013; Mansourabadi *et al.*, 2016).

5.3.7. LCMS analysis of *S. runcinata* extracts in positive mode

The LCMS analysis chromatograms in positive mode for aqueous, acetone and methanol extracts of *S. runcinata* are displayed in the appendices section (appendices 6, 13, and 19). LCMS analysis of *S. runcinata* aqueous, acetone and methanol extract results in positive mode are displayed in Table 5.6. Peak 9 from *S. runcinata* aqueous extracts analysis revealed a compound corresponding to Genistein, an isoflavone with estrogen-like properties initially isolated from *Genista tinctoria* L. - a plant from the Fabaceae. Moreover, it has been reported to have biological effects such as antioxidant, anti-inflammatory, antibacterial, antiviral, antidiabetic, and many other properties (Sharifi-Rad *et al.*, 2021). Canadine a benzyloquinoline alkaloid was detected at peak 10 and RT 11.84. Canadine is known for its cytotoxic and antioxidant effects (Correch  *et al.*, 2008; Kaur and Arora, 2015).

Peak 1 from *S. runcinata* acetone extracts analyses a compound corresponding to Xanthoxylin which was also detected in *S. runcinata* aqueous extracts at RT 5.36. Xanthoxylin is a natural compound reported to possess cytotoxic and fungicidal properties (Bellando *et al.*, 1985). Cirsimaritin was detected at Peak 3 and RT 7.82. Cirsimaritin is known to possess anticancer, antioxidant, antiparasitic, antinociceptive, and anxiolytic properties (Tasdemir *et al.*, 2006; Benali *et al.*, 2022). Salvigenin compound was detected at peak 5 and RT 9.16. Salvigenin is a compound known for its pharmacological properties, which include analgesic, anti-inflammatory, and antitumor effects (Rafatian *et al.*, 2012; Noori *et al.*, 2013; Mansourabadi *et al.*, 2016).

Chlorogenic acid (CGA) was detected from *S. runcinata* methanol extracts analysis at peak 1 and RT 3.43. CGA is a significant and biologically active dietary polyphenol that has a number of therapeutic effects, including antioxidant activity, antibacterial activity, hepatoprotective activity, cardioprotective activity, anti-inflammatory activity, antipyretic activity, neuroprotective activity, anti-obesity activity, antiviral activity, anti-microbial activity, anti-hypertension activity, free radicals scavenger activity, and CNS stimulator activity (Maalik *et al.*, 2016; Naveed *et al.*, 2018; Miao and Xiang, 2020). At Peak 3 and RT 4.17, the analysis revealed a compound corresponding to Secoxyloganin reported to have antibacterial properties (Xiong *et al.*, 2013). A plant flavonoid, Guaijaverin, was detected at peak 4 and RT 4.57. It is known to have antioxidant activities (Bernardi *et al.*, 2007). Rosmarinic acid was detected at peak 6 and RT 5.28. Rosmarinic acid is commonly found in species of the Boraginaceae and Nepetoideae which is a subfamily of Lamiaceae family (Petersen and Simmonds, 2003). This compound has therapeutic biological benefits such as antiviral, antibacterial, anti-inflammatory, cytotoxic, antioxidant and many other benefits (Petersen and Simmonds, 2003; Amoah *et al.*, 2016). Jaceosidin and 6-gingerol were also detected in *S. runcinata* methanol extracts. These compounds are known to have pharmacologically significant properties (Young *et al.*, 2005; Wang *et al.*, 2014).

Table 5.6: Most abundant phytochemical compounds in *S. runcinata* extracts by LCMS analysis in positive and negative mode

Extracts	Peak	Proposed compound	m/z	Tf (min)	[M-H] ⁻	Molecular formula	Ref
Aqueous	2	Chorismic acid isomer	227.129	4.07	+	C ₁₄ H ₂₆ O ₂	Yuan <i>et al.</i> , 2022
	3	Chorismic acid isomer	227.130	4.55	+	C ₁₄ H ₂₆ O ₂	Yuan <i>et al.</i> , 2022
	4	Xanthoxylin	197.118	5.37	+	C ₁₀ H ₁₂ O ₄	Yuan <i>et al.</i> , 2022
	5	Leukotriene B5	335.219	8.46	+	C ₂₀ H ₃₀ O ₄	Yuan <i>et al.</i> , 2022
	6	7 α -hydroxyallyl-royleanone	331.192	9.53	+	C ₂₀ H ₂₆ O ₄	Zao <i>et al.</i> , 2016
	9	Genistein	270.280	11.39	+	C ₁₅ H ₁₀ O ₅	Yuan <i>et al.</i> , 2022
	10	Canadine	362.155	11.84	+	C ₂₀ H ₂₁ NO ₄	Wang <i>et al.</i> , 2017
	1	Glucose or hexose	215,032	0.936	-	C ₆ H ₁₂ O ₆ Cl ⁻	Sans <i>et al.</i> , 2017
	2	Benazeprilat	395.097	3.19	-	C ₂₂ H ₂₄ N ₂ O ₅	Yuan <i>et al.</i> , 2022
	3	Levoletirizine	387.165	4.07	-	C ₂₁ H ₂₅ ClN ₂ O ₃	Yuan <i>et al.</i> , 2022
	4	Gallocatechin	305.070	4.14	-	C ₁₅ H ₁₄ O ₇	Sharma <i>et al.</i> , 2020
	5	Myrostoleic acid	225.114	4.55	-	C ₁₄ H ₂₆ O ₂	El-Banna <i>et al.</i> , 2022; Mrabti <i>et al.</i> , 2022
	6	Rosmarinic acid	359.075	5.29	-	C ₁₈ H ₁₆ O ₈	Zao <i>et al.</i> , 2016
	7	Salvanoic acid A	493.114	5.75	-	C ₂₆ H ₂₂ O ₁₀	Cvetkovikj <i>et al.</i> , 2013
	8	Docosahexanoic acid	327.219	6.67	-	C ₂₂ H ₃₂ O ₂	Yuan <i>et al.</i> , 2022

	9	Jaceosidin	329.232	6.99	-	C ₁₇ H ₁₄ O ₇	Paje <i>et al.</i> , 2022
	10	Tanshindiol	311.223	8.46	-	C ₁₇ H ₂₆ O ₄	Yang <i>et al.</i> , 2006
	11	Carsonol	329.191	8.52	-	C ₂₀ H ₂₈ O ₄	Yuan <i>et al.</i> , 2022
	12	Carsanoic acid	331.191	10.45	-	C ₂₀ H ₂₆ O ₄	Sharma <i>et al.</i> , 2020
	13	Rosmanol	345.204	10.98	-	C ₂₀ H ₂₆ O ₅	Sharma <i>et al.</i> , 2020
Acetone	1	Xanthoxylin	197.118	5.36	+	C ₁₀ H ₁₂ O ₄	Yuan <i>et al.</i> , 2022
	2	Prolithospermic acid derivative	693.365	7.55	+	Unidentified	Kilinic <i>et al.</i> , 2023
	3	Cirsimaritin	315.090	7.82	+	C ₁₇ H ₁₄ O ₆	Jesionek <i>et al.</i> , 2017
	5	Salvigenin	329.105	9.16	+	C ₂₀ H ₂₄ O ₄	Jesionek <i>et al.</i> , 2017
	6	7 α -hydroxyallyl-royleanone	331.192	9.51	+	C ₂₀ H ₂₆ O ₄	Zao <i>et al.</i> , 2016
	9	Miltipolone isomer	301.217	10.98	+	C ₁₉ H ₂₄ O ₃	Ożarowski <i>et al.</i> , 2017
	3	Gibberellin A44	345.169	7.78	-	C ₂₀ H ₂₆ O ₅	Yuan <i>et al.</i> , 2021
	4	Cortisone	359.184	9.40	-	C ₂₁ H ₂₈ O ₅	Yuan <i>et al.</i> , 2021
	5	Verbascoside	659.357	9.50	-	C ₂₉ H ₃₆ O ₁₅ Cl	Irakli <i>et al.</i> , 2021
	6	Carsanoic acid	331.191	10.45	-	C ₂₀ H ₂₆ O ₄	Sharma <i>et al.</i> , 2020
	7	12-O-methyl carnosic acid	345.207	10.95	-	C ₂₁ H ₃₀ O ₄	Jesionek <i>et al.</i> , 2017
	8	12-O-methyl carnosic acid isomer	345.207	11.13	-	C ₂₁ H ₃₀ O ₄	Jesionek <i>et al.</i> , 2017
	1	Chlorogenic acid	353.088	3.43	+	C ₁₆ H ₁₈ O ₉	Kilinic <i>et al.</i> , 2023
	3	Secoxyloganin	403.123	4.17	+	C ₁₇ H ₂₄ O ₁₁	Pferschy-Wenzig <i>et al.</i> , 2022

Methanol	4	Guaijaverin	433.133	4.57	+	C ₂₀ H ₁₈ O ₁₁	Jaiswal et al., 2014
	5	7-Methoxytaxifolin-3-glucoside	479.1	5.10	+	C ₂₁ H ₂₂ O ₁₂	Jaiswal et al., 2014
	6	Rosmarinic acid	359.078	5.28	+	C ₁₈ H ₁₆ O ₈	Zao et al., 2016
	9	Jaceosidin	329.232	6.99	+	C ₁₇ H ₁₄ O ₇	Paje et al., 2022
	10	6-Gingerol	293.212	9.53	+	C ₁₇ H ₂₆ O ₄	Yuan et al., 2021
	11	7-Methyltaxifolin	317.211	10.17	+-	C ₁₅ H ₂₄ O	Chai et al., 2019
	12	Rosmanol	345.206	10.98	+	C ₂₀ H ₂₆ O ₅	Sharma et al., 2020

5.3.8. LCMS analysis of *S. runcinata* extracts in negative mode

The LCMS analysis chromatograms in negative mode for aqueous, acetone and methanol extracts of *S. runcinata* are displayed in the appendices section (appendices 5, 14, and 20). The LCMS analysis of *S. runcinata* aqueous, acetone and methanol extracts in negative mode is displayed in Table 5.6. Peak 6 from *S. runcinata* aqueous extracts analysis revealed a compound corresponding to Rosmarinic acid. Rosmarinic acid was also detected in *S. runcinata* methanol extract analysis in positive mode. Salvanoic acid A was detected at peak 7 and RT 5.75. Salvanoic acid A is a natural phenolic acid of caffeic acid derivative isolated from *Salvia miltiorrhiza* roots. The compound is known for its beneficial properties such as antioxidant, anticancer and hepatoprotective effects (Wu *et al.*, 2007; Zhao *et al.*, 2008; Ma *et al.*, 2019). Peak 5 from *S. runcinata* acetone extracts analysis showed a compound corresponding to Verbascoside known to have pharmacological properties including antioxidant, anti-inflammatory, and antineoplastic properties as well as numerous wound-healing and neuroprotective properties (Alipieva *et al.*, 2014). The presence of Verbascoside compound in *S. runcinata* may explain why the plant has long been used to treat ulcers and throat inflammation (Chapter 4). Carnosic acid was detected at peak 7 and RT 10.45 which is a phenolic diterpene with pharmacologically important properties (Theoduloz *et al.*, 2011, Loussouarn *et al.*, 2017).

5.3.8. LCMS analysis of *S. columbaria* extracts in positive mode

The LCMS analysis chromatograms in negative mode for aqueous, acetone and methanol extracts of *S. columbaria* are displayed in the appendices section (appendices 8, 11, and 21). The LCMS analysis of *S. columbaria* aqueous, acetone and methanol extracts in positive mode is displayed in Table 5.7. LCMS analysis of *S.*

columbaria aqueous extracts revealed a compound corresponding to 4-O-caffeoylquinic acid at peak 2 and RT 3.85. 4-O-caffeoylquinic acid is a phenolic compound known to have health benefits such as antioxidant, anticancer, antiviral, neuroprotective and may other activities (Ganzon *et al.*, 2018; Alcázar Magaña *et al.*, 2021). Canadine was also detected at peak 8 and RT 11.84. Canadine has biological effects such cytotoxic and antioxidant effects (Correche *et al.*, 2008; Kaur and Arora, 2015). Peak 11 and RT 7.80 from *S. columbaria* acetone extract analysis revealed a compound corresponding to Eupatilin, a flavone which has been isolated from a variety of medicinal plants in the *Artemisia* genus. The compound has a variety of biological properties such as anti-cancer, anti-inflammatory, antioxidant, neuroprotective, anti-allergic and cardioprotective activities (Choi *et al.*, 2022; Nageen *et al.*, 2020). Cirsimaritin was detected at Peak 1 and RT 7.82 from *S. columbaria* methanol extracts analysis. The compound is known for its pharmacological properties such as anticancer, antimicrobial, antidiabetic, antiparasitic, antioxidant, and anti-inflammatory effects (Benali *et al.*, 2022).

Table 5.7: Most abundant phytochemical compounds in *S. columbaria* extracts by LCMS analysis in positive and negative mode

Extracts	Peak	Proposed compound	m/z	Tf (min)	Mode	Molecular formula	Reference
Aqueous	2	4-O-caffeoylquinic acid	355.105	3.85	+	C ₁₆ H ₁₈ O ₉	Yuan <i>et al.</i> , 2021
	3	Sweroside	359.135	4.11	+	C ₁₆ H ₂₂ O ₉	Kilinic <i>et al.</i> , 2023
	4	Sweroside isomer	359.135	4.18	+	C ₁₆ H ₂₂ O ₉	Kilinic <i>et al.</i> , 2023
	5	Peonidin-3-O-β-D-glucoside	463.124	4.43	+	C ₂₂ H ₂₃ O ₁₁	Yuan <i>et al.</i> , 2021
	8	Canadine	362.155	11.84	+	C ₂₀ H ₂₁ NO ₄	Wang <i>et al.</i> , 2017
	1	Hexose/glucose	215.035	1.00	-	C ₆ H ₁₂ OCl	Sans <i>et al.</i> , 2017
	3	Chlorogenic acid	353.087	3.45	-	C ₁₆ H ₁₈ O ₉	Kilinic <i>et al.</i> , 2023
	5	Apigenin 6-C-β -xylopyranosyl-8-C-(6'''- O-(3-hydroxy-3- methylglutaroyl)-β-glucopyranoside)	707.179	3.85	-	C ₃₂ H ₃₆ O ₁₈	Wang <i>et al.</i> , 2016
	6	Gentiopicroside	403.123	4.16	-	C ₁₆ H ₂₀ O ₉ FA	Zao <i>et al.</i> , 2016
	7	Gentiopicroside isomer	403.121	4.19	-	C ₁₆ H ₂₀ O ₉ FA	Zao <i>et al.</i> , 2016
	8	2,6-Di-tert-butyl-4-methylphenol	219.085	4.51	-	C ₁₅ H ₂₄ O	Yuan <i>et al.</i> , 2021
	9	Coumarin + 1O + 1MeO, O-Hex-Hex	515.117	5.17	-	C ₂₂ H ₂₈ O ₁₄	Yuan <i>et al.</i> , 2021
	10	Alisol C	521.165	5.49	-	C ₃₀ H ₄₆ O ₅	Zhu <i>et al.</i> , 2020

	11	Amarogentin	585.219	6.33	-	C ₂₉ H ₃₀ O ₁₃	Vipul <i>et al.</i> , 2005
	12	Tricin	329.233	7.00	-	C ₁₇ H ₁₄ O ₇	Bao <i>et al.</i> , 2007
Acetone	1	Eudesmic acid	213.076	3.82	+	C ₁₀ H ₁₂ O ₅	Yuan <i>et al.</i> , 2022
	2	Sweroside	359.135	4.09	+	C ₁₆ H ₂₂ O ₉	Kilinic <i>et al.</i> , 2023
	3	Sweroside isomer	359.134	4.16	+	C ₁₆ H ₂₂ O ₉	Kilinic <i>et al.</i> , 2023
	4	Dihydroxy-triacetoxy-tetratriacontanoic acid	759.273	4.56	+	C ₄₀ H ₇₄ O ₁₀ Na ₂	Pferschy-Wenzig <i>et al.</i> , 2022
	5	Secoxyloganin devivative	403.163	5.10	+	C ₁₇ H ₂₄ O ₁₁	Pferschy-Wenzig <i>et al.</i> , 2022
	6	Xanthoxylin	197.119	5.38	+	C ₁₀ H ₁₂ O ₄	Yuan <i>et al.</i> , 2022
	8	Trihydroxyoctadecenoic acid	331.218	6.40	+	C ₁₈ H ₃₄ O ₅	Pferschy-Wenzig <i>et al.</i> , 2022
	11	Eupatilin	345.099	7.80	+	C ₁₈ H ₁₆ O ₇	Yuan <i>et al.</i> , 2021
	12	Trihydroxy octadecadienoic acid isomer	329.105	9.16	+	C ₁₈ H ₃₄ O ₅	Pferschy-Wenzig <i>et al.</i> , 2022
	14	Miltipolone isomer	301.217	10.17	+	C ₁₉ H ₂₄ O ₃	Ożarowski <i>et al.</i> , 2017
	1	Loganin	389.108	3.66	-	C ₁₇ H ₂₆ O ₁₀	Chai <i>et al.</i> , 2019
	2	Cauloside C derivative	747.232	3.81	-	C ₄₁ H ₆₆ O ₁₃	Kılınç <i>et al.</i> , 2020
	3	Secoxyloganin	403.123	4.18	-	C ₁₇ H ₂₄ O ₁₁	Pferschy-Wenzig <i>et al.</i> , 2022
	4	Guaijaverin	433.133	4.57	-	C ₂₀ H ₁₈ O ₁₁	Jaiswal <i>et al.</i> , 2014
	5	7-Methoytaxifolin-3-glucoside	479.180	5.10	-	C ₂₁ H ₂₂ O ₁₂	Jaiswal <i>et al.</i> , 2014
	6	Laciniatoside V derivative	583.207	5.63	-	C ₁₆ H ₂₀ O ₉	Sarikahya <i>et al.</i> , 2019
	7	Laciniatoside V	585.222	6.32	-	C ₂₇ H ₃₈ O ₁₄	Sarikahya <i>et al.</i> , 2019

	8	Dihydrokaempferol-3-O-(6"-malonyl)-glucoside	331.293	7.99	-	C ₂₄ H ₂₂ O ₁₄	Mascherpa <i>et al.</i> , 2012
	9	Vernolic acid	295.227	10.11	-	C ₁₈ H ₃₂ O ₃	Abdelhafez <i>et al.</i> , 2020
	10	Vernolic acid	295.226	10.48	-	C ₁₇ H ₂₆ O ₄	Yuan <i>et al.</i> , 2021
	11	7-Methyltaxifolin	317.211	10.17	-	C ₁₅ H ₂₄ O	Chai <i>et al.</i> , 2019
Methanol	1	Cirsimaritin	315.090	7.82	+	C ₁₇ H ₁₄ O ₆	Jesioneck <i>et al.</i> , 2017
	2	Trihydroxyoctadecadienoic acid isomer	329.105	9.16	+	C ₁₈ H ₃₄ O ₅	Pferschy-Wenzig <i>et al.</i> , 2022
	3	7 α -hydroxyallyl-royleanone	331.192	9.51	+	C ₂₀ H ₂₆ O ₄	Zao <i>et al.</i> , 2016
	4	Miltipolone isomer	301.217	10.17	+	C ₁₉ H ₂₄ O ₃	Ożarowski <i>et al.</i> , 2017

5.3.9. LCMS analysis of *S. columbaria* extracts in negative mode

The LCMS analysis chromatograms in negative mode for aqueous and acetone extracts of *S. columbaria* are displayed in the appendices section (appendices 7 and 12). The LCMS analysis of *S. columbaria* aqueous and acetone extract results in negative mode are displayed in Table 5.7. LCMS analysis of *S. columbaria* aqueous extracts showed compound Chlorogenic acid (CGA) at peak 3 and RT 3.45. CGA is a significant and biologically active dietary polyphenol with several pharmacologically important effects as mentioned earlier in this chapter. Gentiopicroside was detected at peak 6 and RT 4.16 for the same extracts analysis. The compound is a secoiridoid glycosides isolated from a Chinese medicinal plant *Gentiana macrophylla* Pall associated with the downregulation of inflammatory cytokines such as NO (Nitric oxide), PGE2 (Prostaglandin E2), and IL-6 (Interleukin 6), and the suppression of iNOS (Inducible nitric oxide synthase) and COX-2 (Cyclooxygenase-2) (Zhang *et al.*, 2019). Additionally, it is known for its anticancer and antioxidant properties (Huang *et al.*, 2020, Jin *et al.*, 2020). At Peak 11 and RT 6.33, Amarogenin was detected from the analysis of *S. columbaria* aqueous extracts. Amarogentin is a bitter secoiridoid glycoside present in *Swertia chirata* which is an activator of human bitter taste receptor. This compound is a bitter secoiridoid glycoside found in *Swertia chirata* which activates the human bitter taste receptor (Patel *et al.*, 2019). Pharmacologically, it has been demonstrated to have anti-leishmanial, antibacterial, anti-hepatitis, anticholinergic, and chemo-preventive effects (Patel *et al.*, 2019). Peak 12 and RT 7.00 from *S. columbaria* aqueous extracts analysis revealed a compound corresponding to Tricin. Tricin was reported to have a protective effect against LPS-induced inflammation, antibacterial and antioxidant activities (Shalini *et al.*, 2016; Barrera *et al.*, 2020). Peak 1 and RT 3.66 from *S. columbaria* acetone extracts analysis

corresponded to Loganin, an iridoid glycoside isolated from *Cornus officinalis* which was recently reported to exhibit anti-inflammatory and antioxidant effects (Park *et al.*, 2021).

5.4. CONCLUSION

The phytochemical analysis of *Scambiosa columbaria*, *Hermannia depressa*, *Salvia runcinata*, and *Berkheya onopordifolia* extracts in this chapter has revealed that the plants contain various bioactive compounds that can be classified under alkaloids, tannins, terpenoids, steroids, phenols, glycosides, saponins and flavonoids. These compounds are known to have various therapeutic benefits, which is critical in the development of new drugs in the pharmaceutical industry. Because of their previously investigated properties, the detected plant compounds in this chapter can be used to develop antimicrobial, anticancer, anti-inflammatory, and antioxidant agents which may also be useful in the treatment of STIs. The results of this chapter further validate the use *S. columbaria*, *H. depressa*, *S. runcinata* and *B. onopordifolia* to traditionally treat various ailments due to the presence of these pharmacologically important compounds. Further research into the isolation of these bioactive compounds could lead to the discovery of novel compounds and drugs. Toxicity of medicinal plants is usually caused by the presence of some of the bioactive compounds. Even though it is necessary to investigate medicinal plants therapeutic properties, it is equally important to investigate their safety. The next chapter will investigate the cytotoxicity of *B. onopordifolia*, *H. depressa*, *S. columbaria* and *S. runcinata*.

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CHAPTER 6³

In vitro* cytotoxicity of the extracts of *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata* and *Scambiosa columbaria

ABSTRACT

The toxicity of *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata*, and *Scambiosa columbaria* extracts was evaluated against human cells. The aforementioned plants have a long history of being used to treat a number of diseases including STIs in various South African communities. The investigation of the therapeutic properties of medicinal plants is important, but so is knowing their cytotoxic properties to avoid all negative effects associated with their use. The cytotoxicity of *B. onopordifolia*, *H. depressa*, *S. runcinata*, and *S. columbaria* extracts was evaluated using Hoechst 33342 nuclear dye and propidium iodide exclusion method against African green monkey kidney cell line or Vero cells. All *B. onopordifolia* as well as *S. columbaria* aqueous and methanol extracts had no significant toxic effects against Vero cells at concentrations ranging from 400-100µg/ml. *S. runcinata* methanol extracts showed toxicity by affecting 50% of Vero cells at half maximal cytotoxic concentration (CTC₅₀) = 3.93 µg/ml, while acetone extracts showed toxic effects at CTC₅₀=3.96 µg/ml. At CTC₅₀=6.09µg/ml, *S. columbaria* acetone extracts showed toxic effects against Vero cells. *H. depressa* methanol and acetone extracts showed significant toxic effects against Vero cells at CTC₅₀ = 4.97µg/ml and 4.65 µg/ml respectively. It can be concluded that *S. runcinata*, *S. columbaria* and *H. depressa* need to be used with caution when administered for treatment of STIs and other

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diseases due to the toxic effects they exhibited against the African green monkey kidney cell line.

6.1. INTRODUCTION

Plants have been used by native people around the world for the treatment of diseases for a long time. Botanical medicine is becoming increasingly popular worldwide, especially in developing countries where medicinal plants are available and affordable (Kharchoufa *et al.*, 2018). Medicinal plant users believe that in contrast to synthetic drugs they have less side effects. The widespread belief that herbal medicine is extremely safe and have no negative side effects is misleading (Kharchoufa *et al.*, 2018). While medicinal plants have healing properties, numerous studies have shown that they are not exempt from the development of a wide range of chemical compounds that cause adverse reactions that are carcinogenic, teratogenic, and life-threatening (Tamilselvan *et al.*, 2014; Kuete, 2014; Kharchoufa *et al.*, 2018).

According to the American Association of Poison Control Centre's annual report herbs are the cause of 3–5% of all poisoning in the U.S (Mowry *et al.*, 2013). A survey conducted in a South African hospital revealed that contamination with medicinal plants has been reported to be the second common cause of acute poisoning, accounting for 12.1% of all the cases (Botha and Penrith, 2008). Most cases of poisoning from the use of medicinal plants are not documented because patients may die before they arrive at the hospital, and people are generally reluctant to admit that they use traditional medicine because of the social stigma of their use (Kharchoufa *et al.*, 2018).

Approximately 1.5 million plants have been studied worldwide and most of them have been found to contain potentially toxic secondary metabolites (Mounanga *et al.*, 2015). Plants produce toxic secondary metabolites based on their molecular structure, which include alkaloids, saponins, glycosides, toxic amino acids and terpenoids. The toxicity of a given plant has been shown to depend on various factors such as the strength of toxic secondary metabolites, the amount consumed, the time of exposure, different parts of the plant (root, oil, leaves, stem bark and seeds), individual body chemistry, climate and soil, and genetic differences within the species (Celik, 2012).

Some medicinal plants must be used with caution because most of them can cause different toxicity effects such as acute toxicity, cytotoxicity, cardiotoxicity, genotoxicity, hepatotoxicity and many others (Ramulondi *et al.*, 2019). One of the problems associated with using medicinal plants in rural settings is that they are sometimes used without recommended normal dosages and ultimately lead to overdose (Usman *et al.*, 2014). Acute toxicity due to consumption of plant medicine is common in South Africa and due to undocumented records, the mortality rate is estimated at 80 000 yearly (Meel, 2007; Botha and Penrith, 2008). For South Africa, these statistics are not shocking as the country is rich in plant diversity which includes the use of a variety of medicinal plants with minimal toxicity information.

In South Africa, there are plants used to treat diseases that have already tested positive for various toxicities. Most plants act poisonous when used at higher concentrations but tend to have healing properties at lower concentrations (Ndhlala *et al.*, 2013). For example, the roots of *Adenia gummifera* have tested positive for antiviral activity; however, the plant contains glycosides that may cause vomiting, fits,

liver, and kidney damage (Wink and Van Wyk, 2008). *Aloe Ferox* Mill is traditionally used as a laxative, a relief for arthritis, sinusitis, wound healing, and for the treatment of STIs and many other infections. In higher concentrations the plant can cause intestinal bleeding, heightened menstrual and uterus bleeding (Kambizi *et al.*, 2007). *Artemisia afra* Jacq. ex Willd is also used traditionally to treat colds, asthma, pneumonia, and stomach ailments, amongst others. However, at higher dosages it may cause vomiting, diarrhoea, hallucinations, comas, and seizures (Van Wyk *et al.*, 2009). In Chapter 3, it was mentioned that *Hermannia depressa*, *Scambiosa columbaria*, *Salvia runcinata*, and *Berkheya onopordifolia*, along with other plants, are orally administered for the treatment of STIs such as gonorrhoea, vaginosis, herpes zoster, pubic lice, and symptoms associated with HIV. However, some of these medicinal plants may have adverse effects on human health. Therefore, this chapter aims to specifically investigate the toxicity of *B. onopordifolia*, *H. depressa*, *S. runcinata*, and *S. columbaria*.

6.2. METHODS

6.2.1. Extract preparation

Methanol, acetone, and aqueous extracts of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* were prepared in the same manner described in Chapter 5. Extracts were further reconstituted in dimethyl sulfoxide (DMSO) to give a final concentration of 100 mg/ml. Samples were sonicated if solubility was a problem and stored at 4°C until required.

6.2.2. Cytotoxicity screening

The cytotoxicity of plant extracts was carried out using the Hoechst 33342 nuclear dye and propidium iodide exclusion method according to Valiyari *et al.* (2013) and Bellamakondi *et al.* (2014). The African green monkey kidney cell line (Vero cells) was used for cytotoxicity screening. They were maintained at 37°C in a humidified incubator with 5% CO₂ in 10 cm culture dishes. Complete growth medium consisted of 10% FBS (Fetal bovine serum) supplemented by DMEM (Dulbecco's Modified Eagle's Medium). In each well cells were seeded into 96 well microtiter plates with a density of 4 000 cells/well using a volume of 100 µl. The microtiter plates were incubated for 24 hours at 37 °C, 5 % CO₂ and 100% relative humidity before adding test compounds to allow cell attachment. Cells were treated with 100, 200 and 400 µg/ml of each extract diluted in culture medium. The cells were also treated with the positive control called Melphalan at 100 µg/ml. One hundred microliter aliquots of the diluted extract in fresh medium were used to treat cells. Cells were incubated for a further 48 hours. Treatment medium was aspirated from all wells and replaced with 100 µl of Hoechst 33342 nuclear dye (5 µg/ml) and incubated for 10 minutes at room temperature. Thereafter, cells were stained with propidium iodide (PI) at 100 µg/ml to enumerate the proportion of dead cells within the population. Cells were imaged immediately after addition of PI using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices) with a 10x Plan Fluor objective, DAPI and Texas Red filter cubes. Nine image sites were acquired per well, which is representative of roughly 75% of the surface area of the well. The dye exclusion technique assumes that non-viable cells will absorb the dye whereas viable ones would not (Bellamakondi *et al.*, 2014). After 24 hours of treatment with plant extracts, the total cell count was determined using a haemocytometer. If the live cell number in the presence of the

extract is half or less than half of the untreated control (UT) value, the extract is regarded possibly cytotoxic (Bellamakondi *et al.*, 2014). The percentage growth inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = (\text{Total cells-dead cells} / \text{Total cells}) \times 100.$$

For each cell line, the percentage inhibition was plotted versus concentration, and the **CTC₅₀** (concentration needed to decrease viability by 50%) value was determined. All experiments were carried out in triplicates. The results were calculated as mean $\pm$ standard deviation (SE) values.

