PHYTOCHEMICAL SCREENING, CYTOTOXICITY, ANTIMICROBIAL AND ANTHELMINTIC ACTIVITY OF MEDICINAL PLANTS USED IN THE TREATMENT OF LYMPHATIC FILARIASIS IN THE EASTERN CAPE, SOUTH AFRICA

By

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Dissertation submitted in fulfilment of the requirements for the degree Magister Scientiae in the Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, University of the Free State

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DECLARATION

I, Thumeka Tiwani, 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 Master's Degree qualification in Botany at the University of the Free State 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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Thumeka Tiwani (Miss)

DEDICATION

This work is dedicated to all the members of my family, with special dedication to my son Landa Lonkosi and daughter Qhakazile Usibabale. Those times spent away from you were not in vain. Thank you for your understanding throughout my research period. I thank my mother and grandmother for being my strength and wisdom at all times. Without both of you, there is no me. I know I never even for a minute left your prayers.

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ABSTRACT

Lymphatic filariasis is a disease caused by parasitic filarial nematodes that cause excessive swelling of the limbs, genitalia and breasts due to the distraction of the lymph system. This results in accumulation of lymph and lymphoedema. This disease is one of the neglected tropical diseases found in 38 African countries.

In South Africa, there is no complete system for treatment of this filarial disease. Many patients tend to opt for traditional help to alleviate the suffering caused by the disease. This research was aimed at identifying medicinal plants used as ethnomedicine for treating lymphatic filariasis and to assess the *in vitro* antimicrobial, antifungal, anti-mycobacterial and anthelmintic activity of these medicinal plants. Six plant species (*Platycarpha glomerata*, *Euphorbia gorgonis*, *Ricinus communis*, *Ledebouria* sp., *Rumex obtusifolius* and *Tulbaghia alliacea*) were collected from Raymond Mhlaba and Intsika Yethu municipal areas of the Eastern Cape Province with the assistance of traditional healers and herbalists. Plant extracts were extracted with methanol, ethanol, water and acetone and screened for the presence of phytochemical components, antimicrobial, anthelminthic and cytotoxic properties.

The organic solvent extracts of *R. communis* displayed good inhibitory properties against *K. pneumoniae, S. aureus, E. coli* and *B. pumilus* with MIC values ranging between 0.098 mg/ml and 1.56 mg/ml. All the extracts of *P. glomerata* effectively inhibited the growth of the bacterial strains with MIC values ranging from 0.098 to 1.56 mg/ml except the aqueous extract which displayed poor activity against *K. pneumoniae* with an MIC value of 12.5 mg/ml. All the extracts of *T. alliacea* showed excellent inhibition of bacterial strains with MIC values ranging between 0.098 and 1.56 mg/ml. The best activity was also observed with the organic solvent extracts of *E. gorgonis* by inhibiting the bacterial growth at lowest concentrations of 0.098 and 0.195 mg/ml.

The best antifungal inhibition against *C. albicans* was displayed by the organic solvent extracts of *T. alliacea*, *R. obtusifolius*, *Ledebouria* sp. and *R. communis* with MIC values ranging from 0.098 mg/ml to 1.56 mg/ml.

The highest activity against *M. tuberculosis* was displayed by *R. obtusifolius* extracts with MIC values ranging between 0.098 and 0.78 mg/ml. The ethanol and acetone extracts of *Ledebouria* sp. and *R. communis* displayed good antimycobacterial activity with MIC values ranging between 0.098 and 0.78 mg/ml. Good inhibitory activity was detected with *E. gorgonis* and *T. alliacea* methanol extracts against *M. tuberculosis* (0.78 mg/ml). The best activity was observed with *E. gorgonis* aqueous extract at a concentration of 0.195 mg/ml. *Tulbaghia alliacea* acetone extract exhibited very good activity against *M. tuberculosis* (0.39 mg/ml). *Platycarpha glomerata* aqueous extract was the only extract that displayed good antimycobacterial activity at 0.78 mg/ml.

The exposure of the HuTu cells to the aqueous extracts of *E. gorgonis* displayed high cell viability at 12 and 48 hours. The H4IIE cells were viable for all the concentrations administered at 12 hours. The acetone extracts of *P. glomerata* administered to the HuTu cell lines displayed cell viability that was concentration dependent at 24 hours with high cell viability for the concentration of 0.5 mg/ml. The aqueous extracts displayed exposure time dependent pattern and cell viability was observed at 24 and 48 hours. The H4IIE cell lines on the same dosages also displayed same abovementioned properties.

The acetone extracts of *P. glomerata* administered to the HuTu cell lines displayed cell viability that was concentration dependent at 24 hours with high cell viability for the concentration of 0.5 mg/ml. The aqueous extracts displayed exposure time dependent pattern and cell viability was observed at 24 and 48 hours. The H4IIE cell lines on the same dosages also displayed this reaction. All concentrations of *T. alliacea* extracts were not cytotoxic at 48 hour exposure.

Anthelmintic tests using *Haemonchus contortus* revealed that the acetone extract of *P. glomerata* was the only extract that was active, resulting in larval mortality of *H. contortus* with mortality percentages of 50, 60 and 80 for 0.5, 1 and 2 mg/ml concentrations, respectively.

The ethanol extracts of *T. alliacea* and *P. glomerata* displayed high anthelminthic activity against *Strongylus equinus* with nematode mortality percentages ranging from 50 to 100 percent. The acetone extracts of *R. obtusifolius* displayed average activity

with 50 % mortality while only the 0.5 mg/ml of *T. alliacea* and 1 mg/ml of *P. glomerata* acetone extracts exhibited good activity against *S. equinus*.

This study has shown that medicinal plants assessed in this study have strong *in vitro* antibacterial, antifungal, anti-mycobacterial and anthelmintic efficacy which indicates that they are capable of acting against lymphatic filarial parasite infection. These observations are in agreement with indigenous knowledge provided by traditional healers. However, further *in vivo* studies using mammalian models are required in order to give conclusive evidence that these medicinal plant extracts can be used beyond reasonable doubt for treatment of lymphatic filariasis.

Keywords: Lymphatic filariasis, medicinal plant extracts, antimicrobial, cytotoxicity, anthelmintic, antimcycobacterial.

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LIST OF ABBREVIATIONS

WHO - World Health Organization

MDA - Mass Drug Administration

GPELF- Global Programme to Eliminate Lymphatic Filariasis

DEC – Diethylcarbamazine

MH – Mueller- Hinton

MIC – Minimum Inhibitory Concentration

DMSO – Dimethyl sulfoxide

DMEM – Dulbecco's Modified Eagles Medium

PBS – Phosphate Buffered Saline

INT – *p*-iodonitrotetrazolium violet

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Lymphatic filariasis which is commonly known as elephantiasis, is a condition that causes the limbs and other parts of the body, including genitalia and breasts, to swell excessively due to fluid build-up (lymphoedema) caused by improper functioning of the lymph system (USAID, 2013). It is the second leading cause of long-term and permanent disability in the world (WHO, 2009). The most commonly reported issues relevant to lymphatic filariasis-related disability are its impact on work, stigma within local communities, personal relationships, depression, social events, feelings of shame/humiliation experienced by lymphatic filariasis patients, fear, and reduced social status (Zeldenryk *et al.*, 2012). Some patients, though seldom reported, may suffer from feelings of inadequacy, feeling like a burden, sleeping problems, stigma within the school system, lack of access to appropriate foot-care, unhygienic home conditions, and unhygienic work conditions (Zeldenryk *et al.*, 2012).

Genital elephantiasis is a very serious medical problem in the tropics, affecting young and productive age groups, and is associated with physical disability and extreme mental anguish. The majority of genital elephantiasis cases are due to filariasis; however, a small but significant proportion of patients develop genital elephantiasis due to bacterial and sexually transmitted infections (STIs), mainly lymphogranuloma venereum (LGV) and donovanosis (Gupta *et al.*, 2006). Nonetheless, STI-related genital elephantiasis should be differentiated from elephantiasis due to other causes, including filariasis, tuberculosis, haematological malignancies, iatrogenic, or dermatological diseases (Gupta *et al.*, 2006).

1.2 History of lymphatic filariasis

Lymphatic filariasis history goes back to 600 B.C. along the Nile region. The historical artefacts depicting elephantiasis arabum were detected by Hindu and Persian doctors (Otsuji, 2011). However, according to Chandy *et al.* (2011), the disease may have existed as early as 2000 B.C. The origin of the name *Wuchereria bancrofti* is linked to the findings of two doctors, Joseph Bancroft and Henry Otto Wucherer, who conducted extensive studies on the nematodes (Ayisi-Boateng, 2013). The first recorded observation of microfilariae was by a French surgeon Jean-Nicolas Demarquay. He spotted microfilariae in fluid extracted from a hydrocele of a patient (Chandy *et al.*, 2011). Otto Henry Wucherer discovered the presence of microfilariae in urine in Brazil about 3 years after the first discovery by Demarquay. It was only until Timothy Lewis made the connection between the microfilariae and elephantiasis that the true relationship was discovered. Joseph Bancroft first discovered an adult nematode from the lymphatic abscess and hydrocele of a patient (Otsuji, 2011). In 1900, George Charmichael Low detected the presence of microfilariae on the proboscis of a mosquito, thereby indicating the mechanism of transmission of the filariae from the vectors to human hosts, through a blood meal (Chandy *et al.*, 2011).

Dr. Patrick Manson made a major discovery of lymphatic filariasis when he found microfilaria on the mosquito gut that had a blood meal. This discovery highlighted the beginning of medical entomology (Otsuji, 2011; Palma, 2017). In 1881, Dr. Manson confirmed again that microfilaria appeared in the circulating blood only at night by examining two cases every three hours for 23 days. Between 1878 and 1882, Dr. Manson found that the *Culex quinquefasciatus* was the intermediate host and vector of microfilariae, when he was studying the relationship between microfilariae and elephantiasis (De-Jian *et al.*, 2013).

1.3. Life cycle of lymphatic filariasis parasite

Lymphatic filariasis is transmitted to humans through *Anopheles, Culex, Aedes* and *Mansonia* mosquitoes. The mosquito bites an infected person and absorbs the microfilariae present in the lymph system, which develop inside the mosquito, hatch and migrate to the insect's mouth. The mosquito then bites an uninfected human and releases the hatched larvae on its mouth into the human skin where it gets to the lymphatic system and stays for up to six years. This worm grows into an adult and releases millions of microfilariae into the bloodstream where mosquitoes draw them and the cycle begins again as shown in Figure 1.1 (CDC, 2013).

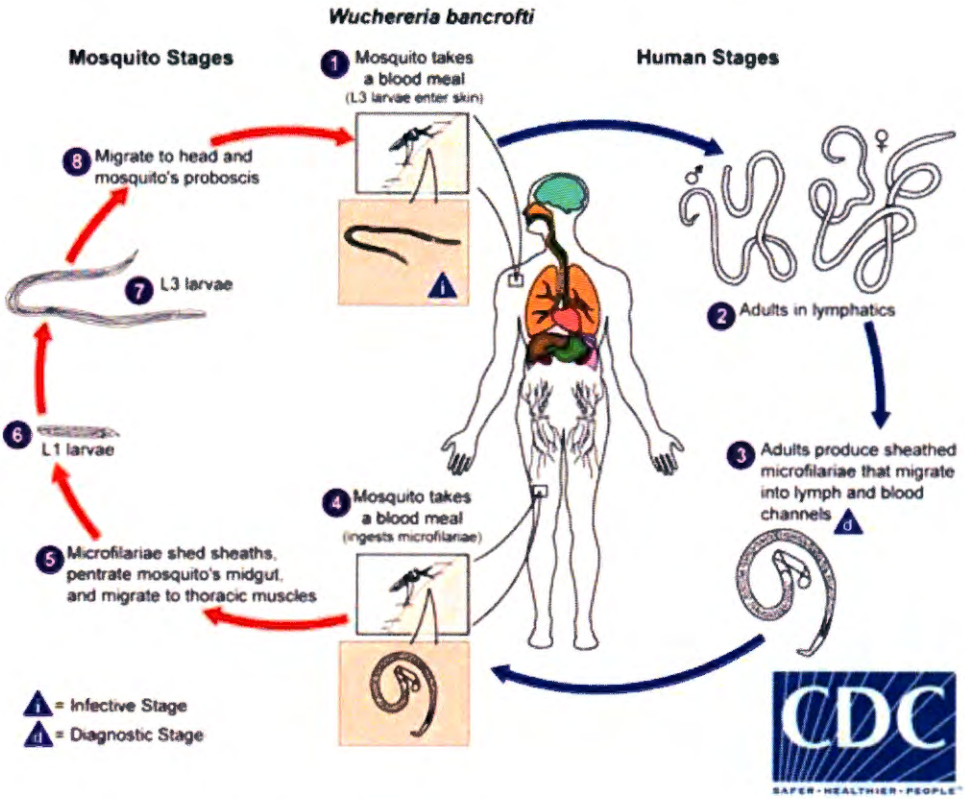

Figure 1.1: Life cycle of *Wuchereria bancrofti* nematodes

Lymphatic filarial parasites survive within the lymphatic vessels for years despite the complex immune environment surrounding them. They adopt various immunomodulatory

strategies, like the release of glutathione-S-transferases (GSTs) that counteract the oxidative free radicals produced by the host (Veerapathran *et al.*, 2009).

1.4. Clinical manifestations of the disease

Lymphatic filariasis may manifest as chronic, acute or even asymptomatic disease. Early stages of lymphatic filarial infection are usually asymptomatic but some or all symptoms may appear as the disease advances. Highly visible symptoms are the swelling and hardening of the skin on the limbs, sometimes severe swelling of the extremities and genitalia/breasts is observed. Men can also develop a condition called a hydrocele, a fluid filled balloon-like enlargement of the scrotum (Global Network, 2014). Tropical pulmonary eosinophilia syndrome may also develop (CDC, 2013). This manifests in the form of cough, shortness of breath and wheezing. High levels of immunoglobulin E and antifilarial antibodies often accompany the aesinophilia (CDC, 2013).

People living in areas where lymphatic filariasis is endemic can be classified into 6 groups, namely:

i. Uninfected but exposed
ii. Clinically asymptomatic but infected
iii. Those with acute filarial infection
iv. Tropical disease without microfilaraemia
v. Those with longstanding chronic infection associated with pathological conditions
vi. Those with tropical pulmonary aesinophilia (Babu and Nutman, 2012).

1.4.1 Chronic stages of lymphatic filariasis

The chronic stages of lymphatic filariasis include lymphoedema (swelling of the limbs and other parts of the body), hydrocele and tropical pulmonary aesinophilia.

1.4.1.1. Lymphoedema

Lymphoedema is an abnormal accumulation of lymph in the lymphatic system, occurring mostly on the lower limbs, due to distraction of the system functionalities (WHO, 2013) This usually results in lack of mobility due to the swollen limbs (Figure 1.2).

Lymphoedema may be filarial or non-filarial, with non-filarial lymphoedema occurring because of breast cancer, surgery radiation and trauma on the limb causing lymph system disruptions. Filarial lymphoedema occurs as a result of accumulation of filaraemia on the lymph system of an infected person (WHO, 2013; Davey, 2014).

This disease manifests in different stages categorized by the appearance of symptoms and reversibility of the oedema. The latent stage is an early stage of lymphoedema (Davey, 2014). At this stage, the lymph system is affected but there are no visible signs of oedema.

