# Pharmacological evaluation of extracts from *Buxus macowanii, Polygala myrtifolia, Scilla* sp. and *Xanthocercis zambesiaca*

By

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Dissertation submitted in fulfilment of the requirements for the degree:

**MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY**

in the

**DEPARTMENT OF HEALTH SCIENCES**

at the

**CENTRAL UNIVERSITY OF TECHNOLOGY, FREE STATE**

**BLOEMFONTEIN**

**2016**

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## Declaration of independent work

I Brian Ngobeni, identity number [redacted] and student number [redacted], do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree Magister Technologiae: Biomedical Technology, is my own independent work. It complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free state. It has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

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**Brian Ngobeni** **Date**

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## Summary

The outbreak of drug resistant pathogens, the high cost of health care, limited accessibility of the conventional drugs and their side effects are problems that make the treatment of infectious diseases difficult all over the world. These challenges have led to the search for novel drugs and drug leads that can surpass the quality of the currently available antimicrobial agents. Medicinal plants are considered to be the best candidates for the discovery of new drugs because of their long history of use in the treatment of various ailments in communities. The current study was aimed at investigating the antimicrobial activity, cytotoxic activity and phytochemical composition of the methanol extracts from *Buxus macowanii, Polygala myrtifolia, Scilla* sp. and *Xanthocercis zambesiaca.*

*Staphylococcus aureus, Clostridium perfringens, Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli, Staphylococcus epidermidis,* and the fungal species *Candida albicans* and *Candida tropicalis* were used to evaluate the antimicrobial activity of the selected plant extracts using the broth Microdilution method. All the plants extracts tested showed no activity against all the bacterial and fungal species except *Buxus macowanii*. *Buxus macowanii* inhibited the growth of *Staphylococcus aureus, Clostridium perfringens, Pseudomonas aeruginosa, Staphylococcus epidermidis, Candida albicans* and *Candida tropicalis* at the MIC of 2.5 mg/ml while *Enterococcus faecalis* and *Escherichia coli* were inhibited at 1.2 mg/ml.

*Buxus macowanii* was selected for further studies because it presented the best antimicrobial properties. Antimicrobial compounds were located using TLC bioautography. Four clear zones possibly flavonoids and alkaloids were detected on the TLC chromatogram. These findings suggest that the antimicrobial activity of *Buxus macowanii* was not attributed to a single compound but to a synergy of compounds. The effect of *Buxus macowanii* on the bacterial cell morphology was also evaluated. Morphological changes such as damage to the cell wall, loss of intracellular contents, incomplete cell

division and shrinkage of the cells were observed using Scanning and Transmission Electron Microscopy. Bacterial cells were affected morphologically after treatment with the extracts of *B. macowanii*.

In order to evaluate the safety of the extracts used in the study, the Sulforhodamine cytotoxicity assay was carried out using the WI-38 cell line (Normal human fetal lung fibroblast). *P. myrtifolia* was inactive against the WI-38 cell line whereas *B. macowanii* and *X. zambesiaca* were found to be moderately hazardous. *Scilla* extracts were found to be hazardous. These results indicate that caution should be exercised when employing plants like *B. macowanii*, *X. zambesiaca* and *Scilla* sp. for treatment of ailments.

The phytochemical screening of *B. macowanii, P. myrtifolia, Scilla* and *X. zambesiaca* using standard methods, TLC and GCMS revealed compounds that have important health benefits. Bioactive compounds such as flavonoids, alkaloids, terpenes, cardiac glycosides, steroids, saponins and tannins were found in most of the extracts and their presence may explain the medicinal usage of the plants. GCMS also revealed compounds such as neophytadiene that was found in the extracts of *Buxus macowanii*, n-hexadecanoic was also found in the extracts of *scilla* sp and *X. zambesiaca*. 2-methoxy-4-vinylphenol was found in the extracts of *P. myrtifolia* and *X. zambesiaca*.

The results obtained in this study show that *B. macowanii* is a promising source of antimicrobial drugs. Further investigation into the isolation and identification of the bioactive compounds as well as *in vivo* screening is recommended.

## Acknowledgements

I would like to sincerely thank the following:

1. God of mount Zion for all his blessings, courage, support, wisdom and perseverance to progress in my project,
2. Prof S. Mashele, Dr I.T Manduna and Dr E van Der Watt for your guidance and support throughout the journey of this project and I have learnt a lot from you.
3. My family (The Mochike's and Ngobeni's) even though you were far away from me, your love and support were a driving force in my life to keep working hard.
4. To my dearest mom and dad you ensured that I get the best in life, I will always make you proud.
5. To my dearest friends Mr R.E Rammutla, Ms R. Mhaladi, Ms E. Ntimane and Ms G. Setlhare your presence in my life helped me to deal with stressful situations much better.
6. The Free State University, Department of Soil, Crop and Climate Sciences for supplying us with plant extracts and the lab equipment that was needed for this project to be a success.
7. Dr Ntsoaki Malebo from Central University of Technology, Department of Life Sciences who assisted with the Microscopy analysis of the samples.
8. The Biomedical Technology programme colleagues for their support and advices.

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## Abbreviations

| Abbreviation | Meaning |
|---|---|
| AChE | Acetylcholinesterase |
| AIDS | Acquired Immune Deficiency Syndrome |
| ATCC | American Type Culture Collection |
| CO2 | Carbon dioxide |
| CNS | Central nervous system |
| CSIR | Council for Scientific and Industrial Research |
| DMSO | Dimethyl sulfoxide |
| EACCC | European Collection of Cell Culture |
| EMEM | Eagle's Minimal Essential Medium |
| FBS | Fetal bovine serum |
| HIV | Human Immunodeficiency Virus |
| INH | Isoniazid |
| GCMS | Gas Chromatographic Mass spectrometry |
| Mg/ml | Milligram per millilitre |
| MIC | Minimum inhibitory concentration |
| NIST | National Institute of Standard Technology |
| PBS | Phosphate Buffer Solution |
| RIF | Rifampicin |
| SEM | Scanning Electron Microscopy |
| SRB | Sulforhodamine B assay |
| TB | Tuberculosis |
| TEM | Transmission Electron Microscopy |
| TIC | Total ion chromatograms |
| TLC | Thin Layer Chromatography |
| WHO | World health Organization |
| WI-38 | Normal human foetal lung fibroblast cell line |

# CHAPTER 1

# 1. INTRODUCTION

## 1.1. The burden of infectious diseases

Infectious diseases continue to kill millions of people every year. They are the second leading cause of death in the world and the leading cause of death in individuals under the age of 50 (Hamburg, 2008). The human immunodeficiency virus (HIV), tuberculosis (TB) and malaria are among the five major infectious diseases that contribute to the death rate of people world-wide (Feachem, 2004). Statistics released by the World Health Organisation (WHO) in 2014 indicated that approximately 36.9 million people live with HIV, while 2 million people were recorded as newly infected (WHO, 2014a). TB has a high rate of drug resistance. In 2013, approximately 9 million people developed the disease and 1.5 million people died from it (WHO, 2014b). Infectious diseases are clearly a worrisome factor in human health.

## 1.2. Challenges in the treatment of infectious diseases

The health care sector implemented different strategies to eradicate infectious diseases, including the development of antibiotics, vaccines, improved hygiene and sanitation, and vector control. This improved the health of many people to such an extent that many people predicted the end of infectious diseases (Hamburg, 2008). However, factors such as an increase in international trade and travel, the movement of people to urban areas, reduced hygiene and sanitation in developing countries, among other factors, interfered with these management strategies, and resulted in the spread of infectious diseases. In recent times, a number of irresponsible practices, such unsafe sex and the careless use of syringes by drug addicts, also contribute to the widespread occurrence of infectious diseases (Hamburg, 2008). These problems are augmented by the development of resistant pathogens as well as difficulties with the accessibility and affordability of treatment. Moreover, drugs that are currently available pose problems such as side effects; toxicity and high production costs (Kisangau et al., 2007;

Adwan et al., 2011; Rawat, 2012). The indiscriminate use of modern drugs or antimicrobial agents usually leads to the emergence of resistant pathogens that may cause nosocomial or hospital-acquired infections (Ibrahim et al., 2011; Daboor and Haroon, 2012).

