PHARMACOLOGICAL SCREENING AND ISOLATION OF BIOACTIVE COMPOUNDS FROM PLANTS USED AGAINST ELEPHANTIASIS IN THE EASTERN CAPE, SOUTH AFRICA

By

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2016203149

A thesis submitted in fulfilment of the requirements for the degree Philosophiae Doctor in Botany in the Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, University of the Free State Qwaqwa, South Africa.

March 2023

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DECLARATION

I, Zanele Adams, declare that the Master's Degree research dissertation or interrelated, publishable manuscripts/published articles, or course work Master's Degree mini-dissertation that I herewith submit for the PhD Degree qualification in Botany at the University of the Free State Qwaqwa Campus is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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Zanele Adams

ACKNOWLEDGEMENTS

I wish to extend my sincere gratitude to God almighty for the precious gift of life and endowing me with the strength, faith to work through my project after granting me the opportunity to enrol for my Ph.D. My gratitude also goes to Prof Komoreng for believing in me and granting me the opportunity to work with her as a research student, to Dr Mojau for his supervision and mentoring through the study and to Prof Thekisoe my co-supervisor for all the support he showed.

To all the traditional healers and herbalists from the OR Tambo District Municipality, Eastern Cape who took part in the ethnobotanical survey, thank you for the help you provided in collection of plant material and knowledge shared during the survey.

I also wish to extend my gratitude to Dr. Suranie Horn and Prof. Rialet Pieters from the North-West University for letting us use their lab and helping with the cytotoxicity experiments. We appreciate Prof. Nomngongo and her research group's assistance with the nuclear magnetic resonance (NMR) spectroscopy from the University of Johannesburg.

My acknowledgments also go to my dear family and my dear friends Olwethu Kutta and Thandeka Dabata for the love, encouragement and support they showed me. For their emotional support and help with lab supplies, I would also want to thank the personnel at the University of the Free-Botany State's Department at the QwaQwa campus, particularly Prof. Ashafa, Dr. Sandy Steenhuisen, Dr. Rudo Ngara, and Mr. Mzizi Ngaka. My beloved colleagues Mrs. Laetitia Voua Otomo, Mr. Victor Mandla Hlongwane, Miss. Thumeka Tiwani, Miss. Sellwane Moloi, Miss. Gloria Lehasa, and Miss. Grace Mochologi deserve a heartfelt thank you for being the best colleagues for the love and support they showed me during this study. I am also very grateful and thankful for the financial support received from the National Research Foundation (NRF) and the UFS tuition fee bursary.

DEDICATION

This dissertation is in honor of my beloved parents. Julian Thembekile Adams and Freedom Nkululeko Adams; my dear brothers and sisters, Vuyiswa, Vuyelwa, Siphelele and the late Phakamile Adams; my beloved nieces and nephews; my beloved daughter Unathi Oratilwe Adams and my late cousin Velile Adams who once said I should be a doctor one day.

ABSTRACT

Elephantiasis, also known as lymphatic filariasis, is a medical condition brought on by parasitic worms that invade the lymphatic system causing obstructions of lymph fluid in the passaged that is associated with excessive swelling of the lower and upper limbs resulting in disability, swelling of genitalia and breasts. The condition is also associated with non-filarial causes including certain sexually transmitted diseases, tuberculosis, leishmaniasis, leprosy and podoconsiasis. The World Health Organization lists the illness as one of the neglected tropical diseases that primarily affects developing nations. Although no precise figures have been provided, South Africa is one of the African nations where incidences of the illness have been reported.

Plants are reported in treating several medical conditions that affect human kind. Traditional medicine has been considered as an alternative to Western medicine as it is easily accessible and the latter being more expansive. The continued use of plant medicine creates the need to identify those substances that are responsible for the biological activity or the healing properties found in plant extracts through scientific validation. Plants are used by indigenous people and traditional healers in different areas to treat the similar or different conditions. South Africa has a wide variety of plants, and traditional plant studies is reported in literature.

In three municipalities of the OR Tambo District Municipality in the Eastern Cape of South Africa, an ethnobotanical survey on medicinal plants used in the management and treatment of lymphatic filariasis was carried out. A total of 29 therapeutic plants from 25 different Angiosperm families were recorded. *Acokanthera oblongifolia Curtisia dentata, Dioscorea sylvatica, Elephantorrhiza elephantina*, *Gunnera perpensa, Hypoxis hemerocallidea, Pentanisia prunellloides* and *R. melanophloeos* were reported as the most used plants in the study to treat elephantiasis and various ailments. The roots followed by the leaves and stem bark were the most used plant parts with infusions and decoctions being reported as the most frequently methods of administration.

In vitro studies such as antimicrobial activity, antioxidant activity, phytochemical analysis, anti-inflammatory activity and cytotoxicity activity on mammalian cells were investigated. Bioactive compounds were also isolated and identified from *K. drepanophylla*.

Antimicrobial screening from the ethnobotanical survey took into consideration the plants which were the most cited in the study and ones which were not studied before for the treatment of lymphatic filariasis. Eight different plant species were evaluated in relation to aqueous and organic extracts (32) for antimicrobial properties using the micro -titre plate dilution plate method. Gram-positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* as well as Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella flexneri* and *Proteus vulgaris* were tested for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). *A. oblongifolia*, *C. dentata*, *D. sylvatica*, *G. perpensa*, *K. drepanophylla*, and *R. melanophloeos* were among the plants whose acetone and ethanol extracts had good MIC and MBC activity against both Gram-positive and Gram-negative bacteria with MIC values ranging from 0.098 to 0.78 mg/ml. *C. dentata* acetone and ethanol extracts dispalyed poor MBC activity against *P. vulgaris*, *S. flexneri*, *K. pneumoniae*, and *E. faecalis*. *G. perpensa* water extract demonstrated higher MBC efficacy to the ethanol extract against *P. vulgaris*, *S. flexneri*, *K. pneumoniae*, and *E. faecalis a*t 6.25 mg/ml and 12.5 mg/ml respectively. At 0.098/ml, 0.098/ml, and 0.39/ml, respectively, the aqueous extracts of *G. perpensa* demonstrated good MICs and MBCs against *E. coli*, *P. aeruginosa* and *P. vulgaris*. At concentrations of 0.098/0.098 mg/ml, 0.195/3.125 mg/ml, and 0.78/0.78 mg/ml, respectively, *K. drepanophylla* water extracts demonstrated MICs and MBCs efficacy against the 3 strains. According to the findings, the effectiveness of the plant extracts against bacteria varied depending on the type of solvent employed. Different bacterial strains are susceptible to the effects of plant extracts and their essential oils in various ways, including by disrupting the phospholipid bilayer of the cell membrane, which makes it more permeable, causing the membrane to lose some of its cellular components, damaging certain cellular enzymes, and destroying or inactivating genetic material (Doughari, 2012).

The microdilution assay was used to assess the plant extracts for antifungal activity against *Candida albicans*, *Candida vulgaris*, and *Trichophyton mucoides*. The acetone extracts of *G. perpensa*, *K. drepanophylla* and *R. melanophloeos* among the studied plant extracts demonstrated outstanding MIC and MFC (minimum fungicidal activity) against all three microorganisms between 0.098 and 0.78 mg/ml. While the MFC values against the three fungi strains were at 3.125 mg/ml, *C. dentata* ethanol extracts inhibited *C. albicans, C. vulgaris*, and *T. mucoides* at 0.195 mg/ml, 0.195 mg/ml, and 0.78 mg/ml, respectively. Aqueous extracts of *A. oblongifolia, C. dentata*, *G. perpensa* and *K. drepanophylla* displayed favorable MIC and MFC activities.

Concerning antimycobacterial activity, *M. tuberculosis* was used as the test strain. Among the plant species tested *A. oblongifolia*, *C. dentata*, *H. albiflos* and *K. drepanopyhlla* exhibited the best activity. The excellent MIC values for the acetone extracts ranged from 0.098 to 0.39 mg/ml. The ethanol extract's MIC ranged from 0.098 to 0.78 mg/ml. Extracts of ethyl acetate exhibited efficacy ranging from 0.39 to 0.78 mg/ml. *H. albiflos* demonstrated the best MIC and MFC activity for the water extracts at 0.098 and 0.195 mg/ml, respectively.

Since phytochemical substances are known to be involved in biological processes such as antibacterial activity and other biological functions, their existence in certain plant species was examined using qualitative and quantitative techniques. All of the studied plant species tested positive for saponins, flavonoids, anthraquinones, and terpenoids, according to the findings of the qualitative analysis. *A. oblongifolia* is the only plant that tested positive for all phytochemicals including alkaloids, tannins, cardiac glycosides and steroids. Cardiac glycosides and steroids were the least present compounds. Quantitative analysis results showed that the tested phytochemicals existed in varying quantities. The most prevalent phytochemicals discovered in the plants were determined to be flavonoids and phenols. The largest concentrations of phenols were found in the acetone, ethanol, and aqueous extracts of *G. perpensa* and *C. dendata*, while the highest concentrations of flavonoids were found in the extracts of *K. drepanophylla* and *G. perpensa*. The acetone extract of *R. melanophloeos* (1.867 mg/g GAE g -1), the ethanolic extract (1.627 mg/g GAE g -1), and the water extract (1.045 mg/g GAE g -1) were found to contain the highest

concentrations of tannins. The extracts of *K. drepanophylla* in acetone (0.364 mg/g QE g-1) and ethanol (0.249 mg/g QE g-1) contained the most flavonols.

The antimicrobial qualities of the analysed plant extracts are thought to be caused by phytochemicals.

The DPPH and ABTS tests were used to evaluate the antioxidant activity. The acetone, ethanol, and water extracts of *K. drepanophylla*, followed by the ethanol and water extracts of *C. dentata*, demonstrated the strongest antioxidant activity, according to the results of the DPPH experiment. *K. drepanophylla* and *C. dentata* demonstrated the best anti-DPPH activity and the best anti-ABTS activity, respectively, for the water extracts. *R. melanophloeos* acetone extract demonstrated the highest level of activity, with an IC_{50} value of 27.40 µg/ml ± 13.73.

Concerning anti-inflammatory activity using 5- Lipoxygenase assay, *C. dentata, K. drepanophylla* and *R. melanophloeos* water extracts displayed higher anti-inflammatory activity than that of NDGA and inhibited 5-LOX. *C. dentata* and *K. drepanophylla* water extracts displayed the best activity at (0.05 ± 0.02 and 0.05 ± 1.10 µg/ml). For ethanol extract, *K. drepanophylla* and *G. perpensa* also exhibited good activity at 0.10 ± 0.11 µg/ml and 0.11 ± 0.07 respectively. The extracts inhibited 5-LOX by showing higher anti-inflammatory activities than NDGA (0.29 ± 0.11 µg/ml).

Cytotoxicity of plant extracts was tested using human duodenum cancer (Hutu-80) cells and rat hepatoma (H4IIE-*luc*) cells. *C. dentata* water extract displayed non-toxicity when administered against Hutu-80 cells at the highest concentration and the H4IIE-*luc* cells managed to proliferate at the lowest concentration. Acetone extract of *K. drepanophylla* revealed that the cells proliferate at high concentrations and the water extract revealed that the plant is non-toxic after a period of 48 h when administered starting at 0.5 mg/ml to 0.03125 mg/ml.

The goal of the study was to separate the active components from the highly active plant extract and then isolate, characterise, and identify the compound using spectroscopic methods, which revealed its structure. Following extraction, column chromatography, thin layer chromatography, and bioautography, isolation was carried

out. An active substance that demonstrated antibacterial activity by preventing the growth of S. aureus was identified after the bioautography assay indicated it. Utilising 1H-NMR, the isolate was characterised. The dimeric anthraquinone 10-hydroxy-10,7-(chrysophanolanthrone)chrysophanol, which was isolated from the roots of K. isoetifolia, showed a proton structure in the spectrum that was 75% comparable to it.

Keywords: elephantiasis, lymphatic filariasis, plant extracts, antimicrobial, phytochemical, antioxidant, anti-inflammatory and cytotoxicity.

PAPERS PUBLISHED FROM THIS THESIS

Adams, Z., Thekisoe, O., & Buwa-Komoreng, L. (2021). An ethnobotanical survey of traditional medicinal plants used against elephantiasis in the OR Tambo district, Eastern Cape, South Africa. Pharmacognosy Magazine, 17, 915-22.

CONFERENCE CONTRIBUTIONS

Poster: Adams, Z., Thekisoe, O., & Buwa-Komoreng, L. An ethnobotanical survey of traditional medicinal plants used against elephantiasis in the OR Tambo district, Eastern Cape, South Africa. **45th Annual SAAB Conference, University of Johannesburg, South Africa.**

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LIST OF ABBREVIATIONS

AIDS- Acquired Immunodeficiency syndrome

ATTC- American Type Culture Collection

ABTS - 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid

Acet - Acetone

$AlCl_3$ - Aluminium chloride

$CDCl_3$ - Duarated chloroform

CHF – Congestive heart failure

COX – Cyclogenase

DEC - Diethylcarbamazine

DMEM- Dubelcco's modified eagle's medium

DMSO – Dimethylsolfoxide

DNA - Deoxyribonucleic acid

DPBS - Dulbecco's phosphate buffered saline

DPPH- 2,2-diphenyl-1-picryl-hydrazyl

EtAH – Ethyl acetate

EtOH – Ethanol

FBS - Foetal bovine serum

FTIR – Fourier-Transform Infared Spectroscopy

GC – Gas Chromatography

GC-MS – Gas Chromatography Mass Spectroscopy

GPELF- Global Program to Eliminate Lymphatic Filariasis

H4IIE-*luc* - Carcinogenic rat haepatoma cells

H^1NMR - Proton nuclear magnetic resonance

HPLC - High Performance Liquid Chromatography

H_2SO_4 – Sulphuric acid

Hutu-80 - Human duodenum cancer cells

IBD – Inflammatory bowel disease

INT - p-Iodonitrotetrazolium violet

LC-MS – Liquid Chromatography Mass Spectroscopy

LF – Lymphatic filariasis

LOX - Lipoxygenase

MBC - Minimum bactericidal concentration

MIC – Minimum inhibitory concentration

MDA – Mass drug administration

MDR – Multidrug-resistant

MFC - Minimum fungicidal concentration

MTT - 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Na_2CO_3 – Sodium bicarbonate

NADPH – Nicotinamide adenine dinucleotide phosphate

NCCLS – National Committee for Clinical Laboratory Standards

NDGA - Nordihydroguaiaretic acid

NMR - Nuclear Magnetic Resonance

NORD – National Organization for Rare Diseases

NSAIDs- Non-steroidal anti-inflammatory drugs

OADC - Oleic Albumin Dextrose Catalase

OR – Oliver Reginald

QE - Quercetin equivalent

R_f - Retention factor

RNA – Ribonucleic acid

RNS – Reactive nitrogen species

ROS – Reactive oxygen species

SC – Solvent control

TB - Tuberculosis

UV – Ultraviolet

WHO - World Health Organisation

XDR – Extremely drug resistant

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Lymphatic filariasis (LF), which has the common name elephantiasis, one of the tropical diseases that are of less concern, usually referred to as Neglected Tropical Diseases (NTDs) due to infection from parasitic worms and transmitted to humans by mosquitoes (WHO, 2010). A condition is characterised by swelling of the lower limbs. WHO (2021) states that lymphatic filariasis impairs the lymphatic system, which results in aberrant body part growth, pain, severe disability, and social shame. Lymphatic filariasis may be asymptomatic, acute or chronic.

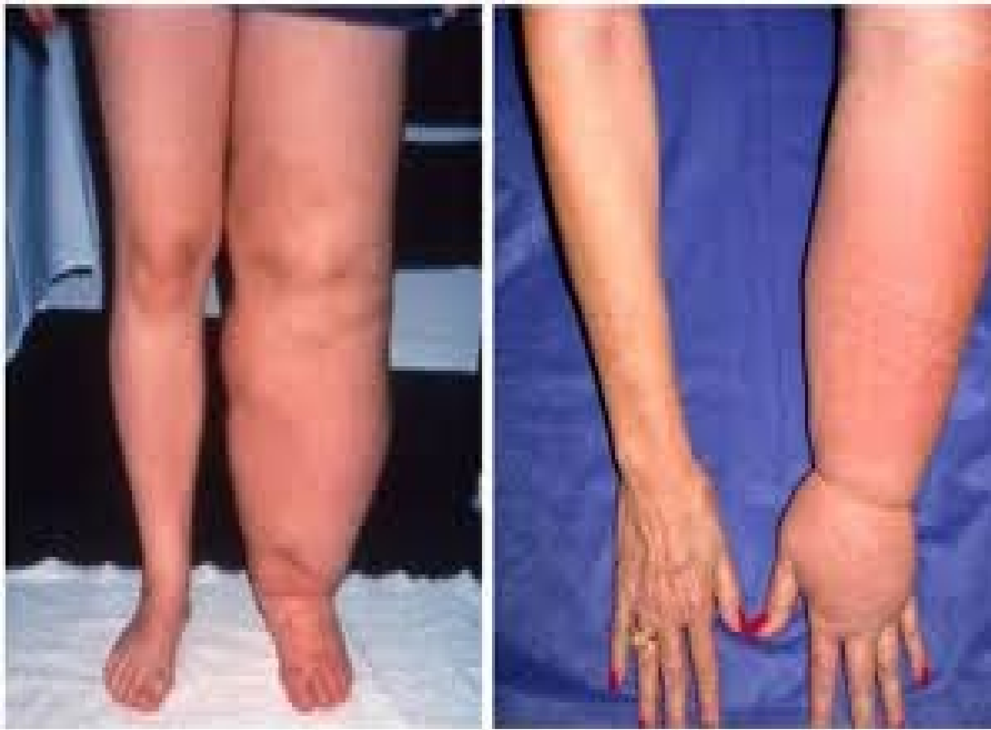

Fig 1.1: Lymphoedema (youtube.com).

Fig 1.2: Manifestation of elephantiasis (daily post. ng).

Fig 1.3: Hydrocele (omicsonline.org).

The asymptomatic infection alters the body's immune system by impairing the lymphatic system and kidneys. Acute infection is associated with fever, pain and tenderness of the affected area corresponding to the inflamed lymphatic channel. A blockage in lymph flow causes increases more fluid around the tissue spaces, resulting in swelling of the part (WHO, 2013). Lymphatic filariasis infections are

classisifed as chronic conditions when there is swelling or skin thickening of the limbs and scrotum (WHO, 2021). The affected body parts include the limbs, breasts, and genitalia. Lymphoedema occurs after progressive oedema and repeated acute attacks. Elephantiasis does not only lead to great personal suffering from its debilitating and disfiguring lesions, but it is also a significant factor in socioeconomic advancement, both locally and nationally (Ottessen et al., 1997). The most common type of elephantiasis is characterised by hydrocele and chronic lymphoedema or swelling of the lower and upper limbs and it affects marginalised persons, in particular those who reside in places with subpar housing and sanitation, are more susceptible to contracting the illness.

South-East Asia has a high prevalence of the disease, therefore strong political initiatives and excellent programs led to the eradication in certain areas. Despite the disease's modest fatality rate, it poses a substantial threat to global public health since it results in significant socioeconomic consequences (WHO, 2005). Neglected tropical disease distribution is due to socio-economic factors, great exposure to vectors, unclean food and water, reservoir hosts and climate.

Lymphatic filariasis is amongst nine infectious diseases aimed to be eliminated globally (Cano et al., 2014). It includes tropical diseases that the World Health Organization had set a target to achieve elimination by the year 2020 which led to increased vaccine and drug innovations and other alternatives for vector control.

1.2 Aetiology of elephantiasis

The disease is classified into filarial and non-filarial elephantiasis. Where no cause has been found, such cases are regarded as idiopathic.

1.2.1 Filarial elephantiasis

Elephantiasis due to lymphatic filariasis may be referred to as "true" elephantiasis in most areas. The three parasitic roundworms *Wuchereria bancrofti*, *Brugia malayi*, and

B. timori are the culprits (WHO, 2010; Simonsen, 2009; Simonsen, 2013; Slatko et al., 2014). According to a report by the World Health Organization (2010), *W. bancrofti* and *B. timori* differ from *B. malayi* in structure, symptoms and in regional extent. 90% of lymphatic filariasis cases are believed to be caused by *W. bancrofti*, whereas the remaining 10% are brought on by Brugian parasites (WHO, 1992). These nematode parasites are spread through various mosquito vector species of the following genera: Aedes, Mansonia, Culex, Anopheles, and Ochlerotatus (Chakraborty et al., 2013; Cano et al., 2014).

These various mosquito species that bite people act as vectors in various places of the world, and biological research into how these vectors interact with their surroundings is crucial for identifying the kinds of environments that can sustain the spread of parasites (Simonsen, 2013).

Lymphatic filariasis is brought on by *W. bancrofti* and spread by Anopheles and Culex mosquitoes in Africa. Southeast Asia is the only region where brugia malayi may be found (WHO, 2013). The huge numbers of minute microfilariae (mf) released by mature, fertilized female worms circulate in the blood and, when consumed by a vector during a blood meal, grow into infectious larvae in about 10–14 days (Simonsen, 2013). These move to the mosquito's proboscis where they may spread to humans during a later blood meal. Throughout their stay in the lymphatic system, adult worms produce millions of microfilariae that move around the blood (Feasy et al., 2009). Thus, the mosquito vectors are crucial to the development and spread of filarial diseases (Simonsen, 2013).

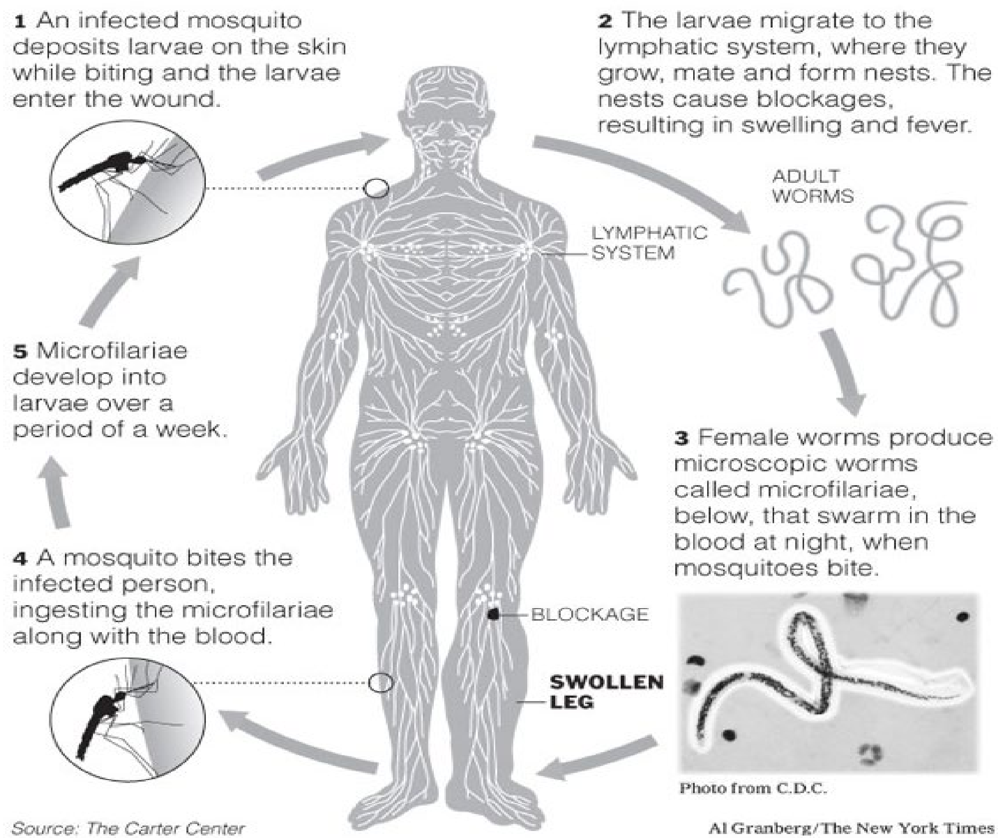

Fig 1.4: Transmission of elephantiasis (The Carter Center).

1.2.2 Wolbachia as an endosymbiont

Wolbachia is an intracellular bacterium that has reportedly been found around the tissues of numerous filarial species in its early stages using electron microscopy (Kozek et al., 1977; McCall et al., 1999; Slatko et al., 2014) was then identified as Wolbachia by molecular methods (Sironi et al., 1995; Slatko et al., 2014). Wolbachia, which was first identified in the sexual organs of Culex mosquitoes, is a member of the Rickettsiales order and is closely linked to Anaplasma, Ehrlichia, and Rickettsia. (Hertig and Wolbach, 1924; Slatko et al., 2014). These endobacteria that are inherited from mothers are obligate mutualists that have coexisted with their filarial hosts throughout evolution. If the Wolbachia bacteria discovered in various hosts or various invertebrate phyla constitute distinct bacterial species or strains, it is still unknown (Lo et al., 2007; Pfarr et al., 2007; Slatko et al., 2014). Wolbachia are present through the life cycle of the filarial worms by rapidly increasing once the nematode is introduced from the insect vector to the mammalian host. The laboratory studies and human trials

together with evidence that Wolbachia is essential for its filarial hosts and prospective novel therapeutic approaches for controlling filarial disease are provided by promising anti-Wolbachia antibiotic therapies in *in-vitro* and *in-vivo* clinical trials. (Slatko et al., 2014).

1.2.3 Non-filarial elephantiasis

In a report by Casteillani (1933), “elephantiasis nostras” is used to desctibe a condition that is clinically not different from filarial elephantiasis, but the filarial pathogenesis can be certainly not be included as patients showed no microfilariae in the blood. The author showed that the condition was from bacteria through the examination of the affected inguinal glands arising from lymphangitis and lymphadenitis attacks. The detected organisms were separated into Gram-positive cocci of the staphylococcus type (*Staphylococcus aureus*, *S. albus*, *S. viscidus*); Gram-positive cocci of the streptococcus group, most strains of *Streptococcus haemolyticus* type; Gram-negative cocci of the *Micrococcus* (coccobacillus) *metamyceticus* type; and Cocci of the *Micrococcus myceticus* type, which at times manifest to be completely Gram-positive, at other times completely Gram-negative, and most times partially Gram-positive (Casteillani, 1933). According to a report by National Organization for Rare Disorders (NORD) (2009), elephantiasis of the sexual organs can also be the result of sexually transmitted diseases, particularly lymphogranuloma venereum and donovanosis. Lymphogranuloma venereum is caused by *Chlamydia trachomatis* whereas the bacterium Calymmatobacterium (Klebsiella) granulomatosis is responsible for donovanosis. Due to the body's immune system's reaction to the bacterium during donovanosis, the lymphatic channels become inflamed and constricted, which results in genital elephantiasis.

Non-filarial elephantiasis is also associated with podoconiosis, which is caused by factors in the environment such as contact with certain minerals in the soil such as silica through barefeet. The growth of inflammatory tissues and nodules in the lymph vessels of the feet and legs is thought to be a result of the immune system's reaction to the mineral compounds (NORD, 2009). Non-filarial elephantiasis also includes a

protozoan condition called leishmaniasis, tuberculosis, leprosy and repeated streptococcal infections. It might also happen following radiation, surgery, or trauma.

1.3 Transmission of elephantiasis

Nematodes carried by insect vectors like *W. bancrofti*, *B. malayi*, and *B. timori* are the causes of lymphatic filariasis. (Simonsen, 2009; Simonsen, 2013; Slatko et al., 2014). *W. bancrofti* causes Bancroftian filariasis and is responsible for ninety percent those affected by lymphatic filariasis whilst the rest are caused by Brugian parasites (WHO, 1992). These two; *W. bancrofti* and *B. timori* distinguish *B. malayi* from both of them in terms of structure, symptomology, and geographic distribution. (WHO, 2010). By depositing infected larvae on human skin, these parasitic organisms spread to people via mosquito vectors, where the larvae then enter the skin and move to the lymphatic arteries, where they finally mature into male and female adult worms over several months. (Simonsen et al., 2013). These nematode species are spread via a variety of mosquito vector species belonging to the following genera; Aedes, Culex, Mansonia, Anopheles and Ochlerotatus (Cano et al., 2014; Chakraborty et al, 2013). When mature female worms are fertilized, a huge number of minute microfilariae (mf) are released into the bloodstream. When a vector consumes the microfilariae during a meal, they develop into infectious larvae in around 10 to 14 days (Simonsen, 2013). Millions of microfilariae that circulate in the blood are produced viviparously by adult worms that lodge in the lymphatic system, where they live for years (Feasy et al., 2009).

1.4 Prevalence of elephantiasis

Elephantiasis, a major public health concern amongst several warm countries having warm climates (Simonsen et al., 2013). Also, it takes second place in causes of disabilities worlwide as about 40 million people who suffer from issues that prevent people from engaging in their jobs, pursuing their education, finding work, and moving around (WHO, 2013a). More than 100 million people in the Americas, Asia, Africa, and

the Pacific are infected with W. bancrofti, the most prevalent lymphatic filarial parasite of humans (Simonsen, 2009). The World Health Organization calculated that more than 1.25 billion individuals in 72 countries and territories are at risk in 2013. It was estimated that around 40 million people had pathologic symptoms from filarial parasites, including the displeasing elephantiasis, and that about 120 million people were affected (Babu and Nutman, 2014). This includes 25 million men with urogenital swelling, including scrotal hydrocele, and 15 million persons with lymphoedema (elephantiasis) (WHO, 2010). A major step that was taken towards the elimination of lymphatic filariasis with only a few implementation units (IUs) having remained in Côte d'Ivoire, Ethiopia, Nigeria, and Zambia, all implementation units (IUs) in the African area had their mapping completed. By the end of 2013, 655 IUs in 17 countries targeted for mapping to determine the need for mass drug administration (WHO, 2013a).

According to research done by Cano et al. (2014), 66 out of 72 countries are said to be currently endemic to lymphatic filariasis. It is in 17 countries where lymphatic filariasis no longer exists but is now found mainly in coastal areas of east and southern Africa (Cano et al., 2014).

Transmission of lymphatic filariasis occurs throughout the Americas region, primarily in north and north-east of South America, Central America, the major Caribbean islands (Haiti and the Dominican Republic), and sporadically in the southern United States (Cano et al., 2014). Twenty countries in the Americas have eliminated lymphatic filariasis, and the only remaining endemic areas are Brazil, Guyana, and Hispaniola (the Dominican Republic and Haiti) (Addiss and Chuke, 2002; Cano *et al.*, 2014). Transmission is anticipated to occur in eastern India, Sri Lanka, much of southeast Asia, southeast China, Papua New Guinea, the northern coast of Australia, and southern Japan in Asia and the western Pacific. LF has been eliminated in China (2007), Japan (the 1980s), and South Korea (2008), but the predicted environmental factors correspond with the known historical and pre-control distribution (Sasa, 1976; Kimura et al., 2005; Tada, 2011; Cano et al., 2014).

In Arica, lymphatic filariasis is also distributed across countries such as Tanzania with an estimation of 6 million people with disabilities because of the disease (Lupenza et al., 2021).

According to immunochromatographic card tests (ICT) and microfilaria (Mf) results, the number of people in Nigeria with lymphatic filariasis was previously believed to be 8.7 million and 3.3 million, respectively. (Eneanya, et al., 2019).

Thirty million individuals in Ethiopia were considered to be at risk for lymphatic filariasis, placing Ethiopia in fourth place (7.8%) among sub-Saharan African countries (Deribe et al., 2012).

The largest prevalence of podoconiosi or non-filarial elephantiasis, is seen in tropical African nations, with 500,000 more individuals living with the disease in Cameroon and about 1 million in Ethiopia. (Deribe et al., 2016). High prevalences of podoconiosis are found in the highlands of Uganda, Tanzania, Kenya, Rwanda, Burundi, Sudan, and Ethiopia (Pietro et al., 2010).

1.4 Management and treatment of elephantiasis (LF)

In order to eradicate lymphatic filariasis, minimize morbidity, and prevent related disabilities, the Global Program to Eliminate Lymphatic Filariasis (GPELF) was established in the year 2000 (WHO, 2010). The GPELF's main goal was giving access to hydrocele surgery, stop the growth of lymphedema and elephantiasis, as well as debilitating and painful episodes of acute adenolymphangitis or acute dermatolymphangioadenitis in areas where lymphatic filariasis is common (WHO, 2010). By using preventive chemotherapy, such as mass drug administration, the provision of anti-filarial medications assisted in the elimination of any remaining worms and microfilariae (WHO, 2013b).

By the year 2014, 73 countries were listed as regions where to lymphatic filariasis is found and 18 countries entered the monitoring phase and only 11 countries had not yet commenced mass drug administration programmes (Molyneux et al., 2016). An

estimate of the year 2014 assessin mass drug administration had suggested that more than 96.71 million cases of the disease had been prevented or treated, yet about 36 million files belonging to hydrocoele and lymphoedema infection had remained (Ramaiah and Ottessen, 2014).

The discovery that Wolbachia species play a substantial role in the life cycles of *B. malayi* and other nematodes has inspired the development of brand-new medications that target the endobacterium. By preventing larvae from moulting and microfilariae from developing, tetracyclines, rifampicin and chloramphenicol demonstrated their efficiency in vitro. Tetracyclines have been shown to produce anomalies in adult worms' embryogenesis and reproduction, which causes the worms to become infertile. Clinical trials have successfully reduced Wolbachia and microfilariae in onchocerciasis and *W. bancrofti* infected patients, and although antibiotics act in a somewhat indirect manner, they represent a source of promising antifilarial medicines (Ottesen et al., 1997). The length of treatment and population-specific contraindications of doxycycline offer obstacles to its application in mass medication administration, necessitating the development of novel anti-Wolbachia medicines (Slatko et al., 2014; Taylor et al., 2014).

Lymphatic filariasis control took a turn-around in the 1990s when advances were made in diagnositic studies about of lasting effective drugs in one-dose administrations which led to the development of a yearly bi-drug, one-dose mass treatment for controlling or elimination of lymphatic filariasis (Ottessen et al., 1997 and Ottessen 2000). The medications chosen for the management of filariasis include ivermectin, albendazole (ALB), and diethylcarbamazine (DEC), which are currently administered in large quantities through national programmes (Ottessen, 2000; Gayen *et al*., 2013; Ramaiah and Ottessen, 2014). In most endemic locations, DEC and ALB are employed; however, in some parts of Africa, where onchocerciasis and bancroftian filariasis are both present, ivermectin and ALB are combined (Molyneux, 2003; Ichimori and Ottesen, 2011). The program's primary approach has been two-fold: to begin mass drug administration tactics in endemic areas in order to completely interfere with transmission, and to provide effective morbidity management in order to lessen suffering in those who have already contracted lymphatic filariasis (Ramaiah and Ottessen, 2014).

Fig 1.5: Treatment for elephantiasis (who. int).

The programme to implement mass drug administration targeting lymphatic filariasis by the GPELF had run a period of 13 years operation by 2012 and it has had a big aim of LF elimination by 2020. Furthermore, the GPELF provides mass drug administration using albendazole + either ivermectin or diethylcarbamazine to whole endemic populations at yearly periods of 4 to 6 years. According to Stolk et al. (2006), if programms like this are conducted in a good way by treating at least 65% of the total population, each MDA could create the possibility of interrupting transmission and eliminating lymphatic filariasis (Stolk et al., 2006).

For microfilaremia, 2 of the main antifilarial medicines in mass drug administration efforts which are DEC and ivermectin were recognised to display significant and more responses against increasing microfilaremia. The anti-microfilarial effect of these drugs is further strengthened when they are taken in combination with ALB, a multi spectrum antihelminth medicine that acts by affecting both adult worm survival and production of microfilariae (Gyapong et al., 2005).

de Kraker *et al*. (2006) stated that 4 to 6 times of one dose of DEC can can result in a reduction of microfilaremia numbers by up to 86% (Bockarie *et al*., 2002; Ramaiah *et al*., 2002). When two principal anti-filarial drugs used in GPELF are combined, (ALB+ DEC) or (ALB+ ivermectin), LF infection levels are highly reduced (Ramaiah and Ottessen, 2014).

However, the main consequences of treatment through anti-filarial drugs are less significant against chronic conditions symptoms than on microfilaremia. Many studies have proven that treatment has a remarkable impact on chronic disease manifestations, starting from the curing of early disease signs and symptoms to the actual curing of some chronic lesions. The presence of adult worms alone is sufficient to cause hydrocele (Dreyer *et al*., 2000). Reducing adult worm burden alone can lead to reducing hydrocele condition numbers. The anti-filarial drugs used in the MDA programme, ALB + ivermectin, as well as DEC alone or with ALB can partially act against adult worms and lessening adult worm problem (Ottessen, 1985; Ismail et *al*., 1998). Ottesen et al. (1997) and Sashidhara et al. (2012) also reported that drugs used may reduce microfilariae numbers but show no effect in killing adult worms. Therefore, these drugs provide only partial benefit to infected patients and usually are associated with adverse reactions.

Regardless of the success of these programs, no doubt that a need exists to develop better and innovative methods for the longer control of adult worms as they are primary causes disease pathogenesis in lymphatic filariasis. New compounds are to be discovered agents that will kill the adult worms (microfilariae), replacing current drugs that might not work in drug resistant worms as reported in onchocerciasis patients against *in vitro* maturation. The discovery of anti-filarial drugs followed by validation through standard experiments is of high importance for the development of screening systems that will lead to new anti-filarial compounds. The gene-sequence encoding for filarial nematodes and application of RNA interference (RNAi) is also of impotance in investigating various gene functions (Singh et al., 2010).

DEC has been reported to cause side effects such as fever, gastrointestinal disturbance, headache, malaise, and a skin rash that reduce patient compliance (Sharma, 1990; Babu et al., 2006). Adverse reactions following treatment with ivermectin usually are not manifestations of direct drug toxicity but result from host inflammatory responses to the very rapid clearance of microfilariae from the blood. The most common side-effects include fever, light-headedness, malaise, and, in extreme cases, postural hypotension which develops between 12-24 h post-treatment and lasts for an additional 24-36 h in approximately half of the microfilaremia patients

treated (Ottesen and Campbell, 1994). Other reported side effects of ivermectin include itching after early administration, acute toxicities such as convulsions after overdosage and deaths that have been reported in patients that were treated for filariasis (Fujimoto et al., 2014).

Treating bancroftian filariasis with a 4 to 6, or 8-week course of 200 mg per day dose of doxycycline will result in long-term sterility and eventual death of the adult worms (Hoerauf, 2008; Bockarie et al., 2009). Furthermore, lab and human trials demonstrate that depletion of *Wolbachia* in filarial parasites by antibiotics like doxycycline and tetracycline work to kill the adult worms in addition to blocking embryogenesis, microfilariae production and worm development (Foster et al., 2013; Slatko; Taylor et al., 2014).

1.6 Medicinal plants in the treatment of elephantiasis

The demand and scarcity of medicine that can prevent disease and the problem of drug-resistant worms has put pressure on an urgent need for cheaper novel anti-filarial drugs with long-term antimicrofilarial or macrofilaricidal activity and less side-effects (Srivastrava et al., 2000; Dharma et al., 2005). Some medicinal products have been discovered from plants which are utilised in traditional medicine. Many medicinal plants containing pentacyclic triterpenes and oleanolic acids have been reported to possess anti-filarial activity (Misra et al., 2007). Plant drugs which can act against filariasis were discovered from plants that are used in traditional medicine.

In the Americas, *Piper aduncum* and *P. elongatum* are in the list of plants used in Brazil for lymphatic filariasis management by acting against filarial worms (Ndjonka et al., 2013). In India, *Butea monosperma, Lantana camara* and *Vitex negundo* have been reported to act against filarial worms (Anil and Talluri, 2015). *Andrographis paniculata* has been reported to be used in greater Asia including China, India and Sri Lanka (Al-abd et al., 2013). In West Africa, *Acacia nilotica* and *Bombax buopozense* are used in Central Nigeria, and *Spathodea campanulate* and *Newbouldia leavis* are used in Ghana (Ndjonka et al., 2013; Twumasi et al., 2020); in East Africa, *Warburgia salutaris* is used in Tanzania (Ndjonka et al., 2013). *Azadirachta indica* is used in India

and West Africa; and *Cardiospermum halicacabum* is used around tropical and subtropical regions of Africa and Asia (Al-abd et al., 2013).

Studies by Al-and et al. (2013) and Komoreng et al. (2017) and reported the use of *Ricinus communis* in South Africa and India for the treatment of the disease. *Euphorbia clavarioides*, *Euphorbia gorgonis*, *Rumex obtusifolius*, *Rhoicissus tomentosa* and *Rhoicissus tridentata* are also used to treat conditions associated with lymphatic filariasis in South Africa (Komoreng et al., 2017).

1.7 Use of medicinal plants in South Africa

Plants have been used as medicine for thousands of years (Samuelsson, 2004; Balunas and Kinghorn 2005). These natural products medicines were in the form of crude extracts such as tinctures, teas, poultices, powders and other herbal formulations (Balick and Cox, 1997; Samuelsson, 2004). South Africa has a long history of traditional plant use for the treatment and mangement of various diseases and conditions (Cunningham, 1993; Van Wyk *et al*., 1997; Amoo *et al*., 2009). Large numbers of populations in second world countries use traditional medicine as a primary healthcare are resource due to the value of Western medicine and healthcare, and because traditional medicines are generally more acceptable from a cultural and spiritual point of view (Addae-Mensah, 1992; Amoo *et al.*, 2009).

80% and more of the world's population are estimated to use use plants as their main source of medicine (Cordell, 1995; Taylor *et al.*, 2003;). In South Africa, 60%–80% of the population mainly or partially use traditional herbal medicines to treat a variety of animal and human diseases (Dausdart, 1990; Shai et *al.*, 2008). Up to 60% of the population consults traditional healers, especially in rural areas where traditional doctors are many and more easily accessible than Western healthcare (van Wyk et al., 1997; Taylor et al, 2003). Large numbers of medicines used on a daily basis in South Africa are derived from plants where large volumes of plants, or their extracts, are so found in both the informal and commercial sectors of the economy (Taylor *et al*., 2009). With the increasing use of traditional medicine by indigenous people, investigating biologically active agents based on traditional use relevant as these

plants possess the potential to provide pharmacologically active compounds (Cragg et al., 1997; Eldeen and van Staden, 2007; Amoo et al, 2009).

Drugs found in plants are an important source of new chemical prroducts with therapeutic effects against pain (Farnsworth, 1889; Gupta *et al*., 2006; Nkomo *et al.*, 2010). Several studies documented over 3000 plant materials which added to the knowledge that includes current ways adaptations and mechanisms with indigenous materials in South African traditional medicine (van Wyk et al., 2009; Philander, 2011). Our country possesses various types of ethnomedicine with medicinal plant having been been documented in various cultures (Philander, 2011). KwaZulu-Natal Province has 1032 plant species recorded as Zulu medicinal plants, and studies from the Eastern Cape discovered that Xhosa people frequently use plants that are of medicinal, cultural or spiritual importance (Hutchings *et al*., 1996; Cocks and Dold, 2002; Dold and Cocks, 2006; Philander, 2011).

1.8 Major groups of plant secondary metabolites

Plants are stationary autotrophs having to adapt to several environmental factors which include manufacturing pollination and seed dispersal, localised fluctuations in nutrient supply they need for food synthesis and their coexistence with herbivores and pathogens in their immediate environment. Plants, therefore evolved secondary biochemical pathways giving them the ability to produce structures of chemicals, often in response to specific environmental stimuli, such as herbivore-induced damage, pathogen attacks or nutrient deprivation (Hermsmeier et al., 2011; Kennedy and Wightman, 2011; Reymond et al., 2000). As a result of these response mechanisms to external stimuli, plants have become a source of natural products that possess the potential to produce new types of drugs that are of great benefit to humans (Karimi et al., 2011) Highly important of these bioactive constituents, which are mainly secondary metabolites, are alkaloids, saponin, flavonoids, tannins, and phenolic compounds, Plant by-products are chemically and taxonomically extremely diverse compounds whose function has not yet been discovered. These compounds are widely used in human therapy, veterinary, agriculture, scientific research, and countless other areas (Yadav and Agarwala, 2011; Vasu et al., 2009). A large number of phytochemicals

belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 2009; Yadav and Agarwala, 2011). The sub-groups of these phytochemicals include phenols, phenolic acids, phenylpropanoids, flavonoids, flavones, glycoflavonones and biflavonols, minor flavonoids, aurones, flavonones, isoflavones, xanthones and stilbenes, hydrolysable and condensed (proanthocyanidins) tannins and quinines (Muchuweti *et al*., 2007 Strack, 1997; Harbone 1998).

1.8.1 Phenolic compounds

Phenolic compounds are secondary plant metabolites that structurally share leastly 1 aromatic hydrocarbon ring with 1 or more hydroxyl groups attached (Kennedy and Wightman, 2011). Phenolic compounds dissolve in water and may occur attached with a sugar molecule, as glycosides (Harborne, 1998; Muchuweti *et al*., 2007). They have a diverse biological activity including toxicity to hormonal mimicry and act as part of cell wall material, colourful attractants for birds and insects helping seed dispersal and pollination, and their compounds also act as defense mechanisms of plants under different environmental stress conditions such as wounding, infection, excessive light or UV irradiation (Harbone, 1998). Phenolics startbfrom simple low-molecular-weight compounds such as the simple phenylpropanoids, coumarins and benzoic acid derivatives, to more complex structures such as flavonoids, stilbenes and tannins. The biological potency of phenolic compounds includes important pharmacological compounds (Ingold, 1960; Muchuweti, 2007). Phenolic compounds at large have long been recognised to possess antiallergenic, anti-inflammatory, antiviral and antiproliferative activities (Muchuweti *et al*., 2007). About 12 000 phenolic compounds have been isolated, a number estimated to be just less than 10 % of the total number (Borris, 1996; Cowan, 1999). The phenolic acids as primary antioxidants having a single or more aromatic rings bearing one or more hydroxyl groups can quench free radicals by forming stabilized phenoxyl radicals ((Takaidza et al., 2018).

1.8.2 Flavonoids

The flavonoids represent the largest and most diverse group of phenols, with about 6000 compounds sharing a common unique structure of two 6-carbon rings. They have low molecular weight. Flavane is an example of a flavonoid and it contains two benzene rings within its chemical composition (Altemimi et *a*l., 2017). Flavones, isoflavones, flavonoids, flavonols, flavanones, anthocyanins and proanthocyanidins are part of flavonoids according to the flavonoid classification. Flavonoids were found to possess several pharmacological properties, including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogens (Scalbert and Williamson, 2000; Manach et al., 2004 & Wulandari et al., 2016).

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones); the addition of a 3-hydroxyl group yields a flavonol (Fessenden and Fessenden, 1982). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection it should not be surprising how they can be effective against antimicrobial substances against a wide range of microorganisms (Dixon et al., 1983; Cowan, 1999). Activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al., 1996). Catechins, which are the most reduced form of the C3 unit in flavonoid compounds, are very relevant in the flavonoid group. These flavonoids were extensively investigated for their presence in green tea, and it was found that teas possessed antimicrobial activity because they have a mixture of catechin compounds (Toda et al., 1989; Cowan, 1999;). The catechins were also reported to have acted against cholera toxin in Vibrio and also inhibited isolated bacterial glucosyltransferases in S. mutans possibly due to complexing activities described for quinones (Nkahara, 1993; Borris, 1996; Cowan, 1999). Flavonoid compounds have inhibitory effects against multiple viruses and numerous studies have documented the effectiveness of flavonoids such as swertifrancheside, glycyrrhizin (from liquorice) and chrysin against human immunodeficiency virus (Pengsuparp, 1995; Critchfield et al, 1996, and Watanbe, 1996). Studies discovered flavone derivatives are inhibitory to the respiratory syncytial virus (RSV) (Kaul et al., 1985; Barnard et al., 1993). A summary of the antiviral effects and modes of action of

quercetin, naringin, hesperetin and catechin in in-vitro cell culture monolayers were also reported by Kaul et al (1985).

1.8.3 Tannins

Tannins are natural products found in many plant families, possessing large numbers of phenolic rings in their structure. Being polymeric substances, they can tann leather or precipitate gelatine from solution, a property known as astringency (Cowan, 1999; Altemimi et al., 2017). They represent the major groups of antioxidant polyphenols found in food and beverages which have drawn a lot of attention in recent years because of their multifunctional properties that are beneficial to human health (Kumari and Jain, 2012). Tannins have earned a lot of attention in recent years as it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a number of diseases (Serafini et al., 1994). Many human physiological processes, such as stimulation of phagocytic cells, host-mediated tumour activity and a wide range of anti-infective actions have been associated with tannins (Haslam, 1996). A part of their molecular actions is to combine with proteins through so-called non-specific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Haslam, 1966; Stern et al., 1996; Cowan, 1999). Thus, their mode of antimicrobial action may be attributed to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins and many other activities (Cowan, 1999). They also form a complex with polysaccharides (Ya et al., 1988). Scalbert (1991) reviewed the antimicrobial properties of tannins and he listed 33 studies that had documented the inhibitory activities of tannins. According to these studies, tannins were reported as toxic to filamentous fungi, yeasts, and bacteria.

They are classified into two groups, hydrolysable and condensed or proanthocyandins. Hydrolysable tannins are a mix of simple phenols with ester linkages in structure based on gallic acid, usually as multiple esters with D-glucose, while the more numerous condensed tannins are derived from flavonoid monomers (Cowan, 1999; Altemini *et al*., 2017). According to Kumar and Jain (2012), hydrolysable tannins are molecules with a polyol (D-glucose) as a central core. The hydroxyl groups of these

carbohydrates are partially or esterified with phenolic groups like gallic acid (gallotannin) or ellagic acid (ellagitannin) and are usually present in low amounts in plants and can also be easily hydrolysed by mild acids and bases to yield carbohydrate and phenolic acids. Condensed tannins possess flavonoid monomers with several degrees of condensation. In nature they occurr as polyphenolic bioflavonoids, specificallly taking the form of oligomers or polymers of polyhydroxy flavan-3-of units, such as (+)-catechin and (-)-epicatechin and flavan-3,4-diols, such as leucoanthocyanidins or a mixture of the two (Porter, 1986; Kumar & Jain, 2012).

Alkaline compounds, mineral acids, and enzymes are some of the factors that can hydrolyse tannins (Altemimi *et al*., 2017). Plant part such as bark, wood, leaves, fruits, and roots possess tannins (Scalbert, 1991; Cowan, 1999 Kumar and Jain, 2012). Condensed tannins can bind cell walls of ruminal bacteria, preventing growth and protease activity (Jones et al., 1994). Condensed tannins are widely distributed in fruits, vegetables, forage, plants, cocoa, red wine, and certain food grains, such as sorghum, finger millets, and legume, possessing cardio-protective, anti-inflammatory, anti-carcinogenic, and antimutagenic, among others. These protective properties are related to their antioxidant capacity to act as free radical scavengers by activating antioxidant enzymes (Kumar and Jain, 2012).

1.8.4 Alkaloids

Alkaloids are heterocyclic nitrogen compounds that are diverse in structure. Over 12 000 cyclic nitrogen-containing compounds are found in over 20% of plant species (Zulak et al., 2006; Kennedy and Wightman, 2011). Although a single classification does not exist, structural similarity distinguishes them i.e., indole alkaloids or a common precursor and benzylisoquinoline, tropane, pyrrolizidine, or purine alkaloids (Kennedy and Wightman, 2011).

Morphine was recorded first in medicinal use as an alkaloid isolated in 1805 from the opium poppy *Papaver somniferum* (Fessenden and Fessenden, 1982; Cowan, 1999). Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae family, are commonly found to have antimicrobial properties (Rahman and Choudary, 1995;

Omulokoli et al*.,* 1997; Cowan; 1999). Berberine as an important example of the alkaloid grouppotenti was discovered to be effective against trypanosomes and plasmodia (Freiburghaus et al., 1996; Omulokoli et al., 1997).

The recorded use of alkaloids for medicinal purposes stretches back some 5000 years ago (Kennedy and Wightman, 2011; Goldman, 2001). This chemical group has contributed to the majority of the poisons, neurotoxins and traditional psychedelics (e.g., atropine, scopolamine, and hyoscyamine, from the plant *Atropa Belladonna* and social drugs e.g., nicotine, caffeine, methamphetamine (ephedrine), cocaine and opiates that are consumed by humans (Zenk and Juenger, 2007; Kennedy and Wightman, 2011). Some herbivorous species evolved to adapt to either tolerate or sequester alkaloids from their host plant. Plant-based alkaloids are however toxic to mammals by their function and chemical nature (Rattan, 2010).

Alkaloids such as isoquinoline, quinolone, and β-carboline type possess several antiviral compounds e.g., sanguinarine and berberine are strong DNA intercalators that have antibacterial, antiviral and cytotoxic properties (Wink, 2020).

1.8.5 Saponins

Saponins are a diverse group of phytochemicals whose chemical structures are composed of a fat-soluble nucleus (aglycone) which is either a triterpenoid (C-30) or a neutral or alkaloid steroid (C-27) attached to one or more water-soluble sugars (glycone) side chains through ester linkages to the aglycone nucleus at different carbon sites (Haralampidis *et* al., 2002; Karimi et al., 2011. Saponins with one sugar molecule attached at the C-3 position are called monodesmoside saponins, and those that have a minimum of two sugars, one attached to the C-3 and one at C-22, are called bidesmoside saponins (Laziszity et al., 1998; Adams, 2014). While steroid saponins are more prevalent in yucca, tomato, and oats, triterpenoid saponins are more prevalent in soybean, alfalfa, and quillaja While steroid saponins are more prevalent in yucca, tomato, and oats, triterpenoid saponins are more prevalent in soybean, alfalfa, and quillaja (Haralampidis et al., 2002; Karimi et al., 2011).

Some biological properties of saponins include haemolytic and antibacterial activities (Sparg *et al*., 2004). As the most popular member of the Araliaceae family, *Panax ginseng* root preparations have a lengthy medical history (Yun, 2001). The putative major active components comprise 40 or more species-specific triterpene saponins known as ginsenosides (Lu et al., 2009). The ginsenosides in plants possess antifungal, viral, bacterial, insecticidal and molluscicidal activity and exert allelopathic and antifeeding effects (Osbourn, 1996; Sparg et al., 2004; Kennedy and Wightman, 2011).

1.8.6 Terpenes

Terpenes are a diverse group of more than 30,000 lipid-soluble compounds. Their structure includes 1 or more 5-carbon isoprene units. They are classified according to the number of isoprene units they contain. Isoprene itself is synthesized and released by plants and if it comprises 1 unit it is classified as a hemiterpene, monoterpenes incorporate 2 isoprene units, sesquiterpenes incorporate 3 units, diterpenes comprise 4 units, sesterpenes include 5 units, triterpenes incorporate 6 units and tetraterpenes 8 units (Kennedy and Wightman, 2011). Terpenes exhibit a level of toxicity that ranges from lethal to entirely edible, and this is in keeping with their wide range of ecological functions, which include pollination, seed dispersal, and secondary protective roles (Kennedy and Wightman, 2011). Terpenoids also exhibit a number of noteworthy pharmacological properties, including anti-inflammatory, anti-cancer, anti-malarial, cholesterol synthesis inhibition, antiviral, and antibacterial properties (Mahato and Sen, 1997; Wadood *et al*., 2013).

1.9 Phytochemicals as antioxidants and their free radical scavenging ability

Free radicals are defined atoms or molecules possessing unpaired electrons. The reactive oxygen species are oxygen-derived free radicals such as superoxide anion (O_2), hydroxyl (OH), hydroperoxyl (ROOH), peroxyl (ROO), alkoxyl (RO) radicals, and non-free radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) and by singlet oxygen (O_2) (Halliwell and Gutteridge, 1999; Paulsamy et al., 2016). They are formed in living organisms endogenously by respiration, peroxisomes stimulation of polymorphonuclear leucocytes and macrophages, and exogenously by

ionizing radiation, tobacco smoke, pollutants, pesticides and organic solvents (Irshad and Chaudhuri, 2002; Paulsamy et al., 2014). Free radicals are produced by our bodies to stabilize the body's natural function, but the problem is that the excess amount can cause cell and tissue damage (Sen et al., 2010). Additionally, they can result in oxidative damage to proteins, lipids, and DNA as well as chronic human disorders like cancer, diabetes, aging, and other degenerative conditions (Aiyegoro and Okoh, 2010).

Antioxidants can further be defined as any chemical that prevents or reduces oxidative damage to a target molecule. defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagishi and Matsui, 2010). Due to their redox hydrogen donors and singlet oxygen quenchers, antioxidants have the potential to scavenge free radicals, which is a defining attribute of an antioxidant (Anokwuru et al, 2011; Wu et al., 2011; Paulsamy et al, 2014). Both natural antioxidants and plants can scavenge free radicals (butylated hydroxyl toluene, butylated hydroxyl anisol and tetra butyl hydro quinone) (Mbaebe et al., 2012). Chemical antioxidants are now being currently replaced by natural antioxidants since the natural ones are considered to have the potential of being safer with less or no side effects (Meenakshi et al., 2011; Paulsamy et al., 2014). Plants include advantageous phytochemicals that can act as natural antioxidants to complement the body's needs (Boots et al., 2008). Many researchers through the years have been interested in the investigation of medicinal plants' phytochemicals for their antioxidant potential to scavenge free radicals that cause human disease.

Many antioxidant compounds are known to be found in fruits and vegetables and these include vitamins A and C, phenolics such as flavonoids, tannins and lignins, carotenoids, anthocyanins and tocopherols (Jakubowski and Bartosz, 1997; Suffredini et al., 2004; Altemimi et al., 2017). Therefore, eating fruits and vegetables has been linked to health advantages due to their therapeutic characteristics and high nutritional content (Valko et al., 2006).

Demand for non-toxic, natural preservatives, many of which are expected to have either antioxidant or antibacterial activity, has increased as awareness of the detrimental effects of synthetic preservatives has grown (Negi et al., 2005; Baharlouei

et al., 2010 and Altemini et al., 2017). Several disorders associated with oxidative stress can be treated with plant-derived antioxidants with free-radical scavenging abilities (Ramchoun et al., 2009). Ascorbic acid, beta carotene, and numerous other phenolics have vital roles in lowering inflammation, slowing the aging process, and avoiding some malignancies (Duthie et al., 1996; Altemini et al., 2017). Plant extracts have been shown in studies to have the ability to scavenge free radicals (Agati et al., 2012; Mbaebie et al., 2012; Adebayo et al., 2015; Tshikalange et al., 2016; Olaokun et al., 2017).