The next stage is characterized by the showing of oedema accumulation that is reversible by elevation of the affected limbs and massaging it to allow free flow of lymph. In stage 3 signs of skin tissue changes start being noticeable. There is an increased risk of fibrosis, infections and skin problems (Davey, 2014). This stage is irreversible but elevation of the limb helps reduce the effects.

The final stage of lymphoedema manifestation is the actual lymphatic filariasis characterized by the hardening and change in colour of the skin. The skin loses its elasticity and hangs in folds. Pappilomas and hyperkeratosis occur and risks of open wounds, bacterial and fungal infections between the folds are extremely high at this stage. This is irreversible and incurable (Dreyer *et al.*, 2002; Ayisi-Boateng, 2013; Davey, 2014).

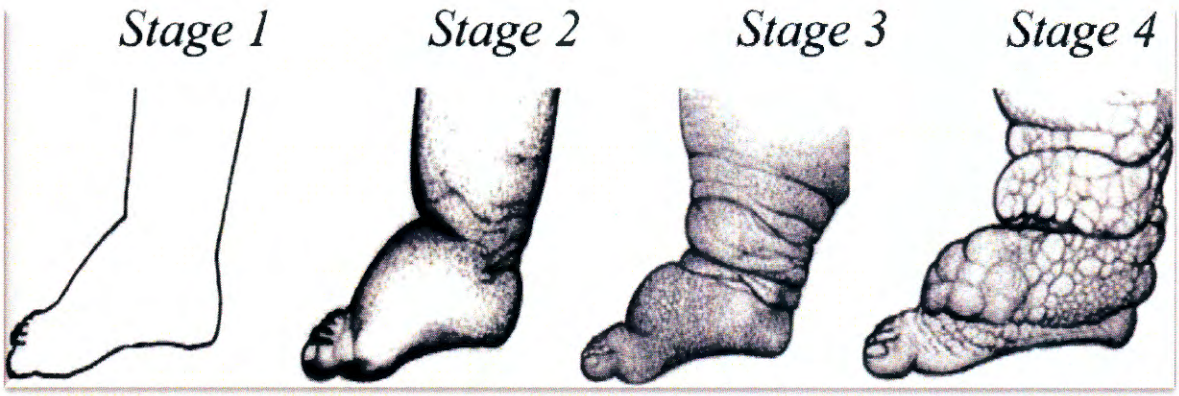

Figure 1.2: Chronic stages of lymphoedema (Addiss, 2010)

1.4.1.2. Hydrocele

Hydrocele (Figure 1.3) occurs due to the accumulation of fluid in the cavity of the scrotal sac (Babu and Nutman, 2012). This manifestation of lymphatic filariasis is not common in young boys but manifests after puberty and it increases with age of the infected persons. In some countries, 60% of infected adult males have hydrocele (Babu and Nutman, 2012).

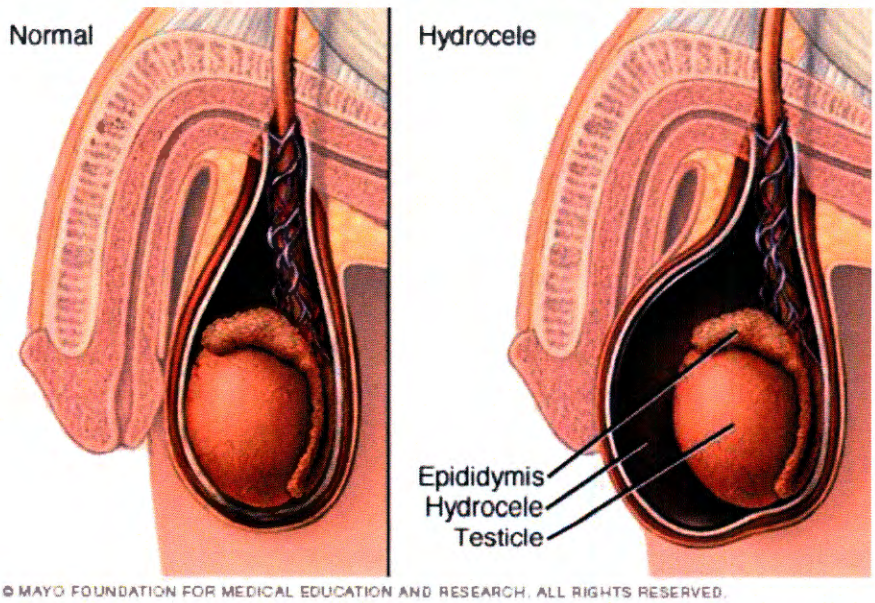

Figure 1.3: Illustration depicting normal testicles compared to testicle with hydrocele (Mayo clinic, 2016)

1.4.1.3. Tropical pulmonary aesinophilia

Tropical pulmonary aesinophilia is a syndrome of wheezing, fever and aesinophilia resulting from infection with filarial parasites *W. bancrofti* and *B. malayi* (Mullerpattan *et al.*, 2013). It is common in young infected males and in age groups of 15 - 40 years. This syndrome primarily affects the lungs and results in respiratory problems including cough, wheezing, breathlessness and chest pains (Mullerpattan *et al.*, 2013).

1.4.2. Acute stages of lymphatic filariasis

These stages include acute adenolymphangitis (ADL) and acute filarial lymphangitis (AFL). The ADL is the most common stage of lymphatic filariasis shown by attacks of fever (Babu and Nutman, 2012). Affected areas are usually painful, tender, warm, red and swollen. Inflammation of the groin and axilla also occurs (Babu and Nutman, 2012).

These acute episodes occur as a result of secondary infection of streptococci bacteria. Fungal infection in the webs of toes, minor injuries, eczema, insect bites or infections may show in the affected limbs. These ADL attacks result in persistent and progressive swelling which is the cause of elephantiasis of the limbs as well as external genitalia and breasts (Palumbo, 2009).

AFL is caused by adult worms, is very rare, and can subside with no treatment. Small tender nodules form at areas where adult worms died in the scrotum or along the lymphatics. Lymph nodes may become tender (Palumbo, 2009).

1.5. Diagnosis

Active infection is easily diagnosed by microscopic examination of the blood for the presence of microfilariae. The blood sample is collected at night because this is the time when the microfilariae are freely flowing in the bloodstream for mosquitoes to take them

with their blood meals. The blood sample should be in the form of a thick smear stained with Giemsa or with hematoxylin-and-eosin (CDC, 2013).

The immunodiagnostic test is a highly specific and sensitive form of test that checks the presence of microfilariae (CDC, 2013). Blood samples do not have to be taken at night for this kind of test. A card test is used to determine the antigens in the blood and it increases the chances of diagnosis and treatment as it can identify those infected but showing no symptoms (Rebollo and Bockarie, 2013). This test requires only a prick on the finger to get the blood sample and there is no need to conduct it in the laboratory. It can therefore be used during the screening programmes in communities (Health24, 2011).

Ultrasonography using a 7.5 or 10 MHz probe is used to detect movements of adult *W. bancrofti* in the scrotum of an asymptomatic male infected with microfilaraemia (Chandy *et al.*, 2011). Lymphoscintigraphy and immunochromatographic tests are also methods used to detect abnormalities caused by microfilariae. Lymphoscintigraphy is able to detect abnormalities caused by *W. bancrofti* while immunochromatography detects and determines the parasite antigens present within 1- 10 minutes (Chandy *et al.*, 2011).

1.6. Treatment, prevention and control of lymphatic filariasis

Global programmes have been established in accordance with World Health Organization (WHO) standards, in trying to eliminate the filariasis, which is endemic in many countries. Due to its significance, medical, social and economic impact, the 50th World Health Assembly passed a resolution to completely eliminate lymphatic filariasis by 2020 (Ottesen, 2000; WHO, 2005). The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has been implemented by means of mass drug administration (MDA) to disrupt parasite transmissions (Ottesen, 2000; Robinson and Zhang, 2011; Maurya *et al.*, 2015). Ivermectin, diethylcarbamazine (DEC) and albendazole are used in MDA to reduce the microfilariae in the bloodstream of the infected person (Taylor *et al.*, 2010). DEC kills the microscopic worms in the blood but has no effect on the adult worms. According to Maurya

et al. (2015), a combination of DEC and albendazole is given in most endemic areas, except in some areas of Africa where bancroftian filariasis and onchocerciasis are present. A combination of ivermectin and albendazole is, therefore, scheduled (Molyneux, 2003; Ichimori and Ottesen, 2011). In some areas of Africa where lymphatic filariasis coexists with LoaLoa, progressive neurological decline and encephalopathy develops in patients within a few days of taking ivermectin. Doxycycline, used to eliminate *Wolbachia* symbiont from lymphatic filarial parasite, has given promising results in these areas and might be an alternative treatment for areas that have lymphatic filariasis co-existing with LoaLoa (Bockarie and Deb, 2010).

The MDA coverage has been expanded from three million people treated in 12 countries in the year 2000, to more than 450 million in 53 countries. This lead to the elimination of lymphatic filariasis in China and Korea, and nine countries no longer require MDA because of a natural decline in transmission intensity in areas of low disease endemicity (Koroma *et al.*, 2013). In addition, the Carter Centre in Nigeria has been performing MDA, giving two anti-parasitic drugs to people in the area and distributing bed nets to avoid insect bites at night (Graitcer, 2013).

According to local newspapers, there are few reported cases of elephantiasis in South Africa though it is not conclusive of its mapping with doctors suggesting that the disease can only be managed and not cured (News24, 2012). Doctors have opted for anti-inflammatory drugs and surgery as a way to combat the effects and burden of elephantiasis (Mbuyazi, 2011; News24, 2012). This gives patients relief as they regain their ability to walk and do their daily chores with much ease. This seems to be one of the most possible solutions though it could not possibly be permanent as the adult nematodes still reside in the lymphatic system of the patient and have high potential for reproduction, thereby causing the same effects all over again.

The best way to prevent filarial infection is to avoid mosquito bites, especially between dusk and dawn as this is the time where the mosquitoes carrying the microscopic worms are usually active (CDC, 2013). The MDA serves to interrupt the transmission of lymphatic filariasis in endemic areas (Keating *et al.*, 2014). Other preventive methods that have

been put in place in the endemic countries include sleeping in air-conditioned rooms or using mosquito nets at night, wearing long sleeves and trousers, and also using mosquito repellents on exposed skin (CDC, 2013). The insecticides can be distributed to households to eliminate the vector mosquitoes before transmission (Abdullahi *et al.*, 2015).

China was once one of the countries heavily burdened by the existence of lymphatic filariasis with a total population of 330 million people at risk of infection (De-Jian *et al.*, 2013). In 2006, China managed to eliminate the burden of lymphatic filariasis using three schemes with DEC for lymphatic filariasis control i.e.:

- Repeated blood surveys and treatments,
- Treatment of microfilaremia cases combined with mass chemotherapy of the whole population in an endemic area, and
- Treatment of microfilaremia cases, integrated with DEC salt.

1.7. Geographical mapping of lymphatic filariasis

Lymphatic filariasis is mosquito-borne, caused by thread-like parasitic nematodes *W. bancrofti, B. malayi* and *B. timori,* of which *W. bancrofti* accounts for 91% of LF infections. The disease is widely distributed in 73 tropical and sub-tropical countries in the world including India, South Asia, the Pacific and the Americas (Gyapong *et al.*, 2005; Ramaiah and Ottesen, 2014). *Anopheles* mosquitoes are the most common vectors for lymphatic filariasis in Africa, while *Culex*, *Mansonia* and *Aedes* are responsible for the transmission of the disease in the Americas, the Pacific and in Asia (CDC, 2013). The *W. bancrofti* is the most common causal agent of the filarial disease in many countries while *B. malayi* is limited to Asia, and *B. timori* is limited to the islands of South Eastern Indonesia (Global Network, 2014).

According to Okon *et al.* (2010), Chu *et al.* (2010), Hotez and Ehrenberg (2010) and Utzinger *et al.* (2010), lymphatic filariasis is endemic in 32 of the world's 38 least developed countries. The disease affects more than 120 million people with 40 million

people seriously incapacitated and disfigured by the disease (Ngwira *et al.*, 2007). According to a number of studies undertaken by Leite *et al.* (2010) and Addiss (2010), one billion people (about 20% of the world population) are estimated to be at risk of infection. About 70% of infected cases are in India, Nigeria, Bangladesh and Indonesia (Chandy *et al.*, 2011).

Africa has one third of people infected with lymphatic filariasis. In this continent, the *Anopheles* mosquitoes are the primary carrier of the disease and malaria (Cano *et al.*, 2014). This disease is endemic to 38 African countries (Ottesen *et al.*, 1997). Nonetheless, while efforts to eliminate lymphatic filariasis are continuing in many parts of the world, a few African countries have yet to complete mapping the geographical distribution of the disease (Mathieu *et al.*, 2008; Shiferaw *et al.*, 2012). Figure 1.4 below shows the mapping of lymphatic filariasis worldwide.

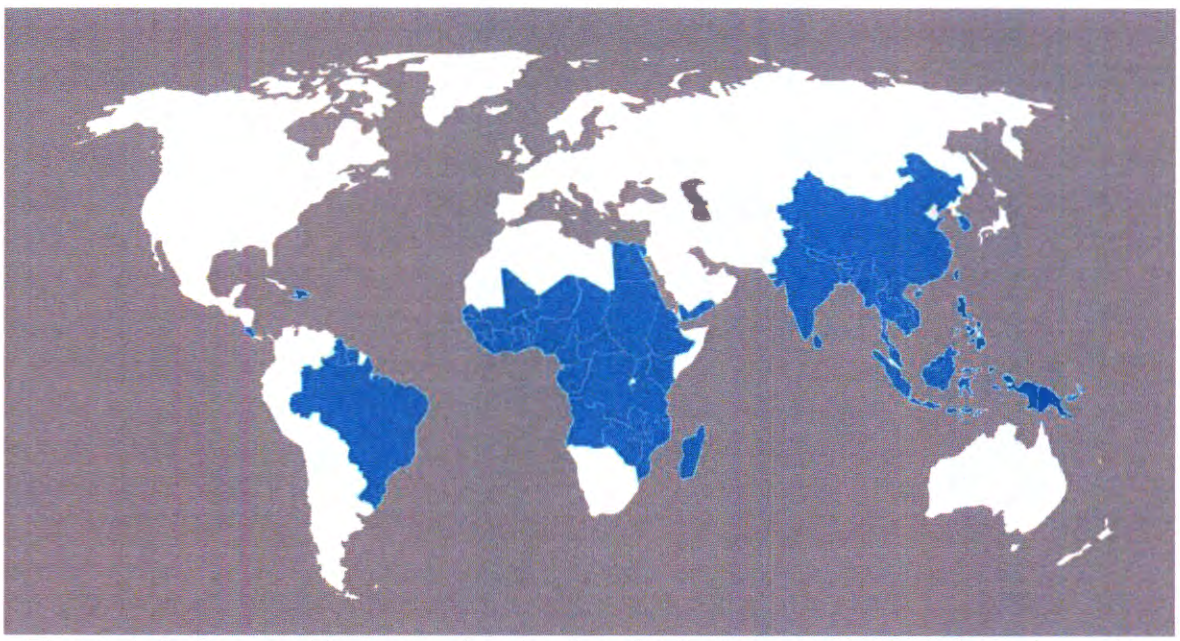

Figure 1.4: Geographical map showing the distribution of lymphatic filariasis worldwide (http://www.bvgh.org/Portals/0/disease_maps/LF_map.gif)

South Africa is one of the African countries where lymphatic filariasis is known to exist, yet data on its mobirdity and geographical distribution is lacking. Moreover, according to a report by eNCA (2013), treating lymphatic filariasis remains a huge problem in South Africa. In a number of reports by Dlamini (2011) and eNCA (2013), some South African public hospitals have turned away patients due to lack of treatment.