### 1.3. Drug-resistant pathogens

Antibiotics were introduced into the health practice some years ago to combat infections or diseases (Hawkey, 2008). Antimicrobial agents such as penicillin, methicillin, streptomycin, vancomycin, chloroquine and many more have been a source of medication for years. However, during the past decades, we have witnessed a major increase in micro-organisms that developed multidrug resistance to antimicrobial agents (Oliphant and Eroschenko, 2015; Roca et al., 2015). Multidrug-resistant pathogens can develop due to mutations that can take place even in the presence of antimicrobial agents. Another driving force that leads to resistance is found in the abuse or misuse of antimicrobials on patients and livestock, or the release thereof into the environment (Oliphant and Eroschenko, 2015). Resistance to penicillin and sulphonamides in particular was reported in the 1940s, followed by resistance to other antimicrobials (Jindal et al., 2015). The pathogens that currently indicate antimicrobial resistance include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, Plasmodium falciparum, Enterobacter* species and *Mycobacterium tuberculosis* (Boucher et al., 2009; Rice, 2008). In 2011b, WHO estimated that approximately 630 000 people were diagnosed with multidrug-resistant TB, and this affected 84 countries (WHO, 2011b). Multidrug-resistant TB strains are responsible for 3.6% of all TB cases, and require two years of treatment using potentially toxic drugs such as floroquinolones and injectable aminoglycosides to replace the resisted Isoniazid (INH) and Rifampicin (RIF) (Winston and Mitruka, 2012). Extensively drug-resistant strains are usually difficult or impossible to cure, and only a few countries are able to detect and isolate those strains (Goldberg et al., 2012).Two hundred and forty million infections and 860 000 deaths caused by malaria are recorded annually (WHO, 2010). Among

all the species that cause malaria, *Plasmodium falciparum* is the most severe species, and accounts for approximately 90% of all malarial infections in Africa (Lee et al., 2013). Malarial pathogens usually develop resistance to antimalarial drugs such as chloroquine and mefloquine, therefore increasing the number of deaths that occur as a result of malaria (Lee et al., 2013; Olliaro and Bloland, 2001). Community and hospital-acquired infections that are caused by methicillin-resistant *Staphylococcus aureus* are on the rise, and occur at an alarming rate, creating a major challenge for clinicians (Motamedi et al., 2010; Jindal et al., 2015). *Shigella* is another species that resists ciprofloxacin, which is the only recommended drug for *Shigella*-related infections (Jindal et al., 2015). Vancomycin-resistant *Enterococci, Pseudomonas, Acinetobacter*, antifungal-resistant fungi and antiviral-resistant viruses significantly increases the number of deaths that occur world-wide. They also contribute to the delay in the healing of patients who suffer infections (Jindal et al., 2015). It is for all these reasons that sources of drugs and drug leads should be found to overcome this major challenge posed by drug resistance.

The problems associated with the antimicrobial drugs that are currently available have prompted the search for new antimicrobial remedies that are effective and present fewer problems (Obeidat et al., 2012). Researchers have turned to natural sources such plants for solutions, and traditional medicinal plants are considered the best option (Adwan et al., 2011).

### 1.4. Plants and traditional medicine

For thousands of years, people have relied on plants to meet their essential needs, such as the need for shelter, food, transport, clothing and medicine (Gurib-Fakim, 2006). Plants have been part of traditional medical systems for thousands of years, and they continue to supply people with remedies to treat various ailments (Normann and Snyman, 1996). Africa has diverse vegetation types, such as the tropical rain forests, coastal and alpines forests, savannas, woodlands and scrublands. In addition, Africa has diverse climatic and geographic factors that contribute to the survival of approximately 68

000 plant species (Cunningham, 1997). Tropical and subtropical Africa contains approximately 45 000 plant species, of which 5 000 are medicinally used and contribute to 25% of the world trade in biodiversity (Iwu, 2014). Due to their availability and natural advantage, African people often use medicinal plants for their health care benefits, such as the treatment of colds, memory enhancement, immunity improvement, etc. (Dzoyen et al., 2013).

It is estimated that approximately 70 – 95% of the world population in developing countries continue to rely on medicinal plants for the treatment of various diseases (Lewu and Afolayan, 2009; WHO, 2011a; Daughari, 2012). In South Africa, there is a rich tradition in the use of medicinal plants, which is based on approximately 3 000 species (Taylor et al., 2001), and approximately 60 – 80% of the country's population depend on medicinal plants to meet their health care and psychological needs (Fuku et al., 2013), this translates to approximately 27 million people (Mander, 1998; Street et al., 2008). People depend on medicinal plants to treat diseases, as it sometimes is the only system available in rural areas (Mabona and Van Vuuren, 2013). Challenges such as the remote location of health care centres and an inability to afford conventional/modern drugs make traditional medicine the first choice in rural areas. The use of traditional medicine is also attributed to the high accessibility of medicinal plants and traditional healers, the low toxicity thereof, the fact that it has less side effects, extensive local knowledge and the affordability thereof (Cheikhyoussef et al., 2011). However, there are indications that indigenous knowledge about medicinal plants is rapidly decreasing as modern education is increasing (Zerabruk and Yirga, 2012). Thus, there is an urgent need for the documentation of the use of medicinal plants in South Africa before this knowledge is lost forever (Masevhe et al., 2015).

### 1.5. The therapeutic potential of secondary metabolites

Medicinal plants are known to contain secondary metabolites that present unlimited opportunities for identifying new drug leads as a result of their supreme chemical diversity (Cos et al., 2006; O'Bryan et

al., 2008). Secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, etc. are usually found in plants where they fulfil a selective and survival function for the plants (Esterhuizen et al., 2006; Manimozhi et al., 2012). Secondary metabolites serve an important purpose for plants, as they protect plants from herbivores and microbial invasions, act as attractants for pollinators and seed-dispensing animals, and protecting plants from ultraviolet (UV) light (Gomez-Caracava et al., 2014). Secondary metabolites are also responsible for the taste, odour and color of plants, and most of them have important applications in pharmacology, chemistry, agricultural and novel drug sectors (Cannes do Nascimento and Fett-Neto, 2010). Various secondary metabolites have the ability to indicate biological properties such as antimicrobial, antioxidant, anti-inflammatory, antifungal, antiviral and anthelmintic activities (Daboor and Haroon, 2012). The effectiveness of medicinal plants as therapeutic agents is established through the presence of these important bioactive constituents (Bedir et al., 2003; Bhat and Karim, 2010). The most important bioactive compounds in plants are alkaloids, flavonoids, tannins, phenols, glycosides and tannins (Kubmarawa et al., 2007).

### 1.5.1. Polyphenolics

Phenols are compounds that occur naturally as colour pigments that are responsible for the colour of fruits and vegetables. They are synthesised from the phenylalanine through the phenylalanine ammonia lyase (PAL) (Doughari, 2012). They fulfil important functions in plants, such as defence mechanisms against pathogens and herbivores, and thus can be considered as potential candidates for the management of pathogenic infections (Doughari, 2012). Polyphenolics with benefits for human health are phenolic acids, flavonoid polyphenolics (flavonones, flavones, xanthones and catechins), lignans and stilbenes (Hooper and Cassidy, 2006). The recent interest in polyphenolics is attributed to their beneficial effects on human health, such as their antioxidant properties. As a result, they are used to combat cancer and heart disease, and can sometimes be used as anti-inflammatory agents (Manach et al., 2004; Fraga et al., 2010; Barbehenn and Constabel, 2011). They were also reported to have

antimicrobial, antiviral, cytotoxic, and vasodilatory effects (Kuete et al., 2012; Kougan et al., 2013; Ngameni et al., 2013).

### 1.5.2. Flavonoids

Flavonoids are an important group of polyphenols that are present in many types of plants. They are structurally composed of more than one benzene ring (Kar, 2007) and over 4 000 flavonoids are known. They are the largest group of phenols, forming half of approximately 8 000 naturally occurring phenols (Harborne and Baxter, 1999). Flavonoids fulfil important functions in plants, such as flower and seed pigmentation; plant fertility and reproduction; and defence from UV light, predators and pathogens (Harborne and William, 2000). For many years, medicinal plants that contain flavonoids have been used to treat human diseases, and isolated flavonoids have also been found to perform important biological activities, such as antifungal and antibacterial activities (Havsteen, 2002; Cushnie and Lamb, 2005).

### 1.5.3. Alkaloids

Alkaloids are usually found in higher plants, lower plants, insects, marine and micro-organisms (Kuete and Efferth, 2010; Wansi et al, 2013). Alkaloids are used as medication, as recreational drugs, or in entheogenic rituals. However, many of them are toxic, and can be used as toxins for weapons (Kuete et al., 2008). They are also used as anaesthetics, stimulants, psychedelics, analgesics, antibacterial agents, anticancer drugs, antihypertensive agents, spasmolysis agents, vasodilators, antiarrhythmia drugs, antiasthma therapeutics and antimalarials (Kuete and Efferth, 2010; Wansi et al., 2013; Zofou et al., 2013).

### 1.5.4. Tannins

Tannins are the most abundant secondary metabolites in plants. They are known to have biological properties such as antifungal, antioxidant, anthelmintic, antidiarrhoeal and healing properties (Zuanazzi and Montanha, 2004). They are also known to defend plant leaves from insect herbivores by toxicity and deterrence (DeGabriel et al., 2009; Barbehenn and Constabel, 2011). Some types of tannins act on the arachidonic acid metabolism in leucocytes, which leads to the reversal of inflammation (Okuda, 2005).

### 1.5.5. Saponins

Saponins are known for their soap-like properties when combined with water. They were named after *Quillaja saponaria*, a plant once used as soap (Doughari, 2012). Basically, saponins are produced by plants, marine animals and some bacteria. They are glycosides, synthesised from the mevalonic acid pathway via the isoprenoid pathway (Gomez-Caravaca et al., 2014). The first group of saponins consists of steroidal saponins common in monocotyledonous angiosperms, while the second group consists of triterpenoid saponins that are found in dicotyledonous angiosperms (Doughari, 2012). Saponins protect plants against attack by pathogens and herbivores (Augustin et al., 2011). They are therefore known to have antimolluscicidal, antidiabetic, antifungal, antiyeast, antibacterial, antimicrobial, antiparasitic, antitumoral and anti-inflammatory activity (Sparg et al., 2004; Elekofehinti, 2015).