1.10 Inflammation and diseases

An organism's physiological response to injury, microbial infections, particulate debris, and malignant cancer cells is inflammation. Prolonged inflammation can cause certain diseases or exacerbate ones that already exist. Acute inflammation arises from an immediate response to the foreign body or microorganism, whereas a delayed, prolonged response typically leads to a chronic disease. The major steps in an inflammatory reaction are initiation of the reaction, progression and termination. Inflammation has been found to further the progression of existing disease conditions. Therefore, understanding the role played by inflammation in these diseases is significant in designing newer therapeutic strategies and disease management in patients (Krishnamoorthy and Honn., 2006).

Acute inflammation occurs quickly and lasts only a short time whereby fluids and plasma proteins are released and leukocytes and neutrophils migrate around the injured area. The acute inflammatory response aims to destroy bacteria, viruses, and parasites while speeding up the healing of wounds (Iwalewa et al., 2007).

When lymphocytes and macrophages are present, chronic inflammation lasts longer and manifests in cell tissues, leading to fibrosis and tissue necrosis. Degenerative diseases like cancer, congestive heart failure (CHF), rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer's, asthma, multiple sclerosis (MS), diabetes, infections (bacteria, fungi, parasites), gout, IBD-inflammatory bowel disease, aging,

and other neurodegenerative disorders are brought on by ongoing chronic inflammation (Dalgleish and Byrne, 2002; Iwalewa et al., 2007).

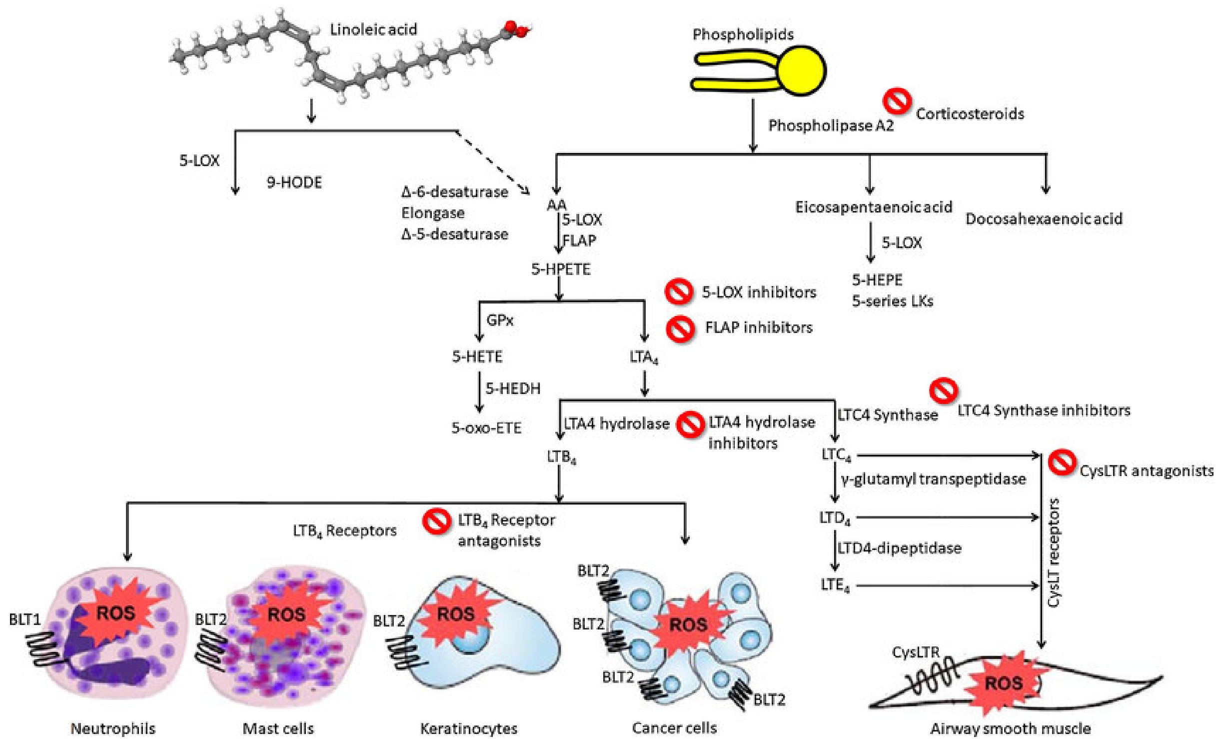

Figure 1.6: The 5-lipoxygenase pathway and therapeutic potential of 5-lipoxygenase inhibitors in GI cancers (www.researchgate.net, 2021).

In a review done by Iwalewa et al (2007), elevated IL-6, ROS and myeloperoxidase in chronic infections served as indicators in viral and bacterial infections. These processes are responsible for injury to the cells through a number of ways including peroxidation of the cell membrane lipids and oxidative damage of proteins or DNA.

These chronic conditions and diseases have been associated with the increased expression of pro-inflammatory mediators, which trigger the production of pro-inflammatory cytokines, NF kappa B, NADPH oxidase, phospholipase A2, COX-1 and -2, 5-LOX, myeloperoxidase, and iNOS, as well as an increase in oxygen consumption and oxygen-free radical production, can eventually result in certain degenerative diseases (Iwalewa et al., 2007).

When the neutrophils are stimulated appropriately, they facilitate the splitting of arachidonic acid from membrane phospholipids and arachidonic acid metabolism takes place via cyclooxygenase (COX) enzyme which is the isoforms, COX-1 and COX-2 either through the LOX route, which results in the production of leukotrienes and hydroperoxy-eicosatetraenoic acids, and prostaglandins and thromboxane A_2 (Bouriche et al., 2005; Akula & Odhav, 2008; Adebayo et al., 2015). Inhibiting the COX enzyme is thought to have played a significant role in the development of anti-inflammatory and anti-nociceptive medications. (Karim, et al., 2019).

1.10.1 Oxidative species as inflammation mediators

When the inflammatory cells are stimulated, they go through a respiratory burst, and ROS such as hydrogen peroxide, superoxide anion and other secondary oxidants are released as well as products of the arachidonic acid cascade. A hypothesis that DNA damage is induced in tissues during inflammatory reactions was developed and that activated neutrophils are responsible for inflammatory diseases (Ward et al., 1994).
ROS and RNS, such as nitric oxide, (NO) are produced by the enzyme NO synthase (NOS) and NADPH oxidase isoform. ROS and RNS have physiological roles and occur at low or moderate concentrations, they have positive impacts in the cells in the cells such as defense against infectious and cellular signalling pathways but excessive stimulation of NADPH or the mitochondrial electron transport chain can lead to oxidative stress, which can be harmful to the cell's structural components, including proteins, DNA, and lipids in cell membranes (Sari et al., 2017). These oxidising substances are produced by neutrophils, monocytes, macrophages, and eosinophils, which invade the tissues (Conner and Grisham, 1996). Mitochondria also produce ROS (Closa and Folch-puy, 2004).

Phagocytes are activated when proinflammatory mediators or bacterial products with specific receptors on the leucocyte plasma membrane interact, resulting in the formation of NADPH oxidase. This enzyme catalyses the production of large amounts of the superoxide anion radical ($NADPH + O_2 \rightarrow NADP + O_2^-$) (Klebanoff, 1992; Conner and Grisham, 1996). Superoxide is relatively unreactive toward most biological substrates and will reacy very rapidly and spontaneously or by attaching to an enzyme

dismutase to yield hydrogen peroxide (H_20_2) and oxygen (02): $O_2^- + O_2^- + 2H^+ \rightarrow H_20_2 + O_2$. Hydroxyl radicals have been shown to peroxidise lipids, oxidize proteins and promote DNA strand splitting (Grisham,1992; Conner and Grisham, 1996). The reaction of the hydroxyl radical with the DNA base deoxyguanosine results in the formation of 8- hydroxydeoxyguanosine which in turn causes an increase in the frequency of misincorporation of DNA bases, suggesting that these mutations in the cells induced by oxygen radicals could play a part in the induction of autoimmunity and, possibly, carcinogenesis (Merry, et al, 1989).

Besides promoting toxicity, reactive oxygen products may also worsen inflammation via the upregulation of several different genes involved in the inflammatory response which may occur by the activation of certain transcription factors, such as nuclear transcription factor-kB (NFkB) (Conner & Grisham, 1996; Closa & Folch-puy, 2004).
NF kappaB (NFkB) is made up of proteins and it binds to DNA to activate gene transcription. The transcription factor NFkB controls the expression of numerous proteins involved in inflammation, including all pro-inflammatory cytokines, chemokines, and enzymes of the arachidonic acid cascade (Iwalewa et al., 2007). Closa and Folch-puy (2004), reported on the beneficial effects of antioxidants such as N-acetylcysteine and pyrrolidine dithiocarbamate on their ability to inhibit NFkB activation.

1.10.2 Lymphoedema and inflammation

Lymphoedema is a chronic condition marked by the buildup of interstitial fluid in tissues as a result of damaged lymphatic vessels, which causes swelling and limb dysfunction. Primary or hereditary lymphoedema is caused by intrinsic abnormalities of the lymphatic system brought on by gene abnormalities involved in the growth and development of the lymphatic vessels. Secondary lymphoedema occurs slowly but progressively resulting from injury, infection, and inflammation. Lymphatic vessels of people in developed countries can also be disrupted by surgical procedures like lymphadenectomy or radiotherapy for cancers such as breast cancer and cancer that require lymph node dissection and radiotherapy for melanoma, sarcoma, neck, and gynaecological cancers (Ly et al., 2017; Yuan et al., 2019). Secondary lymphoedema

pathological features include inflammation, oedema, dermal fibrosis, the development of adipose tissue, and immune system dysfunction, which makes patients more susceptible to infections (Zampell et al.,2012; Yaun et al, 2019). Experimental and clinical studies have associated inflammation with the pathophysiology of lymphedema and rats were used in a study that led to a conclusion to say lymphoedema results in a chronic inflammatory reaction (Ly, et al., 2017). In another study, it was found that genetic variations in some sick patients revealed that inflammatory genes were associated with more symptoms of lymphoedema (Ly, et al., 2017). Increased fluid flow serves as an early sign of inflammation that prompts surrounding fibroblasts to initiate rapid matrix repair through autocrine upregulation of transforming growth factor-1 (TGF-1) and differentiation into myofibroblasts. When fluid accumulation in the interstitial space persists, it contributes to an ongoing cycle of inflammation that eventually results in symptoms of lymphoedema (Ly, et al., 2017).

1.10.3 Medicinal plants as alternatives for inflammatory diseases

Since several epidemiological studies have shown a link between the consumption of fruits and vegetables high in polyphenols like flavonoids and the development of chronic diseases like cancer, cardiovascular disease, and inflammation, the search for natural remedies and phytochemicals with anti-inflammatory activity has significantly increased (Gunathilake et al., 2018). Sari and Katrin (2017), reported on the properties of the flavonoids baicalein and apigenin as inhibitors of lipoxygenase *in vitro.* The antioxidant properties of alkaloids, phenolics, and triterpenoids have also been shown to have anti-inflammatory effects by decreasing the production of O_2- and malondialdehyde (MDA), plasma extravasations, and leukocyte cell migration during radical damage. Lastly, they increase the likelihood of superoxide dismutase (SOD) activity in radical scavenging activity (Nardi et al., 2007). Numerous chemicals having antioxidant capabilities that can combat radical species in vivo, including fatty acids, terpenes, phytosterols, esters, and alcohols, have also been discovered to be present in plants (Oso and Karigidi, 2019). Using herbal drugs with less toxicity and adverse effects from the use of allopathic medications have increased the popularity of certain medications, which has contributed significantly to the development of potential

pharmaceuticals. Additionally, more than 1.5 million practitioners now practice traditional medicine, making it more widely used (Ondua et al., 2016).

1.10.4 Non-steroidal anti-inflammatory drugs (NSAIDs)

The most popular medications used to treat inflammatory and pain diseases are non-steroidal anti-inflammatory medicines (NSAIDs). This comprises the medication families that contain ibuprofen, celecoxib, rofecoxib, meloxicam, indomethacin, and diclofenac (Ong et al., 2007; Karim et al., 2019). NSAIDs' pharmacological effect involves blocking the enzyme cyclooxygenase, which prevents prostaglandin formation (Karim et al., 2019). Without altering the activity of the 5-LOX enzyme, the selective inhibitors prevent prostaglandin production (Mogana, et al., 2013). However, it has been observed that these medications have a number of undesirable side effects, including stomach inflammation that causes gastric ulcers (Gunathilake et al., 2018). The detrimental effects on the cardiovascular system are caused by COX-2 enzyme inhibition, while the gastrointestinal side effects of long-term NSAID use are caused by COX-1 inhibition (Sostress et al., 2010; Karim et al., 2019). Long-term NSAID use has been linked to the development of gastric ulcers because these drugs have large levels of LTB4 in their walls, which draw leukocytes to the stomach and may hasten the development of ulcers. Recently, compounds known as "double inhibitors," which inhibit not only COX-1 and COX-2 but also 5-LOX, have been reported. They have antioxidant properties that may be useful for managing the metabolic processes that cause inflammatory conditions and can lessen adverse effects on the stomach and cardiovascular system by balancing the body's arachidonic acid metabolism. (Mogana, et al., 2013).

Celecoxib is the only coxib that is currently approved by the US Food and Drug Administration (FDA) as it has less COX-2 selectivity than other medications in the same class and has some COX-1 inhibitory effects. Rofecoxib and valdecoxib are two selective inhibitors that were taken off the market because they had relatively high specificity for the COX-2 enzyme and subsequent cardiovascular adverse effects (Sun et al., 2007; Karim et al., 2019).

1.11 Problem Statement

Elephantiasis is a disease that affects many countries, including South Africa. According to a report by Dlamini and eNCA (2013), patients have been turned away from several public hospitals in South Africa because there is no therapy available there or because the illness requires a specialist. The patients can, therefore only lie on their beds watching their legs getting bigger and becoming more and more inactive. Cox (2012) reported that people suffering from elephantiasis are usually bedridden and confined to bleak and dingy surroundings in rundown houses, and they are struggling to get treatment. The patients experience not only physical infirmity but also mental, social, and financial losses that exacerbate poverty and shame.

All of this accounts for the need to give the disease special research that will focus on treatment strategies available in South Africa on how to manage the disease and an assessment of reported cases, especially those people that have been affected been severe lymphoedema.

In collaboration with Dr. Dirk le Roux and his team of physiotherapists, the Netcare Foundation has assisted in the treatment of some of the disease-stricken patients at Netcare Sunninghill Hospital. A few of the severely disabled patients have received therapy with the help of money from the Netcare Foundation, but because treatment takes so long, only a select few patients are eligible because of the program's stringent admission requirements. The patients are typically immobile and severely disabled, with little future prospects unless they can receive therapy. (Netcare Limited, 2013).

There is a tremendous need for coordinated and accessible programs that will help to treat elephantiasis in South Africa. This study as it will focus on medicinal plants that have the potential to cure the disease will bring novelty to plant medicine that can be used to alleviate the symptoms associated with the disease. According to field surveys done on the study, people in communities are familiar with the disease and have mentioned that the people who are affected will be having the signs of the sickness for a very long and just face the burden of living with the disease.

Traditional healers and herbalists do not seem to know the exact cause of the signs of elephantiasis, they will tend to associate it with witchcraft as the disease seems strange and incurable. However, they will try to treat the disease with some plants that are known to help with pain and inflammation which can help the affected person to a considerable extent. The most affected persons are most likely around middle-age. The sick persons will be showing symptoms of lymphoedema which may be a swollen limb or both limbs will be swollen.

1.12 Aim and objectives

1.12.1 Aim

This study aims to examine the presence of antibacterial, anthelmintic, anti-inflammatory, antioxidant, and phytochemical analysis in medicinal plants used by traditional healers, herbalists, and indigenous people in South Africa against elephantiasis and its linked illnesses. Plant extracts were subjected to *in-vitro* cytotoxicity testing using cultured mammalian cells. The active plant compounds were finally isolated in the lab using standard procedures and structure elucidation was done through NMR.

1.12.2 Objectives of the study

The following tasks were completed in order to fulfill the project's goals:

- To conduct an ethnobotanical survey on plants used by traditional healers and herbalists in three municipal areas of the Eastern Cape.
- To determine the quantitative and qualitative phytochemical constituents of the collected medicinal plants.
- To screen plant species for antibacterial activity.
- To screen plant species for antifungal activity
- To screen the plant extracts for antimycobacterial activity.
- To screen plants for antioxidant activity.
- To screen plant species for the presence of anti-inflammatory activity.
- To test medicinal plants for cytotoxicity using *in vitro* and possibly *in vivo* experiments.

- To screen plants for anthelmintic activity.
- To perform an activity-guided bioassay to isolate active compounds from plants showing the best activities.

REFERENCES

Adams, Z. (2014). Comparative phytochemical analyses of *Aloe ferox Mill.* found in Eastern and Western Cape Provinces in South Africa, University of Fort Hare, Alice.

Adebayo, S.A., Dzoyem, J.O., Shai, L.J., & Eloff, J.N. (2015). The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern Africa. BMC Complementary and Alternative Medicine, 15, 159.

Akula, U.S., & Odhav, B., (2008). *In vitro* 5-lipoxygenase inhibition of polyphenolic antioxidants from undomesticated plants of South Africa. *Journal of Medicinal Plant Research*, 2(9), 207–12.

Agati, G., Azarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. Plant Science, 196, 67-76.

Al-abd, N. M., Nor, Z. M., Al-adhroey, A. H., Suhaimi, A., & Sivanandam, S. (2013). *Recent Advances on the Use of Biochemical Extracts as Filaricidal Agents*. Evidence-Based Complementary and Alternative Medicine, 1-14.

Aiyegoro, O.A., & Okoh, A.I., (2010). Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine*, *10*, 21.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., & Lightfoot, D. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*, *6*(4), 42.

Amoo, S. O., Ndhlala, A. R., Finnie, J. F., & Staden, J. Van. (2009). Antibacterial, antifungal, and anti-inflammatory properties *of Burchellia bubalina*. *South African Journal of Botany*, *75*(1), 60–63.

Anil, N., &, & Talluri, V. R. (2015). Lymphatic Filariasis : Drug targets and Nematicidal. *Journal of Pharmaceutical Sciences and Research*, *7*(11), 928–933.

Anokwuru, C.P., Esiaba, I., Ajibaye, O., & Adesuyi, A, O., (2011). Polyphenolic content and antioxidant activity *of Hibiscus sabdariffa* calyx. *Research Journal of Medicinal Plants*, 557-566.

Atta-ur-Rahman & Choudhary, M.I., (1995). Diterpenoid and steroidal alkaloids. *Natural Products Reports*, *12*, 361–379.Baharlouei, A., Sharifi-Sirchi, G.R. & Bonjar, G.H.S., (2010). Identification of an antifungal chitinase from a potential biocontrol agent, streptomyces plicatus strain 101, and its new antagonistic spectrum of activity. Philippine *Agricultural Scientist*, *93*, 439–445.

Balick, M.J., & Cox, P.A. (1997). Plants, People, and Culture: The Science of Ethnobotany. Scientific American Library, New York, NY.

Balunas, M. J., & Kinghorn, A. D. (2005). *Drug discovery from medicinal plants*. *78*, 431–441.

Barnard, D. L., Huffman, J. H., Meyerson, L. R. & Sidwell, R.W., (1993). Mode of inhibition of respiratory syncytial virus by a plant flavonoid. *Chemotherapy* 39, 212–217.

Bockarie, M.J., Taylor, M.J., & Gyapong, J.O. (2009). Current practices in the management of lymphatic filariasis. Expert Review Anti-infective Therapy *7*(5),595-605

Boots, A.W., Haenen, G.R. & Bast, A., (2008). Health effects of quercetin: From antioxidant to nutraceutical. *European Journal of Pharmacology, 585*, 325–337.

Borris, R. P., (1996). Natural products research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacology*, *51*, 29–38.

Bouriche, H., Miles, E.A., Selloum, L., Calder, P.C. (2005). Effect of *Cleome arabica* leaf extract, rutin, and quercetin on soybean lipoxygenase activity and generation of inflammatory eicosanoids by human neutrophils. *Prostaglandins, Leukotrienes & Essential Fatty Acids*. 72, 195–201.

Cano *et al*., (2014). The global distribution and transmission limits of lymphatic filariasis: past and present. *Parasites and Vectors*,*7*, 466.

Closa, D., & Folch-puy, E. (2004). Critical Review Oxygen Free Radicals and the Systemic Inflammatory Response. *Life*, 56, 185–191.

Cocks, M., & Dold, A.P. (2006). Cultural significance of biodiversity: the role of medicinal plants in urban African Cultural Practices in the Eastern Cape, South Africa. *Journal of Ethnobiology*, 26, 60–81.

Cowan, M. M. (1999). *Plant Products as Antimicrobial Agents*. *12*(4), 564–582.

Cragg, G.M., Newman, D.J., & Snader, K.M. (1997). Natural products in drug discovery and development. *Journal of Natural Products*, 60, 52–60.

Critchfield, J. W., Butera, S. T. and Folks, T. M., (1996). Inhibition of HIV activation in latently infected cells by flavonoid compounds. *AIDS Research and Human Retroviruses* 12, 39.

Cunningham, A.B., (1993). African medicinal plants: Setting priorities at the interface between conservation and primary healthcare. People and Plants Working Paper 1. Paris. UNESCO.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 564-582.

Daffre, S, Bulet, P., Spisni, A., Ehret-sabatier, L., Rodrigues, E.G. & Travassos, L.R. (2008). *Bioactive natural peptides.* In: Atta-ur-Rahman (Ed.) *Studies in Natural Products Chemistry*, (35), 597- 691.

Dalgleish, A.G., & O'Byrne, K.J. (2002). Chronic immune activation and inflammation in the pathogenesis of AIDS and cancer. *Advances in Cancer Research*. 84, 231-276.

Das, N.G., Goswami, D., Rabha, B., (2007). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *Journal of Vector-Borne Diseases*; *44* (2),145–8.

Dash, M., Kumar, P.J. & Panda, P., (2008). Phytochemical and antimicrobial screening of extracts of *Aquilaria agallocha* Roxb. *African Journal of Biotechnol*ogy, 3531-3534.

Deribe, K., , Meribo, K., Gebre, T., Hail, A., Ali, A., Aseffa, A., & Davey., G. (2012). The burden of neglected tropical diseases in Ethiopia, and opportunities for integrated control and elimination. *Parasites & Vectors*, 5, 240.

Deribe K., Tekola-Ayele F., Davey G. (2016) Podoconiosis: Endemic Non-filarial Elephantiasis. In: Gyapong J., Boatin B. (eds) Neglected Tropical Diseases - Sub-Saharan Africa. Neglected Tropical Diseases. Springer, Cham, pp 231-249.

Dausdardt, R., (1990). The changing geography of traditional medicine: urban herbalism on the Witwatersrand, South Africa. *Geography Journal,* 22, 275–283.

Dixon, R. A., Dey, P. M. &. Lamb, C.J, (1983). Phytoalexins: enzymology and molecular biology. *Advanced Enzymology, 55*, 1–69.

Duthie, S.J, Ma, A., Ross, M.A. & Collins, A.R., (1996). Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res*earch, *56*, 1291–1295.

Eldeen, I.M.S., & Van Staden, J. (2007). Antimycobacterial activity of some trees used in South African traditional medicine. *South African Journal of Botany*, *73*, 248–251.

Eneanya, O.A., Fronterre, C., Anagbogu, I. *et al.* (2019). Mapping the baseline prevalence of lymphatic filariasis across Nigeria. *Parasites Vectors*, 12, 440.

Farnswort, N.R., (1989). Screening plants for new medicines. In *Biodiversity, Part II* Edited by: Wilson EO. National Academy Press, Washington., pp. 83-97.

Fessenden, R. J., Fessenden, J.S., (1982). Organic chemistry, 2nd ed. Willard Grant Press, Boston, Mass

Freiburghaus, F., Kaminsky, R, Nkunya, M. H. H., & Brun. R., (1996). Evaluation of African medicinal plants for their *in vitro* trypanocidal activity. *Journal of Ethnopharmacology*, *55*,1–11.

Fujimoto, K., Kawasali, Y., Morimoto, K., Kikuchi, I., & Kawana, S. (2014). Treatment for crusted scabies: limitations and side effects of treatment and ivermectin. *Journal of Nippon Medical School*, *81*(3).

Grisham, M.B. (1992). Reactive oxygen metabolism. In: Grisham MB, ed. *Reactive metabolites of oxygen and nitrogen in biology and medicine.* Austin: RG Landers Company, 39.

Gupta, M., Mazumder, U.K., Gomathi, P., & Thamil, S.V., (2006). Anti-inflammatory evaluation of leaves of *Plumeria acuminata. BMC Complementary Alternative medicine*, 6, 36.

Gyapong, J.O., Kumaraswami, V., Biswas, G., & Ottesen, E.A., (2005). Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. *Expert in Pharmacotherapy*; *6* (2), 179–200.

Halliwell, B. &, Gutteridge, J., (1999). Free radicals in biology and medicine. 3rd ed. Oxford: Oxford University Press; p. 23-27.

Haralampidis, K., Trojanowska, M., Osbourn, A., (2002). Biosynthesis of triterpenoid saponins in plants. *Advances in Biochemical Engineering/ Biotechnology, 75*, 31-49.

Haslam, E., (1996). Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *Journal of Natural Products*, *59*, 205–215.

Hermsmeier, D., Schittko, U. & Baldwin, I.T., (2001). Molecular interactions between the specialist herbivore Manduca sexta (Lepidoptera, Sphingidae) and its natural host Nicotiana attenuata. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. *Plant Physiology*, *125*, 683–700.

Hertig, M., & Wolbach, S.B. (1924). Studies on Rickettsia-like micro-organisms in insects. *The Journal of Medical Research*, 44(3), 329-374.

Hoerauf, A. (2008). Filariasis : new drugs and new opportunities for lymphatic filariasis and onchocerciasis. *Current Opinion in Infectious Diseases*, *21*, 673–681.

Irshad, M., & Chaudhuri, P.S., (2002). Oxidant - antioxidant system: role and significance in the human body*. Indian Journal of Experimental Biology*, *40*, 1233- 1239.

Jakubowski, W., & Bartosz, G., (1997). Estimation of oxidative stress in *saccharomyces cerevisae* with fluorescent probes. *International Journal of Biochemistry Cell Biol 29*, 1297–1301.

Jones, G. A., McAllister, T. A., Muir, A. D & Cheng, K.J., (1994). Effects of sainfoin (*Onobrychis viciifolia* scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Applied Environmental Microbiology*, *60*, 1374–1378.

Karim, N., Khan, I., Khan, W., I Khan, I., Khan., A., & Halim, S.A., Khan, H., Hussain, J., & Al-Harrasi, A. (2019). Anti-nociceptive and anti-inflammatory activities of asparacosin A involve selective cyclooxygenase 2 and inflammatory cytokines inhibition : An *in-vitro*, *in-vivo*, and in-silico approach. *Frontiers in Immunology*, *10*, 1–11.

Karimi, E., Jaafar, H. Z. E., & Ahmad, S. (2011). *Phytochemical Analysis and Antimicrobial Activities of Methanolic Extracts of Leaf, Stem, and Root from Different Varieties of Labisa pumila Benth*. 4438–4450.

Kaul, T. N., Middletown, E., Jr., & Ogra, P.L., (1985). Antiviral effect of flavonoids on human viruses. *Journal of Medical Virology*, *15*,71–79.

Kennedy, D. O., & Wightman, E. L. (2011). *Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain Function 1*. 32–50.

Klebanoff, S. (1992). Phagocytic cells: Products of oxygen metabolism. In: Gallen JI, Goldstein IM, Synderman R, eds. *Inflammation-basic principles and clinical correlates. New* York: Raven Press, 541-588.

Komoreng, L., Thekisoe, O., Lehasa, S., Tiwani, T., Mzizi, N., Mokoena, N., Khambule,

N., Ndebele, S., & Mdletshe, N. (2017). *South African Journal of Botany An ethnobotanical survey of traditional medicinal plants used against lymphatic filariasis in South Africa*. *111*, 12–16.

Krishnamoorthy, S., & Honn, K.V. (2006). Inflammation and disease progression. *Cancer Metastasis Review*, 25, 481–491.

Kumari, M., & Jain, S. (2012). *Tannin: An Antinutrient with Positive Effect to Manage Diabetes Tannins.* Research Journal of Recent Sciences, *1*(12), 1-18.

Lasztity, R., Hidvegi, M., & Bata, A., (1998). Saponins in food. *Food Review International*, *14*, 371-390.

Lu, J.M., Yao, Q.Z., & Chen, C.Y., (2009). Ginseng compounds: an update on their molecular mechanisms and medical applications. *Current Vascular Pharmacology*, *7*, 293–302.

Lupenza, E., Gasarasi, D.B. & Minzi, O.M. (2021). Lymphatic filariasis, infection status in *Culex quinquefasciatus* and *Anopheles* species after six rounds of mass drug administration in Masasi District, Tanzania. *Infectious Diseases of Poverty* 10, 20.

Ly, C., L., Kataru, R ., P., &, & Mehrara, B. J. (2017). Inflammatory Manifestations of Lymphedema. *International Journal of Molecular Sciences*, *18*(171), 1–13.

Mahato, S.B., & Sen, S., (1997) Advances in triterpenoid research, 1990-1994. *Phytochemistry*, 44, 1185-1236.

Manach, C., Scalbert, A., Morand, C., Remesy, C. & Jimenez, L., (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical. Nutrition*, *9*, 727-747.

Mander, M., (1998). Marketing of indigenous medicinal plants in South Africa. In: A Case Study in Kwa-Zulu Natal. Food and Agriculture Organisation of the United Nations, Rome.

Masika, P.J. & Afolayan A.J. (2002). Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. *Journal of Ethnopharmacology, 83*, 129–134.

Mbaebie, B.O., Edeoga, H.O., & Afolayan, A.J., (2012). Phytochemical analysis and antioxidant activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine, 2*(2), 118- 124.

Meenakshi, S., Umayaparvathi, S., Arumugam, M. & Balasubramanian, T. (2011). In vitro antioxidant properties of FTIR analysis of two seaweeds of Gulf of Mannar. *Asian Pacific Journal of Tropical Biomedicine*,*1*, 66-70.

Merry, P, Winyard, P.G., Morris, C.J., & Blake, D. R. (1989). Oxygen-free radicals, inflammation, and synovitis : the current status. *Annals of the Rheumatic Diseases*, *48*(3), 864–870.

Misra, N., Sharma, M., Raj, K., & Bhattacharya, S.M., (2007). Chemical constituents and antifilarial activity of *Lantana camara* against human lymphatic filariid *Brugia malayi* and rodent filariid *Acanthocheilonema viteae* maintained in rodent hosts. *Parasitology Research*; *100* (3),439–48.

Mogana, R., Teng-Jin, K., & Wiart, C. (2013). The Medicinal Timber *Canarium patentinervium* Miq. (Burseraceae Kunth .) Is an Anti-Inflammatory Bioresource of Dual Inhibitors of Cyclooxygenase (COX) and 5-Lipoxygenase (5-LOX). *ISRN Biotechnology*, 1–8.

Muchuweti, C.H., Chidewe, C., Ndhlala., A.R., & Benhura, M.A.N.., (2007*). American Journal of Food Technology*, *2*(5), 414-420.

Nakahara, K., Kawabata,S., Ono,H., Ogura,K., Tanaka, T., Toshima, T., & Hamada, S., (1993). Inhibitory effect of oolong tea polyphenols on glucosyltransferases of mutans streptococci. *Applied Environmental Microbiology*, *59*,968– 973.

Nardi, G. M., Junior, J. M. S., Monache, F. D., Pizzolatti, M. G., Ckless, K., & Ribeiro-do-valle, R. M. (2007). Antioxidant and anti-inflammatory effects of products from *Croton celtidifolius* on carrageenan-induced pleurisy in rats. *Phytomedicine*, *14*, 115–122.

Negi, P.S., Chauhan, A.S, Sadia, G.A., Rohinishree, Y.S. & Ramteke, R.S., (2005). Antioxidant and antibacterial activities of various seabuckthorn (*hippophae rhamnoides* l.) seed extracts. *Food Chemistry*., *92*, 119–124.

Ndjonka, D., Rapado, L. N., & Silber, A.M., & Liebau, E., & Wrenger, C. (2013). Natural Products as a Source for Treating Neglected Parasitic Diseases. *International Journal of Molecular Sciences*, 14, 3395–3439.

Nkomo, M., Nkeh-chungag, B. N., Kambizi, L., Jamot, E., & Iputo, J. E. (2010). *Antinociceptive and anti-inflammatory properties of Gunnera perpensa (Gunneraceae)*. *4*(5), 263–269.

Olaokun, O.O., Mkolo, N.M., , Mogale, M.A., & King, P.H. (2017). Phytochemical screening, antioxidant, anti-inflammatory, and glucose utilization activities of

three South African plants used traditionally to treat diseases. Biology and Medicine, 9(5), 1-7.

Oleszek W, Marston A (2000). Saponins in food and medicinal plants. Kluwer academic publishers. New York, pp 1-95.

Omulokoli, E., Khan, B., & Chhabra, S.C., (1997). Antiplasmodial activity of four Kenyan medicinal plants. *Journal of Ethnopharmacology*, 56, 133–137.

Ondua, M., Adebayo, S A, Shai, L. J., & Lebelo, S. L. (2016). The Anti-Inflammatory and Anti-Nociceptive Activities of Some Medicinal Plant Species Used to Treat Inflammatory Pain Conditions in Southern Africa. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(10), 1571–1575.

Ong, C.K., Lirk, P., & Tan, C.H., & Seymour, R.A. (2007). An evidence-based update on nonsteroidal anti-inflammatory drugs. *Clinical & Medicinal Research*, 5, 19–34.

Oso, B.J., Karigidi, K.O. (2019). Inhibitory action of dried leaf of *Cassia alata* (Linn.) Roxb against lipoxygenase activity and nitric oxide generation. *Scientia Agropec*. 10 (2), 185– 190.

Ottesen, E. A., & Campbell, W. C. (1994). Review Ivermectin in human medicine. *Journal of Antimicrobial Chemotherapy*, 34, 195–203.

Ottesen, E.A., Duke, B.O.L., Daram, M., & Behbehani, K., (1997). Strategies and tools for the control/elimination of lymphatic filariasis. *Bulletin of the World Health Organisation*, 75, 491–503.

Philander, L. A. (2011). An Ethnobotany of Western Cape Rasta bush medicine. *Journal of Ethnopharmacology*, *138*(2), 578–594.

Pietro, N., Jan, N., Simon,C., Muylowa,G. K, Davey, G. (2010), Podoconiosis – non-filarial geochemical elephantiasis – a neglected tropical disease? *Journal of the German Society of Dermatology*, *8*(7), 1-7.

Porter L.J., (1986). The Flavonoids. In: Harborne JB, editor. Advances in Research since 1986. London: *Chapman & Hall*, 23–55.

Pengsuparp, T., L. Cai, H. Constant, H. H. Fong, L. Z. Lin, A. D. Kinghorn, J. M. Pezzuto, G. A. Cordell, K. Ingolfsdottir, & Wagne, H., (1995). Mechanistic evaluation of new plant-derived compounds that inhibit HIV-1 reverse transcriptase. *Journal of Natural Products*, *58*, 1024–1031.

Philipson, J.D., & O'Neil, M.J. (1986). Novel antimalarial drugs from plants? *Parasitology Today*, *2*(12), 355-359.

Programme to eliminate lymphatic filariasis, Progress Report 2000–2009 and Strategic Plan 2010–2020; WHO/HTM/NTD/PCT/2010.6

Ramchoun, M., Harnafi, H., Alem, C., Benlys, M., Elrhaffari, L. & Amrani, S., (2009). Study on antioxidant and hypolipidemic effects of polyphenol rich extract from *Thymus vulgaris* and *Lavendula multifida*. *Pharmacognosy Research, 1,106–112.*

Rattan, R.S., (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. *Journal of Crop Protection*, *29*, 913–20.

Samuelsson, G., (2004). Drugs of Natural Origin: a Textbook of Pharmacognosy, 5th Swedish Pharmaceutical Press, Stockholm.

Sari, A.C., Elya, B, Katrin. (2017). Antioxidant activity and lipoxygenase enzyme inhibition assay with total flavonoid assay of *Garcinia porrecta* Laness. Stem Bark extracts. *Pharmacognosy Journal*, 9(2), 257-266.

Shai, L.J., McGaw, L.J., Aderogba, M.A., Mdee, L.K., Eloff, J.N., (2008). Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm. f) C.A. Sm. leaves. *Journal of Ethnopharmacololgy, 119, 238–244*.

Sharma, S., (1900). Vector-borne diseases. *Progress in Drug Research*, *35*, 365.

Simonsen, P.E. (2009) Filariases. In: Cook GC, Zumla AI (eds) Manson's tropical diseases, 22nd edn. Saunders Elsevier, London, 1477– 1513.

Simonsen P.E., & Mwakitalu M.E., (2013). Urban lymphatic filariasis. *Parasitology Research Journal*, *112*, 35–44

Sashidhara, K.V., Singh, S.P., Misra. S., Gupta, J., & Bhattacharya, S.M., (2012). Galactolipids from *Bauhinia racemosa* as a new class of antifilarial agents against human lymphatic filarial parasite*, Brugia malayi*. *European Journal of Medical Chemistry*, *50*, 230–5.

Sarker, S.D., Latif, Z., & Gray, A.I. (2006). Natural Products Isolation. Humana Press Inc, New Jersey.

Scalbert, A., (1991.) Antimicrobial properties of tannins. *Phytochemistry*, *30*, 3875–3883.

Scalbert, A., & Williamson, G., (2000). Dietary intake and bioavailability of polyphenols. *Journal of. Nutrition, 130*, 2073-2085.

Schultz, J. C., (1988). Tannin-insect interactions, In R. W. Hemingway and J. J. Karchesy (ed.), Chemistry and significance of condensed tannins. *Plenum Press*, p 553, New York.

Schultes, R. E. 1978. The kingdom of plants, p. 208. *In* W. A. R. Thomson (ed.), Medicines from the Earth. McGraw-Hill Book Co., New York, N.Y.

Sen, S., Chakaraborty, R., Sridhar, C., Reddy, Y. & Biplab, D. (2010). Free radicals, antioxidants, diseases, and phytomedicines: current status and future prospect. *International Journal of Pharmaceutical Sciences Review and Research*, *3*(1), 91- 100.

Senguttuvan,J., Paulsamy,S., & Karthika, S., (2014*). Asian Pacific Journal of Tropical Biomedicine*, *4*(1), 359-367.

Serafini, M., Ghiselli, A., & Ferro-Luzzi, A., (1994). Red wine, tea, and anti-oxidants. *Lancet*, 344, 626.

Shai, L. J., Mcgaw, L. J., Masoko, P., & Eloff, J. N. (2008). *Antifungal and antibacterial activity of seven traditionally used South African plant species active against Candida albicans*. *74*, 677–684.

Singh, P.K.., Ajay, A., Kushwaha, S, Tripathi, R.P., & Misra-Bhattacharya, S. (2010). Towards novel antifilarial drugs: challenges and recent developments. *Future Medical Chemistry*, *2*(2), 251–283.

Sironi, M., Bandi, C., Sacchi, L., Di Sacco, B, Damiani, G., & Genchi, C. (1995). Molecular evidence for a close relative of the arthropod endosymbiont Wolbachia in a filarial worm*. Molecular and Biochemial Parasitology*, 74(2), 223-237.

Slatko, B.E., Luck, A.N., Dobson, S.L., & Foster. J.M., (2014). Wolbachia endosymbionts and human disease control. *Molecular and Biochemical Parasitology*, *195*, 88–95.

Sostres, C., Gargallo, C.J., Arroyo, M.T., Lanas, A. (2010). Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best Practice & Research Clinical Gastroenterology*, 24, 121–132.

Sparg, S., Light, M., van Staden, J., (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology, 94*, 219-243.

Srivastava, S.K., Chauhan, P.M.S, Bhaduri, A.P., Fatima, N., & Chatterjee, R.K., (2000). Quinolones: novel probes in antifilarial chemotherapy. *Journal of Medical Chemistry, 43, 2275–9.*

Stern, J. L., Hagerman, A. E., Steinberg, P. D & Mason, P. K., (1996). Phlorotannin-protein interactions. *Journal of Chemical Ecology*, *22*, 887–1899.

Stolk, W.A., de Vlas, S.J., & Habbema, J.D.F. (2006) Advances and challenges in predicting the impact of lymphatic filariasis elimination programmes by mathematical modelling. *Filaria Journal,* 5 5,1-10.

Suffredini, I.B., Sader, H.S., Gonçalves, A.G., Reis, A. O. Gales, A.C., Varella, A.D. &Younes, R.N., (2004). Screening of antibacterial extracts from plants native to the Brazilian Amazon rainforest and Atlantic Forest. *Brazilian Journal of Medical Biology. Research*, *37*, 379–384.

Sun, S.X., Lee, K.Y., Bertram, C.T., & Goldstein, J.L. (2007). Withdrawal of COX-2 selective inhibitors rofecoxib and valdecoxib: impact on NSAID and gastroprotective drug prescribing and utilization. *Current Medical Research & Opinion*, 23, 859–66.

Surveswaran, S., Cai, Y., Corke, H &Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chemistry, 102(3), 938-953.

Reymond, P., Weber, H., Damond, M. & Farmer, E.E., (2000). Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. *Plant Cell*, *12*, 707–19.

Takaidza, S., Mtunzi, F., & Pillay, M. (2018). Analysis of the phytochemical contents and antioxidant activities of crude extracts from *Tulbaghia* species. *Journal of Traditional Chinese Medicine*, *38*(2), 272–279.

Taylor, J. L. S., Elgorashi, E. E., Maes, A., Gorp, U. Van, Kimpe, N. De, Staden, J. Van, & Verschaeve, L. (2003). *Investigating the Safety of Plants Used in South African Traditional Medicine: Testing for Genotoxicity in the Micronucleus and Alkaline Comet Assays*. *154*(6), 144–154.

Toda, M., Okubo, S., Ohnishi, R., & Shimamura., T., (1989). Antibacterial and bactericidal activities of Japanese green tea. Japanese *Journal of Bacteriology, 45*, 561– 566.

Tshikalange, T.E., Mamba, P. & Adebayo, S. A., (2016). Antimicrobial, antioxidant, and cytotoxicity studies of medicinal plants used in the treatment of sexually

transmitted diseases. *International Journal of Pharmacognosy and Phytochemical Research*; *8*(11),1891-1895.

Tsuchiya, H., M., Sato, T., Miyazaki, S., Fujiwara, S., Tanigaki, M., Ohyama, T., & Tanaka M., (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology, 50*, 27–34.

Twumasi, E. B., Akazue, P. I., , Kwaku, K., Gwira, T, M., Keiser, J., Cho-ngwa, F., ,Flint, A., Anibea, B., Bonsu, E. Y., Amewu, R. K., Eva, Linda, A., Appiah-, R., Opong, R., & Osei-Safo, D. (2020). Antischistosomal, antionchocercal and antitrypanosomal potentials of some Ghanaian traditional medicines and their constituents. *PLOS Neglected Tropical Diseases*, 1–21.

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. & Mazur, M., (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions Journal*, *160*, 1–40.

Vasu, K., Goud, J.V., Suryam, A., Singara & Chary, M.A. 2009. Biomolecular and phytochemical analyses of three aquatic angiosperms. *African Journal of Microbiology Research*, *3*(8), 418-421.

Van Staden, V., Erasmus, B.F.N., Roux, J., Wingfield, M.J., & van Jaarsveld, A.S., (2004). Modelling the spatial distribution of two important South African plantation forestry pathogens. *Forest Ecology and Management, 187, 61–73.*

Van Vuuren, S.F., (2008). Antimicrobial activity of South African medicinal plants. *Journal of Ethnopharmacology*, *119* (3), 462–472.

Van Wyk, B.E., Van Oudtshoorn, B., & Gericke, N., (1997). Medicinal plants of South Africa. *Briza Publications*, Pretoria, pp 288.

van Wyk, B.E., (2008). A review of Khoi-San and Cape Dutch medical ethnobotany. *Journal of Ethnopharmacology,119*, 331–341.

Wadood, A., Ghufran, M., Jamal, S. B., Naeem, M., Khan, A., & Ghaffar, R. (2013). *Biochemistry & Analytical Biochemistry Phytochemical Analysis of Medicinal Plants Occurring in Local Area of 2*(4), 2–5.

Wink, M. (2020). Potential of DNA intercalating alkaloids and other plant secondary metabolites against SARS-CoV-2 causing covid-19. *Diversity*, *12*(175), 3-10.

Wulandari, L., Retnaningtyas, Y., & Lukman, H. (2016). *Analysis of Flavonoid in Medicinal Plant Extract Using Infrared Spectroscopy and Chemometrics*.

WHO. (1992). Expert Committee on Filariais & World Health Organisation. Lymphatic

filariasis: the disease and its control, fifth report of the WHO Expert Committee on Filarisis, Geneva.

WHO. (2005). Report of the Intercountry meeting of lymphatic filariasis programme managers, South-East Asia Region, New Delhi.

WHO. (2010). Lymphatic filariasis. Progress report 2000–2009 and strategic plan 2010–2020. World Health Organization, Geneva. Managing morbidity and preventing disability in the Global Programme to Eliminate Lymphatic Filariasis, WHO position statement document. WHO/HTM/NTD/PCT/2011.

WHO. (2013a). Global programme to eliminate lymphatic filariasis: progress report.

WHO. (2013b). Morbidity management and disability prevention in lymphatic filariasis.

WHO. (2021). Available online: https://www.who.int Lymphatic filariasis. Last accessed July 2021.

Wu, Y.Y., Li. W., Xu, Y., Jin, E.H., & Tu, Y.Y., (2011). Evaluation of the antioxidant effects of four main thaeflavin derivative through chemiluminescence and DNA damage analyses. *Journal of Zhejiang University Science B*, *12*(9), 744-751.

Wadood, A., Ghufran, M., Jamal, S.B., Naeem, M., Khan, A., Ghaffar, R., (2013). Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. *Biochemistry and analytical Biochemistry*, 2, 4.

Watanbe, H., Miyaji, C., Makino, M., & Abo. T., (1996). Therapeutic effects of glycyrrhizin in mice infected with LP-BM5 murine retrovirus and mechanisms involved in the prevention of disease progression. *Biotherapy*, 9, 209– 220.

Ya, C., Gaffney, S. H Lilley, T. H. & Haslam. E., (1988). Carbohydrate polyphenol complexation, p. 553. *In* R. W. Hemingway and J. J. Karchesy (ed.), Chemistry and significance of condensed tannins. *Plenum Press*, New York, N.Y.

Yamagishi, S., & Matsui, T. (2011). Nitric oxide, a Janus-faced therapeutic target for diabetic microangiopathy - friend or foe? *Pharmacological Research*, *64*,187-194.

Yuan, Y., Arcucci, V., Levy, S. M., &, & Achen, M. G. (2019). Modulation of Immunity by Lymphatic Dysfunction in Lymphedema. *Frontiers in Immunology*, 10, 1–6.

Yun, T., (2001). Brief introduction of Panax ginseng CA Meyer. *Journal of Korean Medical Science*, *16*, 3–5.

Zenk, M.H., & Juenger, M., (2007). Evolution and current status of the phytochemistry of nitrogenous compounds. *Phytochemistry*, *68*, 2757–72.

https://rarediseases.org/rare-diseases/elephantiasis/

Zampell, J.C., Aschen, S., Weitman, E.S., Yan, A., Elhadad, S., De Brot, M., et al. (2012). Regulation of adipogenesis by lymphatic fluid stasis: part I. Adipogenesis, fibrosis, and inflammation. *Plastic and Reconstructive Surgery,* 129, 825–34.

Zulak, K., Liscombe, D., Ashihara, H., Facchini, P., (2006). Alkaloids. Plant secondary metabolism in diet and human health*. Oxford*: *Blackwell Publishing*, p 102–36.

CHAPTER 2

AN ETHNOBOTANICAL SURVEY OF TRADITIONAL MEDICINAL PLANTS USED AGAINST ELEPHANTIASIS IN THE OR TAMBO DISTRICT, EASTERN CAPE PROVINCE, SOUTH AFRICA.

This chapter was published in Pharmacognosy Magazine.

ISSN : 0973-1296

Volume 17 | Issue 76 | October-December 2021

Pharmacognosy Magazine

Publication of Pharmacognosy Network Worldwide

www.phcog.com

Impact Factor® as reported in the 2020 Journal Citation Reports® (Clarivate Analytics, 2021): 1.085

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ORIGINAL ARTICLE

An Ethnobotanical Survey of Traditional Medicinal Plants used against Elephantiasis in the or Tambo District, Eastern Cape, South Africa

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Submitted: 28-Sep-2020 Revised: 24-Feb-2021 Accepted: 22-Apr-2021 Published: 24-Jan-2022

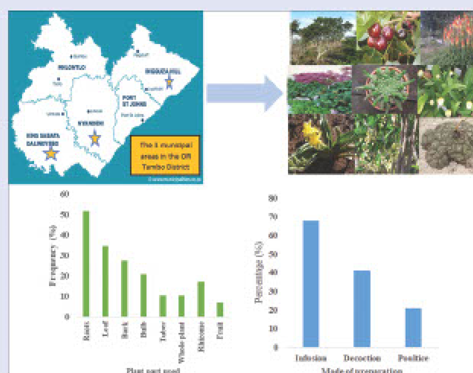
ABSTRACT

Introduction: Elephantiasis is a medical condition associated with skin thickening and excessive swelling of the lower limbs. It is caused by parasitic nematodes which are filarial in nature; hence, it is also known as lymphatic filariasis. The condition is classified into filarial and nonfilarial elephantiasis. Filarial elephantiasis is caused by infection with nematode worms which are transmitted by several genera of mosquitoes. Nonfilarial elephantiasis is a result of cases such as tuberculosis, sexually transmitted infections, leprosy, and repeated streptococcal infections, leading to elephantiasis. South African population is at risk of the disease and treating it is still a problem. This study documents medicinal plants that are used for the treatment of elephantiasis and related infections in the OR Tambo District municipality, Eastern Cape Province, South Africa. **Materials and Methods:** An ethno botanical survey of medicinal plants used against elephantiasis in King Sabata Dalindyebo, Ingquza-Hill and Nyandeni municipal areas in the OR Tambo District of the Eastern Cape Province was conducted using structured questionnaires. The information was gathered from 30 traditional healers, 4 herbalists, and 3 young people with indigenous knowledge. **Results and Discussion:** A total of 29 plant species belonging to 23 families that are used to treat elephantiasis were recorded. *Convolvulaceae (Ipomoea oblongata), Dioscoreaceae (Dioscorea sylvatica), Gunneraceae (Gunnera perpensa), Hypoxidaceae (Hypoxis hemerocallidea),* and *Ranunculaceae (Clematis brachiata)* were the most frequently mentioned in all three municipal areas. The *H. hemerocallidea* had the highest use-value (0.31), followed by *Elephantorrhiza elephantina* (0.27) and *G. perpensa* (0.24). Bark, root, rhizome and leaf decoction and infusion, as well as cooked bark or leaves are commonly used to treat elephantiasis. Some plant parts are ground into powder, mixed with water and applied on affected skin as poultice.

Key words: Elephantiasis, ethnobotany, lymphatic filariasis, medicinal plants, traditional healers

SUMMARY

- The study of medicinal plants used in the treatment and management of elephantiasis in South Africa has not been explored extensively. Research is currently ongoing where the documented plants are further scientifically tested to validate the claims that are made by the traditional healers and herbalists.



Abbreviations used: DEC: Diethylcarbamazine; HIV/AIDS: Human Immunodeficiency virus/Acquired immunodeficiency syndrome; UV: Use-value; SANBI: South African National Biodiversity Institute; WHO: World Health Organization.

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DOI: 10.4103/pm.pm_434_20

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INTRODUCTION

Ethnobotany is the study of relations between people and plants. It looks at how people of a particular culture and region make use of indigenous plants. Based on long-term observations, different cultures have developed their own ethnobotanical methods by making the use of their indigenous plants.[1] Worldwide, medicinal plants have been a resource for healing for thousands of years. Numerous ethnobotanical survey studies documenting different plant species and preserving the indigenous knowledge of various communities worldwide have been undertaken.[2-9] These ethnobotanical studies serve as possible leads for the discovery of effective new drugs that may be used to manage and treat illnesses that pose a serious threat to both human and animal health. Elephantiasis is one such illness.

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Cite this article as: Adams Z, Thekisoe O, Buwa-komoreng L. An ethnobotanical survey of traditional medicinal plants used against elephantiasis in the or tambo district, Eastern Cape, South Africa. Phcog Mag 2021;17:915-22.

Elephantiasis, otherwise medically known as lymphatic filariasis (LF), is a condition that is associated with excessive swelling due to lymph accumulation (lymphedema), leading to disfigurement of the affected body parts which are commonly the lower limbs, arms, breasts, and scrotum in males.[10] The condition is classified into filarial and nonfilarial elephantiasis. Filarial elephantiasis is caused by infection with nematode worms *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*, which are transmitted by several genera of mosquitoes.[11] These worms occupy the lymphatic system, including the lymph nodes and in chronic cases, they can lead to the disease elephantiasis.[12] In most areas, cases such as sexually transmitted diseases (e.g., lymphogranuloma venereum), tuberculosis, an infectious disease called leishmaniasis, repeated streptococcal infections, leprosy, and environmental factors lead to elephantiasis.[13-15]

An estimated 120 million people in 81 endemic countries are affected by the infection with approximately 40 million people having disfiguring symptoms.[11,16] Of the 40 million people, 15 million are suffering from lymphedema and 25 million showing urogenital swelling, particularly scrotal hydrocele.[16] About 30% of people at risk reside in the African region, while 65% in South-East Asia Region and the remainder in other parts of the world.[16] In the African region, elephantiasis is endemic in 39 countries with the population at risk estimated at over 400 million.[16] According to local newspapers, South Africa is also burdened by elephantiasis; however, data on its morbidity are lacking and it has been reported that about 31% of the South African population is at risk of infection.[17-19]

The Global Program to Eliminate LF was launched in the year 2000 with the aim of interrupting the transmission of parasite and reducing morbidity.[16] This could be achieved by taking annual doses of diethylcarbamazine (DEC) or ivermectin together with albendazole. These drugs are effective in reducing microfilariae counts but not effective in killing adult worms.[20,21] As a result, they provide only partial benefit to infected patients and very often are associated with adverse reactions. For example, DEC has been reported to cause side effects such as fever, gastrointestinal disturbance, headache, malaise, and skin rash that reduce patient's compliance.[22,23] According to a study that has been done, antifilarial agents should be free from toxicity, with high cure even with a low dose of the drug, effective against worms, and cost-effective. Unfortunately, the available synthetic drugs do not meet these requirements.[24]

Medicinal plant extracts may be alternative sources of antifilarial agents as they are rich in bioactive compounds. Many researchers have reported on the effectiveness of plant extracts to kill larvae.[24-27] In South Africa, herbal medicine is widely used for the treatment of various diseases and some traditional healers and herbalists have acknowledged the use of medicinal plants to treat elephantiasis and its related symptoms. Komoreng *et al.* reported on a number of medicinal plants that are used by herbalists and traditional healers from certain parts of South Africa to treat elephantiasis.[28] Plant species within the same families may be used in neighboring regions, in different geographical areas, or in other countries to treat various conditions. A study undertaken by Mbuni *et al.* on traditional uses of Kenyan medicinal plants reported on remarkable differences in used plant parts, mode of preparation and the use that has been documented in other regions.[29] It is thus possible to see some of the South African flora in the neighboring countries. Some of those plant species include *Dicoma anomala* Sond., *Gunnera perpensa* L., *Eucomis comosa* (Houtt.) Wehrh., *Euphorbia* sp., *Hypoxis hemerocallidea* Fisch., C.A.Mey. and Avé-Lall., *Elephantorrhiza elephantina* (Burch.) Skeels, *Dioscorea sylvatica* Eckl. and *Pentanisia prunelloides* (Klotzsch) Walp.[6,30-34]

This study reports on the use of medicinal plants in South Africa, particularly in the OR Tambo District municipality in the Eastern Cape Province, to treat and manage elephantiasis.

MATERIALS AND METHODS

Study area

The study was conducted in the Ingquza Hill, Nyandeni and King Sabata Dalindyebo Municipal areas within the OR Tambo District of the Eastern Cape Province, South Africa [Figure 1]. The Eastern Cape is the second biggest Province, covering about 13.8% of the country's total area.[35] It has the second biodiversity hotspot, known as Maputaland-Pondoland Albany, which is dominated by closed shrublands, low forests with evergreens, succulent trees, vines, and shrubs.[36] The climatic conditions are characterized by cold frosty winters and hot summer days.

OR Tambo district municipality is one of the 7 districts of the Eastern Cape province of South Africa. The main town of the area is OR Tambo is Mthatha (formerly known as Umtata). According to the 2011 Census of SA, about 94% of its 1,364,943 people speak isiXhosa.[37] It is within the Wild Coast Region. It is bordered by Alfred Nzo District Municipality to the north, the Joe Gqabi District to the Northwest, the Chris Hani District to the west and the Amathole District to the Southwest.[38] The district is formed by five municipalities, namely King Sabata Dalindyebo, Nyandeni, Mhlontlo, Port St Johns, and Ingquza Hill and covers approximately 80% of what used to be marginalized homeland in the Transkei.[38] The Nyandeni Municipality, which falls within coordinates 31.5284° S and 29.0111° E, is bordered by a 20 km stretch of the coastal belt and falls within the Grassland and Savanna biomes. The communities fall under the Sub-Escarpment Grassland and grassland biome is the second largest in the country.[39] The Ingquza Hill municipality falls within 31.2632° S and 29.6963° E. It accounts for almost a quarter of this district's geographical area. The municipality is located to the north west of the OR Tambo District and was established through the amalgamation of the former Lusikisiki and Flagstaff Transitional Local Councils and the surrounding rural areas. The Municipality has high levels of poverty and underdevelopment.[40] The King Sabata Dalindyebo municipality, which falls between 3.7074° S and 28.5798° E, is the largest of the three municipalities and accounts for a quarter of its total geographical area. The majority population of the municipality resides in the rural areas, where they still practice indigenous cultural lifestyles.[38]

Figure 1: Map of OR Tambo District Municipality showing the study areas (Source: Municipalities.co.za)

Data collection

Ethnobotanical surveys were conducted through semi-structured questionnaires and informal conversations with traditional healers, herbalists, and inhabitants who use medicinal plants. The interviews were conducted in isiXhosa which is the local language in all three municipalities. The recorded information included the age and gender of the informants, common names of the plants they use to treat elephantiasis and related ailments, their local uses, parts of the plants used, the mode of preparation, and forms of administration of the herbal remedies.