6.2.3. Statistical analysis

All experiments were conducted in triplicates and the data was presented as the mean $\pm$ standard deviation. Linear regression analysis was used to calculate IC₅₀ values. Microsoft Excel® was used to enter and capture data. Various graphs and tables were extracted from this data.

6.3. RESULTS AND DISCUSSION

6.3.1. Cytotoxicity of plant extracts against Vero cells

The cytotoxicity effects of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts were evaluated using Hoechst 33342 nuclear dye and propidium iodide exclusion method. All the aqueous, methanol and acetone extracts were tested against normal Vero cells at a concentration ranging from 400-100 $\mu\text{g/ml}$. Table 6.1 displays the cytotoxicity results of *B. onopordifolia*, *H. depressa*, *S. runcinata*, and *S. columbaria* aqueous, methanol, and acetone extracts. The data is presented in terms

of the number of live and dead cells, as well as the CTC₅₀ values obtained after treating the Vero cells with the extracts. The percentage of cell viability is presented in Figure 6.1.

6.3.2. The cytotoxicity of *B. onopordifolia* extracts

B. onopordifolia methanol and aqueous extracts showed no indications of toxicity against normal Vero cells. However, acetone extracts showed toxicity when concentration was increased to 400 µg/ml. In Chapter 3, it was reported that traditional healers usually boil plant samples to prepare for their remedies, therefore, the findings of this chapter promote the use of *B. onopordifolia* methanol and aqueous extracts for the treatment of STIs at concentrations evaluated in this chapter since they showed no sign of toxicity.

Table 6.1: The cytotoxicity results of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts against normal Vero cells

Plant name	Extract	Concentration (µg/ml)	Live cells (Cells/ml) (Mean ±SD)	Percentage cell viability (%)	CTC ₅₀ (µg/ml) ±SD
<i>B. onopordifolia</i>	Acetone	100	1568±115	111.10	5.25±0.35
		200	1027±60	72.80	
		400	668±50	47.34	
	Methanol	100	1919±43	136.00	ND
		200	2113±32	149.75	
		400	2051±104	145.36	
	Aqueous	100	1480±55	105.03	34.32±12.45
		200	1501±78	106.38	
		400	1373±59	97.30	

<i>S. runcinata</i>	Acetone	100	997±54	70.66	3.96±0.11
		200	322±195	22.82	
		400	49±25	3.47	
	Methanol	100	1021±37	72.36	3.93±0.02
		200	394±28	27.92	
		400	13±6	0.92	
	Aqueous	100	1778±77	126.01	21.13±4.93
		200	1616±87	114.52	
		400	1585±41	112.33	
<i>S. columbaria</i>	Acetone	100	1227±96	86.96	6.09±0.99
		200	903±83	63.99	
		400	568±90	40.26	
	Methanol	100	1367±39	96.88	9.56±1.12
		200	1099±81	85.86	
		400	1005±69	71.23	
	Aqueous	100	1640±67	116.23	204.63±228.98
		200	1668±51	118.21	
		400	1556±93	110.28	
<i>H. depressa</i>	Acetone	100	1296±31	91.84	4.65±0.06
		200	698±36	49.46	
		400	346±31	24.52	
	Methanol	100	1649±29	116.87	4.97±0.10
		200	1120±51	79.38	
		400	529±29	37.49	
	Aqueous	100	1708±21	121.05	16.19±3.28
		200	1618±49	114.67	
		400	1455±86	98.91	
UT		100	1411±54	100	ND

Melphalan or positive control (PC)		100	161±12	11.41	16.70±11.81
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ND=not determined; All experiments were conducted in triplicates; All the data was expressed as means ± standard deviation; CTC₅₀= Half maximal cytotoxic concentration

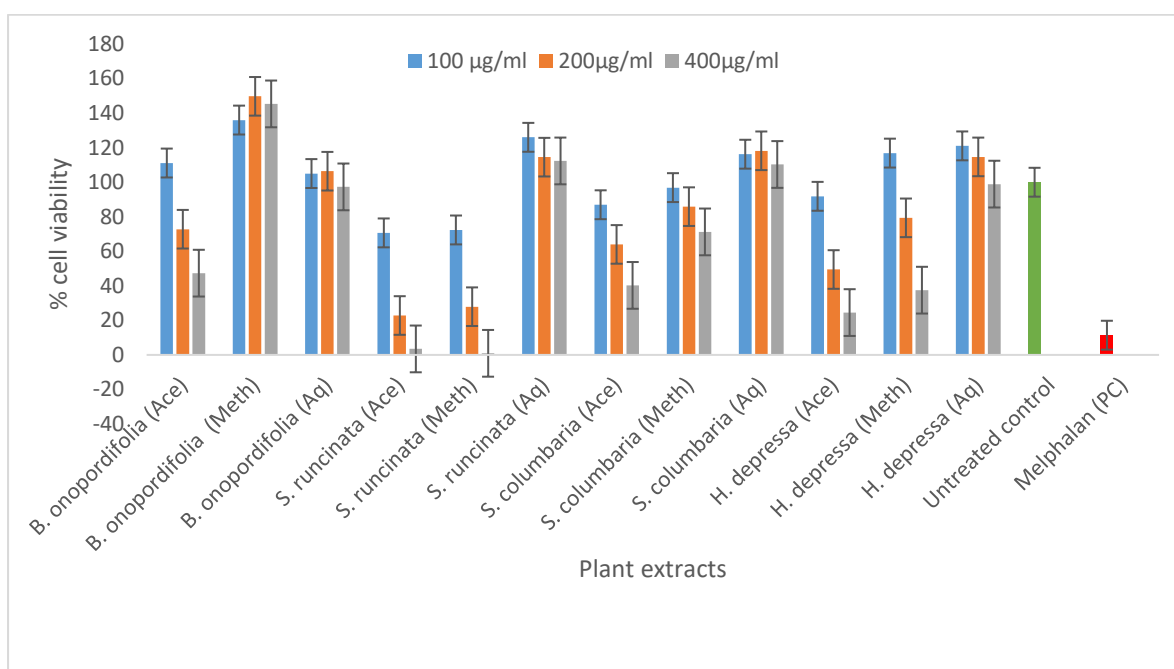


Figure 6.1: Cell viability of Vero cells after exposure to the positive control or different concentrations of *B. onopordifolia*, *S. runcinata*, *S. columbaria* and *H. depressa* extracts

6.3.3. The cytotoxicity of *S. runcinata* extracts

S. runcinata methanol extracts showed toxicity at 200 and 400 g/ml. There was a decrease in cell count from 1411 cell/ml (UT) to 394 cell/ml at 200 µg/ml, which further decreased to 13 cells/ml when the concentration was increased to 400 µg/ml (Table 6.1). The total cell count was reduced by more than 50% cell viability, thus showing an inhibitory action (Figure 6.1). The CTC₅₀ was found to be 3.93 µg/ml which is

extremely low when compared to the positive control CTC₅₀, thus indicating significant toxicity. *S. runcinata* acetone extracts were extremely hazardous to Vero cells at 200 and 400 µg/ml concentrations. There was a decrease in cell count from 1411 cells/ml (UT) to 322 cells/ml at 200 µg/ml. Cell counts further drastically decreased to 49 cells/ml at 400µg/ml. The cell viability decreased by more than 50% when the cells were exposed to *S. runcinata* acetone extracts at 200 and 400 µg/ml (Figure 6.1) *S. runcinata* aqueous extracts showed no signs of toxicity against Vero cells at concentrations ranging 400-100 µg/ml. Plants from the *Salvia* genus such as *Salvia dolomitica*, *Salvia garipensis*, *Salvia lanceolata*, *Salvia muiirii*, *Salvia namaensis*, *Salvia repens* have been previously reported to have toxicity against three human cancer cell lines, such as breast adenocarcinoma (MCF-7), the colon adenocarcinoma (HT-29) and the glioblastoma (SF-268) cell lines, using the sulforhodamine B assay which shows (Kamatou, 2008). *S. runcinata* was also reported positive for the above-mentioned toxicity. This indicates good agreement between sulforhodamine B assay and Hoechst 33342 nuclear dye and the propidium iodide exclusion method. Alkaloids and various phenolic compounds such as flavonoids and phenolic glycosides are common in most *Salvia* species including *S. runcinata*, as reported in chapter 5 (Lu and Foo, 2002; Kamatou, 2006). Alkaloids are known to have toxic effects to both humans and animals (Kamatou et al., 2008). Therefore, the cytotoxicity displayed by *S. runcinata* in this study may be attributed to the presence of alkaloids.

6.3.4. The cytotoxicity of *S. columbaria* extracts

S. columbaria aqueous and methanol extracts indicated no toxicity against Vero cells, indicating that it is safe to use the plant for medicinal purposes including the treatment of STIs. *S. columbaria* acetone extracts exhibited toxicity effects against Vero cells at

400 µg/ml because the cell viability was reduced by more than 50%, where the total cell count was reduced from 1411 cells/ml (UT) to 568 cells/ml. *S. columbaria* was reported to contain compounds such as alkaloids, flavonoids, phenolics, tannins, steroids, terpenoids, and saponins (Sagbo et al., 2020). Various studies have reported the toxicity of flavonoids against various cell lines such as human promyelocytic leukemia cells, human acute myelogeneous leukemia cell, rhesus monkey kidney cells, rat glial tumor cells, bovine leukemia virus-transformed lamb embryo kidney fibroblasts, mouse, and hamster pancreatic b-cells (Matsuo et al., 2005; Kuete et al., 2014; Lay et al., 2014). Therefore, cytotoxicity of *S. columbaria* extracts displayed in this study may be due to the presence of the toxic compounds such as alkaloids and flavonoids found in the plant.

6.3.5. The cytotoxicity of *H. depressa* extracts

No cytotoxicity was observed when normal Vero cells were treated with *H. depressa* aqueous extracts. These findings contradict those of Mofele (2013), who reported that *H. depressa* aqueous extracts were toxic to Brine shrimps at concentrations ranging from 0.2 mg/ml to 0.8 mg/ml. At 400 µg/ml, *H. depressa* methanol extracts demonstrated toxicity against Vero cells, with viable cells decreasing to 37.49% with a low CTC₅₀ when compared to the positive control. *H. depressa* acetone extracts showed cytotoxic activity against Vero cells at concentrations of 400 and 200 µg/ml. The cell count dropped from 1411 cells/ml (UT) to 698 cells/ml at 200 g/ml, showing cytotoxic effects. The cell count was further decreased to 346 cells/ml when the concentration was increased to 400 µg/ml, where in both concentrations the viable cells were decreased by more than 50%. A study by Xaba (2016) reported the presence of tannins, flavonoids, and alkaloids in *H. depressa* aqueous, ethanol,

methanol, and acetone extracts. Anorexia, depression, ruminal atony, hepatic and renal failure, ulcers along the digestive system, and acute gastroenteritis are toxic effects that have been linked to tannins (Frutos *et al.*, 2004). Although alkaloids also offer therapeutic benefits such as antibacterial, antioxidant, anticancer, and anti-inflammatory effects, they may also be toxic if consumed in high amounts (Kaur and Arora, 2015). Acute and chronic toxicity, stomach discomfort, nausea, vomiting, diarrhoea, and oedema, as well as toxic carcinogenic and genotoxic effects are all possible side effects of alkaloids such as alkaloids (Koleva, 2012; Shimshoni *et al.*, 2015; Matsuura and Fett-Neto, 2015). As a result, the presence of alkaloids and tannins may have contributed to the toxicity of *H. depressa* acetone and methanol extracts against Vero cells demonstrated in this chapter.

6.4. CONCLUSION

This chapter investigated the toxicity of different extracts of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria*. All aqueous extracts from all the plants tested in this study showed no toxicity against Vero cells, which supports the use of water to prepare remedies for the treatment of STIs. *S. runcinata* acetone and methanol extracts showed extremely strong toxicity at higher concentrations. Their toxicity is considered physiologically relevant, which may raise concerns with regards to their safety (Gertsch, 2009). *H. depressa* methanol and acetone extracts, and *S. columbaria* and *B. onopordifolia* acetone extracts showed to only exhibit toxicity at the highest concentrations. However, these high concentrations toxicity may be considered physiologically irrelevant (Gertsch, 2009). Although some extracts from *H. depressa*, *S. runcinata*, *S. columbaria* and *B. onopordifolia* have shown to be hazardous to Vero cells, this does not imply that the use of these plants should be

prohibited; however, they should be used with caution. Additional investigations are required to assess the cytotoxic effects of isolated compounds to validate their potential contribution to toxicity or the safety profile of the plants.

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CHAPTER 7⁴

Antimicrobial activity of crude extracts of *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata* and *Scambiosa columbaria*

ABSTRACT

Antimicrobial activity of plants is a current subject of interest for researchers in a quest for the development of new and effective antimicrobial agents against various diseases, including STIs. This chapter evaluates the effectiveness of methanol, acetone, and aqueous extracts of *Berkheya onopordifolia*, *Hermannia depressa*, *Scambiosa columbaria* and *Salvia runcinata* to inhibit the growth of nine bacterial and three yeast species using the broth microdilution method. *H. depressa* methanol extracts inhibited the growth of 3 yeast and eight bacterial species at concentrations ranging from 0.3 to 1.25 mg/ml. Acetone extracts at concentrations ranging from 0.1 to 1.25 mg/ml inhibited the growth of six bacterial and two yeast species. *S. columbaria* methanol extracts exhibited growth inhibition of seven bacterial and two yeast species. Acetone extracts showed growth inhibition of five bacterial and one yeast species. At concentrations ranging from 0.1 to 1.25 mg/ml, *S. runcinata* methanol extracts inhibited the growth of 10 bacterial and three yeast species, while acetone extracts inhibited the growth of eight bacterial species (including colistin resistant *E. coli*) and two yeast species at the same concentration range. *B. onopordifolia* methanol extracts inhibited the growth of 10 bacterial and two yeast species at concentrations ranging from 0.6 to 1.25 mg/ml, while acetone extracts were also effective at concentrations 0.6 mg/ml against four bacterial and two yeast species. Most of the plant extracts exhibited good to moderate activity against the tested microbial species. However, the

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extracts did not display selectivity towards specific microbial species, implying that the observed antimicrobial activity could be associated with potential toxicity of the plants. These plant extracts proved to be potentially effective and can be used as natural alternative agents against microbial diseases. Further studies on plant compounds need to be employed to understand the mechanism of action of the cytotoxicity before ruling out their therapeutic value.

7.1. INTRODUCTION

Every year, millions of people are killed by infectious diseases. They are one of the world's biggest causes of morbidity and mortality rates among people under the age of 50 years, especially in developing countries where infectious diseases account for 25% of worldwide mortality (Ogbole, 2018; Baker et al., 2022). Among the five main infectious diseases that add to the death rate of people globally is sexually transmitted infections (STIs), Tuberculosis (TB) and Malaria (Feachem, 2004). STIs are a significant global public health challenge that can result in short-term sickness, long-term complications, infertility, physical and mental health issues, and even mortality. Additionally, STIs accelerate the proliferation of human immunodeficiency virus (HIV) (Kassie *et al.*, 2019). Statistics published in 2014 by the World Health Organization (WHO) stated that roughly 36.9 million people globally are living with HIV, while two million people are newly infected (WHO, 2015). TB, for instance, has a high drug-resistance rate. Approximately 9 million individuals developed the disease in 2013, resulting in the death of 1,5 million individuals (Murray et al., 2014). Clearly, infectious diseases are a worrying human health factor.

To combat infections or diseases, antibiotics were implemented into health practice years ago (Hawkey, 2008). Antimicrobial agents like penicillin, methicillin, streptomycin, vancomycin, chloroquine, and many others have been used as medication. However, there has been a significant rise in micro-organisms over the previous years that have developed multidrug resistance to antimicrobial agents (Oliphant and Eroschenko, 2015; Roca *et al.*, 2015; Ogbole *et al.*, 2018). Antimicrobial resistance is a major health care challenge. It is estimated that antimicrobial resistances contribute to an estimated 700 000 deaths yearly and given the rise in the world's population and industrialisation, it is also estimated that this number could rise to 10 million deaths by 2050 (Shankar, 2016; Niu and Li, 2019). Given the above-mentioned statistics, urgent action is required to control and limit the growth in the emergence and prevalence of antimicrobial resistance by using innovative tactics required to improve the world's understanding on the mechanism of antimicrobial resistance. Furthermore, there is a need to improve the current efforts to search for new antimicrobial drugs to combat antimicrobial-resistant strains.

Traditional plant-based medicine is the main or only type of affordable primary health care for people in rural Africa in many parts of the developing world (Street and Prinsloo, 2013; Ogbole *et al.*, 2018). This type of medicine is an essential component of the global healthcare system, offering affordable, accessible, and comparatively low-cost herbal medicines with cultural consequences (Erhabor *et al.*, 2019). Traditional medicinal plants are also known to be effective in prevention and treatment of toxicity side effects caused by other synthetic drugs or toxins (Bahmani *et al.*, 2015; Karimi *et al.*, 2015). Moreover, traditional plant medicine is known to have a multitude of secondary bioactive metabolites such as alkaloids, flavonoids, and phenols with a

wide range of health benefits, making them a crucial source of new antimicrobial agents (Bahmani *et al.*, 2015). Plants are also an essential source of medicinal products for the discovery of new drugs, particularly in developing nations, since there has been much effort to find new antimicrobial compounds from different types of natural sources (Arulmozhi *et al.*, 2018).

South Africa has an inimitable and diverse heritage of around 30 000 plant species, 3 000 of which are commonly used for therapy (Van Vuuren, 2008). Available literature in Chapter 4 has indicated that plants such as *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata* and *Scambiosa columbaria* are used traditionally in South Africa to treat various diseases such as infertility, excessive period pains, digestive problems, STIs and many other ailments (Kamatou *et al.*, 2008; Moffet, 2010; Moteetee and Kose, 2016; Moteetee, 2017; Maroyi, 2019; Ngobeni *et al.*, 2023). Therefore, this chapter will focus on studying the antimicrobial activity of these plants and the minimum inhibitory concentration at which the extracts inhibit the growth of bacteria and yeast species.

7.2. METHODS

7.2.1. Extraction preparation and storage

B. onopordifolia, *H. depressa*, *S. runcinata* and *S. columbaria* methanol, acetone and aqueous extracts were prepared in accordance with the method described in Chapter 5. All extracts were stored in the fridge at 4°C until required.

7.2.2. Microbial cultures

The antibacterial activity of the plant extracts was evaluated against *Staphylococcus aureus* (ATCC 11632), *Clostridium perfringens* (ATCC 13124), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 13762), *Escherichia coli* (Resistant) (ATCC 13846), *Enterobacter hormaechei* (ATCC 700323), *Staphylococcus epidermidis* (ATCC 12228), and *Bacillus cereus* (ATCC 13061). The yeast species used were *Candida albicans* (ATCC 90028), *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019). The microbes were selected based on availability and because some are STI-related pathogens. All the control bacterial and fungal species were supplied by the National Health Laboratory Services, Bloemfontein, South Africa and LANCET, Bloemfontein, South Africa. All the microbial species were maintained in Mueller Hinton agar plates at temperatures of 4°C. Prior to treatment with the plant extracts, the bacteria were inoculated in Mueller Hinton broth, and incubated and shaken at 100 revolutions per minute (rpm) for 24 hours, to ensure purity and viability. The bacterial suspensions of test strains were adjusted to 0.5 McFarland standard, which equals to $1-2 \times 10^8$ CFU ml⁻¹ for bacteria and $1-5 \times 10^6$ CFU ml⁻¹ for yeasts.

7.2.3. Minimum inhibitory concentration (MIC) essay

The antimicrobial activity of the plant extracts was conducted using the microdilution method developed by Eloff (1998) with modifications from Santhakumaran *et al* (2018). Eighty microliters of the bacterial suspension were pipetted into the 96 microwell plates already containing 80 µl of diluted plant extract to make a final volume of 160 µl in each well. The concentration of the plant extracts ranged from 0.16 mg/ml to 1.25 mg/ml. The control wells were respectively filled with culture medium only,

bacterial suspension, 5% of the solvent extraction (Nostro *et al.*, 2000; Baris *et al.*, 2006), and plant extract only. Chloramphenicol (0.125 mg/ml) was used as a positive control in bacteria, while amphotericin B (0.03-1 µg/ml) was used for yeast. The microwell plates were incubated for 24 hours for bacteria and 48 hours for yeast. Forty microlitres of four mg/ml Iodonitrotetrazolium salt solution was added to each well. Growth was indicated by a change of colour ranging from pink to violet after 10 to 30 minutes' incubation. All samples were tested in triplicates. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration at which the plant indicated bacterial or fungal growth inhibition.

7.2.4. Selectivity index

The Selectivity Index (SI) was calculated as the ratio logarithm of the cytotoxic concentration (CTC₅₀) and the MIC value for microbial species ($SI = \log [CC_{50}]/[MIC]$), and it was used to determine the relationship between cytotoxicity and antimicrobial activity. Values lower than 1 indicate greater toxicity to Vero cells and lower selectivity to microbial species, while values greater than 1 represent greater selectivity against species of microorganisms than Vero cells (Nunes *et al.*, 2016).

7.3. RESULTS AND DISCUSSION

7.3.1. Evaluation of antimicrobial activity of the selected plant extracts

The antifungal and antibacterial activities of the selected plants are presented in Table 7.1. Plant extracts' antimicrobial activity has been categorised as good when the $MIC < 0.1$ mg/ml, with moderate ($0.1 \leq MIC \leq 0.625$ mg/mL), or weak ($MIC > 0.625$ mg/mL) antimicrobial activity (Eloff, 2004).

Table 7.1: The MIC values of *H. depressa*, *S. columbaria*, *S. runcinata* and *B. onopordifolia* extracts against microorganisms (mg/ml).

		<i>H. depressa</i>			<i>S. columbaria</i>			<i>S. runcinata</i>			<i>B. onopordifolia</i>			CHPL	AMPT B
Microbial species	Gram (+ or -)	M	A	Aq	M	A	Aq	M	A	Aq	M	A	Aq		
<i>Bacillus cereus</i>	+	0.3	1.25	-	0.6	-	-	0.3	-	-	1.25	-	-	<0.1	
<i>Candida albicans</i>	+	0.3	0.1	-	0.6	1.25	-	0.3	0.1	-	0.6	0.6	-		<0.1
<i>Candida krusei</i>	+	1.25	-	1.25	-	-	-	1.25	-	-	-	-	-		<0.1
<i>Candida parapsilosis</i>	+	0.3	1.25	-	0.6	-	-	0.6	1.25	-	0.6	0.6	-		<0.1
<i>Clostridium perfringens</i>	+	0.6	-	-	0.6	-	-	0.3	-	-	0.6	-	-	<0.1	
<i>Enterobacter hormaechei</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	<0.1	
<i>Enterococcus faecalis</i>	+	1.25	-	-	1.25	-	-	0.6	-	-	0.6	-	-	<0.1	
<i>Escherichia coli</i>	-	0.6	1.25	-	0.6	1.25	-	0.6	0.3	-	0.6	0.6	-	<0.1	
<i>Escherichia coli (RESISTANT)</i>	-	-	-	-	-	-	-	-	1.25	-	-	-	-		
<i>Neisseria gonorrhoeae</i>	-	0.6	0.6	-	0.6	0.6	-	0.1	0.3	-	0.6	0.6	-	<0.1	
<i>Pseudomonas aeruginosa</i>	-	1.25	1.25	-	0.6	-	-	1.25	1.25	-	0.6	0.6	-	<0.1	
<i>Staphylococcus aureus</i>	+	0.6	1.25	-	-	1.25	-	0.6	0.6	-	0.6	-	-	<0.1	
<i>Staphylococcus epidermidis</i>	+	0.6	0.6	-	0.6	-	-	0.3	0.6	-	0.6	-	-	<0.1	
<i>Streptococcus pneumoniae</i>	+	0.6	0.6	-	0.6	1.25	-	0.1	0.6	-	0.6	0.6	-	<0.1	



<i>Streptococcus pyogenes</i>	+	0.6	0.6	-	0.6	1.25	-	0.3	0.1	-	0.6	-	-	<0.1	
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Aq=Aqueous extracts; A= Acetone extracts; M= Methanol extracts; CHPL= Chloramphenicol; AMPT B= Amphotericin B; (-) = No antimicrobial activity was found

All aqueous extracts of the selected plants showed no antimicrobial activity against all the microbial organisms, except *H. depressa* aqueous extracts where MIC was recorded at MIC 1.25 mg/ml. One possible explanation is that aqueous extracts may contain an abundance of compounds that may have opposing effects on their overall activity. Moreover, it could be that bioactive compounds found in the plants were difficult to extract in water because most of the bioactive compounds dissolve much better in alcoholic solvents (Moyo *et al.*, 2012; Kalidindi *et al.*, 2015). Methanol extracts of *H. depressa* showed antimicrobial activity against 10 bacterial and three yeast species at MICs ranging from 0.3-1.25 mg/ml. The overall *H. depressa* methanol extract activity had moderate activity against 10 micro-organisms and tested poor against three. *H. depressa* acetone extracts showed antimicrobial activity against eight bacterial and two yeast species at MICs ranging from 0.1-1.25 mg/ml. The activity was considered good against one microbial species, moderate for four microbial species, and poor for five microbial species (Table 7.1). In Chapter 5, we reported the presence of various pharmacologically important compounds such as terpenoids, saponins, tannins, phenols (6-gingerol) and flavonoids (quercetin). Therefore, the antimicrobial activity exhibited by *H. depressa* extracts may be due to the presence of these bioactive compounds.

The antimicrobial activity of *S. columbaria* methanol extracts was moderate against 10 microbial and weak against one microbial species. Acetone extracts showed weak activity against five microbial species and moderate against one microbial species. The presence of compounds such as chlorogenic acid, 4-O-caffeoylquinic acid and Cirsimaritin is known to have antimicrobial properties that have been reported (Chapter 5; Akar, 2021). A study also reported the presence of terpenes, saponins,

phenolic compounds, coumarin, flavonoids and many other bioactive compounds in the *Scambiosa* genus (Hlila *et al.*, 2017). A plant from *Scambiosa* genus, *Scambiosa hymettia* Bois, was reported to have shown antibacterial activity against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. cloacae*, *K. pneumoniae* and *E. coli* (Hlila *et al.*, 2017). The antimicrobial activity shown by *S. columbaria* extracts may be attributed to the presence of the above-mentioned bioactive compounds.

S. runcinata methanol extracts showed good antimicrobial activity against two, moderate activity against nine, and poor activity against two microbial species (Table 7.1). Acetone extracts equally showed good activity against two, moderate activity against five, and weak activity against three microbial species. High presence of sesquiterpenes such as α -Bisabolol, phenolic compounds and alkaloids was reported from *Salvia* species (Kamatou *et al.*, 2008). Sesquiterpenes have been reported to possess medicinal properties such as antimicrobial, cytotoxic, and anti-inflammatory properties (Awouafack *et al.*, 2013). Chapter 5 reported the presence of compounds such as Chlorogenic acid, Genistein, Secoxyloganin, Rosmarinic acid and many other compounds from *S. runcinata* extracts known to have antimicrobial properties. Compounds such as Genistein and Chlorogenic acid exert their effects by blocking or altering the permeability of the cytoplasmic membrane, interfering with nucleic acid synthesis, and disrupting the energy metabolism of bacterial cells (Li *et al.*, 2014; Islam *et al.*, 2020; Ivanov *et al.*, 2022). The antimicrobial activity of *S. runcinata* noted in this chapter may be due to the presence of the above-mentioned compounds. It is important to note that *S. runcinata* acetone extracts inhibited the growth of Colistin resistant *E. coli* species at MIC 1.25 mg/ml. According to the current body of literature, Colistin is acknowledged as the ultimate therapeutic choice for healthcare-associated

infections resulting from multidrug-resistant gram-negative bacteria. This is relevant for infections caused by *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, or *Klebsiella pneumoniae*, *enterobacter*, *Salmonella*, *Shigella*, *Proteus spp.*, *Providencia spp.*, *Morganella morganii*, *Serratia marcescens*, *Moraxella catarrhalis*, *Helicobacter pylori*, and *Brucella* species (Nation and Li., 2009). Colistin is further known to exhibit negative side effects such as renal toxicity, neurotoxicity, kidney damage and gastrointestinal problems such as nausea, vomiting, diarrhoea, and abdominal pain (Spapen *et al.*, 2011). Even though *S. runcinata* antimicrobial activity against this colistin resistant *E. coli* strain is considered poor in this chapter, the plant can potentially be used to treat the above-mentioned infections treated by colistin antibiotic, moreover, the use of isolated compounds may possibly improve the activity. When it comes to treating infections, *S. runcinata* may be a more favourable choice than colistin due to its minimal adverse effects observed in the traditional treatment of STIs. STIs-related microbial strains are currently developing resistance against the currently used antibiotics. Hence, it is crucial to conduct research on the antimicrobial efficacy of *S. runcinata* against different strains that are resistant to drugs. Further investigations can provide additional evidence to confirm its potential effectiveness against resistant strains. This plant could potentially provide a breakthrough in treating infections caused by drug-resistant bacterial species and can be used to develop antimicrobial agents with lesser side effects.