### 1.5.6. Terpenoids and steroids

Terpenes are diverse volatile oily compounds that are derived from isoprene units. They are usually found in essential oils and resins, and include monoterpenes, diterpenes, triterpenes and sesquiterpenoids (Firn, 2010). Examples of monoterpenes include thujone, camphor, eugenol, menthol and terpinen-4-ol. Diterpenes consist of taxols and resins and they are used as anticancer agents (Sandjo and Kuete, 2013). Triterpenes include steroids, sterols and cardiac glycosides. They possess

properties such as anti-inflammatory, sedative and cytotoxicity activity (Chimene et al., 2013; Sandjo and Kuete, 2013). Sesquiterpenes such as betulinic acid, lupeol, oleanic acid and ursolic acid are known to have antimicrobial, antiplasmodial and neurotoxin properties. They act as irritants when applied on human skin and when consumed (Awoufack et al., 2013). Most of the terpenes have activities against cancer, malaria, inflammation and infectious diseases caused by viruses and bacteria. Terpene-based drugs, such as the anticancer drug Taxol and the antimalarial drug Artemisinin, were the most popular drugs; generating approximately US $ 12 billion in 2002 (Wang et al., 2005). Steroids are naturally occurring compounds that are produced biologically from terpenoid precursors. They have therapeutic action on cardiac muscles when injected into the human body (Firn, 2010). Hunters also use them as arrow poisons, indicating that cardiac glycosides should be used with caution, as excessive doses may cause death (Doughari, 2012).

### 1.6. Medicinal plants as a source of novel drugs

The high rate of traditional medicine usage and the variety of indigenous plant species indicate much potential for the discovery of new bioactive compounds that can be used in the discovery of new drugs (Copp and Pearce, 2007). The interest in medicinal plants as potential new drugs is on the rise, and medicinal plants contribute 50% of all the drugs in the clinical world (Gurib-Fakim, 2006). The isolation of bioactive compounds from plants contributed to early discoveries of drugs such as aspirin, morphine, codeine, digoxin, atropine, quinine and artemisinin (Mueller et al., 2000; Samuelsson, 2004). Medicinal plants are considered the best option to overcome the challenges that are associated with the drugs that are currently available (Kaur et al., 2005). Table 1.1 illustrates some of the important drugs that were manufactured from plants.

**Table 1.1:** Plant-derived drugs

| Plant species | Drug | Use | References |
|---|---|---|---|
| *Discorea spp* | Diosgenin | Contraceptive | Sarkar and Nahar, 2007 |
| *Rauwolfia sp* | Reserpine | Antihypertensive | Gurib-Fakim, 2006 |
| *Pilocarpus spp* | Pilocarpin | Treats glaucoma and dry mouth | Gurib-Fakim, 2006 |
| *Galanthus woronowii* | Galantamine | Treats alzheimer's disease | Pirttila et al., 2004 |
| *Artemisia annua* | Arteether | Antimalarial | Graul, 2001 |
| *Callistemon citrinus* | Nitisinone | Treats tyrosinaemia | Frantz, 2004 |

### 1.7. Safety of medicinal plants

Medicinal plants are usually preferred for medicinal purposes, as they are presumed to have fewer side effects and are regarded as safer to use than conventional drugs. However, some species are known to be toxic to humans (Ndhlala et al., 2013). The toxicity of plants is usually attributed to the presence of compounds that can contribute to the survival of plants. Toxic plants can cause irritation or discomfort through skin contact, and serious poisoning when indigested (Van Wyk et al., 2002). Serious poisoning by plants may cause damage to major organs such as the brain, kidneys, central nervous system, lungs and the liver (Ndhlala et al., 2013). Humans have managed to use the toxicity of plants to their benefit for purposes of hunting, war, rituals, murder, suicide and abortion (Doughari, 2012; Ndhlala et al., 2013). The fast-acting cardiac glycosides such as *Acokanthera, Boophone, Strophanthus* and *Adenium* were used by San hunters in Southern Africa to create poisoned arrows (Wink and Van Wyk, 2008). Some poisonous phytochemicals such as saponins (Kar, 2007), alkaloids, phorbol esters, lectins and cyanogenic glycoside possess medicinal properties at lower concentrations (Ndhlala et al., 2013).

Despite the toxicity that cardiac glycosides possess, western doctors still prescribe digoxin from genus *Digitalis* for patients with congestive heart failure. This practice indicates that toxic plants may be used to treat ailments; however, they should be used with caution by regulating the dosage to be administered to a patient to avoid lethal side effects (Botha and Penrith, 2008).

### 1.8. The plants used in this study

Plants such as *Buxus macowanii (B. macowanii), Polygala myrtifolia (P. myrtifolia), Scilla sp. and Xanthocercis zambesiaca (X. zambesiaca)* are traditionally used to treat different ailments. Methanolic extracts from these plants were supplied by the Alternative Crop Development Programme of the University of the Free State (UFS), Bloemfontein, for use in this study.

#### 1.8.1. *Buxus macowanii Oliv.*

*B. macowanii* (Figure 1.1) is commonly known as a "Cape Box". In Xhosa it is called "Umgalagala" or "Igalagala" (Pooley, 1993). It belongs to the family *Buxaceae,* which contains four genera and approximately 100 species (Glen, 1996). It is a small-growing, evergreen plant that is found in the Eastern Cape, Mpumalanga and Limpopo provinces of South Africa. This plant is commonly used by traditional healers to treat wounds, pain, gout, malaria, rheumatism and skin disorders (Wink and Van Wyk, 2008). Plants from the genus *Buxus* are rich in steroidal alkaloids (Ata and Andresh, 2008). Lam et al. (2015) reported the isolation of five new steroidal alkaloids (31-hydroxybuxatrienone, macowanioxazine, 16a-hydroxymacowanitriene, macowanitriene and macowamine) along with another five known steroidal bases ($N_b$-demethylpapillotrienine, moenjodaramine, irehine, buxbodine B and buxmicrophylline C). These aforementioned steroidal alkaloids were reported as having moderate to weak anti–Acetylcholinesterase activity (Rosenbery, 1975; Ata, 2012).

**Figure 1.1:** *Buxus macowanii Oliv.*

### 1.8.2. *Polygala myrtifolia L.*

The *Polygala* genus belongs to the family Polygalaceae, which contains approximately 600 species (Fenner et al., 2005). *P. myrtifolia* (Figure 1.2), commonly known as "Myrtle milkwort" is a leafy perennial shrub usually found in South Africa's coastal and elevated environments between Cape Town and KwaZulu-Natal. Chemical investigation of the genus *Polygala* indicated the presence of secondary metabolites such as xanthones (Cristiano et al., 2003), saponins (Jia et al., 2004; Mitaine-Offer et al, 2003), oligosaccharides (Ikeya et al., 2004), flavonoids (Rao and Raman, 2004; Pizzolatti et al., 2008), coumarins, and styryl pyrones (Pizzolatti et al., 2004). Different biological activities were found on various species of *Polygala* genus, such as antibacterial, anti-inflammatory, trypanocidal and antinociceptive, antifungal and antimycobacterial activity (Lall and Meyer, 1999; Motsei et al., 2003; Pizzolatti et al., 2003; Kou et al., 2006; Pizzolatti et al., 2008; Ribas et al., 2008).

**Figure 1.2:** *Polygala Myrtifolia L.*

### 1.8.3. *Xanthocercis zambesiaca (Baker)*

*Fabaceae* is the third largest family of Angiosperms, consisting of more than 700 genera and approximately 2 0000 species of trees, vines and shrubs world-wide (Stevens, 2001). The family contains approximately 490 medicinal species that are used in traditional medicine (Gao et al., 2010). The boiled stem and roots of *X. zambesiaca* (Figure 1.3), also known as “Umhlwati”, are used by traditional healers to treat stomach complaints and “Nyoko”, an impairment of the gall bladder (Shai et al., 2011). The acetone extracts of *X. zambesiaca* were reported to have antimycobacterial activity (Mmushi et al., 2010). Ntsoelenyane et al. (2014) also reported antibacterial activity of the methanol extracts of the plant.

**Figure 1.3:** *Xanthocercis zambesiaca (Baker)*

### 1.8.4. *Scilla Species*

The genus *Scilla sp.* belongs to the *Hyacinthaceae* family and *Scilloideae* subfamily. Species in *Hyacinthaceae* are known to treat diseases such as urinary diseases, gastrointestinal problems, respiratory problems, headaches, swelling and skin problems, and they are used as internal purifiers (Hutchings., 1996; Louw et al., 2002). Members of this family are known to have traces of phytochemicals such as saponins and homoisoflavanones. However, the presence of alkaloids and steroids in some species may indicate cytotoxicity (Speta, 1998).