1.8. Medicinal plants use

The World Health Organization (2016) explains traditional medicine as the sum total of the knowledge, skills and practices based on theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. Millions of people worldwide use plants for traditional medicines and the plants have been in use for thousands of years. According to the writings, the therapeutic use of plants is as old as 4000 - 5000 B.C. with Chinese using the first natural herbal preparations as medicines (Hosseinzadeh *et al.*, 2015). In India, however, earliest references of use of plants as medicine appear in Rig-Veda, which is said to be written between 1600 - 3500 B.C. (Hosseinzadeh *et al.*, 2015).

In Africa, Asia, Latin America and the Middle East, 70 to 95% of the population is believed to be using traditional medicines for primary healthcare as this is sometimes the only system available in many rural areas (Mabona and Van Vuuren, 2013; Rinaldi and Shetty, 2015). Medicinal therapeutics derived from plants are extracted traditionally, using mainly water and ethanol and these medicines are used in healing many ailments (Maurya and Seth, 2014).

With the development of synthetic organic chemistry in the 19th century and the chromatographic separation methods in the 20th century, isolation and identification of the bioactive principles and compounds in plants have been established and mastered (Barnes and Prasain, 2005). This has led to many plants playing a role in the development of pharmaceuticals. It is estimated that about 25% of the prescription drugs currently used

today by physicians contain at least one active ingredient derived from plants (van Wyk *et al.*, 2009).

Many countries have established efforts of recognizing and aligning traditional medicine with health systems (Tshikalange *et al.*, 2016). Different parts of plants, including roots, leaves, bark, seeds and fruits, are used to prepare the concoctions and infusions for the treatment of many various ailments (Tshikalange *et al.*, 2016).

1.8.1. Use of Medicinal plants in China

Traditional Chinese medicine is still in common use in China. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas (IARC Monographs, 2002). About 5000 traditional remedies are available in China, accounting for approximately one fifth of the entire Chinese pharmaceutical market (IARC Monographs, 2002).

The escalating use of herbal medicines in China has raked in an outstanding USD 83 billion for the year 2012, an increase of above 20% for the year 2011 (Rinaldi and Shetty, 2015). Chinese people from ancient times have used traditional Chinese medicine for centuries. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of more than 12 000 items used by traditional healers, about 500 are in common use (IARC Monographs, 2002).

Botanical products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine (Hosseinzadeh *et al.*, 2015). In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy.

1.8.2. Use of Medicinal plants in India

India has a rich tradition of plant-based knowledge on healthcare. The Indians have a complex Ayurvedic system of treatment that involves physical exercise of the body and

the use of plants in healing of ailments (Kumar et al., 2007). This system is based on empirical knowledge of the observations and the experience over millennia. More than 1200 diseases are mentioned in different classical Ayurvedic texts. Management in various forms of these diseases is made with more than 1000 medicinal plants (89.93%); 58 minerals, metals, or ores (5.24%); and 54 animal and marine products (4.86%) (Biswas and Mukherjee, 2003).

Healing of wounds is one of the important areas of clinical medicines explained in many Ayurvedic texts under the heading "*Vranaropaka*". A large number of plants/plant extracts/decoctions or pastes are equally used by tribal and folklore traditions in India for treatment of cuts, wounds, and burns (Kumar *et al.*, 2007).

1.8.3. Use of medicinal plants in South Africa

South Africa is the third richest country in the world in terms of biodiversity with 68 vegetation types harbouring over 30 000 different flowering plants (Louw *et al.*, 2002; Vasisht and Kumar, 2004) accounting for approximately 10% of the world's higher plant species (van Wyk and Gericke, 2000; Street and Prinsloo, 2012). About 3000 of these species have been found to be used in traditional medicine across the country, with approximately 350 species commonly used and traded (van Wyk *et al.*, 1997; Vasisht and Kumar, 2004).

On rough estimate, 75% of the black South African population use plants from as many as 700 indigenous plant species for traditional medicine or cultural reasons and millions of urban and rural homes use wild edible herbs (Afolayan and Grierson, 1999; Shackleton, 2009). This amounts to about 26.6 million consumers from diverse ranges of age, education levels, religions and occupations (Mander *et al.*, 2007).

The people of the Eastern Cape Province mostly still live according to indigenous traditional lifestyle whereby they rely on plants for medicine, which is also a form of direct/indirect generation of income. According to Dold and Cocks (2002), approximately 525 tonnes of plant materials of around 166 taxa are traded annually in the Eastern Cape.

This trade generates approximately R27 million per annum. This trade industry is crucial in empowering women and the poor people living below the poverty line to sustain their livelihoods (Dold and Cocks, 2002). Plants are sold as crude, unprocessed drugs, but some plants have been commercially developed for the formal market, like *Aloe vera* (van Wyk, 2011).

Medicinal plants are in high demand among the rural people of the Eastern Cape as self-medication is a common practice and wild harvested herbal plants are used regularly as initial response to illness (Dold and Cocks, 2000). These traditional medicines are mostly believed to be effective in treating skin ailments as they contain compounds that can stop the bleeding, speed up wound healing, serve as treatment for burns and alleviate other skin conditions including rashes, acne, etc. (Mabona and Van Vuuren, 2013). Most of the communities, using traditional medicine in the Province, either use decoctions or infusions of the plants and they are mostly administered orally (Masika and Afolayan 2003; Maphosa and Masika, 2010).

The over-exploitation of medicinal plants in South Africa is a serious threat to biodiversity with many plants being listed on the red data list as endangered, rare, vulnerable or extinct (Hoareau and DaSilva, 1999). Due to increased harvesting pressures resulting from growth of traditional medicine popularity, the natural plant supplies have decreased. Approaches towards diversity conservation of medicinal plants have been implemented i.e. conservation of the biodiversity by local community groups and cultivation of medicinal plants (Wiersum *et al.*, 2006).

Regulations and acts have been put in place by South African government aimed at protecting biodiversity and combating ecosystem degradations. These acts are the National Environmental Management Act (NEMA) and National Environmental Management – Biodiversity Act (NEMBA), with the latter directly aimed at dealing with biodiversity issues. The NEMBA Act 10 of 2004 is aimed at providing management and conservation of South African biodiversity, offering protection services for vulnerable species and ecosystems. This act also promotes the sustainable use of indigenous biological resources, the fair and equitable sharing of benefits arising from bioprospecting

involving indigenous biological resources and the establishment and functions of a South African National Biodiversity Institute; and for matters connected therewith (Government Gazette, 2004).

It is important for traditional medicine users and communities to adhere to the conservation of the plants they use and sharing the information for indigenous knowledge records that may lead to further commercialization of these plants. The commercialization of plants contributes to the economy of the country and can serve in poverty alleviation and job creation through the cultivation of medicinal plants (Wiersum *et al.*, 2006).

The current study seeks to document indigenous knowledge on the use of medicinal plants as treatment against lymphatic filariasis in the Eastern Cape Province.

CHAPTER 2

JUSTIFICATION, AIM & OBJECTIVES

2.1. Justification of the study

Lymphatic filariasis is one of the neglected tropical diseases (NTDs) which are a subset of infectious diseases resulting from biologically incomparable groups of pathogens. These pathogens include vector-borne protozoa, bacteria, filarial worms as well as soil transmitted helminths, and the two species of non-tuberculosis mycobacteria that produce *Buruli* ulcer and leprosy (Feasey *et al.*, 2009). According to Gupta *et al.* (2006) and Chris (2015), elephantiasis can be filarial or non- filarial, with non-filarial caused by cases such as tuberculosis, STIs, leprosy, and repeated streptococcal infections.

Lymphoedema of the extremities is a common chronic manifestation of lymphatic filariasis, which progresses to elephantiasis. Once lymphatic filariasis progresses, the lymph may become static due to the malfunctioning of the lymph system (Shenoy, 2008). Bacteria, fungi and mycobacteria, which are responsible for acute attacks of dermatolymphangio-adenitis in filarial limbs, find a way of getting into the infected limbs, especially on the folded areas (Shenoy, 2008). Approximately 36 million cases of lymphoedema and hydrocele have been reported (WHO, 2016).

In Africa, an estimated 406 million people are at risk of infection with lymphatic filariasis. Seven countries (Comoros, Kenya, Madagascar, Malawi, Mozambique, Tanzania and Uganda) have completed mapping of the disease and have identified 90.7 million of the populations to be at risk (Hoerauf *et al.*, 2011). Eleven countries reported 45 463 cases of lymphoedema and 72 548 cases of hydrocele (WHO, 2016). Lymphoedema is prevalent in the Eastern and Southern parts of Africa. There are also reported cases of lymphoedema in South Africa but its mapping is quite impossible as there are few reported cases that are scattered in KwaZulu-Natal, Eastern Cape, Mpumalanga and Free State (Mbuyazi, 2011; News24, 2012; Machogo, 2015). According to reports by Dlamini (2011) and eNCA (2013), treating lymphatic filariasis remains a huge problem in

South Africa as some South African public hospitals turn away patients due to lack of treatment. The GPELF has treatments that have been proven to eliminate the microfilariae, but have no effect on the adult worms of the *W. bancrofti* (Endeshaw *et al.*, 2015). The MDA plays a very crucial role in the elimination of lymphatic filariasis and the modern synthetic drugs used in the programme are effective but with side effects (Lima *et al.*, 2012; Hussain *et al.*, 2014).

It has been claimed that a large number of medicinal plants have good antifilarial activity and less side effects (Aneshwari *et al.*, 2015). The South African government has also taken steps towards the official recognition and institutionalization of African Traditional Medicine as an effort to strengthen and promote traditional medicine and practice, aligning the traditional healthcare practitioners with the official healthcare services (Department of Health, 2016). The formal sector now draws from indigenous knowledge systems to meet international appetites for innovation and new product development. Furthermore, this is aligned with the national government's drive to increase the entrepreneurial spirit in the country towards building the mainstream South African economy. Sustainable utilisation programmes, which benefit the commoditisation of traditionally relevant medicinal plants, would create a new South African prototype (Makunga *et al.*, 2008).

It is estimated that 75% of the South African population use plants for traditional medicine or cultural reasons and millions of urban and rural homes use wild edible herbs (Shackleton, 2009). The Eastern Cape is regarded as one of the poorest Provinces in South Africa and is particularly known for its richness in plant species (Afolayan *et al.*, 2014). The Eastern Cape people are mostly traditional and rely on plants for medicine and direct/indirect generation of income. A minimum of 166 medicinal plants are traded in the Eastern Cape (Dold and Cocks, 2002), generating approximately R27 million per annum. This trade industry is crucial in empowering women and the poor people living below the poverty line to sustain their livelihoods (Mander, 1998; Dold and Cocks, 2002).

Conversely, the current harvesting methods are destructive and unsustainable for many species, especially those harvested from Afromontane Forests (Dold and Cocks, 2002).

Moreover, the knowledge of the healing powers of medicinal plants is passed on to the next generation by word of mouth. Hence, it has become increasingly urgent to document the medicinal use of African plants in South Africa, particularly the Eastern Cape Province. There are numerous publications on the ethnobotanical surveys of Xhosa medicinal plants (Bhat and Jacobs, 1995; Grierson and Afolayan, 1999; Dold, 2005; Dyubeni and Buwa, 2012; Otang *et al.*, 2012; Bhat, 2013; Afolayan *et al.*, 2014).

Plants are known to be rich sources of biologically active compounds, hence, the evaluation of plant extracts has been used in discovering antimicrobial agents. The secondary metabolites like tannins, saponins, flavonoids, alkaloids, cardiac glycosides, etc., are known for their roles in many health attributes including antioxidant activity, antimicrobial effects, modulation of detoxification enzymes, decrease of platelet aggregation and modulation of hormone metabolism, and anticancer property (Saxena *et al.*, 2013). Substances such as flavonoids possess anti-inflammatory properties and are low in toxicity, which make them important for many therapeutic treatments (Borelli and Izzo, 2000).

The development of elephantiasis comes with risks of microbial infection and development of open wounds on the skin folds of the limb (Shenoy, 2008). Antiinflammatory and antimicrobial treatments are administered to patients with elephantiasis to help fight and prevent these bacterial and fungal infections (Shenoy, 2008). It is therefore important to test the plants used in traditional medicines for anti-inflammatory, antibacterial, antifungal and antimycobacterial activities as they are very important in treating the secondary infections of lymphatic filariasis.

Although it is widely acknowledged that medicinal plants have several benefits including their affordability, availability and acceptability, the need for safety and toxicity evaluation remain paramount from a scientific perspective. There is generally limited information on potential mutagenic health hazards resulting from the long-term use of many medicinal plants (Verschaeve and Van Staden, 2008; Mwitari *et al.*, 2013). The use of human gut

cells to determine cytotoxicity is the best way to determine whether the plants are poisonous by ingestion, if so, at what dosages. As a result, the current study sought to assess the anthelmintic, antibacterial, antifungal and antimycobacterial activity as well as cytotoxicity of medicinal plants that are documented to be used against lymphatic filariasis in the Eastern Cape Province.

2.2. Aim

This study was aimed at documenting medicinal plants used in the treatment of lymphatic filariasis by traditional healers and herbalists of the Eastern Cape Province of South Africa and screen them for the presence of antibacterial, antifungal and antimycobacterial properties, anthelminthic activity and cytotoxicity.

2.3. Objectives

- To conduct an ethnobotanical survey on plants used by the traditional healers and herbalists of the Eastern Cape Province in the treatment of lymphatic filariasis.
- To determine the phytochemical constituents of the collected plant species
- To screen plant species for the presence of antibacterial and antimycobacterial properties.
- To screen plant species for the presence of anthelmintic properties.
- To test for *in vitro* cytotoxicity of medicinal plants using cultured mammalian cells.

CHAPTER 3

MATERIALS AND METHODS

3.1. Study Area

The study was conducted in the Intsika Yethu and Raymond Mhlaba local Municipalities of the Eastern Cape Province of South Africa. The Eastern Cape Province (Figure 3.1) is a second biggest Province in South Africa which is approximately 170500 km^2 in size, covering about 13.8 % of the country's total area (South African Government, 2014). It is the most diverse and complex Province, encompassing three biodiversity hotspots such as the Cape floristic region, succulent Karoo, and Maputaland-Pondoland. The Maputaland-Pondoland Albany is the second biodiversity hotspot of South Africa that is dominated by closed shrublands, low forests with evergreens, succulent trees, vines and shrubs (Hamann & Tuinder, 2012).