Plants were collected with the assistance of traditional healers and herbalists and they were identified by their common names and scientific identification was done with the help of Mrs Matitibala who has a book with both common and scientific names. Plants were later validated at the Herbarium of the University of the Free State (QwaQwa Campus) and voucher specimens were prepared and deposited at the herbarium. The data were tabulated to include the botanical name, family, local name, parts used, preparation, and application [Table 1].

Data analysis

Use-value (UV) was used to analyze the data obtained from the questionnaires. The UV is a quantitative index that shows the virtual importance of species known locally.[41] It was calculated as follows:

$UV = \Sigma U / n$

Where UV = UV of a species; U = number of citations per species; n = number of informants.

Intellectual property agreement/ethical approval

The aim and the purpose of the interviews were clarified to each respondent and their consent to record the shared information was obtained. Furthermore, it was verbally agreed that this research shall not be used for commercial purposes, but shall serve as enlightenment to the community about traditional medicinal plants used in the treatment and management of elephantiasis and related ailments in the Eastern Cape Province. This study shall also serve as a means of documenting and preserving South African indigenous knowledge for future use. Ethical clearance for the study was granted by the University of the Free State's Ethics Committee.

RESULTS AND DISCUSSION

The results of the ethnobotanical survey with detailed information are presented in Table 1. A total of 37 informants, comprising 30 traditional healers, 4 herbalists and 3 young people were interviewed. Twenty-six (70%) of the informants were females and eleven (30%) were males with ages ranging between 18 and 75 years. All the informants use medicinal plants to treat and manage elephantiasis, which they commonly refer to as *Umeqo* or *Unyawo lwendlovu*.

In this study, 29 medicinal plants belonging to 25 families of Angiosperms were reported to be used in the treatment and management of elephantiasis and the common symptoms in the 3 municipal areas. The following families were reported as the most commonly used: *Apocynaceae*, *Dioscoreaceae*, *Euphorbiaceae*, *Fabaceae*, *Rubiaceae*, and *Solanaceae* with 2 species each. In another study, Hyacinthaceae family was reported to be the most prominent in the Eastern Cape Province, in Intsika Yethu and Raymond Mhlaba (formerly known as Nkonkobe municipality) municipal areas, with 4 species, followed by *Solanaceae* with 3 species and *Euphorbiaceae* with 2 species.[28] The Eastern Cape Province is known for its richness in plant species with indigenous people having a history of using traditional medicine to treat various diseases.[42] The Xhosa people are said to use herbal medicine since they still believe in their efficacy.[43]

The majority of the reported plants are used in the treatment and management of inflammation, wounds, and pain. Among the reported species are *K. drepanophylla*, *Haemanthus albiflos*, *Pachycarpus concolor*, *Ilex mitis*, *Albuca aurea*, *Microglossa mesipilofia*, *Acorus calamus*, *Chenopodium ambroisoides*, *Ipomoea oblongata*, *Curtisia dentata*, *Elephantorriza elephantina*, *G. perpensa*, *Burchellia bubaline*, *Pentanisia prunelloides*, and *Withania somnifera*. Wube *et al.* reported on the knipholone anthraquinone found in *Kniphofia* species.[44] Knipholone is reported to possess antioxidant and anti-inflammatory properties which justifies the use of the species *K. drepanophylla* by traditional healers to treat pain and swelling, which are the symptoms of elephantiasis.[44] *B. bubaline*, one of the plants used by South African traditional healers to treat and manage inflammation, is reported to possess antimicrobial, antifungal, and anti-inflammatory activities.[45]

The *in vitro* screening of *C. dentata* revealed that the plant possessed good antibacterial, antifungal, and antioxidant properties which could be attributed to ursolic acid, betulinic acid lupeol, and β-sitosterol compounds that were isolated from the plant leaves.[46] *G. perpensa* has been reported to possess a wide range of pharmacological activities including acetylcholinesterase, anthelmintic, antibacterial, antifungal, antinociceptive, anti-inflammatory, antioxidant, antitumor, lactogenic, and uterotonic.[47] These pharmacological properties could be due to the presence of several classes of phytochemicals including alkaloids, benzoquinones, ellagic acids, flavonoids, phenols, proanthocyanidins, and tannins.[47] Tannins were reported to be useful in treating dermatitis and are known to form a protective layer on the skin and mucosa, thereby enhancing tissue regeneration.[48]

Seven of the 29 medicinal plants recorded in this study are reported by Afolayan *et al.* to be used in the management of skin disorders among the Xhosa communities of the Amathole District in the Eastern Cape Province.[49] In this study, *K. drepanophylla* and *Spirostachys africana* are the only plant species that were reported to treat skin ailments. In a study conducted by Amoo *et al.* on phytochemical constituents, antioxidant and acetylcholinesterase-inhibitory properties of stored medicinal plants, *S. africana* displayed a significantly higher phenolic content.[50]

In this study, only four plants (*A. aurea*, *Kniphofia drepanophylla*, *Rapanea melanophloes*, and *Rhoicissus tomentosa*) have been reported to expel parasites. The fruits and leaves of *R. melanophloeos* have been reported to possess anthelmintic activity and are used to treat livestock in East Africa and in South Africa.[51,52]

Of the 29 recorded plants, 28 are indigenous and one is exotic (*Chenopodium ambrosoides*). According to the red data list, *A. aurea*, *H. albiflos*, *K. drepanophylla*, and *M. mesipilofolia* are endemic to South Africa. *K. drepanophylla* is listed as vulnerable, whereas the rest of the plant species is listed as of least concern.[53] Some of the plants reported in this study are said to be poisonous, some parts of *Erythrina caffra*, for example, are said to be poisonous to animals and humans, and the parts of *Aconkanthera oblongifolia* leaves are known to be poisonous.[54,55]

As shown in Figure 2, the most frequently used plant parts reported in this study were roots (51%), followed by leaves (31%) bark (27%), bulb (20%), rhizome (17%), and fruits (6%). Roots and leaves were also reported as the commonly used plant parts.[28] According to a study that has been done, the extensive use of roots leads to complete destruction of plants, which further leads to their extinction.[49] The preference of leaves could be due to the accumulation of phytochemicals which are known to be pharmacologically active against diseases.[56,57] Plants could be used individually or in combination as a decoction and/or as

Table 1: Medicinal plants used for the treatment of elephantiasis in the Eastern Cape, South Africa

Family	Scientific name	Common name	Voucher number	Area	UV	Plant part used	Method of administration
Amaryllidaceae	*Haemanthus albiflos* Jacq.	Umathunga	Adams, 10027	KSD, Nyandeni	0.10	Bulb, roots, leaf	Infusion is taken for pain
Apocynaceae	*Acokanthera oblongifolia* (Hochst)	Intlungunyembe	Adams, 10014	KSD	0.17	Roots, leaves	Infusion is taken orally to drain excess fluid. Swollen feet are soaked in warm mixture
Apocynaceae	*Pachycarpus concolor* E Mey	Itshongwe	Adams, 10015	KSD	0.10	Bulb, roots	Decoction and infusion are used for pain and inflammation
Aquifoliaceae	*Ilex mitis* (L) Radlk	Icimamlilo	Adams, 1004	KSD, IH	0.10	Bark, leaves	Poultice is applied on sores
Asparagaceae	*Albuca aurea* Jacq.	Intelezi	Adams, 1007	KSD, Nyandeni	0.10	Bulb, roots	Infusion is applied on sores
Asphodelaceae	*Kniphofia drepanophylla* baker	Ixonya	Adams, 10016	KSD	0.17	Rhizome, roots	Used to expel worms, treat (poultice) wounds, acne and eczema
Asteraceae	*Microglossa mesipilofia* (less)	Ithyolo	Adams, 10028	KSD	0.10	Leaves, roots	The infusion is taken orally for pain and swelling
Araceae	*Acorus calamus* L.	Ikalmuzi	Adams, 10026	KSD	0.10	Roots, rhizome	Applied to treat sores and swelling
Celastraceae	*Lauridia tetragonia* (L.F)	Umdlavuza	Adams, 10029	KSD	0.14	Bark, leaves	Boiled leaves and bark applied topically to treat swelling and pain and also wounds and blisters
Chenopodiaceae	*Chenopodium ambroisoides* L.	Untsukumbini	Adams, 1002	IH	0.10	Whole plant	Infusion used to treat swollen limbs and to reduce pain
Convolvulaceae	*Ipomoea oblongata* E Mey.	Ubhoqo	Adams, 1005	KSD, Nyandeni, IH	0.10	Bulb, roots	Infusion and decoction is drank to reduce pain and also soak swollen limbs
Curtisiaceae	*Curtisia dentata* (Burm.f.) C.A. Sm	Uzintlwa	Adams, 1006	IH	0.17	Bark, leaves	Bark and leaf infusion is applied topically to treat wounds and infection
Dioscoreaceace	*Dioscorea dregeana* (Kunth) T. Durand and Schinz	Ingcolo	Adams, 10025	KSD	0.10	Rhizome, roots	Grind and boiled to soak feet and reduce swelling
Dioscoreaceae	*Dioscorea sylvatica* (Kunth) Eckl.	Ufudo	Adams, 1008	KSD, IH, Nyandeni	0.17	Bulb, roots	Infusion and decoction is used to treat pain and inflammation
Euphorbiaceae	*Euphorbia gorgonis* Berger.	Nkalimasane	Adams, 10017	KSD	0.14	Whole plant	Infusion is used to treat pain and swelling and crushed plant is applied topically on wounds and sores
Euphorbiaceae	*Spirostachys africana* Sond.	Umthombothi	Adams, 10023	KSD	0.14	Bark	The infusion is used to soak swollen limbs and also to treat skin problems and wounds by applying on skin as poultice
Fabaceae	*Erythrina caffra* Thunb.	Umsintsi	Adams, 1009	KSD, IH, Nyandeni	0.06	Bark	Bark and leave infusion is used to treat sores, wounds, abscesses and arthritis
Fabaceae	*Elephantorriza elephantina* (Burch)	Intolwane	Adams, 10011	KSD	0.27	Rhizome, roots	The infusion is applied topically and root powder is applied on wounds and sores
Gunneraceae	*Gunnera perpensa* (L.)	Iphuzi lomlambo	Adams, 1003	KSD, IH, Nyandeni	0.24	Rhizome, roots	Infusion and decoction taken to drain excess water and dry powder mixed with water is applied on sores and wounds
Hyacinthaceae	*Albuca setosa* Jacq.	Inqwebeba	Adams, 10022	KSD	0.10	Leaves, bulb	Decoction is taken to treat pain and inflammation
Hypoxidaeae	*Hypoxis hemerocallidea* Fisch., C.A Mey. and Avé-Lall.	Inongwe, Ilabatheka	Adams, 10010	KSD, IH, Nyandeni	0.31	Tuber	Weak infusion or decoction is taken orally to reduce swelling and pain and to cleanse the blood
Melianthaceace	*Bersama tysonia* Oliv.	Isibhara	Adams, 10018	KSD	0.13	Bark	Infusion is used to treat pain and venereal diseases and poultice is applied topically to treat sores
Myrsinaceae	*Rapanea melanophloes* (L.) Mez	Umaphipha	Adams, 10012	KSD	0.17	Bark, leaves, fruit	Decoction is drank for pain and worms and infusion is used to soak on sores
Ranunculaceae	*Clematis brachiata* Thunb.	Umvuthuza	Adams, 1001	KSD, IH, Nyandeni	0.10	Whole plant	Infusion or decoction is taken to reduce pain and inflammation
Rubiaceace	*Burchellia bubaline* (L.f.) Sims.	Ithobankomo	Adams, 10019	IH	0.13	Bark, roots	Root and bark cold infusions are used in body wash to reduce pain and inflammation
Rubiaceae	*Pentanisia prunelloides* (Klotzsch ex Eckl. and Zeyh.) Walp.	Icimamlilo	Adams, 10013	KSD, IH	0.17	Tuber, roots	Root decoctions are taken orally or as enemas and also applied externally for sores and wounds
Solanaceae	*Lycium ferocissimum* Miers.	Umbovu, Idywadi	Adams, 10021	KSD	0.10	Leaves, roots	Decoction is applied topically to reduce pain and inflammation or inhaled to reduce pain

Contd...

Table 1: Contd...

Family	Scientific name	Common name	Voucher number	Area	UV	Plant part used	Method of administration
Solanaceae	*Withania somnifera* (L.)	Ubushwa, Ubuvimba	Adams, 10020	KSD, IH	0.13	Leaves, roots	The infusion is drank to heal inflammation and swelling and wounds or soaking the affected areas
Vitaceae	*Rhoicissus tomentosa* (Lam.) Wild and R.B. Drumm.	Isaqoni, Uchitibhunga	Adams, 10024	KSD, IH	0.13	Root tuber	Roots are boiled in milk and given to calves to expel intestinal worms and the plant is also used to reduce inflammation

KSD: King Sabata Dalindyebo; IH: Ingquza-Hill; UV: Use-value

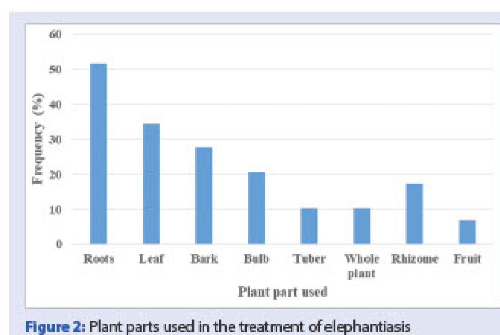


Figure 2: Plant parts used in the treatment of elephantiasis

infusion to prepare the medicine. One traditional healer mentioned that she uses equal portions of boiled powdered *C. ambrosioides* and *G. perpensa* mixed with *krampdruppels* (crampdrops), *rooi poeier* (red powder), entress drops, and camphor blocks in equal proportions in 2 l of water. The mixture is reported to help with improving blood circulation in the veins. A traditional healer from the Eastern Cape was reported to use equal portions of *E. comosa*, *Euphorbia clavarioides*, and *Rumex obtusifolius* to treat elephantiasis.[28] Infusion and decoction were reported as the most commonly used methods of preparation [Figure 3]. Herbal medicines are most applied externally. According to studies undertaken by Yaseen *et al.*[58] and Tshikalange *et al.*,[59] infusion and decoction with water as a solvent are the most common preparation methods that are used by traditional healers.

Analytical tools were also used to determine the use-vale of the plant species to verify the ethnobotanical information about the use of plants in the treatment of elephantiasis and its common symptoms. The overall usefulness of the mentioned medicinal plants was calculated based on the index of a common tool that is used to quantify qualitative data in the biological sciences and other sciences known as the "use value."[60-63]

H. hemerocallidea (*Hypoxidaceae*) was the plant with the highest UV (0.31) [Table 1]. According to a survey on plants that are used as home medicine by families in the Eastern Cape, the plant is used in the treatment of human immunodeficiency virus acquired immunodeficiency syndrome infections, for blood purification, sunburns, as an antibiotic, septic sores, headaches, as a purgative, as an antioxidant, flu, cancer, acne, diarrhea, urinary tract infections, testicular tumors, arthritis, diabetes, prostate gland enlargement, and infertility.[64] Furthermore, the plant is used for healing dizziness, for bladder and urinary disease, as a tonic and for burns.[65] The plant was one of many plants that were screened for genotoxic effects and was among those that did not show positive results after screening.[65] The plant has been recorded in one of the 16 South African traditional medicines that have been partly or fully developed as commercial crops and products and its plant parts are used in traditional medicine as a traditional tonic and for benign prostate hyperplasia include rhizome, tuber or bulb.[66]

The species with the second highest UV (0.27) was *E. elephantina* (*Fabaceae*), locally known as *Intolwane* [Table 1]. It is used in South Africa as a traditional remedy for a wide range of ailments both in humans and livestock.[67] Zulus take an infusion of the inner parts of the roots and administer it as an enema for dysentery and diarrhoea and root decoctions are also taken for diarrhea.[67,68] The roots of *E. elephantina* are reddish and in the Sotho culture, "red medicines" are associated with blood and good health.[69] The San people use red

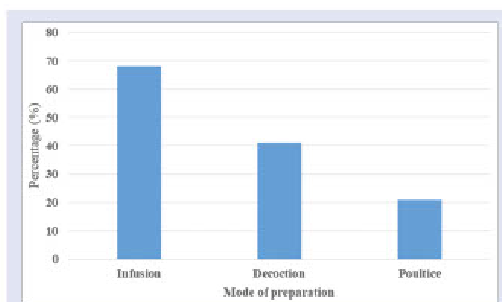


Figure 3: Methods of preparation of the herbal medicine

plant parts to treat anemia, weakness, and to cure fever.[69,70] Animals such as goats are given a decoction from a handful (about 50 g) of root powder mixed with 2 l of water to treat helminthiasis.[69] The plant also showed anthelminthic activity *in vitro* of crude aqueous extracts against *Haemonchus contortus*.[71] The following phytochemicals in certain tissues were found to be present, catechins in rhizomes, which indicated the presence of condensed tannins, as well as flavonoids and other phenolic substances.[72] Tannins were reported to be useful in treating dermatitis and are known to form a protective layer on the skin and mucosa, thereby enhancing tissue regeneration.[73]

The plant with the third highest UV (0.24) was *G. perpensa*. This plant is widely used by the rural population in South Africa for the treatment of several diseases, including dysmenorrhea.[74] It has large rounded leaves hence its common name "river pumpkin."[75,76] Aqueous decoctions of this plant, relieve rheumatoid pain, facilitate childbirth and are believed to treat female infertility.[68,74] Its use in rheumatoid pain treatment supports its use in the treatment of pain and inflammation as mentioned by the traditional healers. A decoction of *G. perpensa* rhizome is also applied on the affected areas to treat wounds and psoriasis.[42,66,68,77] Nkomo *et al.* reported on its antinociceptive and anti-inflammatory properties.[74] Previous studies have shown that *G. perpensa* demonstrated good antibacterial activity, antioxidant properties, and stimulated fibroblast growth in wound healing.[78-80] The leaves are used by the Zulus as an emetic and the stems can be eaten when fresh.[76] Two active compounds, 1,4-benzoquinones and a benzopyran-6-ol, were isolated from the leaves and stems of *G. perpensa* dichloromethane extract and phytol was isolated from the methanol extract. The two benzoquinones and the benzopyran were further examined for antimicrobial properties together with the crude stem, leaf and root extracts.[76]

The plants which had the UV of 0.17, being the fourth highest, were *A. oblongifolia*, *C. dentata*, *D. sylvatica*, *K. drepanophylla*, *P. prunelloides*, and *R. melanophloes*. Three of these plants (*C. dentata*, *K. drepanophylla* and *R. melanophloes*) have been reported to possess anthelminthic activity.[64,81,82] *Kniphofia drepanophylla* is reported to be used for stomach ache, loss of appetite, and gastric worms/parasites.[64] The acetone and dichloromethane extracts and compounds isolated from *C. dentata* leaves were investigated for anthelmintic activity *in vitro* against *Trichostrongylus colubriformis*, *H. contortus*, and *Caenorhabditis elegans* and were found to be active against all nematodes at 160 µg/ml.[82] In Southern Africa, concoctions of the bark of *R. melanophloes* have been reported to be used for wounds, blood purification, muscular pain, and as an anthelmintic and emetic.[73,82] In another study, *R. melanophloes* was reported to possess anthelmintic activity against nematodes.[82] *D. sylvatica* is also used for treating skin problems and rheumatism, like *G. perpensa* in Zimbabwean traditional medicine. The rubbing of fresh peeled rhizome on the skin supports its use for pain and inflammation.[83,84]

CONCLUSION

The study documented significant botanical information on medicinal plants that the people of King Sabata Dalindyebo, Nyandeni and Ingquza-Hill municipalities in the Eastern Cape use to treat and manage elephantiasis and its symptoms. The study documented 29 medicinal plants distributed to 23 families that are used to treat and manage elephantiasis and its symptoms. The families *Apocynaceae*, *Dioscoreaceae*, *Euphorbiaceae*, *Fabaceae*, *Rubiaceae*, and *Solanaceae* were reported as the most commonly used plants represented by two species each. The majority of plants are used to treat and manage inflammation, wounds and pain. Roots, leaves, bark, and bulbs were reported to as the most preferred plant parts. Plants could be used individually or in combination as a decoction or infusion to prepare herbal medicine. *H. hemerocallidea* had the highest UV, followed by *E. elephantina* and *G. perpensa*. The UV is related to their use in the treatment and management of elephantiasis and its related ailments. Work is underway to extensively screen the recorded plants for potential pharmacological activities and for isolation of bioactive compounds.

Acknowledgements

The authors are most grateful to the herbalists, traditional healers and the 3 young people for being part of the study and for sharing their valued time and information.

Financial support and sponsorship

This project was financially supported by Thuthuka grant [grant number U113516] and Block grant [grant number SFH180531337969] of the National Research Foundation, South Africa awarded to L. Komoreng and Z. Adams.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Memariani Z, Gorji N, Moeini R, Farzaei MH. Traditional uses. In: Nabavi SM, Suntar I, Barreca D, Khan H, editors. Phytonutrients in Food: From Traditional to Rational Usage. United Kingdom: Woodhead Publishing; 2020. p. 23-66.
2. Van Wyk BE, De Wet H, Van Heerden FR. An ethnobotanical survey of medicinal plants in the south eastern Karoo, South Africa. S Afr J Bot 2008;74:696-704.
3. Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. J Ethnopharmacol 2008;115:387-408.
4. Jeruto P, Lukhoba C, Ouma G, Otieno D, Mutai C. An ethnobotanical study of medicinal plants used by the Nandi people in Kenya. J Ethnopharmacol 2008;116:370-6.
5. Maroyi A. An ethnobotanical survey of medicinal plants used by the people in Nhema Communal Area, Zimbabwe. J Ethnopharmacol 2011;136:347-54.
6. Seleteng Kose L, Moteetee A, Van Vuuren S. Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho. J Ethnopharmacol 2015;170:184-200.
7. Nortje JM, van Wyk BE. Medicinal plants of the Kamiesberg, Namaqualand, South Africa. J Ethnopharmacol 2015;171:205-22.
8. Mongalo NI, Makhafola TJ. Ethnobotanical knowledge of the lay people of Blouberg area (Pedi tribe), Limpopo Province, South Africa. J Ethnobiol Ethnomed 2018;14:46.
9. Kawarty AM, Behçet L, Çakilcioğlu U. An ethnobotanical survey of medicinal plants in Ballakayati (Erbil, North Iraq). Turk J Bot 2020;44:345-57.
10. World Health Organization. Lymphatic Filariasis. Fact Sheet; c2020. Available from: https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis. [Last accessed on 2020 Sept 23, Last updated on 2020 Mar 02].

11. Molyneux D, Bradley M, Hoerauf A, Kyelem D, Taylor MJ. Lymphatic filariasis (elephantiasis) elimination: A public health success and development opportunity. Filaria J 2003;2:13.
12. Prasad B, Reddy AK, Joy JM, Rasheed A, Dalith D. Natural antifilarial drugs: A review. Int J Pharm Toxicol 2011;1:1-10.
13. Fuller LC. Podoconiosis: Endemic nonfilarial elephantiasis. Curr Opin Infect Dis 2005;18:119-22.
14. Gupta S, Ajith C, Kanwar AJ, Sehgal VN, Kumar B, Mete U. Genital elephantiasis and sexually transmitted infections - revisited. Int J STD AIDS 2006;17:157-65.
15. National Organization for Rare Disorders. Elephantiasis. Rare Disease Database; c2019. Available from: https://rarediseases.org/rare-diseases/elephantiasis/. [Last accessed on 2019 Mar 04].
16. World Health Organization. Progress Report 2000–2009 and Strategic Plan 2010–2020 of the Global Programme to Eliminate Lymphatic Filariasis: Halfway Towards Eliminating Lymphatic Filariasis. Report No. WHO/HTM/NTD/PCT/2010.6; 2010.
17. Elephantiasis Sufferer 'Was Treated'. News 24; February 22, 2012. Available from: https://www.news24.com/News24/Elephantiasis-sufferer-was-treated-20120222. [Last accessed on 2019 March 12].
18. Elephantiasis Patient Takes It in Her Stride. eNCA; October 26, 2013. Available from: https://www.enca.com/south-africa/elephantiasis-patient-takes-it-her-stride. [Last accessed on 2019 March 12].
19. Berger S, editor. Infectious Diseases of South Africa. Global Infectious Diseases and Epidemiology Network (GIDEON). Los Angeles, California: Gideon Informatics Inc.; 2014.
20. Ottesen EA, Duke BO, Karam M, Behbehani K. Strategies and tools for the control/elimination of lymphatic filariasis. Bull World Health Organ 1997;75:491-503.
21. Sashidhara KV, Singh SP, Misra S, Gupta J, Misra-Bhattacharya S. Galactolipids from *Bauhinia racemosa* as a new class of antifilarial agents against human lymphatic filarial parasite, *Brugia malayi*. Eur J Med Chem 2012;50:230-5.
22. Sharma S. Vector-borne diseases. Prog Drug Res 1990;35:365-495.
23. Babu BV, Rath K, Kerketta AS, Swain BK, Mishra S, Kar SK. Adverse reactions following mass drug administration during the programme to eliminate lymphatic filariasis in Orissa State, India. Trans R Soc Trop Med Hyg 2006;100:464-9.
24. Ranjini G, Selvakumari E, Gopal V. Perception of natural antifilarial drugs. Int Res J Pharm 2013;4:27-30.
25. Sahare KN, Singh V. Antifilarial activity of ethyl acetate extract of *Vitex negundo* leaves *in vitro*. Asian Pac J Trop Med 2013;6:689-92.
26. Al-Abd NM, Nor ZM, Al-Adhroey AH, Suhaimi A, Sivanandam S. Recent advances on the use of biochemical extracts as filaricidal agents. Evid Based Complement Alternat Med 2013;2013:986573.
27. Kumar K, Kumar M, Verma M, Yadar AK, Uddin Q. Anti- wolbachia: A herbal approach to the treatment of filariasis. Spatula DD – Peer Reviewed Journal on Complementary Medicine and Drug Discovery 2014;4:101-8.
28. Komoreng L, Thekisoe O, Lehasa S, Tiwani T, Mzizi N, Mokoena N, *et al*. An ethnobotanical survey of traditional medicinal plants used against lymphatic filariasis in South Africa. S Afr J Bot 2017;111:12-6.
29. Mbuni YM, Wang S, Mwangi BN, Mbari NJ, Musili PM, Walter NO, *et al*. Medicinal plants and their traditional uses in local communities around Cherangani hills, Western Kenya. Plants (Basel) 2020;9:331.
30. Balogun FO, Tshabalala NT, Ashafa AO. Antidiabetic medicinal plants used by the Basotho tribe of Eastern Free State: A review. J Diabetes Res 2016;2016:4602820.
31. Moteetee A, Seleteng Kose L. Medicinal plants used in Lesotho for treatment of reproductive and post reproductive problems. J Ethnopharmacol 2016;194:827-49.
32. Mugomeri E, Chatanga P, Chakane N. Medicinal herbs used by HIV-positive people in Lesotho. Afr J Tradit Complement Altern Med 2016;13:123-31.
33. Mujuru L, Jimu L, Mureva A, Mapaura A, Nyakudya IW, Muvengwi J. Diversity of local knowledge on use of wild food and medicinal plants in communities around five biodiversity hotspots in Zimbabwe. Adv Tradit Med 2020;20:663-71.
34. Barbosa F, Hlashwayo D, Sevastyanov V, Chichava V, Mataveia A, Boane E, *et al*. Medicinal plants sold for treatment of bacterial and parasitic diseases in humans in Maputo city markets, Mozambique. BMC Complement Med Ther 2020;20:19.
35. Morris-Paxton AA, Reid S, Ewing RG. Primary healthcare services in the rural Eastern Cape, South Africa: Evaluating a service-support project. Afr J Prim Health Care Fam Med 2020;12:e1-7.
36. Hamann M, Tuinder V. Introducing the Eastern Cape: A Quick Guide to Its History, Diversity and Future Challenges. Stockholm Resilience Centre, Stockholm University, Sweden; 2012.
37. OR Tambo District Municipality. Alchetron; c2020. Available from: https://alchetron.com/OR-Tambo-District-Municipality. [Last accessed on 2019 Apr 12; Last updated on 2018 Mar 16].
38. Municipalities.co.za. OR Tambo District Municipality (DC15); c2012-20. Available from: https://municipalities.co.za/overview/106/or-tambo-district-municipality. [Last accessed on 2020 Sep 24].
39. Mucina L, Rutherford MC, Powrie LW. Vegetation Map of South Africa, Lesotho and Swaziland. Pretoria: South African National Biodiversity Institute; 2005.
40. Ingquza Hill Local Municiplality, c2016. Available from: http://www.ihlm.gov.za/about-us/[Last accessed on 2018 Mar 15].
41. Phillips O, Gentry AH, Reynel C, Wilkin P, Galvez-Durand CB. Quantitative ethnobotany and Amazonian conservation. Conserv Biol 1994;8:225-48.
42. Grierson DS, Afolayan AJ. An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. J Ethnopharmacol 1999;67:327-32.
43. Bhat RB. Medicinal plants and traditional practices of Xhosa people in the Transkei region of Eastern Cape, South Africa. Ind J Trad Know 2014;13:292-8.
44. Wube AA, Bucar F, Asres K, Gibbons S, Adams M, Streit B, *et al*. Knipholone, a selective inhibitor of leukotriene metabolism. Phytomedicine 2006;13:452-6.
45. Van Staden J, Amoo SO, Ndhlala AR, Finnie JF. Antibacterial, antifungal and anti-inflammatory properties of *Burchellia bubaline*. S Afr J Bot 2009;75:60-3.
46. Cox-Georgian D, Ramadoss N, Dona C, Basu C. Therapeutic and Medicinal Uses of Terpenes. Northridge, CA, USA: Department of Biology, California State University; 2019.
47. Maroyi A. From traditional usage to pharmacological evidence: Systematic review of *Gunnera perpensa* L. Evid Based Complement Alternat Med 2016;2016:1720123.
48. Fadipe VO, Mongalo NI, Opoku AR. *In vitro* evaluation of the comprehensive antimicrobial and antioxidant properties of *Curtisia dentata* (Burm.f) C.A. Sm: Toxicological effect on the Human embryonic kidney (HEK293) and human hepatocellular carcinoma (HepG2) cell lines. Excli J 2015;14:971-83.
49. Afolayan AJ, Grierson DS, Mbeng WO. Ethnobotanical survey of medicinal plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. J Ethnopharmacol 2014;153:220-32.
50. Amoo SO, Aremu AO, Moyo M, Van Staden J. Antioxidant and acetylcholinesterase-inhibitory properties of long-term stored medicinal plants. BMC Complement Altern Med 2012;12:87.
51. Bally PR. Native Medicinal and Poisonous Plants of East Africa. Bull Misc. Inform. Kew 1937;1:10-26.
52. Lotter N, Chivandi E, Lembede BW, Ndhlala AR, Nyakudya TT, Erlwanger KH. Anti-oxidant activity, alpha-amylase inhibition and toxicity of leaf extracts of cultivated *Rapanea melanophloeos* (L.) Mez (cape beech). S Afr J Bot 2019;126:261-4.
53. Red List of South African Plants. Threatened Species Programme; c2010-12. Available from: http://redlist.sanbi.org/. [Last accessed on 2018 Feb 03].
54. Taylor JL, Elgorashi EE, Maes A, Van Gorp U, De Kimpe N, Van Staden J, *et al*. Investigating the safety of plants used in South African traditional medicine: Testing for genotoxicity in the micronucleus and alkaline comet assays. Environ Mol Mutagen 2003;42:144-54.
55. Boon R. Pooley's Trees of Eastern South Africa a Complete Guide. Durban: Flora and Fauna Publications Trust; 2010.
56. Tugume P, Kakudidi EK, Buyinza M, Namaalwa J, Kamatenesi M, Mucunguzi P, *et al*. Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda. J Ethnobiol Ethnomed 2016;12:5.
57. Passalacqua NG, Guarrera PM, De Fine G. Contribution to the knowledge of the folk plant medicine in Calabria region (Southern Italy). Fitoterapia 2007;78:52-68.
58. Yaseen G, Ahmad M, Sultana S, Suleiman Alharrasi A, Hussain J, Zafar M, *et al*. Ethnobotany of medicinal plants in the Thar Desert (Sindh) of Pakistan. J Ethnopharmacol 2015;163:43-59.
59. Tshikalange TE, Mophuting BC, Mahore J, Winterboer S, Lall N. AN ethnobotanical study of medicinal plants used in villages under Jongilanga tribal council, Mpumalanga, South Africa. Afr J Tradit Complement Altern Med 2016;13:83-9.
60. Prance GT, BaiZe W, Boom BM, Carneiro RL. Quantitative ethnobotany and the case for conservation in Amazonia. Conserv Biol 1987;1:296-310.
61. Phillips O, Gentry AH. The useful plants of Tambopata, Peru: I. Statistical hypotheses tests with a new quantitative technique. Econ Bot 1993a; 47:15-32.
62. Phillips O, Gentry AH. The useful plants of Tambopata, Peru: II. Additional hypothesis testing in quantitative ethnobotany. Econ Bot 1993b; 47:33-43.
63. Hoffman B, Gallaher T. Important indices in ethnobotany. Ethnobot Res Appl 2007;5:201-18.

64. Sewani-Rusike CR, Mammen M. Medicinal plants used as home remedies: A family survey by first year medical students. Afr J Tradit Complement Altern Med 2014;11:67-72.
65. Elgorashi EE, Taylor JL, Maes A, van Staden J, De Kimpe N, Verschaeve L. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. Toxicol Lett 2003;143:195-207.
66. Van Wyk BE. The potential of South African plants in the development of new medicinal products. S Afr J Bot 2011;77:812-29.
67. Maphosa V, Masika PJ, Bizimenyera ES, Eloff JN. *In-vitro* anthelminthic activity of crude aqueous extracts of *Aloe ferox, Leonotis leonurus* and *Elephantorrhiza elephantina* against *Haemonchus contortus*. Trop Anim Health Prod 2010;42:301-7.
68. Hutchings A, Scott AH, Lewis G, Cunningham A. Zulu Medicinal Plants - An Inventory. South Africa: University of Natal Press Pietermaritzburg, Pietermaritzburg; 1996.
69. Mpofu S, Tantoh Ndinteh D, van Vuuren SF, Olivier DK, Krause RW. Interactive efficacies of *Elephantorrhiza elephantina* and *Pentanisia prunelloides* extracts and isolated compounds against gastrointestinal bacteria. S Afr J Bot 2014;94:224-30.
70. Laidler PW. The magic medicine of the Hottentots. S Afr J Sci 1928;25:433-47.
71. Maphosa V, Masika PJ, Moyo B. Investigation of the anti-inflammatory and antinociceptive activities of *Elephantorrhiza elephantina* (Burch.) Skeels root extract in male rats. Afr J Biotechnol 2009;8:7068-72.
72. Naidoo V. Screening of Four Plants Commonly Used in Ethnoveterinary Medicine for Antimicrobial, Anti-Protozoal and Anti-Oxidant Activity. MSc Dissertation. South Africa: University of Pretoria; 2005. p. 1-130.
73. Van Wyk BE, Van Oudsthoorn B, Gericke N. Medicinal Plants of South Africa. South Africa: Briza Publications Pretoria; 1997. p. 120-8.
74. Nkomo M, Nkeh-Chungag BN, Kambizi L, Jamot E, Iputo JE. Antinociceptive and anti-inflammatory properties of *Gunnera perpensa* (*Gunneraceae*). Afr J Pharmacol 2010;4:263-9.
75. Drewes SE, Khan F, van Vuuren SF, Viljoen AM. Simple 1,4-benzoquinones with antibacterial activity from stems and leaves of *Gunnera perpensa*. Phytochemistry 2005;66:1812-6.
76. Watt M, Breyer-Brandwijk MG. The Medicinal and Poisonous Plants of Southern and Eastern Africa. London, UK: Churchill Livingstone; 1962.
77. Steenkamp V, Mathivha E, Gouws MC, van Rensburg CE. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. J Ethnopharmacol 2004;95:353-7.
78. Buwa LV, van Staden J. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. J Ethnopharmacol 2006;103:139-42.
79. Mabona U, van Vuuren SF. Southern African medicinal plants used to treat skin diseases. S Afr J Bot 2013;87:175-93.
80. Githiori JB, Höglund J, Waller PJ, Baker RL. Anthelmintic activity of preparations derived from *Myrsine africana* and *Rapanea melanophloeos* against the nematode parasite, *Haemonchus contortus*, of sheep. J Ethnopharmacol 2002;80:187-91.
81. Shai LJ, Bizimenyera ES, Bagla V, McGaw LJ, Eloff JN. *Curtisia dentata* (Cornaceae) leaf extracts and isolated compounds inhibit motility of parasitic and free-living nematodes. Onderstepoort J Vet Res 2009;76:249-56.
82. Lukhele T. Isolation, Characterisation and Biological Activity of Some Compounds from *Rapanea melanophloes* (L.) Mez. South Africa: University of Johannesburg; 2012.
83. Gelfand M, Mavi S, Drummond RB, Ndemera B. The Traditional Medical Practitioner in Zimbabwe: His Principles of Practice and Pharmacopoeia. Gweru: Mambo Press; 1985.
84. Cogne AL, Marston A, Mavi S, Hostettmann K. Study of two plants used in traditional medicine in Zimbabwe for skin problems and rheumatism: *Dioscorea sylvatica* and *Urginea altissima*. J Ethnopharmacol 2001;75:51-3.

CHAPTER 3

SCREENING PLANT EXTRACTS FOR ANTIMICROBIAL ACTIVITY

3.1 Introduction

When there is an interaction between a microorganism and its host, the defence of the host usually does not go unchallenged. Based on what the microbe is naturally programmed to do in a given host, there is a successful infection of the host in varying periods. Some rapidly replicating microbes can avoid the primary defences of the host and find another host before certain secondary immune responses develop. Despite the breakthroughs made in medicine, infectious diseases caused by bacteria, fungi, viruses, and parasites pose a severe threat to people's health and wellness. The problem infectious diseases bring is significantly large in developing countries where medicines and drug resistance is emerging (Okeke et al., 2005; Cos et al., 2006). Innovations on new drugs that can act against microbes continues and finding alternative medicine need to be discovered. Natural products continue to play a significant role in the development of novel therapeutics for a variety of ailments, including infectious disorders (Clardy and Walsh, 2004; Cos et al., 2006). The term "antimicrobial activity" refers to all compounds that have the potential to limit bacterial growth, prevent the creation of microbial colonies, and possibly even eliminate germs (Elmogahzy, 2020).

3.1.1 Antibacterial activity

Infections through bacteria are of particular concern globally because of antibiotic resistance. Gram-negative bacteria have acquired resistance over the past 10 years (Pallett and Hand, 2010). In the presence of antibiotic selection processes, they have acquired drug resistance (Boucher et al., 2009; Peleg and Hooper, 2010). The peptidoglycan layer in the periplasmic space, the cell membrane, and the outer membrane make form the multilayered cell surface of the Gram-negative bacterial cell

wall. There are three components that make up the outer membrane: a lipid A region, a core polysaccharide, and an O-specific antigen (Mosier-Boss, 2017). Gram-negative bacteria have efflux pumps that belong to the resistance-nodulation-cell division family of tripartite efflux pumps which are largely involved in multidrug resistance (van Bambeke et al., 2006). Infections linked to oxidative stress in cells, such as cancer, and drug-resistant bacteria including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyrogenes*, and *Candida albicans*, are becoming an alarming concern in South Africa and throughout Africa (Dube et al., 2017). *Acinetobacter baumannii*, *Enterococcus faecium*, *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis* are more bacteria that have developed drug-resistance.(Regiel-Futyra et al., 2017).

The increase of multidrug-resistant bacteria has pushed for the search for novel antibacterial drugs that will fight against resistant phenotypes. This challenge of drug-resistant bacteria creates a need for the use of traditional medicine and new therapeutic procedures (Regiel-Futyra et al., 2017). Due to the variety of secondary chemicals they contain structurally, plant products are a good source of pharmaceuticals (Cowan, 1999; Ndhlala et al., 2013; Ngameni et al., 2013; Tchinda et al., 2017). from Plants may possess antimicrobial compounds that can inhibit bacteria through various mechanisms compared to currently used antibiotics and bring new methods in treating drug resistant strains (Eloff, 1998).

3.1.2 Antifungal activity

Pathogenic fungi cause risk of serious infections in immunocompromised patients. The complex mannoproteins, chitins, and glucans found in the fungal cell wall give it its distinctive structure and are essential to the organism's survival. (Sumthong and Verpoorte, 2007). The resistance to antifungal treatment together with drug resistance during treatment has become a serious problem in the management of diseases (Perea and Patterson, 2002). A number of variables, including the rise in the number of patients who are critically ill and/or immunocompromised, particularly those who are HIV-positive, have contributed to the rise in life-threatening fungal infections in recent years. Other reasons that have contributed to the rise in fungal infections include the

regular use of more invasive medical procedures, therapy with broad-spectrum antibiotics, and glucocorticoids, which are corticosteroids. (Lortholary and Dupont, 1997). Candida species are responsible for a sizable portion of nosocomial fungus infections, with Candida albicans being the most frequent source of fungus bloodstream infections. 90% of nosocomial fungal infections are caused by Candida and Aspergillus infections (Fridkin and Jarvis, 1996; Perea & Patterson, 2002).

Antifungal drugs are classified according to their mechanism of action. Amphotericin acts by altering membrane function, flucytosine acts by inhibiting DNA and RNA synthesis, the azoles (fluconazole, itraconazole, voriconazole, posaconazole and ravuconazole) act by inhibiting ergosterol biosynthesis and, lastly, the echinocandins act by inhibiting glucan biosynthesis (caspofungin, micafungin and anidulafungin) (Perea and Patterson, 2002). However, the problem of drug resistance in numerous common pathogenic fungus was brought about by the overuse and improper application of medications. (Graybill, 1996; Masoko et al., 2005; Picard and Eloff, 2005). Primary resistance is when fungi are found to be intrinsically resistant to antifungal drugs and secondary resistance is due to response to exposure to the drug during treatment (Perea and Patterson, 2002). The growth of antibiotic-resistant human pathogenic fungi necessitates the development of novel antifungal medications. (Masoko et al., 2005).

3.1.3 Antimycobacterial activity

An estimated 1.3 million people will die from tuberculosis (TB), an infectious disease that is a leading cause of illness and death globally. (WHO, 2021). *M.* tuberculosis, the main perpetrator causing the TB globally and threating endemic spread of the disease as drug-resistant strains are rapidly emerging, including MDR and extensively drug resistance (XDR) TB (Jyoti et al., 2016). MDR TB strains are those that are resistant to isoniazid and rifampin, but XDR TB strains are resistant to all of the injectable medicines, including kanamycin, amikacin, and capreomycin, as well as isoniazid, rifampin, fluoroquinolone, and both (Barry et al., 2009; Ganihigama et al., 2015). This pathogen is highly contagious and places a high risk of infection and morbidity rate among immunocompromised patients (Fauci and Group, 2008). Although a person with the dormant form of M. tuberculosis is unlikely to spread the disease, they are

nonetheless at risk for reinfection, especially if they have the HIV, some malignancies, or diabetes (Barry et al, 2009 Koul et al 2011; Ganihigama, et al, 2015). The finding of new drugs is needed to fight against against MDR and XDR-TB (Gillespie, 2002). Ongoing identification of novel drugs may be supported through the screening of natural resources (Copp and Pearce, 2007).

According to Gurib-Fakim et al. (2012), plants may possess needed antibiotics. Remedies that have been derived from herbal or folklore plant extracts and pure compounds that have been isolated from these plants are still being used as traditional medicine around many areas of the world and a remarkable number have been reported as having *in vitro* activity against *M. tuberculosis* (Jyoti et al., 2016) Some natural compounds, such as homoisoflavonoids, sampangine, and cleistopholine, as well as tryptanthrins, have been reported to be effective anti-TB scaffolds for future drug development (Yempala et al., 2012; Claes et al.., 2013; Hwang et al., 2013; Petkova et al., 2014; Stavrakov et al., 2014; Mahajan et al., 2014).

3.1.4. Aim of the study

The chapter's main objective was to carry out dilution bioassays, which are common techniques for comparing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts used to treat elephantiasis in the Eastern Cape of South Africa.

3.2 Materials and methods

3.2.1. Preparation of extracts

From three municipal areas—Ingquza Hill, Nyandeni, and King Sabata Dalindyebo Municipal areas—within the OR Tambo District of the Eastern Cape Province, South Africa, plants were gathered through surveys with traditional healers and herbalists. The harvested plant components were cleaned, reduced in size, and dried at 45 degrees Celsius before being blended (MRC Laboratory Equipment, Durban) (**Figure 3.1**). Figure 3.2 shows how about 25 g of the powdered plant material were extracted using 250 ml each of acetone, ethanol, ethyl acetate, and distilled water (Labcon,

Maraisburg) at room temperature for 24 h (**Figure 3.3**). The extracts were then run through discs made of Whatman No. 1 filter paper (Whatman, United Kingdom) and left to dry at room temperature in front of a fan until a constant dry weight was obtained. To achieve a final concentration of 50 mg/ml stock solution for antibacterial activity and 100 mg/ml for antifungal and antimicrobial activity, the extract residues were re-dissolved in their respective solvents.

Fig 3.1: A blender was used to turn dried plants into a fine powder.

Figure 3.2: For 24 hours, powdered plant material was extracted using the appropriate solvents.

3.2.2 Screening for antibacterial activity

From the Department of Biochemistry and Microbiology at the University of Fort Hare in South Africa, a total of eight bacterial strains were obtained. The following bacterial strains were kept on agar plates: *Escherichia coli* (ATCC 8739), *Enterococcus faecalis* (laboratory isolate), *Klebsiella pneumoniae* (ATCC 25922), *Pseudomonas aeruginosa* (laboratory isolate), *Proteus vulgaris*, *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 6538). Transferring a single colony in 10 ml of previously prepared Mueller-Hinton broth medium revitalized the strains (MH broth) (Oxoid Ltd., Basingstoke, Hampshire, England). The infected medium was then placed in a shaking water bath at 37 °C for 24 hours at 80 rpm (Labcon, Laboratory Consumables Chemical Supplies). To ensure that the bacteria were in the beginning of the log phase when the test started, the saturated bacterial cultures were then diluted with MH broth (1 ml bacteria: 99 ml broth).

For plant extracts having antibacterial activity, the MIC and MBC values were calculated using Eloff's microplate technique from 1998. (Figure 3.1). In 96-well microplates, extracts were first evaluated at a concentration of 12.5 mg/ml. They were

then serially diluted twice, to 0.098 mg/ml, and 100 µl of bacterial cultures were then added to each well. The extracts were tested in triplicates. The suspension cultures were made by combining 20 ml of MH broth with 200 µl of bacterial stock cultures, incubating at 37 °C in a water bath for 24 hours, and then transferring the mixture to an orbital shaker. As a positive control, neomycin (Sigma-Aldrich, Germany) (100 g/ml) (100 ml: 0.4 mg/ml) was utilized. For 24 hours, the microplates were incubated at 37°C. The bacterial growth indicator 40 µl p-iodonitrotetrazolium violet (INT) (Sigma) was dissolved in water at 0.2 mg/ml and added to the wells, and incubated at 37°C for 30 minutes. The MIC values were calculated as the lowest concentrations of the extract, or a clean well, that fully prevented bacterial growth. After 72 hours, the MBC values were captured.

3.2.3 Antifungal screening

Three types of fungi in order to assess the antifungal activity, *Candida albicans*, *Candida vulgaris*, and *Trichophyton mucoides* were utilized. The broth microdilution test using the NCCLS recommended method (M27-P) was modified. (Espinel-Ingroff and Pfaller, 1995). 400 µl milliliters of 24-old fungal cultures received 4 milliliters of sterile saline addition. In order to match the absorbance of a 0.5 McFarland standard solution, the absorbance was measured at 530 nm and corrected with sterile saline. A 1:1000 dilution of the stock cultures in broth (for example, 10 l stock culture: 10 ml broth) was made from the prepared stock cultures. A 96-well microplate had 100 microliters of broth added to each well. The organic solvent extracts were redissolved in dimethyl sulfoxide (DMSO), whereas the aqueous extracts were redissolved in water. One hundred microlitres of the water extract were added to well (A) and serially diluted by taking 100 µl from well (A) and added into well (B). This two-fold dilution was continued down the plate and 100 µl from the last well (H) was discarded. In the case of organic solvent extracts, 25 µl of the extracts were added to 175 µl broth in well (A) and serially diluted by taking 100 µl from well (A) and added to well (B). The final 100 µl of the serial dilution was discarded after continuing down the plate to well (H). For each extract, three duplicates were created. A 100 µl stock culture was added to each well. As a positive control, amphotericin B was utilized, and the controls prepared were wells containing only broth and a fungal strain without extract. At 37°C, the microplates were incubated over night. The wells were filled with 40 µl of INT

dissolved in water at 0.2 mg/ml as a fungal growth indicator, and the mixture was incubated at 37°C for 30 minutes.

3.2.4. Antimycobacterial screening

10% OADC (oleic acid + albumin + dextrose + catalase) Middlebrook 7H9 broth was used to maintain *Mycobacterium tuberculosis* (ATCC 25177). The stock bacterial culture was transferred to supplemented 7H9 broth (Middlebrook 7H9 + 10% OADC) to create the inoculum, which was then cultured for 72 hours on a shaker. Two (5 ml) supplemented 7H9 broths were inoculated with bacterial culture and grown for 72 h. Each culture received a 20% sterile glycerol addition, and 500 µl aliquots were prepared into sterile Eppendorf tubes. These stocks were kept at -30°C and were known as G1 stocks. Middlebrook 7H10 agar (7H10 + 10% OADC) plates supplemented with a single G1 stock were then incubated at 37°C for four days or until growth was visible. A single colony was taken from this culture and used to inoculate 5 ml of enriched 7H9 broth. This was employed in the experiment and was cultivated on a shaker for 72 hours at room temperature.

The MIC and MBC values of the examined plant extracts against *M. tuberculosis* were calculated using the broth microdilution method (Swenson et al., 1982). The organic solvent extract residues were redissolved in DMSO, whereas the aqueous extract residues were redissolved in water. One hundred microliters of the supplemented 7H9 broth were added to all the wells of microtitre plates. All extracts were tested at a concentration of 25 mg/ml and serially diluted to 0.195 mg/ml. At 550 nm, the optical density of the 72-hour broth culture was measured and corrected. Each well of the microtitre plate received 100 microliters of the diluted culture. Middlebrook 7H9 broth alone, the solvent used to dissolve plant extracts, and the antibiotic streptomycin (1.56 mg/ml) were the controls. The plates were covered and kept at 37°C for 72 hours of incubation. Following incubation, 40 µl of an INT solution containing 0.4 mg/ml were applied to each well on the plate. At 37°C, the plates were covered and left incubating for 24 hours. Each extract underwent three separate tests.

3.3 Results and discussion

Crude extracts of each 8 plants, making a total of 32 extracts, were each tested for the presence of antibacterial, antifungal and antimycobacterial properties by screening them against pathogenic strains**.** For antibacterial activity, *H. albiflos* and *C. dentata* water extracts gave the highest yield at 5550 and 4070 mg respectively. *R. melanophloeos* gave the highest yields for acetone and ethanol at 2820 and 2630 mg respectively. For antifungal and antimycobacterial activity, C. brachiata ethanol extract gave the highest yield at 44 190 mg and *A. oblongifolia* and acetone and ethanol extracts were 13 400 and 17 400 mg respectively. The study's high yields for water, ethanol, and acetone extracts may be due to the plant's ability to extract the greatest variety of various compounds and substances. According to Eloff (1998), acetone extracts both polar and nonpolar inhibitory compounds. Water and ethanol were found to extract mostly polar compounds (Ncube, et al., 2012).

3.3.1 Antibacterial activity

Table 5.1 lists the antibacterial properties of crude acetone, ethanol, ethyl acetate, and aqueous extracts. The MIC values and bacteriostatic (MBC) activity values of the organic solvent extracts of *A. oblongifola* showed good inhibitory activities against all bacterial strains and ranged between 0.098 mg/ml and 0.78 mg/ml. Water, ethanol, and ethyl acetate extracts all showed some efficacy at 1.56 mg/ml against *S. aureus* and *P. aeruginosa*, respectively. *P. aeruginosa*, *S. flexneri*, and *E. faecalis* were all targets of the bacteriostatic (MBC) activity.

The bactericidal qualities of crude acetone, ethanol, ethyl acetate, and aqueous extracts are listed in Table 5.1. The organic solvent extracts of *A. oblongifola* demonstrated good inhibitory activities against all bacterial strains, with MIC values and bacteriostatic (MBC) activity values ranging between 0.098 mg/ml and 0.78 mg/ml. At 1.56 mg/ml, the extracts of water, ethanol, and ethyl acetate all demonstrated some effectiveness against *S. aureus* and *P. aeruginosa*. The bacteriostatic (MBC) activity had as its targets *P. aeruginosa*, *S. flexneri*, and *E. faecalis*.

C. dentata acetone and ethanol extracts showed excellent MIC values and bacteriostatic activity against all the test microorganisms but the extracts had no bacteriostatic effects against *P. vulgaris, S. flexneri*, *K. pneumonia* and *E. faecalis*.

The water extract showed poor activity against the strains at 3.125 mg/ml except against *P. aeruginosa* where it showed a good inhibition at 0.98 mg/ml.

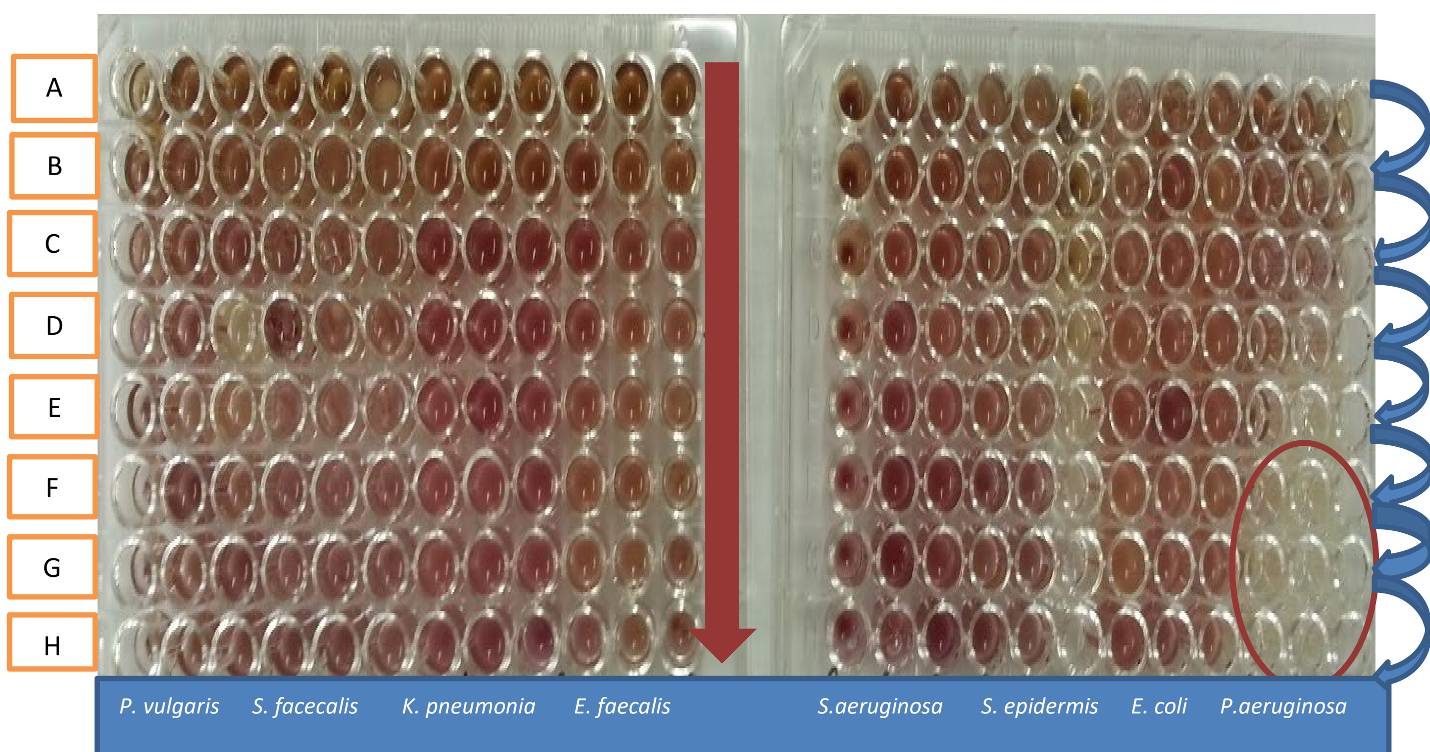


Fig 3.3: Water extracts of *C. dentata* show the lowest inhibition against *P. aeruginosa* at 0.098 mg/ml**.** The orange circle indicates minimum inhibitory concentrations.

All test microorganisms were inhibited by *D. sylvatica* acetone and ethanol extracts, with MIC values ranging from 0.098 to 0.78 mg/ml. Between 0.39 and 1.56 mg/ml, the extracts also exhibited bacteriostatic effects on all test bacteria. MIC values of 1.56 mg/ml for the water extract indicated the least activity. With MBC values ranging from 0.39 to 0.78 mg/ml, the same extract also shown bacteriostatic capabilities against *S. aureus*, *S. epidermis*, *E. coli*, *P. aeruginosa*, and *P. vulgaris*.