## 1.9. Aims and objectives

The aim of the study was to evaluate the antimicrobial activity of *Buxus macowanii, Scilla sp.*, *Polygala myrtifolia* and *Xanthocercis zambesiaca.* This was achieved by meeting the following objectives:

- Determining the antimicrobial activity of methanol extracts from *Buxus macowanii, Polygala myrtifolia, Scilla sp and Xanthocercis zambesiaca.*

- Locating the antimicrobial compounds using Thin Layer Chromatography (TLC).
- Examining the effect of the plant extracts on the microbial cells using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).
- Determining the phytochemical content of *Buxus macowanii, Polygala myrtifolia*, *Scilla sp. and Xanthocercis zambesiaca; and*
- Evaluating the cytotoxicity of the methanol extracts of *Buxus macowanii, Polygala myrtifolia, Scilla sp.* and *Xanthocercis zambesiaca.*

### 1.10. Overview of the study

This study is presented in four sections. In **Chapter 2,** the antimicrobial activity of the plant extracts was evaluated using the microdilution method, TLC bioautography and microscopy. The cytotoxicity of the extracts was tested using the Sulforhodamine B cytotoxicity assay (SRB), the results of which are discussed in **Chapter 3**. **Chapter 4** focused on the determination of the phytochemical contents of the plant extracts using the standard phytochemical screening methods, TLC and Gas Chromatographic Mass Spectrometry (GCMS). **Chapter 5** provides general discussions of the results obtained in this study, conclusions and recommendations. The techniques and procedures used to evaluate the antimicrobial activity of *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* are illustrated in Figure 1.4.

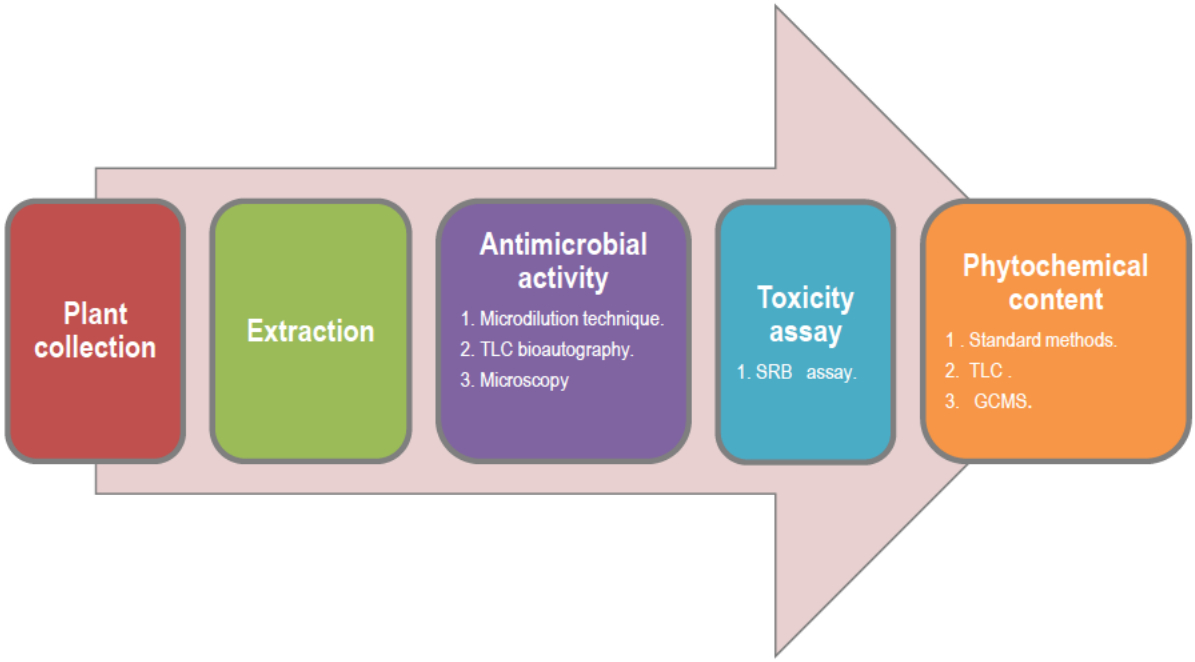



**Figure 1.4:** Flow diagram of the techniques and procedures used to evaluate the antimicrobial activity of *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca.*

## 1.11. References


1. Adwan, G., Salameh, Y., and Adwan, K., 2011. Effect of ethanolic extract of *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans*. Asian Pacific Journal of Tropical Biomedicine: 456-460.
2. Ata, A., and Andersh, B. J., 2008. *Buxus* steroidal alkaloids: chemistry and biology. The Alkaloids: Chemistry and Biology. 66: 191-213.
3. Ata, A., 2012. Novel biomedical agents from plant. In: Orhan IE, editor. Biotechnological production of plant secondary metabolites. Dubai: Bentham Science Publishers: 53–66.
4. Awouafack, M.D., Tane, P., Kuete, V., Eloff, J.N., 2013. 2—Sesquiterpenes from the medicinal plants of Africa. Medicinal plant research in Africa: 33103.
5. Augustin, J. M., Kuzina, V., Anderson, S. B., Bak, S., 2011. Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry. 72: 435–457.
6. Barebehenn, R.V., and Constabel, C.P., 2011. Tannins in plant herbivores interactions. Phytochemistry. 72: 1551-1565.
7. Bedir, E., Abou-Gazar, H., Ngwendson, J.N. and Khan, I.A., 2003. Eurycomaoside: a new quassinoid-type glycoside from the roots of *Eurycoma longifolia*. Chemical and pharmaceutical bulletin. 51(11): 1301-1303.
8. Bhat, R. and Karim, A.A., 2010. *Tongkat Ali* (*Eurycoma longifolia Jack):* a review on its ethnobotany and pharmacological importance. Fitoterapia. 81(7): 669-679.
9. Botha, C.J., Penrith, M.-L., 2008. Poisonous plants of veterinary and human importance in Southern Africa. Journal of Ethnopharmacology. 119: 549–558
10. Boucher, H., Talbot, G.H., Bradley, J.S. 2009. Bad bugs, no drugs: no ESKAPE! An Update from the Infectious Diseases Society of America. Clinical Infectious Diseases. 425: 1–12.
11. Cannes do Nascimento, N., Fett-Neto, A.G., 2010. Plant secondary metabolism and challenges in modifying its operation: An overview. Humana Press Inc., New York: 1–13.

12. Cheikhyoussef, A., Shapi, M., Matengu, K., and Ashekele, H.M.U., 2011. Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. Journal of Ethnobiology and Ethnomedicine. 7: 10.

13. Chimene, M.K., Okunji, C.O., Iwu, M.M., Kuete., V., 2013. 1—Monoterpenes and related compounds from the medicinal plants of Africa. In: Kuete V, editor. Medicinal plant research in Africa. Oxford: Elsevier: 132

14. Cristiano, R., Pizzolatti, M. G., Monache, F. D., Rezende, C. M., Branco, A., 2003. Two xanthones from *Polygala paniculata* and confirmation of the 1-hydroxy-2, 3, 5-trimethoxy-xanthone at trace level by HRGC-MS. Zeitschrift für Naturforschung C. 58(7-8): 490-494.

15. Copp, B.R., and Pearce, A.N., 2007. Natural product growth inhibitors of *Mycobacterium tuberculosis*. Natural products reports. 24: 278-297.

16. Cos, P., Vlietinck, A.J., Berghe, D.V., Maes, L., 2006. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. Journal of Ethnopharmacology. 106: 290–302.

17. Cunningham, A. B., 1997. An Africa-wide overview of medicinal plant harvesting, conservation and health care. Medicinal plants for forest conservation and health care. 11: 116-129.

18. Cushnie, T. T., and Lamb, A. J., 2005. Antimicrobial activity of flavonoids. International journal of antimicrobial agents. 26(5): 343-356.

19. Daboor, S.M., and Haroon, A.M., 2012. *In vitro:* Antimicrobial potential and phytochemical screening of some Egyptian aquatic plants. Egyptian Journal of Aquatic Research. 38: 233-239.

20. DeGabriel, J. L., Moore, B. D., Foley, W. J., Johnson, C. N., 2009. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology. 90(3): 711-719.

21. Doughari, J. H., 2012. Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents. INTECH Open Access Publisher.

22. Dzoyen, J.P., Tshikalange, E., Kuete, V., 2013. Medicinal Plants Market and Industry in Africa. Medicinal Plant Research in Africa. 859-890.

23. Elekofehinti, O. O., 2015. Saponins: Anti-diabetic principles from medicinal plants–A review. Pathophysiology. 22(2): 95-103.

24. Esterhuizen, L.L., Meyer, R., and Dubery, I.A., 2006. Antimicrobial compounds from *Coleonema album (Rutaceae).* Journal of Biosciences. 61. (7-8): 489-498.

25. Feachem, R. G., 2004. Editorial: the research imperative: fighting AIDS, TB and malaria. Tropical Medicine and International Health. 9(11): 1139-1141.

26. Fenner, R., Sortino, M., Rates, S.M.K., Dall'Agnol, R., Ferraz, A., Bernardi, A.P., Albring, D., Nör, C., Von Poser, G., Schapoval, E., Zacchino, S., 2005. Antifungal activity of some Brazilian *Hypericum* species. Phytomedicine. 12: 236-240.

27. Firn, R., 2010. Nature's Chemicals. Oxford University Press, Oxford: 74-75.

28. Frantz, S., 2004 Approvals: The demise of the blockbuster? Nature Reviews Drug Discovery. 4(2): 93-94.

29. Fraga, C. G., Galleano, M., Verstraeten, S. V., Oteiza, P. I., 2010. Basic biochemical mechanisms behind the health benefits of polyphenols. Molecular Aspects of Medicine. 31(6): 435-445.

30. Fuku, S., Al-Azzawi, A.M., Madamombe-Manduna, I.T., and Mashele, S., 2013. Phytochemistry and Free Radicals Scavenging activity of *Asparagus laricinus.* International Journal of Pharmacology. 9(5): 312-317.