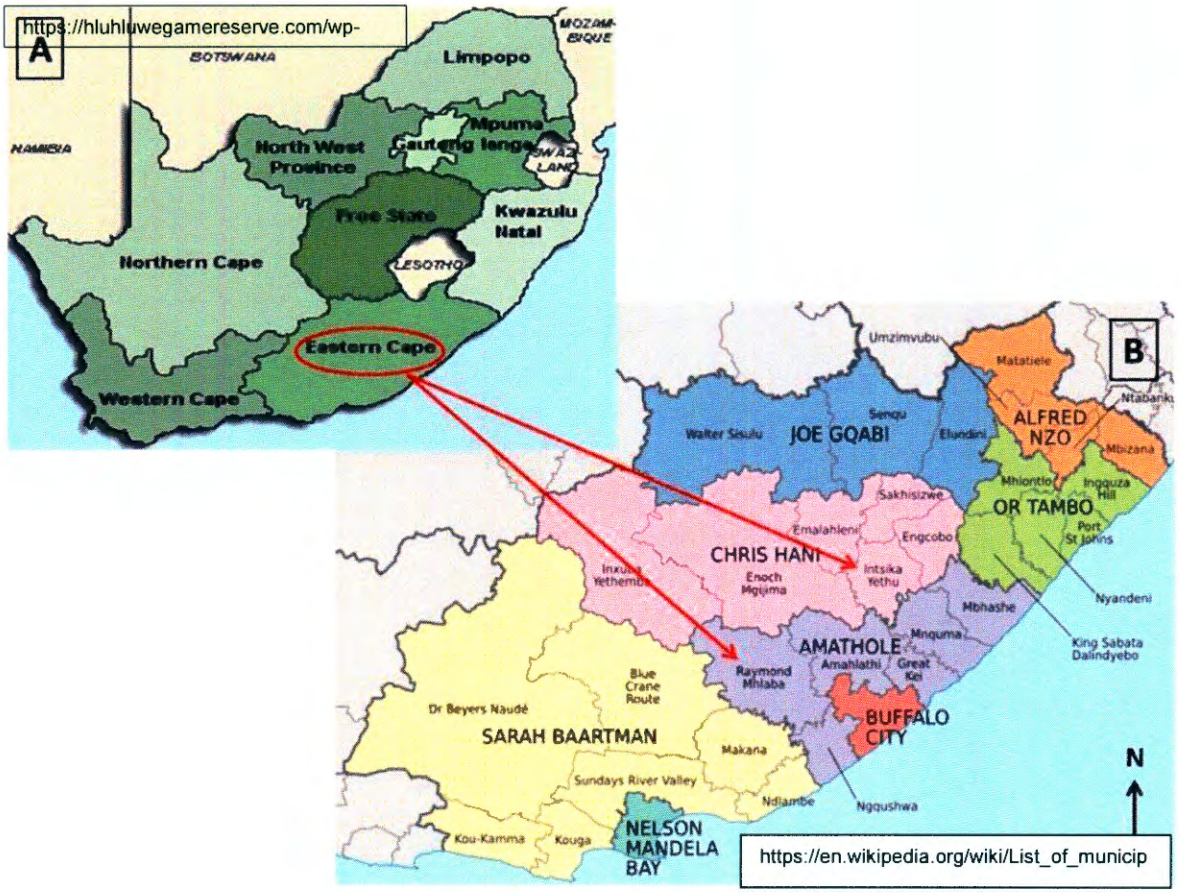

Figure 3.1: A; Map of South Africa showing the nine Provinces. **B**; Eastern Cape Province with all its districts and local municipalities including the sampled Intsika Yethu and Raymond Mhlaba local municipalities

The Eastern Cape has a number of endangered ecosystems with a total of 316 threatened plant species, one fifth of these is found in the thicket biome (Hamann & Tuinder, 2012). The forest and fynbos contain high numbers of threatened plant species. In addition, the Province is home to 4 endemic freshwater fish species, 8 threatened marine fish species, 6 threatened frog species, 4 of which are endemic, and 19 threatened reptile species, 18 of which are endemic (Hamann and Tuinder, 2012).

The Eastern Cape is characterised by southern Drankensberg Mountains, ragged cliffs, northern tropical forest and dense bushes. Most of the area's natural biome has been converted to agricultural lands; these farmlands have sheep and goat (stock) or olive nurseries, maize and sorghum (crops), pineapple and chicory plantations (Hamann & Tuinder, 2012). Indigenous forest plants include yellowwoods, white stinkwood and many exotic plants (Hamann & Tuinder, 2012). Climate conditions are characterised by cold frosty winters and hot summer days. This region has a good summer rainfall ranging between 401 and 600 mm per annum, average annual temperatures are between 18 and 21°C (Moyo and Masika, 2009).

The Raymond Mhlaba Local Municipality is a countryside municipality found at the foot of the Winterberg mountain range (Figure 3.1). It covers towns and villages of Alice, Fort Beaufort, Hogsback, Middledrift and Seymour. This municipality is 3725 km^2 in size with 327119.1 km^2 remaining as natural ecosystems with protected areas comprising11 nature reserves, covering the beautiful biodiversity this Province has (Nkonkobe municipality IDP, 2014).

Intsika Yethu Municipality (Figure 3.1) is one of the eight municipalities found within the Chris Hani district. It is a purely rural municipality with a population of about 194 000 people in its 23 wards. The economy of this municipality is mainly generated through agriculture and farming and tourism (Local government, 2017).

3.2. Ethnobotanical survey and plant collection

An ethnobotanical survey was conducted at Intsika Yethu and Raymond Mhlaba Local Municipalities in the Eastern Cape. The survey was conducted in the form of questionnaires (Appendix 1). Eight people, including elderly people with indigenous knowledge of medicinal plants, *sangomas* and herbalists, were consulted and the plant specimens were collected directly from their natural habitats. The information that was

gathered included plants used against lymphatic filariasis, plant parts used, the common names of the plants and methods of preparation and use. Collected plants were identified by Dr. E. Sieben of the University of Free State and Mr. A.P. Dold of the Selmar Schönland Herbarium in Grahamstown. Voucher specimens for each plant were prepared and deposited at the herbarium of the University of the Free State, QwaQwa Campus.

3.3. Plant preparation and extraction

The steps followed in preparation and extraction of the plant materials are shown in Figure 3.2 below. The collected plant material was oven dried at 26°C, thereafter it was ground to fine powder using a blender. The material was then stored in sealed clear-plastic honey jars in the dark at room temperature until further processing and extraction.

The powdered plant material was extracted with ethanol, water, methanol and acetone. This was done by shaking 1 g powdered plant material in 10 ml of the solvent for 24 hours, at room temperature. The plant extracts were filtered through Whatman No.1 filter paper discs and left to dry in front of a fan. The dry extracts were kept in sealed containers inside a refrigerator at 4°C until they were used in experiments.

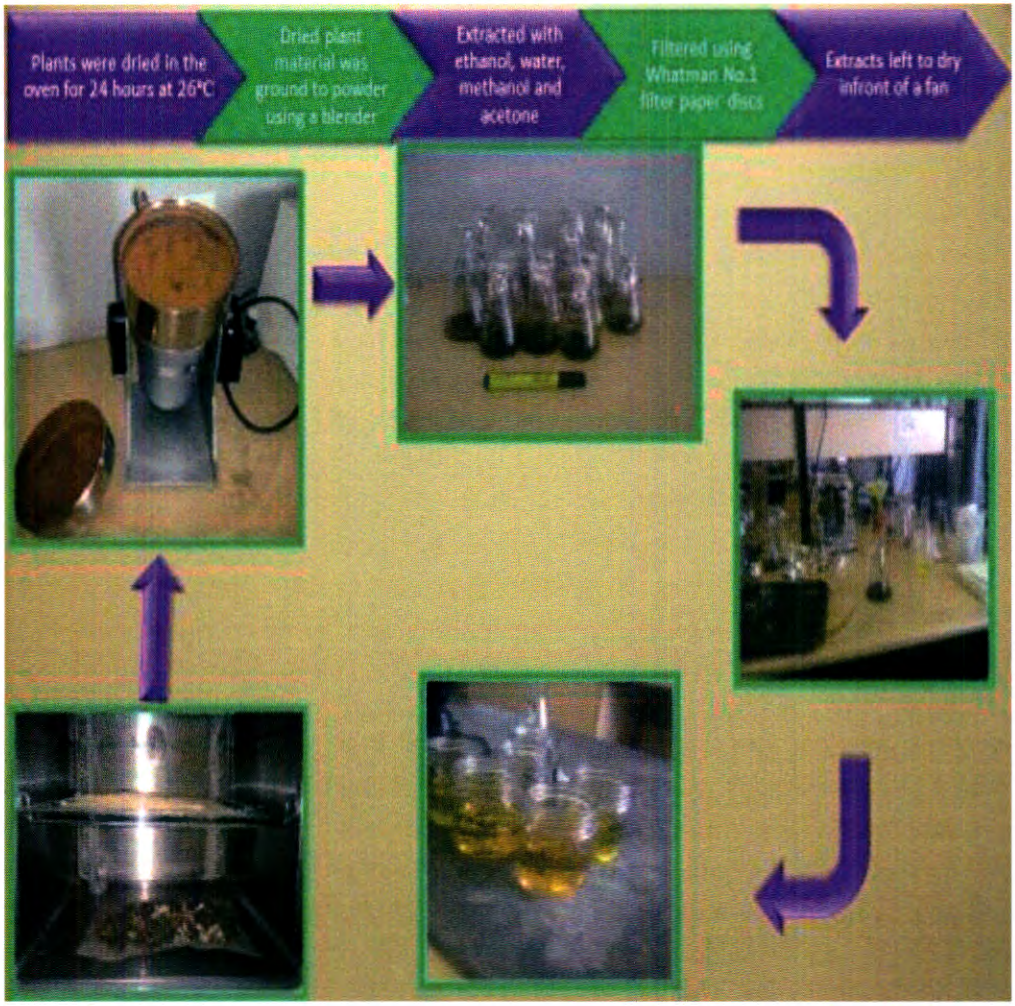

Figure 3.2: Plant collection and preparation of plant extracts

3.4 Phytochemical analysis

The presence of phytochemical constituents such as tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, anthraquinones and alkaloids was determined using the standard procedures described by Harborne (1973), Trease and Evans (1989) and Sofowara (1993). The test for the secondary metabolites was based on the visual observation of colour change or through the formation of the precipitate after the addition of the specified reagent(s). For the phytochemical analysis 10 grams of the plant material was extracted with 100 ml of the extracting solvent.

3.4.1. Test for alkaloids

Two millilitres of the prepared plant extract was stirred in 5 ml of 1% aqueous hydrochloric acid and heated in a water bath. One millilitre of the filtrate was then treated with few drops of Mayer's reagent and a second portion was treated with Dragendroff's reagent. Turbidity of precipitation with either of those reagents was taken as preliminary evidence for the presence of alkaloids in the extract (Harborne, 1973).

3.4.2. Test for tannins

In the test for tannins, 2 ml of the plant extract was boiled for a few minutes in a water bath. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue black colouration as indication of the presence of tannins (Sofowara, 1993).

3.4.3. Test for saponins

Two millilitres of the plant extract was boiled in 20 ml of distilled water in a water bath and then filtered. Thereafter, 10 ml of the filtrate was mixed with 5 ml of distilled water and then shaken vigorously and observed for a stable persistent froth. The frothing was then mixed with 3 drops of olive oil, shaken and observed for the formation of emulsion as indication of the presence of saponins (Harborne, 1973).

3.4.4. Test for flavonoids

One millilitre of the plant extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was shaken vigorously with 1 ml of diluted ammonia solution. A development of yellow colouration was an indication of the presence of flavonoids (Sofowara, 1993).

3.4.5. Test for steroids

Two millilitres of acetic anhydride was added to 1 ml of the plant extract with a 2 ml concentrated H_2SO_4. The colour change from violet to blue indicated the presence of steroids.

3.4.6. Test for terpenoids

Five millilitres of plant extracts was added in 2 ml chloroform and 3 ml H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was an indication of the presence of terpenoids (Harborne, 1973).

3.4.7. Test for cardiac glycosides

In this test, 5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated H_2SO_4. A brown ring on the interface served as indicator of the presence of a deoxysugar characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer, a greenish ring may form throughout the thin layer (Trease & Evans, 1989).

3.4.8. Test for anthroquinones

Five millilitres of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for pinkish red colour changes which is an indication of the presence of anthroquinones (Harborne, 1973).

3.5 Antimicrobial Screening

3.5.1 Antibacterial activity

Escherichia coli (ATCC 8739), *Klebsiella pneumoniae* (ATCC 13047) *Bacillus pumilus* (ATCC 14884) and *Staphylococcus aureus* (ATCC 6538) were maintained on Mueller-Hinton (MH) agar plates and invigorated for bioassay by culturing a single colony in 2 ml MH broth for 24 h. The saturated bacterial cultures were then diluted with MH broth (1 ml bacteria: 99 ml broth), to make certain that the bacteria is at the start of the log phase when the test commences.

The microplate method of Eloff (1998) was used to determine the minimal inhibitory concentration (MIC) values for plant extracts with antibacterial activity. Residues of plant extracts were dissolved at 50 mg/ml with the extracting solvents. All extracts in well A were tested at 12.5 mg/ml in 96-well microplates where 100 µl of the plant extract was added and serially diluted two-fold to 0.098 mg/ml, after which 100 µl of bacterial cultures were added to each well. Controls included the antibiotic neomycin, extract-free solutions and extracting solvents. The microplates were incubated overnight at 37°C. As an indicator of bacterial growth, 40 µl *p*-iodonitrotetrazolium violet (INT) (0.2 mg/ml) dissolved in water was added to the wells and incubated at 37°C for 30 min. MIC values were recorded as the lowest concentration of the extract that completely inhibited bacterial growth, i.e. a clear well. The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms (Eloff, 1998). Where bacterial growth was inhibited, the solution in the well remained clear after incubation with INT. All extracts were tested in triplicates.

3.5.2. Antifungal activity

A standard strain of *Candida albicans* was obtained from the University of Fort Hare and maintained on nutrient agar. A modification of the NCCLS proposed method (M27-P) broth microdilution test was performed (Espinel-Ingroff *et al.*, 1995). Four millilitres of sterile saline were added to approximately 400 µl of 24 hour old *Candida* culture. The absorbance was read at 530 nm and adjusted with sterile saline to match that of a 0.5

McFarland standard solution. From the prepared fungal culture, a 1:1000 dilution with broth (e.g. 10 µl fungal culture: 10 ml broth) was prepared.

The water extract residues were dissolved in water and the organic solvent extract residues were dissolved in dimethyl sulfoxide (DMSO). All extracts were dissolved to a concentration of 100 mg/ml. Water extracts were tested at a concentration of 25 mg/ml whereas organic solvent extracts were tested at 6.25 mg/ml, for well A. One hundred microlitres of broth were added to each well of a 96-well microplate. One hundred microlitres of the water extract were added to well (A) and serially diluted from (A) by taking 100 µl into (B). This two-fold dilution was continued down the plate and 100 µl from the last well (H) were discarded. In case of organic solvent extracts 25 µl of the 100mg/ml extracts were added to 175 µl broth and diluted serially. Three replicates were prepared for each extract. All the wells were then filled with 100 µl of stock yeast culture. Amphotericin B was used as a reference for this experiment and the following controls were prepared: wells containing broth only, fungal strain with no extract and solvent used to dissolve plant extracts. The microplates were incubated overnight at 37 °C. As an indicator of fungal growth, 40 µl of 0.2 mg/ml INT dissolved in water were added to the wells and incubated at 37°C for 30 min.