With the exception of *S. epidermis*, which was inhibited at 0.78 mg/ml, the *D. sylvatica* ethyl acetate extract had weak MIC activity against the bulk of the test bacterial strains.

At 0.098 mg/ml, *G. perpensa* demonstrated excellent MIC activity against all bacteria, while bacteriostatic activity was seen at 0.78 mg/ml. Ethanol extract also showed good MIC activity but it displayed no bacteriostatic effect. Ethyl acetate and water extracts showed good inhibition against all the test strains but no bacteriostatic effects were observed.

While acetone extract had low action against the strains between 3.125 mg/ml and 6.25 mg/ml for MBC, *H. albiflos* water and ethanol extracts showed no activity against certain bacteria. At 0.098 mg/ml and 0.39 mg/ml, respectively, only ethyl acetate extract demonstrated efficacy against *S. flexneri* for MIC and MBC.

With MIC values ranging from 0.098 to 0.39 mg/ml and bacteriostatic activity values of 0.098 mg/ml, the acetone and ethanolic extracts of *K. drepanophylla* demonstrated good activity against all bacterial strains. According to Mahachi's (2013) investigation into the antibacterial properties of *K. drepanophylla*, water extracts were active against all five Gram-negative bacteria tested—*Citrabacter freundii*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Proteus mirabilis*; while methanol inhibited the strains at concentrations of 5.0, 10.0, 5.0, 7.0, and 1.0 At 0.5 mg/ml, acetone extract only inhibited *C. freundii* and *P. mirabilis*.

With MIC values ranging from 0.098 to 0.78 mg/ml, *R. melanophloeos* acetone, ethanolic, and water extracts shown good efficacy by inhibiting all test bacterial strains. The bacteriostatic activities against *P. vulgaris*, *S. flexneri*, *K. pneumoniae*, and *E. faecalis* were good (0.098 mg/ml) and moderate (1.56 mg/ml).

In a study by Mostafa and Afolayan (2012), the water extract of *C. brachiata* showed antibacterial activity against *P. aeruginosa* and *S. flexneri*, while the acetone extract of *C. brachiata* inhibited the growth of all test microorganisms between 1.0 and 3.0 mg/ml, while the methanol extract inhibited the growth of all test microorganisms between 2.0 and 3.0 mg/ml. In Mexico, the leaves of *C. drummondi* are used as an antibiotic and a disinfectant. (Cantrell et al., 1998; Mostafa et al., 2018).

In additional research, acetone extracts of *C. dentata's* leaves, twigs, and stem bark showed effectiveness against *C. albicans*, *E. coli*, *S. aureus*, *P. aeurginosa*, and *S. feacalis* as well as suppression of parasitic and free-living nematodes (Shai et al., 2008; 2009; Doughari et al., 2011). Compared to other extracts, acetone extracts of C. dentata were found to have significant antibacterial activity against Gram-negative bacteria (Wintola and Afolayan, 2017). The herb has historically been used by people to treat stomach problems, diarrhea, blood thinning, and as an aphrodisiac. (Hutchings et al., 1996; Pujol 2000). According to Nielsen et al. (2012), *C. dentata* stem bark extracts have MIC values of 156.25 g/ml against -lactamase, *E. coli*, ampicillin-resistant *K. pneumoniae*, chloramphenicol-resistant *Citrobacter*, methicillin-resistant *S. aureus*, and carbenicillin-resistant *P. aeruginosa*. Betulinic acid was discovered to have the strongest antibacterial action when compared to other substances that were discovered, including ursolic acid, lupeol, and -sitosterol (Fadipe et al., 2015).

There have been reports of the antibacterial activity of the 2, 6-dimethoxy-1, 4-benzoquinone molecule that was isolated from the roots of *G. perpensa*. Colds, endometritis, gonorrhea, bladder issues, and syphilis can all be treated using decoctions or infusions of the root or rhizome (Mc Gaw, et al., 2005; Buwa & Van Staden, 2006; Nzue, 2009; Maroyi, 2016).

Due to the large number of serious infections it is associated with and the issue with the lack of effective therapies, *K. pneumoniae* had lately come to be known as a resistant infectious bacterium. Due to these worrying circumstances, new strains of *K. pneumonia* have emerged that are either hypervirulent (HV) or antibiotic-resistant due to the genetic features they have acquired (Bagley, 1985; Merino et al, 1992; Paczosa & Rock et al., 2014; Mecsas, 2016). The bacterium is gaining momentum of antibiotic resistance, including resistance to cephalosporins and β-lactams which are broad-spectrum antibiotics (Wu & LI, 2015; Gouby et al., 1994. As a result, *Klebsiella* infections frequently result in high death rates, especially in those with compromised immune systems who are afflicted (Stahlhut et al, 2012; Wu & Li, 2015).

K. drepanophylla aqueous extract inhibited *E. coli* and *P. aeruginosa* at 0.098 and S. *aureus* and *S. epidermis* at 0.39 mg/ml which are good MIC values concerning an aqueous extract since most of the traditional healers and herbalist use water for the extraction methods. *P. aeruginosa* is commonly found in soil and water as well as in plants and humans. Pseudomonas bacteria are believed to be one of only a few true pathogens for plants. Importantly, *P. aeruginosa* has become an emerging opportunistic pathogen in clinics. Recent epidemiological investigations, particularly those involving strains with elevated antibiotic resistance, show its nosocomial pathogen status. One of the most significant categories of opportunistic infections in humans is *P. aeruginosa*. Although it seldom affects unaffected organs, the bacteria can attack any tissue that is immunodeficient. In particular in individuals with severe burns, tuberculosis, cancer, and AIDS, it causes infection in the urinary tract, respiratory system, dermis, soft tissue, bacteremia, bone, joint, gastrointestinal, and blood. *P. aeruginosa* has resistance to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics the bacterium is naturally resistant to many antibiotics. The Gram-negative outer membrane of the bacteria provides a permeability barrier that makes it inherently resistant to several drugs. These characteristics of *P. aeruginosa* are crucial to its ecological success, which explains the organism's ubiquity and significance as a nosocomial infection. The genus Kniphofia is a rich source of pharmacologically active monomeric and dimeric anthraquinones, anthrones, phenylanthraquinones, and oxanthrones (Shenkute, 2018).

R. melanophloes extracts demonstrated good antibacterial activity against the six bacterial strains examined, including *B. cereus*, *E. coli*, *E. faecalis*, *K. pneumoniae*, and *S. epidermidis*, according to Lukhele et al. (2011).

Table 3.1: Antibacterial activity of traditional medicinal plant extracts used against elephantiasis in the Eastern Cape (MIC and MBC values in mg/ml).

Plant Name and plant part	Extract	Extract yield (mg)	Bacterial strain							
			Sa	*Se*	*Ec*	*Pa*	*Pv*	*Sf*	*Kp*	*Ef*
			MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
A. oblongifolia	Acet	1884	0.78/0.78	0.098/0.098	0.39/0.39	0.098/0.098	0.78/0.098	0.78/0.39	0.39/0.098	0.098/0.098
	EtoH	2070	0.78/0.78	0.098/0.098	0.78/0.78	1.56/1.56	0.39/0.39	0.098/0.098	0.78/0.78	0.098/0.098
	ETAH	612	1.56/1.56	0.098/0.098	0.39/0.098	0.098/0.098	3.125/3.125	0.39/0.098	12.5/12.5	0.098/0.098
Leaves	H_2O	1834	1.56/1.56	0.098/0.098	3.125/3.125	3.125/0.098	3.125/3.125	3.125/0.098	3.125/3.125	3.125/0.098
C. brachiata	Acet	819	1.56/0.39	0.098/0.098	0.098/0.098	0.098/0.098	0.195/0.195	3.125/3.125	3.125/3.125	3.125/0.098
	EtoH	1715	3.125/3.125	0.195/0.39	0.78/0.78	1.56/0.098	1.56/1.56	3.125/3.125	3.125/3.125	3.125/3.125
Leaves	ETAH	596	0.098/0.195	0.098/0.098	0.098/0.098	0.098/0.098	3.125/3.125	0.098/0.098	3.125/3.125	0.098/0.098
	d H_2O	930	0.78/0.78	0.098/0.098	6.25/6.25	0.39/0.39	3.125/3.125	12.5/12.5	6.25/6.25	3.125/3.125
C. dentata	Acet	1504	0.098/0.098	0.098/0.098	0.098/0.098	0.098/0.098	0.098/12.5	0.195/12.5	0.195/12.5	0.195/12.5
	EtoH	1600	0.098/0.098	0.195/0.195	0.098/0.098	0.195/0.195	0.098/12.5	0.098/12.5	0.098/12.5	0.098/12.5
Bark	ETAH	693	6.25/12.5	0.195/12.5	1.56/12.5	0.098/6.25	0.78/12.5	0.39/12.5	0.39/0.39	0.39/0.39
	d H_2O	4070	3.125/3.125	3.125/3.125	3.125/3.125	0.098/0.39	3.125/3.125	3.125/3.125	3.125/3.125	3.125/0.39

Plant Name and plant part	Extract	Extract yield (mg)	Bacterial strain							
			Sa	*Se*	*Ec*	*Pa*	*Pv*	*Sf*	*Kp*	*Ef*
			MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
D. sylvatica Stem tuber	Acet	380	0.098/0.78	0.098/0.78	0.098/0.78	0.098/0.78	0.195/0.39	0.195/0.39	0.78/1.56	0.195/0.39
	EtoH	910	0.098/0.78	0.098/0.78	0.098/1.56	0.098/0.78	0.39/0.78	0.098/0.78	0.78/0.78	0.39/0.78
	ETAH	250	1.56/6.25	0.78/3.125	1.56/6.25	6.25/6.25	3.125/6.25	3.125/6.25	3.125/3.125	3.125/3.125
	d H_2O	410	1.56/0.39	1.56/0.39	1.56/0.39	1.56/0.78	1.56/0.78	1.56/1.56	1.56/1.56	1.56/1.56
G. perpensa Rhizome tuber	Acet	1030	0.098/0.78	0.098/0.78	0.098/0.78	0.098/0.78	0.39/0.39	0.195/0.78	0.39/0.78	0.098/0.78
	EtoH	1790	0.098/12.5	0.39/6.25	0.098/6.25	0098/6.25	0.098/12.5	0.195/12.5	0.195/12.5	0.195/12.5
	ETAH	250	0.78/0.78	0.78/0.78	0.78/0.78	0.098/1.56	0.39/0.78	0.195/0.39	0.39/0.78	0.78/1.56
	d H_2O	1780	0.195/6.25	0.78/6.25	0.098/6.25	0.098/6.25	0.39/6.25	0.195/6.25	0.098/6.25	0.195/6.25

Plant Name and plant part extracted	Extract	Extract yield (mg)	Bacterial strains							
			Sa	*Se*	*Ec*	*Pa*	*Pv*	*Sf*	*Kp*	*Ef*
			MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
H. albiflos	Acet	160	3.125/3.25	3.125/6.25	6.25/3.125	3.125/6.25	3.125/6.25	3.125/6.25	3.125/12.5	6.25/12.5
	EtoH	897	12.5/NA	NA/NA	12.5/NA	NA/NA	NA/NA	NA/NA	12.5/NA	12.5/NA
Bulb, Roots	ETAH	80	12.5/NA	12.5/NA	12.5/NA	12.5/NA	3.125/6.25	0.098/0.39	6.25/12.5	3.125/6.25
	D H_2O	5550	NA/NA	NA/NA	12.5/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
K. drepanophylla	Acet	371	0.39/0.098	0.195/0.098	0.39/0.098	0.39/0.098	0.195/0.098	0.78/0.098	0.78/0.098	0.78/0.098
	EtoH	1280	0.78/0.098	0.39/0.098	0.39/0.098	0.39/0.098	0.098/0.098	0.098/0.098	0.098/0.098	0.098/0.098
	ETAH	335	6.25/6.25	0.39/0.39	6.25/6.25	1.56/6.25	1.56/1.56	0.098/0.098	3.125/3.125	0.39/0.39
Rhizome	d H_2O	3600	0.39/0.39	0.39/0.39	0.098/0.098	0.195/0.195	0.78/0.78	1.56/1.56	1.56/1.56	0.098/0.098
R. melanophloes	Acet	2820	0.39/3.125	0.39/3.125	0.78/3.125	0.195/3.125	0.195/1.56	0.195/1.56	0.39/1.56	0.39/1.56
	EtoH	2630	0.195/3.125	0.195/3.125	0.195/3.125	0.098/3.125	0.098/0.098	0.39/0.098	0.195/0.098	0.78/0.098
	ETAH	440	6.25/6.25	6.25/6.25	6.25/6.25	6.25/3.125	3.125/6.25	3.125/3.125	3.125/3.125	6.25/3.125
Bark	d H_2O	3330	0.195/6.25	0.195/3.125	0.39/3.125	0.39/3.125	0.39/6.25	0.78/3.125	0.78/3.125	0.195/3.125
Neomycin(µg/ml)			3.125/3.125	0.39/0.39	0.78/0.78	0.78/0.78	3.125/3.125	0.39/0.39	0.78/0.78	3.125/3.125

S.a., Staphylococcus aureus, S.e., E.c., Escherichia coli, P.a., Pseudomonas aeruginosa P.v., Proteus vulgaris, S.f., Staphylococcus flexneri, Klebsiella pneumoniae, E.f, Escherichia faecalis
Ace., acetone, EtOH., ethanol, ETAH., Ethyl acetate., d H20., distilled water.

3.3.2 Antifungal activity

In Table 3.2, the antifungal activity results are shown. MIC and MFC values between 0.098 mg/ml and 0.78 mg/ml were judged to indicate very active extracts, 1.56 mg/ml indicated moderate activity, and 3.125 to 6.25 mg/ml indicated poor activity.

In contrast to the water extract of *A. oblongifolia*, which showed action against the three fungi strains at 0.78 mg/ml for MIC and MFC, *A. oblongifolia* and *C. brachiata* extracts showed inadequate inhibition against the three fungal strains between 3.125 and 6.25 mg/ml. At 10 mg/ml, the acetone extract of C. brachiata completely inhibited the growth of the fungi *C. albicans*, *A. flavus*, *P. notum*, and *A. niger*, according to Mostafa and Afolayan (2013).

Although no MFC activity was found, *C. dentata* aqueous and ethanolic extracts shown outstanding efficacy against all three fungal strains. According to reports, extracts from *C. dentata* leaves have the ability to fight against *C. albicans* and a number of other fungi (Shai 2007; Shai, McGaw, Masoko & Eloff 2008). The isolated compound betulinic acid has the lowest MIC value of 0.008 and 0.02 for MFC against *C. albicans* in a study by Fadipe et al. (2015) when compared to the other isolated compounds. Extracts of *D. sylvatica* showed poor inhibition of the fungal strains but water extracts showed good inhibition of *C. vulgaris* and *T. mucoides* at 0.78 mg/ml.

Between 0.098 mg/ml and 0.39 mg/ml, *G. perpensa* acetone and water extracts showed excellent efficacy against all three fungal strains. Only the water extract had MFC activity between 0.39 mg/ml and 0.78 mg/ml against the fungi strains. The antifungal activity of *G. perpensa* rhizome extracts in water, petroleum ether, dichloromethane, and 80% ethanol against *C. albicans* was examined using the microdilution technique in a related study by Ndhlala et al. (2011). The MIC and MFC values for the extracts ranged from 0.093 to 6.25mg/ml, while ethanol had the strongest antifungal action, with MIC and MFC values of 0.78mg/ml and 0.093mg/ml, respectively.

Acetone and aqueous extracts of *K. drepanophylla* had the best MIC values, 0.39 mg/ml for *C. vulgaris* and *T. mucoides* and 0.098 mg/ml against *C. albicans*. MFC values ranged from 0.098 mg/ml to 0.39 mg/ml for all three test fungal strains.

A good level of antifungal activity was shown by *R. melanophloeos* acetone and ethanol extracts against *C. albicans* at 0.098 mg/ml and 0.78 mg/ml against *C. vulgaris* and *T. mucoides*, respectively. No MFC actions were seen against the fungi strains.

H. albiflos extracts showed no signs of action. Aqueous and organic extracts of both bulbs and leaves that were tested against two clinical isolates of *C. albicans* from some afflicted patients were shown to be ineffective in a study on the antifungal properties of the Amaryllidaceae family. According to research, *C. albicans* is more resistant to the majority of plant extracts (Heisey and Gorham, 1992; Buwa and Van Staden, 2006; Ncube et al., 2011). Among HIV/AIDS patients, candidiasis is a prevalent opportunistic illness and one of the leading causes of death in underdeveloped nations. (Reichart, 2003; Ncube et al., 2012). The antifungal activity of *H. albiflos* leaf and bulb extracts tested against two clinical isolates from certain infected patients was shown not to exhibit antifungal activity in a study by Nair and van Staden (2017).

Table 3.2: Antifungal activity of traditional medicinal plants used against elephantiasis in the Eastern Cape (MIC and MFC values in mg/ml).

Plant Name and plant part	Extract	Extract yield (mg)	Fungal strains		
			Ca	*Cv*	*Tm*
			MIC/MFC	MIC/MFC	MIC/MFC
A. oblongifolia	Acet	13400	6.25/6.25	3.125/3.125	6.25/6.25
	EtoH	17440	6.25/6.25	6.25/6.25	6.25/6.25
Leaves	ETAH	6880	6.25/6.25	6.25/6.25	6.25/6.25
	d H_2O	1250	0.78/0.78	0.78/0.78	0.78/0.78
C. brachiata	Acet	5510	6.25/6.25	3.125/3.125	3.125/3.125
	EtoH	44190	6.25/6.25	6.25/6.25	6.25/6.25
Leaves	ETAH	4210	3.125/3.125	3.125/3.125	3.125/3.125
	d H_2O	2683	3.125/3.125	3.125/3.125	3.125/3.125
C. dentata	Acet	1430	1.56/3.125	0.39/3.125	1.56/3.125
Bark	EtoH	1360	0.195/3.125	0.195/3.125	0.78/3.125
	ETAH	580	0.78/12.5	0.39/12.5	0.39/12.5
	d H_2O	4050	0.098/3.125	0.098/1.56	0.098/3.125

Plant Name and plant part	Extract	Extract yield (mg)	Fungal strains		
			Ca	*Cv*	*Tm*
			MIC/MFC	MIC/MFC	MIC/MFC
D. sylvatica Stem tuber	Acet	310	1.56/1.56	1.56/1.56	1.56/1.56
	EtoH	860	3.125/3.125	1.56/3.125	1.56/3.125
	ETAH	190	1.56/3.125	1.56/3.125	1.56/3.125
	H_2O	370	1.56/1.56	0.78/1.56	0.78/1.56
G. perpensa Rhizome tuber	Acet	940	0.195/1.56	0.195/1.56	0.195/1.56
	EtoH	1480	1.56/3.125	0.78/1.56	0.78/1.56
	ETAH	140	0.78/1.56	0.78/0.78	0.78/0.78
	d H_2O	1620	0.195/0.39	0.098/0.39	0.39/0.78

Plant Name and plant part extracted	Extract	Extract yield (mg)	Fungal strains		
			Ca	*Cv*	*Tm*
			MIC/MFC	MIC/MFC	MIC/MFC
H. albiflos	Acet	121	3.125/6.25	3.125/6.25	6.25/12.5
	EtoH	740	12.5/NA	6.25/12.5	NA/NA
Bulb, Roots	ETAH	50	3.125/6.25	6.25/12.5	12.5/NA
	d H_2O	4100	6.25/12.5	6.25/12.5	NA/NA
K. drepanophylla	Acet	322	0.098/0.78	0.39/0.78	0.39/0.78
	EtoH	1088	0.78/1.56	0.78/1.56	1.56/1.56
	ETAH	280	3.125/0.78	3.125/0.78	3.125/0.78
Rhizome	d H_2O	1400	0.098/0.0195	0.39/0.39	0.39/0.39
R. melanophloeos	Acet	1750	0.098/6.25	0.78/6.25	0.78/6.25
	EtoH	2560	0.78/12.5	0.78/12.5	0.39/12.5
Bark	ETAH	330	1.56/6.25	0.78/6.25	1.56/6.25
	d H_2O	3220	1.56/12.5	0.39/6.25	0.39/6.25
Amphotericin B *(µg/ml)*			0.039	0.039	0.039

Ca: Candida albicans, Cv: Candida vulgaris, Tm.: Trichophyton mucoides. Ace: acetone, EtOH: ethanol, ETAH., ethyl acetate., d H_20., distilled water

Figure 3.4. Figure showing water extracts of *G. perpensa* active against *C. vulgaris* with the lowest MIC value at 0.098 mg/ml and the arrow shows ethyl acetate extract showing the MIC value for all the fungal strains (*C. albicans*, *C. vulgaris*, *T. mucoides*) at 0.78 mg/ml.

3.3.3 Antimycobacterial activity

The results for the antimycobacterial activity are represented in table 5.2.

Only the aqueous extract of A. oblongifolia, with a good MFC value of 0.78 mg/ml, demonstrated good antimycobacterial action against the TB strain.

C. brachiata, acetone, ethanol and water showed moderate activity at 1.56 mg/ml and ethyl acetate extract showed better activity at 0.39 mg/ml but it displayed no bacteriostatic effect.

The TB strain was resistant to the *C. dentata* acetone, ethanol, and ethyl acetate extracts at 1.56 mg/ml with excellent MIC values and a mild bacteriostatic impact. The aqueous extract showed moderate MIC activity at 1.56 mg/ml.

Concerning *D. sylvatica*, only ethanol and ethyl acetate extracts showed displayed good inhibition.

Water extract of *G. perpensa* had weak action, ethyl acetate had moderate inhibition at 1.56 mg/ml, and the acetone and ethanol extracts of the organism showed good inhibition.

The best MIC value was shown by *H. albiflos* water extracts, which were 0.195 mg/ml for MBC and 0.098 mg/ml for MIC. The bacteriostatic action of the extracts was seen at 1.56 mg/ml, while acetone, ethanol, and ethyl acetate extracts also had good activity between 0.39 mg/ml and 0.78 mg/ml.

The acetone and ethanol extracts' antimycobacterial effectiveness against *K. drepanophylla* was determined to be maximum at 0.195 mg/ml and 0.78 mg/ml for MBC, respectively. The TB strains' growth was decreased by ethyl acetate and aqueous extract at a concentration of 0.78 mg/ml, with the water extract also having a bacteriostatic effect.

Only the *R. melanophloeos* ethyl acetate extract, at 1.56 mg/ml, had moderate efficacy against the TB strain.

In several areas of southern Africa, medicinal plants are utilized to treat TB-related symptoms like coughing and chest pain (McGaw and Eloff, 2008; Dzoyem et al., 2016).

The rhizome of *K. drepanophylla* is used to heal wounds, pimples, acne, eczema, and ringworms, according to a study on plants that are used to cure skin conditions (Mahachi, 2013). According to Famewo et al. (2017), *K. drepanophylla* roots and *R. melanophloes* bark are two components of a polyherbal utilized as a herbal remedy for tuberculosis (Maroyi, 2019).

It is significant to note that while some *H. albiflos* extracts showed no efficacy against specific bacterial strains, an acetone extract of this plant inhibited *M. tuberculosis* at a concentration of 0.39 mg/ml. One of the most frequently utilized herbs to cure

tuberculosis was said to be *H. albiflos* (Lawal et al., 2014; Singh et al., 2015). Despite reports that the bulb was the plant part used in traditional anti-TB therapy in the eastern part of the OR Tambo district, the leaf and bulb parts extracts for the plant had shown comparable results in terms of antimycobacterial activity, with the acetone extracts showing MIC values of 0.63 mg/ml against two Mycobacterium strains (Madikizela and McGaw, 2018).

Table 3.3: Antimycobacterial activity of traditional medicinal plants used against elephantiasis in the Eastern Cape (MIC and MBC values in mg/ml).

Plant Name and plant part	Extract	Extract yield (mg)	Antimycobacterial strain
			M. tuberculosis
			MIC/MBC
A. oblongifolia Leaves	Acet	13400	0.195/3.125
	EtoH	17440	0.195/6.25
	ETAH	6880	0.39/3.125
	d H_2O	1250	0.39/0.78
C. brachiata Leaves	Acet	5510	1.56/1.56
	EtoH	44190	1.56/6.25
	ETAH	4210	0.39/6.25
	d H_2O	2683	1.56/1.56
C. dentata Bark	Acet	1580	0.098/1.56
	EtoH	1420	0.195/1.56
	ETAH	517	0.098/3.125
	d H_2O	3995	1.56/12.5
D. sylvatica Stem tuber	Acet	290	3.125/6.25
	EtoH	860	0.78/3.125
	ETAH	190	0.39/1.56
	d H_2O	150	6.25/12.5
G. perpensa	Acet	1010	0.39/0.39
	EtoH	1480	0.78/0.78
	ETAH	140	1.56/3.125

Rhizome tuber	d H_2O	170	3.125/12.5

Plant Name and plant part	Extract	Extract yield (mg)	Antimycobacterial strain
			M. tuberculosis
			MIC/MBC
H. albiflos Bulb, Roots	Acet	110	0.39/1.56
	EtoH	550	0.78/1.56
	ETAH	30	0.78/1.56
	d H_2O	3400	0.098/0.195
K. drepanophylla Rhizome	Acet	300	0.195/0.78
	EtoH	6660	0.195/0.78
	ETAH	305	0.78/1.56
	d H_2O	3350	0.78/0.78
R. melanophloeos Bark	Acet	3140	6.25/6.25
	EtoH	2970	12.5/12.5
	ETAH	416	1.56/6.25
	d H_2O	2963	12.5/12.5
Amphotecerin 0.098 μg/ml			

Acet., acetone, EtoH., ethanol., ETAH., ethyl acetate., d H2O., distilled water.

3.4 Conclusions

According to the study's findings, the studied plant species' extracts had good efficacy against both Gram-negative and Gram-positive bacteria, fungal strains, as well as the antimycobacterial strain. The results have also revealed that the plants have antibacterial, antifungal, and antimycobacterial properties. It can further be deduced that these plants contain bioactive compounds due to the presence of the phytochemicals that were tested. Acetone and ethanol were the most active solvents followed by the aqueous extracts and ethyl acetate. Minimal inhibition was detected for *H. albiflos* against the antibacterial strains but the plant was active against *M. tuberculosis*. These findings support the traditional healers' assertions that a variety of ailments can be treated using medicinal plants.

References

Bagley, S.T. (1985). Habitat association of *Klebsiella* species. *Infection Control* 6, 52–58.

Barry 3rd, C.E., Boshoff, H.I., Dartois, V., Dick, T., Ehrt, S., Flynn, J., Schnappinger, D., Wilkinson, R.J., Young, D. (2009). The spectrum of latent tuberculosis: rethinking the biology and intervention strategies, *Nature Reviews Microbiology*, 7, 845-855.

Buwa, L.V. & van Staden, J. (2006). Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *Journal of Ethnopharmacology*, 103, 139-42.

Claes, P., Cappoen, D., Mbala, B.M., Jacobs, J., Mertens, B., Mathys, V., Verschaeve, L., Huygen, K. & De Kimpe, N. (2013). Synthesis and antimycobacterial activity of analogues of the bioactive natural products sampangine and cleistopholine, *European Journal of Medical Chemistry*, 67, 98-110.

Clardy, J., & Walsh, C., (2004). Lessons from natural molecules. *Nature*, 432, 829–837.

Copp, B.R., & Pearce, A.N. (2007). Natural product growth inhibitors of Mycobacterium tuberculosis. *Natural Products Report*, 24(2), 278-297.

Dube, P., Meyer, S., & Marnewick, J. L. (2017). Antimicrobial and antioxidant activities

of different solvent extracts from fermented and green honeybush (Cyclopia intermedia) plant material. *South African Journal of Botany,* 110, 184– 193.

Dzoyem, J. P., Aro, A. O., Mcgaw, L. J., & Eloff, J. N. (2016). Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments. *South African Journal of Botany,* 102, 70–74.

Eloff, J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology, 60*(1), 1–8.

Ganihigama, D. U., Sureram, S., Sangher, S., & Hongmanee, P. (2015). Antimycobacterial activity of natural products and synthetic agents : Pyrrolodiquinolines and vermelhotin as anti-tubercular leads against clinical multidrug-resistant isolates of *Mycobacterium tuberculosis*. *European Journal of Medicinal Chemistry,* 89, 1–12.

Jyoti, M. A., Nam, K. W., Jang, W. S., Kim, Y. H., Kim, S. K., Lee, B. E., & Song, H. Y. (2016). Antimycobacterial activity of methanolic plant extract of *Artemisia capillaris* containing ursolic acid and hydroquinone against Mycobacterium tuberculosis*. Journal of Infection and Chemotherapy, 22*(4), 200–208.

Koul, A., Arnoult, E., Lounis, N., Guillemont, J., & Andries, K. (2011). The challenge of new drug discovery for tuberculosis, *Nature,* 469, 483-490.

Lawal, I.O., Grierson, D.S., & Afolayan, A.J. (2014). Phytotherapeutic information on the plants used for the treatment of tuberculosis in Eastern Cape Province, South Africa. *Evidence-Based Complementary and Alternative Medicine,* Article ID 73542, 1-11.

Mahajan, A., Hans, R., Chibale, K., & Kumar, V. (2014). Synthesis and medicinal chemistry of selected antitubercular natural products and natural product derivatives. *RSC Advances 4,15180-15215.*

Maroyi, A. (2019). A review of botany, medicinal uses, and biological activities of Pentanisia Prunelloides (Rubiaceae). *Asian Journal of Pharmaceutical and Clinical Research, 12(8), 1–5.*

Masoko, P., Picard, J., & Eloff, J. N. (2005). Antifungal activities of six South African Terminalia species (Combretaceae). J*ournal of Ethnopharmacology*, *99*(2), 301–308.

Mcgaw, L. J., Jager, A. K., & Staden Van, J. (2000). Antibacterial, anthelmintic and

anti-amoebic activity in South African medicinal plants. *Journal of Ethnopharmacology*, *72*(1-2), 247–263.

Mosier-Boss, P. A. *(2017). Review on SERS of Bacteria. Biosensors, 7(51), 1–26.*

Nair, J.J., & van Staden, J. (2017). Antifungal activity-based studies of Amaryllidaceae plant extracts. Natural Product Communications, *12*(12), 1953-1956.

Ncube, B., Finnie, J. F., & Van Staden, J. (2012). In vitro antimicrobial synergism within plant extract combinations from three South African medicinal bulbs. *Journal of Ethnopharmacology, 139(1), 81–89.*

Paczosa, M. K. & Mecsas, J. (2016). *Klebsiella pneumoniae* : Going on the Offense with a Strong Defense. *Microbiology and Molecular Biology Reviews*, 80(3), 629–661.

Perea, S., & Patterson, T. F. (2002). Antifungal Resistance in Pathogenic Fungi. *Clinical Infectious Diseases*, 35(9), 1073–1080.

Petkova, Z.,Valcheva, V., Momekov, G., Petrov, P., Dimitrov, V., Doytchinova, I., Stavrakov, G., & Stoyanova, M. (2014). Antimycobacterial activity of chiral amino alcohols with camphane scaffold. *European Journal of Medicinal Chemistry,* 81, 150-157.

Regiel-Futyra, A., Dąbrowski, J. M., Mazuryk, O., Śpiewak, K., Kyzioł, A., Pucelik, B., Brindell, M., &, & Stochel, G. (2017). Bioinorganic antimicrobial strategies in the resistance era. *Coordination Chemistry Reviews,* 351, 76–117.

Shenkute, K. (2018). Phytochemical Investigation and Antibacterial Evaluation of Roots of *Kniphofia schimperiana*. Dissertation. Adama Science and Technology University.

Singh, B., Jain, M., Singh, S. V, Dhama, K., Aseri, G. K., Jain, N., Datta, M., Kumar, N., Yadav, P., Jayaraman, S., & Gupta, S. (2015). Plants as Future Source of Anti-Mycobacterial Molecules and Armour for Fighting Drug Resistance. *Asian Journal of Animal and Veterinary Advances, 10(9), 443–460.*

Stavrakov, G., Philipova, I., & Valcheva, V., & Momekov, G. (2014). Synthesis and antimycobacterial activity of novel camphane-based agents. *Bioorganic & Medicinal Chemistry Letters, 24,165-167.*

Tchinda, C. F., Voukeng, I. K., Beng, V. P., & Kuete, V. (2017). Antibacterial activities of the methanol extracts of *Albizia adianthifolia*, *Alchornea laxiflora*, *Laportea ovalifolia* and three other Cameroonian plants against multi-drug resistant Gram-negative bacteria. *Saudi Journal of Biological Sciences, 24(4), 950–955.*

WHO. (2021). *Global Tuberculosis Report*. ISBN 978-92-4-003702-1

Wintola, O. A., & Afolayan, A. J. (2017). Aspects of Medicinal Activities of the Stem Bark Extracts of Curtisia dentata (Burm . F .) *International Journal of Pharmacology, 13(3), 237-246.*

Wu, M., & Li, X. (2015). *Klebsiella pneumoniae and Pseudomonas aeruginosa*. *Molecular Medical Microbiology (Second edition).* 3, 1547-1564.

Yempala, Y., Sriram, D., Yogeeswari, P. & Kantevari, S. (2012). Molecular hybridization of bioactives: synthesis and antitubercular evaluation of novel dibenzofuran homoisoflavonoids via Baylis-Hillman reaction*. Bioorganic &. Medicinal Chemistry Letters*, 22, 74.

CHAPTER 4

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS

4.1 Introduction

Phytochemicals are described as specialised metabolites that are produced by plants as mechanisms that assist the plants to cope with environmental stressors and as a source of several medicinal compounds (Defossez, et al., 2021). Plant phytochemicals also serve as a defense mechanism against microorganisms (Soliman et al., 2021). Plant phytochemicals offer a source of chances for creating novel immune system-attacking illness treatments. Because they originated as secondary biochemical routes for the production of a variety of chemical compounds from the basic metabolic functions of plants, secondary metabolites are a rich source of diverse substances found in natural plant-derived products (Yadav and Agarwala, 2011). The response is brought on by particular environmental triggers such as food scarcity, herbivore-induced damage, and pathogen attacks against bacteria, fungi, and viruses (Kennedy and Wightman, 2011; Saxena et al., 2013). These secondary metabolites account for the taste and colour of plants (Cowan, 1999).

Numerous phytochemicals have been shown to have positive biological effects, including those against cancer, bacteria, allergies, inflammation, diarrhea, and wound healing (Clarice et al., 2017). Ethnopharmacology was created as a result of the wide structural diversity of natural chemicals that can supply key components for therapeutic improvement, even through molecular alteration and microbial resistance to chemically synthesized medications (Chirumbolo, 2012; Inge et al., 2017). Understanding the pharmacological characteristics of medicinal plants is essential for understanding their phytochemistry, which entails the study of plant secondary metabolites thought to be responsible for a variety of biological functions (Aremu, 2009). The discipline of ethnopharmacology has been producing new medicines and

bio-prospecting, however elucidating found phytochemicals is time-consuming and expensive (George et al., 2001).

Similar to many other developing nations, South Africa has a rich floral biodiversity that serves as a natural resource for the production of herbal medicine, therefore medicinal plants and herbal formulations play vital roles in meeting people's daily healthcare needs (WHO, 1996; Mahomed and Ojewale, 2006). Over 4000 plant taxa are utilized nationwide in herbal preparations (Mulholland, 2005). Plant phytochemicals have therapeutic benefits because they protect against oxidative stress caused by free radicals and lack the negative side effects of synthetic antioxidants (Atala et al., 2009; Subedi et al., 2014; Takaidza, et al., 2018).

Proanthocyanidins, flavonoids, flavonols, phenols, and other substances have been shown in numerous studies to quench free radicals and prevent the generation of peroxides (Oyedemi et al., 2010; Mbaebie et al, 2012). Some plant constituents such as saponins, alkaloids, glycosides, and tannins have also been documented to exhibit various biological activities including anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities (Aiyegoro and Okoh, 2010; Mbaebie et al., 2012). The outer layers of the various plant tissues frequently contain the highest concentrations of phytochemicals, especially color compounds (Seethal et al., 2017). The action of flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, and isocatechins accounts for the majority of the antioxidant activity (Aqil et al., 2006; Nariya et al., 2013).

4.2 Aim of the study

In order to discover the key phytoconstituents present in plant extracts used to treat elephantiasis in the OR Tambo District, Eastern Cape Province, South Africa, the chapter conducted qualitative and quantitative phytochemical analyses.

4.3 Materials and methods

4.3.1 Plant collection and extraction

In Umtata, Lusikisiki, and Libode, three settlements in the Eastern Cape Province, plants were gathered with the aid of herbalists and traditional healers. The plant components were collected, dried for 24 hours at 50°C in an Ecotherm oven made by Labotec in South Africa, then ground in a blender (MRC Laboratory Equipment, Durban). Each plant material was collected and kept in sealed jar containers, labelled and then kept in a dry environment.

4.3.2 Qualitative phytochemical analysis

With some modifications from the methods previously reported by Harborne (1973) and Sofowara (1993), collected plant material was examined for the presence of secondary metabolites. The secondary metabolites were tested using either visual color change observation or the precipitate that formed after adding the designated reagent (s).

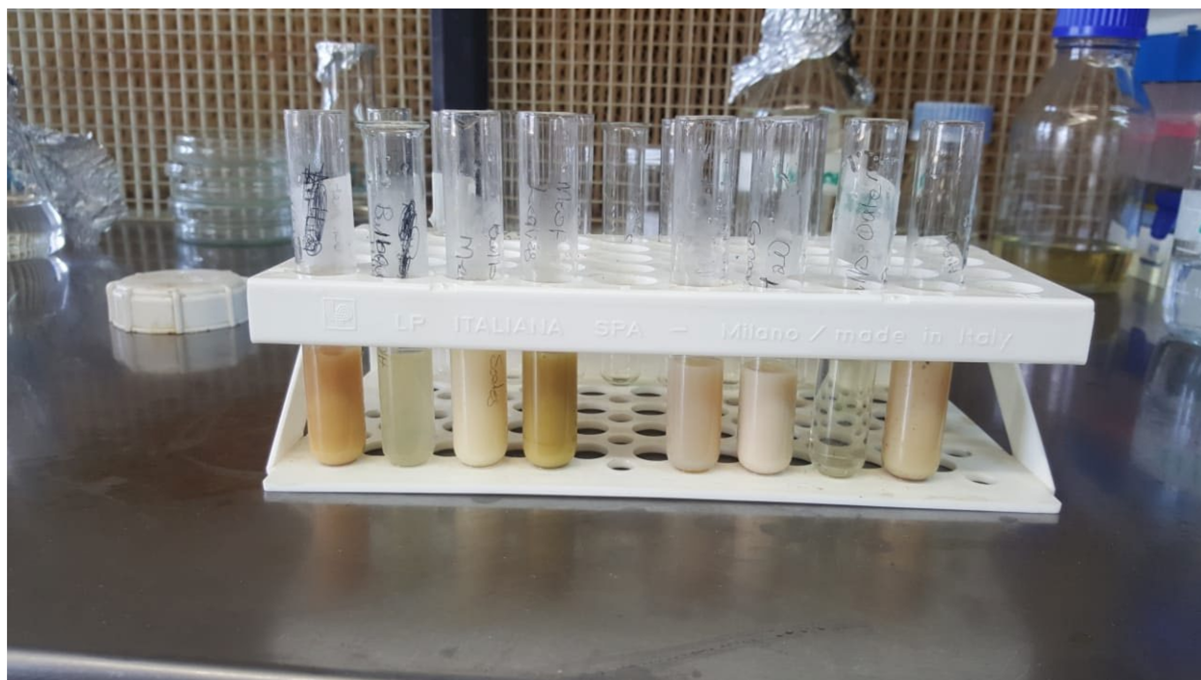


Figure 4.1: Analyses of phytochemistry displaying test tubes with various plant extracts.

4.3.2.1 Alkaloids test

In order to test for alkaloids, 0.5 g of the powdered plant material was agitated for 5 minutes in a steam bath with 5 ml of 1% aqueous hydrochloric acid solution. The resulting mixture was then run through Whatman No. 1 filter paper for filtration.

Dragendoff's reagent was then applied to one millimeter of the filtrate. After adding Dragendoff's reagent, an orange color in a precipitate indicated the presence of alkaloids. It was assumed that the turbidity of the precipitation with any of those reagents was indicative of the presence of alkaloids in the extract (Harborne, 1973).

4.3.2.2 Flavonoids test

1 g of the powdered substance was cooked for 3 minutes in a steam bath with 10 ml of ethyl acetate to produce flavonoids. After filtering the mixture, 1 ml of diluted ammonia solution and 4 ml of the filtrate were briskly shaken together. The appearance of yellow coloration was a sign that flavonoids were present (Sofowara, 1993).

4.3.2.3 Tannins test

0.5 g of the dried powdered sample was cooked in 20 ml of water in a test tube before being filtered to determine the sample's tannin content. When a few drops of 0.1% ferric chloride were applied, the presence of tannins was determined by looking for a brownish green or blue-black coloration (Sofowara, 1993).

4.3.2.4 Anthraquinones test

About 5 g of the powdered plant material was dissolved in 10 ml of benzene to test for anthraquinones, and the mixture was agitated until it was homogeneous. Following a filtering process, the liquid was mixed with 5 ml of chloroform. One milliliter of diluted ammonia was then pipetted into the newly created chloroform layer in another test tube. The pink color of the resultant solution was intended to serve as a positive indicator for the presence of anthraquinones (Harborne, 1973).

4.3.2.5 Saponins test

20 ml of distilled water was used to boil 2 g of powdered material in a water bath, and the mixture was then filtered. The filtrate was then combined with 5 ml of distilled water at a ratio of 10 ml, forcefully shaken, and checked for the presence of a stable, long-lasting foam. After adding three drops of olive oil to the foam, it was shaken, and the production of an emulsion was watched for as a sign of the presence of saponins (Harborne, 1973).

4.3.2.6 Terpenoids test

The plant extract was initially diluted in distilled water and filtered at a concentration of around 0.5 g. Three milliliters of pure H_2SO_4 were carefully added to five milliliters of plant extract, which had been dissolved in two milliliters of chloroform, to create a layer. Terpenoids were responsible for the interface's reddish-brown coloring (Harborne, 1973).

4.3.2.7 Cardiac glycosides (Keller-Killani test)

The plant matter was filtered after being dissolved in distilled water in an amount of around 0.5 g. Using 2 ml of glacial acetic acid and 1 drop of ferric chloride solution, 5 ml of the extract was processed. A 1ml layer of concentrated H_2SO_4 was placed underneath this. The interface displayed a brown ring that appeared to be a deoxysugar element common to cardenolides. Below the brown ring, a violet ring appears, and in the thin acetic acid layer, a greenish ring may grow gradually (Trease & Evans, 1989).

4.3.2.8 Steroids test

To check for steroids, 0.5 g of an ethanolic extract with a 2 ml concentrated H_2SO_4 was mixed with 2 ml of acetic anhydride. Steroids were present because the violet color turned blue (Harborne, 1973).

4.3.3 Quantitative phytochemical analysis

4.3.3.1 Process of making plant extracts

With the help of herbalists and traditional healers, plant material was gathered in three municipal regions of the OR Tambo District Municipality in the South African province of the Eastern Cape. The plant materials were cleaned, and an Ecotherm oven was used to dry them for 24 hours (Labotec, South Africa) at a temperature of 50°C and pulverized using a blender (MRC Laboratory Equipment, Durban). A shaker (Labcon, Maraisburg) was used to extract 25 g of dried plant material individually in 250 ml of distilled water, ethanol, and acetone for 48 hours at room temperature. The next step was to filter the extracts using a Buchner funnel and Whatman No. 1 filter paper (Whatman, United Kingdom). In front of a fan, the filtrates were separately

concentrated to complete dryness, and each extract was weighed until a steady weight was achieved.

4.3.3.2 Determination of total phenolic content

The Folin-phenol Ciocalteu's technique was used to determine the total phenolic content of the extracts. (Adedapo et al., 2009; Wolfe et al, 2003). Five milliliters of the extract were combined with four milliliters (75 g/l) of Na_2CO. The tubes were vortexed for 15 seconds before being left at 40°C for 30 minutes to develop their color. The Hewlett Packard UV-vis spectrophotometer was then used to measure absorbance at 765 nm. Plant extract samples were tested at a 0.1 mg/ml final concentration. Based on the calibration curve, the total phenolic content was expressed as mg/g tannic acid equivalent using the equation below: $y = 0.1216x$, $R^2 = 0.9365$, where y was the absorbance and x was the concentration (Adedapo et al., 2009).

4.3.3.3 Determination of total flavonoid content

The method outlined by Ordon Ez et al. was used to calculate total flavonoid contents (2006). The sample solution was mixed with 2% $AlCl_3$ ethanol in a volume of 0.5 ml. After an hour at room temperature, the absorbance at 420 nm was measured. The presence of flavonoids was indicated by the yellow color. The final concentration of the extract samples was 0.1 mg/ml. On the basis of the calibration curve, the following equation was used to determine the total flavonoid content as quercetin (mg/g): $y = 0.0255x$, $R^2 = 0.9812$, where x was the absorbance and was the quercetin equivalent (mg/g).

4.3.3.4 Determination of total tannin content

According to Biswas et al. (2017), 0.1 ml of the sample extract was first diluted with 7.5 ml of distilled water to evaluate its total tannin concentration. Test tubes containing the diluted extract solution were added 0.5 ml of the Folin-Ciolateu phenol reagent. After that, 10 mL of distilled water were added to the mixture in order to dilute 1 ml of a 35% sodium carbonate solution. After shaking, the mixture was let to stand at room temperature for 30 minutes. Using a spectrophotometer, the absorbance at 510 nm was measured in comparison to a blank and compared to a produced standard curve of gallic acid solutions in methanol. Using the calibration curve, total tannin content

was reported as mg/g of gallic acid equivalent: y = 0.0593x – 0.0485, R^2 = 0.9826, where x is the absorbance and y is the gallic acid equivalent.

4.3.3.5 Determination of total flavonol content

The method of Kumaran and Karunakaran was used to estimate the total flavonols present in the plant extracts (2007). 2% AlCl3 ethanol and 3.0 mL (50 g/l) sodium acetate solutions were added to 2.0 ml of sample (standard), respectively. After 2.5 hours at 20°C the absorbance was at 440 nm. The final concentration of the extract samples was 0.1 mg/ml. On the basis of the calibration curve, the following equation was used to determine the total flavonoid content as quercetin (mg/g): y = 0.0255x, R^2 = 0.9812, where x was the absorbance and was the quercetin equivalent (mg/g).

4.4 Statistical analysis

Three replicates' mean and standard error of the mean (SEM) were used to express the results. The data were subjected to a one-way analysis of variance (ANOVA), and Duncan's Multiple Range test was used with Prism 5_2. to identify variations between samples. *P* values were considered significant at < 0.05 and very significant at < 0.01.

4.5 Results and discussion

4.5.1 Qualitative analysis

Table 3.1 displays the findings of qualitative study of secondary metabolites found in the investigated plant species. All plant species tested positive for the presence of flavonoids, anthraquinones, saponins and terpenoids. Cardiac glycosides and steroids followed by alkaloids were discovered as least abundant (Tonisi, et al., 2020). A study by Xaba (2016) also revealed the presence of anthraquinones in *D. sylvatica* extracts. With over 700 different compounds, anthraquinones are the biggest class of naturally occurring pigments. They are present in all sections of plants, including the roots, rhizomes, fruits, flowers, and leaves (Duval et al., 2016). Their derivatives are widely employed in dyes, as additives in the paper and pulp industry, in seed treatment procedures, and as pesticides. They are aromatic carbon-based complexes that are

found in a few herbs and food plants either as bianthrones or anthrones (Khan, 2019). Many anthraquinones also possess antiparasitic, antifungal, and insecticidal properties and act as anticancer agents (Khan, 2019; Yadav et al., 2019). Knipholone and knipholone anthrone which are phenyl anthraquinone derivatives are found in *Kniphofia* species and a few other genera including *Bulbine* (Dagne and Steglich, 1984; Dagne and Yenesew, 1993; Bringmann, et al., 2008; Feilcke et al., 2019). These two phenyl compounds were isolated from *K. foliosa* and were found different pharmacological activities such as antibacterial, antiplasmodial, anthelmintic, anti-HIV-1c and cytotoxic effects in several human cell lines and primary cells (Feilcke et al., 2019). Though no literature was found on the pharmacological properties of *K. drepanophylla*, this plant could also possess phenyl anthraquinone derivatives owing to its healing properties that have been reported by traditional healers.

Saponins have many industrial and commercial applications due to their wetting, emulsifying and foaming properties. They possess antimicrobial, antioxidant, insecticidal, nematicidal and molluscicidal activities (Singh, B. & Kaur, 2017). Additionally, they have been shown to exhibit cytotoxic, hemolytic, anti-inflammatory, antibacterial, antifungal, anticancer and antiviral activities (El Aziz et al., 2019).

Terpenoids are biologically active compounds that vary in structure, possess strong odours and are used largely in pharmaceuticals as they are found in the plant essential oils and fixed oils (Perveen, 2018). Terpenoids of the composite kind aid in protecting plants from infections and herbivores (Yazaki, et al., 2017). Terpenoids have been shown to have hepatoprotective, anti-inflammatory, anti-cancer, anti-nociceptive, anti-foaming and carminative, and antibacterial properties (Ludwiczuk et al., 2017).

Alkaloids were found in *A. oblongifolia, D. sylvatica, G. perpensa* and *K. drepanophylla*. They are amongst the most diverse and therapeutically significant plant substances. Alkaloids are considered an important group of phytochemicals that exhibit diverse biological activities including antimalarial activity (Uzor, 2020). Despite being highly poisonous, they have a considerable therapeutic impact when used sparingly (Roy, 2017). They are crucial for medical application in antibacterial, analgesic, and antispasmodic activity because they play a vital role in plant defense

against bacteria, fungus, insects, herbivores, and other plants through compounds with allelopathic activity (Roy, 2017). Alkaloids, cardiac glycosides and steroids were undetected for *C. brachiata*. Mostafa and Afolayan (2013) reported that methanol, acetone, and water extracts of *C. brachiata* included tannins, flavonoids, and terpenoids but did not contain any steroids, glycosides, or alkaloids.

Roots of *C. brachiata* are used as a decoction for treating malaria in Tanzania and Kenya and South Africa, the stem and leaves are utilised to cure schistosomiasis with some areas of the Eastern part of the country using the leaf extract as medicine for the eye, skin infections and wounds (Chhabra et al., 1991; Spang et al., 2000; Koch et al., 2005; Pendota et al., 2008; Mostafa and Afolayan, 2013).

In the plant world, tannins are the most prevalent classes of polyphenols. They have a diverse range of physiological, biochemical, and pharmacological activities, such as antioxidant, antibacterial, antiviral, anticancer, and enzyme inhibition (Shahat and Marzouk, 2013). Condensed tannins have been reported extensively as potential defences against pests and pathogens, and for their beneficial impact on human health as they are known to possess high *in vitro* antioxidant capacity (Gourlay and Constabel, 2019).

Cardiac glycosides, like cardenolides and bufadienolides, are substantial steroid substances with a variety of natural sources that are involved in sodium pumps. A variety of cellular activities, including maintaining cellular volume, co-transporting extracellular nutrients and ions, and preserving membrane excitability, depend on the sodium pump for maintaining an electrochemical gradient across cellular membranes (EL-mallakh et al., 2019; Ayogu and Odoh, 2020). Some cardiac glycosides that were isolated in plants were discovered to treat dropsy, oedema, congestive, cancer and inflammation (EL-mallakh et al., 2019; Ayogu and Odoh, 2020).

Plant steroids are another diverse group of plant secondary compounds that possess several biochemical and pharmacological activities including anti-tumour, antiprotozoal, anti-inflammatory, antimicrobial, antihypercholesterolemic,

hepatoprotection, and anticancer activity antihelminthic, cytotoxic and cardiotonic activity (Patel and Savjani, 2015; Dembitsky et al., 2018). A study by Tarkowská (2019) lists several plant steroids that are synthesized by plants.

Flavonoids, tannins, anthraquinones, saponins, terpenoids, cardiac glycosides, and steroids were present in *C. dentata*, while alkaloids were absent. Tannins, flavonoids, saponins, anthraquinones, steroids, and glycosides presence was initially reported to be found in various extracts of *C. dentata* (Doughari et al., 2010; Doughari et al., 2011). In South Africa's Eastern Cape Province, stem bark is used as an aphrodisiac, to purify the blood, and in veterinary medicine (van Wyk & Prinsloo, 2021). Phytochemical screening previously by Doughari et al. (2012) reported on alkaloids in the roots and leaves of *C. dentata* but no trace of alkaloids in the stem bark of various extracts. Four antibacterial and antifungal triterpenoids called betulinic acid, ursolic acid, and lupeol were reported as isolated through Bioactivity-guided fractionation and bioautogram studies (Shai et al., 2007; 2008; Fadipe et al., 2015). Phytosteroid β-sitosterol which is used to treat high cholesterol and other disorders was also isolated from *C. dentata* (Fadipe et al., 2015). The plant has been reported for skin-associated infections in traditional use (Dold and Cocks, 2001; Shai et al., 2009). Additionally, the plant has been linked to reports of antibacterial action and motility inhibition in nematodes, both parasitic and free-living (Shai et al., 2008; 2009).

Anthraquinones, alkaloids, saponins and flavonoids were all present in *D. sylvatica*. In Zimbabwe, the plant is used to the treat skin problems and rheumatism is by rubbing fresh rhizome on the skin (Cogne, et al., 2001). The tuber is put in cold water for several days and then boiled to remove toxic alkaloids called dioscorin and dihydrodioscorin (Neuwinger, 1994; Cogne et al., 2001).

Alkaloids, flavonoids, tannins, anthraquinones, saponins, and terpenoids were all present in *G. perpensa*. Phytochemical analysis of extracts from methanol, dichloromethane, and water in a study by Mfengwana et al. (2019), *G. perpensa* rhizome demonstrated the presence of flavonoids, tannins, saponins, terpenoids, and alkaloids saponins. Alkaloids, flavonoids, tannins, anthraquinones, saponins, and

terpenoids were all present in *G. perpensa*. Chigor (2014) also extracted phenols, flavonoids, flavonols, proanthocyanidins, alkaloids, and tannins from *G. perpensa* leaf and rhizome extracts in aqueous, acetone, and methanol. There are several known medicinal uses for the leaves, rhizomes, roots, and stems of *G. perpensa* in the treatment and management of several human and animal conditions (Maroyi, 2016). The root or rhizome is used as a detoxifier, to treat impotence, infertility, cancer, colds, earaches, endometritis, heart disorders, hypertension, poor appetite, rheumatic pains, scabies, syphilis, and urinary infections (Maliehe, 1997; Van Wyk and Gericke, 2000; McGaw et al, 2005; Buwa and Van Staden, 2006; Lwalewa, 2007; Mwale and Masika, 2009; Nzue, 2009; Masafu et al., 2016; Mugomeri et al, 2016).

While alkaloids were not found, screening of *R. melanophloeos* revealed the presence of flavonoids, tannins, anthraquinones, saponins, terpenoids, cardiac glycosides, and steroids. Additionally, flavonoids, tannins, saponins, terpenoids, alkaloids, cardiac glycosides, and phlobatannins were reported by Mehrbod et al. (2018). Mosa et al. (2011) previously found the availability of saponins, tannins, terpenoids, flavonoids, alkaloids, cardiac glycosides, phlobatannins, steroids and anthraquinones were undetected. *R. melanophloeos* has been used to treat various illnesses and conditions including respiratory problems, stomach and heart ailments, and it has been used in tuberculosis treatment (Gibango, et al., 2020). The grey bark has been used medicinally for a variety of conditions (Mosa et al., 2011). Molluscicidal, antifungal, and anthelmintic characteristics have all been documented for it (Ohtani, et al, 1993; Githiori, et al, 2002).

Table 4.1: Qualitative analysis of the phytochemical constituents of the plants used against elephantiasis in the Eastern Cape Province, South Africa.

Plant Name	Alkaloids	Flavonoids	Tannins	Anthraquinones	Saponins	Terpenoids	Cardiac glycosides	Steroids
A. oblongifolia	+	+	+	+	+	+	+	+
C. brachiata	-	+	+	+	+	+	-	-
C. dentata	-	+	+	+	+	+	+	+
D. sylvatica	+	+	-	+	+	+	-	-
K. drepanophylla	+	+	+	+	+	+	-	-
G. perpensa	+	+	+	+	+	+	-	-
R. melanophloeos	-	+	+	+	+	+	+	+

+Present; -Absent

4.5.2 Quantitative analysis

Total phenolic, flavonoid, tannin, and flavonol contents of the bark of *C. dentata* and *R. melanophloeos* and rhizome of *K. drepanophylla* and *G. perpensa* were determined and are presented in Table 3.2.

Most plants are used for medicinal purposes because they possess phytochemicals that were reported for medicinal properties. Varying solvent extracts had considerably different phytochemical concentrations from one another showing that the solvents possess different extracting capacities. The phenol content, followed by flavonoids in some of the plant extracts, was found to be high in the acetone extracts followed by ethanol or water which may account for their good antioxidant activity. *C. dentata* acetone extract was with the highest concentration of phenols followed by *G. perpensa* ethanol and acetone extracts. *K. drepanophylla* extracts demonstrated a lower concentration of phenols. The highest concentration for phenols in *G. perpensa* was found in the ethanol extract at 493.837 mg/ml and the lowest at 438.215 for the water extract with no significant difference ($P > 0.05$).