31. Gao, T., Yao, H., Song, J., Liu, C., Zhu, Y., Ma, X., Pang, X., Xu, H., Chen, S., 2010. Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. Journal of Ethnopharmacology. 130(1): 116–121.

32. Glen, H.L., 1996. FSA contributions 5: Buxaciae. Bothalia. 26(1): 37-40.

33. Goldberg, D. E., Siliciano, R. F., Jacobs, W. R. 2012. Outwitting evolution: fighting drug-resistant TB, malaria, and HIV. Cell. 148(6): 1271-1283.

34. Gómez-Caravaca, A. M., Verardo, V., Segura-Carretero, A., Fernández-Gutiérrez, A., Caboni, M. F., 2014. Phenolic compounds and saponins in plants grown under different irrigation regimes. Polyphenols in Plants: Isolation, Purification and Extract Preparation: 37-52.

35. Graul, A. I., 2001. The year's new drugs. Drug News Perspectives. 14: 12-31.

36. Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine. 27(1): 1-93.

37. Havsteen, B. H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacology and Therapeutics. 96(2): 67-202.

38. Hawkey, P.M., 2008. Molecular epidemiology of clinically significant antibiotic resistance genes. British Journal of Pharmacology. 153: 406-413.

39. Hamburg, M.A., 2008. Considerations for infectious disease research and practice. Technology in Society. 30: 383– 387.

40. Harborne, J. B., and Baxter, H., 1999. The handbook of natural flavonoids. Volume 1 and Volume 2. John Wiley & Sons.

41. Harborne, J.B., William, C.A., 2000. Advances in flavonoids research since 1992. Phytochemistry. 55: 481-504.

42. Hooper, L. and Cassidy, A., 2006. A review of the health care potential of bioactive compounds. Journal of the Science of Food and Agriculture. 86(12): 1805-1813.

43. Hutchings, A., 1996. Zulu medicinal plants: an inventory. University of Natal Press.

44. Ibrahim, T.A., Opawale, B.O., Oyinloye, J.M.A., 2011. Antibacterial activity of herbal extracts against multi drug resistant strains of bacteria from clinical origin. Life Sciences Leaflets. 15: 490-498.

45. Ikeya, Y., Takeda, S., Tunakawa, M., Karakida, H., Toda, K., Yamaguchi, T. and Aburada, M., 2004. Cognitive improving and cerebral protective effects of acylated oligosaccharides in *Polygala tenuifolia*. Biological and Pharmaceutical Bulletin. 27(7): 1081-1085.

46. Iwu, M. M., 2014. Handbook of African medicinal plants. CRC press.

47. Jia, H., Jiang, Y., Ruan, Y., Zhang, Y., Ma, X., Zhang, J., Zhang, D., 2004. Tenuigenin treatment decreases secretion of the Alzheimer's disease amyloid β-protein in cultured cells. Neuroscience letters. 367(1): 123-128.

48. Jindal, B.A.K., Pandya, M.K., Khan, M.I.D., 2015. Antimicrobial resistance: A public health challenge. Medical Journal Armed Forces India. 71: 178 -181.

49. Kar, A., 2007. Pharmaocgnosy and Pharmacobiotechnology (Revised-Expanded Second Edition). New Age International Limited Publishers New Delhi: 332-600.

50. Kaur, S., Micheal, H., Arora, S., Harkonen, P.L., Kumar, S., 2005. The *In vitro* cytotoxicity and apoptotic activity of Triphala-an Indian herbal drug. Journal of Ethno pharmacology. 10: 15-20.

51. Kisangau, D.P., Lyaruu, H.V.M., Hosea, K.M., Joseph, C.C., 2007. Use of traditional medicines in the management of HIV/AIDS opportunistic infections in Tanzania: a case in the Bukoba rural district. Journal of Ethnobiology and Ethno medicine. 3 (29).

52. Kou, J., Si, M., Dai, G., Lin, Y., Zhu, D., 2006. Antiinflammatory activity of *Polygala japonica* extract. Fitoterapia. 77(6): 411-415.

53. Kougan, G.B., Taboada, T., Kuete, V., Verpoorte, R., 2013. Simple phenols, phenolic acids, and related esters from the medicinal plants of Africa. Medicinal plant research in Africa: 225-49.

54. Kubmarawa, D., Ajoku, G.A., Enwerem, N.M. and Okorie, D.A., 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. African Journal of Biotechnology, 6(14): 1690-1696.

55. Kuete, V., Wansi, J. D., Mbaveng, A. T., Sop, M. K., Tadjong, A. T., Beng, V. P., Lall, N., 2008. Antimicrobial activity of the methanolic extract and compounds from *Teclea afzelii* (Rutaceae). South African Journal of Botany. 74(4): 572-576.

56. Kuete, V., and Efferth, T., 2010. Cameroonian medicinal plants: pharmacology and derived natural products. Frontiers in Pharmacology. 1.

57. Kuete, V., Eichhorn, T., Wiench, B., Krusche, B., Efferth, T., 2012. Cytotoxicity, anti-angiogenic, apoptotic effects and transcript profiling of a naturally occurring naphthyl butenone, guieranone A. Cell division. 7(1): 1-12.

58. Lall, N., and Meyer, J.J., 1999. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. *Journal Ethno pharmacology*. 66: 347-354.

59. Lam, C.W., Wakeman, A., James, A., Ata, A., Gengan, R.M., Ross, S.A., 2015. Bioactive steroidal alkaloids from *Buxus macowanii Oliv*. Steroids. 95: 73–79.

60. Lee, S.J., Seo, E., Cho, Y., 2013. Proposal for a new therapy for drug-resistant malaria using Plasmodium synthetic lethality inference. International Journal for Parasitology: Drugs and Drug Resistance. 3: 119–128.

61. Lewu, F.B., Afolayan, A.J., 2009. Ethnomedicine in South Africa: the role of weedy species. African Journal of Biotechnology. 8 (6): 929–934.

62. Louw, C.A.M., Regnier, T.J.C., Korsten, L., 2002. Medicinal bulbous plants of South Africa and their traditional relevance in the control of infectious diseases. Journal of Ethno pharmacology. 82: 147-154.

63. Mabona, U., and Van Vuuren, S.F., 2013. Southern African medicinal plants used to treat skin diseases. South African Journal of Botany. 87: 175–193.

64. Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L., 2004. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition. 79(5): 727-747.

65. Mander, M., 1998. Marketing of indigenous medicinal plants in South Africa: A case study in KwaZulu-Natal.

66. Manimozhi, D.M., Sankaranarayanan, S., and Sampathkumar, G., 2012. Evaluating the antibacterial activity of flavonoids extracted from *Ficus benghalensis*. International Journal of Pharmaceutical and Biological Research. Vol 3, ISSN: 0976-285X.

67. Masevhe, N.A., McGaw, L.J., Eloff, J.N., 2015. The traditional use of plants to manage *candidiasis* and related infections in Venda, South Africa. Journal of Ethno pharmacology. 168: 364–372.

68. Mitaine-Offer, A. C., Miyamoto, T., Laurens, V., Delaude, C., Lacaille-Dubois, M. A. 2003. New acylated triterpene saponins from *Polygala arenaria*. Helvetica chimica acta. 86(7): 2404-2413.

69. Mmushi, T.J., Masoko, P., Mdee, L.k., Mokgotho, M.P., Mampuru, L.J., and Howard, R.L., 2010. Antimycobacterial evaluation of fifteen medicinal plants in South Africa. African Journal of Traditional, Complementary and Alternative Medicines. 7 (1): 34–39.

70. Motamedi, H., Mirzabeigi, H., and Shirali, T., 2010. Determining of antibiotic resistance profile in *Staphylococcus aureus* isolates. Asian Pacific Journal of Tropical Medicine. 3(9): 734-737.

71. Motsei, M.L., Lindsey, K.L., van Staden, J., Jager, A.K., 2003. Screening of traditionally used South African plants for antifungal activity against *Candida albicans.* Journal of Ethno pharmacology. 86: 235–241.

72. Mueller, M. S., Karhagomba, I. B., Hirt, H. M., Wemakor, E. 2000. The potential of *Artemisia annua L.* as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. Journal of Ethno pharmacology. 73(3): 487-493.

73. Ndhlala, A.R., Ncube, B., Okem, A., Mulaudzi, R.B., Van Staden, J., 2013. Toxicology of some important medicinal plants in southern Africa. Food and Chemical Toxicology. 62: 609–621.

74. Ngameni, B., Fotso, G. W., Kamga, J., Ambassa, P., Abdou, T., Fankam, A. G., Kuete, V., 2013. 9 Flavonoids and Related Compounds from the Medicinal Plants of Africa. Medicinal Plant Research in Africa: Pharmacology and Chemistry, 301.

75. Normann, H., and Snyman, I. 1996. Indigenous knowledge and its uses in southern Africa (Vol. 61). HSRC Press.

76. Ntsoelenyane, P.H., Mashele, S.S., Manduna, I.T., 2014. The anticancer, antioxidant and phytochemical screening of *Philenoptera violacea* and *Xanthocercis zambesiaca* leaf flower and twig extracts. International Journal of Pharmacological Research. 4 (3): 100-105.