B. onopordifolia methanol extracts showed moderate antimicrobial activity against 11 and poor activity against one microbial species. Acetone extracts showed moderate activity against 6 microbial species. Chapter 5 reported the presence of various compounds such as alkaloids, terpenoids, steroids and glycosides. Compounds such

as Skimmin, 14-Methylmonocrotaline-like (Pyrrolizidine alkaloids), 3',4'-Dimethoxyluteolin, Isosakuranetin and many others were also reported to be present in *B. onopordifolia* extracts. Thus, the compounds present in *B. onopordifolia* may have influenced the antimicrobial activity noted in this chapter.

Methanol and acetone extract of *H. depressa*, *S. columbaria*, *S. runcinata* and *B. onopordifolia* showed moderate to good antibacterial activity against *N. gonorrhoea*, which is a microorganism responsible for an STI, gonorrhoeae. It was reported that the estimated global prevalence of gonorrhoea was found to be 0.9% in women and 0.7% in men, which equates to 30.6 million cases worldwide per year (Kularatne *et al.*, 2018; Kirkcaldy *et al.*, 2019). Given the above-mentioned statistics, the activity of the selected plants against *N. gonorrhoeae* is of utmost importance and may provide a breakthrough against challenges associated with the treatment of STIs in Thaba 'Nchu, South Africa and the world.

7.3.2. Selectivity index

The selectivity index results are presented in Table 7.2 calculated using the cytotoxicity CTC₅₀ results imported from Chapter 6. All selectivity index values for all microorganisms were below 1, which indicates that none of the plant extracts demonstrated selectivity against all the microbial strains used in this study. The antimicrobial activity demonstrated against the chosen microbial species may have been influenced by the plant's cytotoxicity. There may be no therapeutic value for plant extracts that are more toxic to cells than to bacteria or fungi. The cytotoxicity could also be caused by a metabolic toxin that affects both animal and microbial cells generally (Makhafola *et al.*, 2012). Using just one or even several cell lines, however,

could lead to incorrect conclusions about the cytotoxicity and usefulness of a plant extract. Before drawing any strong conclusions, the toxicity must also be assessed in *in vivo* studies. It might be counterproductive to discard a crude extract because it initially had cytotoxic effects on cell lines. The antibacterial compound may or may not be the cytotoxic substance. It is theoretically possible to separate a potent, non-toxic novel metabolite from harmful crude plant extracts (Elisha *et al.*, 2017). Isolation of antimicrobial compounds from the antimicrobial promising extracts in this study is therefore recommended to determine whether the antimicrobial activity is influenced by their toxicity or otherwise.

Table 7.2: Cytotoxicity and selectivity index of *H. depressa*, *S. columbaria*, *S. runcinata* and *B. onopordifolia* extracts against 15 bacterial strains calculated by dividing cytotoxicity by MIC

	<i>H. depressa</i>			<i>S. columbaria</i>			<i>S. runcinata</i>			<i>B. onopordifolia</i>		
Extracts	M	A	Aq	M	A	Aq	M	A	Aq	M	A	Aq
Cytotoxicity (mg/ml)	0.005	0.005	0.016	0.010	0.006	0.205	0.004	0.004	0.021	ND	0.005	0.034
<i>Bacillus cereus</i>	0.017	0.004	-	0.017	-	-	0.013	-	-	-	-	-
<i>Candida albicans</i>	0.017	0.05	-	0.017	0.005	-	0.013	0.04	-	-	0.008	-
<i>Candida krusei</i>	0.004	-	0.013	-	-	-	0.003	-	-	-	-	-
<i>Candida parapsilosis</i>	0.017	0.004	-	0.017	-	-	0.007	0.003	-	-	0.008	-
<i>Clostridium perfringens</i>	0.008	-	-	0.017	--	-	0.013	-	-	-	-	-
<i>Enterobacter hormaechei</i>	-	-	-	-	-	-	--	-	-	-	-	-
<i>Enterococcus faecalis</i>	0.004	--	-	0.008	-	-	0.007	-	-	-	-	-
<i>Escherichia coli</i>	0.008	0.004	-	0.017	0.005	-	0.007	0.013	-	-	0.008	-
<i>Escherichia coli</i> (RESISTANT)	-	-	-	-	-	-	-	0.003	-	-	-	-
<i>Neisseria gonorrhoeae</i>	0.008	0.008	-	0.017	0.01	-	0.04	0.013	-	-	0.008	-
<i>Pseudomonas aeruginosa</i>	0.004	0.004	-	0.017	-	-	0.003	0.003	-	-	0.008	-
<i>Staphylococcus aureus</i>	0.008	0.004	-	-	0.005	-	0.007	0.007	-	-	-	-
<i>Staphylococcus epidermidis</i>	0.008	0.008	-	0.017	-	-	0.013	0.007	-	-	-	-



<i>Streptococcus pneumoniae</i>	0.008	0.008	-	0.017	0.005	-	0.04	0.007	-	-	0.008	-
<i>Streptococcus pyogenes</i>	0.008	0.008	-	0.017	0.005	-	0.013	0.04	-	-		-

Aq=Aqueous extracts; A= Acetone extracts; M= Methanol extracts; (-) = No antimicrobial activity was found; ND= Not determined

7.4. CONCLUSION

S. runcinata, *H. depressa*, *S. columbaria* and *B. onopordifolia* methanol and acetone extracts showed good, and moderate to weak antimicrobial activity against 15 microbial species. *S. runcinata* extracts showed antibacterial activity against colistin resistant *E. coli*. Therefore, the plant is a potential candidate to address the challenges caused by drug-resistant species, including those that may be related to STIs. The results of this chapter further validate the use of *S. runcinata*, *H. depressa*, *S. columbaria* and *B. onopordifolia* for the treatment of STIs, since they exhibited good to moderate antibacterial activity against *Neisseria gonorrhoeae*. The plants are promising candidates that can be used to discover novel antimicrobial compounds and for the development of new and affordable antimicrobial agents. STIs are usually characterised by inflammation to the affected area, and some STIs such as Human Papilloma Virus (HPV), Herpes Simplex Virus (HSV) and Human Immunodeficiency Virus (HIV) usually proliferate to cancer if not well managed. The next chapter will investigate the anti-inflammatory and antioxidant activity of *S. runcinata*, *H. depressa*, *S. columbaria* and *B. onopordifolia* extracts.

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CHAPTER 8⁵

Anti-inflammatory and antioxidant activity of *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata* and *Scambiosa columbaria*

ABSTRACT

Sexually transmitted infections can trigger inflammation and oxidative stress in the body. Inflammation is a natural response of the immune system to fight infections, however, if it becomes chronic, it can cause tissue damage and increase the risk of chronic diseases. STIs can also cause oxidative stress, which occurs when there is an imbalance between the production of reactive oxygen species and the ability of the body to neutralise them. This chapter evaluates the anti-inflammatory and antioxidant capacities of *Berkheya onopordifolia*, *Hermannia depressa*, *Scambiosa columbaria* and *Salvia runcinata* extracts. Nitric oxide (NO) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the Ferric Reducing Ability of Plasma (FRAP) methods were used to evaluate the anti-inflammatory and antioxidant activity of the plant extracts respectively. To ensure that the anti-inflammatory activity of the plant extracts was not influenced by their toxicity, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used. *H. depressa* (acetone), *S. columbaria* (methanol) and *S. runcinata* (methanol) had promising anti-inflammatory properties. The plant extracts demonstrated strong inhibitory action against more than 60% nitric oxide release, which has been linked to the etiology of many inflammatory conditions. The anti-inflammatory activity of *S. runcinata* and *S. columbaria* acetone extracts and *B. onopordifolia* methanol extracts were ruled out due to the high cytotoxic effects they

⁵Some parts from this chapter were presented at African Traditional and Natural Product Medicine Conference hosted by the University of Limpopo at the Protea Hotel Ranch Resort from 19-21 October 2022.

demonstrated. *H. depressa* aqueous ($IC_{50} = 9.081 \mu\text{g/ml}$), methanol ($5.197 \mu\text{g/ml}$) and acetone ($3.576 \mu\text{g/ml}$) and *B. onopordifolia* acetone ($9.834 \mu\text{g/ml}$) extracts showed the strongest DPPH scavenging activity when compared to ascorbic acid ($5.69 \mu\text{g/ml}$) and Trolox ($6.166 \mu\text{g/ml}$). *B. onopordifolia*, *H. depressa*, *S. columbaria* and *S. runcinata* extracts can be used to treat inflammatory disorders and as natural antioxidants in primary prevention of inflammatory processes in STIs conditions.

8.1. INTRODUCTION

Inflammation can be characterised as a defensive, non-specific immune response to harmful stimuli such as damaged cells, irritants, free radicals, and pathogens (Jisha *et al.*, 2019). Inflammation development is primarily due to the combined effect of innate and adaptive immune response where it requires a sequence of initial responses to infection from the immune system which directs immune cells such as macrophages, dendritic cells, lymphocytes, neutrophils etc. to the injury site (Waisman *et al.*, 2015; Azeem *et al.*, 2010; Jisha *et al.*, 2019). Various inflammatory mediators such as interferons, interleukins, tumour necrosis factor, prostaglandins, leukotrienes, cytokines etc. are often released upon the activation of different inflammatory responses (Vignali and Kuchroo, 2012).

Free radical overcrowding, activation of complex enzymes, release of inflammatory and pro-inflammatory mediators, etc. may contribute to the advancement of acute inflammatory responses (Ward *et al.*, 2022). One of the inflammatory mediators during the immuno-inflammatory response is nitric oxide (NO). An effective defence against invasive microbes is aided by adequate NO levels, whereas an inability to produce NO makes a person seriously and even fatally susceptible to infections (Ondua *et al.*,

2019). Additionally, an excess of NO has been linked to the pathogenesis of numerous degenerative diseases including cancer, inflammatory autoimmune diseases, neurodegenerative diseases, atherosclerosis, and neurodegenerative diseases (Ghuman *et al.*, 2019).

Oxidative stress is also a key causative factor in the advancement and progression of aging and degenerative diseases (Boligon *et al.*, 2014; Moldogazieva *et al.*, 2019). ROS, including singlet oxygen ($^1\text{O}_2$), hydroxyl radicals ($\text{OH}^\cdot$), superoxide anion ($\text{O}_2^\cdot$), and hydrogen peroxide (H_2O_2), are well known to play a significant role in the onset of oxidative stress. The link between oxidative stress and inflammation is that oxidative stress can trigger inflammation, and inflammation can exacerbate oxidative stress. Sexually transmitted infections (STIs) can trigger both inflammation and oxidative stress in the body (Agarwal *et al.*, 2018). Inflammation is a natural response of the immune system to STIs, but if it becomes chronic, it can cause tissue damage and increase the risk of chronic diseases (Paulis *et al.*, 2018). STIs can also cause oxidative stress, which occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of the body to neutralise them (Garcia-Sanchez *et al.*, 2020).

Steroidal and non-steroidal anti-inflammatory drugs are currently being used for the management of various inflammatory disorders (Moore *et al.*, 2019). Long term use of these anti-inflammatory drugs is associated with adverse effects such as gastric ulcers, renal failure, and gastrointestinal detriments (Jahnavi *et al.*, 2019; Jisha *et al.*, 2019). In addition, these drugs have shown limited success in treating many anti-inflammatory conditions, so there is a need to develop safe, affordable, non-toxic, and

active anti-inflammatory drugs. To treat oxidative stress, many synthetic antioxidants have also been developed (Menezo *et al.*, 2016). The high cost, lack of availability and side effects, however, continue to be significant obstacles in the fight against oxidative stress (Pickering *et al.*, 2018). The use of natural antioxidants is a growing field of interest in complementary therapy since the currently used synthetic antioxidants are hazardous to human health (Peanparkdee and Lwanoto, 2019).

Antioxidants can be found naturally in plants, and they play an essential role in preventing the production of free radicals and are usually present in fruits, vegetables, grains, etc. (Sridhar and Charles, 2019; Shahidi and Zhong, 2015). Plants produce compounds such as vitamins, flavonoids and polyphenols that possess effective antioxidant activity (Sridhar and Charles, 2019). The use of plants for antioxidant defence purposes is attributed to their availability, less after-use side effects and relative low toxicity (Comert and Gokmen, 2018). Therefore, natural antioxidants have been the subject of a wide variety of research studies to identify plants with potentially safe, effective, and cheap antioxidants.

Numerous plants have been used traditionally to treat diseases in South Africa and other developing countries and they tested positive for anti-inflammatory activity. Okoli *et al.* (2003) and Iwalewa *et al.* (2007) are amongst several authors who reviewed plants that are used to treat pain-related inflammatory disorders in South Africa. Several plants used for inflammatory diseases by the people of South Africa include *Setaria megaphylla*, *Athrixia phyllicoides*, *Peltophorum africanum*, *Sclerocarya birrea*, *Acacia sieberiana*, *Aloe vera* Linn and many more. Some of these plants have been tested for various biological properties such as antimicrobial, antioxidant and anti-

inflammatory activities (Iwalewa *et al.*, 2007; Naidoo *et al.*, 2004) due to the rapidly increasing involvement of pharmaceutical companies in the investigation of the anti-inflammatory and antioxidant activity of medicinal plants and scientific research into the discovery of new therapeutic compounds (Fawole *et al.*, 2010). It is for this reason that this chapter will focus on evaluating the anti-inflammatory and antioxidant activities of *Berkheya onopordifolia*, *Hermannia depressa*, *Salvia runcinata* and *Scambiosa columbaria* extracts.

8.2. METHODS

8.2.1. Sample preparation

B. Onopordifolia, *H. depressa*, *S. runcinata* and *S. columbaria* methanol, acetone and aqueous extracts were prepared in the same manner as described in Chapter 5.

8.2.2. Nitric oxide production and viability of LPS-activated RAW 264.7 macrophages

RAW 264.7 cell lines were obtained from Cellonex (South Africa), seeded into 96-well plates at a density of 1×10^5 cells per well and allowed to attach overnight. The extracts respectively were dissolved in DMEM complete medium to give final concentrations of 50, 100 and 200 $\mu\text{g/mL}$ and stored at room temperature until needed. To assess the anti-inflammatory activity, 50 μl of Lipopolysaccharide (LPS) (final concentration of 500 ng/ml) containing the medium was added to the corresponding wells. Aminoguanidine (AG) was used as the positive control for this experiment. Cells were incubated for a further 18 hours.

To quantify nitric oxide (NO) production, 50 µl of supernatant from each well of cell culture plates was transferred to a new 96-well plate and 50 µl Griess reagent added. Absorbance was measured for 10 min at 540nm, and the results expressed relative to the appropriate untreated control. A standard curve using sodium nitrite dissolved in culture medium was used to determine the concentration of NO in each sample. Percentage inhibition was calculated based on the ability of extracts to inhibit nitric oxide formation by cells compared with the control (cells in media without extracts containing triggering agents and Dimethyl sulfoxide [DMSO]), which was considered as 0% inhibition (Dzoyem and Eloff *et al.*, 2015).

8.2.3. Cell viability

A cytotoxicity assay was also conducted to observe that nitric oxide inhibition was not due to cytotoxic effects using a method as previously described by Mosmann (1983) and with slight modifications by Dzoyem and Eloff *et al.* (2015). This was done by removing the remaining medium and treatments in each well and replacing these with a medium containing 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) and further incubated for 30 minutes at 37°C. Thereafter, MTT was removed and 200 µL DMSO was added to each well to solubilise the formazan crystals. Absorbance was read at 540 nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). The percentage of cell viability was calculated with reference to the control (cells without extracts containing LPS taken as 100% viability). All the tests for measurement of nitric oxide inhibition were conducted in triplicates. The absorbance of pink colour formazan formed by reduction of MTT solution by the presence of mitochondrial dehydrogenase in viable cells was used to

measure the percentage cell viability. The IC₅₀ value denotes the lowest concentration of plant extract that inhibits 50% of cells (Nemudzivhadi and Masoko, 2014).

8.2.4. DPPH radical scavenging assay

The DPPH method was used to test radical scavenging activity of the aqueous, methanol, and acetone extracts of the selected plants. The method was adopted from Sridhar and Charles (2019), Thaipong *et al.*, 2006 and Locatelli *et al.* (2009), with slight modifications. The stable free radical DPPH was dissolved in methanol to give a 0.1mM solution; 10 µL of extract samples in methanol (or methanol itself as blank control) was added to 175 µL of the methanol DPPH solution. For each test compound, different concentrations were tested (200, 100, 50, 25, 12.5, 6.25, 0 µg/mL) for extracts. After further mixing, the decrease in absorbance was measured at 520nm after 20 minutes. The actual decrease in absorption induced by the test sample was calculated by subtracting that from the control. The antioxidant activity of each test sample was expressed as an IC₅₀ value, i.e., the concentration in µg/ml that inhibits DPPH absorption by 50% and was calculated from the concentration effect linear regression curve.

8.2.4.1. Calculations

The % DPPH inhibition was calculated using the formula: $(A_0 - A_1)/A_0 \times 100$ where A₀ is the absorbance of the control and A₁ is the absorbance of the test sample. Ascorbic Acid and Trolox were used for positive control. Trolox equivalents was calculated as the IC₅₀ of the test sample relative to that of Trolox expressed as a dry mass.

8.2.5. Ferric ions reducing antioxidant power (FRAP) method

The FRAP method was used to evaluate the antioxidant capacity of plant extracts with slight modifications from Popović et al (2012), which involves the presence of extracts to reduce the ferric iron complex to the ferrous form. The FRAP reagent was prepared by mixing 20ml acetate buffer (pH 3.6), 2ml 2,4,6-Tripyridyl-S-triazine (TPTZ) (10mM in 40mM HCl), 2ml 20mM ferric chloride and 2.4ml water. The extract sample (10µl) or positive control (Trolox or Ascorbic acid) was mixed with 200ml of FRAP reagent and absorbance was measured at 30 min after vortexing at 37°C. The increase in absorbance of reaction mixture was measured for each sample on UV–Visible spectrophotometer at 593 nm. All samples were measured in three replicates. The results were compared with Ascorbic acid and Trolox as positive controls and FeSO₄ was used for calibration. FRAP activity was calculated as ferrous equivalent (FE) in µM.

8.2.5.1. Calculations

Trolox equivalents were calculated as a linear slope of the absorbance versus concentration for each sample relative to Trolox.

8.2.6. Selectivity index

The Selectivity Index (SI) was calculated as the ratio logarithm of the cytotoxic concentration (CTC₅₀) and the FRAP IC₅₀ ($SI = \log [CTC_{50}] / [FRAP/DPPH IC_{50}]$), and it was used to determine the relationship between cytotoxicity and antioxidant activity. Values lower than 1 indicate greater toxicity to Vero cells while values greater than 1 represent greater selectivity by exhibiting antioxidant activity with less effect to Vero cells (Adamu et al., 2014).

8.2.6. Statistical analysis

All experiments were conducted in triplicates and the data was presented as the mean $\pm$ standard deviation. Linear regression analysis was used to calculate IC₅₀ values. Microsoft Excel® was used to enter and capture data. Various graphs and tables were extracted from this data.

8.3. RESULTS AND DISCUSSION

8.3.1. Anti-inflammatory activity of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts

LPS-activated malignant macrophages cell line (RAW 264.7) was used to evaluate the Nitric Oxide (NO) production inhibitory activity of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* methanol, acetone, and aqueous extracts. The anti-inflammatory activity of the plant extracts was considered significant when NO is reduced by more than 50%. As shown in Figure 8.1, *S. columbaria* methanol, *H. depressa* acetone, *S. runcinata* methanol and *H. depressa* methanol extracts showed dose dependent inhibition of NO production from concentrations of 50, 100 and 200 μ g/ml. At the highest concentration (200 μ g/ml) all the above-mentioned plant extracts showed NO percentage inhibition greater than 60%. *H. depressa* acetone and *S. runcinata* methanol extracts had the most potent NO inhibition percentage of 77.5% and 88.2% at concentrations of 200 μ g/ml, respectively. *H. depressa* and *S. columbaria* methanol extracts showed NO percentage inhibition of 62.6% and 61.2% at a concentration of 200 μ g/ml. This is an indication that the *S. columbaria*, *H. depressa* and *S. runcinata* extracts showed strong anti-inflammatory activity by inhibiting NO production on LPS-activated malignant macrophages cell line. *B. onopordifolia*, *S.*

runcinata, *S. columbaria* and *H. depressa* aqueous extracts showed less than 50% NO inhibition at higher concentrations as compared to other extracts. It can thus be concluded that these extracts showed insignificant anti-inflammatory activity.

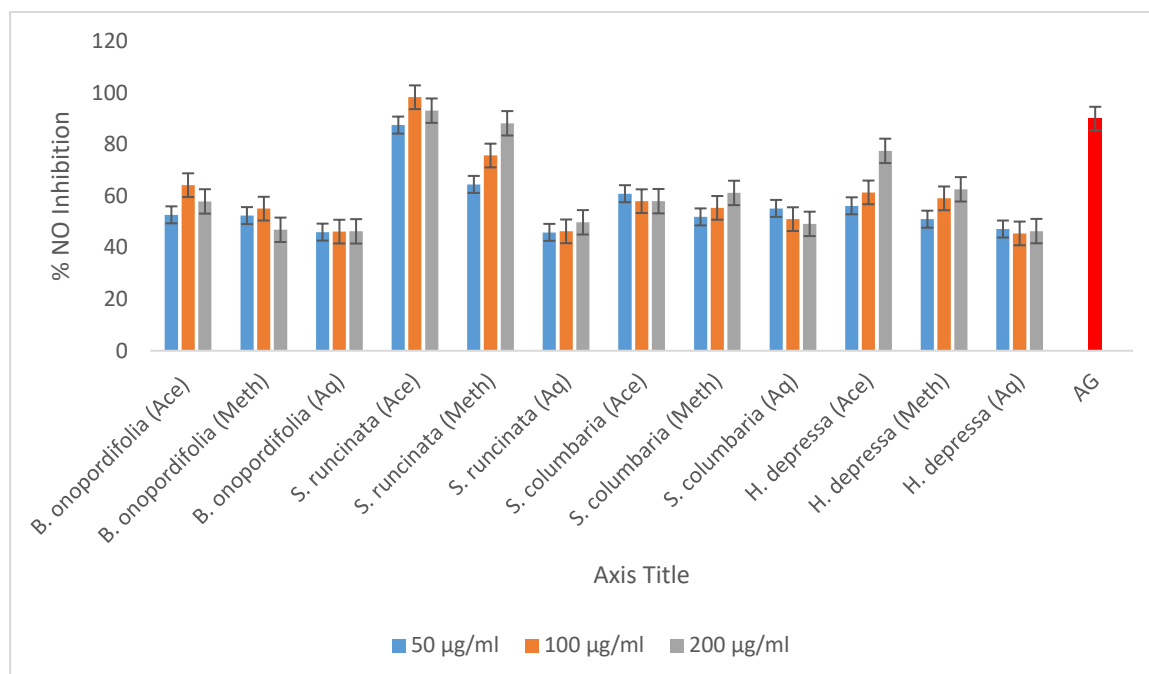


Figure 8.1: The nitric oxide percentage inhibition of *B. onopordifolia*, *S. runcinata*, *S. columbaria* and *H. depressa* extracts

Plants from *Salvia* genus have shown to contain bioactive compounds such as terpenoids and phenolics. *Salvia* spp. have antiseptic, antibacterial, anti-inflammatory, antiviral, cytotoxic, anticonvulsant and antimycobacterial properties according to ethnobotanical, phytochemical, and pharmacological studies (Kamatou *et al.*, 2008; Coisin *et al.*, 2012; Çadirci *et al.*, 2015; Alimpić *et al.*, 2017). In chapter 5, *S. runcinata* extracts were reported to contain alkaloids, terpenoids, tannins, glycosides, and flavonoids. It was further reported in the same chapter that the extracts contained compounds such as Genistein, Salvigenin, chlorogenic acid and many other compounds with anti-inflammatory properties. The anti-inflammatory activity of *S.*

runcinata in this chapter may be due to various bioactive compounds found in the plant.

Alkaloids, flavonoids, phenolics, tannins, terpenoids and saponins were reported to be present in *S. columbaria* (Chapter 5; Table 5.1). According to Pinto *et al.* (2018), a diverse range of secondary metabolites have been detected, confirming that the *Scambiosa* genus is high in flavonoids and terpenoids. The detected compounds are known to possess various biological properties such as antimicrobial, anticancer, anti-inflammatory, antioxidant, and many other activities (Majouli *et al.*, 2017; Brodowska, 2017; Sagbo *et al.*, 2020). Chapter 5 further reported the presence of compounds such as Eupatilin, Gentiopicroside, Tricin, cirsimaritin and many other compounds known to have anti-inflammatory properties. Cirsimaritin is known to have anti-inflammatory effect by causing blockage of the production of different cytokines such as interleukin 6, interleukin 10, interferon gamma (IFN- γ), and tumor necrosis factor alpha (TNF- α), as well as NO (Benali *et al.*, 2022). Tricin show anti-inflammatory activity by inhibiting the activation of proinflammatory mediators like TNF α , IFN- γ , MCP 1 by modulating nuclear factor kappa-B (NF- κ B) and Mitogen-activated protein kinases (MAPK) signalling pathways (Shalini *et al.*, 2016). The anti-inflammatory activity of *S. columbaria* noted in this chapter could be due the presence of the above-mentioned bioactive compounds. The results of this chapter may further validate the use of *S. columbaria* to traditionally treat wounds, period pains and colic traditionally as reported by Sagbo *et al.* (2020).

In South Africa, *H. depressa* and other members of the *Hermannia* genus are traditionally used to manage and treat a variety of illnesses such as heartburn, colic,

internal aches, wounds, and cuts (Moffet, 2010; Hlongwane, 2016). Hlongwane (2016) reported the presence of tannins, flavonoids, saponins and cardiac glycosides in *H. depressa* extracts. Furthermore, compounds such as Waltherione C, Tricin and quercetin were reported in Chapter 5. The bioactive compounds are known to have anti-inflammatory properties. The anti-inflammatory activity of *H. depressa* extracts in this chapter may be due to the ability to inhibit inflammatory mediators of bioactive compounds mentioned above (Wu *et al.*, 2020). These results support the use the plants traditionally used for internal aches, colic, wounds, and cuts. The current results indicate the efficacy of *H. depressa*, *S. columbaria*, *S. runcinata* as effective potential therapeutic agents in the treatment of inflammatory conditions caused by STIs.

8.3.2. Cytotoxicity screening of the *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts

MTT cytotoxicity assay was conducted to ensure that the anti-inflammatory activity was not due to toxicity effects of the plant extracts. The results of the cell viability after treatment of the cells with the plant extracts at various concentrations are displayed in Table 8.1. *S. runcinata*, *S. columbaria* acetone and *B. onopordifolia* methanol extracts' anti-inflammatory activity was considered as significant because the extracts showed concentration dependant cytotoxicity. At the highest concentration (200µg/ml), cells treated with *S. runcinata* acetone extracts exhibited the lowest live cells survival percentage of 35.35%. Cells treated with *S. columbaria* acetone extracts showed a low survival percentage with an increase in concentration. The cell viability of cells treated with *S. columbaria* acetone extracts at the highest concentration of 200µg/ml was 54.27%. Cells treated with *B. onopordifolia* methanol extracts showed low survival

with an increase in concentration. The cell viability of cells treated with *B. onopordifolia* methanol extracts at the highest concentration was 64.88%.

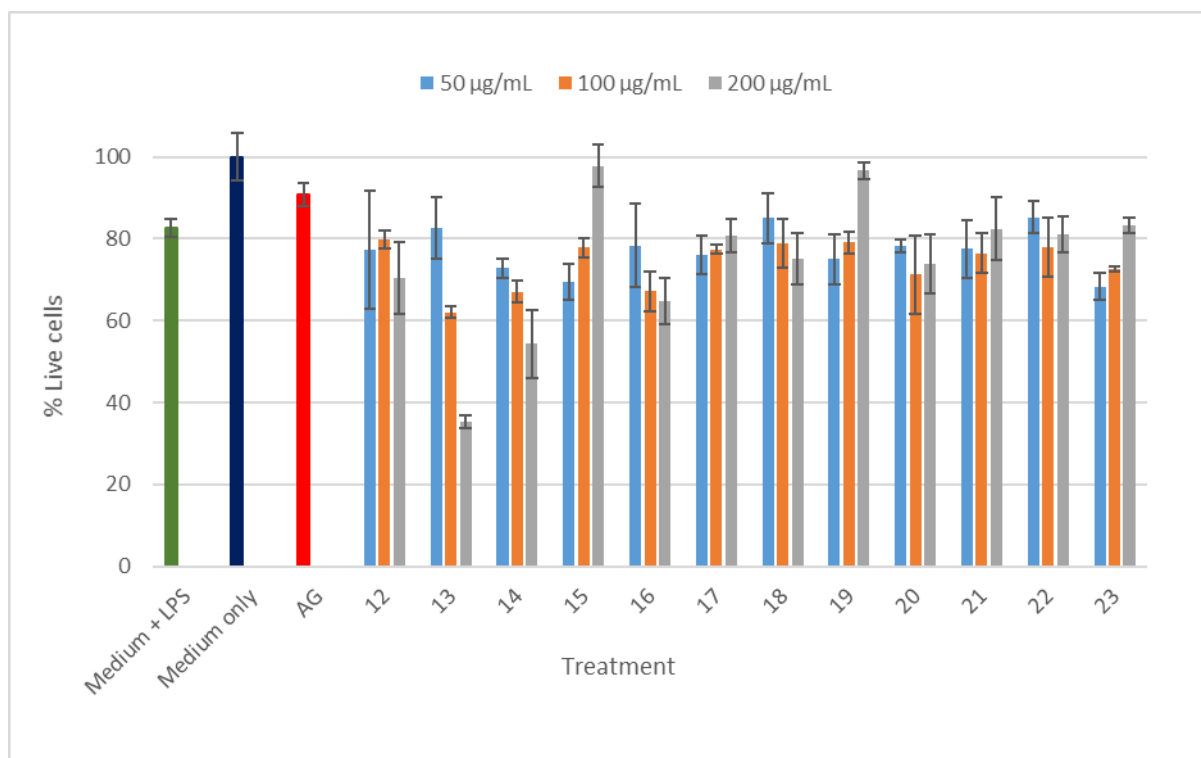


Figure 8.2: Cell viability (%) of LPS activated macrophages after 24 hours of exposure to the plant extracts. Bar graph represents triplicate values of one experiment and error bars represent the standard deviation of the mean. **Taxon abbreviations:** 12= *B. onopordifolia* (Acetone); 13= *S. runcinata* (Acetone); 14= *S. columbaria* (Acetone); 15= *H. depressa* (Acetone); 16= *B. onopordifolia* (Methanol); 17= *S. runcinata* (Methanol); 18= *S. columbaria* (Methanol); 19= *H. depressa* (Methanol); 20= *B. onopordifolia* (aqueous); 21= *S. runcinata* (Aqueous); 22= *S. columbaria* (aqueous); 23= *H. depressa* (Aqueous). **AG**=Aminoguanidine

The *Salvia* genus has plants that have been previously reported to be cytotoxic against various cell lines. Plants from the *Salvia* genus were reported to have compounds such as terpenoids (monoterpenoids, diterpenoids, triterpenoids etc) and phenols (Jassbi *et al.*, 2016). *Salvia limbata*, *Salvia hypoleuca* and *Salvia aethiopis* from the

Salvia genus showed significant cytotoxic activities against HL-60 (promyelocytic leukemia), K562 (chronic myelogenous leukemia) and MCF-7 (breast adenocarcinoma) cells (Jassbi *et al.*, 2016). *S. runcinata* extracts were reported to contain cytotoxic compounds such as Xanthoxylol, canadine and Rosmaric acid. The cytotoxicity of *S. runcinata* extracts against RAW 264.7 macrophages in this chapter may be due to the cytotoxic compounds detected and isolated from other plants from the *Salvia* genus.