In a similar study, total polyphenol contents of methanol extract were found to be 248.45 mg/ml (Simelane et al., 2010). In a similar study, total phenolic content for *C. dentata* was discovered as less for the acetone, chloroform and ethanol extracts between ranges of 24 – 40 mg/ml (Doughari et al., 2012). In our study, phenol composition was within the range of 380 – 500 mg/ml. This variation may result from the various approaches that were used in the two studies as a lower concentration (0.05 mg/ml) of the stock solution of the extracts was used in the former study as compared to a higher concentration (5 mg/ml) of extract solutions which were used in our study. Similarly, Odeyemi et al. (2012) determined the total phenol composition of the hydroalcoholic extracts by using a stock solution of a lesser concentration of mg/ml and reported lower results. The age, growth, habitat, and location are other factors to be considered when determining the phytochemical composition of the same plant within different studies as the flavonoid composition of the hydroalcoholic extracts of *C. dentata* in a similar study by Oyedemi et al. (2012) were found to be considerably much lesser as compared to our study. The results for tannins between the two studies were found to be lower and similar as compared to phenols and flavonoids which are (0.51% composition and 0.560 mg/ml).

K. drepanophylla demonstrated the highest concentration of flavonoids in the order: acetone > aqueous > ethanol and *G. perpensa* extracts also displayed a higher concentration of flavonoids than *R. melanophloeos* and *C. dentata* in the order: water > ethanol > acetone. Significant differences were in the extracts' concentrations ($p \leq 0.05$).

Tannins were found to be lower as compared to phenols and flavonoids. They were discovered to be noticeably higher in *R. melanophleoes* acetone extract followed by ethanol and water extracts. *C. dentata* water extract and *K. drepanophylla* water and acetone extracts also displayed a good concentration of flavonoids.

Flavonols were found to be the highest in *K. drepanophylla* acetone and ethanol extracts followed by *C. dentata* and *G. perpensa* acetone extracts. *C. dentata,* and lastly *R. melanophloes* with the least concentration. Flavanols were discovered to be the least concentrated plants and they were more in the acetone extracts. This variation may be due to solvent polarity which is responsible for the type and quantity of the extracted compounds.

Genus *Kniphofia* is rich in anthraquinones, flavonoids, and alkaloids which are known for their broad range of bioactivities, including anticancer and antimalarial activities (Abdissa et al., 2020). The genus *Kniphofia* has been well explored but no studies on the phytochemical investigation of *K. drepanophylla* have been conducted previously. Studies that were conducted on the bioactivity of *G. perpensa* revealed compounds such as ellagic acid lactone (0.33%), 1,1'-biphenyl-4,4'-diacetic acid (0.11%), p-hydroxybenzaldehyde (1%), and Z-methyl lespedezate (8.9%) (Mammo et al., 2017; Brookes and Dutton 2007). The polyphenolic compounds caffeic acid, ellagic acid, and ellagitannins which were isolated from two *Gunnera* species demonstrated antibacterial activity (Mcgaw et al., 2005). Reports found in literature state that in biological systems, phenolic compounds show free radical activity, peroxide breakdown, metal inactivation, or oxygen scavenging, and they guard against oxidative illnesses like heart disease, cancer, diabetes, and neurological disorders diseases (Aryal et al., 2019).

Table 4.2. Phytochemical constituents identified in the extracts of the selected medicinal plants.

Plant name	Extracts	Total Phenolics GAE (mg/g)	Total Flavonoids QE (mg/g)	Total Tannins GAE (mg/g)	Total Flavonols QE (mg/g)
C. dentata	**H_20**	440.377[a]	85.173[a]	0.557[a]	0.044[a]
	Ethanol	382.773[b]	36.773[b]	0.316[b]	0.150[b]
	Acetone	499.847[a]	35.302[b]	0.293[b]	0.223[c]
G. perpensa	**H_20**	438.512[a]	246.018[a]	0.073[a]	0.130[a]
	Ethanol	493.837[b]	163.256[b]	0.284[b]	0.177[b]
	Acetone	492.387[b]	126.087[c]	0.329[c]	0.222[c]
K. drepanophylla	**H_20**	238.762[a]	287.868[a]	0.414[a]	0.079[a]
	Ethanol	183.229[b]	261.529[b]	0.115[b]	0.249[b]
	Acetone	287.456[a]	436.814[c]	0.335[c]	0.364[c]
R. melanophloeos	**H_20**	367.854[a]	97.875[a]	1.045[a]	0.045[a]
	Ethanol	382.773[a]	48.538[b]	1.627[b]	0.045[a]
	Acetone	465.242[b]	40.115[b]	1.867[c]	0.120[b]

Values with the same letter superscript within the same constituent are not significantly different ($P > 0.05$).

Z-venusol a phenylpropanoid alkaloid pure compound possessing anti-tumour activity was isolated from *Gunnera perpensa* and revealed as an important component of the plant (Khan et al., 2004; Mathibe et al., 2017).

R. melanophloeos displayed the same trend as the previous plants possessing more phenols followed by total flavonoids, tannins, and lastly flavonols. Similar to this, Lotter et al. (2019) found that the methanolic extract had considerably more total phenolics, flavonoids, and condensed tannins than the aqueous extract. Water is a polar solvent and will dissolve polar substances more easily than the less polar solvents like acetone, ethanol, and methanol and the latter will easily dissolve the non-polar substances.

4.6 Conclusions

Plants include a variety of phytochemical substances, each with unique properties and concentrations, according to the phytochemical screening of plant extracts. The variation in concentration could be attributed to the types of solvents that were used as solvents to extract different compounds based on polarity. Screening for phytochemical presence and activity is very important as the plant compounds have been said to offer therapeutic benefits

References

Abdissa, D., Faye, G., Fayera, M., & Demissie, S. (2020). Extraction and Isolation of Compound from Roots of *Kniphofia pumila*, and Its Antibacterial Activities in Combination with Zinc Chloride. *Walailak Journal of Science & Technology*, *17*(7), 631–638.

Adedapo, A.A., Jimoh, F.O., Koduru, S., Masika, J.P., & Afolayan, A.J., (2009). Assessment of the medicinal potentials of the methanol extracts of the leaves

and stems of *Buddleja saligna. BMC Complementary and Alternative Medicine, 9, 9-2.*

Aiyegoro, A.O. and Okoh, A.I. (2010). Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary Alternative Medicine*, 10, 21.

Aqil, F., Ahmed, I., Mehmood, Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish Journal of Biology, 30,177-8.*

Aremu, A.O. (2009). Pharmacology and phytochemistry of South African plants used as anthelmintics. MSc Thesis, University of KwaZulu-Natal.

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., &, & Koirala, N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, *8*(96), 1–12.

Atala, E., Vásquez, L., Speisky, H., Lissi, E. & López-Alarcón, C. (2009). Ascorbic acid contribution to ORAC values in berry extracts: an evaluation by the ORAC-pyrogallol red methodology. *Food Chemistry*, *113*(1), 331-335.

Biswas, N.N., Acharzo, A.K., Anamika, S., Khushi, S., & Bokshi, B. (2017). Screening of natural bioactive metabolites and investigation of antioxidants, antimicrobial, and hyperglycaemic, neuropharmacological, and cytotoxicity potentials of *Litsea polyantha* Juss. Ethanolic root extract. *Evidence-Based Complementary and Alternative* Medicine, ID 3701349.

Brookes, K. B., & Dutton, M.F. (2007). Bioactive Components of the Uteroactive Medicinal Plant, *Gunnera Perpensa* (or Ugobo): Research in Action. *South African Journal of Science, 103* (5 & 6): 187–189.

Bringmann, G., & Mutanyatta-comar, J., Knauer, M., & Abegaz, B. M. (2008). Knipholone and related 4-phenylanthraquinones : structurally, pharmacologically, and biosynthetically remarkable natural products. *Natural Product Reports*, *25*, 696–718.

Buwa, L.V., & Van Staden, J. (2006). Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *Journal of Ethnopharmacology*, *103*(1), 139–142.

Chhabra, S.C., Mahunnah, R.L.A. & Mshiu, E. N. (1991). Plants used in traditional medicine in Eastern Tanzania. V. Angiosperms (Passifloraceae to Sapindaceae). *Journal of Ethnopharmacology*, 33, 43-157.

Chirumbolo, S. (2012). Plant phytochemicals as new potential drugs for immune disorders and cancer therapy: really a promising path? *Journal of the Science of Food and Agriculture*; 92: 1573–1577.

Clarice, C. V., Soares, G. L., Perez, A. C., Rodrigues, V.G., &, & Silva, F. C. (2017). Pharmacological potential of Maytenus species and isolated constituents, especially tingenone , for treatment of painful inflammatory diseases. *Brazilian Journal of Pharmacognosy*, *27*(4), 533–540.

Cogne, A., Marston, A., Mavi, S., & Hostettmann, K. (2001). *Study of two plants used in traditional medicine in Zimbabwe for skin problems and rheumatism : Dioscorea syl 6 atica and Urginea altissima*. *75*, 51–53.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12, 564-582.

Dagne, E. & Steglich, W. (1984). *Phytochemistry*, 23, 1729–1731.

Dagne, E., & Yenesew, A. (1993). Knipholone anthrone from *Kniphofia foliosa*. *Phytochemistry*, 34, 1440–1.

Defossez, E., Pitteloud, C., Descombes, P., Glauser, G., & Allard, P., Wlaker, T.N.W., Pilar, F.Wolfender, J., Pllissier, L. & Rasmann, S. (2021). Spatial and evolutionary predictability of phytochemical diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(3), 1–7.

Dembitsky, V. m., Savidov, N., Poroikov, V. ., Gloriozova, T.A., &, & Imbs, A. B. (2018). Naturally occurring aromatic steroids and their biological activities. *Applied Microbiology and Biotechnology*, 1–12.

Dold, A.P., & Cocks, M.L. (2001). Traditional veterinary medicine in the Alice district of the Eastern Cape Province, South Africa. South Africa. *Journal of Science*, 97, 375-379.

Doughari, J.H., Ndakidemi, P.A., Human, I.S., & Benade, S. (2010). Antimicrobial susceptibility profile and effect of stem bark extracts of *Curtisia dentata* on multi-drug resistant verotoxic *Escherichia coli* and *Acinetobacter* spp. isolates obtained from water and wastewater samples. Conference Abstracts, *International Conference on Antimicrobial Research* (ICAR), 2010, Valladolid, Spain.

Doughari, J.H., Ndakidemi, P.A., Human, I.S., & Benade, S. (2012). Antioxidant, antimicrobial, and antiverotoxic potentials of extracts of *Curtisia dentata*. *Journal of Ethnopharmacology*, 141, 1041– 1050.

Duval, J., Pecher, V., Poujol, M., & Lesellier, E. (2016). Research advances for the extraction, analysis and uses of anthraquinones : A review. *Industrial Crops & Products*, 94, 812–833.

El-mallakh, R. S., Brar, K.S., &, & Yeruva, R. R. (2019). Cardiac Glycosides in Human Physiology and Disease : Update for Entomologists. *Insects*, *10*(102), 1–11.

El Aziz, M. M. A., & Ashour, A.S., Melad, A. S. . (2019). A Review on Saponins from Medicinal Plants : Chemistry, Isolation, and Determination A review on saponins from medicinal plants : chemistry, isolation, and determination. *Journal of Nanomedicine Research*, *8*(1).

Fadipe, V.O., Mongalo, N.I., & Opuku, A.R. (2015). In vitro evaluation of the comprehensive antimicrobial and antioxidant properties of *Curtisia dentata* (Burm. F) CA Sm: toxicological effect on the human embryonic kidney. *EXCLI Journal*, 14, 971-983.

Feilcke, R., Arnouk, G., Raphane, B., Richard, K., Tietjen, I., Andrae-marobela, K., Erdmann, F., Schipper, S., Becker, K., Arnold, N., Frolov, A., Reiling, N., Imming, P., &, & Fobofou, S. A. T. (2019). Biological activity and stability analyses of knipholone anthrone, a phenyl anthraquinone derivative isolated from Kniphofia foliosa. *Journal of Pharmaceutical and Biomedical Analysis*, *174*, 277–285.

George, J., Laing, M.D., Drewes, S.E., (2001). Phytochemical research in South Africa. *South African Journal of Science*, 97, 93-105.

Gibango, L., &, Oosthuizen, C.B., &, & Lall, N. (2020). South African Journal of Botany Potential implications of the use of Rapanea melanophloeos (L .) Mez against mycobacteria. *South African Journal of Botany*, *132*, 388–394.

Githiori, B. J., Höglund, J., Waller, P.J., & Baker, L.R., (2002). Anthelmintic activity of preparations derived from *Myrsine Africana* and *Rapanea melanophloeos* against the nematode parasite, *Haemonchus contortus*, of sheep. *Journal of Ethnopharmacology.,* 80, 187-191.

Gourlay, G, &, & Constabel, C. P. (2019). Condensed tannins are inducible antioxidants and protect hybrid poplar against oxidative stress. *Tree Physiology*, *39*, 345–355.

Khan, F, Peter, X.K., Mackenzie, R.M., Katsoulis, L., Gehring, R., Orde, Q. Munro, O.Q., Fanie R van Heerden, F.R., & Drewes, S.E. (2004). Venusol from *Gunnera perpensa*: structural and activity studies. *Phytochemistry, 65* (8), 1117–1121.

Khan, N. T. (2019). Anthraquinones-A Naturopathic Compound. *Journal of New Developments in Chemistry*, *2*(2), 25–28.

Koch, A., Tamez, P., Pezzuto, J. & Soejarto, D. (2005). Evaluation of plants for antimalarial treatment by the Masai of Kenya. *Journal of Ethnopharmacology*, 101, 95-99.

Kumaran,2007). A., & Karunakaran, R.J., (2007). *In vitro* antioxidant activities of methanol extracts of Phyllanthus species from India. *Lebens-Wiss Technologie*, 40, 344-352.

Lotter, N., Chivandi, E., Lembede, B. W., Ndhlala, A. R., Nyakudya, T. T., & Erlwanger, K. H. (2019). South African Journal of Botany Anti-oxidant activity, alpha-amylase inhibition and toxicity of leaf extracts of cultivated Rapanea melanophloeos (L .) Mez (cape beech). *South African Journal of Botany*, *126*, 261–264.

Ludwiczuk, A., Skalicka-Wo´zniak, ., &, & Georgiev, M. I. (2017). *Plant Metabolites: Their Chemistry.Terpenoids, Chapter 11*.

Mahomed IM, Ojewole JA (2006) Anti-convulsant activity of *Harpagophytum procumbens* DC [pedaliaceae] secondary root aqueous extract in mice. *Brain Research Bulletin*, *69*(1), 57-62.

Maliehe, E.B. (1997). Medicinal Plants and Herbs of Lesotho, Mafeteng Development Project, Roma, Italy.

Mammo, F., Mohanlall, V., & Shode, F. (2017). Gunnera perpensa L .: A multi-use ethnomedicinal plant species in South Africa. *African Journal of Science Technology Innovation and Development*, *January 2018*.

Maroyi, A. (2016). *From Traditional Usage to Pharmacological Evidence : Systematic Review of Gunnera perpensa L*. Evidence-Based Complementary and Alternative Medicine, Article ID 1720123.

Masafu, M.M., Mbajiorgu, C.A., Nemadodzi, L.E., & Kabine, E.S. (2016). A study of natural habitats and uses of medicinal plants in Thulamela and JS Moroka Municipalities, South Africa. *Indian Journal of Traditional Knowledge,15*(3), 363–369.

Mathibe, L.J., Botha, J., & Naidoo., S. (2017). Effects of Z-venusol isolated from *Gunerra perpensa* l., on the IL-6 and cAMP activity in human breast cancer cells *in vitro*. *South African Journal of Botany*, 108, 96-99.

Mbaebie, B.O., Edeoga, H.O. & Afolayan, A.J. (2012). *Asian Pacific Journal of Tropical Biomedicine*, *2*(2), 118-124.

McGaw, L.J., Gehring, R., Katsoulis, L., & Eloff, J.N. (2005). Is the use of Gunnera perpensa extracts in endometritis related to antibacterial activity? *Onderstepoort Journal of Veterinary Research*, 72, (2), 129–134.

Mcgaw, L. J., & Fasina, F. . (2018). Immunomodulatory properties of quercetin-3-O- α -L-rhamnopyranoside from Rapanea melanophloeos against influenza a virus. *BMC Complementary and Alternative Medicine*, *18*(184), 1–10.

Mehrbod, P., Abdalla, M. A.,Njoya, E.M., Ahmed, A. S., Fotouhi, F., Heidarzadeh, Farahmand, B., Gado, D.A., Tabatabaian, M., Fasanmi, O.G., Eloff, J. N., McGaw, L.J. & Fasina, F.O. (2018). South African medicinal plant extracts active against influenza A virus. *BMC Complementary and Alternative Medicine*, 1*8*(112), 1-10.

Mfengwana, P. H., Mashele, S.S.., Manduna, I.T., (2019). *In-vitro* Antibacterial, Antioxidant, and Anti-Inflammatory Effects of *Senecio asperulus* and *Gunnera perpensa* from Mohale's Hoek, Lesotho. *Pharmacognosy Journal*, *11*(4), 730-9.

Mosa, R.A., Lazarus, G.G., Gwala, P.E., Oyedeji, A.O., Opoku, A.R. (2011). *In Vitro* Anti-platelet Aggregation, the antioxidant and cytotoxic activity of extracts of some Zulu medicinal plants. *Journal of Natural Products*, 4,136-146.

Mostafa, M., & Afolayan, A.J. (2013). Antimicrobial activity of Clematis brachiata Thunb leaf extract. *International Journal of Pharmaceutical Sciences and Research*, *4*(1): 243-247.

Mulholland, D.A. (2005) The future of ethnopharmacology a South African perspective. *Journal of Ethnopharmacology*,100, 124-126.

Mugomeri, E., Chatanga, P., Raditladi, T., Makara, M., & Tarirai, C. (2016). Ethnobotanical study and conservation status of local medicinal plants: towards a repository and monograph of herbal medicines in Lesotho. *African Journal of Traditional, Complementary and Alternative Medicines*, *13*(1), 143– 156.

Mwale, M., & Masika, P.J. (2009). Ethno-veterinary control of parasites, management, and role of village chickens in rural households of Centane district in the Eastern Cape, South Africa. *Tropical Animal Health and Production*, *41*(8), 685–1693.

Nariya, P. B., Bhalodia, N. R., Shukla, V. J., Acharya, R., & Nariya, M. B. (2013). *In-vitro* evaluation of antioxidant activity of *Cordia dichotoma* (Forst f.) bark. *AYU,* 34 (1): 124–128.

Neuwinger, H.D., (1994). Afrikanische Arzneipflanzen und Jagdgifte. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, p. 384.

Nzue, A. P. M. M. (2009). Use and conservation status of medicinal plants in the Cape Peninsula, Western Cape Province of South Africa [M.S. thesis], Stellenbosch University, Stellenbosch, South Africa.

Ohtani, K., Maw, S., & Hostettmann, H. (1993): Molluscicidal and antifungal triterpenoid saponins from *Rapanea melanophloeos* leaves. *Phytochemistry*, 33, 83-86.

Ordon Ez, A.A.L., Gomez, J.D., Vattuone, M.A., & Isla, M.I., (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chemistry*, 97, 452-458.

Oyedemi, S.O., Bradley, G., Afolayan, A.J. (2010). In vitro and in vivo antioxidant activities of aqueous extract of *Strychonos henningsii* Gilg. *African Journal of Pharmaceutical Pharmacology*, *4*(2): 70-78.

Oyedemi, S.O., Oyedemi, B.O., Arowosegbe, S., Afolayan, A.J., (2012). Phytochemicals Analysis and Medicinal Potentials of Hydroalcoholic Extract from *Curtisia dentata* (Burm. f) C.A. Sm Stem Bark. *International Journal of Molecular Sciences***,** 13, 6189-6203.

Pendota, S.C., Grierson, D.S. & Afolayan, A.J. (2008). An ethnobotanical study of plants used for the treatment of eye infections in the Eastern Cape, South Africa. *Pakistan Journal of Biological Science*, 11, 2051-2053.

Perveen, S. (2018). Terpenes and terpenoids. DOI: 10.5772/intechopen.71175

Roy, A. (2017). A Review on the Alkaloids an Important Therapeutic Compound from Plants. *International Journal of Plant Biotechnology*, *3*(2), 1–7.

Seethal, B., Gajalakshmi, R., Mohan, A.C., Nisha, K. (2017). Screening of antioxidant and antibacterial activity in methanol extract of *Toddalia Asiata* L. *European Journal of Biomedical and Pharmaceutical Sciences*, *4*(6),612- 616.

Shahat, A.A., &, & Marzouk, M. S. (2013). 13 Tannins and Related Compounds from Medicinal Plants of Africa. In *Medicinal Plant Research in Africa*. Elsevier Inc.

Shai, L.J. (2007). Characterization of compounds from *Curtisia dentate* (Cornaceae) active against *Candida albicans*. *Ph.D. Thesis*, Department of Paraclinical Sciences, University of Pretoria, South Africa.

Shai, L.J., McGaw, L.J., Aderogba, M.A., Mdee, L.K., & Eloff, J.N. (2008). Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm. f) C.A. Sm. leaves. *Journal of Ethnopharmacology*, 119, 238-244.

Shai, L.J., McGaw, L.J., & Elof,f J.N. (2009). Extracts of the leaves and twigs of the threatened tree *Curtisia dentata* (Cornaceae are more active against *Candida albicans* and other microorganisms than the stem bark extract. *South African Journal of Botany*, 75, 363-366.

Shai, L.J., Bizimenyera, E.S., Bagla, V., McGaw, L.J., & Elof,f J.N. (2009). *Curtisia dentata* (Cornaceae) leaf extracts and isolated compounds inhibit the motility of parasitic and free-living nematodes. Onderstepoort. *Journal of Veterinary Research*, *76*(2), 49-256.

Simelane, M. B. C., Lawal, O. A., Djarova, T. G., & Opoku, A. R. (2010). *In vitro antioxidant and cytotoxic activity of Gunnera perpensa L . (Gunneraceae) from South Africa*. *4*(21), 2181–2188.

Singh, B., &, & Kaur, A. (2017). Control of insect pests in crop plants and stored food grains using plant saponins: A review. *LWT - Food Science and Technology*, *10.10*, 1–35.

Soliman, S. S. . M., Balsam, Q. S., Elseginy, S. A., Al-marzooq, F., Ahmady, I. M., El-keblawy, A.A., &, & Hamdy, R. (2021). Chemico-Biological Interactions Critical discovery and synthesis of novel antibacterial and resistance-modifying agents inspired by plant phytochemical defense mechanisms. *Chemico-Biological Interactions*, *333*(October 2020), 1–11.

Spang, S.G., Van Staden, J. & Jäger, A.K. (2000). Efficiency of traditionally used South African plants against schistosomiasis. *Journal of Ethnopharmacology*, 73, 209–214.

Takaidza, S., Mtunzi, F., & Pillay, M. (2018). Analysis of the phytochemical contents and antioxidant activities of crude extracts from *Tulbaghia* species. *Journal of Traditional Chinese Medicine*, *38*(2), 272–279.

Tarkowská, D. (2019). Plants are Capable of Synthesizing Animal. *Molecules*, *24*(2585), 1–13.

Tonisi, Si., Okaiyeto, K., Hoppe, H., Mabinya, L.V., &, & Nwodo, U. U. (2020). Chemical constituents, antioxidant and cytotoxicity properties of *Leonotis leonurus* used in the folklore management of neurological disorders in the Eastern Cape, South Africa. *3 Biotech*, *10*(3), 1–14.

Uzor, P. F. (2020). Alkaloids from Plants with Antimalarial Activity : A Review of Recent Studies. *Evidence-Based Complementary and Alternative Medicine*, *2020*, 1–17.

van Wyk, B.E & Gericke, N. (2000). *People's Plants: A Guide to Useful Plants of Southern Africa*, Briza, Pretoria, South Africa.

van Wyk, A. S. &, & Prinsloo, G. (2021). NMR-based metabolomic analysis of the seasonal and regional variability of phytochemical compounds in Curtisia dentata stem bark. *Biochemical Systematics and Ecology*, *94*(January), 104197.

Wolfe, K., Wu, X., & Liu, R.H., (2003). Antioxidant activity of apple peels. *Journal of Agriculture and Food Chemistry*, 51, 609-614.

World Health Organization (1996). WHO Guideline for the Assessment of Herbal Medicines. WHO Expert Committee on the specification for pharmaceutical preparation. Technical Report Series no. 863, Geneva.

Xaba, V.M. 2016. Pharmacological screening of traditional medicinal plants used to treat skin ailments in the Free State Province of South Africa (Doctoral dissertation).

Yadav, R.N.S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology, 3*(12), 10-14.

Yadav, A.N., Kour, D., Rana, K.L., Yadav, N., Singh, B., Chauhan, V.S., Rastegari, A.S., Hesham, A.E., & Gupta, V.K. (2019). Metabolic Engineering to Synthetic Biology of Secondary Metabolites Production. *New and Future Developments in Microbial Biotechnology and Bioengineering,* 279-320.

Yazaki, K., Gen-ichiro, A., &, Toshiyuki, O. (2017). *' Hidden ' Terpenoids in Plants : Their Biosynthesis, Localization and Ecological Roles*. *58*(August), 1615–162.

CHAPTER 5

IN VITRO ANTIOXIDANT ACTIVITY OF THE PLANT EXTRACTS

5.1 Introduction

The disease elephantiasis poses several conditions like pain and swelling due to lymphatic vessel blockage which is associated with oxidative stress. The cells of the body may not be able to fight reactive substances that are released during oxidative stress which eventually causes the malfunction of cells. According to reports, lymphatic dysfunction causes inflammatory responses in the skin and subcutaneous tissues, which eventually leads to lymphoedema and thus fastens the development of elephantiasisIt has also been reported that lymphatic dysfunction exposes infected individuals to bacterial and fungal infections (Nutman, 2013). Human cells have antioxidant defense mechanisms that forage and counterbalance oxidants that are produced exogenously and endogenously during normal physiological processes (Gulumian et al., 2018). They are essential compounds that can shield the organism from oxidative stress damage brought on by free radicals (Nariya et al., 2019).

It is possible to limit oxidative damage and slow the progression of disease with antioxidant supplementation because oxidants like reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in many diseases and the antioxidant defenses are somehow inadequate in fighting these diseases (Benabbou et al 2018; Dunnill et al., 2015; Moasser et al., 2018; Gulumian, et al, 2018). The pathogenesis of a number of physiological conditions, such as cellular injury, diabetes mellitus, aging, cancer, hepatic and renal disorders, cardiovascular, atherosclerosis, arthritis, and neurodegenerative diseases, is significantly influenced by the generation of ROS with a single unpaired electron (Madamanchi et al., 2005; Nariya, et al., 2013; Losada-Barreiro and Bravo-Díaz, 2017; Aryal et al., 2019).

Antioxidants act as electron donors to maintain ROS below the physiologically necessary levels, preventing them from stealing electrons from other molecules, ultimately causing them to be destroyed (Kurahashi & Fujii, 2015; Gulumian et al., 2018).

Environmental pollutants, radiation, chemicals, poisons, deep-fried and spicy foods, as well as physical stress, are some of the variables that cause the production of ROS, which in turn causes the synthesis of aberrant proteins and depletes the immune system's supply of antioxidants (Agrawal et al., 2011; Aryal, 2019).

In addition to various forms of active oxygen, ROS such as superoxide anion radicals (O2), hydroxyl radicals (OH), and non-free radicals such as H2O2 and singlet oxygen (O2) are involved in various physicochemical processes in the body. Aging derivatives of oxygen are also continuously produced inside the human body. The antioxidants in cells detoxify the generated ROS, but excessive ROS production and insufficient antioxidant defenses can easily affect and cause oxidative damage to a variety of bimolecular substances, including proteins, peroxidation of the membrane lipids, lipoproteins, tissue membranes, DNA, and enzymes (Husain et al., 1987; Farber, 1994; Aiyegoro and Okoh, 2009; Nariya et al., 2013).

The human body has developed several complex antioxidant defense systems that use antioxidant enzymes which include glutathione peroxidase, superoxide dismutase and catalase and non-enzymatic antioxidants which include glutathione, vitamins E and C, thiol antioxidants, melatonin and carotenoids (Takaidza et al, 2018).

In order to defend themselves against oxidative damage, organisms have antioxidant and repair systems that deactivate free radicals and sustain healthy cellular processes (Kurutas, 2016; Takaidza et al., 2018; Aryal, 2019). Dietary antioxidants may be required because endogenous antioxidants may not be adequate to sustain optimal cellular functions under increased oxidative stress (Rahman, 2007; Aryal, 2019).

5.1.1 Plant products as a source of antioxidants

Approximately 20 000 plant species are utilised as medicines, according to the World Health Organization (Gullece et al., 2006; Maregesi et al., 2008). The widespread usage of medicinal plants around the world may be caused by a variety of factors, such as high pricing, the scarcity of Western medicine, and the widespread perception that herbal medications are significantly less dangerous (Maregesi et al., 2008; Mbeng-Otang and Afolayan, 2017). Standardised plant extracts or pure compounds

as natural products can serve as alternatives for new drug developments due to their distinctive structural variety (Cos et al., 2006; Duraipandiyan & Ignacimuthu, 2011; Mbeng-Otang and Afolayan, 2017).

Wild vegetables are not only a source of high nutritive value but their different parts which include the leaf, fruit and root are a source of medicine. The healing properties of plant products are justifiable due to the biological activities of their phytochemical and antioxidant compounds such as phenols, proanthocyanin, vitamins, carotenoids, flavonoids and saponins (Francis et al., 2002; Dinda et al., 2007; Abifarin et al., 2019). The use of natural antioxidants to combat the harmful effects of free radicals in the human body has gained popularity recently, and because of their efficiency and safety, many medical applications utilise the antioxidant properties of medicinal plants (Zaouli et al., 2010; Al-Snafi, 2016; Abifarin et al., 2019). A higher amount of natural antioxidants in the diet may serve as protection against several types of life-threatening diseases like cancer as indicated by epidemiological studies (Bunea et al., 2011; Zeghad et al., 2019). Natural antioxidants are typically preferred over synthetic antioxidants because the latter are thought to either induce or promote detrimental health consequences (Calviello et al., 2005; Gulumian et al., 2018). Rutin, gallic acid esters, butylated hydroxyl anisole, butylated hydroxyl toluene, tertiary butylated hydroquinone, and other commercially available synthetic antioxidants that have been linked to atherosclerosis, toxicity, cell damage and other negative outcomes in both animals and people (Robak, 1995; Saha et al., 2008; Wintola and Afolayan, 2011; Subedi et al., 2014; Takaidza et al., 2018).

Plant secondary metabolites known as phenolic and flavonoid chemicals hold an aromatic ring with at least one hydroxyl group, and some of them promote the formation of endogenous antioxidant molecules in cells (Côté et al., 2010; Tungmunnithum et al., 2018; Aryal et al., 2019). Numerous studies have shown that phenolic compounds can inhibit oxidative illnesses, free radicals, peroxide breakdown, metal inactivation, and oxygen scavenging in biological systems (Oberoi and Sandhu, 2015; Aryal et al., 2019).

5.2 Aim of the study

This chapter aimed to determine the antioxidant activity of the selected plant extracts by performing 1-1-diphenyl-2-picryl hydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays.

5.3 Materials and methods

5.3.1 DPPH radical scavenging activity

DPPH free radical scavenging activity was assessed using Liyana-Pathiranan and Shahidi's technique (2005). One milliliter of an extract in methanol containing between 0.02 and 0.5 mg of extract was combined with one milliliter of a solution of 0.135 mM DPPH in methanol. The reaction mixture was completely vortexed and kept at room temperature for 30 minutes in the dark. A spectrophotometer was used to determine the mixture's absorbance at 517 nm. The standard utilised was ascorbic acid. The percentage of scavenging inhibition of DPPH of the extract was calculated using the following equation: Inhibition (%) = $\frac{Abs\ control - Abs\ sample}{Abs\ control}$ *X 100*

where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract or standard.

5.3.2 ABTS radical scavenging activity

The Adedapo et al. (2008) method was used to assess the plant extracts' capacity to scavenge ABTS radicals. Equal parts of 2.4 mM potassium persulfate solution and 7 mM ABTS solution in water were combined to create a stock solution. The mixture was then allowed to react for 12 to 16 hours at room temperature and in the dark to produce a stable oxidative state. Then, methanol was used to dilute the working solution to an initial absorbance of 0.700 0.020 (Abscontrol) at 734 nm. After that, 1 mL of the sample solution and 1 mL of the ABTS solution were combined, and after 7 minutes, the absorbance at 734 nm was measured using methanol as the reference. Every measurement was made three times. As a positive control, trolox was used. The percentage of scavenging inhibition of ABTS of the extract was calculated using the following equation: Inhibition (%) = $\frac{Abs\ control - Abs\ sample}{Abs\ control}$ *X 100.*

The IC_{50} values of plant extracts were also determined.

5.4 Results and discussion

Table 5.1 and Figures 5.1 to 5.4 exhibit the findings regarding antioxidant activity of plant extracts.5.4.1 DPPH radical scavenging activity.

Several plant extracts indicated good antioxidant activity as several of them showed low IC_{50} values. In comparison to the control, ascorbic acid, which had an IC_{50} value of 52. 772 µg/ml, the ethanol and acetone extracts of *K. drepanophylla* showed lower IC_{50} values of 36.58 µg/ml and 39.34 µg/ml, respectively. At 141.43 µg/ml, the water extract showed less antioxidant activity.

According to Wube et al. (2006), *K. foliosa*-isolated knipholone displayed a lower level of DPPH radical scavenging action than *K. drepanophylla*, with an IC50 value of 355 µM. For quantitative analysis, *K. drepanophylla* showed the highest composition of flavonoids for acetone extract, followed by water and ethanol extract, and this may be the reason for its strong DPPH radical scavenging action.

Table 5.1. DPPH and ABTS scavenging activity of the medicinal plants extracts.

Plant name	Extract	DPPH (µg/ml	ABTS (µg/ml)
C. dentata	Ethanol	368.8 ± 5.20	92.11 ± 13.88
	Acetone	538.37 ± 5.87	76.94 ± 8.13
	H_20	389.89 ± 5.31	86. 40 ± 8.75
G. perpensa	Ethanol	392. 94 ± 2.85	48.02 ± 5.34
	Acetone	392. 94 ± 7.90	60.44 ± 9.30
	H_20	518.23 ± 0.52	119.41 ± 8.67
K. drepanophylla	Ethanol	36.58 ± 15.76	49.46 ± 7.23
	Acetone	39.34 ± 22.10	56.23 ± 42.21
	H_20	114.43 ± 18.95	136.88 ± 1.39
R. melanophloeos	Ethanol	876.952 ± 0.66	541. 86 ± 3.32
	Acetone	361.81 ± 5.56	27. 40 ± 13.73
	H_20	705.95 ± 8.49	343.77 ± 1.42
Controls		Ascorbic acid 52.772 ± 18.34	Trolox 58.167 ± 10.19

K. *drepanophylla* water extract activity was 141.431 µg/ml which was higher than that of ethanol and acetone extracts (36.583 and 39.342 µg/ml) and the extracts had demonstrated higher phenols and flavonoids concentrations than the water extract. In a study by Mahachi (2013), antioxidant activity of the methanol extract for *K. drepanophylla* was not determined, contrary to our investigation, all of the analysed extracts showed action against DPPH.

The ethanol extract of *C. dentata* had an IC_{50} value of 368.80 µg/ml, while the water extract's was 389.89 µg/ml, with acetone extract showing the least amount of activity. Compared to the acetone extract, *C. dentata* water and ethanol extracts had higher flavonoid contents and lower IC_{50} values, indicating better antioxidant action. The percentages of inhibition for the *C. dentata* ethanol, acetone, and water extracts in our study were 39.38%, 33.16%, and 32.64%, respectively.

R. melanophloeos acetone extract demonstrated the lowest IC_{50} values than ethanol and water extracts and the results for phytochemical analysis demonstrated acetone extract had a higher concentration of phenols than ethanol and water extracts. According to traditional healers, the plant can be used to treat wounds, worms, and pain.

While the acetone extract of *R. melanophloeos* performed better than that of *G. perpensa*, the ethanol and water extracts of *G. perpensa* demonstrated better activity than *R. melanophloeos* extracts by presenting lower IC_{50} values of 392.94 µg/ml and 518.23 µg/ml, respectively. At 500 µg/ml, the water extract showed a 60% percentage inhibition in a study by Mfengwana et al. (2019), however in our investigation, the inhibition was 45%. The increased concentration of the extract (500 µg/ml) may have contributed to the higher scavenging activity, which was 60%. At 500 µg/ml, the dichloromethane extract of *G. perpensa* exhibited a percentage inhibition of 37–38% (Mfengwana et al., 2019). According to Fadipe et al. (2015), ascorbic acid completely inhibited DPPH, at higher concentrations of 1 and 2 mg/100 ml activity of *G. perpensa* acetone extract was at 56.3% and 64.1%, respectively.

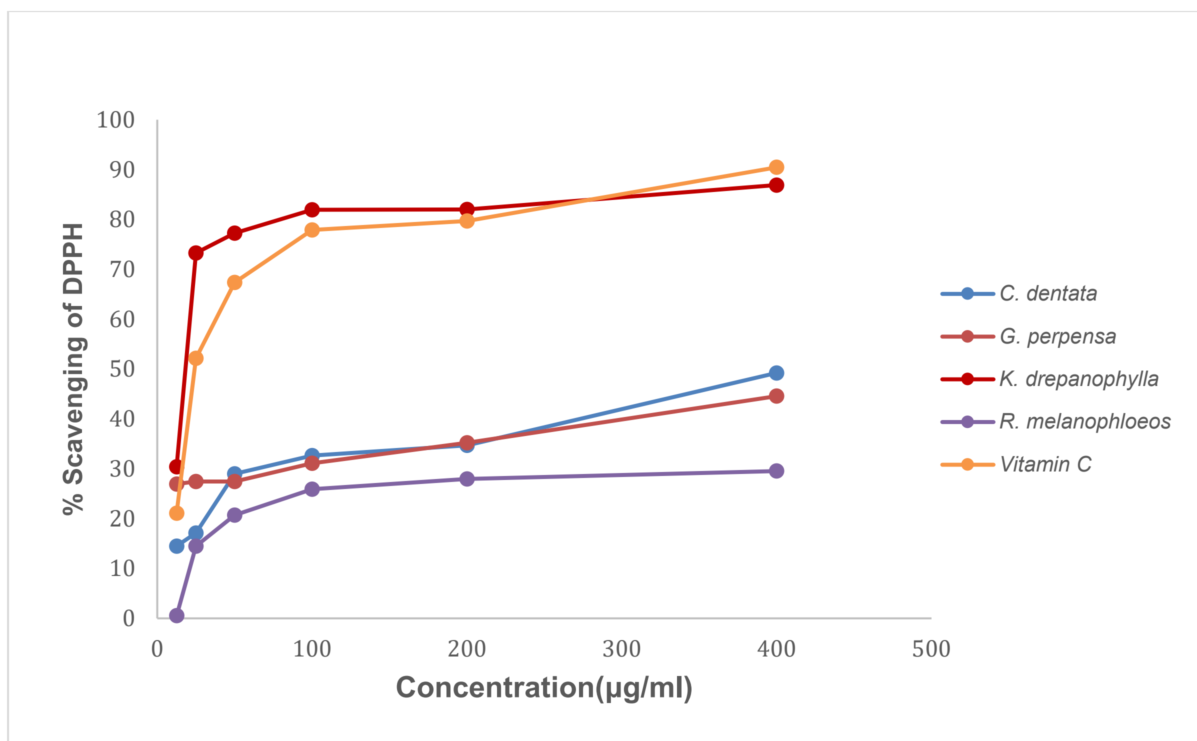


Figure 5.1. DPPH scavenging activity of the water extracts of the medicinal plants.

Phytochemical antioxidants like flavonoids have the ability to reduce oxidative stress by neutralizing these ROS. Plant antioxidants are considered to be safer and active antioxidants with fewer side effects when used *in vivo* (Wagner and Elmadfa, 2003; Mfengwana et al., 2019). The phytochemical content's relationship to its capacity for radical scavenging of extracts was established whereby extracts that had demonstrated higher amounts of phenols or flavonoids for phytochemical analysis showed higher radical scavenging as compared to extracts that had demonstrated lower contents for total phenols and flavonoids.

The phytochemical analysis of *G. perpensa* had revealed higher concentrations of phenols and flavonoids for the ethanol and acetone extracts than for water extract and the extracts demonstrated lower IC_{50} values of 392.94 and 392.94 µg/ml respectively. *R. melanophloeos* acetone extract revealed the most content for phenols and the extract had the lowest IC_{50} of 361.813 µg/ml.

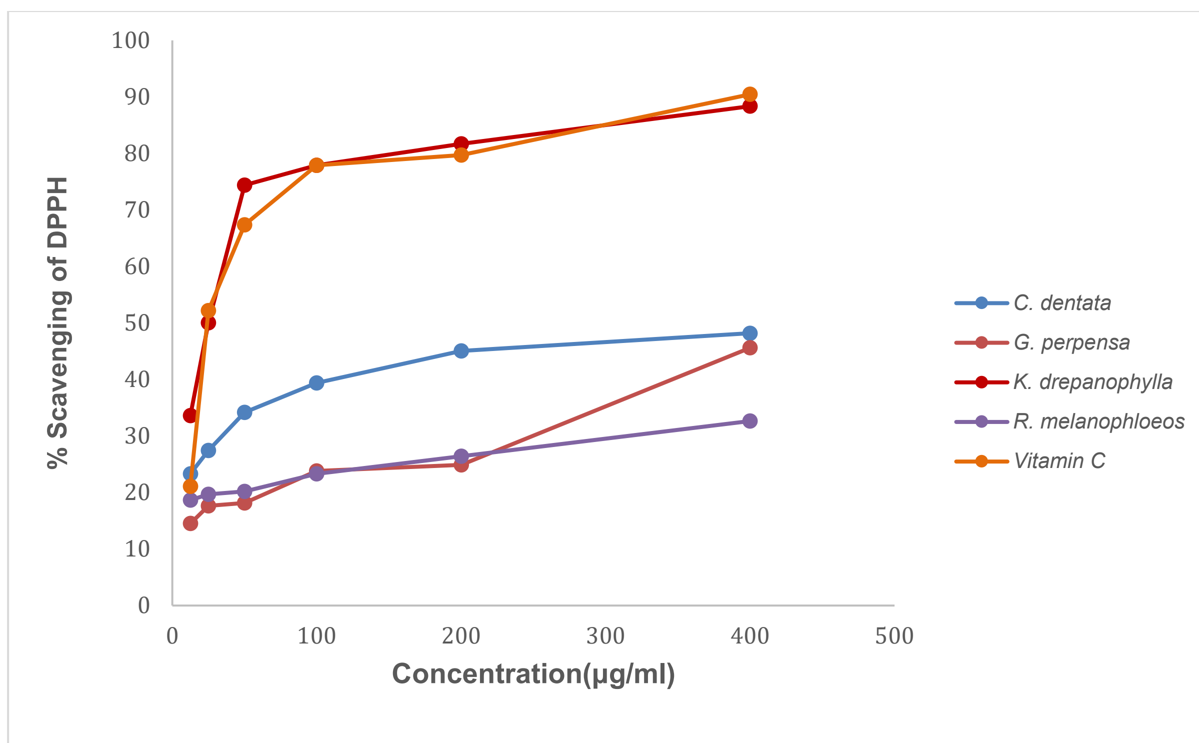


Figure 5.2. DPPH scavenging activity of the ethanol extracts of the medicinal plants.

The scavenging capacities of plant extracts are highly associated with the overall polyphenol contents of the plant, according to a study by Zeghad et al. (2019). The phenolic chemicals found in plant extracts may be responsible for the DPPH radical scavenging activity because they act by giving the radical a hydrogen atom (Tung et al., 2009). Antioxidants are hypothesized to affect DPPH because of their capacity to donate hydrogen, and DPPH functions by taking an electron or a hydrogen radical to transform into a stable diamagnetic molecule (Nariya et al., 2013). Purple-coloured radical with a stable N atom in DPPH is reduced by natural antioxidants to pale-yellow coloured hydrazine that acts by reducing the compounds (Mohd Azma et al., 2013). Antioxidants are hypothesized to affect DPPH because of their capacity to donate hydrogen, and DPPH functions by taking an electron or a hydrogen radical to transform into a stable diamagnetic molecule (Nariya et al., 2013). Purple-coloured radical with a stable N atom in DPPH is reduced by natural antioxidants to pale-yellow coloured hydrazine that acts by reducing the compounds.

The kind of solvent is a further consideration for DPPH radical scavenging activity and the reagents that were used during the assay as plant extracts that had demonstrated

a higher content of phenols or flavonoids or flavonols could have a lower IC_{50} value than a solvent extract that had a lower phytochemical content of the plant phenols.

In a study by Marinova and Batchvarov (2011), the results were found to indicate that sample/ solvent reagent ratio had a significant influence on the accuracy of the DPPH method which is probably due to one solvent having a greater affinity to extracting more antioxidant substances than another solvent. The outcomes of the radical scavenging activity were shown to follow a concentration-dependent pattern and to be statistically different from one another ($p < 0.01$) in a study by Akter et al. (2016), and this was also true in our work.

5.4.2 ABTS radical scavenging activity

The antioxidant capacity of the plant extracts was also evaluated using the protonated radical ABTS. An essential characteristic that pertains to the ability of extracts to serve as antioxidants is their capacity to scavenge protonated radicals (Wolf et al. 2016).

The IC_{50} for *C. dentata* acetone extract (76.94 μg/ml) wasn't as high as that of the standard Trolox (58.167 μg/ml) demonstraing better antioxidant activity. Similarly, the observations for DPPH scavenging activity, the ethanolic and acetone extracts of *K. drepanophylla* had lower IC_{50} values than the standard of 49.46 and 56.23 μg/ml, respectively, representing good ABTS scavenging activity. No report on the antioxidant activity of *K. drepanophylla* against ABTS has been found in the literature.

K. foliosa was said to have monomeric and dimeric anthraquinones and phenylanthraquinones, which demonstrated a variety of biological activity as well as anti-plasmodial effects of some isolated compounds, and to be used for treating abdominal cramps and wound healing (Induli et al., 2013).

Since the IC_{50} for the ethanolic extract of *G. perpensa* was 48.02 μg/ml, it showed somewhat more scavenging activity than *K. drepanophylla* ethanolic extract.

Acetone extract of *R. melanophloeos* demonstrated the highest ABTS scavenging activity, with a concentration of 27.40 μg/ml. The ethanolic extract of *R. melanophloeos*

showed the least amount of scavenging activity at 541.86 µg/ml, while the aqueous extract of the plant had an IC_{50} of 343.77 µg/ml.

At the highest concentrations of 200 g/ml, the water extracts of the four plants showed larger percentage inhibitions than the ethanol and acetone extracts. *K. drepanophylla* water extract had the highest percentage inhibiton against ABTS of 89%. *C. dentata* and *G. perpensa* water extracts inhibited ABTS at 85% and 83% respectively. *R melanophloeos* water extract inhibited the compound at 80%.

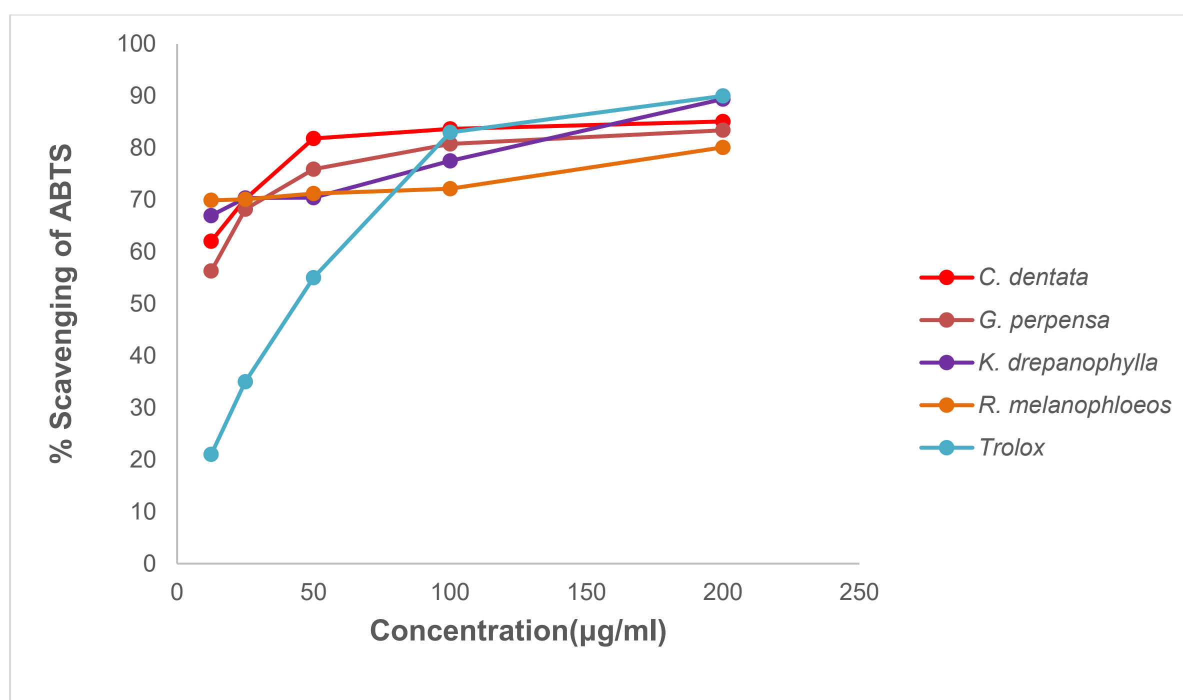


Figure 5.3. ABTS scavenging activity of the water extracts of the medicinal plants.

The ABTS IC_{50} values for the *R. melanophloeos* extracts in chloroform, ethyl acetate, and methanol in a study by Mosa et al. (2011) were lower than the outcomes for DPPH activity. In contrast, it was shown that the plant extracts in this investigation had stronger ABTS scavenging activities than DPPH scavenging activities as the IC_{50} values of the plant extracts were lower. This could be caused by the reason that the plant compounds react with ABTS radicals and oxidise them better than they do for DPPH activity.

The leaves of *R. melanophloeos* were reported in treating fungal infections, molluscs, and worms, the bark and fruit in treating gastrointestinal, respiratory, and nervous system disorders and the bark extracts have been reported to show anti-platelet aggregation activity *in vitro* (Lotter et al., 2019).

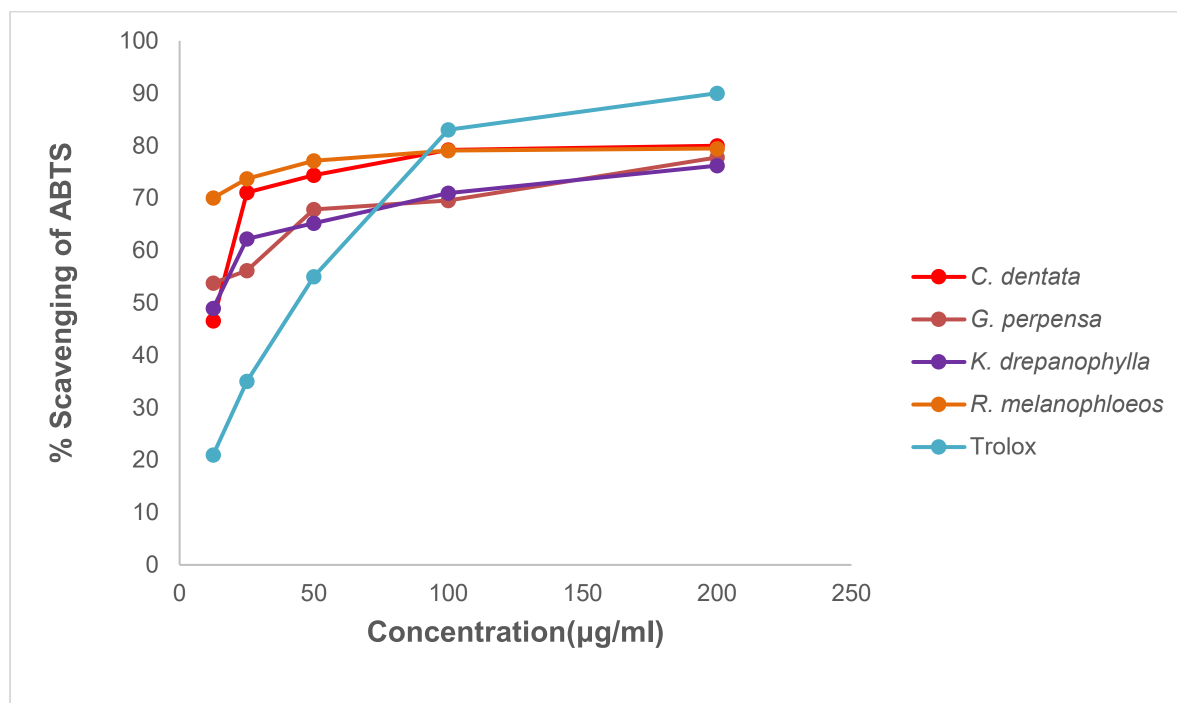


Figure 5.4. ABTS scavenging activity of the ethanol extracts of the medicinal plants.

With substantial differences at $p < 0.01$ for each extract, the ability to neutralize the radical cation ABTS was demonstrated. The scavenging activity of the ABTS radical by the extracts was found to be higher than that of the DPPH radical, and this difference may be due to many variables including the radicals' reactivity with the compounds in plant extracts and the solvents' polarity.

The phytochemicals that were studied for phytochemical analysis indicated that plant extracts possessed antioxidant properties. It is well known that flavonoids function as antioxidants by foraging or by removing the toxic species (Zou et al., 2016).

5.5 Conclusions

The DPPH and ABTS experiments performed on the plant extracts revealed strong antioxidant activity. As a result of the findings, it can be said that *K. drepanophlla, G. perpensa*, *C. dentata* and *R. melanophloeos* can be used as natural antioxidants that can help to fight various diseases. The reference compounds, ascorbic acid and trolox were either less or higher than the extracts regarding their radical scavenging activity or IC_{50} values. Further investigation of the active plant compounds that displayed the best antioxidant activity needs to be conducted. The ethanol and acetone extracts of the tested plants showed percentage inhibtions between 74% to 79% at 200 µg/ml. The IC_{50} values for *K. drepanophylla* extracts were comparatively lower to those of other plant extracts.

REFERENCES

Adedapo, A.A., Jimoh, F.O., Afolayan, A.J, & Masika, P.J. (2008). Antioxidant activities and phenolic contents of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera*. *BMC Complementary and Alternative Medicine*, 8, 54.

Agrawal, S., Kulkarni, G.T. & Sharma, V.N. (2011). A comparative study on the antioxidant activity of methanolic extracts of *Terminalia paniculata* and *Madhuca longifolia. Free Radicals and Antioxidants*, *1*, 62–68.

Akter, K., Barnes, E. C., Brophy, J. J., Harrington, D., Elders, Y. C., Vemulpad, S. R., & Jamie, J. F. (2016). Phytochemical Profile and Antibacterial and Antioxidant Activities of Medicinal Plants Used by Aboriginal People of New South Wales, Australia. *Evidence-Based Complementary and Alternative Medicine*, Article ID 4683059.

Aryal, S., Baniya, K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic Content, Flavonoid Content, and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, *8*, 96.

Brookes, K. B., & Dutton, M. F. (2007). Bioactive components of the uteroactive medicinal plant, *South African Journal of Science*, *103*, 187–189.

Fadipe, V.O., Mongalo, N.I., & Opoku, A.R. (2015). In Vitro evaluation of the comprehensive antimicrobial and antioxidant properties of *Curtisia dentata* (Burm . f) C. A . Sm : Toxicological effect on the Human Embryonic Kidney (HEK293) and Human Hepatocellular. EXCLI Journal, 14, 971–983.

Gulumian, M., Yahaya, E. S., & Steenkamp, V. (2018). African Herbal Remedies with Antioxidant Activity : A Potential Resource Base for Wound Treatment. *Evidence-Based Complementary and Alternative Medicine*, Article ID 4089541.

Induli, M., Gebru, M., Abdissa, N., Akala, H., Wekesa, I., Byamukama, R., Heydenreich, M., Murunga, S., Dagne, E., and Yenesew, A. (2013). Antiplasmodial Quinones from the Rhizomes of Kniphofia foliosa. *Natural Product Communications*, *10*, 8–11.

Lotter, N., Chivandi, E., Lembede, B. W., Ndhlala, A. R., Nyakudya, T. T., & Erlwanger, K. H. (2019). Anti-oxidant activity, alpha-amylase inhibition, and toxicity of leaf extracts of cultivated *Rapanea melanophloeos* (L .) Mez (cape beech). *South African Journal of Botany*, *126*, 261-264.

Mahachi, J. (2013). Medicinal properties of some plants used for the treatment of skin disorders in the OR Tambo and Amathole Municipalities of the Eastern Cape Province, South Africa (MSc Dissertation, Walter Sisulu University).

Mfengwana, P., H., Mashele, S., & Manduna, I.T, (2019). *In-vitro* Antibacterial, Antioxidant and Anti-Inflammatory Effects of Senecio asperulus and Gunnera perpensa from Mohale ' s Hoek, Lesotho. *Pharmacognosy Journal*, *11*(4), 730-739.

Nariya, P. B., Bhalodia, N. R., Shukla, V. J., Acharya, R., & Nariya, M. B. (2013b). In vitro evaluation of the antioxidant activity of *Cordia dichotoma* (Forst f.) bark. *Ayu*, *34*(1), 124–128.

Nutman, T.B., (2013). Insights into the Pathogenesis of Disease in Human Lymphatic Filariasis. Lymphatic research and biology,*11*(3), 144-148.

Takaidza, S., Mtunzi, F., & Pillay, M. (2018). Analysis of the phytochemical contents and antioxidant activities of crude extracts from *Tulbaghia* species. *Journal of Traditional Chinese Medicine*, *38*(2), 272–279.

Wube, A. A., Bucar, F., Asres, K., Gibbons, S., Adams, M., & Streit, B. (2006). Knipholone, a selective inhibitor of leukotriene metabolism. *Phytomedicine*, *13*, 452–456.

Zeghad, N., Ahmed, E., Belkhiri, A., Heyden, Y. Vander, & Demeyer, K. (2019). Antioxidant activity of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium,* and *Opuntia ficus* indica fruits cultivated in Algeria. *Heliyon*, *March*, e01575.

Zou, Z. Xi, W. Hu, Y., & Nie, C. (2006). Antioxidant activity of Citrus fruits. *Food Chemistry*, 196(1), 885-896.