77. Obeidat, M., Shatnawi, M., Al-alawi, M., Enas, A., Hanee, A., Masia, A., El-Quadah, J., Otr, I., 2012 Antimicrobial activity of crude extracts of some plant leaves. Research Journal of Microbiology. 7:59-67.

78. O'Bryan, C.A., Crandall, P.G., Ricke, S.C., 2008. Organic poultry pathogen control from farm to fork. Foodborne pathogens and disease. 5: 709–720.

79. Okuda, T., 2005. Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry*. 66(17): 2012-2031.

80. Oliphant, C. M., and Eroschenko, K., 2015. Antibiotic Resistance, Part 1: Gram-positive Pathogens. The Journal for Nurse Practitioners. 11(1): 70-78.

81. Olliaro, P. L., and Bloland, P. B., 2001. Clinical and public health implications of antimalarial drug resistance. In Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery (ed. P. J. Rosenthal). Totowa, NJ: Humana Press: 65-83.

82. Pirttilä, T., Wilcock, G., Truyen, L., Damaraju, C. V., 2004. Long-term efficacy and safety of galantamine in patients with mild-to-moderate Alzheimer's disease. multicenter trial. European Journal of Neurology.11(11): 734-741.

83. Pizzolatti, M.G., Koga, A.H., Grisard, E.C., Steindel, M., 2003. Trypanocidal activity of extracts from Brazilian Atlantic Rain Forest plant species. Phytomedicine. 10: 422-426.

84. Pizzolatti, M. G., Cunha, A., Pereira, W. S., Delle Monache, F., 2004. A new styryl-2-pyrone derivative from *Polygala sabulosa* (Polygalaceae). Biochemical systematics and ecology. 32(6): 603-606.

85. Pizzolatti, M. G., Mendes, B. G., Cunha Jr, A., Soldi, C., Koga, A. H., Eger, I., Steindel, M., 2008. Trypanocidal activity of coumarins and styryl-2-pyrones from *Polygala sabulosa* AW Bennett *(Polygalaceae).* Revista Brasileira de Farmacognosia. 18(2): 177-182.

86. Pooley, E., 1993. The complete guide to trees of Natal, Zululand and Transkei. Durban: Natal Flora Publication Trust.

87. Rao, M.S., and Raman, N.V., 2004. A novel flavonoid from *Polygala chinensis*. *Biochemical systematics and ecology*. *32*(4): 447-448.

88. Rawat, A., 2012. Gaps and shortages in South Africa's health work force. Back- grounder. 31:1–6.

89. Rosenberry, T.L., 1975. Acetylcholinesterase. Advanced Enzymology Related Areas of Molecular Biology. 43: 103–218.

90. Ribas, C. M., Meotti, F. C., Nascimento, F. P., Jacques, A. V., Dafre, A. L., Rodrigues, A. L. S., Santos, A. R. 2008. Antinociceptive effect of the *Polygala sabulosa* hydroalcoholic extract in mice: evidence for the involvement of glutamatergic receptors and cytokine pathways. Basic & clinical pharmacology & toxicology. 103(1): 43-47

91. Rice, L. B., 2008. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. Journal of Infectious Diseases. 197(8): 1079-1081.

92. Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., Vila, J., 2015. The global threat of antimicrobial resistance: science for intervention. New Microbes and New Infections.

93. Rao, M. S., & Raman, N. V., 2004. A novel flavonoid from *Polygala chinensis*. Biochemical systematics and ecology. 4(32): 447-448.

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

95. Sarker, S., and Nahar, L., 2007. Chemistry for pharmacy students: general, organic and natural product chemistry. John Wiley & Sons.

96. Sandjo, L.P., Kuete, V., 2013. 4—Triterpenes and steroids from the medicinal plants of Africa. Medicinal plant research in Africa: 135-202.

97. Shai, L.J., Magano, S.R., Lebelo, S.L., and Mogale, A.M., 2011. Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. Journal of Medicinal Plants Research. 5(13): 2863-2867.

98. Sparg, S., Light, M. E., Van Staden, J., 2004. Biological activities and distribution of plant saponins. Journal of ethno pharmacology. 94(2): 219-243.

99. Speta, F., 1998. Hyacinthaceae. In Flowering Plants· Monocotyledons. Springer Berlin Heidelberg: 261-285.

100. Stevens, P. F., 2001. Onwards. Angiosperm Phylogeny Website. Version 12, July 2012 and more or less continuously updated since.

101. Street, R.A., Stirk, W.A. and Van Staden, J., 2008. South African traditional medicinal plant trade challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology. 119(3): 705-710.

102. Taylor, J.L.S., Rabe, T., McGaw, L.J., and Van Staden, J., 2001. Toward the scientific validation of medicinal plants. Plant Growth Regulations. 34: 23-37.

103. Van Wyk, B. E., Heerden, F. V., Oudtshoorn, B. V., 2002. Poisonous plants of South Africa. Briza Publications.

104. Wansi, J. D., Devkota, K. P., Tshikalange, E., and Kuete, V., 2013. Alkaloids from the medicinal plants of Africa. Medicinal Plant Research in Africa: 557-605.

105. Wang, G., Tang, W., and Bidigare, R. R., 2005. Terpenoids as therapeutic drugs and pharmaceutical agents. In Natural products (pp. 197-227). Humana Press.

106. Wink, M., Van Wyk, B.-E., 2008. Mind-Altering and Poisonous Plants of the World. Briza Publications, Pretoria.

107. Winston, C. A., and Mitruka, K., 2012. Treatment duration for patients with drug-resistant tuberculosis, United States. Emerging infectious diseases. 18(7): 1201.

108. World Health Organisation., 2010. Global Tuberculosis report: WHO report 2010. World Health Organization

109. World Health Organisation (WHO), 2011a. The world medicines situation traditional medicines: global situation, issues and challenges. Geneva. Available at:

http://www.who.int/medicines/areas/policy/world_medicines_situation/WMS_ch18_wTraditionalMed.pdf (Accessed on: 11/01/2013).

110. World Health Organization. World Health Day 2011b: Policy Briefs. Geneva: WHO; 2011. Available at: http://www.who.int/ world-health-day/2011/policybriefs/en/index.html. Accessed Nov 2012.

111. World Health Organisation., 2014a. Global summary of the AIDS epidemic. WHO- HIV department. World Health Organisation. Global Tuberculosis report 2014. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf?ua=1

112. World Health Organisation., 2014b. World Malaria Report. WHO Library Cataloguing-in-Publication Data, ISBN 978 92 4 156483 0.

113. Zerabruk, S., and Yirga, G., 2012. Traditional knowledge of medicinal plants in Gindeberet district, Western Ethiopia. South African Journal of Botany. 78: 165-169.

114. Zofou, D., Kuete, V., Titanji, V. P. K., 2013. Antimalarial and other antiprotozoal products from African medicinal plants. Medicinal Plant Research in Africa: Pharmacology and Chemistry. Elsevier Inc., London, UK.

115. Zuanazzi, J. A. S., and Montanha, J. A., 2004. Flavonóides In: Farmacognosia da Planta ao medicamento. Porto Alegre/Floriano polis, (In Portuguese).

# CHAPTER 2

# ANTIMICROBIAL ACTIVITY OF *BUXUS MACOWANII, POLYGALA MYRTIFOLIA*, *SCILLA* SP AND *XANTHOCERCIS ZAMBESIACA*

## 2.1. Introduction

The use of western medicine to treat infectious diseases has always been associated with a number of problems that make the treatment of infectious diseases more challenging. The use of antimicrobial drugs that are currently available include problems such as limited accessibility for people living in rural areas or places remote to health care facilities, high purchase prices, and side effects (Bodeker and Graz, 2013). The emergence of drug-resistant pathogens and an increase in opportunistic infections in people with HIV/AIDS and those receiving chemotherapy are additional problems that make the treatment of infectious diseases very difficult (Fargana et al., 2014). These problems have encouraged researchers and scientists to devise new alternative manners of treatment, which can lead to the discovery of novel drugs that will overcome the challenges associated with the drugs that are currently used.

For thousands of years, plants have been used by millions of people as a source of food and for medicinal purposes (Brouwer et al., 2005). An estimated 80% of the world's population, and 72% of black South Africans, still rely on traditional medicine for their primary health care needs (Gurib-Fakim, 2006). Traditional medicine has not only gained popularity due to its effectiveness against diseases, but because it is sometimes the only available system in rural areas (Mabona and Van Vuuren, 2013). The use of traditional medicine is further attributed to advantages such as accessibility, affordability, fewer side effects, and extensive knowledge and expertise thereof by people within communities (Fennell et al., 2004; Runyoro et al., 2006).

Medicinal plants, especially those used by traditional healers, have become the focus of research, and are considered as the best option for the production of novel drugs, as they have an unmatched chemical diversity (Cos et al., 2006; O'Bryan et al., 2008). They contain bioactive compounds such as alkaloids, terpenoids, tannins, flavonoids, peptides and phenolic compounds that are known to have antimicrobial, antiviral, antifungal, anti-inflammatory, anthelmintic and antioxidant activity (Kubmarawa et al., 2007). Moreover, medicinal plants are known to work on different target sites than those targeted by conventional drugs (Kimberlin and Whitley, 1996). They are also cheaper and have fewer side effects, and thus medicinal plants remain an important and better alternative source from which new therapeutic agents can be manufactured.