Alkaloids and flavonoids are some of the toxic compounds that have been identified in *S. columbaria* (Sagbo *et al.*, 2020; Tapas *et al.*, 2008). Canadine and Chlorogenic acid are also an addition to the toxic compounds found in the extracts of *S. columbaria* (Chapter 5). The toxicity of compounds detected in *S. columbaria* extracts may have also contributed to the anti-inflammatory activity shown by the plant in this chapter. Chapter 5 reported the presence of various toxic compounds such as Pyrrolizidine alkaloids (14-Methylmonocrotaline-like), flavones (3',4'-Dimethoxyluteolin), and Azelaic acid in *B. onopordifolia* extracts. Therefore, the cytotoxicity effects of *B. onopordifolia* demonstrated in this chapter may be due to the presence of the above-mentioned toxic compounds; hence, the extracts were ruled out as the best candidate for treatment of inflammatory conditions.

8.3.3. The antioxidant activity of plant extracts *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts

8.3.3.1. DPPH and FRAP

Antioxidant capacities of acetone, methanol, and aqueous extracts of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* were measured by DPPH assay. The

sample concentration needed to inhibit DPPH by 50% was determined using the IC_{50} values. The antioxidant activity of the samples is considered significant when the IC_{50} values are lower. Table 8.2 shows the IC_{50} values of the *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts. *B. onopordifolia* acetone extracts showed strong free radical scavenging activity at a low IC_{50} of $9.834 \pm 0.31 \mu\text{g/ml}$. *H. depressa* acetone ($IC_{50} = 3.576 \pm 0.44 \mu\text{g/ml}$), methanol ($5.197 \pm 0.10 \mu\text{g/ml}$), and aqueous ($9.081 \pm 1.94 \mu\text{g/ml}$) extracts showed strong antioxidant activity. *H. depressa* methanol and acetone extracts showed stronger antioxidant capacity as compared to the synthetic antioxidant ascorbic acid ($IC_{50} = 6.569 \pm 0.98 \mu\text{g/ml}$) and Trolox ($IC_{50} = 6.166 \pm 0.43 \mu\text{g/ml}$). Therefore, *H. depressa* can be considered as a promising plant-based antioxidant in the context of the DPPH method. Figures 8.3 and 8.4 show DPPH percentage inhibition of plant extracts with low IC_{50} values or strong antioxidant activity as compared to positive controls Trolox and Ascorbic acid. *B. onopordifolia* aqueous, *H. depressa* acetone, methanol, and aqueous extracts in figures 8.3 and 8.4 showed better DPPH inhibition at lower concentrations as compared to Trolox and ascorbic acid, which is a further indication that these extracts are potentially good antioxidants. This warrants further investigations into bioactive compounds.

The FRAP assay measures the reducing ability of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex to produce a coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ) where the free radical chain breaking takes place through donating a hydrogen atom at an acidic pH of about 3.6. The FRAP value represents the equivalent concentration of Trolox that would produce the same antioxidant capacity or reducing capacity as the plant extract being tested. A sample is considered a strong antioxidant when its FRAP value is either slightly higher, equal, or lower than that of the positive

control. *H. depressa* acetone, methanol and aqueous extracts showed good antioxidants. Acetone extracts from *H. depressa* exhibited significant antioxidant activity, as shown by their ability to achieve an antioxidant effect at a lower concentration ($0.87 \pm 0.03 \mu\text{g/ml}$), compared to the positive control Trolox ($1.00 \pm 0.02 \mu\text{g/ml}$) (Table 8.1). *H. depressa* methanol extracts also showed strong antioxidant potential as compared to Trolox by producing a slightly higher concentration ($1.05 \pm 0.04 \mu\text{g/ml}$) required to produce an antioxidant effect. Based on the results of both the DPPH and FRAP methods, it can be affirmed that the acetone and methanol extracts from *H. depressa* exhibit strong antioxidant properties. Therefore, this warrants further investigations on isolated compounds to determine the possible leads on the use of the plant to develop antioxidant agents.

Table 8.1: Antioxidant activity evaluation of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts using the DPPH and FRAP method.

Plant name	Extracts	DPPH IC ₅₀ (μg/ml)	FRAP IC ₅₀ (μg/ml)
<i>B. onopordifolia</i>	Acetone	9.83±0.31	ND
	Methanol	172.00±6.18	17.34±0.88
	Aqueous	302.13±28.87	28.83±2.25
<i>S. runcinata</i>	Acetone	48.52±1.98	8.14±0.52
	Methanol	19.10±0.34	3.18±0.02
	Aqueous	15.02±0.054	3.90±0.13
<i>S. columbaria</i>	Acetone	76.75±1.95	12.13±0.39
	Methanol	33.68±1.78	6.58±0.06
	Aqueous	41.19±0.72	8.94±0.61
<i>H. depressa</i>	Acetone	3.58±0.44	0.87±0.03
	Methanol	5.20±0.10	1.05±0.04
	Aqueous	9.10±1.94	2.31±0.09

Ascorbic acid		6.57±0.98	ND
Trolox		6.17±0.43	1.00±0.02

ND=not determined; All experiments were conducted in triplicates; All the data was expressed as means ± standard deviation; IC₅₀= half maximal inhibitory concentration.

The presence of bioactive compounds such as saponins, tannins and phenols in *H. depressa* aqueous and acetone extracts was reported by Molefe (2013). Saponins were reported to have various properties including antioxidant activity (Desai *et al.*, 2009). Therefore, the antioxidant activity demonstrated by *H. depressa* aqueous, acetone and methanol in this chapter may be due to the presence of saponins because of their ability to inhibit the formation of reactive oxygen species (ROS) (Tatiya *et al.*, 2007). Tannins have also been found to have antioxidant and radical scavenging effects in the gastrointestinal system when consumed (Seranno *et al.*, 2009). Tannins have shown *in vitro* to prevent lipid peroxidation and lipoxygenases, and there is evidence indicating their capacity to scavenge radicals such as hydroxyl, superoxide, and peroxy, which are known to be significant in cellular prooxidant states (Haslam, 1996; Gyamfi and Aniya, 2002). Furthermore, some of the bioactive compounds with antioxidant properties found in *H. depressa* extracts include 4-O-caffeoylquinic acid, Tricin, quercetin, Jaceosidin, and 6-Gingerol (Chapter 5). Therefore, the antioxidant activity of *H. depressa* noted in this chapter may be attributed to the presence of the above-mentioned bioactive compounds.

B. onopordifolia extracts were reported to have compounds such as 3',4'-Dimethoxyluteolin, Isosakuranetin, Formononetin, azelaic acid and other antioxidant compounds. A plant belonging to the *Berkheya* genus (*Berkheya bergiana*) was found to contain compounds such as flavonoids and terpenoids (Kumar and Pandey, 2013).

Flavonoids and terpenoids are also known to display antioxidant properties (Yang *et al.*, 2020; Karak, 2019). Therefore, the antioxidant activity displayed in this chapter by *B. onopordifolia* extracts may be due to the presence of the above-mentioned compounds. Flavonoids, saponins, tannins etc. are currently being used in nutraceutical, pharmaceutical, medicinal, and cosmetic applications (Chiocchio *et al.*, 2021). *H. depressa* and *B. onopordifolia* may be considered as promising antioxidant sources.

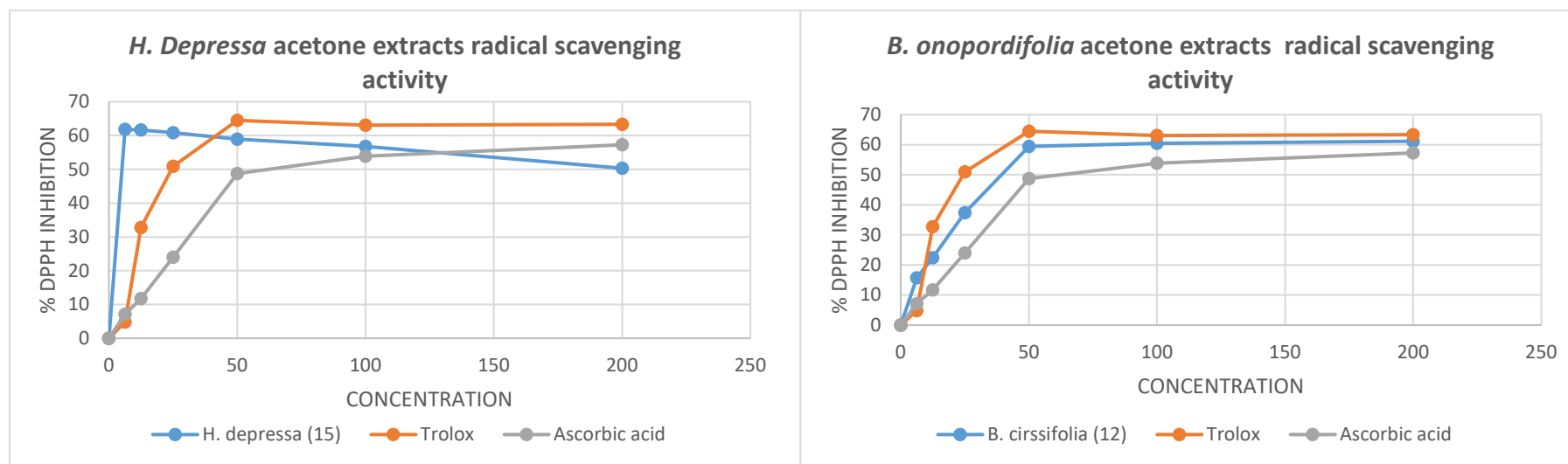


Figure 8.3: The percentage of antioxidant activity of *H. depressa* and *B. onopordifolia* acetone extracts. Ascorbic acid and Trolox were included as positive controls. Antioxidant activity was measured by the inhibition of DPPH radicals, and each value is expressed as the mean $\pm$ standard deviation.

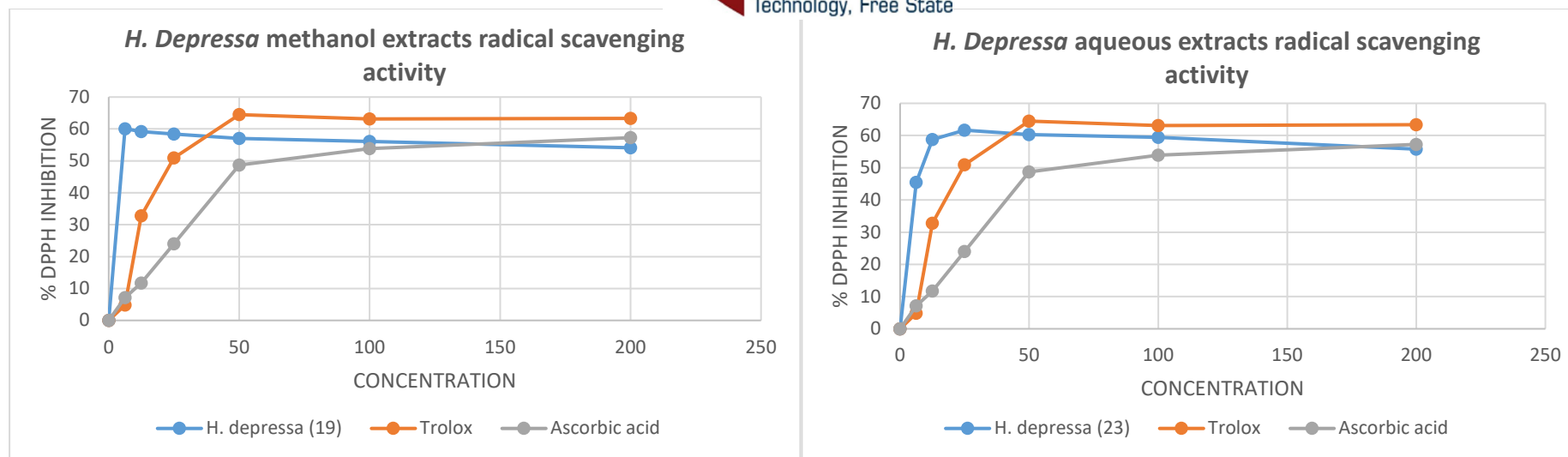


Figure 8.4: The percentage of antioxidant activity of *H. depressa* methanol and aqueous extracts. Ascorbic acid and Trolox were included as positive controls. Antioxidant activity was measured by the inhibition of DPPH radicals, and each value is expressed as the mean $\pm$ standard deviation.

8.3.4. Selectivity index of the most antioxidant plant extracts

To ensure the safety of plant extracts for potential use as antioxidants, a cell-based toxicity test was conducted to assess their cytotoxic effects on Vero monkey kidney cells, as described in Chapter 6. The selectivity index values for all antioxidant active plant extracts were less than 1, suggesting that none of the plant extracts exhibited selectivity against the Vero cells employed in this study (Table 8.2). According to the In Vitro cytotoxicity criteria outlined by Syarifah et al (2011), a compound or plant extract is categorized as having low toxicity if the CTC₅₀ is equal to or greater than 50 µg/ml, moderately active if the CTC₅₀ falls between 10 µg/ml and 50 µg/ml, and as highly toxic if the CTC₅₀ is less than or equal to 10 µg/ml. The antioxidant activity of the most antioxidant plant extracts maybe due to the toxicity effect of the extracts. Hence, it is essential to conduct investigations on isolated compounds from *H. depressa* to identify the specific compounds that could be responsible for the toxicity observed in the plant extracts.

Table 8.2: The cytotoxicity (CTC₅₀) of the most antioxidant plant extracts and their selectivity index values against normal Vero cells.

Plant	Extract	CTC ₅₀ (µg/ml)	DPPH	FRAP
<i>H. depressa</i>	Acetone	5	0.15	0.76
	Methanol	5	-0.01	0.68
	Aqueous	16	0.25	0.84

8.4. CONCLUSION

H. depressa acetone, *S. columbaria* methanol and *S. runcinata* methanol extracts have promising anti-inflammatory activity by demonstrating strong inhibitory action against Nitric Oxide release, which has been linked to the etiology of many inflammatory conditions. However, *S. runcinata*, *S. columbaria* acetone extracts and *B. onopordifolia* methanol extracts' anti-inflammatory activity was ruled out due to the high cytotoxic effects they showed against RAW 264.7 macrophages. *H. depressa* aqueous, methanol and acetone and *B. onopordifolia* acetone extracts showed the strongest DPPH scavenging activity at various concentrations. The antioxidant activity of *H. depressa* and *B. onopordifolia* is strong enough that the plants can be used as alternative natural sources of antioxidants in food as compared to ascorbic acid and Trolox. However, due to their non-selectivity against Vero cells, the antioxidant activity could be due to their toxicity. Therefore, further investigations need to be conducted on isolated compounds. *H. depressa*, *S. columbaria* and *S. runcinata* are promising plants that may be used in the treatment of inflammatory conditions that may be linked to STIs. *H. depressa* and *B. onopordifolia* have also indicated to be strong antioxidants that may be used to prevent oxidative stress caused by STIs.

8.5. REFERENCES

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CHAPTER 9

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

9.1. INTRODUCTION

Due to ineffective preventive and treatment methods, sexually transmitted infections (STIs) pose a serious threat to public health. STIs are one of the top five disease categories treated by healthcare professionals, according to data from the World Health Organization (WHO). Every day, more than a million people are thought to have STIs (Date and Destache, 2016). Furthermore, 80% of Africans, according to the WHO, use traditional medicine for their main healthcare requirements, which includes treating STIs despite the advancements made in modern scientific medicine (Chinsembu, 2016). This reliance on medicinal plants by some STI patients can be attributed to traditional beliefs, cultural barriers, low socio-economic status, stigma, lack of confidentiality, and inadequate user-friendly facilities (Tsobou *et al.*, 2016).

Moreover, medicinal plants have been introduced as the main source of the novel biomolecules, which can hypothetically be used in treating multiple life-threatening illnesses such as STIs (Zeidali *et al.*, 2021). There are various plants already being used traditionally to treat STIs and many other diseases across communities in South Africa and the world. Documentation of medicinal plants used to treat STIs through ethnobotanical studies is important for preservation of indigenous knowledge and conservation of plant resources (Zeidali *et al.*, 2021). Documentation of traditional knowledge on medicinal plants has contributed to the development of drugs. Therefore, this study focused on documentation of plants used to treat STIs and to

further investigate the pharmacological properties, safety, and the phytochemical properties of the commonly used plants in the study area.

9.2. SUMMARY OF THE RESULTS AND DISCUSSION

9.2.1. Ethnobotanical survey of medicinal plants used for the treatment of STIs in Thaba 'Nchu

One of the objectives of this study was to document the medicinal plants used for the treatment of STIs in Thaba 'Nchu through an ethnobotanical survey. The ethnobotanical survey presented information in **Chapter 3** on the type of STIs that local traditional healers treat and the medicinal plants they use to treat those infections. According to the analysis of ethnobotanical survey data, it was found that medicinal plants are frequently employed in the treatment of various conditions such as Herpes zoster, Vaginitis, pubic lice, HIV/AIDS, and Gonorrhoea in the study area. It was further revealed that 35 medicinal plants from 24 families were used to treat STIs in the community by traditional healers. Most of the plants reported for the treatment of STIs are from Asteraceae, Fabaceae, Solanaceae and Hyacinthaceae families, while *Bulbine narcissifolia*, *Pentanisia prunelloides*, *Hypoxis hemerocallidea*, *Scabiosa columbaria*, *Xysmalobium undulatum*, *Rumex lanceolatus*, *Eucomis autumnalis*, *Dicoma anomala* and *Salvia runcinata* were listed among the plants that are frequently used to treat STIs in Thaba 'Nchu. Among the plant parts used for preparing various remedies, the roots were found to be the most utilized. The preferred method of remedy preparation was decoction. The results of this study indicate the impact of STIs on the health of the residents of Thaba 'Nchu. Even though there are treatment and preventative methods currently being used to address challenges posed by STIs, this study indicates that medicinal plants play an important role in the

treatment of STIs. Some of the medicinal plants mentioned in the current study have previously been reported in other ethnobotanical studies on medicinal plants for the treatment of STIs in other areas (Njoroge and Bussmann, 2009; Van Vuuren and Naidoo, 2010; Semanya *et al.*, 2013; Naidoo *et al.*, 2013; Tariq *et al.*, 2018; Maema *et al.*, 2019). Furthermore, some have also been reported for bioactivities which may justify their roles as natural remedies for the treatment of STIs in this study (Kamatou *et al.*, 2008; Van Vuuren and Naidoo, 2010; Seleteng-Kose *et al.*, 2019, Otang-Mbeng and Sagbo, 2020). The documentation of this knowledge through ethnobotanical studies is important in conservation and utilisation of medicinal plant resources. The ethnobotanical information of this study will serve as a base for more scientific pharmacological investigations on the plants and to validate their traditional use in the treatment of STIs. For further investigations four most favoured medicinal plants were selected namely *Hermannia depressa* (roots), *Berkheya onopordifolia* (roots), *Salvia runcinata* (whole plant) and *Scambiosa columbaria* (whole plant).

9.2.2. Phytochemical screening of *H. depressa*, *B. onopordifolia*, *S. runcinata* and *S. columbaria* extracts

Phytochemicals are known to be the main contributors to the pharmacological value of all medicinal plants hence their traditional use in the treatment of various diseases. To phytochemical profile of the selected plants was evaluated through standard phytochemical screening methods, Folin-Ciocalteu, aluminium chloride colorimetric method and LCMS in **Chapter 5**. The overall phytochemical screening results post analysis of *H. depressa*, *B. onopordifolia*, *S. runcinata* and *S. columbaria* extracts are presented in Table 9.1. The methanol, acetone and aqueous extracts of the selected medicinal plants were subjected to screening using standard methods. Important

phytochemicals such as alkaloids, steroids, glycosides, terpenoids, phenols, saponins appeared across all the plant extracts. These important phytochemicals are scientifically known to have various pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory and many other activities (Abdulaziz *et al.*, 2019). Therefore, the presence of such phytochemicals may explain why the extracts are used to traditionally treat various diseases, including STIs.

The Folin-Ciocalteu method was used to determine the concentration of total phenolic content (TPC) of the plant extracts. Amongst all the extracts evaluated for TPC, *H. depressa* acetone (8.45 ± 0.006 mgGAE/g), methanol (7.65 ± 0.004 mgGAE/g) and aqueous (5.66 ± 0.002 mgGAE/g) extracts contained the highest concentration of phenolic compounds. This study provides for the first time *H. depressa* quantitative phenolic compounds analysis. Further phytochemical analysis using Gas chromatography Mass Spectrometry (GCMS) and isolation of phenolic compounds is recommended for the development of new drugs. Total flavonoid content (TFC) was conducted using the aluminium chloride colorimetric method. *B. onopordifolia* methanol (11.77 ± 0.081 mgQE/g), *S. runcinata* methanol (5.15 ± 0.01 mgQE/g) and *S. columbaria* methanol (3.56 ± 0.01 mgQE/g) extracts contained the highest concentration of flavonoid compounds. Methanol extracts in this study had a high concentration of flavonoids. This could be due to the solubility of polyphenols in methanol. This is an indication that methanol could be a suitable solvent to consider when isolating flavonoids for further investigations on bioactivity of plants using compounds.

LCMS managed to isolate and identify various compounds in negative and positive mode. 3',4'-Dimethoxyluteolin, Azelaic acid, Naringenin, Isosakuranetin, Carsonic acid, Jaceosidin, Skimmin, 14-Methylmonocrotaline-like, and Formononetin are some of the compounds detected across methanol, acetone, and aqueous extracts of *B. onopordifolia*. The compounds are known to have various pharmacological properties such as anti-inflammatory, antidiabetic, antimicrobial, antiviral, anti-allergic, antioxidant, hepatoprotective, antihypertensive, cytotoxic and many other biological activities (**Chapter 5**). *H. depressa* extracts were found to contain compounds such as Methoxyestradiol, Jaceosidin, 6-Gingerol, 4-O-caffeoylquinic acid, Matairesinol, Pinoresinol, Pinoresinol, Salvigenin, Waltherione C, Tricin and Quercetin. The compounds are also known to have pharmacological properties which explains the use of *H. depressa* to treat various diseases as well as STIs in Thaba 'Nchu.

Xanthoxylin, Cirsimaritin, Salvigenin, Verbascoside, Carnosic acid, Chlorogenic acid, Secoxyloganin Guaijaverin, Rosmarinic acid, Genistein, Canadine acid and Salvanoic acid are compounds that were identified from *S. runcinata* extracts using LCMS in negative and positive mode. Carsonic acid and Rosmarinic acid were also detected in high levels from *S. runcinata* extracts using high-performance liquid chromatography, UV, and Mass spectroscopy (Kamatou, 2006). These compounds have a range of pharmacological properties amongst others antioxidant, anti-inflammatory, IgE-suppressive, anti-cancer, immunomodulatory, cytotoxic, antinociceptive, antidepressant and anxiolytic, antibacterial, antiviral, cardioprotective, hepatoprotective and neuroprotective properties. The presence of these compounds

in *S. runcinata* may contribute to the pharmacological value of the plant; hence, the traditional use in the treatment of diseases.

S. columbaria extracts LCMS analysis yielded bioactive compounds such as Eupatilin, Loganin, Cirsimaritin, 4-O-caffeoylquinic acid, Canadine, Chlorogenic acid, Gentiopicroside, Amarogenin and Tricin. Chlorogenic acid was also reported as one of the major compounds from the flowers and leaves of *Scambiosa columbaria*. The compounds detected in *S. columbaria* are also known to have important pharmacological properties. It is for this reason that the plant is used traditionally to treat diseases, including STIs.

Table 9.1. A summary of the phytochemicals found in *B. onopordifolia*, *H. depressa*, *S. runcinata*, and *S. columbaria* extracts.

Plants	Extracts	Standard methods	LCMS
<i>B. onopordifolia</i>	Acetone	Alkaloids, steroids	3',4'-Dimethoxyluteolin, Azelaic acid, Naringenin
	Methanol	Alkaloids, steroids, glycosides	Isosakuranetin, Carsonic acid, Jaceosidin
	Aqueous	Terpenoids, glycosides	Skimmin, 14-Methylmonocrotaline- like, Formononetin
<i>H. depressa</i>	Acetone	Terpenoids, tannins, phenols, saponins	Methoxyestradiol, Jaceosidin, 6- Gingerol,
	Methanol	Alkaloids, terpenoids, tannins, phenols, saponins	4-O-caffeoylquinic acid, Matairesinol, Pinoresinol, Pinoresinol, Salvigenin
	Aqueous	Terpenoids, tannins, saponins	Waltherione C, Tricin, Quercetin
<i>S. runcinata</i>	Acetone	Alkaloids, terpenoids, tannins, glycosides, flavonoids	Xanthoxylin, Cirsimaritin, Salvigenin, Verbascoside, Carnosic
	Methanol	Alkaloids, glycosides	Chlorogenic acid, Secoxyloganin Guaijaverin, Rosmarinic acid,

	Aqueous	Alkaloids, terpenoids, tannins, phenols, saponins	Genistein, Canadine, Rosmarinic acid, Salvanoic acid
<i>S. columbaria</i>	Acetone	Alkaloids, terpenoids, tannins, glycosides	Eupatilin, Loganin
	Methanol	Alkaloids, terpenoids, glycosides	Cirsimaritin
	Aqueous	Alkaloids, terpenoids, tannins, phenols, saponins	4-O-caffeoylquinic acid, Canadine, Chlorogenic acid, Gentiopicroside, Amarogenin, Tricin

9.2.3. The toxicity analysis of *H. depressa*, *B. onopordifolia*, *S. runcinata* and *S. columbaria* extracts

The toxicity of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* methanol, acetone and aqueous extracts was evaluated using the Hoechst 33342 nuclear dye exclusion method and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Whilst using the Hoechst 33342 nuclear dye exclusion method, it was found that *S. runcinata* methanol (CTC₅₀ = 3.93 µg/ml) and acetone (CTC₅₀ = 3.96 µg/ml), and *H. depressa* methanol (CTC₅₀ = 4.97 µg/ml) and acetone (4.65 µg/ml) extracts showed toxicity against the African green monkey kidney cell line (**Chapter 6**). The summary of toxicity evaluation results is displayed in Table 9.2.

The extracts were considered toxic due to the low CTC₅₀, and their use should be under dosage regulation to avoid exposure to negative side effects. *Salvia runcinata*, *Scambiosa columbaria* acetone extracts and *Berkheya onopordifolia* methanol extracts showed concentration-dependent toxicity against RAW 264.7 cell line (**Chapter 8**). The cell viability decreased with an increase in concentration. The results from both methods were considered since both methods have advantages and disadvantages. **Chapter 5** reported the presence of some compounds with both therapeutic and toxic properties. Pyrrolizidine alkaloids, tannins, 3',4'-Dimethoxyluteolin, Azelaic acid, Matairesinol, Salvigenin, Xanthoxylin and Rosmarinic acid are some of the toxic compounds detected across the plant extracts reported to be toxic in this study. Therefore, the toxicity displayed in this study may be due to the presence of the toxic compounds. The need to be used with caution to avoid exposure to their poisonous effects.

9.2.4. The antimicrobial activity of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts

The antimicrobial activity of the *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts was carried out using the broth microdilution method against bacterial species *Staphylococcus aureus*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* (Colistin-resistant), *Enterobacter hormaechei*, *Staphylococcus epidermidis*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Bacillus cereus*. Yeast species were *Candida albicans*, *Candida krusei*, and *Candida parapsilosis* (See **Chapter 7**). The summary of antimicrobial activity results of the plant extracts is displayed in Table 9.2.

H. depressa methanol extracts showed antimicrobial activity against three yeast and eight bacterial species at concentrations ranging from 0.3 to 1.25 mg/ml, while the acetone extracts of the same plant showed activity against six bacterial and two yeast species. The antibacterial activity noted in this study correlate to the results demonstrated by Hlongwane (2016), where *H. depressa* methanol, acetone and aqueous showed antibacterial activity against *Klebsiella pneumoniae*, *Bacillus pumilus*, *Escherichia coli* and *Staphylococcus aureus*. However, in the current study *H. depressa* aqueous extracts did not show any activity against all the microbial species it was tested against. This could be due to the absence of enough antimicrobial compounds in aqueous extracts to exert the activity against the microbial species used in this study. It is noteworthy that *H. depressa* methanol and acetone extracts showed activity against *Neisseria gonorrhoeae* and *Candida albicans* which are microbial species usually implicated with STIs. Therefore, the antimicrobial activity

results of this study warrant the use of this plant for the traditional treatment of STIs. However, more tests against other STIs causing microorganisms such as *Chlamydia trachomatis*, *Treponema pallidum*, *Mycoplasma genitalium* and *Haemophilus ducreyi* are required.