CHAPTER 6

ANTI-INFLAMMATORY ACTIVITY OF MEDICINAL PLANTS EXTRACTS

6.1 Introduction

Inflammation is a localized immunological reaction of cells or tissues of the body to allergic or chemical irritation, injury, and/or infections and acts as an adaptive response to those stimuli or conditions triggered by harmful stimuli and conditions (Iwalewa et al., 2007; Medzhitov, 2008). The process is sped up by the release of chemical mediators from damaged cells or tissues and migrating cells. It is also referred to as the body's response to inactivate or eradicate invasive stimuli or organisms, remove the irritants, and prepare the foundation for tissue restoration (Gunathilake, et al., 2018). Acute pain, heat, redness, swelling, and eventual tissue repair with the production of scars are all signs of inflammation (Schmid-Schonbein, 2006).

A disease associated with inflammation of the lower and upper limbs, elephantiasis can lead to lymphoedema and hydrocele, ultimately causing damage and dysfunction of the lymphatic system (Shrivastava et al., 2016).

As the body recognizes the injury and gets ready to repair the damage, the blood vessels dilate, resulting in an increase in blood supply, and intercellular spaces expand, causing the movement of leukocytes, proteins, and fluids into the inflamed regions, resulting in the manifestation of diseases and disorders through inflammatory responses (Iwalewa et al., 2007).

Chemical mediators are inflammatory compounds that are produced by cells like neutrophils, platelets, mast cells, and monocytes/macrophages or that are released as plasma proteins. Injury, allergies, chemical irritants, and infections are the causes of them (Iwalewa et al., 2007). These chemicals may improve vascular permeability,

encourage neutrophil chemotaxis, stimulate smooth muscle contraction, boost direct enzymatic activity, cause discomfort, and/or mediate oxidative damage by binding to specific target receptors on the cells (Coleman, 2002; Iwalewa et al., 2007).

Ahmed et al. (2013) found that cytokines from activated inflammatory cells can release significant amounts of nitrogen species, including proteases and arachidonic acid metabolites (prostaglandins from cyclooxygenase pathways and leukotrienes from lipoxygenase), as well as toxic oxygen molecules, such as hydrogen peroxide, peroxide anion, and hypochlorous acid.

By stimulating cytokines and activating pro-inflammatory enzymes including lipoxygenase, hyaluronidase, inducible nitric oxide synthase, and xanthine oxidase are as a result of excess production of reactive oxygen species, leading to inflammation (Perera et al., 2018). Lipoxygenases, in turn, produce lipid mediators, leukotrienes, and prostaglandins which have been reported to cause several chronic diseases. Reactive oxygen species (ROS), mostly produced and released quickly by neutrophils, are the hallmark of an oxidative burst. The inhibition of oxidative burst is recognized as being important in the search for anti-inflammatory drugs despite the fact that ROS molecules play an important role in the defense mechanism during phagocytosis. Higher levels of ROS during an oxidative burst can cause severe tissue injury and inflammation (Perera et al., 2018).

6.1.1 Role of arachidonic acid

Arachidonic acid and other polyunsaturated fatty acids are oxidized by lipoxygenases (LOXs) (AA). Phospholipases like phospholipase A1 release arachidonic acid from cell membranes in response to different cytokines, polypeptides, and growth factors. AA produces a mechanism responsible for addressing inflammation and wound healing whilst metabolites produced by AA oxidation cause inflammation (Zheng et al., 2020).

As lipid-peroxidizing enzymes that catalyze the addition of molecular oxygen to unsaturated fatty acids like linoleic and arachidonic acids, lipoxygenases are implicated in the manufacture of leukotriene from arachidonic acid, which is a mediator

of inflammatory and allergic reactions (Porta and Rocha-Sosa, 2002). The four primary varieties of lipoxygenase enzymes are 5-LOX, 8-LOX, 12-LOX, and 15-LOX, and they differ according on where unsaturated fatty acids are oxidized (Porta and Rocha-Sosa, 2002).

Leukotrienes are a factor in several chronic diseases and the selective inhibition of LOX is a crucial step in the management and therapy of many illnesses since it may offer effective methods for controlling inflammatory processes and allergic reactions. (Dzoyem et al., 2014; Adebayo et al., 2015).

6.2 Aim of this study

This chapter examined the prevalence of anti-inflammatory characteristics in traditional medicinal plants used to treat secondary infections and elephantiasis.

6.3. Materials and methods

6.3.1. Lipoxygenase assay

The 5-lipoxygenase (5-LOX) enzyme activity was determined according to the method of Evans (1987) and modified by Baylac & Racine (2003) and Trouillas et al. (2003). An anti-Arachidonate 5-LOX antibody (Sigma-Aldrich, Germany) produced in rabbits was used in the 5-LOX activity assay. Each sample test with various plant extracts in five different concentrations (0.02, 0.05, 0.1, 0.2, and 0.4 mg/ml) were prepared and 12 µl of the ice-cold buffer (potassium phosphate) was mixed with 12 µl (100 U) of the thawed enzyme. At 234 nm, the conjugated dienes' synthesis was timed over a period of ten minutes. As a positive control, nordihydroguaiaretic acid (NDGA) was utilised (enzyme inhibition). The production of the conjugated dienes was measured over 10 min at 234 nm. Nordihydroguaiaretic acid (NDGA) was used as a positive control (enzyme inhibition). Graphpad Prism was used to calculate the IC50 value (concentration at which 50% of the enzyme was inhibited) for each sample test. By comparing enzyme activity to the controls (a mixture of Tween® 20 and DMSO), the percentage of inhibition of the enzyme activity was obtained. Three duplicates of the experiment were performed.

6.4. Results and discussions

Table 6.1 presents the findings for the 5-LOX enzyme inhibitory activity of plant extracts, and Figures 6.1 and 6.2 show the percentage inhibition of the extracts relative to NDGA. One of the plant polyphenols that inhibit LOXs because of their antioxidant characteristics is NDGA. These substances are believed to bind in the active site and inhibit the enzyme from being activated by the conversion of Fe^{2+} to Fe^{3+} (Gilbert et al., 2020). The anti-inflammatory effect increased as the plant extract concentration increased.

With IC_{50} values of 0.05 g/ml respectively, the aqueous extracts of *C. dentata* and *K. drepanophylla* showed the strongest inhibitory efficacy against 5-LOX. The activity of the ethanol extract from *K. drepanophylla* was greater than that of *C. dentata*, at 0.10 and 0.11 µg/ml, respectively. Traditional medicine uses *C. dentata* as part of a combination to treat stomach issues, diarrhea, and heart-water in cattle, among other conditions (Pujool, 2000, Dold & Cocks, 2001). According to Fink-Gremmels (2010), the phytochemical substances that were examined for this plant, such as anthraquinones, cardiac glycosides, phenols, steroids, saponins, and tannins, act to prevent the invasion of bacteria, fungi, and illnesses. Essential oils were discovered to be present in an ethanol extract of the roots, stem, and leaves of *C. dentata* in a study by Doughari et al. (2012). These oils have been reported to have medicinal properties including anti-inflammatory, antiviral, antitumor, antimicrobial, antiseptic, and decongestant effects.

Table 6.1: The inhibitory activity of water and ethanol extracts against the 5-lipoxygenase enzyme represented by IC_{50} values.

5-lipoxygenase assay (µg/ml)		
Plant species	**H_20**	**Ethanol**
C. dentata	0.05 ± 0.02	0.33 ± 0.14
G. perpensa	0.18 ± 0.05	0.11 ± 0.07

K. drepanophylla	0.05 ± 1.10	0.10 ± 0.11
R. melanophloes	0.08 ± 0.03	0.27 ± 0.20
NDGA 0.29 ± 0.11		

IC_{50}: mean of triplicate assays, mean ±SEM.

At 0.08 µg/ml and 0.27 µg/ml, respectively, *R. melanophloeos* water extract showed more efficacy against 5-LOX enzyme than the ethanol extract. At concentrations of 0.18 µg/ml and µ0.11 g/ml, respectively, the *G. perpensa* water extract showed less activity than the ethanol extract.

The percentage inhibitions were determined and higher inhibition accounts for good activity against 5-LOX. *K. drepanophylla* water extract had the highest percentage inhibition at 195% and the ethanol extract was at 87%. Similar to our study, Wube et al. (2006) showed that knipholone isolated from the roots of *K. foliosa* inhibited leukotriene metabolism at 100% + at a concentration of 20 µM in an *ex vivo* setting using human platelets.

The compound knipholone was initially discovered in *K. foliosa* and then in 5 other species of the genus *Kniphofia* following the discovery of the compound from *K. foliosa*. Knipholone is a naturally occurring phenylanthraquinone with just minor work done on its pharmacological activities such as selective inhibition of leukotriene metabolism, antimalarial activity, and antitumor properties (Bringmann et al., 2008). Since the extracts of *K. drepanophylla* have shown excellent activity against DPPH and ABTS for quantitative phytochemical analysis, these qualities of the plant may be linked to their antioxidant activities to scavenge free radicals.

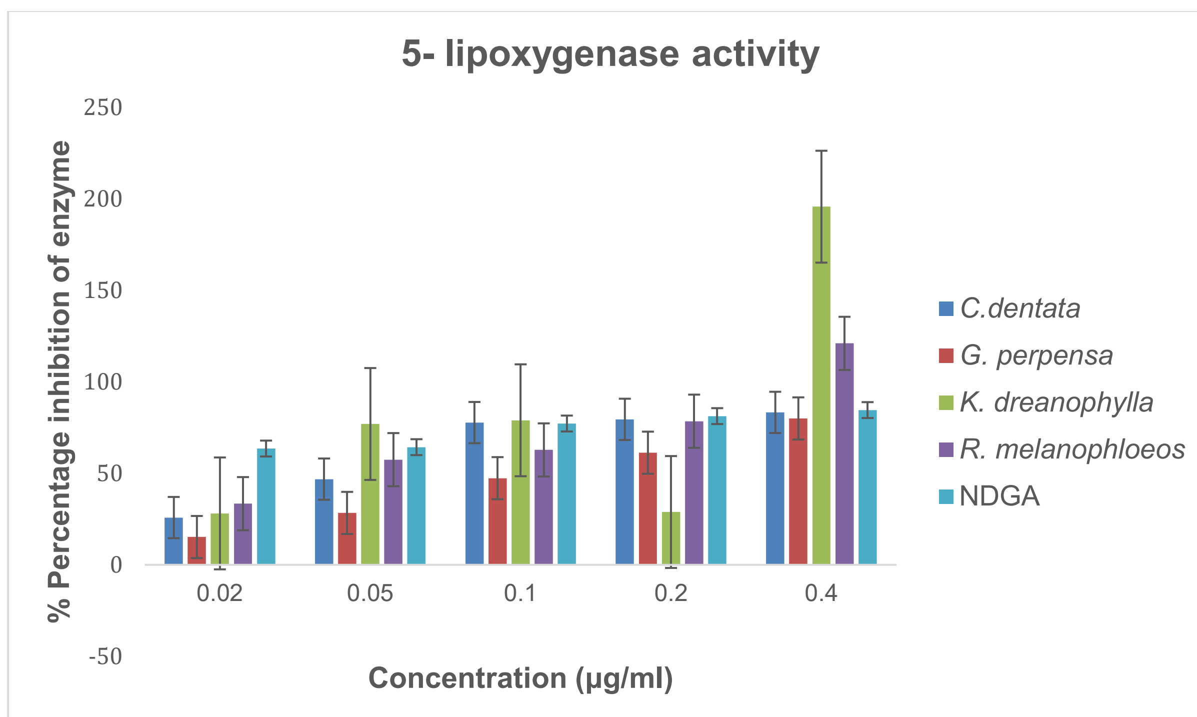


Figure 6.1: The percentage inhibition (%) of the 5-lipoxygenase enzyme by the water extracts and the reference drug Nordihydroguaiaretic acid (NDGA. The error bars represent the standard deviation.

R. melanophloeos water extract inhibited the enzyme at 121% whilst the ethanol extract inhibited the enzyme at 50%.

A study by Madikizela et al. (2012) showed the water extract from *R. melanophloeos* inhibited COX-1 at a level of 60% and the ethanol extract at 100%. The two extracts had different effects on the COX-2 enzyme, with the water extract inhibiting the enzyme at a level of about 65% and ethanol at less than 25%. This demonstrates that the water extract can inhibit the COX-2 enzyme far more effectively than the ethanol extract, and that both extracts can inhibit COX-1 metabolism much more effectively than COX-2 metabolism. The results of Dzoyem et al. (2016) about *R. melanophloeos*' activity against *M. aurum* and *M. tuberculosis* species could support its usage in conventional medicine to treat respiratory issues. By inhibiting the enzyme alpha-amylase, the plant is also used to treat diabetes, one of the chronic diseases linked to inflammation. At 1000 µg/ml, the aqueous extract exhibited a 77.32% inhibition of -

amylase, whereas at the same dosage, the methanolic extract showed a 51.62% inhibition (Lotter et al., 2019).

C. dentata water extract demonstratred a percentage inhibition at 80% and ethanol extract acted against the enzyme at 77%. *G. perpensa* water extract demonstrated an inhibition of 83% and ethanol extract inhibited 5-LOX at 50%.

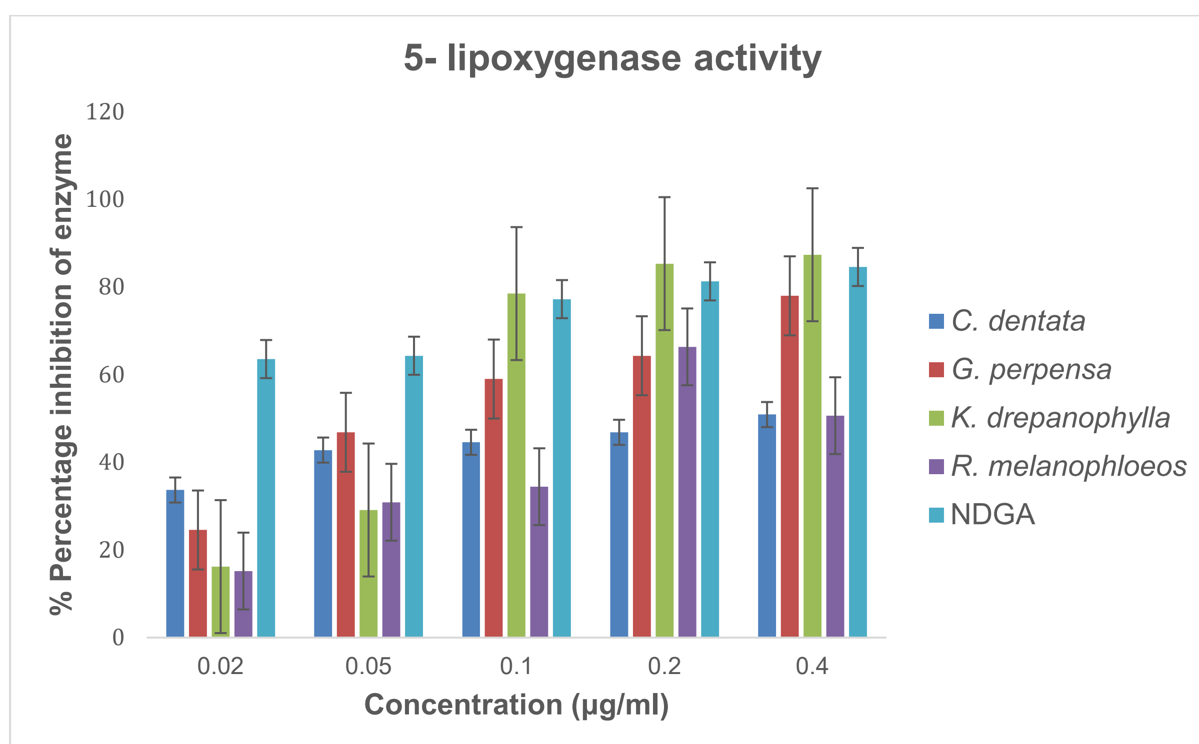


Figure 6.2: The percentage inhibition (%) of the 5-lipoxygenase enzyme by the ethanol extracts and the reference drug Nordihydroguaiaretic acid (NDGA. The error bars represent the standard deviation.

After 48 hours of exposure, Z-venusol, a substance isolated from the roots of G. perpensa, showed a percentage inhibition of 51% at 150 µg/ml against MCF-7 human breast cancer cells in the IL-6 assay (Mathibe et al., 2017). It was determined that interleukin-6, one of the pro-inflammatory cytokines, may be inhibited by G. perpensa in the traditional treatment of inflammatory disorders like hemorrhoids (Hutchings et al., 1996; Mathibe et al., 2017). The aqueous extract of *G. perpensa*, which inhibited

COX-1 and COX-2 by over 70%, showed stronger inhibitory action in the COX-2 bioassay when compared to the COX-1 bioassay, indicating that the extract was selective for the COX-2 enzyme, according to a review (Maroyi, 2016).

For quantitative phytochemical analysis, *R. melanophloeos* followed by *C. dentata* were found to possess a high number of tannins. Several tannins have been found to have pharmacological properties, including the ability to suppress carcinogenesis, lipid peroxidation, and lipoxygenase (Chung et al., 2016). A high intake of tannins though can harm the body and so a limited consumption of tannin possessing foods is needed (Chung et al, 2016).

Because ROS is regarded to be an underlying component that causes oxidative stress and inflammatory cytokines, plant extracts with strong antioxidant activity are thought to contain powerful anti-inflammatory potential. A wide class of phytochemicals known as polyphenols, which includes flavonoids and phenolic acids, has been linked to a number of biological activities, including antioxidant, anti-inflammatory, and anti-diabetic effects (Olaokun, et al., 2017). Activity of plant extracts on the 5-lipoxygenase enzyme can be attributed to their antioxidant activity as demonstrated. The therapeutic properties of phytochemical compounds such as flavonoids such as antioxidative, antimicrobial, antiviral, anti-allergic, anti-cancer and anti-inflammatory have been widely reported (Doughari et at., 2012).

6.5 Conclusions

As evidenced by their lower IC_{50} values, which were lower than NDGA, six extracts displayed greater anti-inflammatory activity against the 5-LOX enzyme, according to our results. he most active extracts were the water and ethanol extracts of *K. drepanophylla* and *G. perpensa* and the water extracts of *C. dentata* and *R. melanophloeos*. Two plant extracts which displayed lower inhibitory activity than NDGA could be active at other inflammatory sites other than 5-LOX activity. Plant

extracts that displayed antioxidant activity against DPPH and ABTS could be considered to have potential against an inflammatory activity as free radicals are associated with inflammatory activity or diseases. The plant compounds found in the plant extracts such as those belonging to the phenolic group are phenolic compounds such as flavonoids and tannins that donate electrons to free radicals by breaking the chain reaction of oxidation. The role of free radicals in inflammation is stimulating cytokines which are inflammatory mediators in the body responsible for initiating anti-inflammatory responses. All these pharmacological properties justify the use of these plants against pain and inflammatrion in traditional medicine.

REFERENCES

Adebayo, S. A., Dzoyem, J. P., Shai, L. J., & Eloff, J. N. (2015). The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. *BMC Complementary and Alternative Medicine*, *15*(159), 1–10.

Ahmed, A. S., Mcgaw, L. J., & Eloff, J. N. (2013). Evaluation of pharmacological activities, cytotoxicity, and phenolic composition of four *Maytenus* species used in Southern African traditional medicine to treat intestinal infections and diarrhoeal diseases. *BMC Complementary and Alternative Medicine*, *13*(100), 1-15.

Akula, U.S., & Odhav, B., (2008). *In vitro* 5-lipoxygenase inhibition of polyphenolic antioxidants from undomesticated plants of South Africa. *Journal of Medicinal Plant Research*, 2(9), 207–12.

Baylac, S., & Racine, P. (2003). Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. *International Journal of Aromatherapy*, *13*(2-3), 138-142.

Bringmann, G., Mutanyatta-comar, J., & Abegaz, B. M. (2008). Knipholone and related 4-phenylanthraquinones : structurally, pharmacologically, and biosynthetically remarkable natural products. *Natural Products Report*, *25*, 696–718.

Chung, K., Wong, T. Y., Wei, C., Huang, Y., Lin, Y., Chung, K., Wong, T. Y., Wei, C., & Huang, Y. (2016). Tannins and Human Health : A Review Tannins and Human

Health : A Review. *Critical Reviews in Food Science and Nutrition*, *38*(6), 421–464.

Coleman, J.W. (2002). Nitric oxide: a regulator of mast cell activation and mast cell-mediated inflammation. *Clinical & Experimental Immunology*, 129, 4-10.

Conner, M., & Grisham, B. (1996). Inflammation, Free Radicals, and Antioxidants. *Nutrition*, *12*(4), 274–277.

Dzoyem, J.P., Kuete, V., McGaw, L.J., & Eloff, J.N. (2014). The 15- lipoxygenase inhibitory, antioxidant, antimycobacterial activity, and cytotoxicity of fourteen ethnomedicinally used African spices and culinary herbs. *Journal of Ethnopharmacology*, 156, 1-8.

Dzoyem, J. P., Aro, A. O., Mcgaw, L. J., & Eloff, J. N. (2016). Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments. *South African Journal of Botany*, *102*, 70–74.

Gilbert, N. C., Gerstmeier, J., Schexnaydre, E. E., Börner, F., Garscha, U., Neau, D. B., Werz, O., & Newcomer, M. E. (2020). Structural and mechanistic insights into 5-lipoxygenase inhibition by natural products. *Nature Chemical Biology*, *16*, 783–790.

Gunathilake, K. D. P. P. Ranaweera, K. K. D. S., Vasantha Rupasinghe, H. P. (2018). In Vitro Anti-Inflammatory Properties of Selected Green Leafy Vegetables. *Biomedicines*, 1–10.

Iwalewa, E. O., Mcgaw, L. J., Naidoo, V., & Eloff, J. N. (2007). Inflammation : the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal of Biotechnology*, *6*(25), 2868–2885.

Lotter, N., Chivandi, E., Lembede, B. W., Ndhlala, A. R., Nyakudya, T. T., & Erlwanger, K. H. (2019). South African Journal of Botany Anti-oxidant activity, alpha-amylase inhibition, and toxicity of leaf extracts of cultivated *Rapanea melanophloeos* (L .) Mez (cape beech). *South African Journal of Botany*, *126*, 261–264.

Madikizela, B., Ndhlala, A.R., & Finnie, J.F., & Van Staden, J. (2012). Ethnopharmacological study of plants from Pondoland used against diarrhoea. *Journal of Ethnopharmacology*, 141, 61– 7.

Maroyi, A. (2016). *From Traditional Usage to Pharmacological Evidence : Systematic*

Review of Gunnera perpensa L. Evidence-Based Complementary and Alternative Medicine, Article ID, 1720123

Mathibe, L. J., Botha, J., & Naidoo, S. (2017). South African Journal of Botany Effects of Z -venusol, isolated from Gunnera perpensa L ., on IL-6 and cAMP activity in human breast cancer cells in vitro ☆. *South African Journal of Botany*, *108*, 96–99.

Medzhitov, R. (2008*).* Origin and physiological roles of inflammation. *Nature*, 454, 428-435.

National Organization for Rare Disorders-Rare Disease Database. (2021, March, 2022). Elephantiasis. https://rarediseases.org./rare-disease/ele

Olaokun, O.O., Mkolo, N.M., Mogale, M.A., & King, P. H. (2017). Phytochemical Screening, Antioxidant, Anti-inflammatory, and Glucose Utilization Activities of Three South African Plants Used Traditionally to Treat Diseases. *Biology and Medicine*, *9*(5).

Oso, B.J., & Karigidi, K. O. (2019). Inhibitory action of dried leaf of Cassia alata (Linn.) Roxb against lipoxygenase activity and nitric oxide generation. *Scientia Agropecuaria*, *10*(2), 185–190.

Porta, H., Rocha-Sosa, M., (2002). Plant lipoxygenases, physiological and molecular features. *Plant Physiology*, 130, 5–21.

Rajasekaran, S., Anuradha, S., Manokaran, G., & Bethunaickan, R. (2017). An overview of filariasis lymphedema. *Lymphology*, 50, 164-182.

Schmid-Schonbein, G.W. (2006). Analysis of Inflammation. *Annual Reviews in Biomedical Engineering*, 8, 93–15.

Shrivastava, S. Gidwani, B., Gupta, A. & Kaur, C.D. (2016). Ethnopharmacological approaches to treat lymphatic filariasis. *International Journal of Pharmacy and Analytical Research*, *5*(3), 1-16.

Trouillas, P., Calliste, C.A., Allais, D.P., Simon, A., Marfak, A., Delage, C., & Duroux, J.L. (2003). Antioxidant, anti-inflammatory, and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. *Food Chemistry*, *80*(3), 399-407.

Wube, A. A., Bucar, F., Asres, K., Gibbons, S., Adams, M., & Streit, B. (2006). Knipholone, a selective inhibitor of leukotriene metabolism. *Phytomedicine*, *13*, 452–456.

Zheng, Z., Li, Y., Jin, G., Huang, T., Zou, M., & Duan, S. (2020). The biological role of arachidonic acid 12-lipoxygenase (ALOX12) in various human diseases. *Biomedicine & Pharmacotherapy* , 129, 1-11.

CHAPTER 7

IN VITRO CYTOTOXICITY ACTIVITY OF MEDICINAL PLANTS EXTRACTS

7.1 Introduction

The definitions of cytotoxicity vary, depending on the nature of the study as it depends if the cells are killed or if their metabolism has been affected (Freshney, 2005). Cytotoxic assays are divided into 5 classes which include, viability assays, survival, metabolic, transformation and irritancy assays (Freshney, 2005).

When cytotoxic anticancer medications induce direct cellular damage, toxicity occurs within the cells. It can also happen externally at the application site or other places, physiologically via membrane transport in the kidneys or neurologically in the brain. The majority of experiments are performed at the cellular level, or cytotoxicity because it is difficult to assess systemic and physiological effects of toxicity in vitro (Freshney, 2005).

Cytotoxicity assays using plant extracts are run to test if there is any possibility a plant could be poisonous or toxic to human cells. There is a danger that documented herbal medicinal products can go undiscovered for toxicity compared to conventional drugs research and development, the problem is that the toxicity of traditional herbal medicines is tested. Most people conclude that the herbal products have been used so far and so most probably they are free from toxicity (Ouedraogo et al., 2012). Only the history of their clinical use and a few studies have given a warning about the potential for hazardous side effects connected with plant medicine, therefore there is a dearth of scientific proof regarding the safety and efficacy of herbal medications to provide the desired outcomes (Raynor et al., 2011; Neergheen-Bhujun, 2013; Bae et al., 2015).

According to one study, herbal medicine and drug-induced liver illness are related (Bae et al., 2015). The genetic makeup of the plant, ambient contaminants, and the

herb's direct harmful effects can all contribute to plant toxicity (Haller et al.,2002; Bae et al., 2015).

A plant's toxicity from a toxicological standpoint depends on the dose, and certain organs and systems, such as the liver, kidney, digestive system, neurological system, and cardiovascular system, may be affected (Woo et al., 2012). Before conducting clinical trials, it is said that in vivo or animal toxicity studies are crucial for determining the safe drug dosages and for forecasting side effects (Bae et al., 2015).

According to Hübsch et al. (2014), traditional interactions are still poorly understood and should be handled with caution when using natural products in combination with commonly prescribed medications.

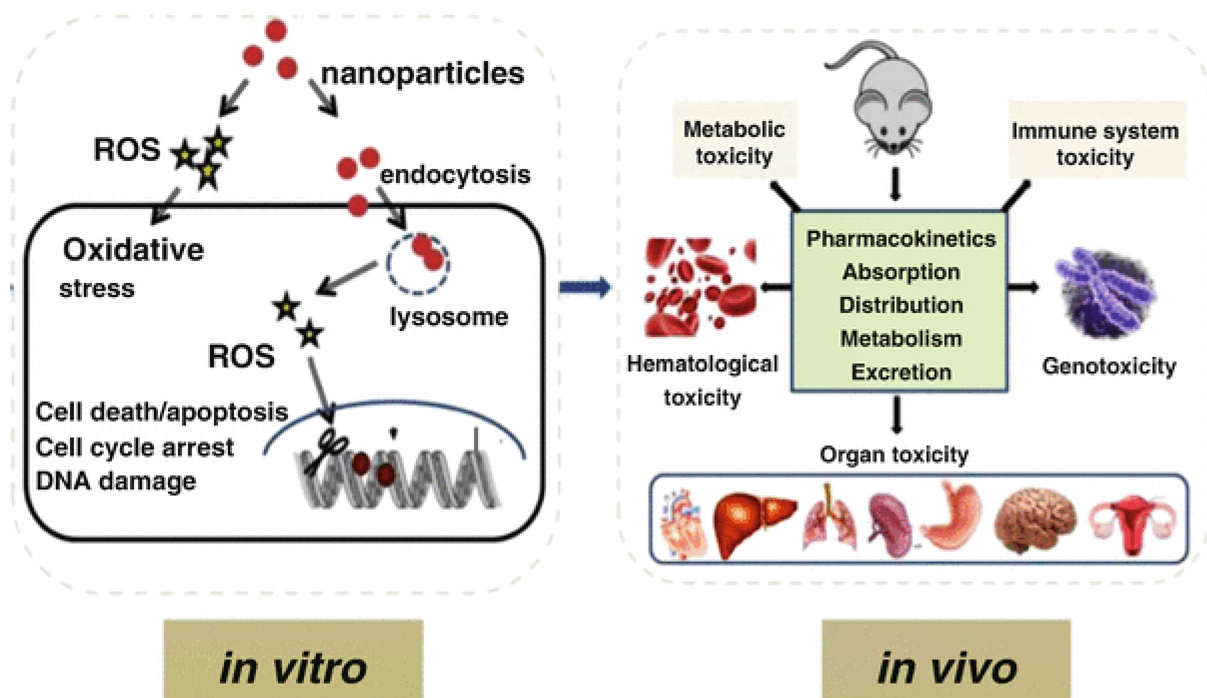


Figure 7.1: Illustration of *in vitro* and *in vivo* toxicity (link. Springer.com).

In vitro cytotoxicity assays are much more beneficial compared to *in vivo* cytotoxicity assays in terms of convenience, direct detection of toxicity, costs and duration (Malich et al., 1997).

If a substance contains significant amounts of biologically harmful extractables, cytotoxicity tests using cell cultures are a quick, standardized, sensitive, and affordable way to find out. The high sensitivity of the tests is caused by the isolation of the test cells in cultures and the absence of the protective mechanisms that cells find in the body (Masoko, 2007).

The majority of human intestinal cell lines are taken from the colon and most of them are cancerous and the only widely used small intestinal human cell line which is isolated from the duodenum is HUTU-80 (human duodenum adenocarcinoma) but is also cancerous (Brosnahan & Brown, 2012).

An *in vitro* test called the H4IIE rat hepatoma cell line bioassay (H4IIE bioassay) is used to find certain types of pollutants in the environment, including in soil, water, and the cells or tissues of living things (Whyte & Tillitt, 2002). Ibrahim et al. (2015) published a study on H4IIE-luc cells, which were first created as a reporter gene assay to detect and semi-quantify the concentrations of specific classes of persistent organic pollutants. Planar halogenated hydrocarbons (PHHs) and chemically related substances that have accumulated in wild creatures can also be screened for using the H4IIE cell bioassay (Whyte et al.,2004). Due of its superior growth characteristics and low basal aryl hydrocarbon hydroxylase (AHH) activity, the H4IIE rat hepatoma cell line is advised (Whyte et al.,2004).

The MTT assay is one of the most popular methods for determining cytotoxicity *in vitro*. Cell viability, proliferation, and activation are measured using the sensitive, accurate, and trustworthy MTT test. 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide (MTT), a yellow water-soluble substrate, can be changed by mitochondrial dehydrogenase enzymes into the dark blue formazan product, which is insoluble in water. While cells that have suffered toxic damage are incapable of reducing the yellow MTT under tetrazolium ring cleavage to a water-insoluble purple-blue compound that precipitates in the cellular cytoplasm and can be eliminated after cell lysis, viable cells can (Mosmann, 1983; Senthilraja & Kathiresan, 2015). When a detergent is added, the cells become solubilized, which liberates the crystals that are solubilized. The amount of surviving cells is directly correlated with the amount of formazan product that was generated (Mosmann, 1983; Masoko, 2007). The formed

product is proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. A spectrophotometer is used to measure the color (Mosmann. 1983). The endoplasmatic reticulum and the mitochondrial dehydrogenase enzymes are involved in the physiological factors that cause the reaction (Fotakis and Timbrell, 2006; Senthilraja & Kathiresan, 2015).

7.2 The study's objective

The chapter targeted on employing mammalian cell lines to examine the cytotoxicity of *C. dentata*, *K. drepanophylla*, and *R. melanophloeos* water, ethanol, and acetone extracts that are used by traditional healers to treat lymphatic filariasis.

7.3. Materials and methods

7.3.1. Plant collection and extraction

In three municipal regions, King Sabatha Dalindyebo, Ingquza Hill, and Nyandeni, three municipal regions of the OR Tambo District Municipality, plants were gathered with the aid of traditional healers and herbalists. The collected plant materials were air dried between 24 and 48 hours at room temperature before being blended (MRC Laboratory Equipment, Durban). 10g of dried plant material was extracted using 100 ml each of water, acetone, and ethanol. This was accomplished by shaking the materials using a platform shaker for 24 hours at room temperature (Labcon, Maraisburg). The extracts were then reduced to complete dryness in front of a fan after being filtered using Whatman No. 1 filter 59 paper discs (Whatman, United Kingdom). All extracts were weighed daily until a constant weight was reached.

7.3.2. Plant extracts serial dilution

A sterile single-use syringe (SURGI PLUS, 10 ml) was connected with a sterile syringe filter (PALL, AcrodiscR 0.22 m PN 4187, NY) to filter the plant extracts, which were then separated into individual 1.5 mL amber glass vials. The filtered extracts were serially diluted twice down to 0.03125 mg/mL (1, 0.5, 0.25, 0.125, 0.0625, 0.03125

mg/mL) from an initial concentration of 1 mg/mL. Dilutions were made using water, ethanol, or acetone as the appropriate extraction solvent.

7.3.3 The maintenance of tissue cultures

The cells were kept up to date using the Prinsloo et al. stated technique (2013). Hutu-80 (cancer cells from the human duodenum) and H4IIE-luc (cancer cells from the rat liver) cell lines were employed. The Canadian University of Saskatchewan gave the University of Saskatchewan the H4IIE-luc cell line. The American Type Culture Collection (ATCC) was where the HuTu-80 cells were obtained (Manassas, VA, USA). The cells were first cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, D55231-1KG, Germany), and then 0.08 mol of sodium bicarbonate (NaHCO3) (Sigma-Aldrich, S5761-1KG) was added, along with NaOH to bring the pH to 7.1. Inside the biosafety hood, the stock media was sterile filtered using a 0.22 m bottle top filter coupled to a 1 L autoclaved Schott bottle. The media can be kept for three months at 4 °C.

A bottle top filter was used to sterilely filter the DMEM growth medium after it had been prepared and supplemented with 10% foetal bovine serum (FBS) (Biowest, S181G-500 G), an antibiotic/antimycotic mixture at 1000 times dilution (Gibco cat number 15420). For 14 days, this material can be kept at 4 °C. In tissue culture trays (90 mm x 20 mm) in a humid incubator with 5% CO2 and 37°C, H4IIE-luc and HuTu-80 cells were cultured. Every three days, media were changed. When confluent, cells were trypsinized and moved (passaged) to new tissue culture dishes.

7.3.4 Bio-assay

With slight adjustments, the bio-assays were carried out in accordance with the procedure outlined by Vogt et al. (2019). The cells were moved from the tissue culture dishes to the 96-well microtitre plates on the first day (TPP, 92096). Dulbecco's phosphate buffered saline (DPBS: Sigma-Aldrich cat no D5652-10L) was used to wash tissue culture dishes confluent with cells three times. Trypsinization of the cells took place at 37°C for 2 minutes using Trypsin EDTA 10X Biowest, X0930-100. 12 mL of enriched DMEM media were added when the trypsin was removed from the dish.

Using a haemocytometer, the cell density was manually measured, and a solution containing 80 000 cells/mL was made. The 96-well plates' inner 60 wells each received 250 µl of the cell solution. PBS was placed in the 96-well plates' outer wells to uniformly distribute the plate's humidity. With the exception of row 12, which was also given cells that were methanol-killed at the conclusion of the test to serve as the negative control (0% viability). Before adding the plant extracts, the 96-well plates were incubated at 37°C and 5% CO_2 for 24 hours. 2.5 L of the serially diluted extracts were added to the cells in their corresponding wells. Cells were exposed in triplicate. Wells that had only been dosed with solvent were regarded as the solvent control (SC), where 100% cell growth was anticipated, while wells that had only been dosed with cells were regarded as the blank control. After that, the plates were incubated for 12, 24, and 48 hours at 37°C and 5% CO_2. The MTT assay was run after the incubation period.

7.3.5 The MTT cell viability test

Promega CellTiter 96 Aqueous Non-Radioactive Cell Proliferation (Promega 2005) and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were used in the cell viability experiment. (Mosmann, 1983). The cells were visually viewed under an inverted microscope (Nikon eclipse TS100, ELWD 0.3/0075, T1-SNCP) to assess the state of the cells after the exposure period (12 h, 24 h, and 48 h). The cells were visually viewed under an inverted microscope (Nikon eclipse TS100, ELWD 0.3/0075, T1-SNCP) to assess the state of the cells after the exposure period (12 h, 24 h, and 48 h). In order to kill the cells and obtain an absorbance value for wells with medium and dead cells, The medium of the negative control wells (row 12) was removed and substituted with 200 L of methanol to kill the cells and have an absorbance value of wells with medium and dead cells. Thiazolyl blue tetrazolium bromide from Sigma-Aldrich, M5655-1G, was given to the cells along with 100 µl of PBS, and the cells were then incubated for 30 minutes at 37°C and 5% CO_2. Cell growth media was prepared with the MTT solution. Following 30 minutes, the media was disposed and 200 µl of dimethyl sulfoxide (DMSO) (Chromasolv® Plus, for HPLC, 99.7%) were added. To dissolve the blue formazan crystals, Riedel-de Haan®. 34869-1L (Honeywell) was added to each well. The plates were then incubated for 30 minutes at room temperature before the absorbance at 560 nm was determined using the MikroWin

2000 program and a spectrophotometer (Berthold, Tristar LB 941). The number of live cells per well is directly proportional to the optical density (OD) of the solubilized formazan. The mean OD of all the exposed cells was calculated by subtracting the mean OD of the negative control (MeOH dead cells). The percentage viability was estimated using this value expressed in terms of the OD values of the solvent control (100 percent viable cells). Non-parametric tests were run on viability percentages to see if these samples were statistically significantly cytotoxic.

7.3.6 Statistical analysis

Using the SPSS statistical software, version 24, statistically significant differences for these replies were examined (IBM). The responses obtained from the exposed cells were tested for normality using the Kolmogorov-Smirnov test, and the homogeneity of the variance was determined using Levine's test. A non-parametric test (Mann-Whitney U) had to be run because of the sample size, unequal variance, and data that were not normally distributed (significance = $p < 0.05$). Mann-Whitney U was used to evaluate whether there were statistically significant variations in each exposure compound's viability ($p < 0.05$).

7.4. Results and discussion

Cytotoxicity levels of the plant extracts were assessed using HuTu-80 and H4IIE – *luc* cell survival model of MTT assay and the plant extracts were considered cytotoxic if the mean percentage viability value was less than 80%.

HuTu-80 cells proliferated in *C. dentata* water extracts at concentrations as low as 0.03125 mg/ml and as high as 1 mg/ml, which was 116.40% and 118.37%, respectively (Figure 1c). For H4IIE- *luc* cells, cell proliferation was displayed at 1mg/ml and 0.03125 mg/ml which was 86.22% and 128.69% respectively after 48 h (Figure 10c). The water extract is the choice of solvent that is usually chosen by traditional healers and herbalists. In a study by Fadipe et al. (2015), identified chemicals from the ethanolic extracts of C. dentata, betulinic acid, ursolic acid, and -sitosterol, showed high LD50 values, indicating that they are less harmful to the HEK 293 (human

embryonic kidney) and Hep G2 (human hepatocellular carcinoma) cell lines. Higher LD_{50} implies that it would take higher quantities of the extract to cause toxic responses, whilst smaller LD_{50} values mean high toxicity and the possibility of danger (Okeleye et al., 2013; Fadipe et al., 2015).

Male Wistar rats were dosed with large quantities of C. dentata water extract in another in vivo investigation, however there were no symptoms of toxicity or mortality in either the acute or subacute phases, and there were no appreciable differences between the extract-fed and control rats ((Wintola & Afolayan, 2018). Another study found that compared to the standards of vincristine sulfate and cyclophosphamide, with LC_{50} values of 0.52 g/ml and 16.3 g/ml respectively, the hydroalcoholic extracts of *C. dentata* had no harmful effects on brine shrimp eggs with LC_{50} values of 0.302 mg/ml (Oyedemi et al., 2012).

After 48 hours of exposure to high concentrations of 1 and 0.5 mg/ml, the acetone extract of *K. drepanophylla* demonstrated significant cell vitality; the percentage viability was more than 100% for both Hutu-80 and H4EII-luc cells (Figures 5c and 14c). The water extract demonstrated that the plant's water extract is hazardous at 1 mg/ml after 24 and 48 hours, but that it can become non-toxic when supplied starting at 0.5 mg/ml and decreasing. After 48 hours of exposure, the HuTu-80 cells were less cytotoxic to the acetone extracts of *K. drepanophylla* and *R. melanophloeos* than they were to the ethanol and water extracts.

At high concentrations, the water, acetone and ethanol extracts of *R. melanophloeos* were highly toxic against HuTu-80 cells after 24 and 48 hours (Figures 3,6, and 9) and they were also toxic against H4EII – *luc* cells after 48 h (Figures 12c, 15c and 18c).

K. drepanophylla acetone extract is the only extract of acetone where the cells proliferated (118.80%) at high concentrations against H4EII – *luc* cells after 48 h (Figure 14c). When utilizing this plant as medication, lowering the concentration of the extracts may be advantageous because all of the extracts were discovered to be poisonous to HuTu -80 cells at 1 mg/ml after 24 hours, despite the fact that the cells appeared to have improved vitality after 48 hours (Figure 5a and 5b).

Knipholone and knipholone anthrone, two compounds isolated from *K. foliosa*, had different effects on melanoma and leukemic monocyte/macrophage cell lines, with Knipholone being between 70 and 480 times less toxic to cancer cells. Biochemical and morphological studies also revealed that knipholone anthrone's cytotoxicity was linked to membrane dysfunction that resulted in necrosis leading to discovery of a new natural anticancer drug. *K. drepanophylla* might also possess a class of these compounds that were found in *K. foliosa* which are either toxic or less toxic to HuTu and H4EII cells.

Five compounds isolated from the roots of *K. foliosa*, 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene,10-(chrysophanol-7′-yl)-10-(ξ)-hydroxychrysophanol-9-anthrone, chryslandicin, knipholone, and chrysophanol with knipholone being found as the most cytotoxic and 10-(chrysophanol-7′-yl)-10-(ξ)-hydroxychrysopanol-9-anthrone and chryslandicin displayed very low toxicity ED_{50} (effective dose) values of 104 and 90 µg/mL respectively against human epidermoid carcinoma (KB) cells (Wube et al., 2005). Since the acetone extract of *K. drepanophylla* was shown to be less hazardous against H4EII cells at high concentrations, the kind of solvent used may have an impact on the sorts of chemicals discovered in the various extracts utilized in the study.

Acetone has good properties according to Eloff (1998), it can dissolve hydrophilic and lipophilic compounds and has low toxicity. Isolation of the compounds of this plant and testing them for cytotoxicity like in previous studies mentioned above could be good to find how safe the plant is for medicinal purposes.

The results demonstrated that *R. melanophloeos* ethanol, acetone and water extracts showed the highest cell survival after 12 h in H4EII-*luc* cell line exposures but the viability decreased after 24 and 48 hours (Figures 12, 15 and 18). *R. melanophloeos* extracts, with the exception of the acetone extract at 0.5 mg/ml in Hutu-80 cell exposure, showed toxicity in Hutu-80 and H4EII- luc cell exposures at greater doses of 1 and 0.5 mg/ml. However, the extracts were less hazardous at lower concentrations.

The water and acetone extract of *K. drepanophylla* and *C. dentata* water and ethanol extracts displayed non-toxicity when administered against H4EII-*luc* cells after 48 h exposures (Figures 10c, 11c, 14c and 16c). The cytotoxic activity of *R. melanophloeos* extracts in chloroform, ethyl acetate, hexane, methanol, and water was assessed using the brine shrimp lethality test. These extracts were found to have LC_{50} values values greater than 250 μg/ml and were not regarded as toxic to the eggs because extracts with LC_{50} values less than 250 μg/ml are considered significant (Mosa, et al., 2011).

In accordance with Gwala (2011), a triterpene compound that was isolated from *R. melanophloeos* was identified as 3-Hydroxylanosta-9, 24-dien-21-oic acid when it was tested for cytotoxicity on the HEK293 and HEPG cell lines. The LC_{50} values were 851.5 μg/ ml and 796.0 μg/ ml, respectively, and extracts of the plant were considered to be safe for medicinal use.

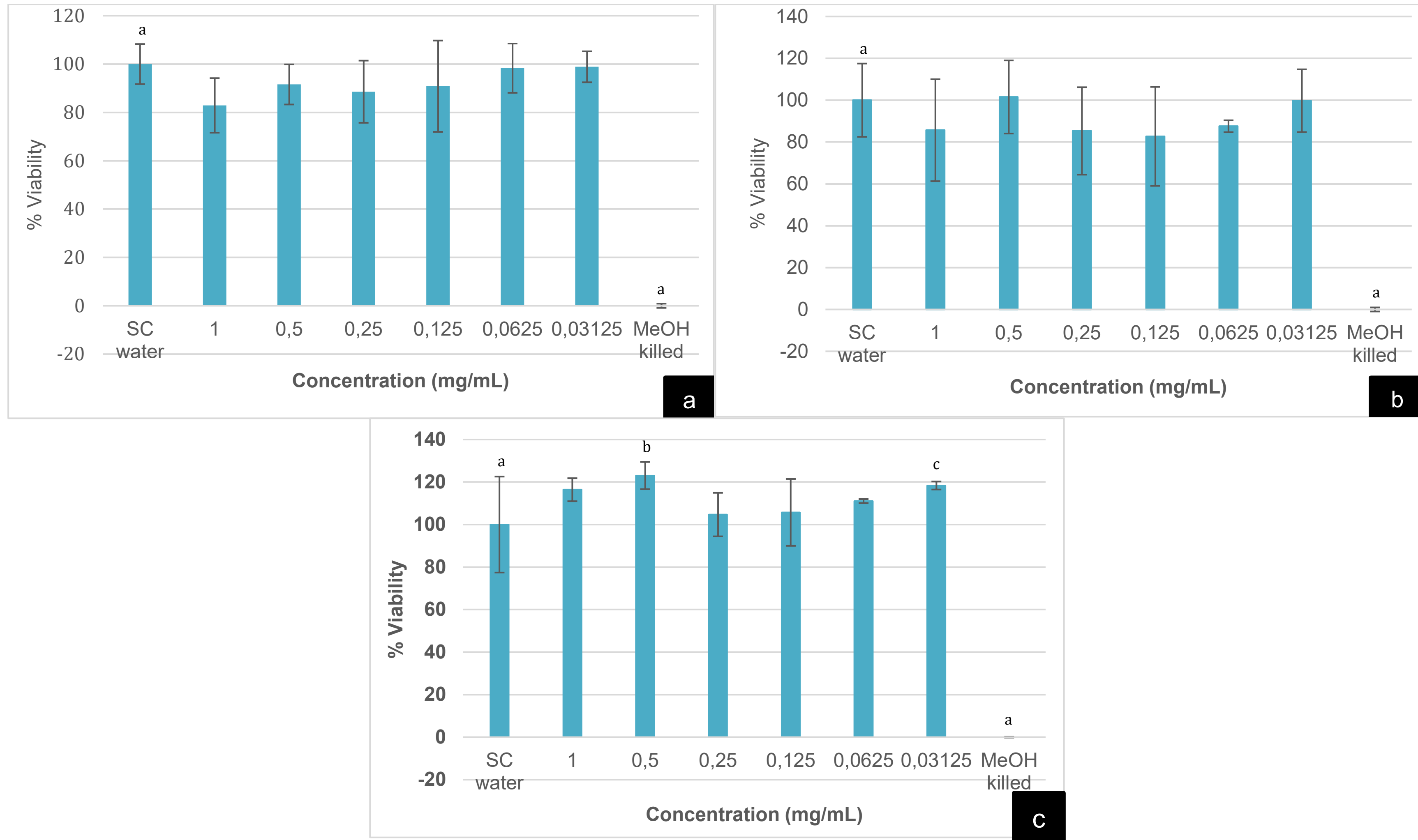


Figure 7.2: Cytotoxicity effects of *C. dentata* water extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

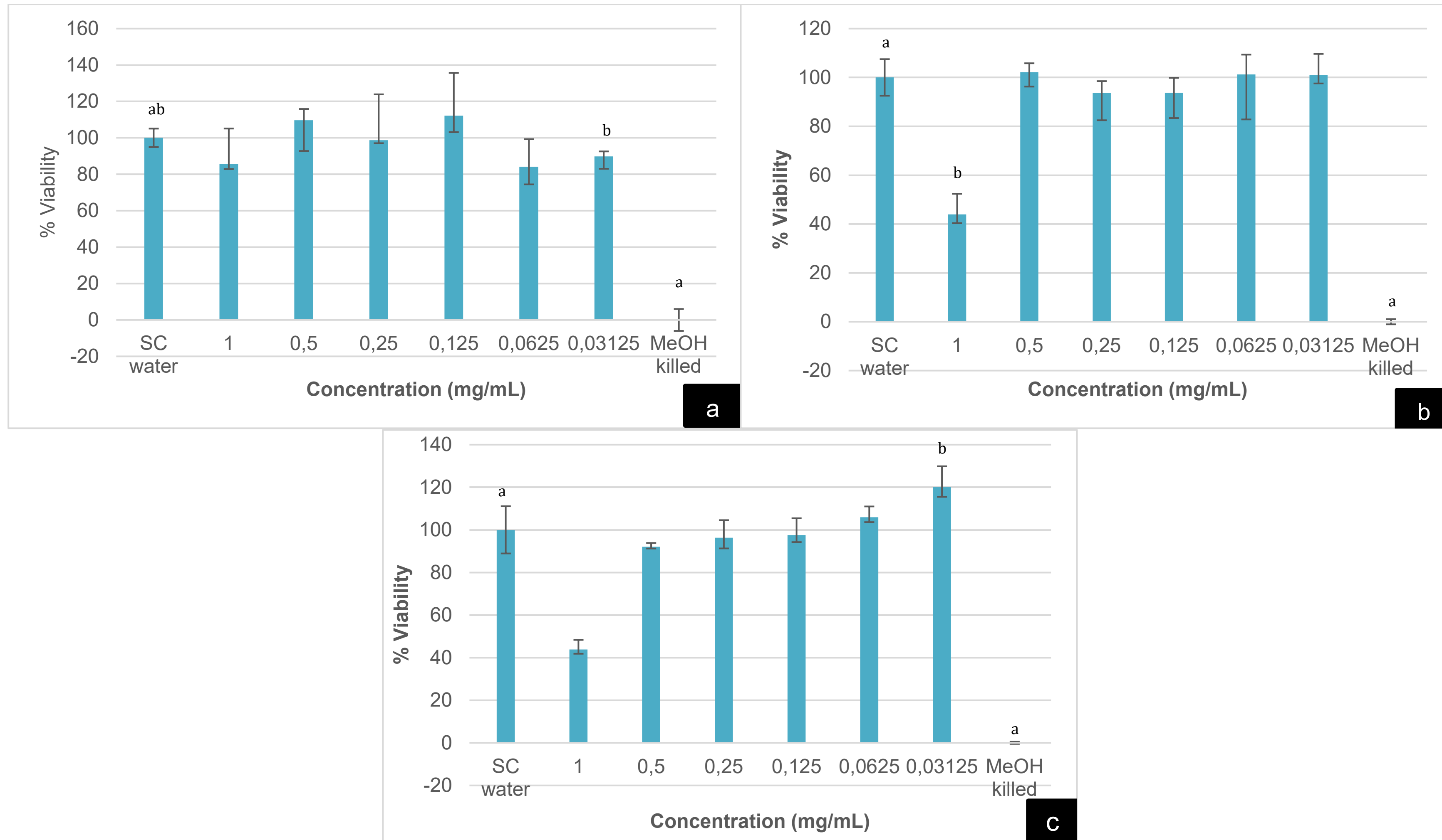


Figure 7.3: Cytotoxicity effects of *K. drepanophylla* water extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

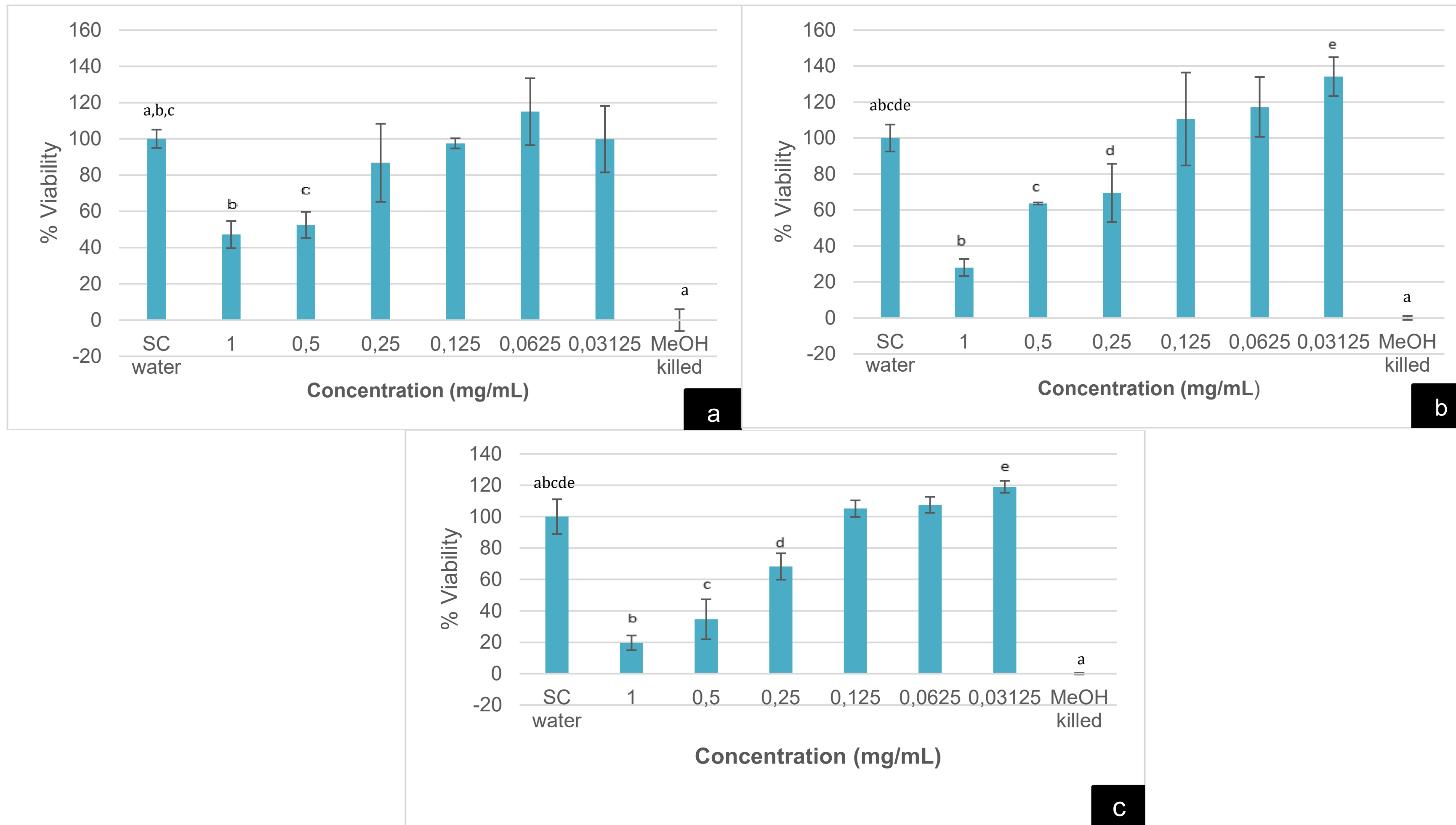


Figure 7.4: Cytotoxicity effects of *R. melanopholoeos* water extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