Traditional knowledge and ethnobotany help researchers to identify medicinally relevant plants that can lead to the discovery and manufacturing of new drugs. Available literature indicated that plants such as *B. macowanii*, *P. myrtifolia*, *X. zambesiaca* and other *Scilla sp.* are traditionally used for the treatment of wounds, skin disorders, stomach problems, back problems, fractures and other major diseases, such as cancer (Pooley, 1993; Hutchings et al., 1996; Crouch et al., 1999, Shai et al., 2011). It is therefore necessary that this chapter focuses on studying the antimicrobial activity of these plants, and the minimum concentration at which the extracts inhibit the growth of bacteria and fungi, by using the microdilution method.

## 2.2. Methods

### 2.2.1. Plant extracts

*Buxus macowanii Oliv* (Buxuceae), *Polygala myrtifolia L.* (Polygalaceae), *Scilla sp. (*Asparagaceae) and *Xanthocercis zambesiaca (Baker) (*Fabaceae) were selected for the investigation of their antimicrobial activity against bacterial and fungal species. Methanol plant extracts and information about the plants

(as presented in Table 2.1) were supplied by the Department of Soil, Crop and Climate Sciences at the University of the Free State (UFS) because they were the only type of extracts available.

**Table 2.1.** Information about plant extracts

| **Plant name** | **Plant part** | **Collection number** | **Extract number** | **Place of collection** |
|---|---|---|---|---|
| *B. macowanii Oliv* | Leaves and twigs | RB 829 a (Brand 795) | 1544 | Mpumalanga |
| *Scilla sp.* | Whole plant | RB 512 (Brand 614) | 1272.2 | Eastern Cape |
| *Scilla sp.* | Roots and bulbs | JV 9852b (Venter 251) | 1163 | Free State |
| *P. myrtifolia L* | Leaves and twigs | RB 801 a (Brand 753) | 1561 | Eastern Cape |
| *X. zambesiaca (Baker)* | Stem | - | 1584 | Mpumalanga |

### *2.2.2.* Extract preparation and storage

The plants were collected, washed, oven dried at 40ºC for 72 hours, and ground to powder. The dry, powdered material of the plants was extracted with 100% methanol because the solvent is easy to evaporate and does not affect results of any screening. The methanol was evaporated at 40ºC, under a vacuum, using a Buchi Rotavapor. All extracts were stored in the fridge at -20ºC until required (Zaidan et al., 2005).

### 2.2.3. Microbial cultures

The antibacterial activity of the plant extracts was evaluated against *Staphylococcus aureus* (ATCC 25923), *Clostridium perfringens* (ATCC 13126), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Staphylococcus epidermidis* (ATCC 12228). The fungal species used were *Candida albicans* (ATCC 90028) and *Candida tropicalis* (ATCC 756). *Bacillus cereus* (ATCC 13061) was selected for examination of the cell wall after treatment with the plant extract, which exhibited the best antibacterial activity using microscopy. All the bacterial and fungal species were supplied by the National Health Laboratory Services, Bloemfontein, South Africa. All the microbial species were maintained in Mueller Hinton agar plates at temperatures of 4ºC. Prior to treatment with the plant extracts, the bacteria were inoculated in Mueller Hinton broth, and incubated and shaken at 100 revolutions per minute (rpm) for 24 hours, to ensure purity and viability. Thereafter, one millilitre of the culture was diluted in 100 ml of Mueller Hinton broth (1:100) (Meyer and Afolayan, 1995).

### 2.2.4. Antimicrobial activity

The antibacterial and antifungal activity of five plant extracts was investigated using the microdilution method developed by Eloff (1998). The microdilution method was selected because it is easier to perform, and it can determine the minimum inhibitory concentration of plant extracts, as opposed to the agar diffusion method. One hundred microliters of the bacterial suspension was pipetted into the 96 microwell plates already containing 100 µl of diluted plant extract to make a final volume of 200 µl in each well. The concentration of the plant extracts ranged from 0.16 mg/ml to 2.5 mg/ml. The control wells were respectively filled with culture medium only, bacterial suspension, 5% dimethyl sulfoxide (DMSO) (Nostro et al., 2000; Baris et al., 2006), and plant extract only. Chloramphenicol (0.125 mg/ml) was used as a positive control in bacteria, while amphotericin B (0.03-1 µg/ml) was used in fungi because it is one of the antifungal agents that were used to treat fungal infections (Wanger et al., 2005;

Andrews, 2001). The microwell plates were incubated for 24 hours, where after 40 µl of 4 mg/ml Iodonitrotetrazolium salt solution was added to each well. Growth was indicated by a change of colour ranging from pink to violet after 10 to 30 minutes' incubation. All samples were tested in triplicates. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration at which the plant indicated bacterial or fungal growth inhibition.

### 2.2.5. Thin Layer Chromatography (TLC) bioautography

The bioautography method was used to screen and identify compounds with antibacterial activity present in the plant extracts of *B. macowanii*. The detection of antimicrobial compounds was performed using aluminium-coated DC-fertigfolien alugram Xtra Sil G/ Uv 254 TLC plates. The plant that exhibited the best antibacterial activity was loaded onto three TLC plates, and eluted using a mobile polar solvent system Toluene, Chloroform and Ethanol (5.7:11.4:2.9), which provided the best separation of the compounds. The developed plate was left to dry for 24 hours to remove traces of the solvent on the plate. The prepared TLC plate was sprayed with the *Enterococcus faecalis* bacterial suspension until wet, whereafter it was incubated overnight at 37ºC and at 100% relative humidity for 8 hours (Esterhuizen et al., 2006). The plate was sprayed with 4 mg/ml solution of Iodonitrotetrazolium chloride, and further incubated for eight hours in a sealed container for colour development. The antibacterial compounds were identified as white areas against a violet- or pink-coloured background, which indicated bacterial growth. On the other two plates, the separated alkaloids were detected using Dragendorff's reagent, and flavonoids were detected using natural product-polyethylene glycol reagent. The detected alkaloids and flavonoids were visualised under ultraviolet light at a wavelength of 365 nm. All the TLC plates were run in duplicates, and one was used as a reference chromatogram.

### 2.2.6. The microscopic analysis of the bacterial cell wall.

The effect of *B. macowanii* on bacterial morphology was examined using scanning and transmission electron microscopy. *B. cereus* was treated with different concentrations (0.2 to 2.5 mg/ml) of the methanol extracts of *B. macowanii*. The untreated samples (control) and the treated samples were incubated at 37ºC for 24 hours. After incubation, the cells were washed twice with 0.1 M phosphate buffer solution (PBS, pH 7.0), were subjected to fixation using 3% glutardialdehyde and 1% osmiumtetroxide, and were kept for two hours at -4ºC. Thereafter, fixation of the cells were further subjected to dehydration in ethanol at successive concentrations of 50%, 70%, 95% and 100%, followed by critical point-drying using Carbon dioxide ($CO_2$) to remove the ethanol. The samples were finally mounted on a specimen stub and coated with gold under vacuum, followed by microscopic examination using SEM (Moosavy et al., 2008; Lv et al., 2011). For TEM-dehydrated bacterial cells, cells were embedded by replacing ethanol with epoxy to make slim sections suitable for microscopic examination. Samples were further embedded using epoxy for eight hours at 70ºC in a special mould. The samples were cut into sections using ultramicrotome, and were stained with 6% Uranyl and lead citrate, followed by TEM examination (Joshi et al., 2010; He et al., 2014).

## 2.3. Results and discussion

### 2.3.1. The antimicrobial activity of the selected plant extracts

*P. myrtifolia*, Scilla sp. and *X. zambesiaca* showed no activity against all the microbial strains against which they were tested. Previous studies documented antimicrobial activity of *X. zambesiaca* (Mmushi et al., 2010; Masoko, 2013) and antifungal activity of *P. myrtifolia* (Motsei et al., 2003). A factor such as the choice of solvent might have affected the antimicrobial activity of extracts from *X. zambesiaca* and *P. myrtifolia,* because bioactive phytochemicals are affected by factors such as solvent polarity. The extracts of *B. macowanii* showed antimicrobial activity against *S. aureus*, *S. epidermidis*, *C. albicans*, *C. tropicalis*, *C. perfrengens* and *P. aeruginosa*, where the minimum inhibitory concentration (MIC) was 2.5

mg/ml. *B. macowanii* also showed antimicrobial activity against *E. faecalis* and *E. coli* (MIC:1.2 mg/ml). Usually one may expect a variation in the inhibitory activity against Gram-negative and Gram-positive bacteria due to their differences in cell wall composition; however, it was found that both bacteria were affected in the same way. The inhibition of Gram-negative bacteria, Gram-positive bacteria and Candida is therefore of critical importance, because of their drug resistance to current antibacterial and antifungal agents. The *B. macowanii's* inhibitory activity against Gram-negative bacteria, Gram-positive bacteria and *Candida* may indicate a possible breakthrough in the fight against the challenges caused by drug-resistant micro-organisms. Numerous studies have reported that most species in the Buxus genus are a rich source of steroidal alkaloids. It can be suggested that the antimicrobial activity *B. macowanii* extracts can be attributed towards alkaloids, which, according to literature, are abundantly found in most plants that belong to the Buxus genus (Loru et al., 2000; Atta-ur-Rahman, et al., 2001; Babar et al., 2006; Ata and Andresh, 2008). Thus *B. macowanii* was considered the best option for further investigation.