S. columbaria methanol extracts showed antimicrobial activity against seven bacterial and to yeast species. Acetone extracts of the same plant showed antimicrobial activity against five bacterial and one yeast species. The antimicrobial activity of *S. columbaria* could be due to the antimicrobial properties of phytochemicals detected in the extracts. In **Chapter 5** Van Vuuren and Naidoo (2010) evaluated antibacterial activities of aqueous and a mixture of methanol and dichloromethane leaf and root extracts of *S. columbaria*. The antimicrobial activities were evaluated against bacterial species associated with STIs which included *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, *Oligella ureolytica*, *Candida albicans* and *Ureaplasma urealyticum* using the microdilution method (Maroyi, 2019). The extracts showed antimicrobial activity at MICs ranging from 2.0 to 16 mg/ml, which is weaker than the MICs demonstrated in the current study (0.6-1.25 mg/ml). This is the first study to report on the antimicrobial activity of *S. columbaria* whole plant extracts. However, antimicrobial screening using isolated compounds against more STIs causing microorganisms is required. The result of this study is an indication that *S. columbaria* can be used to treat STIs, and it has the potential to be used to develop antimicrobial agents.

S. runcinata methanol extracts showed antimicrobial activity against 10 bacterial and three yeast species, while acetone showed activity against eight bacterial species

(including colistin resistant *E. coli*) and two yeast species - both extracts at concentrations ranging from 0.1 to 1.25 mg/ml. The antimicrobial activity displayed in this study was also demonstrated by Kamatou *et al.* (2005) where *S. runcinata* essential oils exhibited activity against *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis* at MIC greater than 1.6 mg/ml. It is important to note that *S. runcinata* extracts showed antimicrobial activity against colistin resistant *E. coli*. This could indicate a breakthrough in the treatment of drug-resistant infections such as gonorrhoea. There is limited evidence on the antimicrobial activity of compounds isolated from *S. runcinata*. Therefore, further studies on the antimicrobial activity of *S. runcinata* using isolated compounds and more microbial species especially the STI-causing microbes are required.

B. onopordifolia methanol showed antimicrobial activity extracts inhibited against 10 bacterial and two yeast species at concentrations ranging from 0.6 to 1.25 mg/ml, while acetone extracts were effective at concentrations of 0.6 mg/ml against four bacterial and two yeast species. The antimicrobial activity of *B. onopordifolia* displayed in this study may be attributed to the presence of antimicrobial compounds in the extracts, as indicated in **Chapter 5**. This plant needs to be tested against more STI-causing microbes; however, the results of this study warrant the use of this plant in the treatment of STIs due to activity demonstrated against *N. gonorrhoeae*.

9.2.5. Anti-inflammatory and antioxidant activity of *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts

STIs are mostly characterised by inflammation of the reproductive organs. Inflammation is a natural response of the immune system and can be beneficial in fighting off infections. However, if left untreated, STIs can cause chronic inflammation, which can lead to serious health problems such as infertility, chronic pelvic pain and oxidative stress which usually causes cancer. To address the above problems, *B. onopordifolia*, *H. depressa*, *S. runcinata* and *S. columbaria* extracts were evaluated for their anti-inflammatory and antioxidant activities. The anti-inflammatory evaluation was carried out using the Nitric Oxide assay, while antioxidant activity was evaluated using the DPPH assay (**Chapter 8**). The summary of anti-inflammatory and antioxidant activity of all the extracts are displayed in Table 9.2.

H. depressa acetone, *S. columbaria* methanol and *S. runcinata* methanol extracts showed good anti-inflammatory activity by demonstrating more than 60% inhibitory action against the release of Nitric Oxide, which is an inflammatory mediator linked to the progression of inflammatory conditions. The anti-inflammatory activity of *S. runcinata*, *S. columbaria* acetone and *B. onopordifolia* methanol extracts were ruled out due to the high cytotoxic effects they demonstrated using MTT assay, as reported in **Chapter 8**. *S. runcinata* aerial parts essential oils showed anti-inflammatory activity by inhibiting 15.93% of cyclooxygenase-2 (COX-2), which is also an inflammatory mediator (Kamatou *et al.*, 2005). The *S. runcinata* anti-inflammatory activity results from this study are a contribution to the body of knowledge that currently exists. The results further indicate that the plant can be used to treat inflammatory conditions related to STIs. However, the anti-inflammatory activity evaluation of isolated

compounds needs to be conducted. *S. columbaria* extracts' anti-inflammatory activity demonstrated in this study may be attributed to the presence of anti-inflammatory compounds reported to be present in the extracts (**Chapter 5**). The current study is the first to report *B. onopordifolia* extracts' anti-inflammatory activity. The anti-inflammatory activity of *B. onopordifolia* may be due to the presence of anti-inflammatory compounds in the extracts, which were also reported in **Chapter 5**. Therefore, both *S. columbaria* and *B. onopordifolia* can be used in the treatment of inflammation related to STIs and can be used as a basis to develop agents to treat inflammatory conditions.

H. depressa aqueous, methanol and acetone and *B. onopordifolia* acetone extracts showed the strongest antioxidant activity in this study. *H. depressa* methanol and acetone extracts showed the best antioxidant activity when compared to Trolox and ascorbic acid. The results indicate that *H. depressa* is the best candidate with the potential to be used as a plant-based antioxidant to help prevent oxidative stress caused by STIs. Antioxidant activity evaluation needs to be conducted using isolated compounds from *H. depressa* to increase the chances of the plant being developed into an antioxidant agent.

S. columbaria whole plant extracts did not show antioxidant activity in the current study. However, in a study conducted by Akar (2021), *S. columbaria* aerial parts' methanol + dichloromethane extracts showed strong antioxidant activity using DPPH, Ferric-reducing antioxidant power (FRAP), Cupric-reducing antioxidant capacity (CUPRAC) and ABTS⁺ radical scavenging capacity methods. The difference in results

may be because of the choice of extracting solvents; therefore, a change in extracting solvents may yield better results.

S. runcinata also showed poor antioxidant activity in the current study. However, in a study conducted by Kamatou *et al.* (2005), methanol extracts showed good antioxidant activity (IC_{50} values $\leq 16 \mu\text{g/ml}$) using the DPPH method. The antioxidant activity of *S. runcinata* may be attributed to the antioxidant compounds reported to be present in the extracts of *S. runcinata* such as rosmarinic acid, caffeic acid and carnosic acid (Kamatou *et al.*, 2010; **Chapter 5**). Based on the latter information, *S. runcinata* and *S. columbaria* still qualify to be used as plant-based antioxidants and can contribute to preventing oxidative stress that may be caused by STIs.

Table 9.2: Summary of the biological experiment results obtained in this study.

Plants	Extracts	Cytotoxicity analysis	Antimicrobial activity	Anti-inflammatory activity	Antioxidant activity
<i>B. onopordifolia</i>	Acetone	Weak	+	-	+
	Methanol	Toxic	+	-	-
	Aqueous	Weak	-	-	-
<i>H. depressa</i>	Acetone	Toxic	+	+	+
	Methanol	Toxic	+	-	+
	Aqueous	Weak	-	-	+
<i>S. runcinata</i>	Acetone	Toxic	+	-	-
	Methanol	Toxic	+	+	-
	Aqueous	Weak	-	-	-
<i>S. columbaria</i>	Acetone	Toxic	+	-	-
	Methanol	Weak	+	+	-
	Aqueous	Weak	-	-	-

9.4. GENERAL CONCLUSION

The outcome from this study provides a full ethnopharmacological profile of *H. depressa*, *B. onopordifolia*, *S. runcinata* and *S. columbaria* extracts as plants used to treat STIs in Thaba 'Nchu. All methanol and acetone extracts of *H. depressa*, *B. onopordifolia*, *S. runcinata* and *S. columbaria* were found to have significant antimicrobial activity against various microbes at various MICs. However, *B. onopordifolia* methanol, *H. depressa* and *S. runcinata* methanol and acetone, and *S. columbaria* acetone extracts' antimicrobial activity was ruled out due to the toxicity effects of the extracts against African green monkey kidney cell line and RAW 264.7 cell line. *B. onopordifolia* acetone and *S. columbaria* methanol extracts were found to be the most promising source for the development of antimicrobial drugs for the treatment of STIs. Both the extracts are considered safe to use due to non-toxicity effects against African green monkey kidney cell line and RAW 264.7 cell line. *S. columbaria* methanol extracts were considered the best to use for the development of anti-inflammatory agents for inflammatory conditions caused by STIs. The anti-inflammatory activity by *S. runcinata* methanol and *H. depressa* acetone extracts in this study was ruled out due to their toxicity effects against African green monkey kidney cell line and RAW 264.7 cell line. *B. onopordifolia* acetone and *H. depressa* aqueous extract were found to be the most promising plant-based antioxidant. These plants can play a role in preventing oxidative stress caused by STIs. *H. depressa* acetone and methanol extracts' antioxidant activity was ruled out due to their toxicity effects. All the tested plant extracts used to treat STIs in Thaba 'Nchu had pharmacological properties (antimicrobial, antioxidant, and anti-inflammatory activities). However, caution needs to be exercised when using the extracts that showed toxicity effects against the tested cell lines. Furthermore, the pharmacological

properties and the toxicity of the plants need to be tested using isolated compounds to confirm bioactive compounds that contribute to the therapeutic value of the plants.

9.5. LIMITATIONS OF THE STUDY

- It was not possible to subject the plant extracts to more STI-related microorganisms due to limited resources and the absence of a license to handle some pathogenic strains in our facilities.
- It was also not possible to further subject the plant extracts against more drug-resistant bacterial species due to a lack of resources.
- Most of the mentioned plants were not available in the study area; therefore, the selection of the plants to investigate further was limited to their availability.
- All the experiments were conducted *In Vitro*.

9.6. RECOMMENDATIONS FOR FUTURE RESEARCH

- Antimicrobial activity of the plant extracts and isolates compounds needs to be evaluated against more drug-resistant strains and more bacterial and fungal pathogens related to STIs.
- Bio-guided fractionation, isolation, purification, and identification of compounds are recommended to increase the chances of developing new drugs for the treatment of STIs and related ailments.
- Toxicity of all the isolated compounds needs to be investigated.
- The use of boiled water extracts to increase the possibility of positive results regarding antimicrobial activity evaluation is recommended, since the traditional healers usually boil the plant material for the preparation of the remedies.

- Synergistic effects of medicinal plants used for treating sexually transmitted infections (STIs) need to be investigated, as many traditional healers typically use a combination of plants to treat STIs.
- Further research is necessary to determine the structure of the unidentified compounds found in *Berkheya onopordifolia*, *Salvia runcinata*, *Hermannia depressa*, and *Scambiosa columbaria* using NMR techniques, following their detection through LCMS analysis in the current study.
- The experiments carried out in this study should be examined using animal studies.
- Development of an antimicrobial product using the most potent plant of the study is recommended.

9.7. REFERENCES

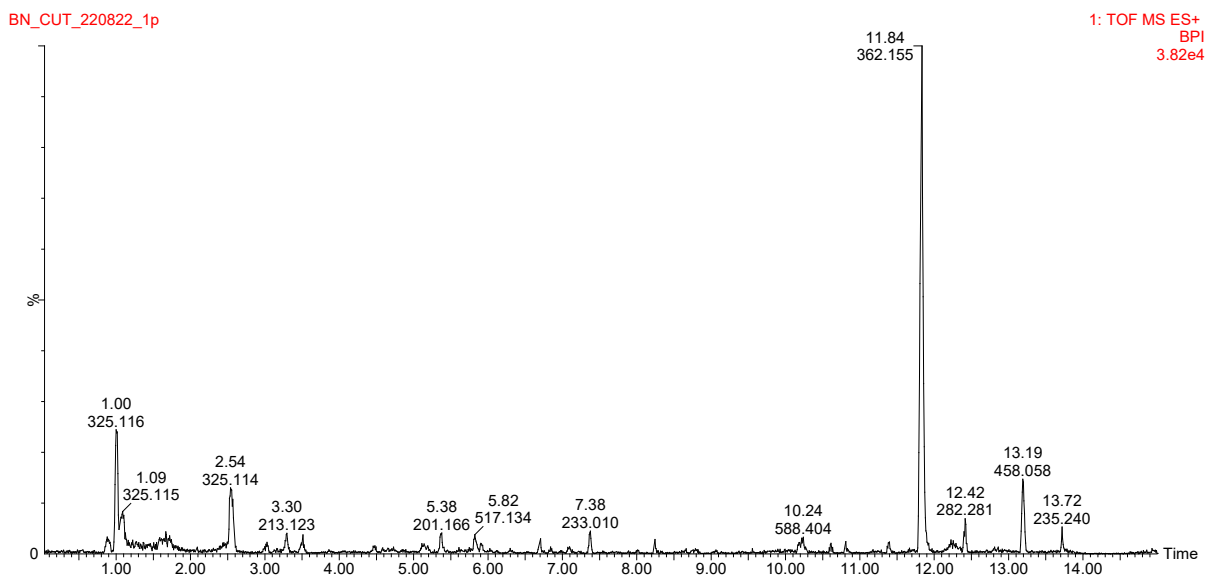
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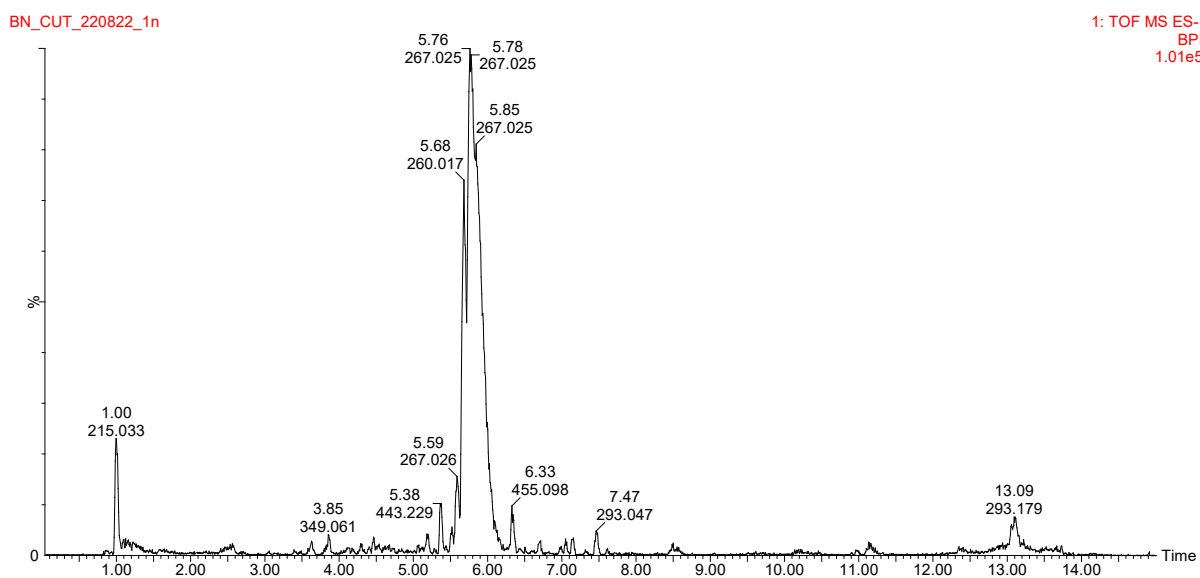
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APPENDICES

1. LCMS Chromatograms of plant extracts.



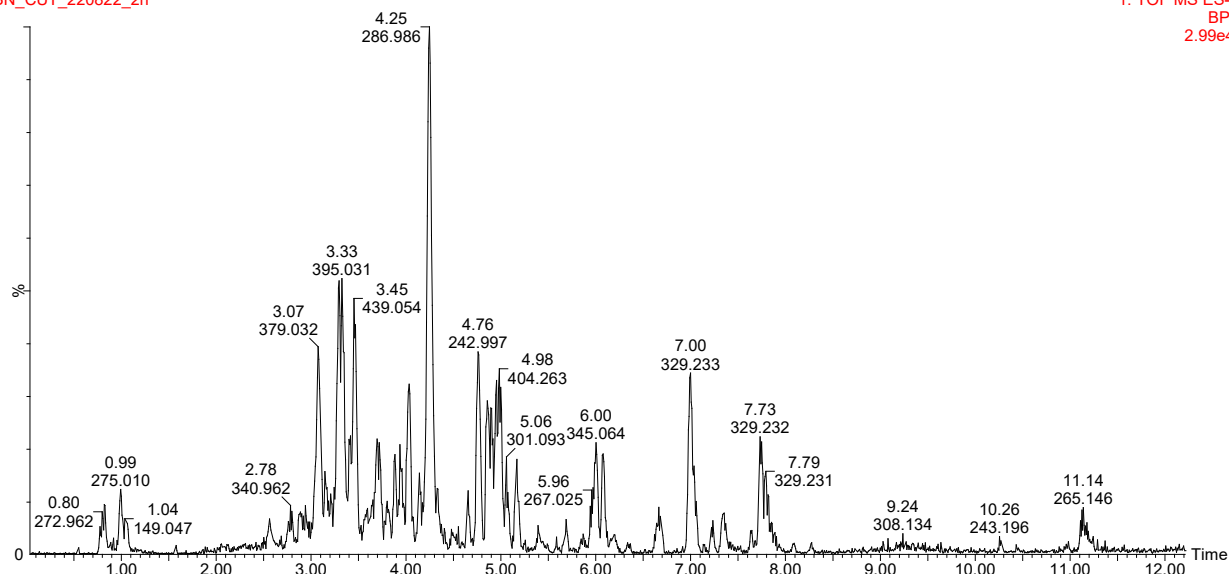
Appendix 1.1: LCMS chromatogram of *B. onopordifolia* aqueous extracts in positive mode.



Appendix 1.2: LCMS chromatogram of *B. onopordifolia* aqueous extracts in negative mode.

BN_CUT_220822_2n

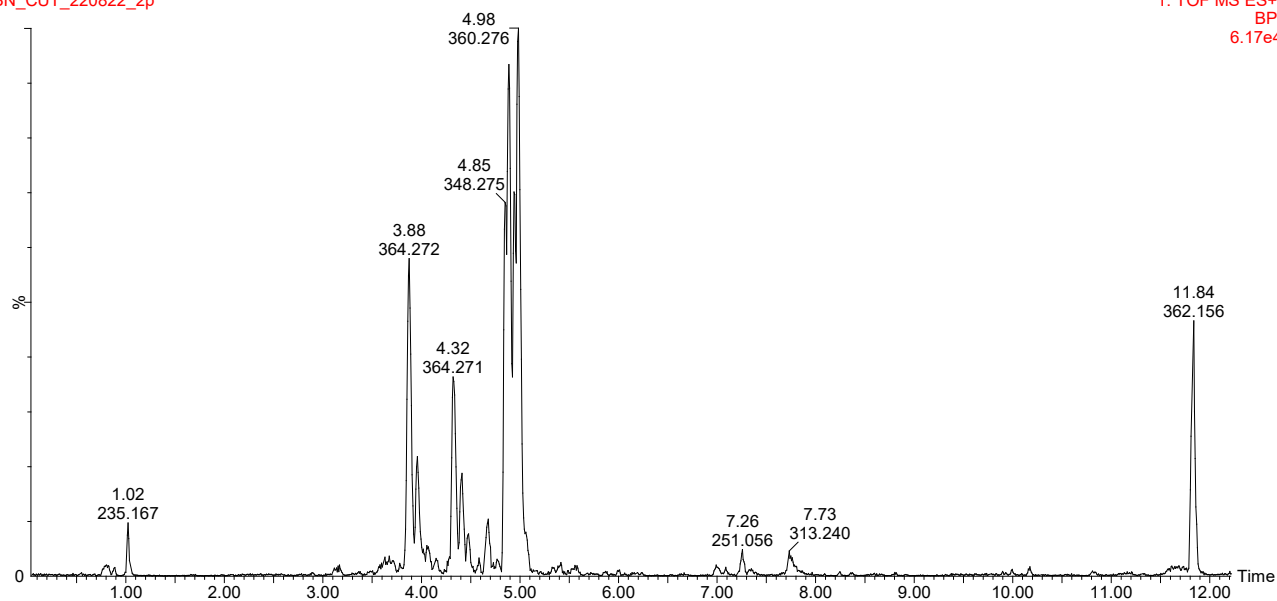
1: TOF MS ES-
BPI
2.99e4



Appendix 1.3: LCMS chromatogram of *H. depressa* aqueous extracts in negative mode.

BN_CUT_220822_2p

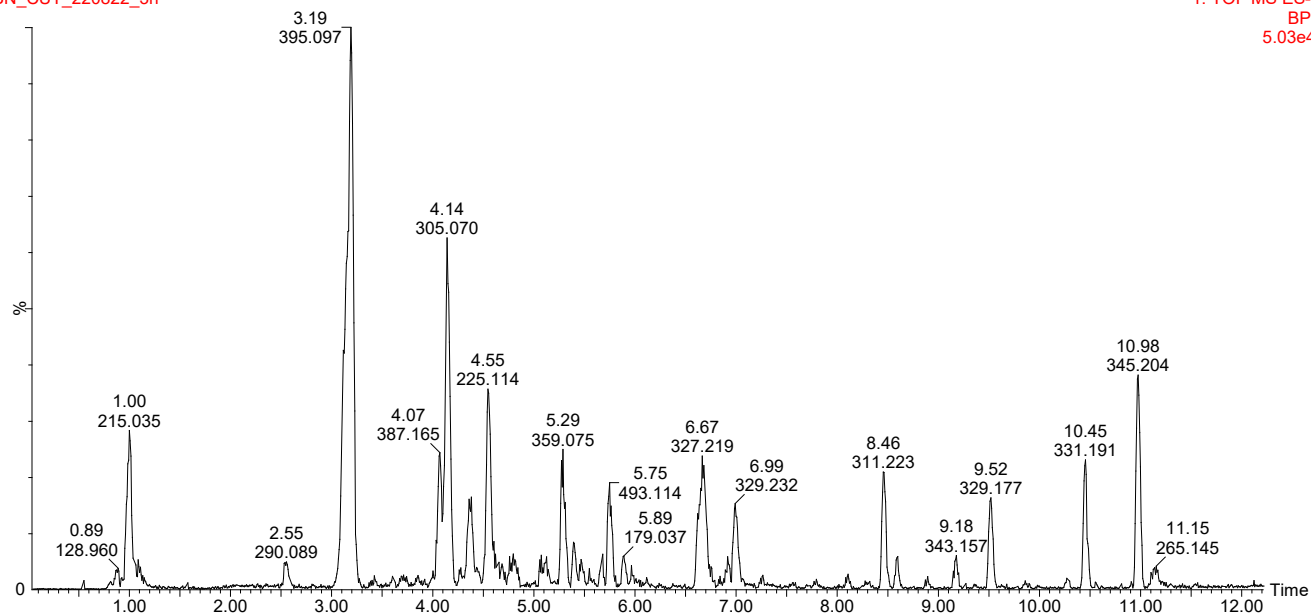
1: TOF MS ES+
BPI
6.17e4



Appendix 1.4: LCMS chromatogram of *H. depressa* aqueous extracts in positive mode.

BN_CUT_220822_3n

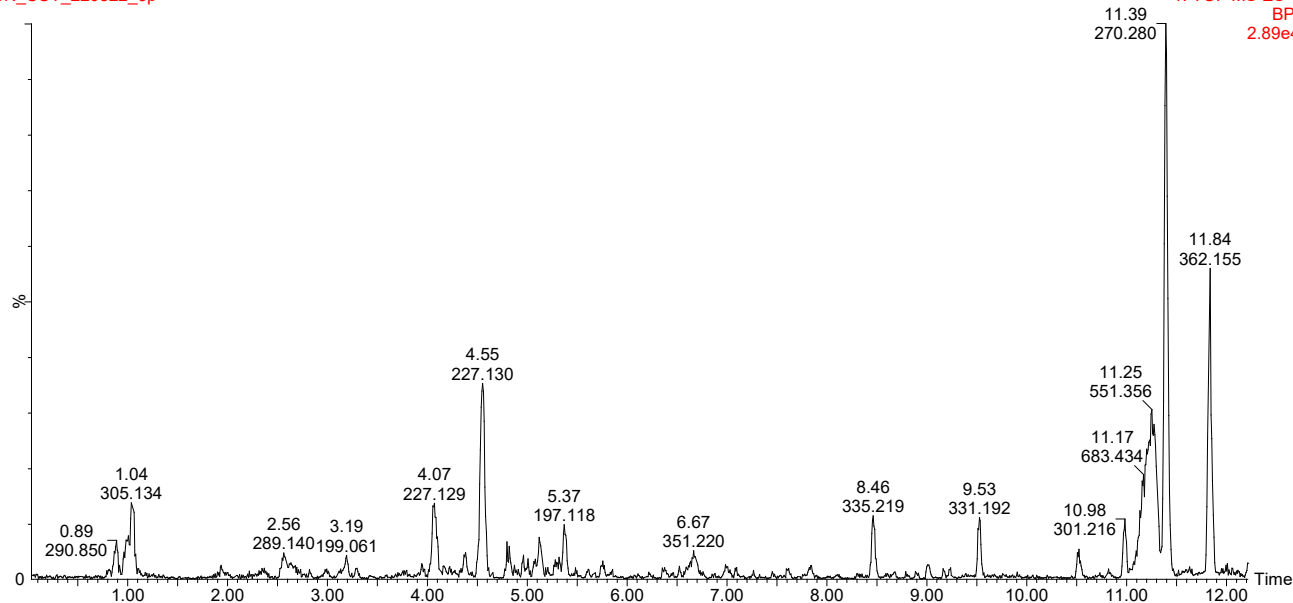
1: TOF MS ES-
BPI
5.03e4



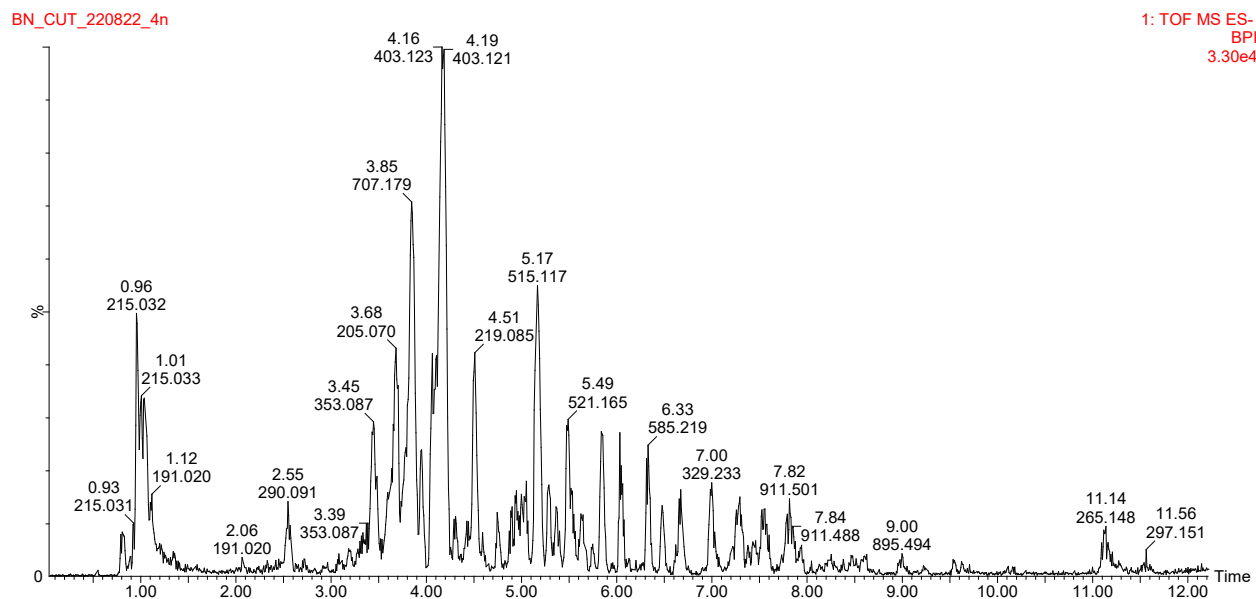
Appendix 1.5: LCMS chromatogram of *S. runcinata* aqueous extracts in negative mode.

BN_CUT_220822_3p

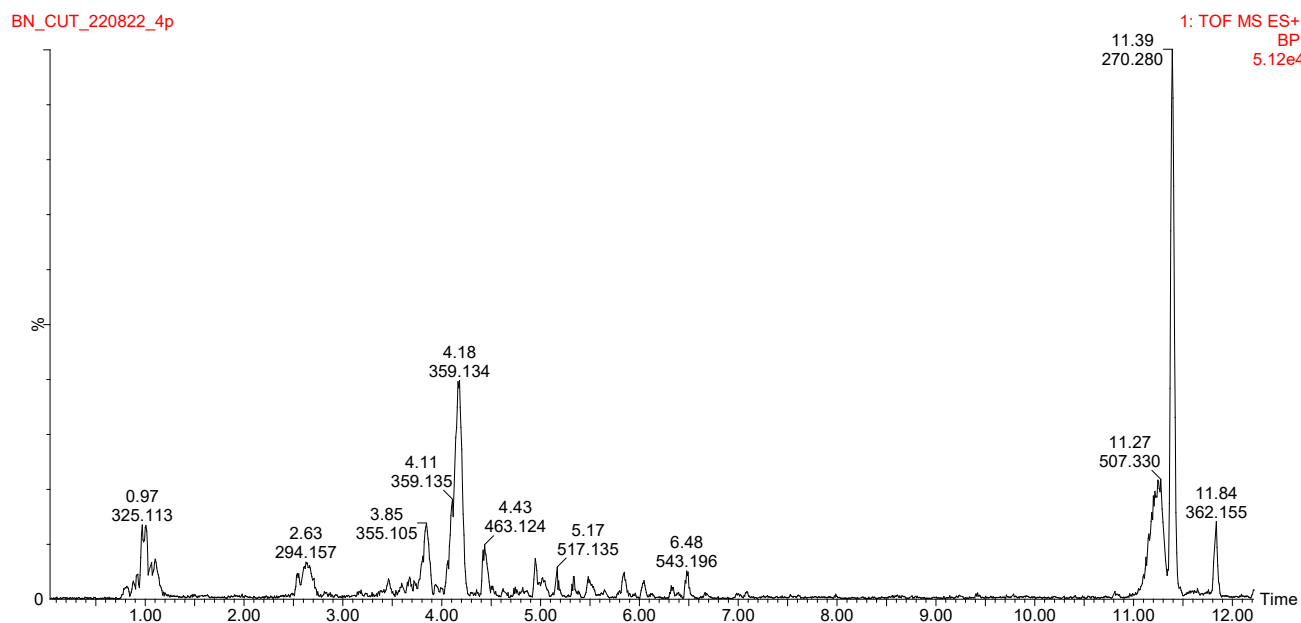
1: TOF MS ES+
BPI
2.89e4



Appendix 1.6: LCMS chromatogram of *S. runcinata* aqueous extracts in positive mode.