3.5.3. Antimycobacterial activity

Mycobacterium tuberculosis (ATCC 25177) was maintained in Middlebrook 7H9 broth containing 10% OADC (oleic acid + albumin + dextrose + catalase). Inoculum was prepared by transferring the stock mycobacterial culture to supplemented 7H9 broth (Middlebrook 7H9 + 10% OADC) and grown for 72 h on a shaker. Two (5 ml) supplemented 7H9 broths were inoculated with the mycobacterial culture and grown for 72 h. Twenty percent sterile glycerol was added to each culture and 500 µl aliquots were made into sterile Eppendorf tubes. These stocks were named G1 stocks and were stored at -30°C. A single G1 stock was used to inoculate supplemented Middlebrook 7H10 agar (7H10 + 10% OADC) plates and incubated at 37°C for four days or until growth was observed. From this culture, a single colony was used to inoculate 5 ml supplemented

7H9 broth. This was grown on a shaker at room temperature for 72 h and used for the experiment.

The broth microdilution method (Swenson et al., 1982) was used to determine the MIC values for plant extracts against *M. tuberculosis*. The aqueous extract residues were re-dissolved in water and other extract residues were dissolved in absolute DMSO. All extracts were dissolved to a concentration of 100 mg/ml. One hundred microliter of the supplemented 7H9 broth was added to all the wells of microtitre plates. All extracts were tested at a concentration of 25 mg/ml in well A and serially diluted to 0.195 mg/ml. The optical density of the 72 h broth culture was determined and adjusted at 550 nm. One hundred microliter of the diluted culture was added to every well of the microtitre plate. The controls included the solvent used to dissolve plant extracts, Middlebrook 7H9 broth alone, and the antibiotic streptomycin (1.56 mg/ml) as a positive control. The plates were covered and incubated at 37°C for 72 h. After incubation, 40 µl of 0.4 mg/ml solution of INT was added to each well of the plate. The plates were covered and incubated for 24 h at 37°C. All extracts were tested in triplicates.

3.6. Anthelmintic screening

Nematodes were obtained from cattle and horse faecal samples collected by Mr. Jacob Mabena (The Veterinary Technician from Department of Zoology and Entomology, University of the Free State). The faecal samples were collected according to the method described by Reinecke (1973). The faecal culture technique was used to hatch the eggs and larval identification was done according to van Wyk *et al.* (2004).

For the assay, the method of Rasoanaivo and Ratsimamanga-Urverg (1993), modified by McGaw et al. (2007) was used with some modifications. Ten grams of each faecal sample was weighed and incubated with 10 grams of vermiculite at 26°C for 7- 10 days. The L_3 larvae were harvested from the *in vitro* cultures prepared and transferred into a single petri dish. One hundred and fifty microlitres of the solution, containing about 10 - 15

nematodes was incubated with 0.5, 1 and 2 mg/ml of plant extracts respectively for 2 h at 25 °C in the dark. The anthelmintic levamisole was used as a positive control, and solvent blanks were included. The percentage of living nematodes was estimated using a stereomicroscope.

All live and motile L_3 stage larvae in each petri dish were counted and a percentage inhibition of larval development was calculated using the formula described by Coles *et al.* (1992) and Bizimenyera *et al.* (2006) with slight changes:

$$Inhibition\ percentage\ (\%) = \frac{1 - X_1}{X_2}\ X\ 100$$

Where X_1 is the initial number of larvae in test extracts pre-treatment, and X_2 is the number of larvae obtained post-treatment.

3.7. Cytotoxicity screening

The HuTu and H4IIE cells were obtained from the American Type Culture Collection (ATCC). The cells were removed from the incubator and the observance of the cells was performed microscopically. Cells were grown in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS), 100 U/ml penicillin and µg/ml streptomycin in a humidified atmosphere of 5% CO_2 and 95% air at 35°C. The cells were cultured using cell culture petri dishes.

Cells were shaken gently on the platform in order to assess viability and 70% ethanol was thoroughly sprayed on the dishes and flask containing DMEM before introduced into the fume hood. Cultured cells from the petri dish were discarded into a waste container; cells were rinsed 3 times with 5 ml phosphate buffer saline (PBS) (without salt) to remove all debris and leave cells attached to the surface of dish. After washing the cells with PBS, 1.5 ml of Trypsin was applied to the dish to detach the cells and was incubated for 3 minutes. One and half millilitre of Trypsin was carefully added to

a 15 ml tube containing 10.5 ml of supplemented media to make a working volume of 12 ml.

3.6.1. Cell count

From 12 ml of DMEM supplement solution containing FBS and stock media, 100 µl was pipette into Eppendorf tube. Thereafter, 10 µl of the solution was pipette into two sides of hematocytometer slide and counted to 20 000 cell/ml under light microscope. Ninety six well plates were filled with 250 µl of PBS (without salt) and 250 µl supplement media and incubated for 12, 24 and 48 hours. After every exposure period, different concentrations of plant extracts (1, 0.5, 0.25, 0.125, 0.0625 and 0.03 mg/ml) were administered. Plates also contained blank control (Plant extract), solvent control (water, acetone or ethanol) and methanol killed cells.

3.6.2. MTT assay

Assessment of cell viability was carried out using Promega CellTiter 96 Aqueous Non-Radioactive Cell Proliferation (MTT) assay to determine the number of viable cells in culture Mosmann (1983) and Promega (2005). At the end of each exposure period, some cells were killed using 200 µl of undiluted methanol and incubated for 10 minutes. After 10 minutes of incubation, cells from the 96 well plate were discarded and washed three times with PBS (with salt); then 100 µl of 3 -(4,5-dimethylthiazol-2-yl)-2,5- diphenyll tetrazolium bromide was added into each well and the cells were further incubated for 30 minutes. Thereafter, cells were lysed with 100 µl of DMSO for 30 min in a dark place at room temperature to allow them to dissolve the formazan crystals.

The formation of purple colour (formazan) was measured with a microtitre plate spectrophotometer (Tristar LB 941) at 560 nm. Results were read using Mikro 2000 software. The percentage of cell viability was calculated using the formula below:

$$Percentage\ cell\ viability = \frac{Mean\ Abs_{sample}}{Mean\ Abs_{control}}\ X\ 100$$

The minimum lethal concentrations were determined from the mean % viability with 80% and above of live cells considered the viable cells

CHAPTER 4

RESULTS

4.1. Ethnobotanical survey

Four male herbalists and two community members with indigenous knowledge, with ages ranging between 35 and 70 years, were consulted and interviewed about medicinal plants used against lymphatic filariasis in the study areas. A total of six plant species belonging to the families of *Asparagaceae, Euphorbiaceae, Polygonaceae, Asteraceae* and *Alliaceae* were collected and identified (Table 4.1). Most of the information given by the informants pointed out to the same plants, with different names by locality. There were no conservation methods employed by most of these herbalists, except one member who cultivates the medicinal plants within his home yard for personal use and helping those close to him as well as animals. The whole plant, leaves, bulbs and roots or rhizomes were the most commonly used plant parts in the study areas with mainly aqueous extraction methods used for decoctions and infusions.

The pictures of the plants collected are displayed in Plates 4.1 to 4.6 below. These plants were collected directly from their natural environments with the assistance of the consultants.

Table 4.1: A list of plants collected as guided by the ethnobotanical survey and their detailed uses

Family	Scientific names	Common names	Plant part used	Medicinal uses	Applications and preparations
Asparagaceae	*Ledebouria sp.* Roth	*Umathunga*	Bulb	Used to cure non-healing wounds and to lighten thick blood clots.	A concoction of this plant is prepared by grinding the plant and adding boiling water to make a drinkable solution. Half a mug three times a day is a normal dose given to people with wounds and blood clots.
Euphorbiaceae	*Ricinus communis* L.	*Umhlamvuthwa, mhlabawuvuthwa*	Leaves and young roots.	Multiple uses ranging from healing headaches, pains and relieving clots from the body.	The leaves and roots are fused into paste and applied on the affected areas of the body. For headaches, the leaves are usually heated and put against the forehead until the pain subsides.
Euphorbiaceae	*Euphorbia gorgonis* A. Berger	*Nkalimasane, ntsema*	Whole plant	Used to treat wounds, swelling and skin problems	The plant parts are used in bathing water to get rid of the skin problems
Polygonaceae	*Rumex obtusifolius* L.	*Idolo lenkonyana, inkunzane*	Whole plant	For relief of pain associated with swelling of limbs and other body parts.	This plant is ground to paste, mixed with other plants to make a drinking concoction. The ground paste is also added in warm water to wash the swollen parts of the body.
Asteraceae	*Platycarpha glomerata* Thunb. Less.	*Umthuma*	Root only	Treatment for wounds, swelling and pain.	Its roots are ground to powder and immersed in boiling water. It can also be mixed with the root of *R. obtusifolius* to heal skin rashes, sores and cleansing the

					blood. The mixture is boiled and cooled, and half a mug is taken 3 times daily before meals
Alliaceae	*Tulbaghia alliacea L.f.*	*Mwelela, Isivimbampunzi*	Bulb	This plant is used for bathing, as a drinking concoction in the treatment of swellings and wounds. It also cleanses the body from inside out.	The patient takes half a mug of this medicine twice before meals. Another half a mug is added in bathing water to rinse the affected area.

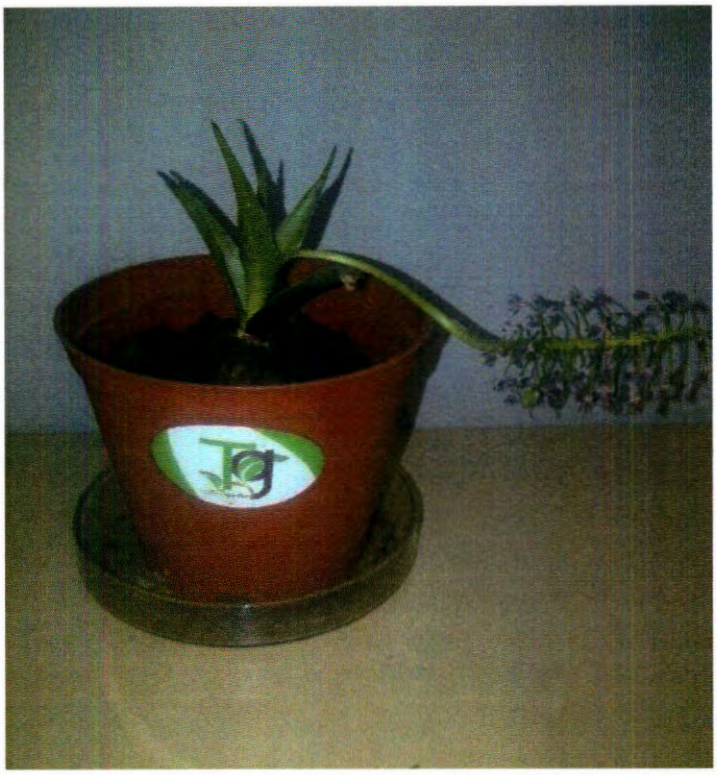

Plate 4.1: *Ledebouria* sp. showing the purple flowers

Plate 4.2: *Rumex obtusifolius*

Plate 4.3: *Tulbaghia alliacea* (circled)

Plate 4.4: *Euphorbia gorgonis*

Plate 4.5: *Ricinus communis* showing the fruits at blooming season

Plate 4.6: *Platycarpha glomerata* with purple flowers at the central base of the leaves

4.2. Phytochemical screening and antimicrobial activities of the plant extracts

4.2.1. Qualitative analysis

Table 4.2 below shows the phytochemical constituents available in the plants selected and extracted with different solvents. The test for tannins displayed that most of the plant extracts contained the tannins, except the acetone extract of *P. glomerata* and *Ledebouria sp.* The organic extracts of *T. alliacea* also displayed lack of tannins.

The presence of saponins was observed in all the aqueous plant extracts except *R. obtusifolius* which displayed the presence of saponins in the acetone extract. The presence of fasflavonoids was observed in the extracts of *E. gorgonis* and *Ledebouria* sp., while the ethanolic extracts of *Ledebouria* sp. and *R. communis* were the only extracts that displayed the presence of steroids. Ethanol and acetone extracts of all the plants displayed the presence of cardiac glycosides. The *T. alliacea* had cardiac glycosides present in all the extracts. The presence of anthraquinones was observed in the methanol extracts of *T. alliacea* and *R. communis.* Alkaloids presence was displayed by all the extracts of *T. alliacea* as well as methanol extracts of *R. obtusifolius*, *E. gorgonis* and *Ledebouria* sp., and acetone extracts of *Ledebouria* sp.

Table 4.2: Phytochemical analysis of the plant extracts.

Plant names	Extraction solvents	Tannins	Saponins	flavonoids	steroids	Terpenoids	Cardiac glycosides	Anthraquinones	Alkaloids
R. communis	Water	+	+	-	-	+	-	-	-
	Ethanol	+	-	-	+	-	+	-	-
	Methanol	+	-	-	-	+	-	+	-
	Acetone	+	-	+	-	-	+	-	-
R. obtusifolius	Water	+	-	-	-	+	-	-	-
	Ethanol	+	-	-	-	-	+	-	-
	Methanol	+	-	-	-	-	-	-	+
	Acetone	+	+	-	-	-	+	-	-
E. gorgonis	Water	+	+	+	-	+	-	-	-
	Ethanol	+	-	+	-	-	+	-	-
	Methanol	+	-	-	-	-	-	-	-
	Acetone	+	-	+	-	-	+	-	+
P. glomerata	Water	+	+	-	-	-	-	-	-
	Ethanol	+	-	+	-	-	+	-	-
	Methanol	+	-	-	-	-	-	-	-
	Acetone	-	+	+	-	+	+	-	-
Ledebouria sp.	Water	-	+	-	-	-	-	-	-
	Ethanol	+	+	-	+	-	+	-	-
	Methanol	+	-	+	-	-	-	-	+
	Acetone	-	+	+	-	+	+	-	+
T. allliacea	Water	+	+	+	-	+	+	-	+
	Ethanol	-	-	-	-	+	+	-	+

	Methanol	-	-	-	-	+	+	+	+
	Acetone	-	-	-	-	+	+	-	+

+ = Present; - = Absent

4.3. Antimicrobial screening

4.3.1. Antibacterial activity

The organic solvents extracts of *R. communis* displayed good inhibitory properties against *K. pneumoniae, S. aureus, E. coli* and *B. pumilus* with MIC values ranging between 0.098 mg/ml and 1.56 mg/ml (Table 4.3). The aqueous extracts of *R. communis* exhibited poor antibacterial activity at 6.25 mg/ml against all the tested strains.

All the extracts of *P. glomerata* effectively inhibited the growth of the bacterial strains with MIC values ranging from 0.098 to 1.56 mg/ml except the aqueous extract which displayed poor activity against *K. pneumoniae* with an MIC value of 12.5 mg/ml (Plate 4.7).

The aqueous extract of *R. obtusifolius* showed no activity against the bacterial strains tested. The acetone extract exhibited good inhibition against *K. pneumoniae* and *S. aureus* at 0.78 mg/ml. Ethanol extract showed minimum inhibition against all strains (1.56 mg/ml). Poor activity was observed with *R. obtusifolius* methanol extract.

All the extracts of *T. alliacea* showed excellent inhibition of bacterial strains with MIC values ranging between 0.098 and 1.56 mg/ml. The best activity was observed with the organic solvent extracts of *E. gorgonis* by inhibiting the bacterial growth at lowest concentrations of 0.098 and 0.195 mg/ml. The aqueous extract displayed no activity at all.

Poor activity was exhibited by the extracts of *Ledebouria* sp. The methanol and ethanol extracts of the plant displayed poor activity against *K. pneumoniae*, *S. aureus* and *B. pumilus*. No activity was detected with acetone and aqueous extracts.