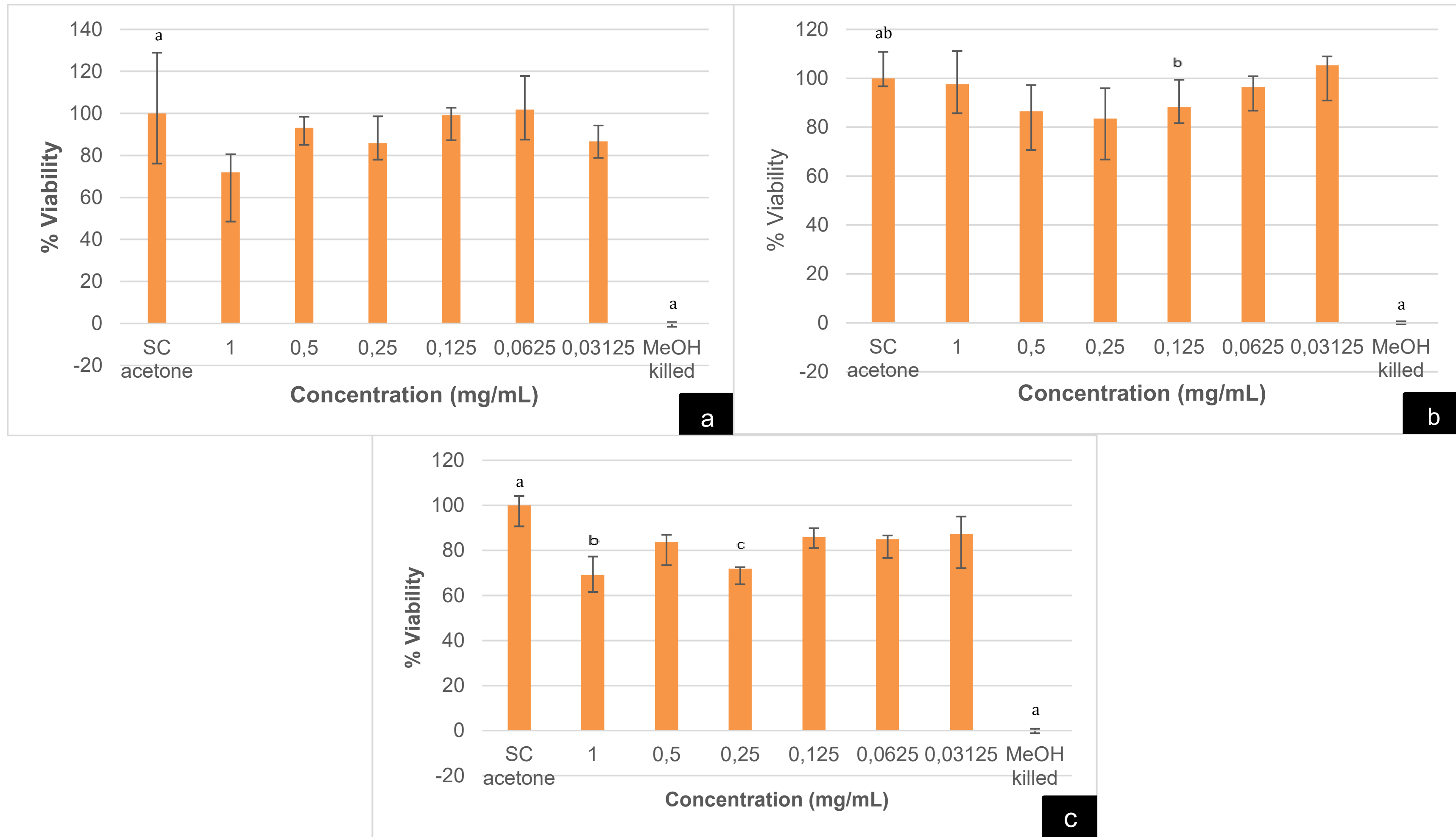


Figure 7.5: Cytotoxicity effects of *C. dentata* acetone extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

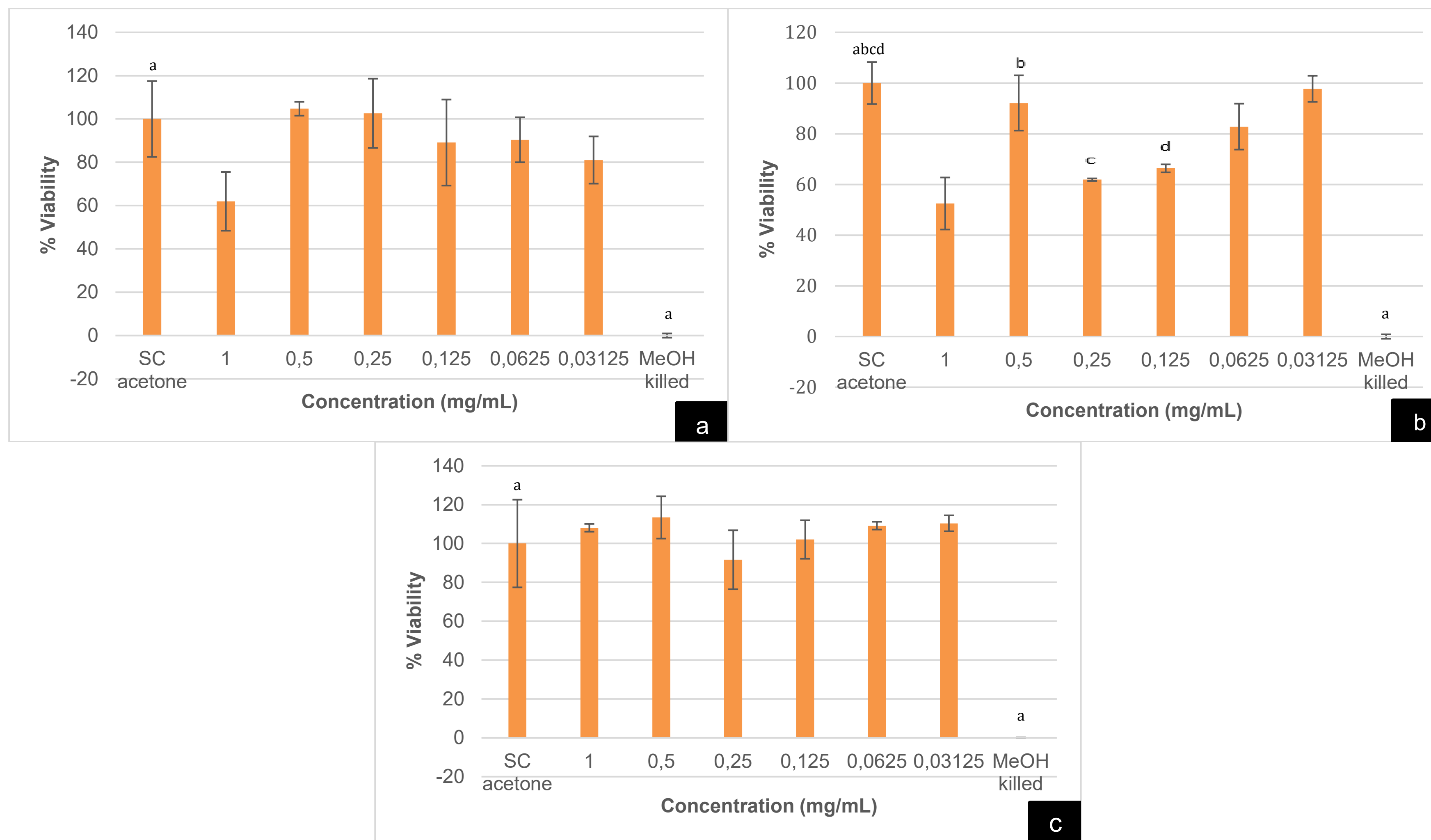


Figure 7.6: Cytotoxicity effects of *K. drepanophylla* acetone extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

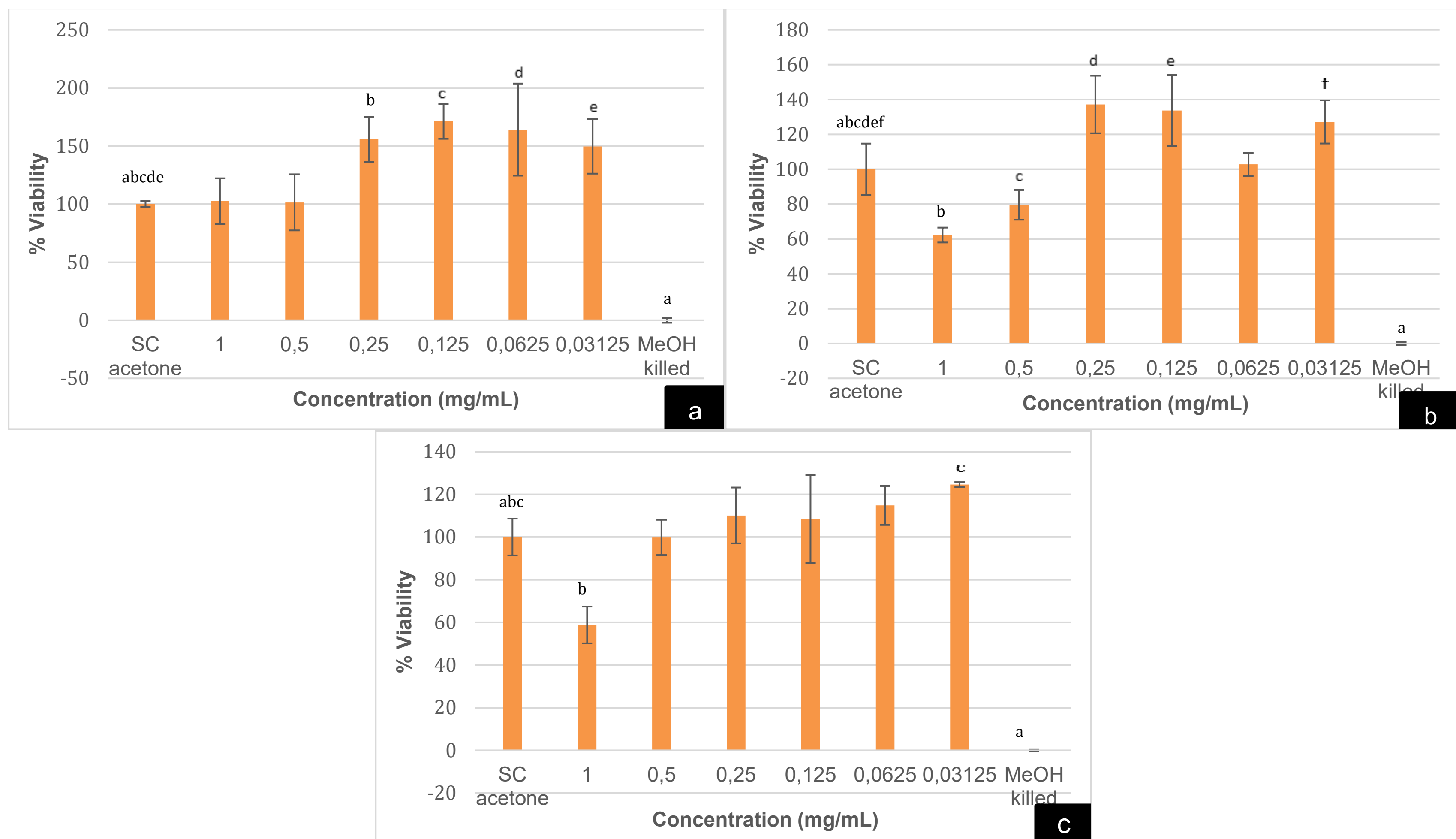


Figure 7.7: Cytotoxicity effects of *R. melanophloeos* acetone extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

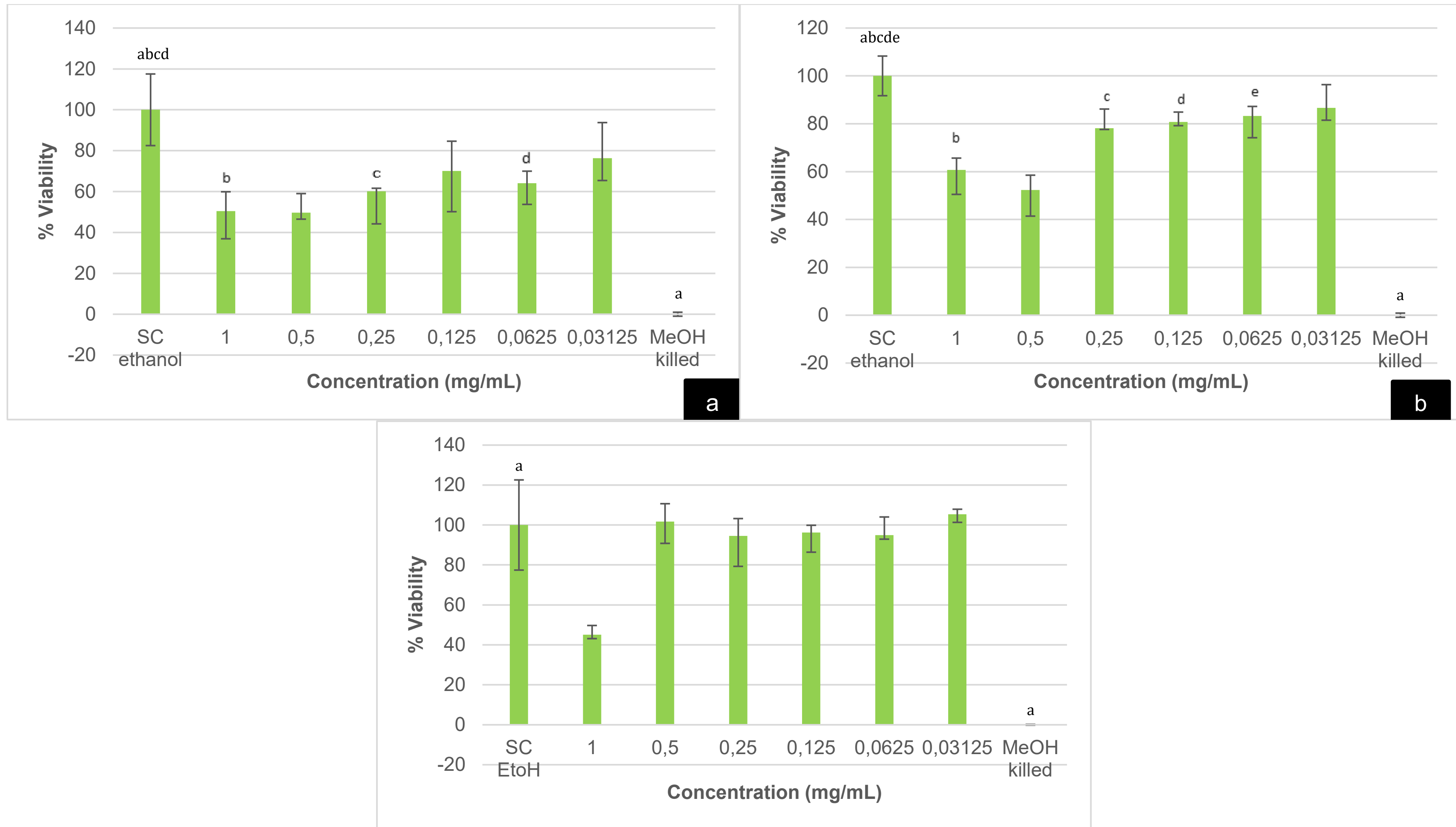


Figure 7.8: Cytotoxicity effects of *C. dentata* ethanol extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

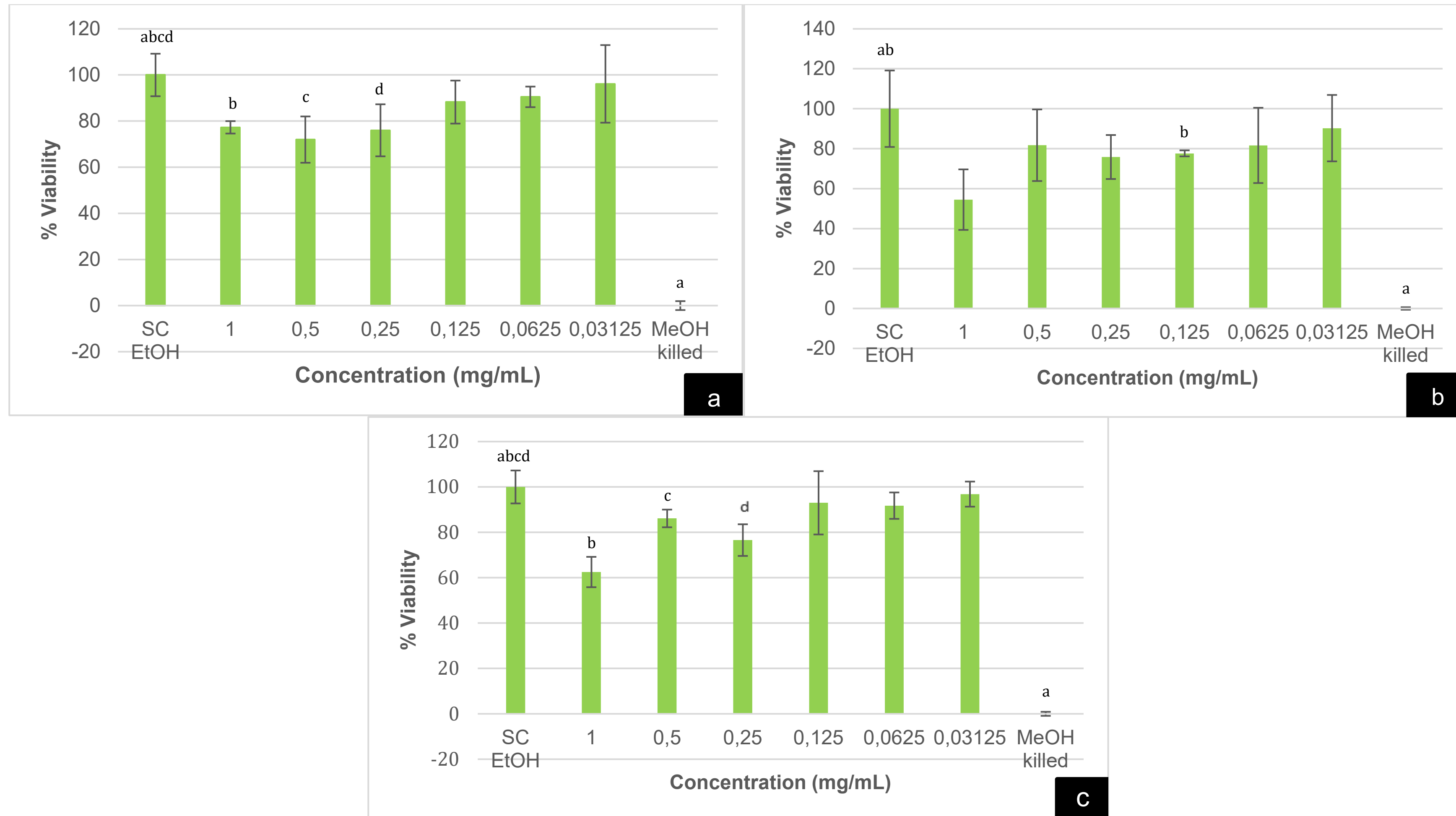


Figure 7.9: Cytotoxicity effects of *K. drepanophylla* ethanol extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

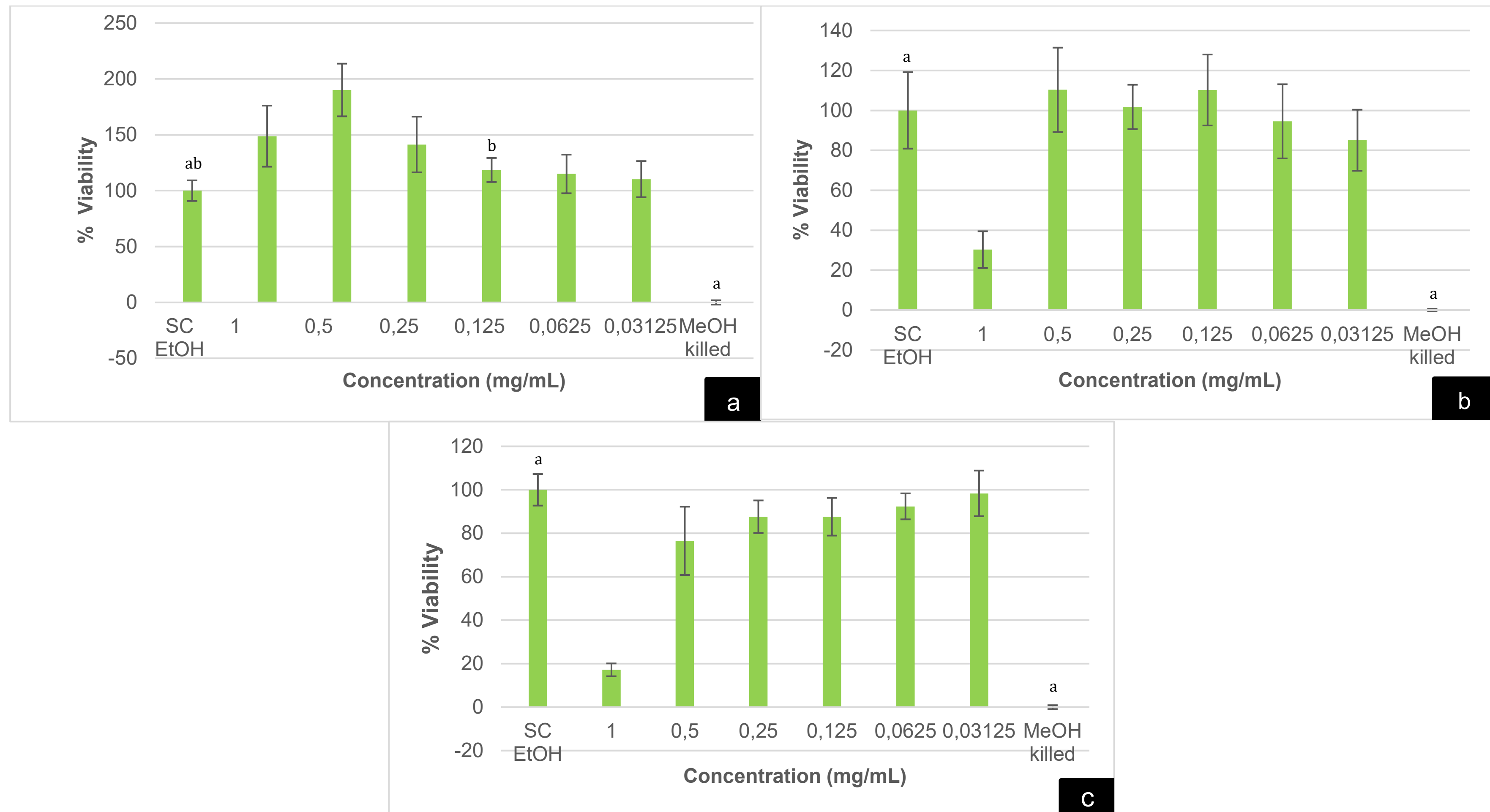


Figure 7.10: Cytotoxicity effects of *R. melanophloeos* ethanol extract's cytotoxicity to Hutu-80 cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

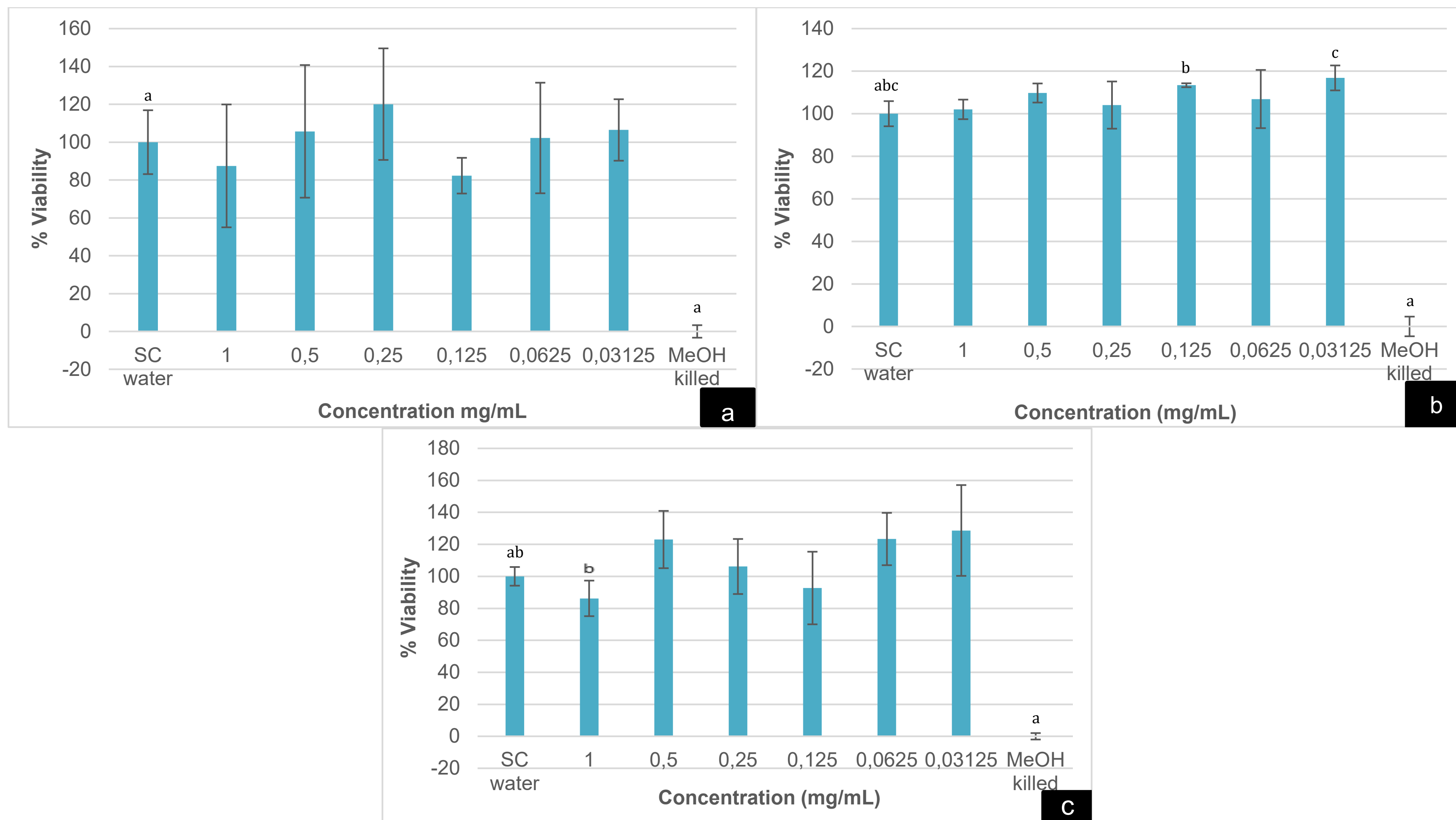


Figure 7.11: Cytotoxicity effects of *C. dentata* water extract's cytotoxicity to H4IIE-luc cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

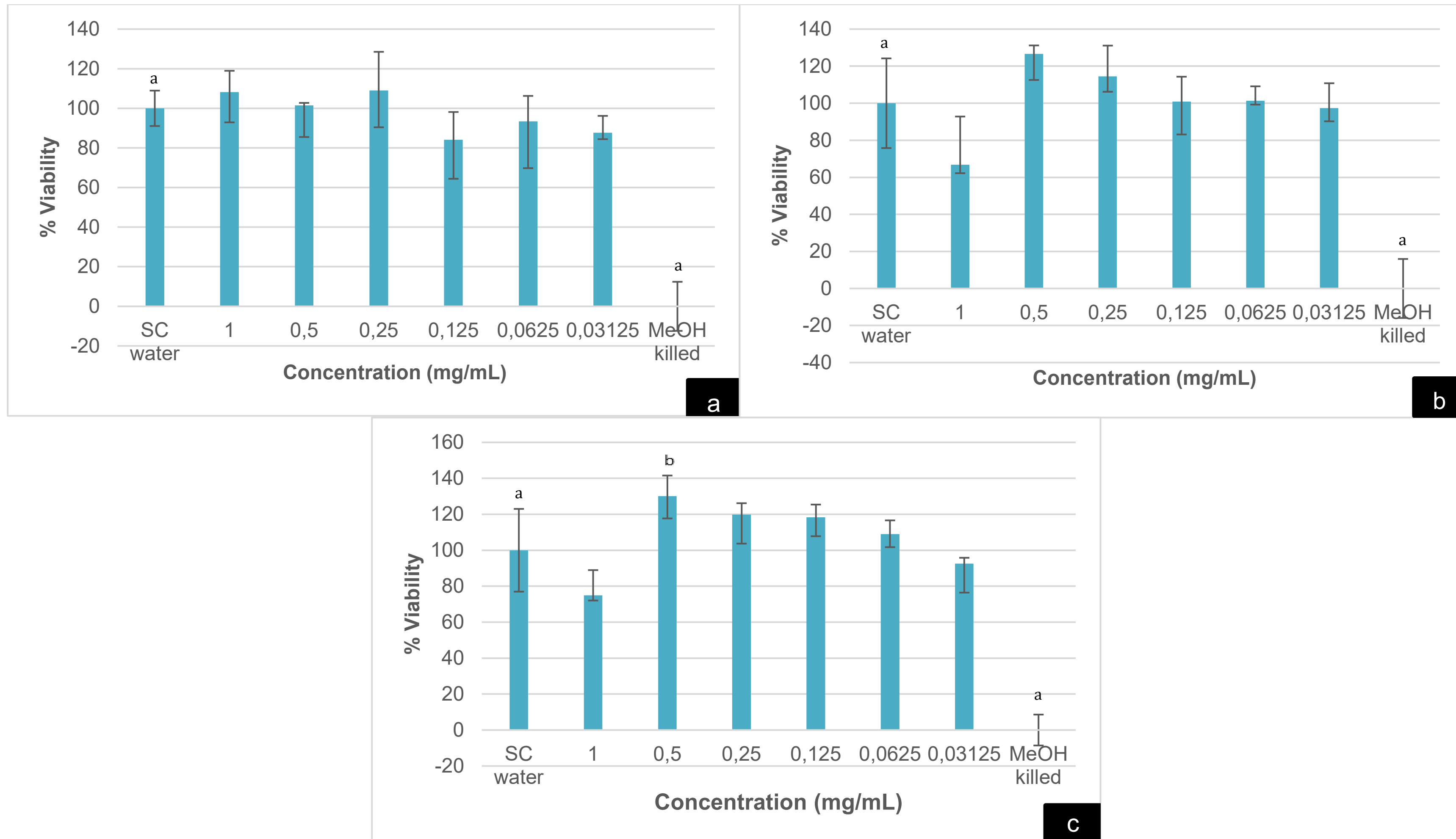


Figure 7.12: Cytotoxicity effects of *K. drepanophylla* water extract's cytotoxicity to H4IIE-luc cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

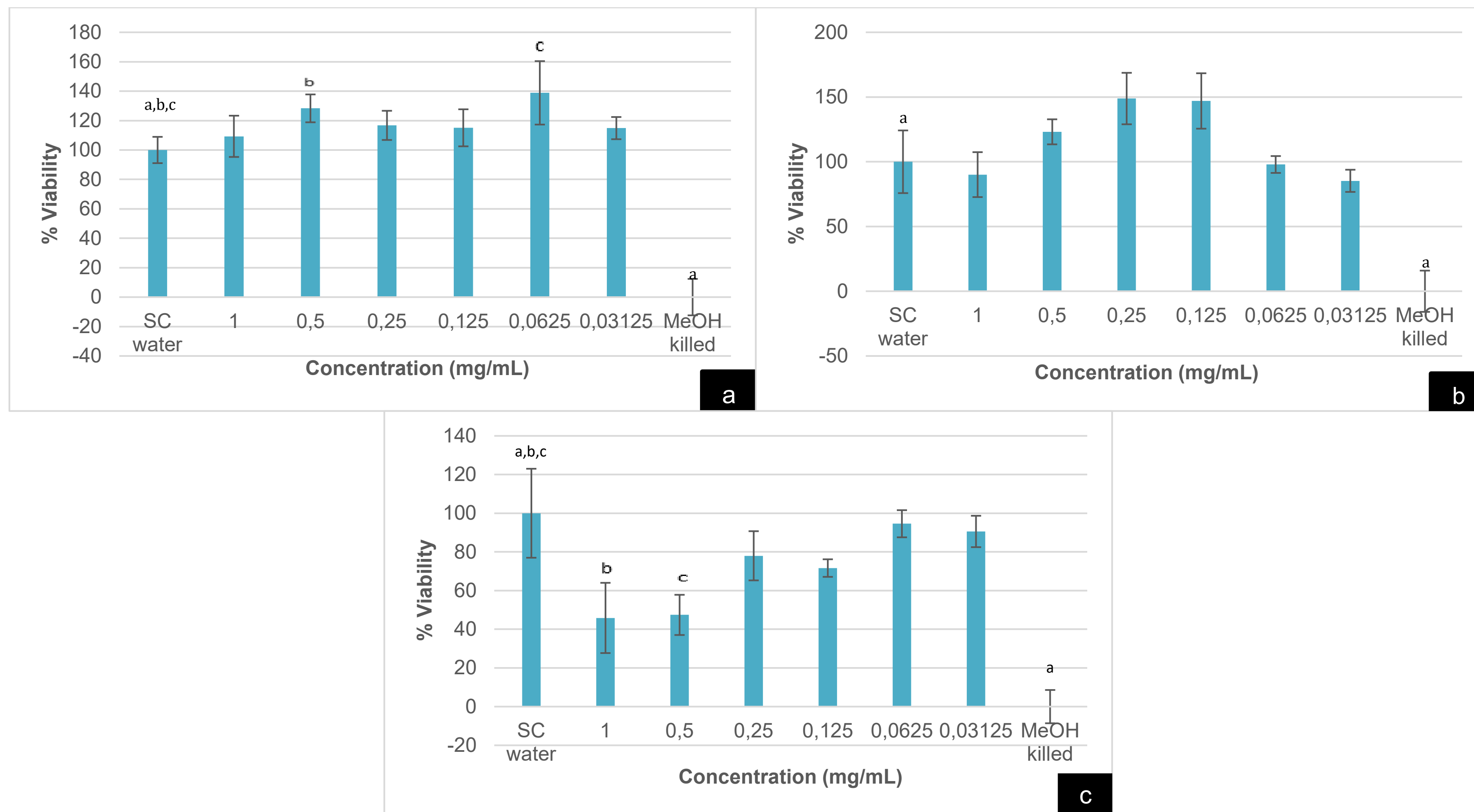


Figure 7.13: Cytotoxicity effects of *R. melanophloeos* water extract's cytotoxicity to H4IIE-luc cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

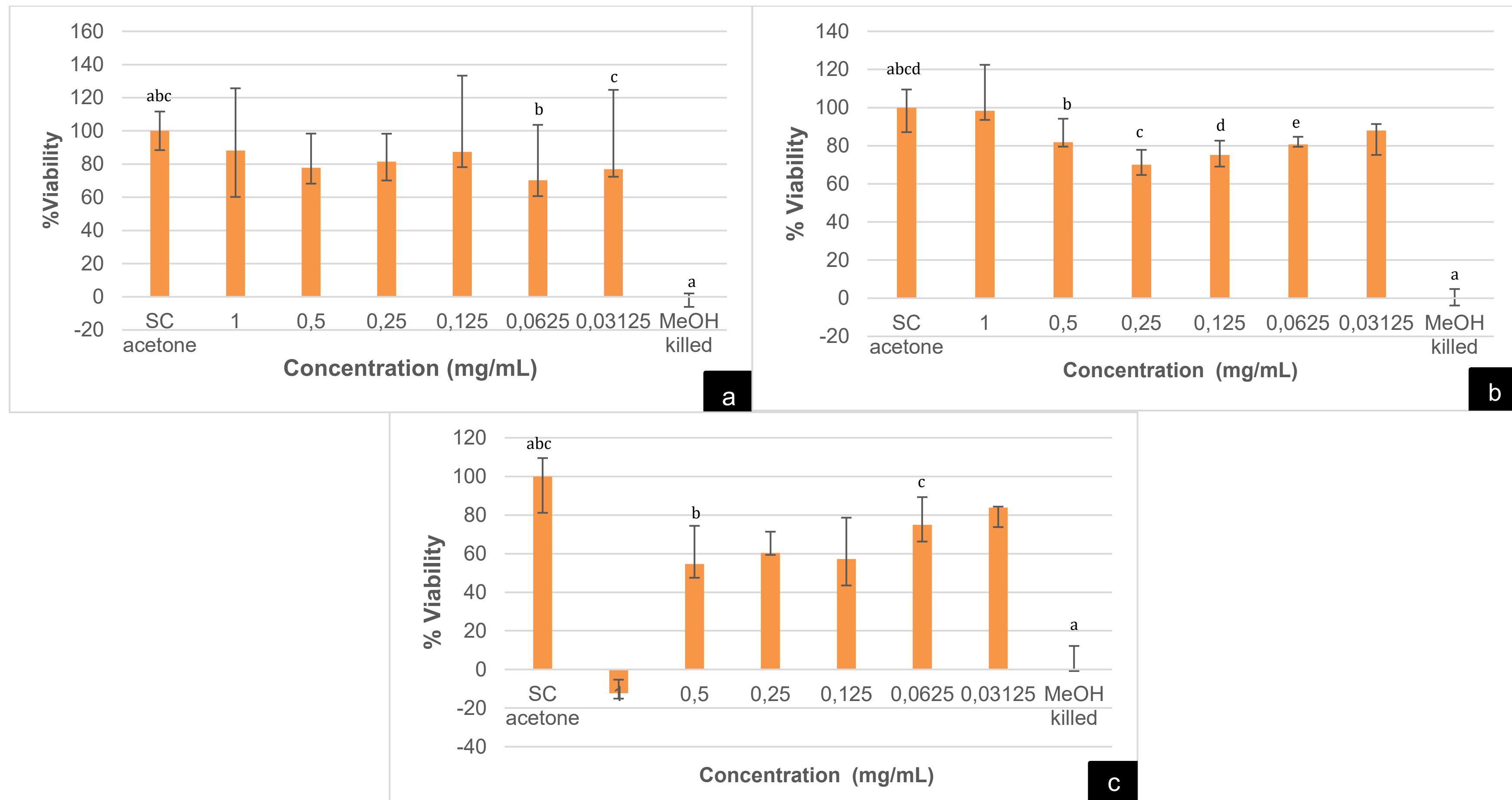


Figure 7.14: Cytotoxicity effects of *C. dentata* acetone extract's cytotoxicity to H4IIE-luc cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

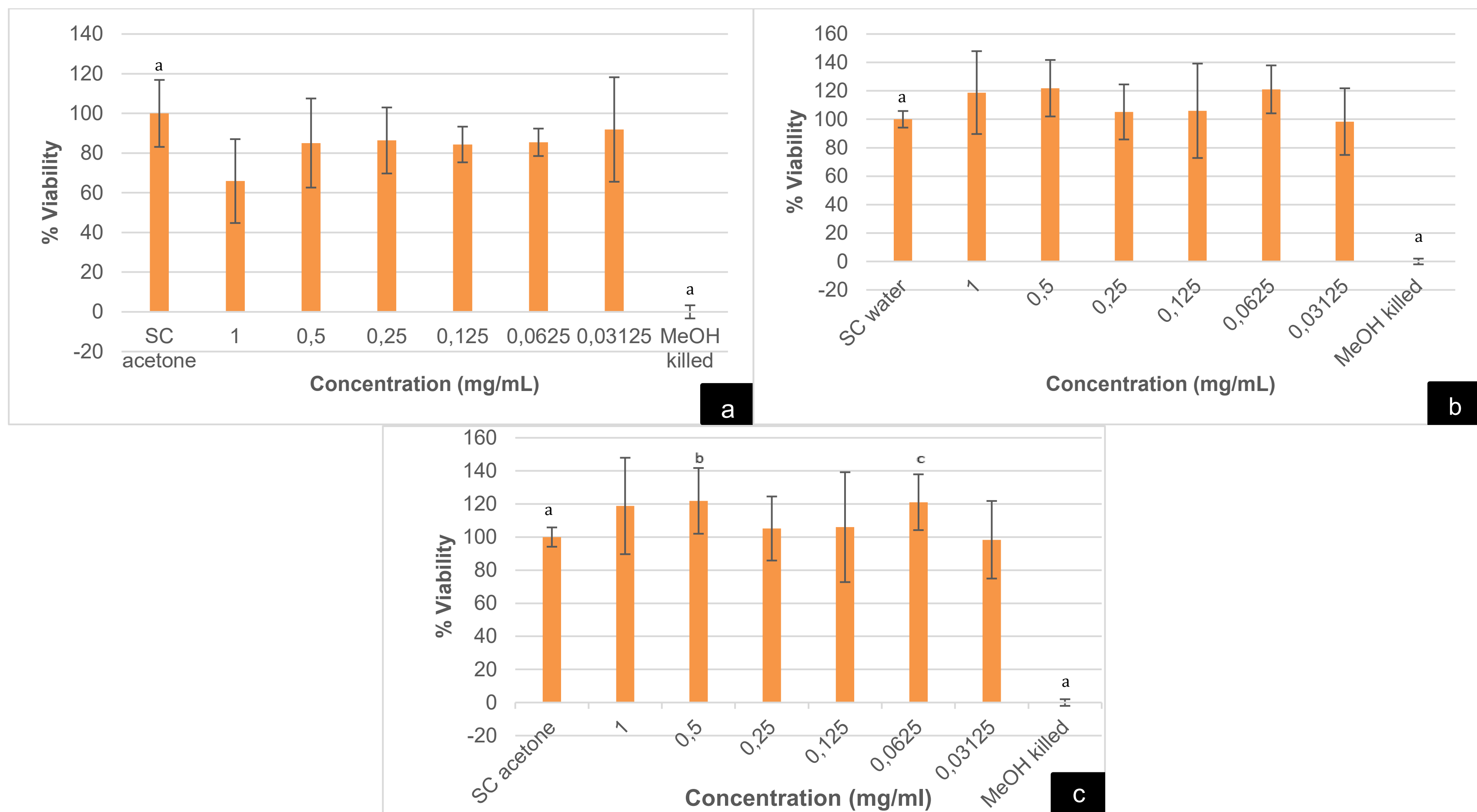


Figure 7.15: Cytotoxicity effects of *K. drepanophylla* acetone extract's cytotoxicity to H4IIE-luc cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, $p \leq 0.05$). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

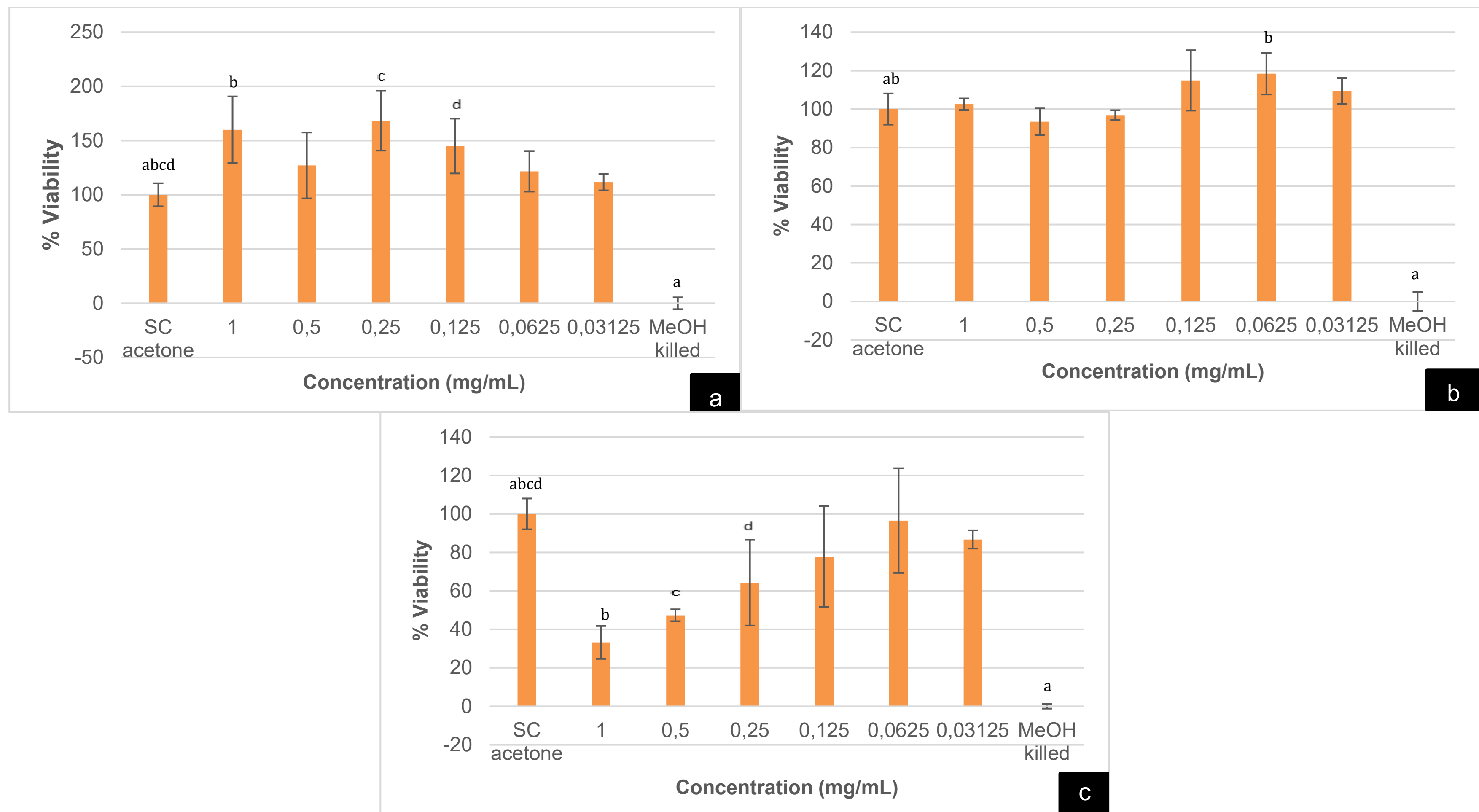


Figure 7.16: Cytotoxicity effects of *R. melanophloeos* acetone extract's cytotoxicity to H4IIE-luc cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

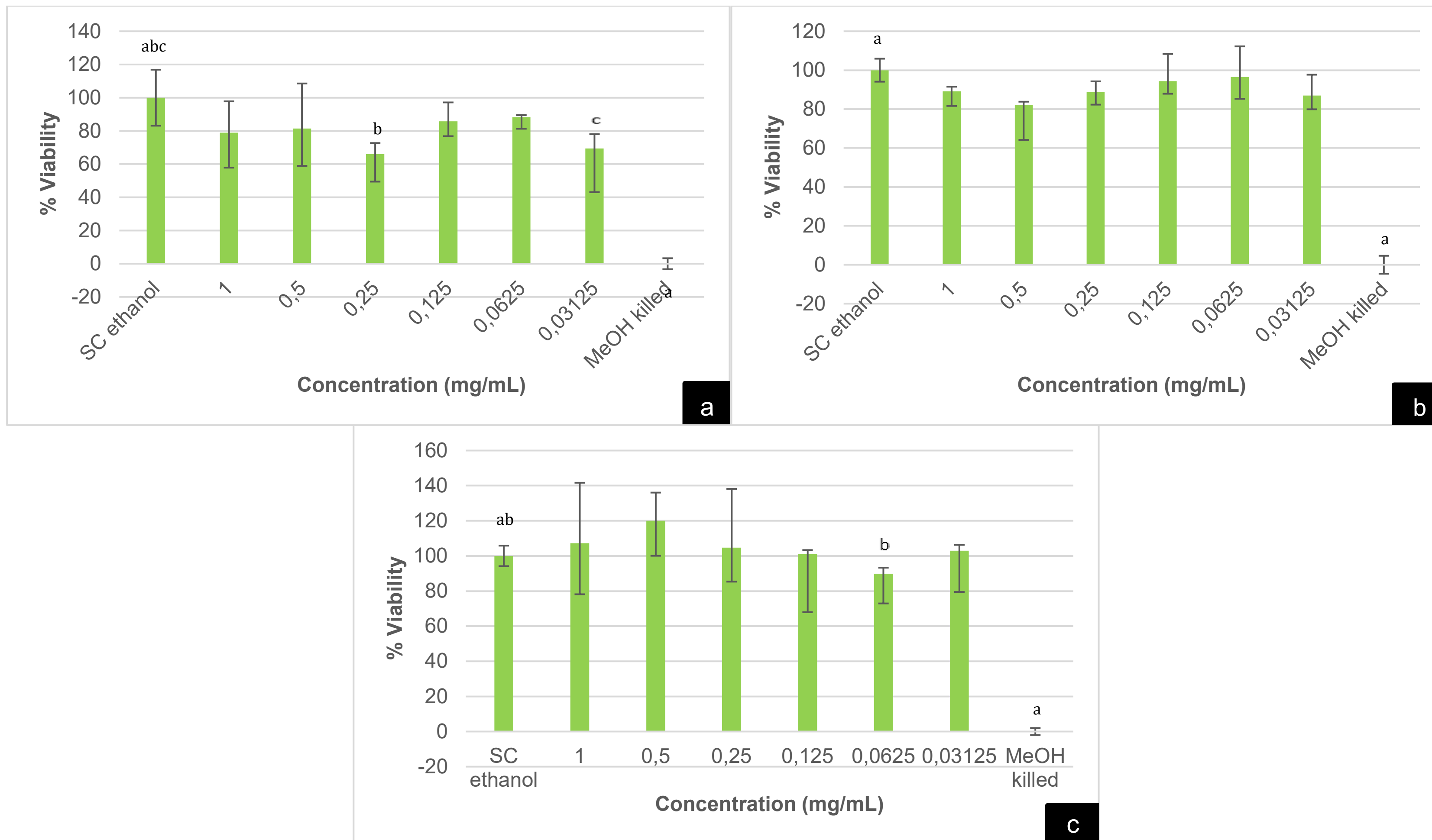


Figure 7.17: Cytotoxicity effects of *C. dentata* ethanol extract's cytotoxicity to H4IIE-luc cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

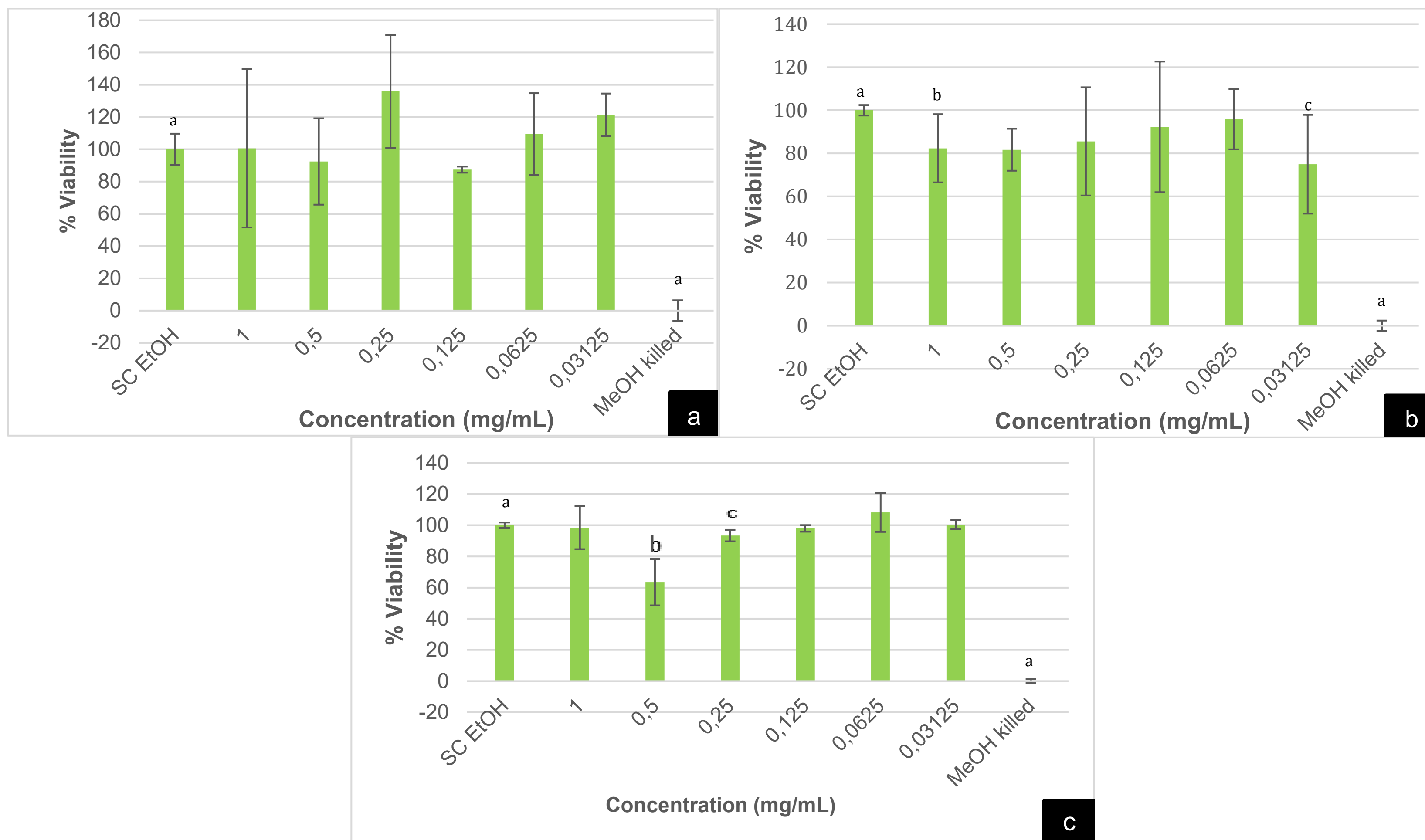


Figure 7.18: Cytotoxicity effects of *K. drepanophylla* ethanol extract's cytotoxicity to H4IIE-luc cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

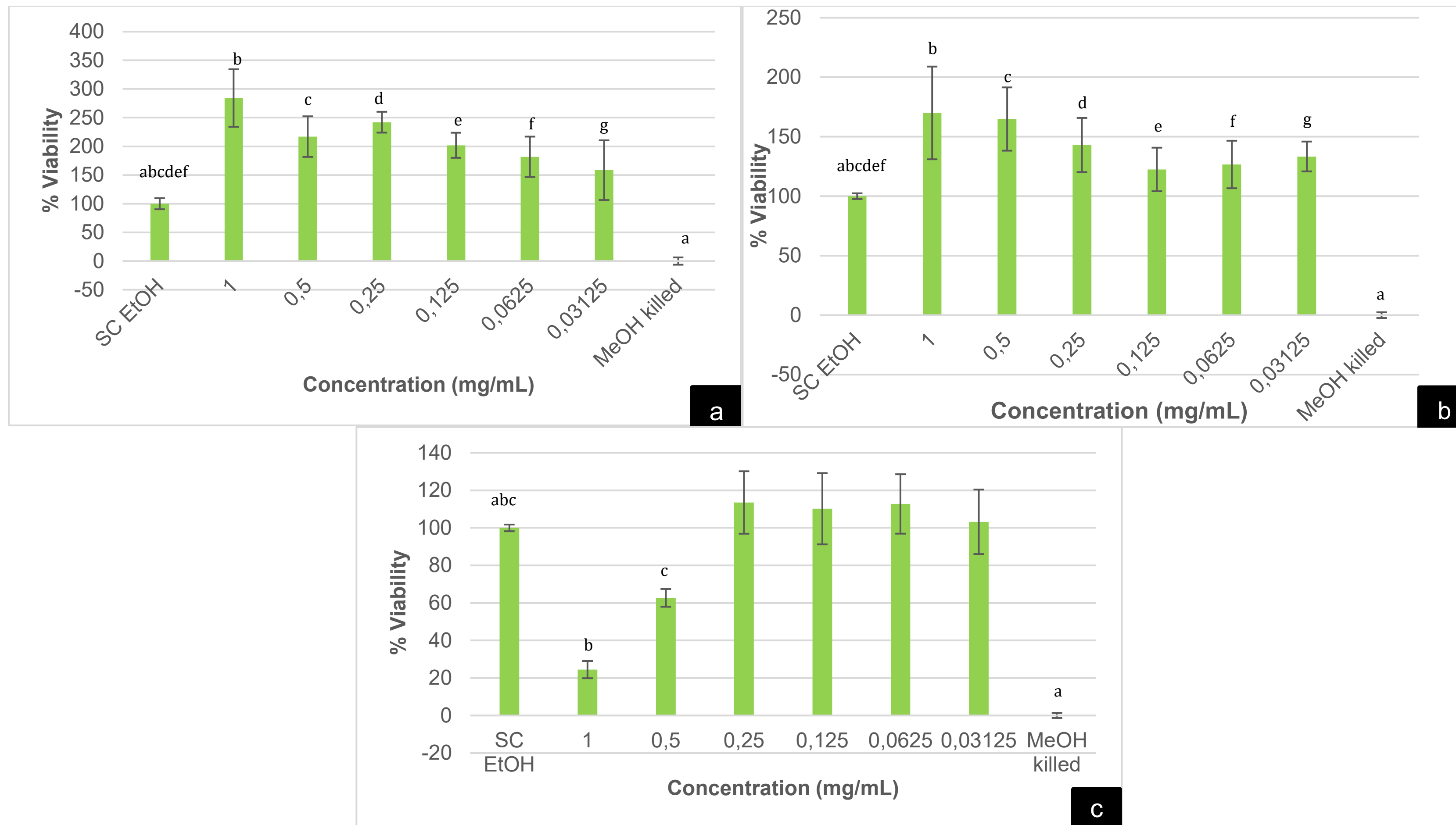


Figure 7.19: Cytotoxicity effects of *R. melanophloeoes* ethanol extract's cytotoxicity to H4IIE-l*uc* cells after (a) 12 hours, (b) 24 hours, and (c) 48 hours. The same letter superscript denotes a statistically significant difference between the control and treated cells (Mann-Whitney, p≤0.05). Bars represent means (n=3). The solvent control using SC displays 100% cell viability. MeOH is the negative control showing 0% cell viability.

7.5 Conclusions

The findings demonstrated that acetone is a good and more viable extractant than ethanol because, after 48 hours, extracts of acetone from *K. drepanophylla* were less hazardous to H4IIE-*luc* cells. The water, acetone, and ethanol extracts of *C. dentata* and *K. drepanophylla* were shown to be less hazardous at high concentrations than the extracts of *R. melanophloeos*.

References

Adwan, G., Abu-Shanab, B., Adwan, K., 2010. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant *Pseudomonas aeruginosa* strains. *Asian Pacific Journal of Tropical Medicine* 3, 266–269.

Bae, J., Kim, D., Lee, W., Kim, H., & Son, C. (2015). Characterizing the human equivalent dose of herbal medicines in animal toxicity studies $. *Journal of Ethnopharmacology*, *162*, 1–6.

Brosnahan, A. J., & Brown, D. R. (2012). Porcine IPEC-J2 intestinal epithelial cells in microbiological investigations. *Veterinary microbiology*, *156*(3-4), 229-237.

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, *64*(08), 711-713.

Fadipe, V.O., Mongalo, N.I., & Opoku, A.R. (2015). *In Vitro* evaluation of the comprehensive antimicrobial and antioxidant properties of *Curtisia dentata* (Burm. f) C.A. Sm: Toxicological effect on the Human Embryonic Kidney (HEK293) and Human Hepatocellular carcinoma (HepG2) cell lines. *EXCLI Journal*, 14, 971-983.