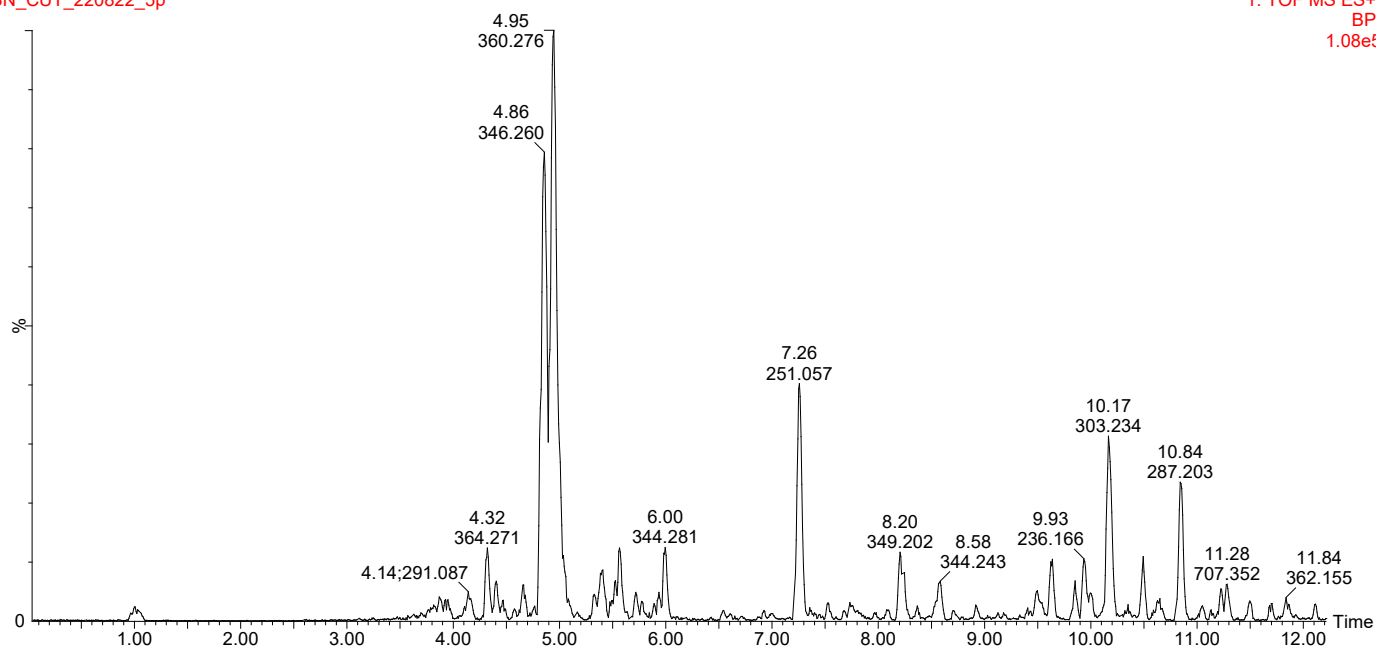
Appendix 1.7: LCMS chromatogram of *S. columbaria* aqueous extracts in negative mode.



Appendix 1.8: LCMS chromatogram of *S. columbaria* aqueous extracts in positive mode.

BN_CUT_220822_5p

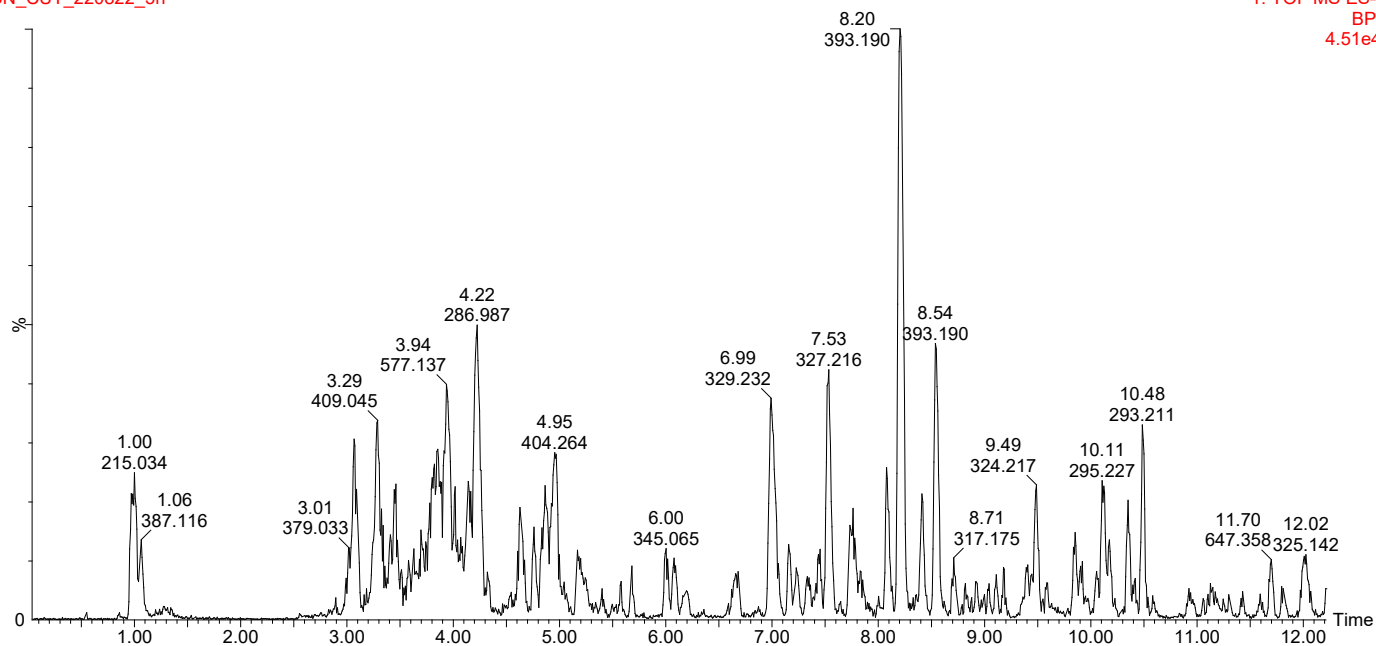
1: TOF MS ES+
BPI
1.08e5



Appendix 1.9: LCMS chromatogram of *H. depressa* acetone extracts in positive mode.

BN_CUT_220822_5n

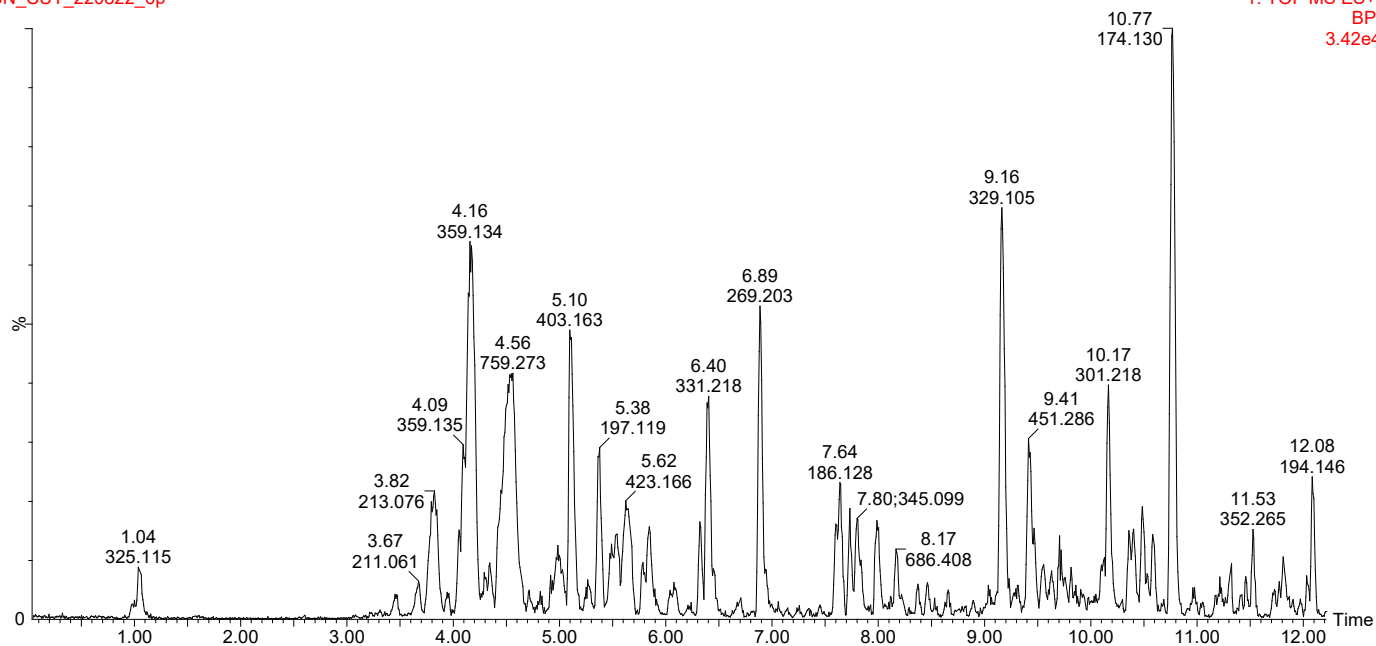
1: TOF MS ES-
BPI
4.51e4



Appendix 1.10: LCMS chromatogram of *H. depressa* acetone extracts in negative mode.

BN_CUT_220822_6p

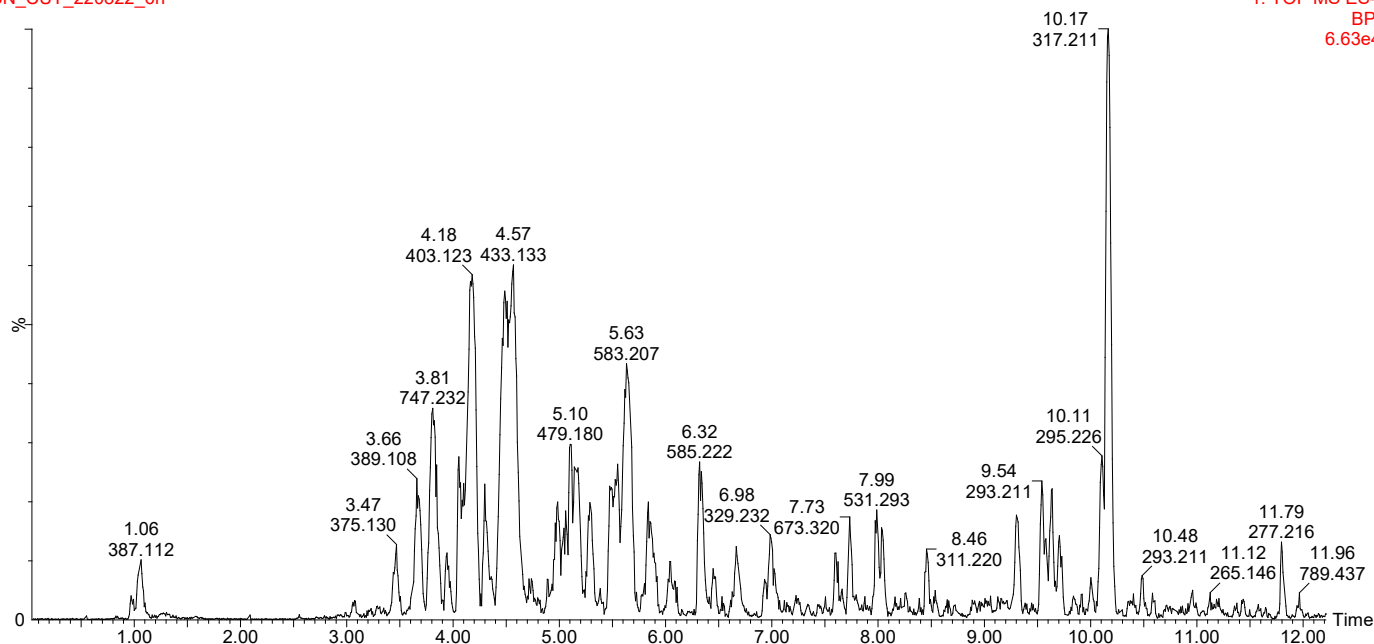
1: TOF MS ES+
BPI
3.42e4



Appendix 1.11: LCMS chromatogram of *S. columbaria* acetone extracts in positive mode.

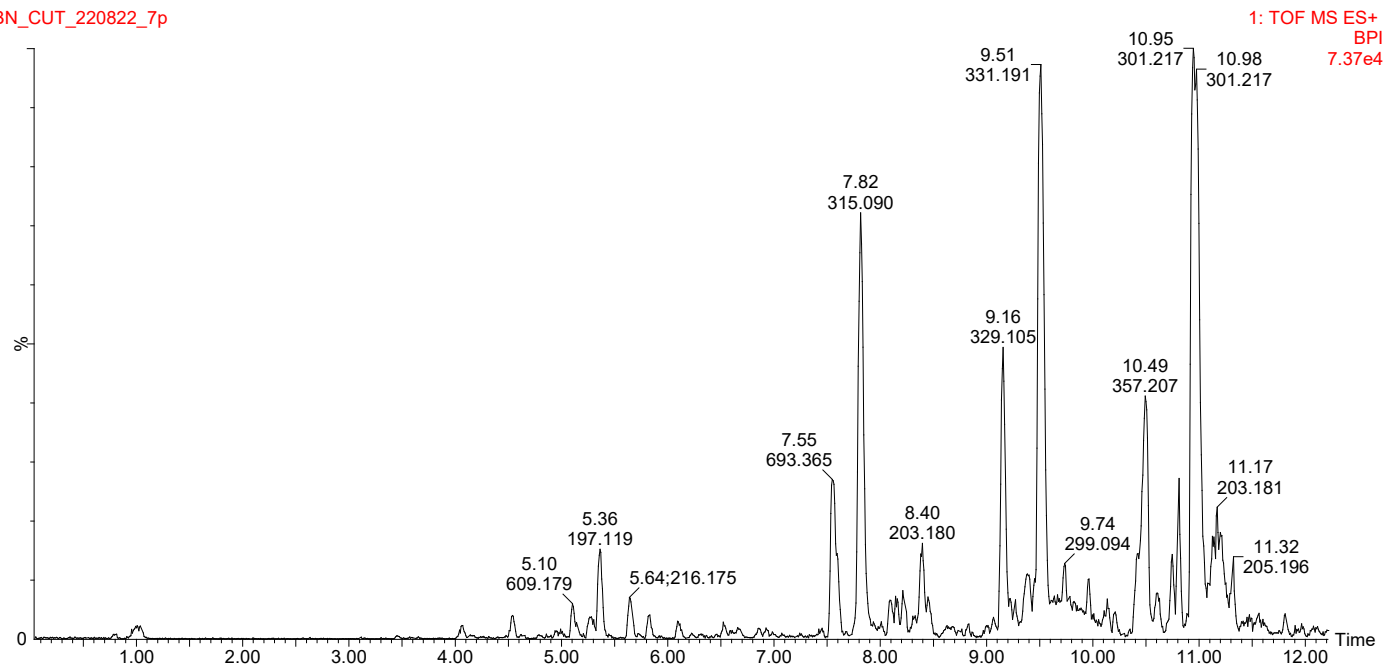
BN_CUT_220822_6n

1: TOF MS ES-
BPI
6.63e4



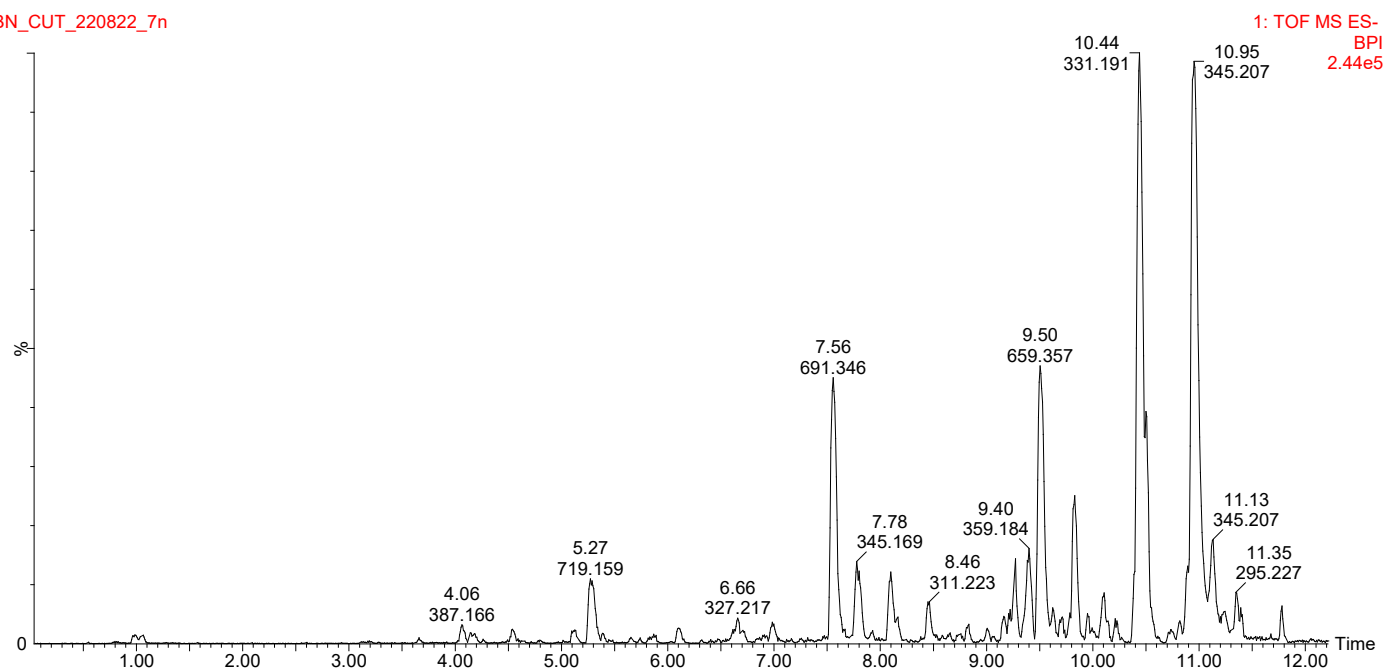
Appendix 1.12: LCMS chromatogram of *S. columbaria* acetone extracts in negative mode.

BN_CUT_220822_7p



Appendix 1.13: LCMS chromatogram of *S. runcinata* acetone extracts in positive mode.

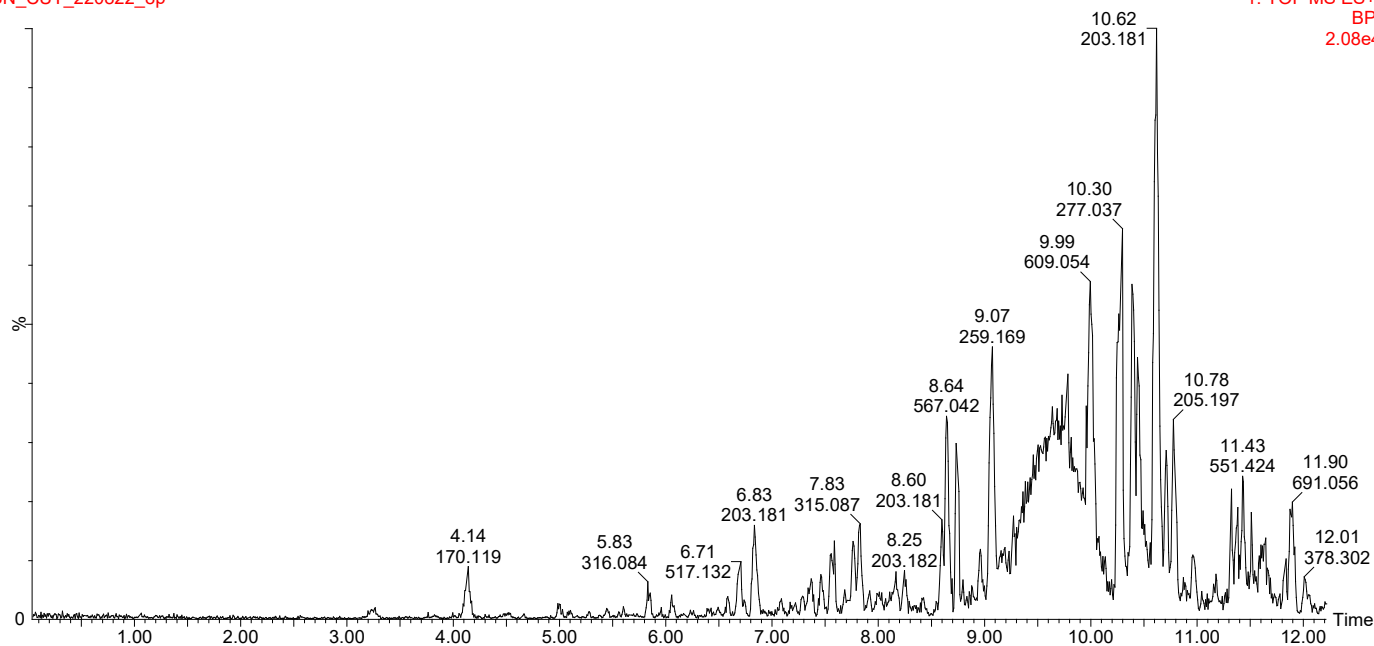
BN_CUT_220822_7n



Appendix 14: LCMS chromatogram of *S. runcinata* acetone extracts in negative mode.

BN_CUT_220822_8p

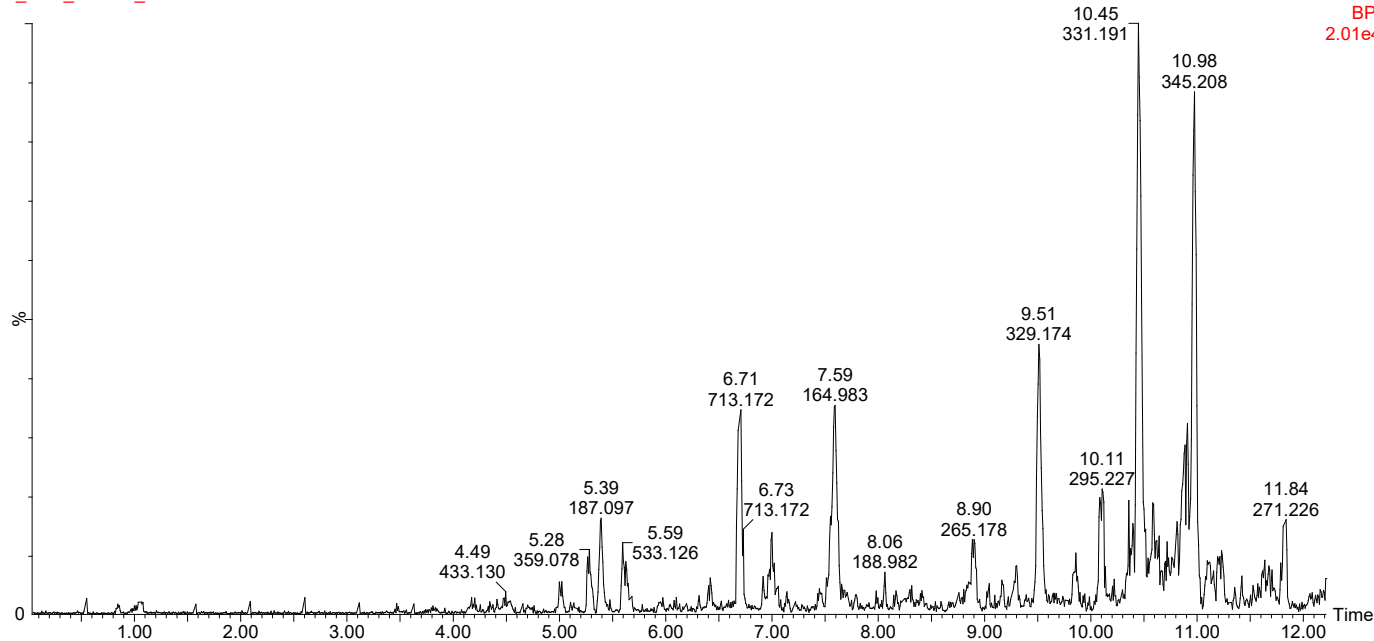
1: TOF MS ES+
BPI
2.08e4



Appendix 1.15: LCMS chromatogram of *B. onopordifolia* acetone extracts in positive mode.

BN_CUT_220822_8n

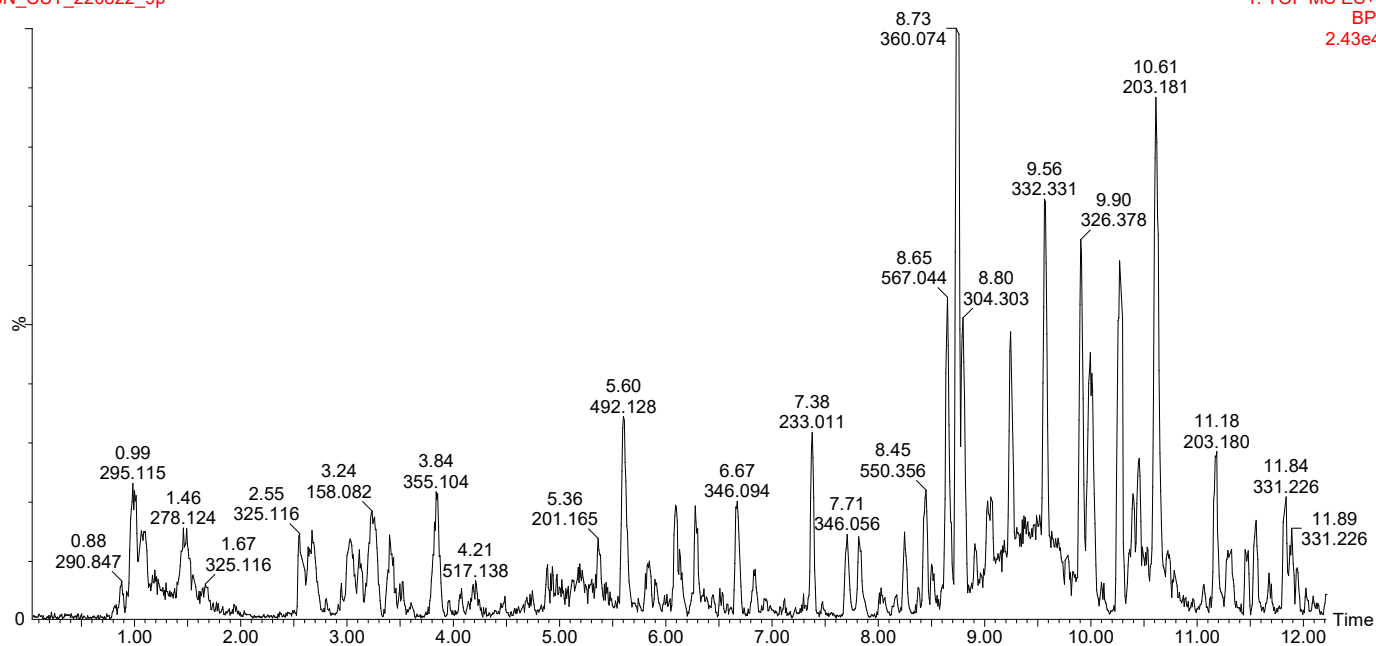
1: TOF MS ES-
BPI
2.01e4



Appendix 16: LCMS chromatogram of *B. onopordifolia* acetone extracts in negative mode.

BN_CUT_220822_9p

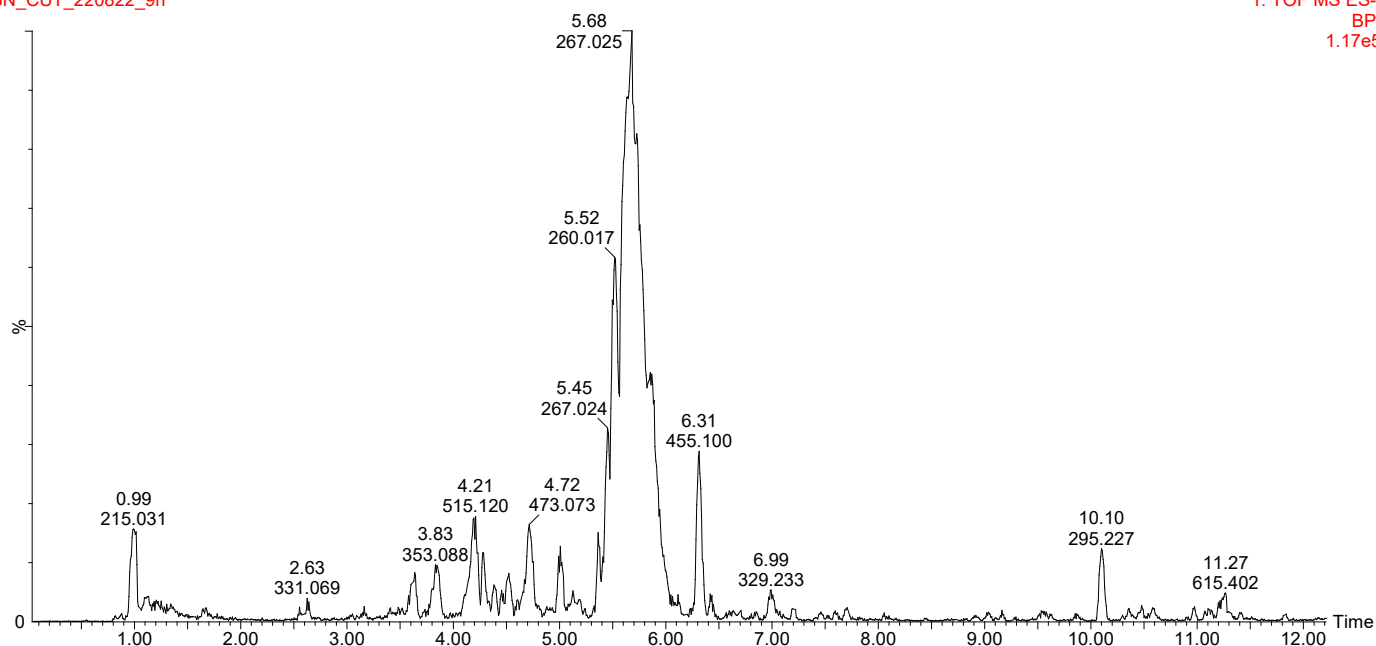
1: TOF MS ES+
BPI
2.43e4



Appendix 1.17: LCMS chromatogram of *H. depressa* methanol extracts in positive mode.