The negative controls were performed using 100% solvent extracts which had no inhibitory effects on the bacterial growth.

4.3.2. Antifungal activity

The results for antifungal activity are presented in Table 4.4 below. The highest activity against *C. albicans* was displayed by the organic solvent extracts of *T. alliacea*, *R. obtusifolius*, *Ledebouria* sp. and *R. communis* with MIC values ranging from 0.098 mg/ml to 1.56 mg/ml. The organic solvent extracts of *E. gorgonis* and *P. glomerata* displayed poor activity against the fungal strain with MIC values ranging from 3.12 mg/ml to 6.25 mg/ml. The aqueous extracts of all the plants proved ineffective against *C. albicans* with poor or no activity.

4.3.3. Antimycobacterial activity

The results for antimycobacterial activity are displayed in Table 4.5. The highest activity against *M. tuberculosis* by all the extract solvents was displayed by *R. obtusifolius* extracts with MIC values ranging between 0.098 and 0.78 mg/ml.

The methanol extracts of *E. gorgonis* and *T. alliacea* displayed good inhibition for this extract solvent with MIC values of 0.78mg/ml. Of the ethanol extracts, *Ledebouria* sp., *R. obtusifolius* and *R. communis* displayed the best antimycobacterial activity with MIC values ranging between 0.195 and 0.78 mg/ml.

The aqueous extracts of *R. obtusifolius*, *E. gorgonis* and *P. glomerata* displayed high mycobacterial inhibition with MIC values of 0.195 to 0.78 mg/ml.

The acetone extracts of the plants displayed the best inhibition with MIC values ranging between 0.098 and 0.78 mg/ml, with the exception of *E. gorgonis* which displayed good activity with an MIC value of 1.56 mg/ml.

Table 4.3: Antibacterial activity of plant extracts (MIC in mg/ml)

Plant Names	Extraction solvents	Extract yield (mg)	Bacteria strains			
			K. pneumoniae	***S. aureus***	***E. coli***	***B. pumilus***
P. glomerata	Methanol	40	0.098	0.098	0.098	0.78
	Ethanol	300	0.39	0.098	0.098	1.56
	Acetone	50	0.098	0.098	0.098	0.78
	Water	650	12.5	0.19	0.19	0.19
R. obtusifolius	Methanol	300	3.12	3.12	3.12	1.56
	Ethanol	160	1.56	1.56	1.56	1.56
	Acetone	200	0.78	0.78	1.56	1.56
	Water	270	-	-	-	-
R. communis	Methanol	440	0.098	0.098	0.098	0.098
	Ethanol	340	1.56	1.56	0.098	0.098
	Acetone	120	0.098	0.098	0.098	0.19
	Water	1040	6.25	6.25	6.25	6.25
T. alliaceae	Methanol	320	1.56	1.56	1.56	1.56
	Ethanol	260	0.78	0.78	0.098	0.78
	Acetone	80	0.19	0.39	0.39	0.19
	Water	140	0.39	0.78	0.19	0.39
E. gorgonis	Methanol	550	0.19	0.098	0.098	0.098
	Ethanol	390	0.098	0.098	0.098	0.098
	Acetone	390	0.098	0.098	0.098	0.098
	Water	760	-	-	-	-
Ledebouria sp.	Methanol	300	12.5	-	-	6.25
	Ethanol	200	6.25	6.25	-	-
	Acetone	300	-	-	-	-
	Water	170	-	-	-	-
Control (µg/ml)	Neomycin		0.098	0.098	0.098	0.098

-: No activity

Table 4.4: Antifungal activity of plant extracts (MIC values in mg/ml)

Plant names	Extraction solvents	Extract yield (mg)	*C. albicans*
Ledebouria sp.	Methanol	430	1.56
	Ethanol	250	1.56
	Acetone	150	1.56
	Water	390	-
R. communis	Methanol	270	3.12
	Ethanol	150	0.78
	Acetone	200	0.39
	Water	420	6.25
R. obtusifolius	Methanol	100	0.78
	Ethanol	50	0.78
	Acetone	60	0.78
	Water	900	-
P. glomerata	Methanol	2900	3.12
	Ethanol	380	3.12
	Acetone	210	1.56
	Water	4260	-
E. gorgonis	Methanol	420	3.12
	Ethanol	320	6.25
	Acetone	290	6.25
	Water	610	25
T. alliacea	Methanol	2100	0.195
	Ethanol	290	0.098
	Acetone	100	0.098
	Water	5660	6.25
Control (µg/ml)	Amphotericin B		0.049

-: No activity

Table 4.5: Antimycobacterial activity of plant extracts (MIC values in mg/ml)

Plant names	**Extraction solvents**	**Extract yield (mg)**	***M. tuberculosis***
Ledebouria sp.	Methanol	70	3.12
	Ethanol	30	0.78
	Acetone	30	0.098
	Water	180	6.25
R. communis	Methanol	290	6.25
	Ethanol	80	0.78
	Acetone	90	0.39
	Water	180	3.12
R. obtusifolius	Methanol	150	0.78
	Ethanol	130	0.195
	Acetone	100	0.098
	Water	150	0.195
P. glomerata	Methanol	140	3.12
	Ethanol	60	1.56
	Acetone	30	1.56
	Water	460	0.78
E. gorgonis	Methanol	500	0.78
	Ethanol	110	3.12
	Acetone	80	1.56
	Water	1430	0.195
T. alliacea	Methanol	140	0.78
	Ethanol	60	1.56
	Acetone	30	0.39
	Water	460	-
Control (µg/ml)	Streptomycin		0.098

- = No Activity

4.4. Anthelminthic activity

In the absence of laboratory stabilates of *W. bancrofti,* the nematode causing lymphatic filariasis, the stage 3 larvae of *Haemonchus contortus* and *Strongylus equinus* were used as model nematodes in this study. The *H. contortus* were selected for this study due to their anatomical resemblance to *W. bancrofti.* The positive control, Levamisole, was found to have 100% larval mortality while the solvent control of 70% ethanol did not kill any nematodes. The 100% acetone solvent control displayed toxicity to the *H. contortus* leading to 50% acetone being used to re-dissolve the acetone-based extracts, which resulted in no larval mortality.

The *P. glomerata* displayed the best anthelmintic activity against *H. contortus* with both the ethanol and acetone extracts resulting in 50 to 100% nematode mortalities. Only the ethanol extracts of *T. alliacea* and *R. obtusifolius* displayed the best activity against *H. contortus* with larval mortality of 90 to 100 percent (Figure 4.7).

Anthelminthic activity of the plant extracts against *S. equinus* is shown in Figures 4.8 and 4.9 which display the mean percentages of larval mortality. The ethanol extracts of *T. alliacea* and *P. glomerata* displayed high anthelminthic activity against *S. equinus* with nematode mortality percentages ranging from 50 to 100 percent.

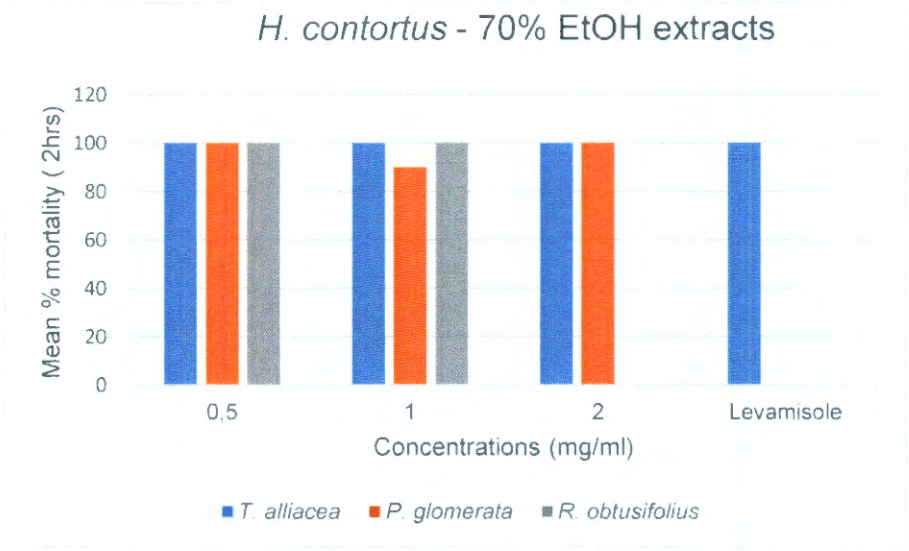

Figure 4.1: Larval mortality assay of ethanol plant extracts against *Haemonchus contortus* at 2 hours exposure. EtOH - Ethanol

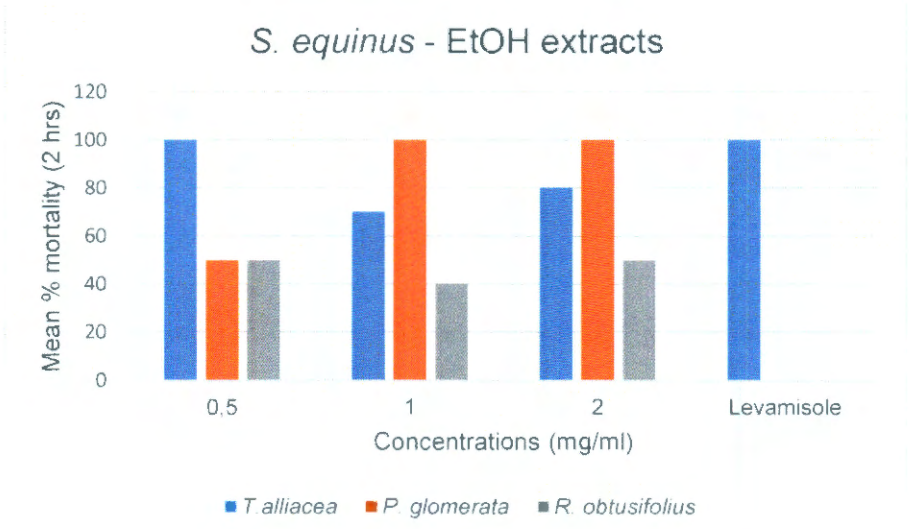

Figure 4.2: Larval mortality assay of ethanol plant extracts against *S. equinus* at 2 hours exposure (recorded in % Mean). EtOH - Ethanol

4.4. Cytotoxicity tests

The three plants that have not been extensively studied were selected for the cytotoxicity assays. These plants were *E. gorgonis*, *T. alliacea* and *P. glomerata*. Furthermore, *E. gorgonis* was included for cytotoxicity tests because traditional healers and herbalists claimed that the plant is generally poisonous and should not be ingested but should only be used topically. This test was to determine the lowest concentration at which the plant might be effective by ingestion.

Euphorbia gorgonis

Exposure of the HuTu cells to the aqueous extract of *E. gorgonis* displayed the best viability at 12 hours, with live cell percentages above 80% in all concentrations, with cell viability decreasing as exposure times increased. The acetone extract exhibited cytotoxic trends as the concentrations decreased, while the ethanol extract displayed decreased cell viability at the lowest concentration (Figure 4.1). The H4IIE cells dosed with acetone extract exhibited decreased viability as the concentration decreased while the aqueous extracts of *E. gorgonis* displayed high percentage (80 and above) of cell viability at 12 and 24 hour exposures (Figure 4.2).

Platycarpha glomerata

HuTu cells exposed to the acetone extract of *P. glomerata* displayed no toxicity to cells with cell viability of 80% and above at 24 hours exposure. The 12 and 48 hour exposures displayed increasing cell viability as the concentrations decreased. The aqueous extract of the plant exhibited high cell viability at 12 and 24 hours while at 48 hours it displayed cytotoxicity to the cells (Figure 4.3).

The exposures of H4IIE cells to the acetone and water extracts have shown that the plant is toxic at 48 hours for 2, 1 and 0.5 mg/ml, while it is not toxic for the acetone extract at 12 hours for all concentrations, and for 1 and 0.5 mg/ml at 24 hours. The ethanol extract displayed toxicity to the H4IIE cells (Figure 4.4).

Tulbaghia alliacea

All tested concentrations of *T. alliacea* acetone extracts exhibited non-toxic properties to HuTu cells at 12, 24 and 48 hours exposure although 1 mg/ml at 48 hours and 0.5 mg/ml at 12 hours displayed slight toxicity with cell viability falling a little under 80%. The aqueous extract of *T. alliacea* displayed no toxicity to HuTu cells at 12 and 24 hours exposure. At 48 hours, only the 1 mg/ml concentration of the aqueous extract was not toxic. Ethanol extracts were not toxic at 12 hours exposure for all concentrations and only 2 mg/ml was not toxic to the cells at 24 hours exposure (Figure 4.5).

The H4IIE cells displayed decreased cell viability when dosed with *T. alliacea* plant extracts. Treatment with acetone extract for 24 hours showed that 1 mg/ml and 0.5 mg/ml of the plant extract are not toxic. Furthermore, all tested concentrations were not toxic at 12 hours exposure.

Treatment of the H4IIE cells with acetone extract for 24 hours showed that at 12 hours the viability of cells is decreasing with concentration decrease while at 24 and 48 hours cell viability is increased with decrease in the concentration of the extract. Cell viability of more than 80% was also observed on the H4IIE cells dosed with aqueous extracts at 12 hours for all concentrations (Figure 4.6).

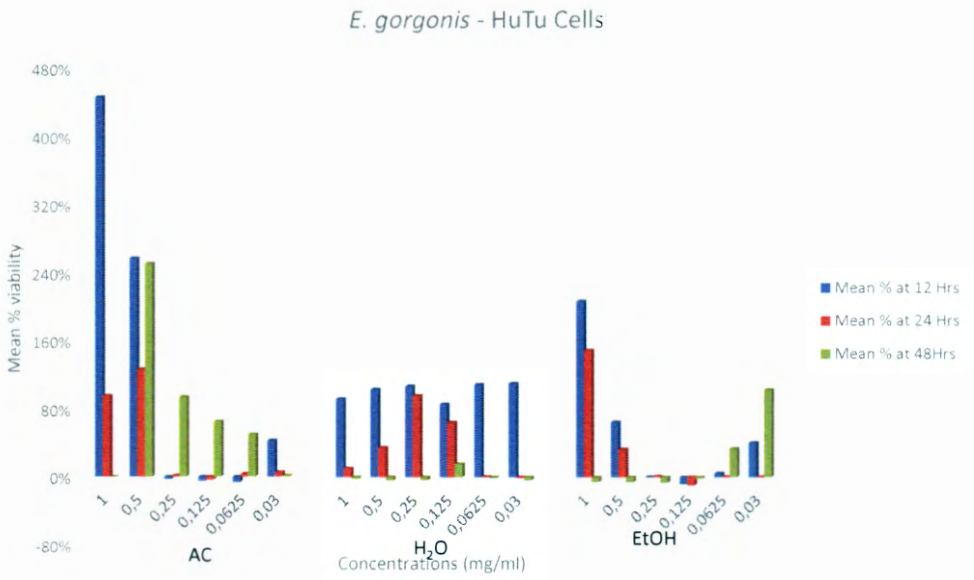

Figure 4.3: Cytotoxicity results of HuTu cells dosed with *E. gorgonis* acetone, water and ethanol extracts at 12, 24 and 48-hour intervals. AC = Acetone, H_2O = Water, EtOH = Ethanol.