Fennell, C.W., Lindsey, K.L., McGraw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., & Van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of Ethnopharmacology* 94, 205–217.

Fotakis, G., & Timbrell, J. A. (2006). In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT, and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicology Letters*, *160*(2), 171-177.

Freshney, R. I. (2005). Culture of Animal Cells: A Manual of Basic Technique, Fifth Edition, John Wiley & Sons, Inc.

Gwala, P. E. (2011). The anti-platelet aggregation activity of *Rapanea melanophloeos* -A Zulu medicinal plant. (MSc Dissertation, University of Zululand).

Habtemariam, S. (2010). Fitoterapia Knipholone anthrone from Knipho fi a foliosa induces a rapid onset of necrotic cell death in cancer cells. *Fitoterapia*, *81*(8), 1013–1019.

Haller, C.A., Dyer, J.E., Ko, R., Olson, K.R.,2002. Making a diagnosis of herbal-related toxic hepatitis. The Western Journal of Medicine176,39–44.

Hübsch, Z., Zyl, R. L. Van, Cock, I. E., & Vuuren, S. F. Van. (2014). South African Journal of Botany Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. *South African Journal of Botany*, *93*, 185–197.

Ibrahim, M.I.M., Pieters, R., Abdel-Aziem, S.H., van der Walt, A.M., Bezuidenhout, C.C., Giesy, J.P., Abdel-Wahhab, M.A. (2015). Protective effects of *Amaranthus hybridus* against aflatoxin B1 and fumonisin B1-induced genotoxicity in H4IIE-*luc* cells. *Hepatoma Research*, *1*(3), 136-146.

Malich, G., Markovic, B., & Winder, C. (1997). The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. *Toxicology*, *124*(3), 179-192.

Mosa, R.A; Lazarus, G.G; Gwala, P.E.; Oyedeji, A. Opoku, A. R. (2011). Journal of Natural Products In Vitro Anti-platelet Aggregation, Antioxidant and Cytotoxic Activity of Extracts of Some Zulu Medicinal Plants. *Journal of Natural Products*, *4*, 136–146.

Mosmann, T., (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity tests. *Journal of immunological methods*, 65, 55–63.

Masoko, P. (2007). *In vitro* cytotoxicity tests of the developed extracts. (Doctoral dissertation, University of Pretoria).

Neergheen-Bhujun, V.S., 2013. Underestimating the toxicological challenges associated with the use of herbal medicinal products in developing countries. BioMed Research International 2013, 9 (Article ID 804086).

Okeleye, B.I., Mkwetshana, N.T., & Ndip, R.N. (2013). Evaluation of the antibacterial and antifungal potential of *Peltophorum africanum*: Toxicological effect on human chang liver cell line. Sci World J. Article ID 878735, pp 9.

Ouedraogo, M., Baudoux, T., Stévigny, C., Nortier, J., Colet, J., Efferth, T., Qu, F., Zhou, J., Chan, K., Shaw, D., Pelkonen, O., & Duez, P. (2012). Review of current and " omics " methods for assessing the toxicity (genotoxicity, teratogenicity, and nephrotoxicity) of herbal medicines and mushrooms. *Journal of Ethnopharmacology*, *140*(3), 492–512.

Oyedemi, S. O., Oyedemi, B. O., & Arowosegbe, S. (2012). Phytochemicals Analysis and Medicinal Potentials of Hydroalcoholic Extract from *Curtisia dentata* (Burm . f) C . A . Sm Stem Bark. *International Journal of Molecular Sciences*, *13*, 6189–6203.

Promega, C. (2005). 96® aqueous one solution cell proliferation assay. *Technical Bulletin*.

Raynor, D.K., Dickinson, R., Knapp, P., Long, A.F., Nicolson, D.J., 2011. Buyer beware? Does the information provided with herbal products available over the counter enable safe to use? *BMC Medicine* 9, 94.

Senthilraja, P., & Kathiresan, K. (2015). In vitro cytotoxicity MTT assay in Vero, HepG2, and MCF -7 cell lines study of Marine Yeast. *Journal of Applied Pharmaceutical Science*, *5*(03), 80–84. Whyte, J. J., & Tillitt, D. E. (2000). H4IIE bioassay. Biomonitoring of Environmental Status and Trends (BEST) Program: Selected Methods for Monitoring Chemical Contaminants and Their Effects in Aquatic Ecosystems. *US Geological Survey*, 9-12.

Whyte, J.J., Schmitt, C.J., & Tillitt, D.E. (2004). The H4IIE Cell Bioassay as an Indicator of Dioxin-like Chemicals in Wildlife and the Environment. *Critical reviews in toxicology*, *34*(1):1–83.

Wintola, O. A., & Afolayan, A. J. (2018). Toxicity assessment of aqueous extract of *Curtisia dentata* (Burm . f) C . A . Sm : stem bark in male Wistar rats. *Tropical Journal of Pharmaceutical Research*, *17*(January), 97–104.

Woo, S. S. J., Lau, J.S.H., & El-Nezami, H. (2012). Herbal Medicine: Toxicity and Recent Trends in Assessing Their Potential Toxic Effects. *Advances in Botanical Research*, 62, 365-384.

Wube, A. A., Bucar, F., Asres, K., Gibbons, S., Rattray, L., & Croft, S. L. (2005). Antimalarial Compounds from Kniphofia foliosa Roots. *Phytotherapy Research*, *19*(2004), 472–476.

CHAPTER 8

ISOLATION AND IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM *KNIPHOFIA DREPANOPHYLLA*

8.1 Introduction

Interest in science has increased to find the type of compounds found in medicinal plants due to known qualities of new plant-derived drugs and the rise in concerns about the side effects of modern medicine hence the need to find new molecular structures found in plants has led to the search of novel drugs. Over 50% of clinical medications used worldwide are made from plant-based substances because they are efficient and offer a wide range of pharmacological activity (Abdissa et al., 2020). Many natural substances that have been extracted from plants and have special bioactive qualities like fungicides, insecticides, growth regulators, or antioxidants have been discovered. Natural resource extracts, such as those from terrestrial or marine plants, are inevitably complex since each extract can contain hundreds of different chemicals, and the concentration of each compound in an extract can vary widely, necessitating careful study (Duval, et al., 2016).

There are 70 species in the genus Kniphofia (family Asphodelaceae), with South Africa being a diversity centre (Duval, et al., 2016; Bekele et al., 2020). Furthermore, extensive research demonstrates the wide spectrum of biological activities, including anticancer, antibacterial, anti-inflammatory, immunosuppressive, antioxidant, and other capabilities, that are present in natural anthraquinones (Duval et al., 2016). Kniphofia is well recognized for having a variety of anthraquinones, such as monomeric and dimeric anthraquinones and phenylanthraquinones, which have a variety of pharmacological actions, in terms of phytochemistry (Induli et al., 2013; Meshesha et al., 2017). As many people have been researching phenylanthraquinones from various plant sources through isolation, the number of isolated plant-derived phenylanthraquinones has increased quickly (Bringmann et al., 2008).

For detecting the total concentration of plant chemicals like flavonoids, several analytical methods have been developed, including gas chromatography (GC), mass spectrometry, thin layer chromatography (TLC), UV spectroscopy, and high-performance liquid chromatography (HPLC) (Wulandari et al., 2016). TLC is a rapid, easy, and affordable method that provides the scientist with a quick answer regarding how many components are present in a mixture. When a compound's R_f value is contrasted with the R_f value of a known chemical, TLC can also be used to identify a compound in a mixture (Sasidharan et al., 2011). Many areas of plant research, including plant biochemistry, food chemistry, chemotaxonomy, and quality control of medicinal plants, have effectively used LC-MS as a fingerprinting tool. If ideal MS circumstances are present, mass spectrometry's principal benefit is its great sensitivity, which enables the detection of low molecular weight substances at concentrations below the nanogram per milliliter level (Safer et al., 2011).

The identity of the molecules in a sample is found in NMR spectra based on the fact that NMR can be used to identify and quantify metabolites in samples of biological origin (Krishnan et al., 2005). NMR spectroscopy has a long history of being used to evaluate the quality and quantity of secondary plant metabolites, and the combination of 1H NMR spectroscopy with multivariate statistics has emerged as a popular method for metabolic fingerprinting. When compared to LC-MS investigations, 1H NMR allows for the detection of constituents that, for example, cannot be seen when there is inadequate ionization. Unmatched repeatability is another key benefit when compared to other analytical methods.

The main disadvantages of NMR spectroscopy are its relatively low sensitivity compared to contemporary mass spectrometry instrumentations and that low concentration molecules may not be identifiable with NMR. Additionally, when analysing items like plant extracts, signal overlapping is frequently an issue if more than one component is present in an NMR sample (Safer et al., 2011).

When compared to other analytical techniques, sample preparation for NMR is quite straightforward, and it is simple to achieve high sample throughput with minimum instrument drift.

With other nuclei, particularly 13C and 15N, more details can be learned about the metabolites. Additionally, liquid chromatography (LC)-NMR, 2D NMR, heteronuclear NMR, and stable isotope labeling investigations are also used (Ward et al., 2007).

Column chromatography is frequently used to separate, identify, and purify the components of a mixture for qualitative study because different bioactive chemicals can be discovered in plant extracts (Coskun, 2016).

There are no records of the isolation of bioactive chemicals from *Kniphofia drepanophylla* Baker, but only from other species in the genus.

8.2 Materials and methods

8.2.1 Plant collection and bulk extraction

Dried plant material (1.5 kg) of *K. drepanophylla* rhizome was collected with the assistance of a traditional healer from King Sabata Dalindyebo Municipality, Eastern Cape Province. The plant material was thoroughly cleaned with clean water, let to air dry, and then processed through a blender (MRC Laboratory Equipment, Durban). The dried plant material was extracted with 90% ethanol over 5 days. This was performed by shaking at room temperature using a platform shaker (Labcon, Maraisburg). The extract was subsequently filtered using Whatman No. 1 filter paper discs (Whatman, United Kingdom), and the filtrates were concentrated at a controlled temperature and decreased pressure (60–70 oC) in a rotary evaporator (LabTech, EV400, Italy). The extract was then weighed while being held in front of a fan until a consistent weight was achieved.

8.2.2 Solvent-solvent extraction

The ethanol extract was solubilised in hydromethanol and re-dissolved in methanol and *n*-hexane, chloroform, and ethyl acetate were used to partition (Figure 8.2). The obtained fractions from the separation funnel were transferred into pre-weighed beakers then air-dried under a fan until constant weights were achieved.

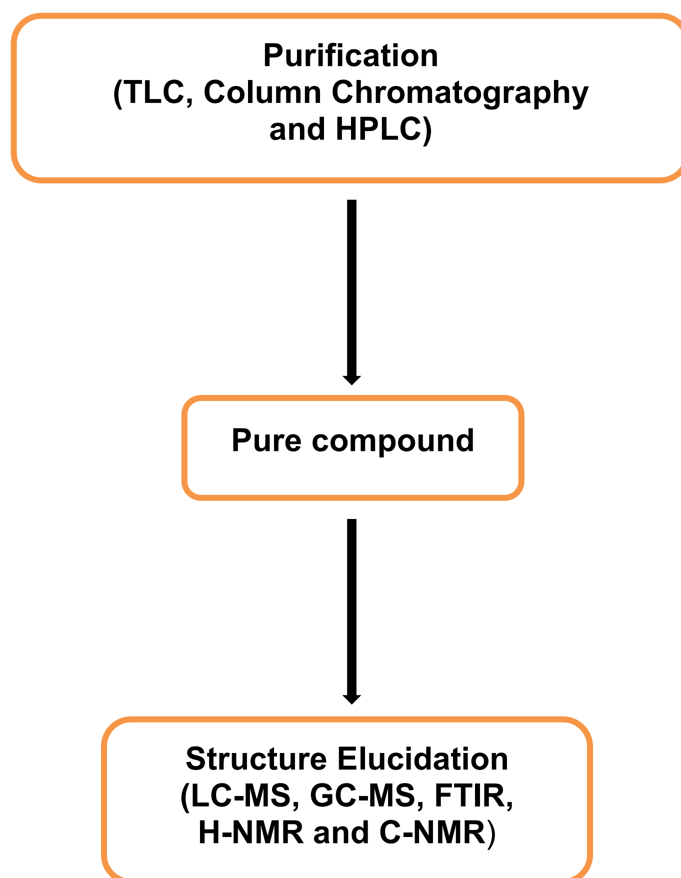


Figure 8.1: An overview of the common methods for extracting, identifying, and characterising bioactive chemicals from plant extracts (Sasidharan et al.,2011).

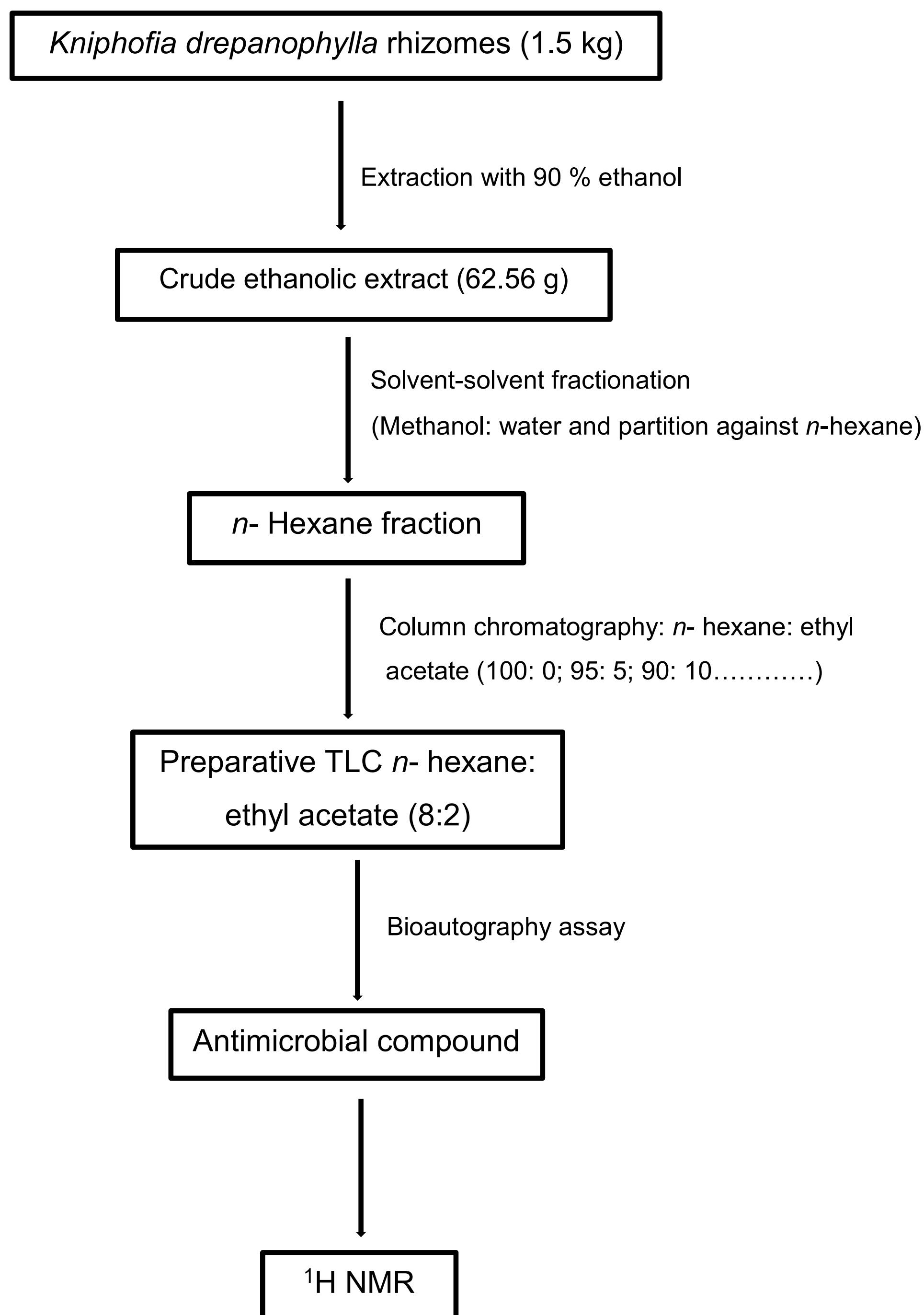


Figure 8.2: Bio-activity guided isolation process (fractions which showed antimicrobial activity on the bio-autography assay was further purified).

8.2.3. Column chromatography

A little amount of hexane was used to suspend the silica gel (Merck, 9385, 230-400 mm, Mesh Particle Size, Germany), which was then swiftly poured into a 48.5 cm-long column while being continually swirled to remove air bubbles. Re-dissolved in n-hexane, the extract was then gradually added to the column. It was fractionated using solvents with varying degrees of polarity; 500 ml of the solvent mixtures (n-hexane: ethyl acetate, 100%, 95%, 90%, 85%, 75%, 70%, 65%, 60%, 55%, 50%, 0%) were run down each column. At 5-minute intervals, fractions were collected as the extract formed bands along the column. After that, the solvent was slowly introduced and allowed to pass through the column until it was clear above the silica.

8.2.4. Preparative TLC

By loading roughly 20 mg of each fraction in a 1 cm band on precoated silica gel aluminum plates, fractions from column chromatography were analyzed (60F254, 0.22 mm, 20x10 cm, Merck, Germany). Fractions with similar spot development were blended further in a beaker, decreased in front of a fan, and then ran once more over the TLC plate. The mobile phase was developed in n-hexane: ethyl acetate (8:2) in a glass chamber saturated with the mobile phase and the development was carried out twice with the same mobile phase for a better resolution. For improved resolution, the mobile phase was produced twice with the identical mobile phase in n-hexane: ethyl acetate (8:2) in a glass chamber saturated with the mobile phase. The plates were air-dried and dried, the solvent front was noted, and the separated components were observed under visible and ultraviolet light (UV254 and UV366 nm). The plates were then heated for ten to fifteen minutes while anisaldehyde (AS) spray reagent was used to stain them and develop the colors of several previously invisible components. To test for antibacterial activity, a bioautography experiment was carried out, and the active fractions were taken off the TLC plate by scraping and eluting from the silica with n-hexane. To get rid of silica, the extracts were filtered through glass cotton wool. Results were documented by photographing various chemical profiles of the various fractions while they were exposed to UV light.

8.2.5. Bioautography

S. aureus (ATCC 12600) was employed as the test organism for the bioautographic assay (Martin & Eloff, 1998). In an MH broth medium, a culture was created overnight (3 X 20 ml). The cultures were centrifuged at 3000 g for 10 min and the supernatant medium was discarded. In 10 ml of brand-new broth, the bacterial cell pellets were mixed and reconstituted. After that, a TLC plate was coated with the broth culture and incubated for 24 hours at 37°C with 100% humidity. The plates were incubated, and after a brief period of drying, they sprayed with a 2 mg/ml solution of p-iodonitrotetrazolium violet (INT). To allow for color development, the plates were then re-incubated for a further 30 to 60 minutes. Zones of inhibition, which were represented by white specks on a pink background, indicated the presence of active compounds. A retention factor (Rf) was calculated using the clear spots that were observed on the TLC plate.

8.2.6. NMR Spectroscopy

The structure of the isolated chemical was sent for analysis using proton nuclear magnetic resonance (H-NMR) to the University of Johannesburg, Department of Chemistry.

8.3. Results and Discussion

8.3.1. Bulk extraction

K. drepanophylla rhizome was extracted in bulk with ethanol, producing a thick, brown residue of mass 62.56 g crude extract.

8***.3.2. Solvent-solvent extraction***

In solvent-solvent fractionation, 1 fraction belonging to *n*-hexane was obtained at the bottom of the separating flask. Hydromethanol and chloroform fractions could not be determined and separated which could be due to the mixing of the solvents together. For structural elucidation or bio-assay guided isolation, it is considered to perform solvent partitioning as the first and most necessary step (Liu, 2011; Mabona 2013). This is done to divide the chemical compounds into several groups based on chemical and physical compatibilities, where compounds with varying polarities can be

partitioned into various fractions which are achieved by the principle that states that like substances dissolve like substances (Liu, 2011; Mabona 2013).

8.3.3. Column Chromatography

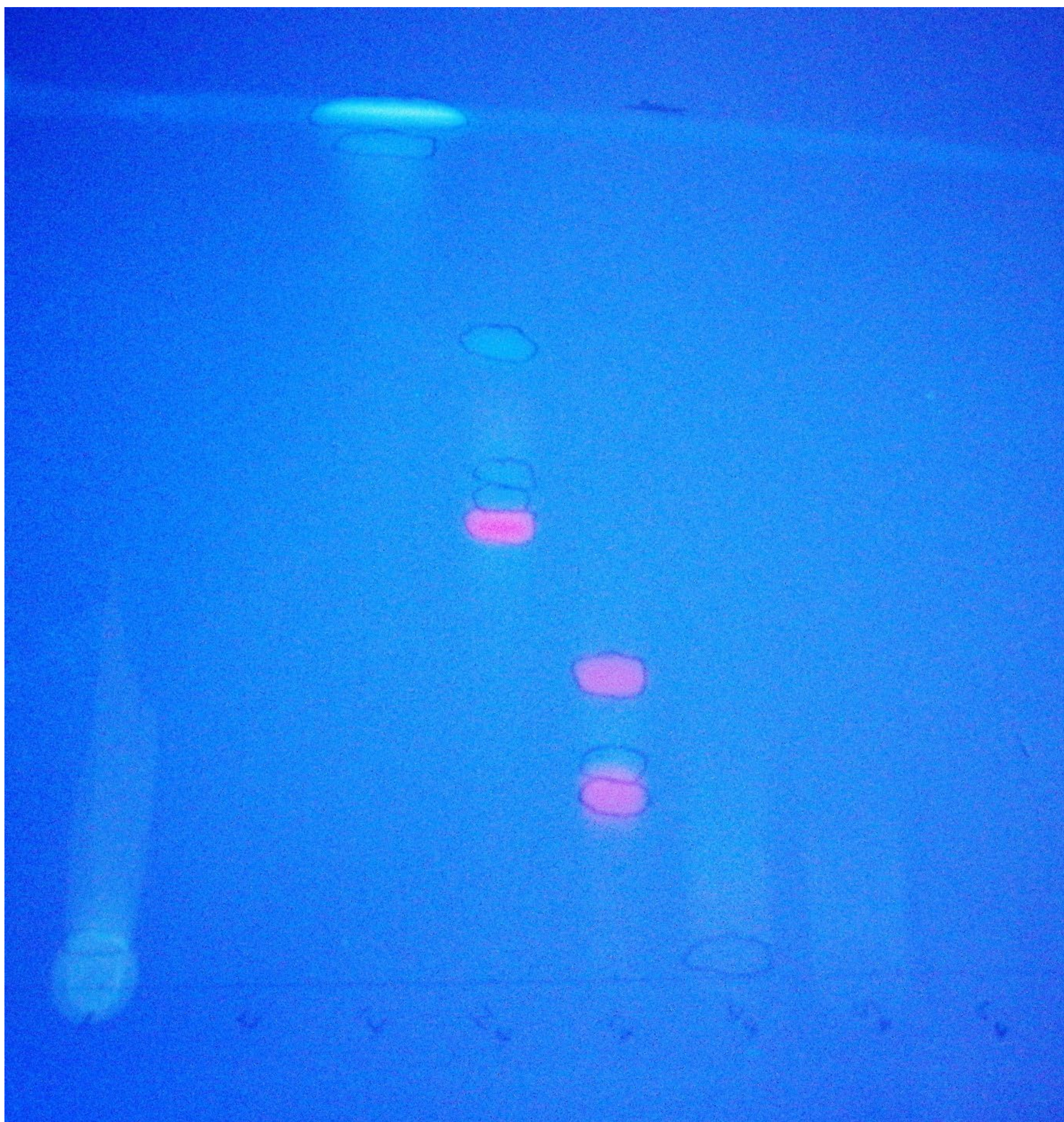
From the *n*-hexane fraction, 84 fractions were collected from the column chromatography and spotted on a TLC plate (Figure 8.3). Fractions 1-14, 15-34, 35-54, and 55-84 were combined and spotted onto TLC plates and then sprayed with (Figure 8.4). Figure 8.5 shows a TLC chromatogram of combined fractions (1-34) under (UV_{254} and UV_{366}) and after spraying with an anisaldehyde spray reagent. The combined fractions (1-34) which were spotted as a long band are presented in (Figure 8.6). A bright blue fluorescing colour was observed under UV_{366}.

With regards to the bioautography test, the combined fractions showed bacterial inhibition at an R_f value of 0.7 (Figure 8.7). The bioautography assay was employed in this investigation to identify and monitor *K. drepanophylla's* antibacterial activity during the isolation procedure. The bioautography results revealed one unique clear zone, indicating that a certain compound in that zone was inhibiting bacterial growth. There was a 20 mm zone of growth inhibition on the isolated chemical. *S. aureus*, *E. faecalis*, *P. aeruginosa*, and E. coli, four pathogenic bacterial strains, are known to be resistant to first-line medications (Meshesha, et al., 2017). A 25 mm zone of inhibition was seen for the isolated substance 10-Hydroxy-10,7-(chrysophanolanthrone)chrysophanol against S. aureus. There was a 20 mm zone of growth inhibition on the isolated chemical. *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli*, four pathogenic bacterial strains, are known to be resistant to first-line medications (Meshesha, et al., 2017). A 25 mm zone of inhibition was seen for the isolated substance 10-Hydroxy-10,7-(chrysophanolanthrone)chrysophanol against *S. aureus* (Meshesha, et al., 2017). For the antimicrobial analysis, *K. drepanophylla* extracts had shown good antibacterial activity against *S. aureus*.

8.3.4. Preparative TLC

Findings for preparative TLC plates are presented in Figure 8.6. Preparative TLC on *n*-hexane resulted in a compound that displayed antibacterial activity against *S. aureus*

(observed as a violet-blue fluorescing band under UV_{366}) and a greenish colour after spraying with anisaldehyde) with an R_f value of 0.7 with a mass of 0.012 g. In a study by Abdissa et al. (2020), they discovered an R_f value of 0.8 cm in 50% ethyl acetate in *n*-hexane.



wFigure 8.3: TLC chromatogram of n-hexane fractions from the rhizome of *K. drepanophylla* under ultraviolet light. The column chromatography's n-hexane fractions were collected, spotted on a TLC plate, and developed using an 8:2 n-hexane: ethyl acetate solvent system.

Figure 8.4: After being sprayed with anisaldehyde reagent, the n-hexane combined fractions from the column chromatography were spotted on a TLC plate and developed with n-hexane: ethyl acetate (8:2) solvent system.

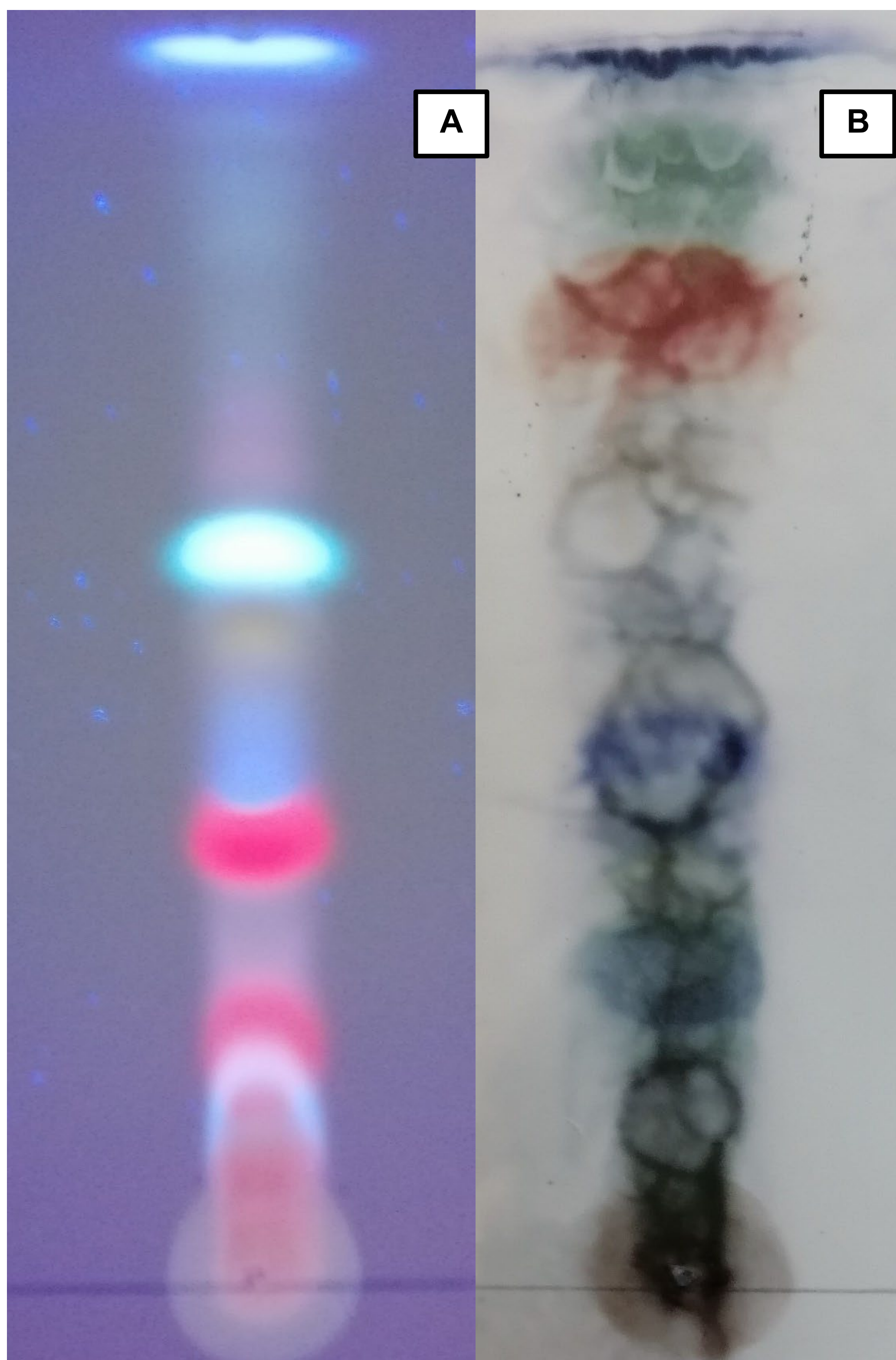


Figure 8.5: The *n*-hexane combined fractions (1-34) from column chromatography were spotted on a TLC plate and developed with *n*-hexane: ethyl acetate (8:2) solvent system. **(A)** Reference TLC plate under UV light and **(B)**

TLC plate.

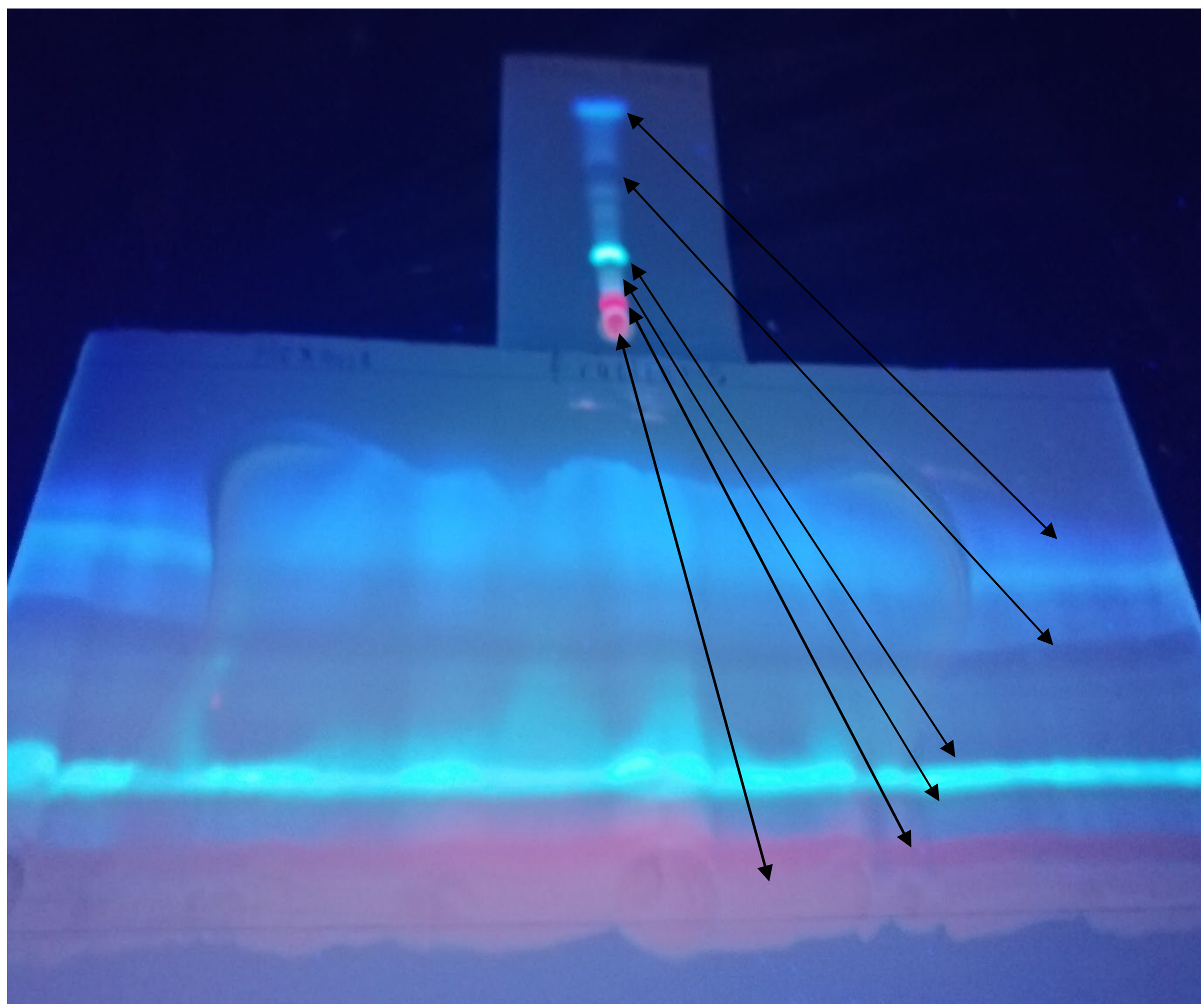


Figure 8.6: The combined fractions (1-34)'s preparative TLC chromatogram was spotted as a long band onto a TLC plate. an arrow indicating to the area that was scraped. A solvent system of 8:2 n-hexane to ethyl acetate was used to develop the plate.

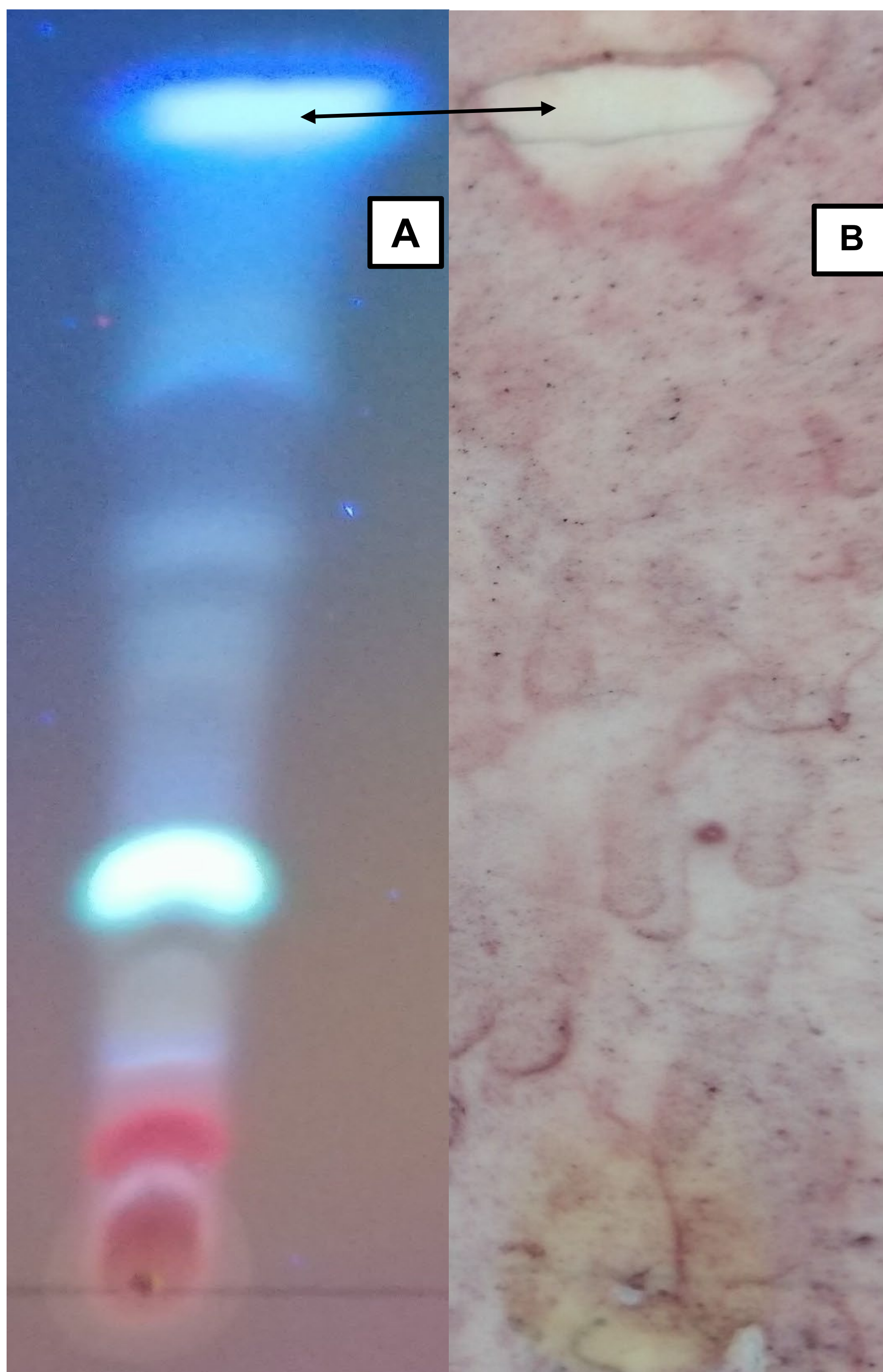


Figure 8.7: TLC plates of the n-hexane fraction of the rhizome of *K. drepanophylla*. Fractions 1 through 34 that were obtained using silica column chromatography were spotted on TLC plates and developed using an 8:2 n-hexane: ethyl acetate solvent system. TLC plate with *S. aureus* bacterial overlay and reference plate illuminated by UV light, respectively. The arrow points to a clear area or location.

8.3.5. NMR analysis

By comparing the spectral data with information from the literature, 1 dimension (1D) 1H NMR (1D-1H-"PowerScan") was used to estimate the structure of the n-hexane fraction. The calibration of the measurements was done using solvent signals in deuterated chloroform (CDCl3).

The ^{1}H NMR spectrum of the isolated compound revealed signals at δ 2.29 (carbonyl proton), δ 2.69 (ester proton), δ 2.80, δ 3.66 (ether proton), and δ 4.17 (alkyl fluoride proton). The spectrum showed signals at δ 5.35, δ 5.77, and δ 5.87 indicating protons of amide groups. The spectrum also revealed four singlet peaks of phenol groups showed at δ 6.14, δ 6.29, δ 6.43, δ 6.55 and δ 6.93. The last two signals in the spectrum were found at δ 7.06 and δ 7.25, indicating protons of phenols and aromatic protons respectively.

After comparing the spectral structure with spectra already reported in literature through analyses of their NMR spectral data the isolated compound in our study had an ^{1}H NMR spectrum with 75% similarity to 10-hydroxy-10,7'(chrysophanolanthrone)chrysophanol, a dimeric anthraquinone compound that was isolated from the roots of *K. isoetifolia* after purification on column chromatography on silica gel using ethyl acetate in *n*-hexane with protons that resonated at δ 2.26, δ 2.44, δ 6.61, δ 6.78, δ 6.95, δ 7.04, δ 7.41, δ 7.63, δ 8.0, δ 8.64 and δ 2.42 (Meshesha, et al., 2017). The isolated compound, 10-hydroxy-10,7'-(chrysophanolanthrone) chrysophanol showed a good growth inhibition against *S. aureus* at 25 mm (Meshesha, et al., 2017). Our isolated compound had inhibited *S. aureus* at 20 mm for the bioautographic assay.

In Africa, the genus Kniphofia contains roughly 70 species, the majority of which are located in the continent's Southern regions. Traditional medicine has employed plants from this genus to treat a variety of conditions, including menstrual pain, infertility, abdominal cramps, wounds, malaria, and chest complaints, as well as some sexually transmitted diseases like gonorrhoea and hepatitis (Meshesha et al., 2017).

Bringmann et al. (2008) claims that the proton signals for the methoxy groups in the 1H NMR spectra of various knipholone compounds, such as phenylanthraquinones, resonate at values like 3.98, 4.00, 3.35, 3.33, 3.77, and 4.00, and 3.68, respectively.

The amount and position of the phenolic hydroxyl groups of anthraquinones affect their antibacterial effectiveness, with aloe-emodin having more additional phenolic hydroxyl groups than chrysophanol had better antibacterial activity against *S. aureus* and *P. aeruginosa* strains at lower concentrations. (Meshesha, et al., 2017). The compounds found in the crude extracts and isolated compounds of Kniphofia species must have different types of phenolic hydroxyl groups that act differently on various bacterial pathogens similarly to how the isolated compound acted against *S. aureus*.

8.4 Conclusions

The antibacterial activity that was seen with the plant extracts from *K. drepanophylla* is justified by a pure component that was isolated from the roots and rhizome that showed growth inhibition of *S. aureus* during TLC. When the 1H-NMR spectra of the substance were analyzed, it was discovered that 75% of the structure of the antibacterial molecule 10-Hydroxy-10,7-(chrysophanolanthrone)chrysophanol was represented in the spectrum. Further characterisation of the isolated compound using HMBC NMR, COSY NMR and GC-MS gas chromatography-mass can be done to give a further and definite identification of the compound.

REFERENCES

Abdissa D., Faye. G., Fayera, M., & Demissie, S. (2020). Extraction and isolation of compound from roots of *Kniphofia pumila,* and its antibacterial activities in combination with zinc chloride*. Walailak Journal of Science & Technology*, *17*(7), 631-638.

Bekele, E. T., Gonfa, B. A., Zelekew, O. A., Belay, H.H., &, & Sabir, F. . (2020). Synthesis of Titanium Oxide Nanoparticles Using Root Extract of *Kniphofia foliosa* as a Template, Characterization, and Its Application on Drug Resistance Bacteria. *Journal of Nanomaterials*, (Article ID 2817037), 1–10.

Bringmann, G., & Mutanyatta-comar, J., Knauer, M., & Abegaz, B. M. (2008). Knipholone and related 4-phenylanthraquinones : structurally, pharmacologically, and biosynthetically remarkable natural products. *Natural Product Reports*, *25*, 696–718.

Coskun, O. (2016). Separation techniques: chromatography. *Northern clinics of Istanbul*, *3*(2), 156.

Dai, Y., Harinantenaina, L., Bowman, J.D., Da Fonseca, I. 0., Brodie, P.J., Goetz, M., Cassera, M.B., & Kingston, D. G. I. (2014). Isolation of antiplasmodial anthraquinones from *Kniphofia ensifolia*, and synthesis and structure-activity relationships of related compounds. *Bioorganic & Medicinal Chemistry*, *22*(1), 269–276.

Duval, J., Destandau, E., & Pecher, Vi., & Poujol, M., Tranchant, J., & Lesellier, E. (2016). super/subcritical fluid extraction and centrifugal partition. *Journal of Chromatography*, 1–13.

Duval, J., Pecher, V., Poujol, M., &, & Lesellier, E. (2016). Research advances for the extraction, analysis and uses of anthraquinones : A review. *Industrial Crops & Products*, *94*, 812–833.

Induli, M., Gebru, M., Abdissa, N., Akala, H., Wekesa,I., Robert Byamukama,R., Heydenreich, M., Murunga, S., Dagne ,E., & Yenesew, A. (2013). Antiplasmodial Quinones from the Rhizomes of *Kniphofia foliosa*. *Natural Product Communications*. *8*(9),1261 - 1264.

Krishnan, P., Kruger, N.J., &, & Ratcliffe, R. G. (2005). Metabolite fingerprinting and profiling in plants using NMR. *Journal of Experimental Botany*, *56*(410), 255–265.

Liu, W.J.H., 2011. Traditional Herbal Medicine Research Methods: Identification, Analysis, Bioassay, and Pharmaceutical and Clinical Studies. Wiley, Hoboken, New Jersey, United States of America.

Mabona, U., (2013). Antimicrobial activity of southern African medicinal plants with dermatological relevance University of the Witwatersrand, Johannesburg.

Meshesha, M., Deyou, T., Tedla, A, & Abdissa, N. (2017). Chemical constituents

of the roots of *Kniphofia isoetifolia* Hochst. and evaluation for antibacterial activity. *Journal of Pharmacy & Pharmacognosy Research*, *5*(6), 345–353.

Safer, S., Cicek, S. S., Pieri, V., Schwaiger, S., Schneider, P., Wissemann, V., &, & Stuppner, H. (2011). Metabolic fingerprinting of *Leontopodium* species (Asteraceae) by means of 1H NMR and HPLC-ESI-MS. *Phytochemistry*, *72*(11–12), 1379–1389.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation, and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, *8*(1), 1-10.

Ward, J. L., Baker, J.M., &, & Beale, M, H. (2007). Recent applications of NMR spectroscopy in plant metabolomics. *The FEBS Journal*, *274*, 1126–1131.

Wulandari, L., & Retnaningtyas, Y., & Lukman, H. (2016). Analysis of Flavonoid in Medicinal Plant Extract Using Infrared Spectroscopy and Chemometrics. *Journal of Analytical Methods in Chemistry*, *2016*, 1–6.

CHAPTER 9

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

9.1 General discussion

Lymphatic filariasis is a tropical disease that affects the lymphatic system leading to swelling and immobility in extreme cases. Three parasitic nematode worms, *W. brancofti*, *B. malayi*, and *B. timori*, are primarily responsible for the disease and are transmitted to humans by infected mosquitoes (Rajasekaran et al., 2017). Some people may experience no symptoms whilst others may suffer from inflammation, swelling or fever. Filariasis is associated with lymphedema which is the retention or build-up of fluid, hydrocele which is the swelling of the male genitalia and the swelling of the upper and lower limbs. Chronic manifestations of the lymphatic filariasis lead to social and economic burdens as the disease is common amongst the poor who are often marginalised (Adamu, et al., 2020). Africa, India, the Americas, and Asia all have high rates of the illness. 512 million people in sub-Saharan Africa are thought to be at risk of infection, while the disease is known to affect 28 million individuals and is one of the main causes of disability (Adamu, et al., 2020). The disease is referred to as 'elephantiasis' due to the thickening of skin and the disfigurement that results from the excessive swelling.

In this study, bioactive chemicals were isolated from plants used to treat elephantiasis in the Eastern Cape of South Africa and subjected to pharmacological screening.

The ethnobotanical study indicated that elephantiasis is treated in three municipal regions of OR Tambo District Municipality in the Eastern Cape, South Africa, with 29 medicinal plants from 25 Angiosperm families that are found in 29 families. Traditional healers and herbalists most frequently used the plants they indicated to cure pain, wounds, worms, swelling, and inflammation. The plants were used as poultices on the skin to treat wounds. As a decoction and/or infusion, plants can be used singly or in combination.

Plants that were reported to have a good use-value from the were *H. hemerocallidea*, *E. elephantina* and *G. perpensa*. *A. oblongifolia*, *C. dentata*, *D. sylvatica*, *K. drepanophylla*, *P. prunelloides* and *R. melanophloeos* had the fourth highest use-value. Based on the use-values that were discovered in the ethnobotanical survey, the plants were chosen for screening.

To ascertain the plants' effectiveness against bacterial, antifungal, and mycobacterium species, antimicrobial screening was carried out. The selected plants were *A. oblongifolia*, *C. brachiata*, *C. dentata*, *D. sylvatica*, *G. perpensa*, *H. albiflos*, *K. drepanophylla*, and *R. melanophloeos* because there had never been any studies on plants used to treat elephantiasis utilizing them.

Amongst 12 documented plant species, *E. elephantina*, *P. prunelloides* and *D. sylvatica* were most frequently used plants and were selected for screening of anthelmintic, anti-inflammatory, cytotoxicity activities and for isolation and characterisation of bioactive compounds. Some of the plants that act against the filarial parasites have been mentioned in previous studies. The seeds of *Ricinus communis* methanol extract were reported to act against *B. malayi* (Al-abd et al., 2013). In South Africa, it has also been cited that the plant's leaves and roots can heal ailments linked to lymphatic filariasis (Komoreng et al., 2017).

Main functions of the lymphatic system are to balance the fluid levels in the body and to protect it from diseases. Damage to the lymphatic system will render the body to infections as the immune system of the affected person will grow weaker or vulnerable.

Bacterial and fungal infections eventually result from a weakened immune response. The study's focus was on the plant extracts' antimicrobial properties because elephantiasis is linked to a weakened immune system. Elephantiasis Nostras Verrucosa (ENV), a rare clinical disorder linked with chronic non-filarial lymphedema brought on by bacterial or non-infectious lymphatic blockage, is a form of lymphatic system disease. Lymphedema and skin alterations such hyperkeratotic, verrucous, and papillomatous lesions are its defining features (Moussa et al., 2019). Bacteria have been highly resistant to antibiotics and so the use of novel compounds to treat bacterial and fungal conditions is very important in the treatment of elephantiasis. *S.*

aureus and *S. pyogenes* are one of the bacteria that are associated with skin infections.

Erysipelas, a streptococcus species infection known also as St. Anthony's fire, is an erythematous infection with distinct raised edges and frequently occurs around the legs and face. It is also sometimes accompanied by lymphatic streaking. The disorder might impact patients with lymphedema or weakened immune systems. (Daniel et al.., 2002). Damage to the lymphatic system makes the skin of those who are afflicted vulnerable to bacterial infections from staphylococcus and streptococcal pathogens.

The ability of *K. drepanophylla* aqueous extract to inhibit *S. aureus* and *S. epidermis* at 0.39 mg/ml and *E. coli* and *P. aeruginosa* at 0.098 mg/ml supports the use of the plant by traditional healers to treat skin infections. Since lymphatic drainage associated with elephantiasis is also caused by certain sexually transmitted infections and repeated streptococcal infections, the tested which showed good antibacterial activity can be used to treat the disease and the plants can be used to test their activity against other bacterial species that are associated lymphedema. The skin can also be attacked by fungal infections due to the condition. *G. perpensa* and *K. drepanophylla* acetone and aqueous extracts as well as *C. dentata* ethanol and aqueous extracts revealed good antifungal activity against *C. albicans*, *C. vulgaris* and *T. mucoides*.

Alkaloids, flavonoids, tannins, anthraquinones, saponins, and terpenoids were the phytochemical components seen in the study, whereas cardiac glycosides and steroids were the least prevalent. Xanthine oxidase, cyclo-oxygenase, lipoxygenase, and phosphoinositide are just a few of the enzymes that flavonoids have the potential to inhibit in addition to having a wide range of pharmacological activity including antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties. (Panche, et al., 2016). Acetone showed the highest phenolic content for quantitative analysis. Plant polyphenols are a huge family of plant-derived compounds possessing one or more phenol ring. According to the nutritional classification, polyphenols are grouped in flavonoids and research has revealed their antioxidant activity, anti-inflammatory activity and cell signalling pathways (Vetrani et al., 2020). Terpenoids are modified terpenes that have antibacterial, antifungal, antiviral, anticancer,

antihyperglycemic, analgesic, anti-inflammatory, and antiparasitic activities (Cox-Georgian et al, 2019).

Antioxidant activity of the medicinal plant extracts might be linked to the presence of phenolic compounds, flavonoids, and saponins, according to the results of the DPPH and ABTS antioxidant assays. According to our findings, acetone extracts have good antioxidant activity as they had showed the highest number of phenols. The screening further showed that ethanol was more effective than aqueous solvent, as it had lower IC_{50} values as compared to the aqueous extracts for *G. perpensa* and *K. drepanophylla*. Antioxidants work to combat ROS and oxidative stress, which aids in the treatment of chronic conditions like inflammation, diabetes, cardiovascular disease, and early aging (Saranya et al. 2014; Ijaz & Hasnain, 2015).

Elephantiasis is linked to anti-inflammatory reactions; hence anti-inflammatory analysis was done. The 5-lipoxygenase (5-LOX) enzyme inhibition was assessemento evaluated the anti-inflammatory effect of the plant extracts. Attacks by the filarial worms *W. bancrofti* and *B. malayi* through mosquitoes causes the adult worms to block the lymph vessels causing blockage lymphatic fluid flow back to the blood and initiating an anti-inflammatory response (Rajasekaran, 2017). Small soil minerals that enter through bare feet in non-filarial elephantiasis podoconiosis trigger an immune system reaction ultimately leading to the development of inflammatory masses of nodules (granulomas) in the lymphatic capillaries of the feet and legs (National Organization for Rare Disorders, 2021). All tested plants *C. dentata*, *G. perpensa*, *K. drepanophylla* and *R. melanophloeos* displayed good anti-inflammatory activity against 5-LOX. Concerning *C. dentata* ethanol extracts, slightly poor activity against 5-LOX was observed with an IC_{50} value higher than the positive control (NDGA).

Tetrazolium salt-based assay was utilized in the cytotoxicity investigation of plant extracts in order to quantify cytotoxicity or cell proliferation (Mosmann,1983; Karakas, 2017). The MTT assay was utilized; it is a straightforward and quick colorimetric experiment that produces quantitative results (Karakas, 2017). The results revealed that at high concentration the extracts of *R. melanophloeos* were toxic against both Hutu-80 and H4IIE-*luc* cells after 48 h. Cardiac glycosides and steroids during phytochemical analysis were found to be present in this plant which could attribute to

its toxicity at higher concentrations. *K. drepanophylla* extracts showed non-toxicity against the cells after 48h and the plant had excellent antimicrobial, antioxidant and anti-inflammatory results and therefore it was the plant of choice for isolation.

A study to isolate the ethanol extract of *K. drepanophylla* was conducted so as to determine the compounds that were responsible for good activity of the plant's extracts. To try and isolate a pure chemical, silica gel column chromatography, TLC, and bioautography were used. After isolation, the fraction showed good antibacterial activity by preventing *S. aureus* from growing with a 20 mm inhibition zone. The isolated substance was only partially characterized by 1H-NMR. The functional groups that shared a 75% identity with the dimeric anthraquinone compound 10-hydroxy-10,7-(chrysophanolanthrone)chrysophanol were identified according to the acquired spectra. However, additional characterisation using 13C-NMR is crucial for concluding the analysis and identification.

9.2 Conclusions and recommendations

Overall, medicinal plants chosen for the pharmacological screening for treatment against elephantiasis and its related disorders have shown extremely strong antibacterial, antioxidant, anti-inflammatory, and less cytotoxic levels against Hutu-80 and H4IIE-luc cells.

The findings of the study support the use of these botanicals in conventional medicine. The examined phytochemicals, such as phenols, flavonoids and saponins, are what give plant extracts their antioxidant capabilities. Elephantiasis and its related disorders could be treated in South Africa with the assistance of newer medications made from the plants thanks to their newly identified pharmacological qualities.

There are very few studies on traditional South African medicine used to control and treat elephantiasis. Therefore, more research on the plants that are used to treat elephantiasis is required. This research will also advance in-vitro studies by utilizing

more antibacterial and antifungal strains, antioxidant assays, and in-vivo testing of plant extracts against conditions related to filarial and non-filarial elephantiasis.

Since it was found that some plant extracts revealed a level of toxicity at high concentrations but can be safe to use if the dosage can be reduced, initiatives such as awareness programmes to teach traditional healers, herbalists, and people with indigenous knowledge about the safe practice of traditional medicine through community leaders are crucial. The standardisation of herbal medicine is needed by developing safe dosages of the herbal formulations.

Using various chemical techniques, such as HPLC, further isolation research on *K. drepanophylla* can be carried out, advancing our understanding of the chemicals behind the extracts' potent activity. Traditional healers' use of the plant to treat elephantiasis can then be further validated by using the separated chemicals to test for pharmacological action.

References

Cox-Georgian, D, Ramadoss, N., & Dona, C., & Basu, C. (2019). Therapeutic and Medicinal Uses of Terpenes. © Springer Nature Switzerland AG. N Joshee et al. (eds.), *Medicinal Plants*.

Daniel, L. Stulberg, M.D., Marc, A. Penrod, M.D., Richard, A., & Blatny, M.D. (2002). Common Bacterial Skin Infections. *American Family Physician*, *66*(1), 119-124.

Ijaz, S., & Hasnain, S. (2015). Antioxidant potential of indigenous cyanobacterial strains in relation with their phenolic and flavonoid contents. Natural Products Research, 1-4.

Karakas, D., Ari, F. & Ulukaya, E. (2017). The MTT viability assay yields strikingly false-positive viabilities although the cells are killed by some plant extracts. Turkish Journal of Biology, 41: 919-925.

Moussa, Y., Moussa, Y., &, Chakra, A.B. (2019). Penoscrotal Elephantiasis nostras verrucosa: A case report and literature review. International Journal of Surgery Case Reports, 65, 127-130.

National Organization for Rare Disorders-Rare Disease Database. (2021, March, 2022). Elephantiasis. https://rarediseases.org./rare-disease/ele

Panche, A.N., Diwan, A.D., & Chandra, S.R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*(47), 1-15.

Saranya, C., Hemalatha, A., Parthiban, C., & Anantharaman, P. (2014). Evaluation of antioxidant properties, total phenolic and carotenoid content of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*. International Journal of Current Microbiology and Applied Science, 3, 365–377.

Vetrani, C., Costabile, G., Vitale, M., & Giacco, R. (2020). (Poly)phenols and cardiovascular diseases: Looking in to move forward. Journal of Functional Foods, 7,1-4.