BN_CUT_220822_9n

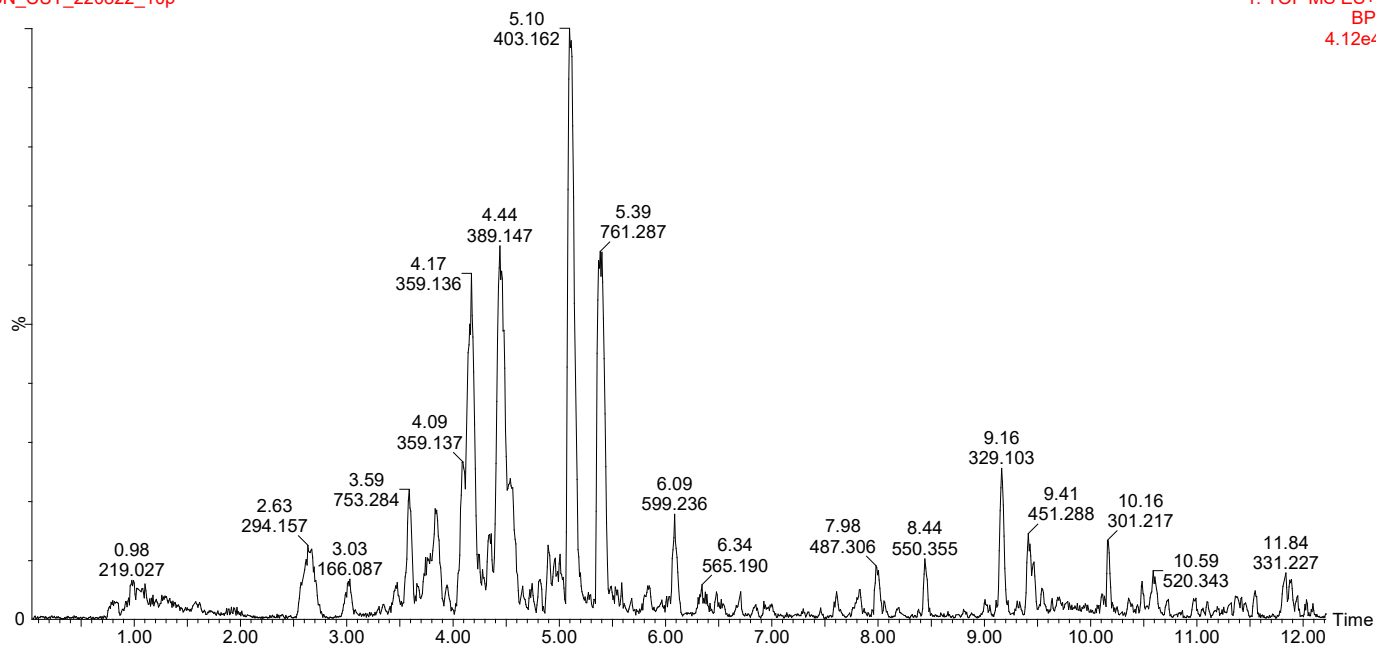
1: TOF MS ES-
BPI
1.17e5



Appendix 1.18: LCMS chromatogram of *H. depressa* methanol extracts in negative mode.

BN_CUT_220822_10p

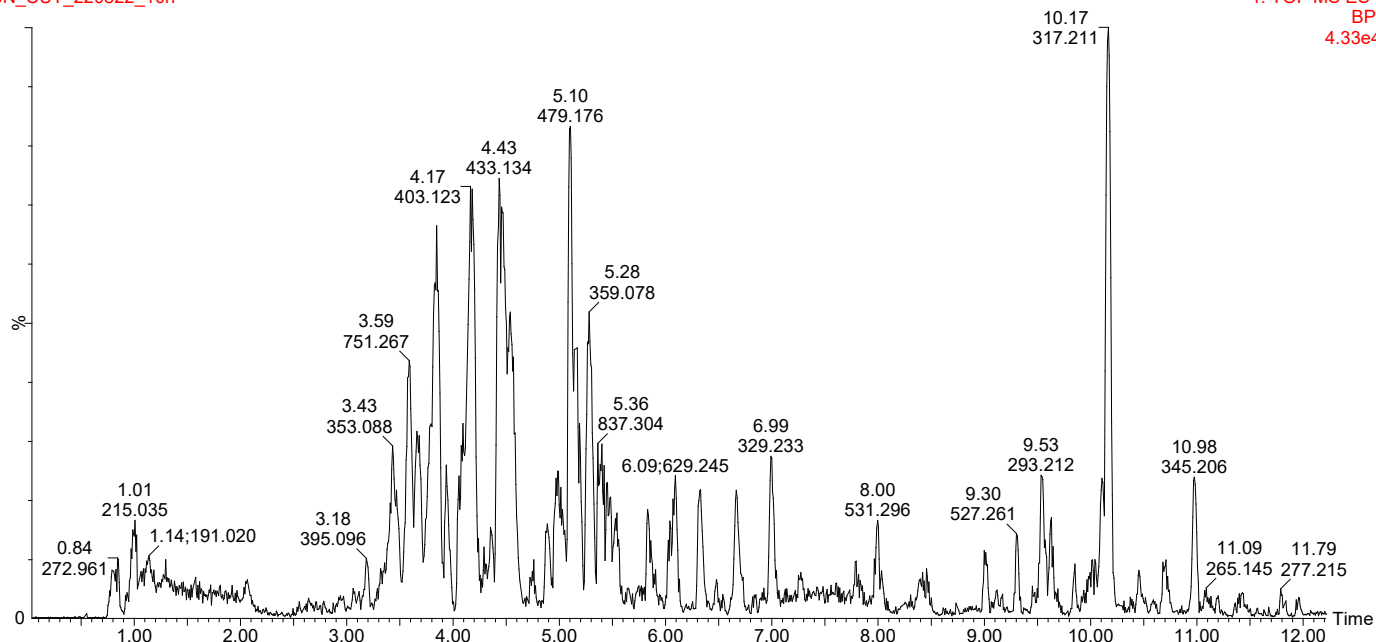
1: TOF MS ES+
BPI
4.12e4



Appendix 1.19: LCMS chromatogram of *S. runcinata* methanol extracts in positive mode.

BN_CUT_220822_10n

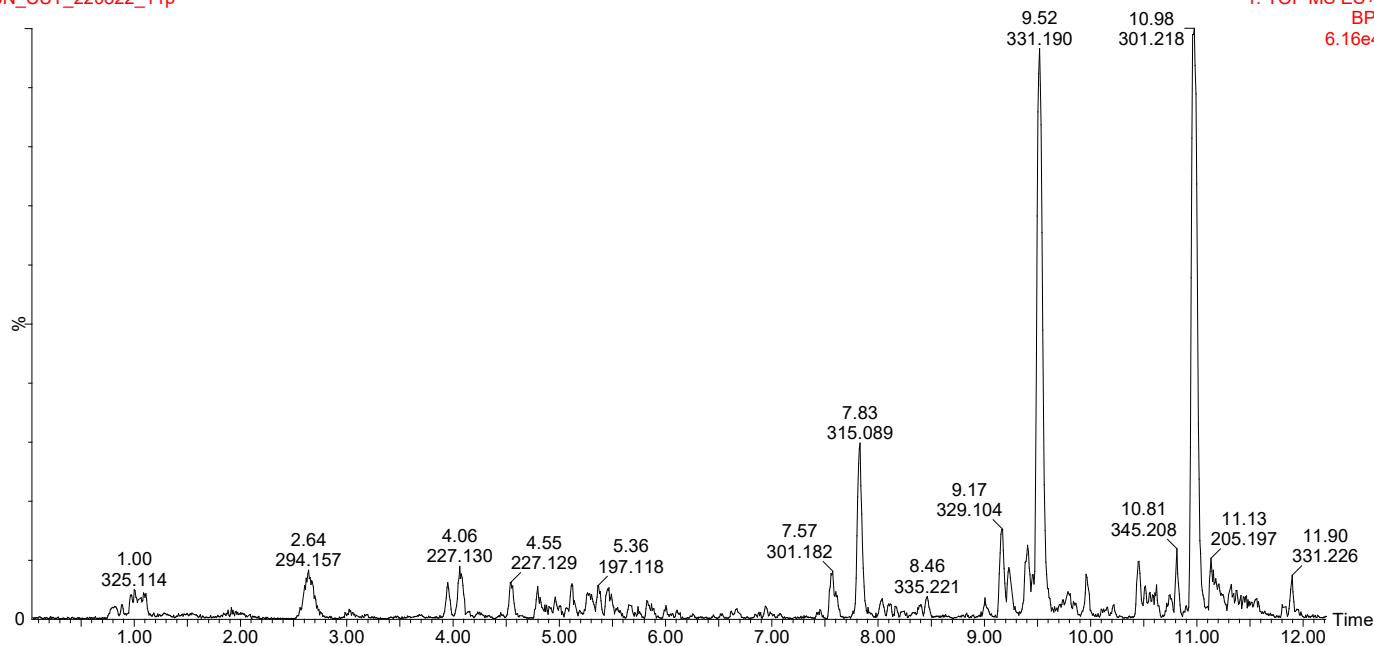
1: TOF MS ES-
BPI
4.33e4



Appendix 1.20: LCMS chromatogram of *S. runcinata* methanol extracts in negative mode.

BN_CUT_220822_11p

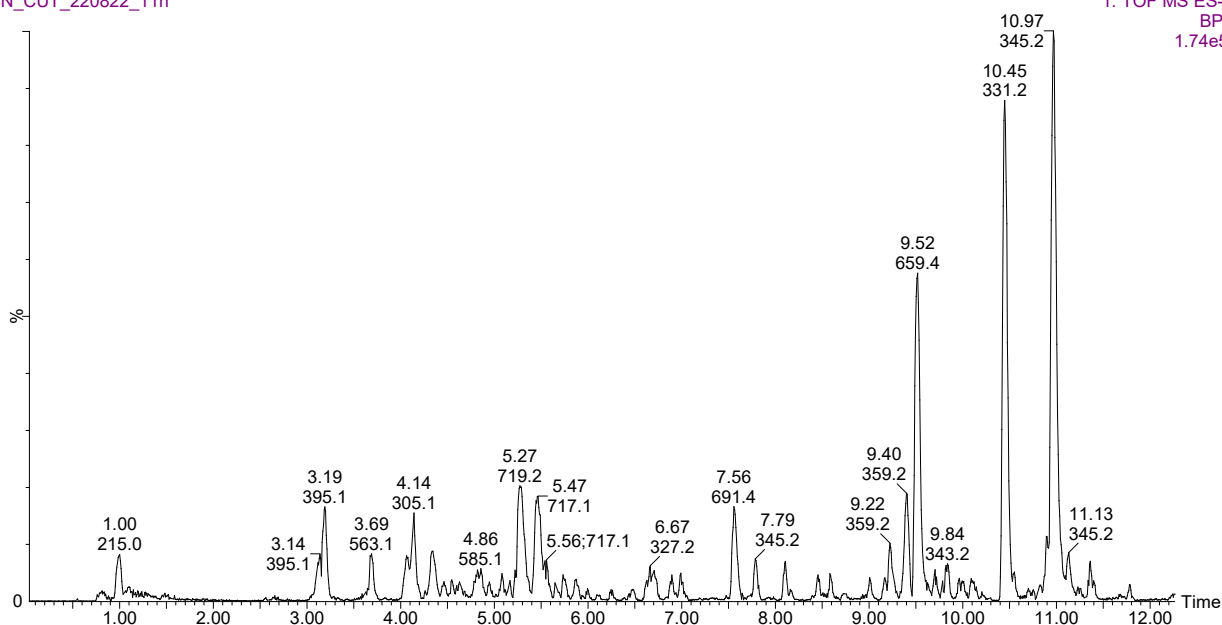
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BPI
6.16e4



Appendix 1.21: LCMS chromatogram of *S. columbaria* methanol extracts in positive mode.

BN_CUT_220822_11n

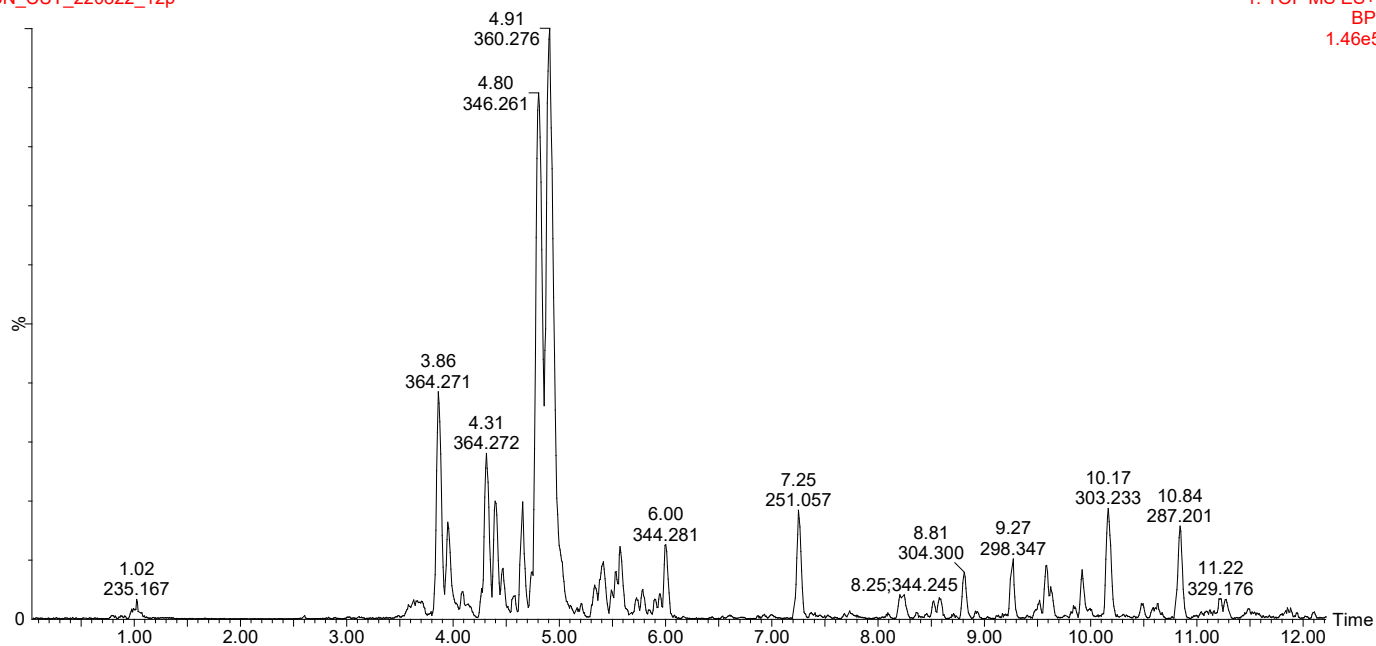
1: TOF MS ES-
BPI
1.74e5



Appendix 1.22: LCMS chromatogram of *S. columbaria* methanol extracts in negative mode.

BN_CUT_220822_12p

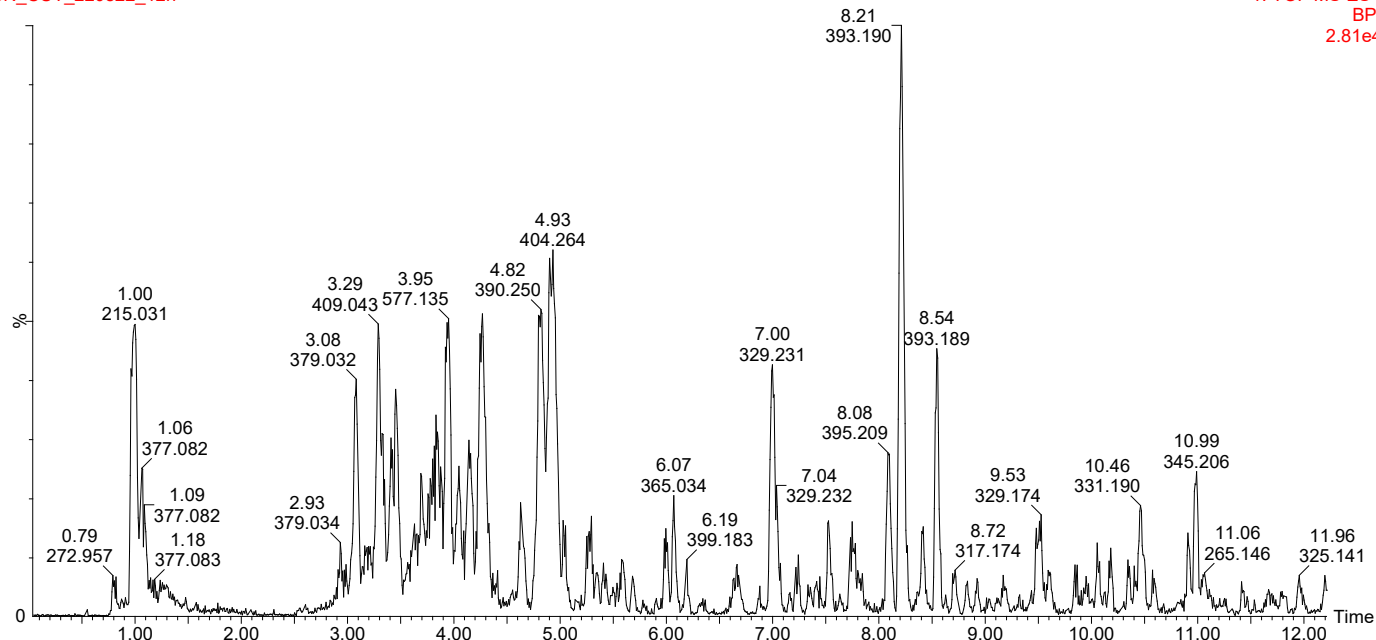
1: TOF MS ES+
BPI
1.46e5



Appendix 1.23: LCMS chromatogram of *B. onopordifolia* methanol extracts in positive mode.

BN_CUT_220822_12n

1: TOF MS ES-
BPI
2.81e4



Appendix 1.24: LCMS chromatogram of *B. onopordifolia* methanol extracts in negative mode.

2. Published article from this study.

Pharmacogn J. 2023; 15(1): 21-30

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Original Article

Phytotherapy for Sexually Transmitted Infections In Thaba 'Nchu, Free State Province, South Africa

Brian Ngobeni^{1,4}, Idah Tichaidza Manduna^{2,*}, Ntsoaki Joyce Malebo³, Samson Sitheni Mashele¹

Brian Ngobeni^{1,4}, Idah Tichaidza Manduna^{2,*}, Ntsoaki Joyce Malebo³, Samson Sitheni Mashele¹

¹Department of Health Sciences, Central University of Technology, Free State, Private Bag X20539, 9300, SOUTH AFRICA.

²Centre for Applied Food Sustainability and Biotechnology, Central University of Technology, Free State, Private Bag X20539, 9300, SOUTH AFRICA.

³Department of Life Sciences, Central University of Technology, Free State, Private Bag X20539, 9300, SOUTH AFRICA.

⁴Department of Clinical Sciences, Central University of Technology, Free State, Private Bag X20539, 9300, SOUTH AFRICA.

Correspondence

Idah Tichaidza Manduna

Centre for Applied Food Sustainability and Biotechnology, Central University of Technology, Free State, Private Bag X20539, 9300, SOUTH AFRICA.

E-mail: imanduna@cut.ac.za

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ABSTRACT

Medicinal plants have been prescribed by traditional healers for the treatment of Sexually Transmitted Infections STIs for years. This study documents therapeutic plants used against STIs in Thaba 'Nchu, South Africa. Information was gathered through semi-structured interviews with traditional medical practitioners between March and December 2017. Information on medicinal plants, their local names, and their uses against STIs was gathered. The use value (UV) of the plant species, frequency of citation (FC) and the informant consensus factor (ICF) were calculated for the plants and STIs included in the study. Plants were used to treat infections such as Herpes zoster (ICF=0.4) as gonorrhoea (ICF=0.11), vaginosis (ICF=0.2), symptoms of HIV/AIDS (ICF=0.1) and pubic lice (ICF=0.1). A total of 35 plant species were identified for the treatment of STIs. Most medicinal plants in the study area came from the families Asteraceae (21%), Fabaceae (17%), Solanaceae (13%) and Hyacinthaceae (13%). *Bulbine narcissifolia*, *Pentstemon prunellifolius*, *Hypoxis hemerocallidea*, *Scabiosa columbaria*, *Xysmalobium undulatum*, *Rumex lanceolatus*, *Eucomis autumnalis*, *Dicoma anomala* and *Salvia runcinata* were frequently used. The preferred method for preparation of remedies were decoctions made from the roots (72%), whole plant (17%), leaves (7%), fruits (2%) and bark (2%) which were consumed orally. This study contributes to the documentation of plants used for STIs to conserve the information for future generations. A selection from the most frequently mentioned plants for treatment of STIs may be used for further phytopharmacological investigations and subsequently be used for the development of new antimicrobial agents for global use and for the socioeconomic development of local communities.

Key words: Ethnobotany, Medicinal plants, Sexual transmitted infections, Thaba 'Nchu, Traditional Healers.

INTRODUCTION

The World Health Organization reported an estimated 357.9 million cases of sexually transmitted infections STIs that occur annually worldwide. South Africa has high rates (7.9 million in 2017) of HIV infections and millions of residents are co-infected with other STIs. The burden of these sexually transmitted infections is a global cause for concern because of the negative health, economic and social implications.¹⁻³ Addressing the burden of STIs requires concerted efforts from multiple angles including traditional knowledge and medicinal plants.

STIs are usually transferred from one person to the other through sexual contact. The most common pathogens responsible for STIs are *Treponema pallidum*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis*,^{4,5} which are treated with antimicrobial agents such as penicillin, azithromycin, amoxicillin, quinolone ciprofloxacin, metronidazole, famciclovir and many other drugs.⁶ However, most patients in developing countries especially from rural areas fail to seek therapy from official health care centres therefore they turn to traditional healers for help.⁷ Traditional healers are usually the first caregivers in rural areas because of traditional beliefs, poverty, and patient stigmatization.^{7,8} Additionally, limited accessibility to health care facilities, long waiting queues, lack of laboratories, shortages of drugs and poor attitudes of health workers make traditional medicine more appealing.⁹⁻¹¹

Traditional medicine especially medicinal plants, play an important role in the treatment and management of human diseases and ailments in South Africa.¹² Plants are the greatest source of remedies for many health problems as about 71 % of new drugs that have been approved since 1981 have directly or indirectly been derived from natural products.¹³ The need for basic scientific investigations on medicinal plants using indigenous knowledge has become more relevant because they can lead to discovery of novel compounds that will contribute to development of new drugs.¹⁴ However, some traditional communities have become less concerned about local resources and have adopted modern lifestyles, resulting in the loss of indigenous plant knowledge.¹⁵ Urbanization, modernization of public services including education systems, and globalization of trade and belief systems, and the rapid loss of natural habitats also contributes to the loss of knowledge.¹⁶ Furthermore, ethnobotanical knowledge of plant uses is typically passed down across generations through oral tradition which can lead to distortion.¹⁷⁻¹⁹ As a result, documenting traditional medicinal plant applications has become essential and critical.

Information on medicinal plant use in the Free State Province of South Africa is readily available in literature²⁰⁻²⁸ however; none is focused on the treatment of STIs in this particular region. Similar studies in South Africa have been carried out in other provinces for example, ethnic groups like Bapedi and Vhavenda in Limpopo,²⁹⁻³⁴ Zulu people in Kwa-Zulu Natal province,^{35,36} Tsonga people in

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Mpumalanga province.³⁷ Xhosa people in the Eastern and Western Cape province.³⁸⁻⁴⁰ Similarly, other studies have documented plants used by Basotho for treatment of various diseases in the Free State and Lesotho.⁴¹⁻⁴⁵ Therefore, the aim of this study is to document the medicinal plants used for treatment of STIs in Thaba 'Nchu located in the Free State province of South Africa. STIs were the most treated by traditional healers in this area.

Study area

Thaba 'Nchu is dominated by BaSotho and BaTswana who represent 80% of the overall population of the area. People in the area make a living through informal trade, agriculture, and some find employment in nearest city Bloemfontein.⁴⁶ It is one of the emerging towns of the Free State that has yet to be studied in terms of ethnobotany. Thaba 'Nchu is in the central portion of the Free State province, covering an area of roughly 704 km², and is about 67 km east of Bloemfontein (29.2040° S and 26.8204° E) (Figure 1). The study area is in the grassland biome, where the dominant plants are grasses, geophytes, and other small flowering plants. Thaba 'Nchu receives about 435 mm of rain annually, with most rainfall occurring mostly during the summer. It receives the lowest rainfall (3mm) in June and the highest (73mm) in March. The average midday temperatures range from 15.4°C in June to 28.5°C in January. The region is the coldest during July where temperatures drop to 0.1°C on average during the night.⁴⁷

METHODS

Data collection

The protocol for this study was approved by the University Research and Innovation Committee (URIC) of the Central University of Technology, Free State and all ethical considerations associated with research in traditional/indigenous communities were observed. The traditional practitioners in the survey were identified and selected based on their willingness to participate using purposive sampling through a traditional healer's association found in the area. Before commencement, the study was explained by the researcher to the members of the association in the local Sesotho language. Thereafter, semi-structured interviews were conducted after obtaining informed consent from willing participants between March and December 2017. Prior to the main individual interviews, a focus group with traditional healers was held to test the reliability and validity of the questions. Twenty-four traditional healers were individually interviewed for information about medicinal plants used to treat STIs in the Thaba 'Nchu. The data included the local names of the plants, the parts used, the mode of preparation, route of administration and the diseases treated. Plant specimens were collected with guidance from the traditional healers from the surrounding grasslands and from some home gardens. Plants were identified first by their vernacular names and later taken to SANBI (South African National Biodiversity Institute)



Figure 1: Geographical location of the study area.

in Bloemfontein for validation. Voucher specimens were deposited in Centre of Applied and Food Sustainability and Biotechnology (CAFSaB), Central University of Technology.

Data analysis

Following the interviews, the local importance of each plant species reported by traditional healers in the study area for treating STIs was calculated using the Informant Consensus Factor (ICF) and the Use value (UV).

Informant consensus factor

The consensus of informants within a community and between cultural groups discloses which plants are important and regularly used. This is relevant for conservation purposes and aids in plant selection for pharmacological and phytochemical research.⁴⁸ ICF was used to identify plants with cultural significance as well as consensus on how to use plants. The Informant Consensus factor was calculated using the formula:

$$ICF = \frac{Nur - Nt}{Nur - 1}$$

Where:

Nur = the number of use citations in each disease category.

Nt = the number of species used in each disease category.

The ICF is used to ensure that the information obtained is homogeneous. A high value (near #1) indicates that a specific plant species is used by the majority of informants interviewed for treating a disease category, whereas a low value (0 or close to 0) indicates that each informant used a different plant species and expresses disagreement among informants regarding the treatment of that disease category by a specific plant species.⁴⁹

Frequency of citation (FC)

FC was used to evaluate the most preferred plants or more used plant species for treatment of STIs. The FC of the species of plants being utilized was evaluated using the following formula:³⁷

For diseases treated:

$$FC = \frac{\text{Number of times a particular disease was mentioned}}{\text{Total number of times that all the diseases were mentioned}} \times 100$$

For plant species used:

$$FC = \frac{\text{Number of times a particular species was mentioned}}{\text{Total number of times that all species were mentioned}} \times 100$$

Use value (UV)

The Use Value is a quantitative method that describes the importance of a plant based on its frequency of mention by respondents and was calculated according to the following formula:⁵⁰

$$UV = U/N$$

Where:

UV = is the use value of a species.

U = to the number of citations per species.

N = to the number of informants.

A high UV score for a plant indicates that there are many usage reports for that plant, which may also be considered an important plant in the community, whereas a low score indicates that informants reported less usage reports for that plant.

RESULTS AND DISCUSSION

The participants

An ethnobotanical survey was conducted to document plants used for STIs by traditional healers of Thaba 'Nchu. The results of the study showed that the overall age of the participants ranged between 20 and 80 years. More females (79.2%) than males (20.8%) participated in the study (Table 1). This kind of female dominance is contrary to findings in Lesotho⁵¹ where most of the traditional healers were men because of the belief that women should manage home duties such as taking care of the families, including children and the elderly. In this study, there were more women, which conforms to the general setup of a community dominated by female-headed households in the Free State province.⁵¹ They practice traditional healing to earn an income and provide for their families. Additionally, men from the community usually find work in places that are far away from Thaba 'Nchu, therefore they no longer participate in traditional healing in the area. The trends that were observed in this study also adhere to the Age, Gender, and Dynamics of Knowledge Hypotheses,^{52,53} which states that women and older people tend to have more knowledge of local medicinal plants. Most traditional healers who participated in the study were between the ages of 40-59, accounting for 45% of all participants, with the remainder being either young or old (Table 1). Most traditional healers had a traditional calling to practice traditional healing while some used it as their primary source of income to support their families due to unemployment.