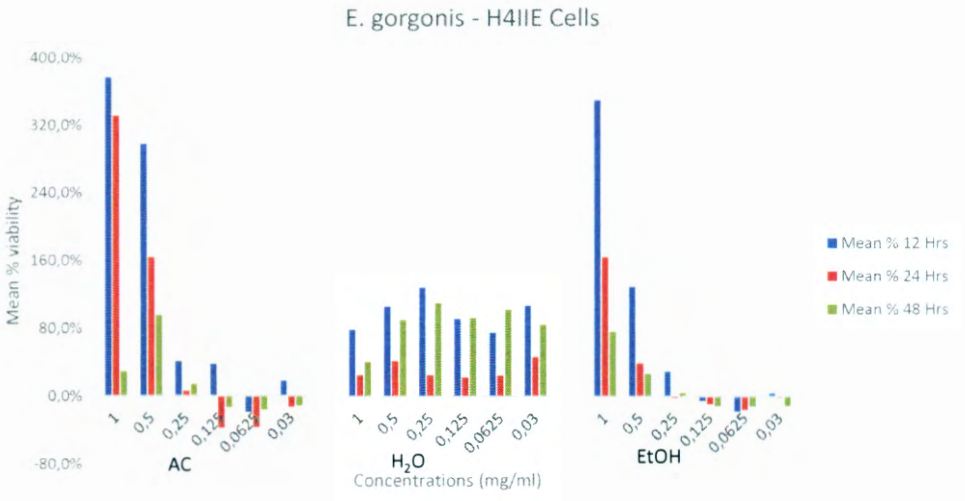

Figure 4.4: Cytotoxicity results of H4IIE cells dosed with *E. gorgonis* acetone, water and ethanol plant extracts at 12, 24 and 48-hour intervals. AC = Acetone, H_2O = Water, EtOH = Ethanol.

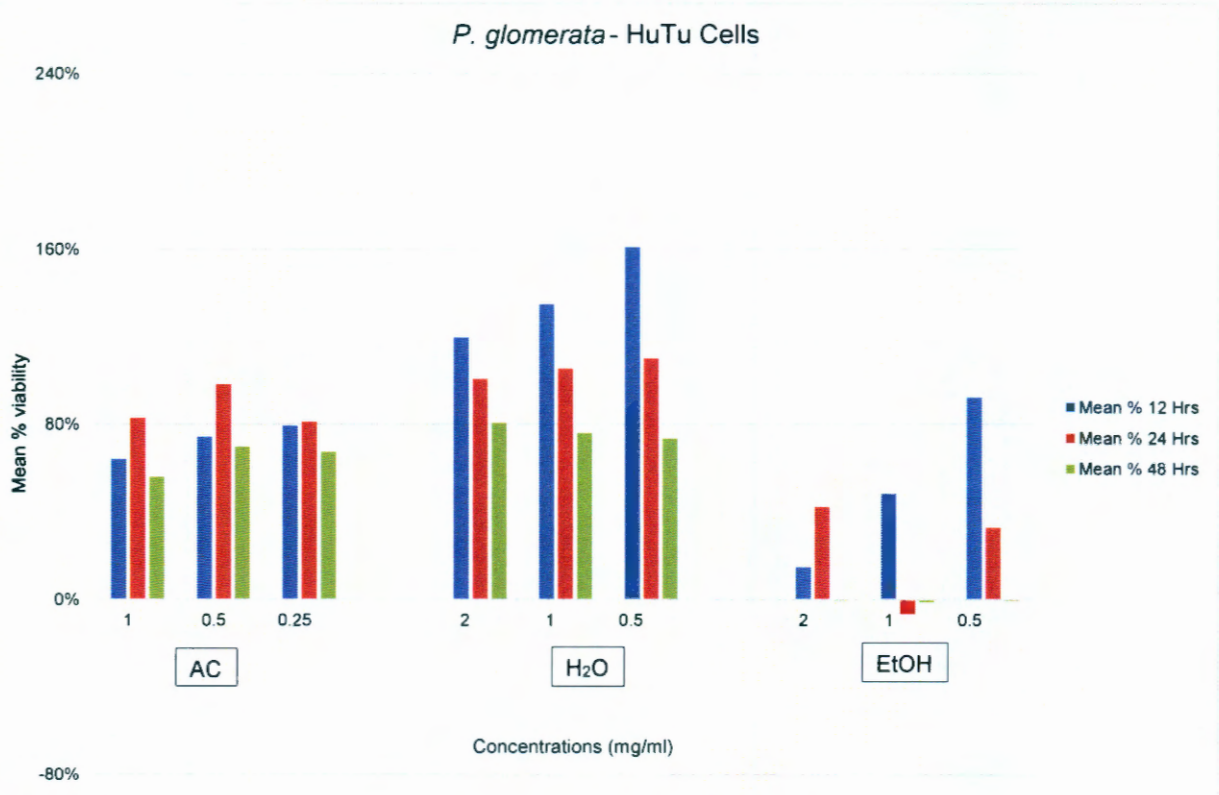

Figure 4.5: Cytotoxicity results of HuTu cells dosed with *P. glomerata* acetone, water and ethanol plant extracts at 12, 24and 4h hours. AC = Acetone, H_2O = Water, EtOH = Ethanol.

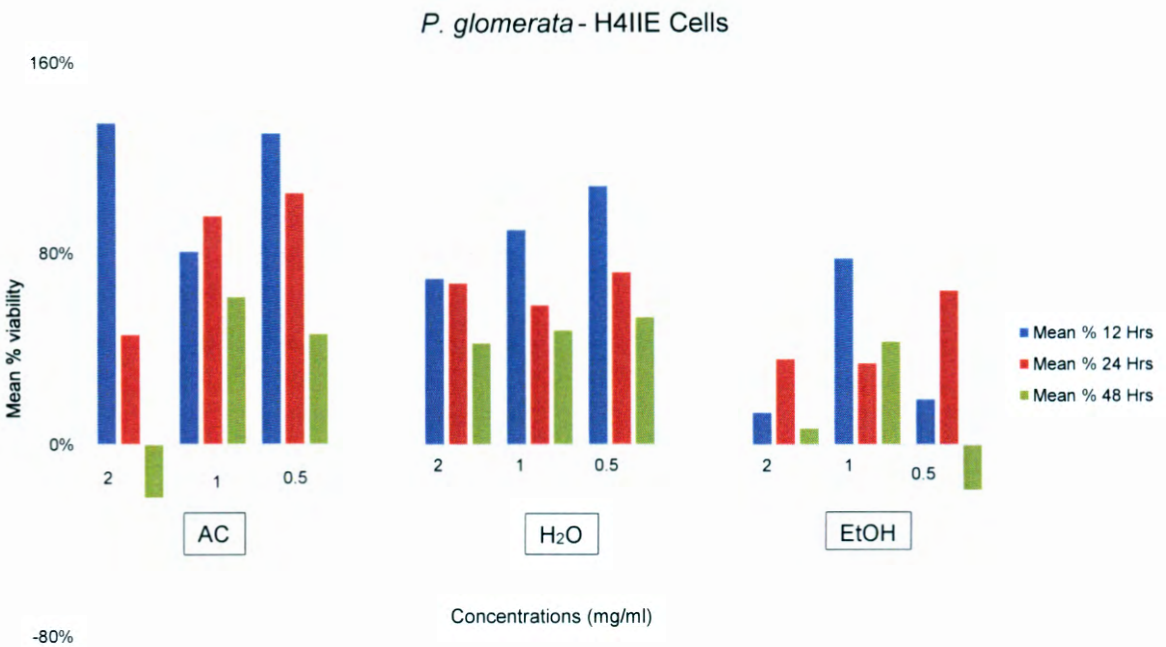

Figure 4.6: Cytotoxicity results of H4IIE cells dosed with *P. glomerata* acetone, water and ethanol plant extracts at 12, 24 and 48-hour intervals. AC = Acetone, H_2O = Water, EtOH = Ethanol.

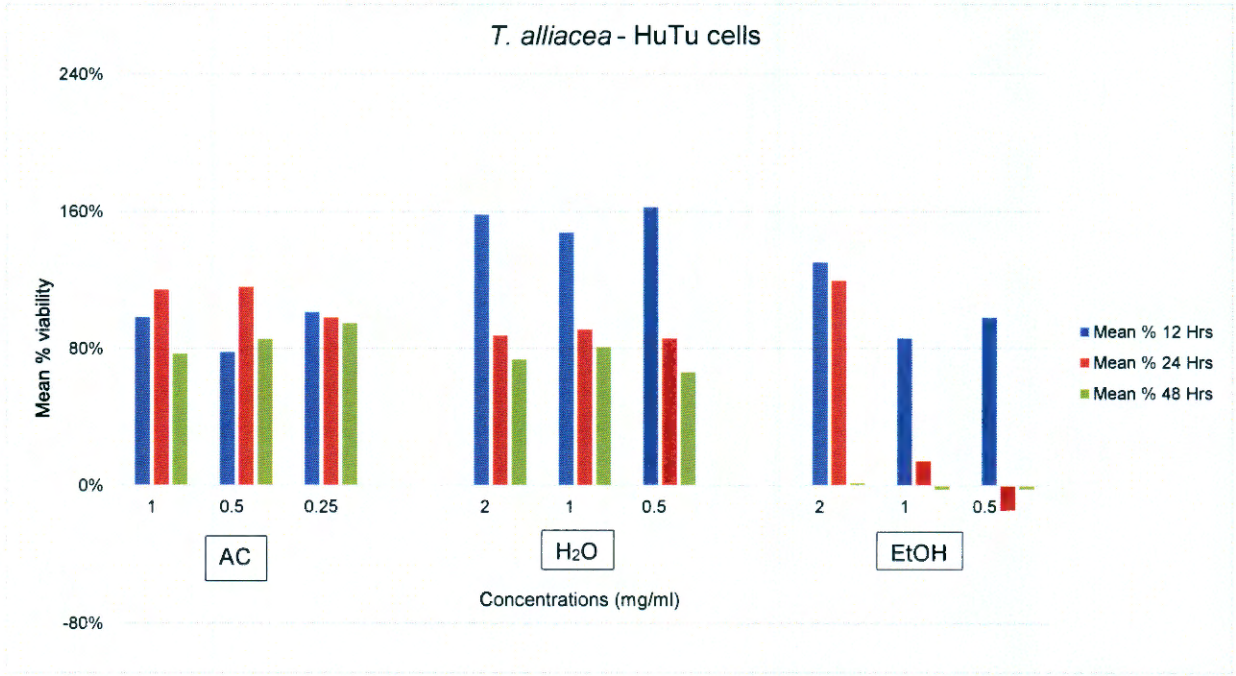

Figure 4.7: Cytotoxicity results of HuTu cells dosed with *T. alliacea* acetone, water and ethanol plant extracts at 12, 24 and 48-hour intervals. AC = Acetone, H_2O = Water, EtOH = Ethanol.

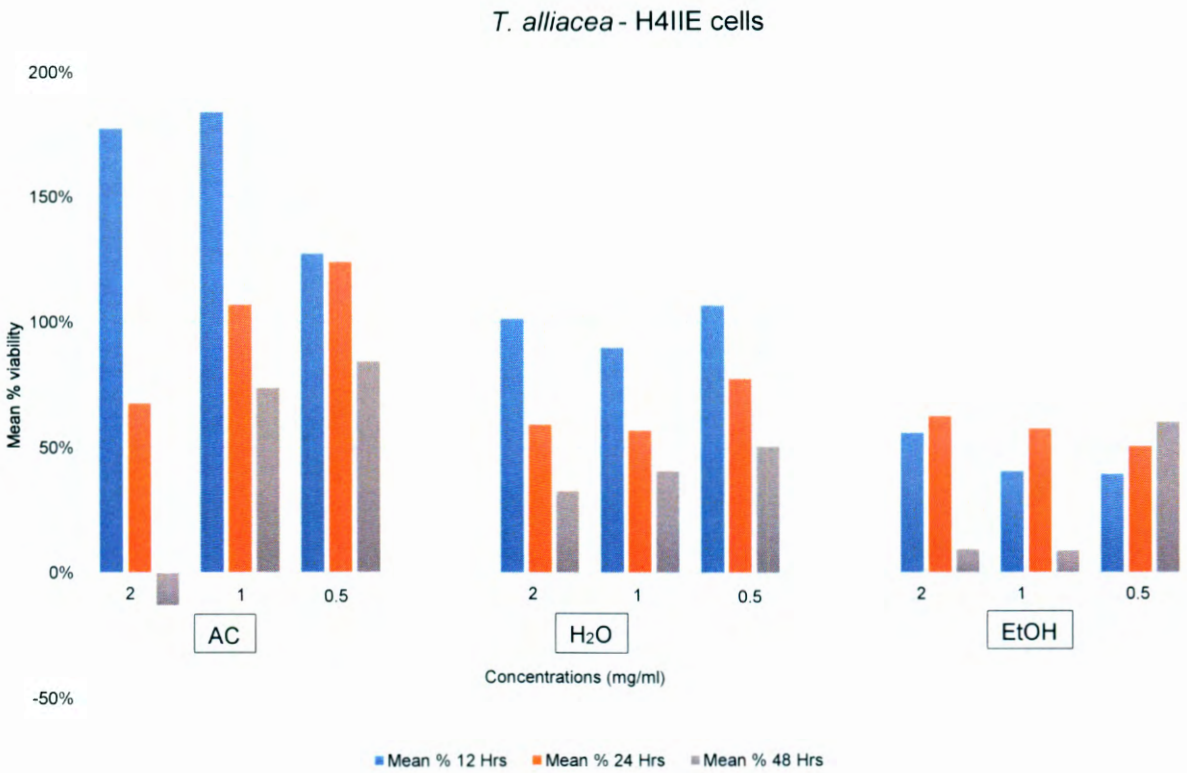

Figure 4.8: Cytotoxicity results of H4IIE cells dosed with *T. alliacea* acetone, water and ethanol plant extracts at 12, 24 and 48-hour intervals.AC = Acetone, H_2O = Water, EtOH = Ethanol.

CHAPTER 5

DISCUSSION

5.1. Ethnobotanical survey

The ethnobotanical survey documented six medicinal plants (*R. obtusifolius, R. communis, P. glomerata, Ledebouria* sp., *E. gorgonis* and *T. alliacea*) that are commonly used by traditional healers and herbalists from the Eastern Cape Province to treat and manage lymphatic filariasis. These plants are believed to heal many ailments when used separately or in combination with other plants as a concoction, infusion or paste. Infusion and decoction are reported to be the most commonly used methods of preparation with water often used as a solvent system (Ahmad *et al.*, 2014; Tshikalange *et al.*, 2016). According to Komoreng *et al.* (2017), in the Eastern Cape, a mixture containing equal portions of *R. obtusifolius*, *Eucomis comosa* and *Euphorbia clavarioides* is used in the treatment and management of the disease. The aerial parts of *R. obtusifolius* are often used to treat constipation, and an infusion of all plant parts are used for sores, antidote to nettle, blisters, burns, cancer and tumours (Harshaw *et al.*, 2010; Vasas *et al.*, 2015).

The whole plant, leaves, bulbs and roots or rhizomes were reported to be the most commonly used plant parts in this study. Underground plant parts were reported to be frequently used in traditional medicine, with herbalists believing that they possess the highest concentration of healing agents (Shale *et al.*, 1999; Appidi *et al.*, 2008; Komoreng *et al.*, 2017). In other studies, the leaves were shown to be the most frequently used plant part in treating skin-related ailments (Saikia *et al.*, 2006; Afolayan *et al.*, 2014). According to De Wet *et al.* (2013) and Afolayan *et al.* (2014), this is encouraging for sustainable use of medicinal plants. With the exception of one indigenous informant who cultivates the medicinal plants in the garden for personal use, there are no conservation methods in

place that are used to conserve these plant species as they are directly harvested from the natural habitats.