STIs commonly treated using medicinal plants in the study area

The participants in Thaba 'Nchu commonly treated STIs such as gonorrhoea, herpes zoster, HIV, pubic lice, and vaginosis. However, some traditional healers did not differentiate STIs and generally used the term "Mafu a thobalano" to refer to any diseases that affect the reproductive organs obtained from having sex. According to the frequency of mention (Figure 2) and frequency of citation (Table 2), herpes zoster, followed by gonorrhoea and vaginosis were the most treated STIs in the study area. The ICF ranged from 0.4 to 0 for all the disease categories treated in the community. This showed weak consensus (heterogeneity) in across all the reported diseases and indicates that multiple plant resources are used to treat the same STI.

Table 1: Demographic characteristics of individual participants (n=24).

Age (years)	Female	Male	Number	Percentage (%)
20-29	2	1	3	12.5
30-39	3	1	4	16.7
40-49	4	1	5	20.8
50-59	6	0	6	25.0
60-69	1	1	2	8.3
70+	3	1	4	16.7
Total	19	5	24	100

Table 2: Frequency citations and ICF values of the STIs treated using medicinal plants in Thaba 'Nchu.

STIs	Local name	FC (%)	ICF
Vaginosis	'Seso'	19.23	0.2
HIV/AIDS	'Phamokate'	7.7	0.1
Herpes zoster	'Lebanta'	30.8	0.4
Pubic lice	'Dintatsasenana'	7.7	0.1
Gonorrhoea	'Lerothodi'	22.1	0.11
Unspecified STIs	'Mafu a thobalano'	11.5	0

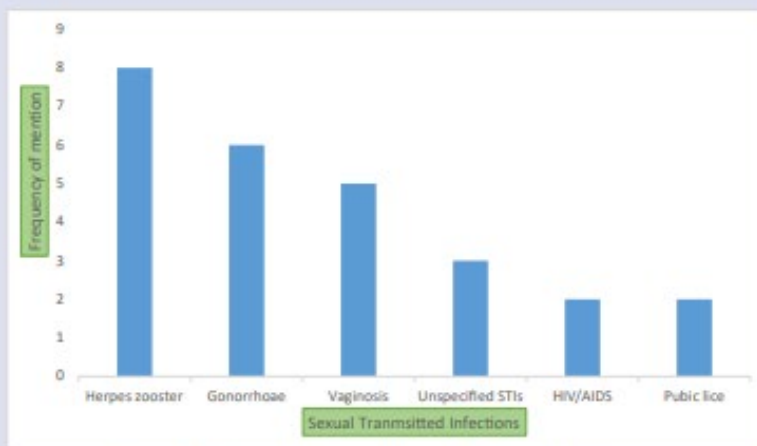


Figure 2: Frequency of mention of STIs treated with medicinal plants.

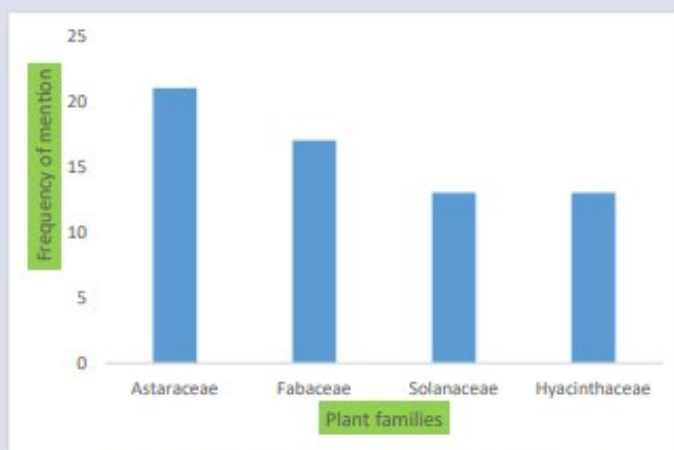


Figure 3: The most cited plant families in area of study in percentages.



Figure 4: The most cited plants by informants in the study area.



Table 3: Medicinal plants used to treat STIs in Thaba 'Nchu.

Scientific name	Local name	Family	Voucher specimen number	Plant parts	STIs treated	Companion Plants	Method of preparation and route of administration.	UV	FC (%)
<i>Bulbine narcissifolia</i> Salm-Dyck	Khomo-ea-balisa	Asphodelaceae	B001	Leaves	Gonorrhoea	<i>Rumex lanceolatus</i> , <i>Leucosidea sericea</i> , <i>Metalsia muricata</i> , <i>Gunnera perpensa</i> , <i>Eucomis autumnalis</i>	Decoction (Orally)	0.29	8.3
				Flowers	Vaginitis				
<i>Euclea coriacea</i> A.DC	Monna-motsi	Ebenaceae	B002	Roots	Gonorrhoea	<i>Hypoxis hemerocallidea</i>	Decoction (Orally)	0.04	1.19
<i>Pentstemon prunellifolius</i> (Klotzsch ex Eckl. & Zeyh.) Walp.	Setima-mollo	Rubiaceae	B003	Whole plant	Herpes zoster	<i>Pentstemon prunellifolius</i> , <i>Xysmalobium undulatum</i> , <i>Dicoma anomala</i> , <i>Turbinia oblongata</i> , <i>Metalsia muricata</i> , <i>Salvia runcinata</i> and <i>Nasturtium officinale</i> .	Decoction (Orally)		
					Gonorrhoea	<i>Bulbine narcissifolia</i> , <i>Bulbine narcissifolia</i> , <i>Solanum aculeatissimum</i> , <i>Berkheya montana</i> and <i>Hypoxis hemerocallidea</i> .	Plant powder (Topically)	0.33	9.52
						<i>Bulbine narcissifolia</i> , <i>Hypoxis hemerocallidea</i>	Decoction		
						<i>Xysmalobium undulatum</i> , <i>Nasturtium officinale</i> , <i>Cussonia paniculata</i>	Bath		
<i>Dicoma anomala</i> Sond. subsp. <i>circioides</i> (Harv.)	Hloenya	Asteraceae	B004	Roots	HIV/AIDS		Decoction (Orally)	0.17	4.76
<i>Scabiosa columbaria</i> L.	Selomi	Dipsacaceae	B005	Whole plant	Gonorrhoea	<i>Xysmalobium undulatum</i> and <i>Hermaniadepressa</i> .	Decoction (Orally)	0.17	4.76
				Roots	Unspecified STIs	<i>Scabiosa columbaria</i> , <i>Hermaniadepressa</i> , <i>Helichrysum caespititium</i>	Decoction (Orally)		
<i>Asparagus microrhaphis</i> (Kunth) Baker	Lereratau	Asparagaceae	B006	Roots	HIV	<i>Scabiosa columbaria</i> and <i>Agave Americana</i>	Decoction (Orally)	0.04	1.19
<i>Hermaniadepressa</i> N.E.Br.	Seletjane	Sterculiaceae	B007	Roots	Gonorrhoea		Decoction (Orally)	0.08	2.4
					Unspecified STIs	<i>Scabiosa columbaria</i> and <i>Salvia runcinata</i> .	Decoction (Orally)		
<i>Xysmalobium undulatum</i> (L.)	Poho-Tsiehla	Apocynaceae	B008	Roots	HIV	<i>Dicoma anomala</i> and <i>Nasturtium officinale</i> .	Decoction (Orally)	0.17	4.76
<i>Helichrysum caespititium</i> (DC)	Phate-ea-ngaka	Asteraceae	B009	Whole plant	Unspecified STIs	<i>Scabiosa columbaria</i> and <i>Hermaniadepressa</i> .	Decoction	0.04	1.19
<i>Drimys depressa</i> (Baker) Jessop	Moretele	Hyacinthaceae	B0010	Roots	Herpes zoster	<i>Vimbela</i> and coarse salt.	Ashes (Topically)	0.17	4.76
<i>Withania somnifera</i> (L.) Dunal	Mofera-ngope	Solanaceae	B0011	Roots	Gonorrhoea	A decoction is prepared and mixed with the roots of <i>Xysmalobium undulatum</i> for treatment of Gonorrhoea.	Decoction (Orally)	0.08	1.19
<i>Rumex lanceolatus</i> Thunb	Khamane	Polygonaceae	B0012	Roots	Gonorrhoea	<i>Eucalyptus</i> spp	Decoction (Orally)	0.17	4.76
					Vaginitis	<i>Lessertia depressa</i> , <i>Dicoma anomala</i> , <i>Eucomis autumnalis</i> and <i>Euphorbia clavarioides</i> .			
<i>Leucosidea sericea</i> Eckl. & Zeyh.	Cheche	Rosaceae	B0013	Leaves	Vaginitis	<i>Bulbine narcissifolia</i> , <i>Rumex lanceolatus</i> , <i>Metalsia muricata</i> , <i>Gunnera perpensa</i> , <i>Eucomis autumnalis</i> .	Decoction (Orally)	0.04	1.19
<i>Euphorbia clavarioides</i> Boiss	Sehlooko	Euphorbiaceae	B0014	Roots	Vaginitis	<i>Lessertia depressa</i> , <i>Dicoma anomala</i> , <i>Eucomis autumnalis</i>	Decoction (Orally)	0.04	1.19
<i>Eucomis autumnalis</i> (Mill.)	Khapumpu/Mathethebale	Hyacinthaceae	B0015	Roots	Vaginitis	<i>Lessertia depressa</i> , <i>Dicoma anomala</i> , <i>Euphorbia clavarioides</i>	Decoction (Orally)	0.17	4.76
<i>Metalsia muricata</i> R.Br.	Tee-ae-baroa	Asteraceae	B0016	Whole plant	Vaginitis	<i>Bulbine narcissifolia</i> , <i>Rumex lanceolatus</i> , <i>Leucosidea sericea</i> , <i>Gunnera perpensa</i> , <i>Eucomis autumnalis</i>	Decoction (Orally)	0.04	1.19
<i>Salvia runcinata</i> L.f	Mosisili	Lamiaceae	B0017	Roots	Pubic lice	<i>Rumex lanceolatus</i> , <i>Hypoxis hemerocallidea</i> , <i>Eucomis autumnalis</i> , <i>Selaginella caffrorum</i> , <i>Bulbine narcissifolia</i> , <i>Drimys depressa</i> , <i>Drimys depressa</i> and <i>Aloe ferox</i> .	Decoction (Orally)	0.17	4.76
						<i>Bulbine narcissifolia</i> , <i>Rumex lanceolatus</i> , <i>Leucosidea sericea</i> , <i>Eucomis autumnalis</i>	Bath (Topically)		
<i>Gunnera perpensa</i> L.	Qobo	Gunneraceae	B0018	Whole plant	Vaginitis		Decoction (Orally)	0.04	1.19
<i>Malva parviflora</i> L.var	Tikamotse	Solanaceae	B0019	Leaves	Herpes zoster		Decoction (Orally)	0.08	2.4
<i>Berkheya montana</i> (DC)	Mohatollo	Asteraceae	B0020	Roots	Gonorrhoea	<i>Solanum aculeatissimum</i> , <i>Berkheya montana</i> , <i>Hypoxis hemerocallidea</i> , <i>Bulbine narcissifolia</i> .	Decoction (Orally)	0.04	1.19
<i>Achyranthes aspera</i> Linn.	Lemanamana	Fabaceae	B0021	Roots	Pubic lice	<i>Achyranthes aspera</i> , 'Madubula' antiseptic	Bath (Topically)	0.04	1.19



<i>Solanum aculeatissimum</i> Jacq	Thola	Solanaceae	B0022	Roots	Gonorrhoea	<i>Solanum aculeatissimum</i> , <i>Berkheya montana</i> , <i>Hypoxis hemerocallidea</i> , <i>Bulbine narcissifolia</i> .	Decoction (Orally)	0.08	2.4
<i>Agave americana</i> L. subsp. <i>americana</i>	Lekhala	Agavaceae	B0023	Whole plant	Vaginosis	<i>Drimys depressa</i> , <i>Eriosephalus punctatus</i> , <i>Salvia runcinata</i> , <i>Hypoxis hemerocallidea</i> , <i>Eucomis autumnalis</i>	Decoction (Orally)	0.04	2.4
<i>Eucalyptus</i> spp	Boloukomo	Myrtaceae	B0024	Bark	Gonorrhoea	<i>Rumex lanceolatus</i>	Decoction (Orally)	0.04	1.19
<i>Lessertia depressa</i> Harv.	Mmusapelo	Fabaceae	B0025	Roots	Vaginosis	<i>Dicoma anomala</i> and <i>Eucomis autumnalis</i> , <i>Euphorbia clavarioides</i>	Decoction (Orally)	0.04	1.19
<i>Schizocarphus gerrardii</i> (Baker) Van der Merwe	Letjoetlane	Hyacinthaceae	B0026	Roots	Vaginosis	<i>Bulbine narcissifolia</i>	Decoction (Orally)	0.04	1.19
<i>Turbina oblongata</i> A. Meeuse	Mothukho	Convolvulaceae	B0027	Whole plant	Herpes zoster	<i>Xysmalobium undulatum</i> and <i>Dicoma anomala</i> , <i>Pentstemon prunelloides</i> , <i>Metastasia muricata</i> , <i>Salvia runcinata</i> and <i>Nasturtium officinale</i> .	Decoction (Orally)	0.04	1.19
<i>Selaginella affrorum</i> (Milde)	Moriri-oo-matlapa	Selaginellaceae	B0028	Roots	Pubic lice	<i>Rumex lanceolatus</i> , <i>Hypoxis hemerocallidea</i> , <i>Eucomis autumnalis</i> , <i>Salvia runcinata</i> , <i>Bulbine narcissifolia</i> , <i>Drimys depressa</i>	Bath (Topically)	0.04	1.19
<i>Elephantorrhiza elephantina</i> (Burch) Skeels	Mositsane	Fabaceae	B0029	Roots	Vaginosis	<i>Agave americana</i> , <i>Drimys depressa</i> , <i>Eriosephalus punctatus</i> , <i>Salvia runcinata</i> , <i>Hypoxis hemerocallidea</i> , <i>Eucomis autumnalis</i> .		0.08	2.4
<i>Eriosephalus punctatus</i> DC	Sehalahala-sa-matlaka	Asteraceae	B0030	Roots	Vaginosis	<i>Agave americana</i> , <i>Drimys depressa</i> , <i>Salvia runcinata</i> , <i>Hypoxis hemerocallidea</i> , <i>Eucomis autumnalis</i> , <i>Elephantorrhiza elephantina</i> .	Decoction (Orally)	0.04	1.19
<i>Melolobium microphyllum</i> (L.F.)	Mofahlatoeba	Fabaceae	B0031	Whole plant	Unspecified STIs		Decoction (Orally)	0.04	1.19
<i>Cussonia paniculata</i> Eckl. & Zeyh. Subsp. <i>Sinuate</i>	Mots'se'se	Araliaceae	B0032	Roots	HIV	<i>Heteromorpha trifoliata</i> .	Decoction (Orally)	0.08	2.4
<i>Heteromorpha trifoliata</i> (H. L. Wendl.) Eckl. & Zeyh.	Monkhoane	Apiaceae	B0033	Roots	HIV	<i>Cussonia paniculata</i>	Decoction (Orally)	0.04	1.19
					Gonorrhoea	<i>Solanum aculeatissimum</i> , <i>Berkheya montana</i> , <i>Bulbine narcissifolia</i> .			
<i>Hypoxis hemerocallidea</i> syn.	Moli	Hypoxidaceae	B0034	Roots	Vaginosis	<i>Agave americana</i> , <i>Drimys depressa</i> , <i>Eriosephalus punctatus</i> , <i>Salvia runcinata</i> , <i>Eucomis autumnalis</i> and <i>Elephantorrhiza elephantina</i> .	Decoction (Orally)	0.25	7.14

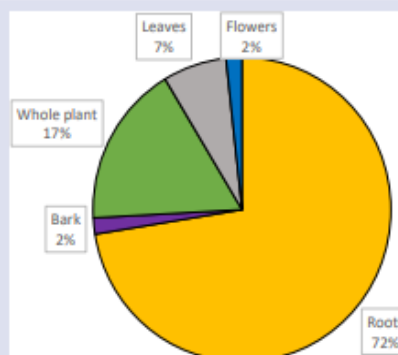


Figure 5: Plant parts used for preparation of remedies.

Plants used to treat sexually transmitted infections

Information on plants species used to treat STIs, local names (in Sesotho), families, scientific names, use value as well as frequency of citation was reported by traditional healers during ethnobotanical interviews is presented in table 3. The ethnobotanical survey revealed that 35 plant species that belong to 24 families are used for various STIs in the study area. The family with the largest number of useful species was from Asteraceae which amounted to 21% of all the mentioned species, followed by Fabaceae at 17% (Figure 5a). Asteraceae was also found to be one of the dominant families in an ethnobotanical study conducted in Limpopo province on plants used for STIs by Bapedi people.²⁰ In contrast to the findings of this study, Fabaceae was found to have the greatest number of plant species utilized to treat various diseases in earlier ethnobotanical studies.¹⁷⁻⁵⁵ Plants of the Asteraceae family have been used as astringents, antipyretics, anti-inflammatory, hepatoprotective, diaphoretic in fevers, smooth muscle relaxants, nerve tonics, laxatives, and for the treatment of wounds, bleedings, headaches, pains, and spasmodic illnesses, flatulence, dyspepsia, dysentery, lumbago, leucorrhoea, haemorrhoids, ulcer, and disorders causing cachexia.⁵⁶ Additionally, other species such as *Achillea millefolium*, *Artemisia absinthium*, *Matricaria recutita*, *Parthenium hysterophorus*, *Inula crithmoides* from the family were found to contain bioactive compounds such as flavonoids (apigenin), phenolics (myricetin, ferulic acid, quercetin), Sesquiterpene compounds, terpenoids, alkaloids and cardiac glycosides known to have various biological activities such as antioxidant, antimicrobial, anti-inflammatory and many other properties⁵⁷ which explains the dominance of plants from Asteraceae family in the current study.

The 10 most mentioned plants were *Bulbine narcissifolia*, *Pentstemon prunelloides*, *Dicoma anomala*, *Scabiosa columbaria*, *Xysmalobium undulatum*, *Rumex lanceolatus*, *Eucomis autumnalis*, *Salvia runcinata* and *Hypoxis hemerocallidea* (Figure 4). Most of the species mentioned by the traditional healers in Thaba Nchu were also mentioned in previous studies for the treatment of STIs.¹⁷⁻⁶⁰ Plants such as *E. autumnalis*, *B. narcissifolia*, *P. prunelloides*, *S. runcinata* have biological properties such as antimicrobial, anti-inflammatory, antioxidant activities.⁶¹⁻⁶⁵ Therefore, the selection of these plants for preparation of remedies used to treat STIs by the informants may be attributed to the above-mentioned biological activities and indicates that the therapeutic properties of the plants are reliable and could thus possibly lead to the discovery of new bioactive compounds and products.⁶⁶

Use value

The use value (UV) across all the mentioned plants ranged between 0.33 and 0.04. The UV for *P. prunelloides* (0.33), *B. narcissifolia* (0.29), *H. hemerocallidea* (0.25), *S. runcinata* (0.17), *E. autumnalis* (0.17), *Dicoma anomala* (0.17), *R. lanceolatus* (0.17), *S. Columbaria* (0.17), *X. undulatum* (0.17), *D. depressa* (0.17) were the highest values (Table 3). Plants that were mentioned in most disease categories by many informants often have a high use value, whereas species that are only used by a few informants usually have a low use value.⁶⁶ This implies that the above-mentioned plant species are frequently used to treat a variety of STIs in the study area. The high use value of these plants indicates their extensive usage in the study area by traditional healers. These may also be due to their wide distribution in the area. High use values are also useful in indicating the importance of plants in treating STIs in the study area and can be selected in determining their safety, pharmacological effects and can influence the development of plant-derived products that can be used to treat STIs in the study area.

The plant parts used for remedy preparation

The plant parts that are usually used for the preparation of remedies used to treat STIs are shown in figure 5. Roots were reported to be

the most used plant part, followed by whole plant and leaves. This corroborates the Optimal Defence Theory^{62,67} which states that humans select medicinal plants to optimize secondary chemistry and are likely to select plant organs that are less likely to be attacked by herbivores. However, the excessive use of roots and whole plants has a negative impact on the growth of plant populations, whereas the use of aerial plant parts would be more sustainable.^{68,69} For example, El Houssine Bouiamrine *et al* (2017), Swemmer *et al* (2019)^{70,71} and many other studies showed the importance of harvesting plants sustainably and further recommended the establishment programs of conservation and sensitization of rural populations on the protection of biodiversity. In addition, future research and interventions may also advocate for sustainable use of medicinal plants in the Thaba Nchu through the development of medicinal plant gardens and applying the measures recommended in the above-mentioned studies to reduce misuse and avert extinction.

Plant collection and method of preparation

The informants mentioned that they personally collect most of the plants they use to treat their patients and only buy and cultivate those that are difficult to access. For storage purposes, the plants are usually dried out and ground into fine powder. The informants mentioned that plants can be stored up to 5 years when ground into fine powder. Grinding the plants allows the solvent to have better contact with the plant material thus allowing better compound extraction and therefore increase efficacy in treatment of diseases.⁷² The informants utilized most of the plants used for STI treatment in the research region individually or in combination. The use of a combination of plants species against a particular type of ailment may imply that the ailment is prevalent in that area of study.⁷³ Decoctions are the most often utilized remedy preparation method in the research area, possibly due to their simplicity of preparation and administration (Table 3). Herbal administration was mostly oral, and some plants were topically applied especially when treating skin infections (Table 3). This is consistent with other studies that previously documented that most remedies used for treatment of STIs were prescribed orally.³⁰⁻⁷⁵

CONCLUSION

The study documented the indigenous knowledge of medicinal plants used to treat symptoms of STIs in Thaba Nchu, Free state province, South Africa. The results showed that an overall of 35 medicinal plants from 24 families are used for treatment of various STIs such as Herpes zoster, vaginosis, pubic lice, HIV/AIDS, and Gonorrhoea. Majority of plants used by traditional healers for treatment of STIs are from Asteraceae, Fabaceae, Solanaceae and Hyacinthaceae families. *Bulbine narcissifolia*, *Pentstemon prunelloides*, *Hypoxis hemerocallidea*, *Scabiosa columbaria*, *Xysmalobium undulatum*, *Rumex lanceolatus*, *Eucomis autumnalis*, *Dicoma anomala* and *Salvia runcinata* were frequently used for treatment of STIs in the study area. Roots were the most used plant parts for preparation of different remedies and decoction is the common method of remedy preparation. Medicinal plants in the area might be endangered or go extinct in a long run due to the collection method and the use of roots for remedy preparation. Sustainable harvesting and use of medicinal plants to avoid further loss of plants needs to be encouraged. Many of these plants have previously been reported for antimicrobial activities which may justify their roles as natural remedies for sexually transmitted infections in this study. Further studies are needed to determine the dosages, minimum inhibitory concentrations, biological activities, and toxicities, and characterize the active phytochemicals. The plants recorded in this study can serve as a basis for further research of novel drugs that can be used to treat STIs and other microbial infections.

CONFLICTS OF INTEREST

All the authors declare no conflicts of interest.

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3. Informed consent

Project title

Ethnopharmacology of a selection of plants used for sexually transmitted infections in Thaba 'Nchu, Free State.

PROMOTER Dr IT Manduna Centre for Applied Food Security and – Biotechnology (CAFSaB) Department of Health Sciences Central University of Technology Bloemfontein Free State 9301 051 507 4023 imanduna@cut.ac.za	RESEARCHER Brian Ngobeni DM Selemela Road, Qalo village Bloemanda Bloemfontein Free State 9323 0670353017 ngobenibrian1@gmail.com/bngobeni@cut.ac.za
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Introduction (purpose of study)

You are being asked to take part in a research study. To participate, you must be 18 years (older). Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information. Please take whatever time you need to discuss the study with your family and friends, or anyone else you wish to. The decision to join, or not to join, is up to you. In this research study, we are investigating and evaluating medicinal plants used to treat sexual transmitted infections.

What is involved in the study?

If you decide to participate in the study you will be asked to undergo an interview where you will be sharing what you know and experience about sexual transmitted infections and the medicinal plants used to treat them, the way in which you prepare the plant for treatment and also the researcher participating in the preparing process if possible, and also telling where the plant is collected, or sold. This will consist of me asking you questions and taking notes on the same time.

For collected plants, in our second time of interaction, the plant will be brought and presented to you to avoid misidentification of the plant.

Ethnobotanical inventory/field interview

You are also asked to accompany the researcher to the area of plant collection or tell the researcher where the plant is purchased. This consist of me walking with you in the field, listening to you talking about medicinal plants used for treatment of sexual transmitted infections, and taking notes. This is to avoid misidentification of the plant.

Time frame

You are requested to indicate when you will be available for the interview not later than 31 November 2017, which we think will take you 60 minutes, at the comfort of your home and in the field, if necessary, in two different interactions.

Benefits

We can't guarantee that you will personally experience benefits from participating in this study. It is reasonable to expect future benefits like the following from the information we find in this study:

- Help in creating an ecostor (area for product development) for the medicinal plants used for sexual transmitted infections.
- Fundamental knowledge on medicinal plants used for treatment of sexual transmitted infections.
- Full access to research processes and results
- Full access to the document used to document various medicinal plants used to sexual transmitted infections in the study area.
- Preserving the knowledge for the future generation on the use of medicinal plants, their safety and efficacy
- Influence on planting medicinal plants in gardens

Risks

From this research, the risks or discomforts to the participant will be minimal or non- existent. The study is all about day to day, time to time experience the respondent get when treating sexual transmitted infections. The respondent will never be put in a position where they feel awkward, self-unconscious, or ashamed. There will be no damage to financial standing, employability, or reputation.

Confidentiality

We will take the following steps to keep information about you confidential, and to protect it from unauthorized disclosure, tampering, or damage:

Individuals and agencies who will have access to the data and records is the participating group of the IKS laboratory at the Central University of Technology. The data will be kept in locked cabinets, and also on a computer that requires passwords getting into the system.

Indirect quotes will be used to avoid tracing it back to the individual to describe if data will be published. The data will be aggregated.

Your rights as a research participant

Participation in this study is completely voluntary and anonymous. Information gathered during the research will be used solely for the purpose of this study and all efforts will be made to ensure the confidentiality of participants' personal information. Please note that while your name will be recorded with the data, it will not be used in the report. All identifiable data will be stored securely on a computer with password-restricted access and only the researcher (and supervisor if applicable), and ethics committee members will have access to it. All identifiable information will be destroyed at the end of the study or after 15 years, whichever comes first.

Please take time to read this entire form and ask questions before deciding whether to take part in this research project. If you decide not to participate there will not be any negative consequences. Please be aware that if you decide to participate, you may withdraw from the study at any time and your data will be returned to you or destroyed. You may also decide not to answer any specific question.

Contacts for questions or problems?

Call the following people if you have questions or concerns about your rights as a research participant:

Dr I Manduna 051 507 4023
Mr B Ngobeni 0670353017

CONSENT FORM

Thank you for your participation. By submitting this form, you are indicating that you have read the description of the study, are over the age of 18, and that you agree to the terms as described in the short questionnaire that follows:

I have read and I understand the provided information and have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without cost. I understand that I will be given a copy of this consent form. I voluntarily agree to take part in this study. **I have had all my questions answered to my satisfaction.**

Yes ☐

No ☐

I grant permission for the research to be recorded and saved for purpose of review by the researcher, supervisor / principal investigator, and ethics committee.

Yes ☐

No ☐

I grant permission for the research recordings to be used in presentations or documentation of this study.

Yes ☐

No ☐

Participant's Name: _____

Address: _____

Contact details: _____

Signature _____ Date _____

Investigator's signature _____ Date _____

Verbal consent (*Applicable when participants cannot read or write*)

I hereby declare that I have read explained and clarify the contents of the data sheet to the research participant. The nature and purpose of the study were explained. The possible risks and benefits of the study were also explained. The research participant has undoubtedly showed that he/she is aware of the right to withdraw from the study at any time, for any reason and without threatening his/her relationship with the research team. I hereby certify that the research participant has verbally agreed to participate in this study.

Participant's Name: _____

Address: _____

Contact details: _____

Signature _____ Date _____

Investigator's signature _____ Date _____

Contact

If you have any questions at any time about this study or the procedures, you may contact the researcher on 0670353017.

4. Questionnaire

QUESTIONNAIRE

1. Demographic information

1.1 Name and Surname

1.2 Gender

Male		Female	
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1.3 Location/Residence

1.4 How old are you?

18 – 35	35 – 55	55 – 75
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1.5 What is your marital status?

Single	Married	Divorced	Widowed
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1.6 What is your employment status/ occupation?

1.7 Religion

1.8 Traditional healer?

Yes	No
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If not a traditional healer, then state

2. Knowledge attribute

2.1 Knowledge about medicinal plants

2.2 How long is your experience of the use for medicinal plants to treat sexual transmitted infections?

2.3. What type of sexual transmitted infections do you treat? Name the disease.

2.4. How do you identify the infection? Symptoms?

2.5 What are the plants that you know that are used for the treatment of sexual transmitted infections? (Local name in Sotho)

2.6 Basic description of the plant Habitat (Tree/ Herb/ Shrub/Climber)

2.7 Where is the plant collected? (Sources of the plants)

2.9 When is the plant harvested? (Season and time of the day)

2.10 What plant part(s) do you use?

2.11 Is the plant used individually or with a mixture of other plants?

2.11. How do you prepare the plant of the remedies?

2.13 Do you use it fresh or dry?

If dry, how do you store it after collection and for how long?

2.14 What is the mode of administration?

2.15 How much dosage should they take, when and how long does the treatment last?

2.16 What are the Contraindications?

2.17 What are the side effects that can be observed?

2.18 Cultural observations.