5.2. Phytochemical analysis

Plants are sources of many organic components that are used to heal many ailments through their medicinal uses. These bioactive components are known to act as antimicrobial, anti-inflammatory, antispasmodic and anticancer compounds (Tiwari *et al.*, 2011). The phytochemical screening of medicinal plants is a very important step in detecting the presence and quantity of bioactive components in the plants (Yadav *et al.*, 2014).

Tannins are known to have multiple biological activities against microbial pathogens. These include antifungal and antibacterial activities (Compean and Ynalves, 2014). Tannins were present in both organic and aqueous extracts of *R communis*, *R. obtusifolius*, *E. gorgonis*, *P. glomerata*, organic extracts of *Ledebouria* sp. and in the aqueous extract of *T. alliacea.* This explains their high antimicrobial activity.

The aqueous extracts of *R. communis*, *E. gorgonis*, *P. glomerata*, *Ledebouria* sp., and *T. alliacea* as well as the acetone extracts of *R. obtusifolius*, *P. glomerata* and *Ledebouria* sp. displayed the presence of saponins. While saponins are known to play a role in the haemolytic and anti-inflammatory studies, antibacterial, antiparasitic and antifungal activities have been observed in isolated saponins of many plants (Compean and Ynalves, 2014).

According to a study undertaken by Aremu and van Staden (2013), the genus *Tulbaghia* is characterised by the relative absence of alkaloids. In this study, the presence of alkaloids was detected in the aqueous extract of *T. alliacea*, which contradicts results by Aremu and van Staden (2013). This variation may be attributed to many different

environmental, genetic, ecological and physical factors that affect plants harvested from the wild habitats (Bopana and Saxena, 2007). Plant age, seasonal variation and geographical deviation in harvest site are contributing factors towards variation in concentration of biological components (Street et al., 2008). Furthermore, according to Rastogi *et al.* (2014), alkaloids are relatively insoluble in water as compared to other solvents.

The aqueous extract of *T. alliacea* was the only extract that possessed tannis, saponins, flavonoids, terpenoids, cardiac glycosides as well as alkaloids.

Flavonoids are important constituents of the human diet and are found in several plants used in traditional medicine around the world (Borelli and Izzo, 2000). The presence of phytochemicals in plants is indicative of their medicinal properties as these phytochemicals are the bioactive components that assist in the treatment of diseases. According to Li *et al.* (2015), underground plant parts are commonly known to possess higher amounts of bioactive compounds while leaves and fruits are less frequently used. In this study, the rhizomes of *T. alliacea* and *P. glomerata* displayed the presence of most of the tested phytochemicals.

5.3. Antimicrobial activity

Platycarpha glomerata

All extracts of *P. glomerata* displayed the best activity with MIC values ranging between 0.098 mg/ml and 0.78 mg/ml. Poor activity was detected with *P. glomerata* water extract against *K. pneumoniae* with an MIC value of 12.5 mg/ml. Members of the Asteraceae family possess antimicrobial properties (Chovanova *et al.*, 2013). The extracts from the Asteraceae family displayed good activity against some of the test microorganisms in studies conducted by Nino *et al.* (2006) and Chetan *et al.* (2012). In another study, members of the Asteraceae family showed small inhibition zones in a disc-diffusion assay (Chovanova *et al.*, 2013).

The organic solvents extracts of *P. glomerata* displayed poor activity against the fungal strain tested in this study except the acetone extract, which displayed a minimum activity at a concentration of 1.563 mg/ml. The aqueous extracts displayed poor or no activity. Water is not an effective solvent at extracting the active components from the plants (Buwa and Afolayan, 2009). Although traditional healers most commonly use water to extract bioactive compounds from plants, the organic solvents extracts have been found to give consistent antimicrobial activity compared to water extracts (Tiwari *et al.*, 2011).

Acetone is probably a preferable solvent as compared to water, ethanol and methanol due to its volatility, miscibility and relatively low toxicity to microorganisms (Eloff, 1998b).

P. glomerata aqueous extracts displayed the best activity (0.78 mg/ml) against *M. tuberculosis* compared to organic solvents extracts. Minimum activity was detected with *P. glomerata* ethanol and acetone extracts at a concentration of 1.563 mg/ml. *Platycarpha glomerata* possesses tannins and flavonoids which have been proven to have antimicrobial activities.

Tulbaghia alliacea

The acetone, ethanol and water extracts of *T. alliacea* displayed good antibacterial activity against all the test microorganisms with MIC values ranging between 0.098 mg/ml and 0.78 mg/ml. In a study undertaken by Buwa and Afolayan (2009), *T. violacea* ethanol and water extracts displayed poor activity; good antibacterial activity was only detected with dichloromethane extract. According to a study by *Takaidza et al.* (2015), there is no consistency in the findings of researchers when it comes to the antimicrobial activities of the genus *Tulbaghia*. The presence of alkaloids indicates the possible use of the plant in the control of bacterial and fungal infections (Asl *et al.*, 2008; Tenikotan et al., 2013).

The highest antifungal activity against *C. albicans* was displayed by the organic solvents extracts of *T. alliacea* with MIC values ranging between 0.098 mg/ml to 0.195 mg/ml. This is also supported by the findings of Lindsey and van Staden (2004) which indicate that the *Tulbaghia* family possess antifungal properties. A review of Aremu and van Staden (2013) reported that *T. alliacea* methanol and water extracts displayed high activity

against the fungal strains including *C. albicans*. The antifungal activity is suspected to be due to the presence of allicin (Motsei *et al.*, 2003; Aremu and van Staden, 2013). Allicin is an organic compound, found in the *T. alliacea* species, that possesses antibacterial, antifungal, antiviral, antiprotozoal and antioxidant effects. It has high reactivity and high membrane permeability that allows it to rapidly penetrate different cell membranes (Marchese *et al.*, 2016).

The methanol and acetone extracts of *T. alliacea* displayed the best activity against *M. tuberculosis* with MIC values of 0.78 and 0.39 mg/ml, respectively. These findings are in agreement with Thamburan *et al.* (2006) who stated that *T. alliacea* phytotherapy is antimycobacterial and modulates IFN-γ, which is vital in fighting TB infection. Furthermore, van Wyk *et al.* (1997) and Aremu and van Staden (2013) indicated that the bulbs of *T. alliacea* are used as a remedy for pulmonary tuberculosis as well as anthelminthic and aphrodisiac medicine. Buwa and Afolayan (2009) reported the activity of the genus *Tulbaghia* against *M. aurum* which is a sister strain to *M. tuberculosis*.

Rumex obtusifolius

In this study, *R. obtusifolius extracts* have been found to contain antimicrobial agents as they displayed high activity against *M. tuberculosis* with MIC values ranging between 0.098 and 0.78 mg/ml. The plant also displayed good fungal growth inhibition. In a study by Harshaw *et al.* (2010), *R. obtusifolius* displayed antibacterial activity against the strains of *E. coli*, *S. aureus* and *Salmonella typhii*. The best antibacterial activity of *R. obtusifolius* was displayed by the acetone extract against the strains of *K. pneumoniae* and *S. aureus*. According to Eloff (1998b), acetone is the best extractant of antimicrobial compounds.

Ricinus communis

The essential oils extracted from the leaves of *R. communis* showed strong antimicrobial activity against *Bacillus subtilis, Staphyllococcus aureus* and *Enterobacter cloacae*

(Jenna and Gupta, 2012; Zarai *et al.*, 2012). This plant is known to have alkaloids, flavones and glycosides, among other phytochemicals (Jena and Gupta, 2012). This plant has been found to have wound-healing properties, which are attributed to the presence of tannins, saponins and flavonoids (Jena and Gupta, 2012). In this study, *R. communis* contains tannins in all the extraction solvents while saponins were present in aqueous extract and flavonoids in acetone extract. Aqueous extract displayed minimal bacterial growth inhibition while the ethanol and acetone extracts displayed the best antifungal activity.

Euphorbia gorgonis

The plant *E. gorgonis* contains tannins, saponins, alkaloids and flavonoids. High antibacterial activity was observed in the organic solvents extracts. The aqueous extracts had no activity at all regardless of having tannins, saponins and flavonoids, which are believed to have antimicrobial activities.

All the extracts of this plant displayed minimal to poor antifungal activity. The antimycobacterial assays of *E. gorgonis* extracts displayed high inhibition of mycobacterial growth that corresponds with the phytochemical composition of the plant.

Ledebouria sp.

The ethanol extract of *Ledebouria* sp. displayed the presence of tannins, saponins, steroids and glycosides. Saponins were present in the ethanol and acetone extracts, while flavonoids were found in the methanol and acetone extracts. Regardless of this phytochemical composition, the extracts of this plant had no significant activity against all the bacterial strains tested in this study. Antifungal activity was consistent in the organic solvent extracts displaying fungal growth inhibition.

The best antimycobacterial activity was exhibited by the ethanol and acetone extracts of *Ledebouria* sp. *Ledebouria ovatifolia*, a relative of the tested *Ledebouria* sp. in this study,

displayed high antibacterial activity in a study reported by Buwa and van Staden (2006). The methanol and acetone extracts of *Ledebouria revolute* displayed good activity against *E. coli* (Muleya *et al.*, 2014).

5.4. Anthelminthic activity

The results for anthelminthic acitivity indicated that the 50% EtOH extracts of *T. alliacea* and *P. glomerata* were effective in the mortality of L_3 larvae of *Haemonchus contortus* with larval mortality ranging between 90 and 100 percent. The larval mortality was not concentration dependent. The activity of the ethanolic extracts against *Strongylus equinus* larvae concentration dependent results and the lowest concentration (0.5 mg/ml) displayed minimal inhibition. The 0.5 mg/ml concentration of *T. alliacea* displayed high larval mortality.

Plants with typical anthelminthic properties contain alkaloids, amino acids, saponins, flavonoids and other phenolic compounds (Maphosa *et al.*, 2010). Hossain *et al.* (2012) stated that phenolic compounds contain anthelmintic activity since phenols are able to disrupt energy generation in parasitic worms by uncoupling the oxidative phosphorylation, which is the process to generate energy in organisms. The acetone extract of *P. glomerata* was found to possess both saponins and flavonoids; furthermore, the ethanol extract had both tannins and flavonoids which are effective against helminths, hence, the high anthelminthic activity. The results for the ethanol extracts were not concentration based as the plants showed effectiveness across all tested concentrations.

Haemonchus contortus are the blood-sucking parasitic worms found mostly in the abomasum of sheep (Saccareau *et al.*, 2017). *Wuchereria bancrofti* and *H. contortus* follow similar life patterns. Ivermectin and albendazole are medicines used in the control of both these parasitic nematodes, with similar results. Based on this, it could be possible to obtain the similar results for the plant extracts used when testing them against the larvae of *W. bancrofti*.

5.5. Cytotoxicity

The exposure of the HuTu cells to the aqueous extracts of *E. gorgonis* displayed high cell viability at 12 and 48 hours while the acetone and ethanol extracts were not viable. The H4IIE cells were viable for all the concentrations administered at 12 hours. The cell viability in these extracts was exposure time dependent with 12 hours being the most viable period for all concentrations. The 48-hour exposures of the acetone extract on HuTu cells displayed that the viability was concentration dependent and cell viability decreased as the concentration decreased. The milky latex on the Euphorbia plant species is highly toxic and can irritate the skin and eyes (Basak *et al.*, 2009). Similarly, the traditional healers interviewed in this study mentioned that they consider *E. gorgonis* as toxic and they encourage their patients use it topically.

The acetone extracts of *P. glomerata* administered to the HuTu cell lines displayed cell viability that was concentration dependent at 24 hours with high cell viability for the concentration of 0.5 mg/ml. The aqueous extracts displayed exposure time dependent pattern and cell viability was observed at 24 and 48 hours. The H4IIE cell lines on the same dosages also displayed this reaction.

At 48 hours, all concentrations of *T. alliacea* extracts proved not to be cytotoxic. The *T. alliacea* acetone extract (0.5 mg/ml and 0.25 mg/ml) and water extract (1 mg/ml) did not have cytotoxic effects on the cells. The rhizomes of *T. alliacea* tested negative in a study conducted by Bamuamba *et al.* (2008). The plant is generally considered to be non-toxic as its doses are usually taken orally as infusions or concoctions (Tunzi, 2015; Gustav *et al.*, 2016).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The main objectives of this study have been achieved and the results from this study highlighted the role these plants play in the treatment of lymphatic filariasis, and related ailments, in South Africa. The ethnobotanical survey revealed that traditional healers and herbalists from the Eastern Cape Province use indigenous medicinal plants to treat elephantiasis and related diseases. This paved a way towards identifying the plants that are more scientifically reliable in this purpose through the study of their scientific components and the ethnopharmacological studies conducted on these plants. Of the six medicinal plants that have been validated in this study, *P. glomerata* and *T. alliacea* have been identified as plants that need extensive study for the purpose of developing novel drugs for the treatment of lymphatic filariasis and the related ailments. There is paucity of documented information on the medicinal values of these two plant species. This gap needs to be filled extensively by further studying the full potential of these medicinal plant species.

This study has served as initial step to scientifically validate the use of traditional medicinal plants for the treatment and management of secondary infections, which are a result of microbial infections. The plants used against lymphatic filariasis in the Eastern Cape Province have displayed significant antibacterial, antifungal and antimycobacterial activities which explains and justifies their use by the traditional healers and herbalists.

Qualitative analysis of phytochemicals was also conducted, and it displayed the presence of compounds that are essential in fighting against pathogens that are responsible for

secondary infections in people affected by lymphatic filariasis. Cytotoxicity tests were conducted in order to determine the safety of the plant species. This information will contribute in indigenous knowledge which can be used to help the communities that rely on traditional medicine to properly use and consume herbal preparations in order to avoid adverse reactions. The results revealed that some of the plants possessed cytotoxic properties that could be harmful. These plants should, therefore, be handled with care.

In vitro anthelminthic activity assay displayed the potential of *T. alliacea*, *P. glomerata* and *R. obtusifolius* to kill parasitic nematodes that are related to *W. bancrofti* that cause lymphatic filariasis.

6.2. Recommendations

- There is lack of statistical data about morbidity caused by lymphatic filariasis, and its prevalence, in South Africa. Geographical mapping of the disease in the country is, therefore, necessary to develop statistical data that will determine the populations at risk and the extent of infections.

- An integrated approach that would look at knowledge about the disease, its prevalence and causes is also needed. The causes of lymphatic filariasis in South Africa need to be extensively studied to establish whether it is filarial or non-filarial, which will result in better treatment prospects for the patients. A community-based approach will help bring together healthcare practitioners, traditional healers and patients in an attempt to fight the disease.

- Further studies on quantitative analysis of secondary metabolites/phytochemicals, isolation and characterisation of bioactive compounds are recommended for plants that displayed the best activities and less or no toxicity.

- *In vivo* antifilarial, anti-inflammatory and cytotoxicity studies are also recommended to further evaluate the effects of crude plant extracts from this study using animal models.

- There is also a need for indigenous knowledge forum where between scientists can give feedback of scientific research to traditional herablists about medicinal plants used for various ailments.

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