### 2.3.2. TLC bioautography

Antimicrobial compounds from the extracts of *B. macowanii* were identified using the TLC bioautography. The clear zones on the TLC chromatogram indicated that the extracts of *B. macowanii* contain compounds that have antibacterial activity against *E. faecalis* using a solvent system of Toluene, Chloroform and Ethanol (5.7:11.4:2.9) (Figure 2.1). Four clear zones were identified on the bioautography chromatogram, suggesting that the antibacterial activity of *B. macowanii* cannot only be attributed to one compound. A yellowish-orange colour under visible light showed that the extracts of *B. macowanii* have alkaloids, as indicated in Figure 2.2. Figure 2.3 showed traces of flavonoids in *B. macowanii* by a dark yellow, green or blue fluorescence colour under UV light at a wavelength of 354 nm. Some studies reported over 200 or more steroidal alkaloids isolated in various species belonging to the genus *Buxus* (Ata et al., 2002; Meshkatalsadat et al., 2006; Babar et al., 2006; Ata et al., 2010;

Matochko et al., 2010). Flavonoids have also been reported to have antibacterial and antifungal properties (Manimodzi et al., 2012). Therefore, the presence of alkaloids and flavonoids may explain the antibacterial effects of *B. macowanii*.

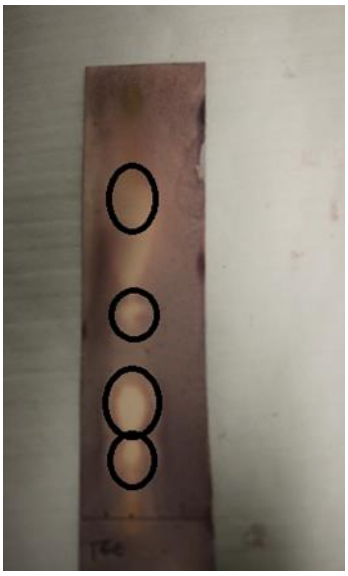


**Figure 2.1:** Chromatogram developed with Toluene/Chloroform/Ethanol (5.7:11.4:2.9) indicates the antibacterial activity of the plant *B. macowanii* against *E.faecalis.*

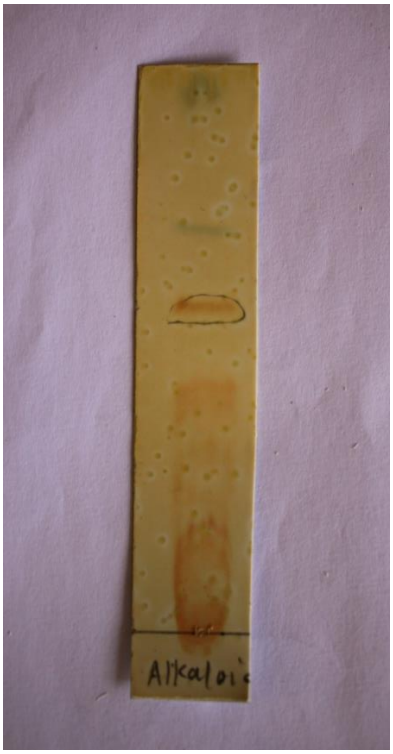



**Figure 2.2:** A chromatogram developed with Toluene/Chloroform/Ethanol (5.7:11.4:2.9) indicates the presence of alkaloids in the extracts of *Buxus macowanii.* An orange colour indicates the presence of alkaloids.

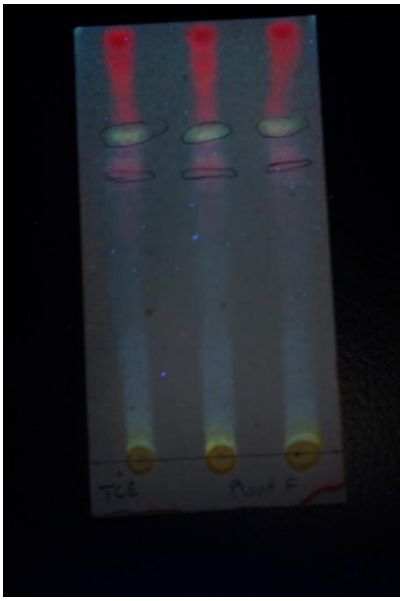


**Figure 2.3**: A chromatogram developed with solvent system Toluene/Chloroform/Ethanol (5.7:11.4:2.9) indicates the presence of flavonoids in the extracts of *Buxus macowanii* under UV light. A green-blue colour indicates the presence of flavonoids.

### 2.3.3. The effect of *Buxus macowanii* on the cell morphology

The effect of the extracts of *B. macowanii* on the bacterial cell morphology was examined using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). SEM was used to examine the morphology of the cells of *Bacillus cereus* treated with extracts of *B. macowanii,* for 24 hours at 37ºC. The untreated samples of *B. cereus* were considered as a control. Under normal conditions, the cells of *B. cereus* under a microscope appear as long bacilli cells (Figure 2.4 (A)). The cells of *B. cereus* that were treated with the extracts of *B. macowanii* at the MIC concentration of 2.5 mg/ml in SEM showed major structural changes compared to the untreated cells that lead to cell death (Figure 2.4 (B) and (C)). Figure 2.4 (C) indicates some damage to cell walls (DCW), which is evident by holes on the surface of the cell. The extracts also caused incomplete cell division (ICD), and swollenness of the cells (SC), caused by the penetration of the plant extracts into the cell. The swelling of the cell lead to cytoplasmic membrane damage, which eventually resulted in loss of the intracellular contents (LCC), which were observed outside the cells. The loss of cellular or cytoplasmic contents resulted in shrinkage of the cell (SC), which was explained by the wrinkled surface of the cell (Figure 2.4 (B) and (C)). The effects of *B. macowanii* on the morphology of the bacterial cells also demonstrated some damage in the structure of the cells because treated cells examined using TEM showed cell-wall distortion, which resulted in increased membrane permeability (Figure 2.5(B)). When cell-membrane permeability increased, the loss of intracellular or cytoplasmic contents occurred. Incomplete cell division, roughness of the cell and separation of the cytoplasmic membrane from the cell wall were some of the morphological changes that were caused by the extracts of *B. macowanii* on the bacterial cells. Vancomycin is one of the drugs that are used to treat infections that are caused by

*Bacillus cereus.* It usually inhibits the second stage of cell-wall synthesis, alter the cell-membrane permeability, and inhibits ribonucleic acid synthesis (Watabakunakorn, 1981). Given the results that were obtained via SEM and TEM, *B. macowanii* was identified as the best treatment and may be the best option for the development of a new drug that can control resistant pathogens causing infectious diseases.[1]

[1] This section has been submitted for publication with the title: Antimicrobial activity of *Buxus macowanii* and its morphological effects on Bacillus cereus. (See annexure B).

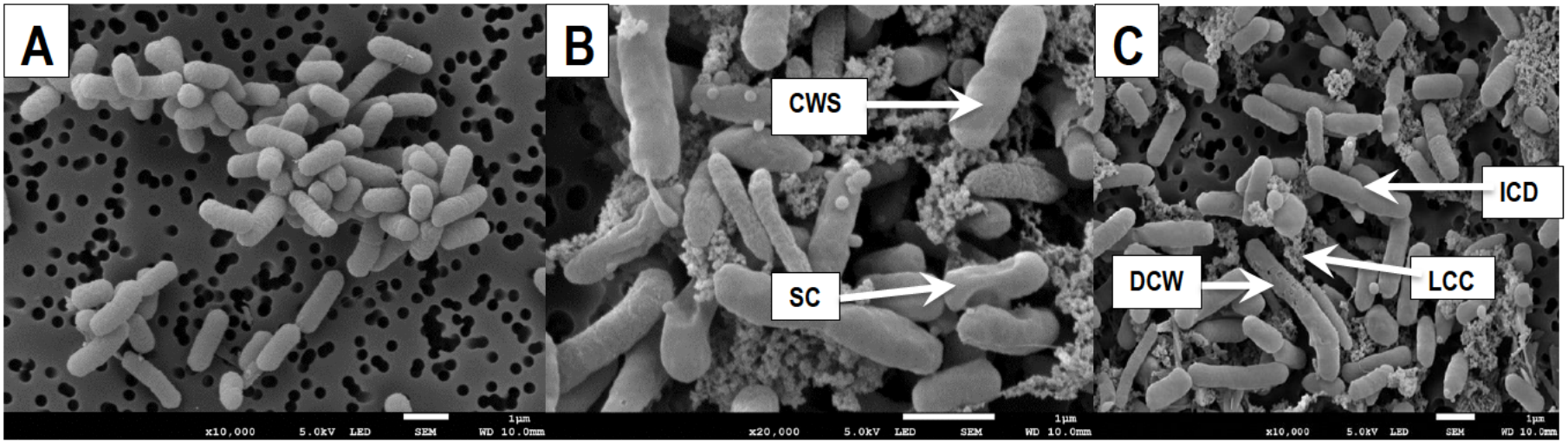



**Figure 2.4:** (A) Control cells (untreated) and (B and C) different effects of *B. macowanii* on the bacterial cell wall and structure. Damaged cell wall (DWC) with the formation of holes on the cell surface; loss of cellular contents (LLC); incomplete cell division (ICD); cell wall completely swollen (CWS); and shrinkage of the cell (SC).

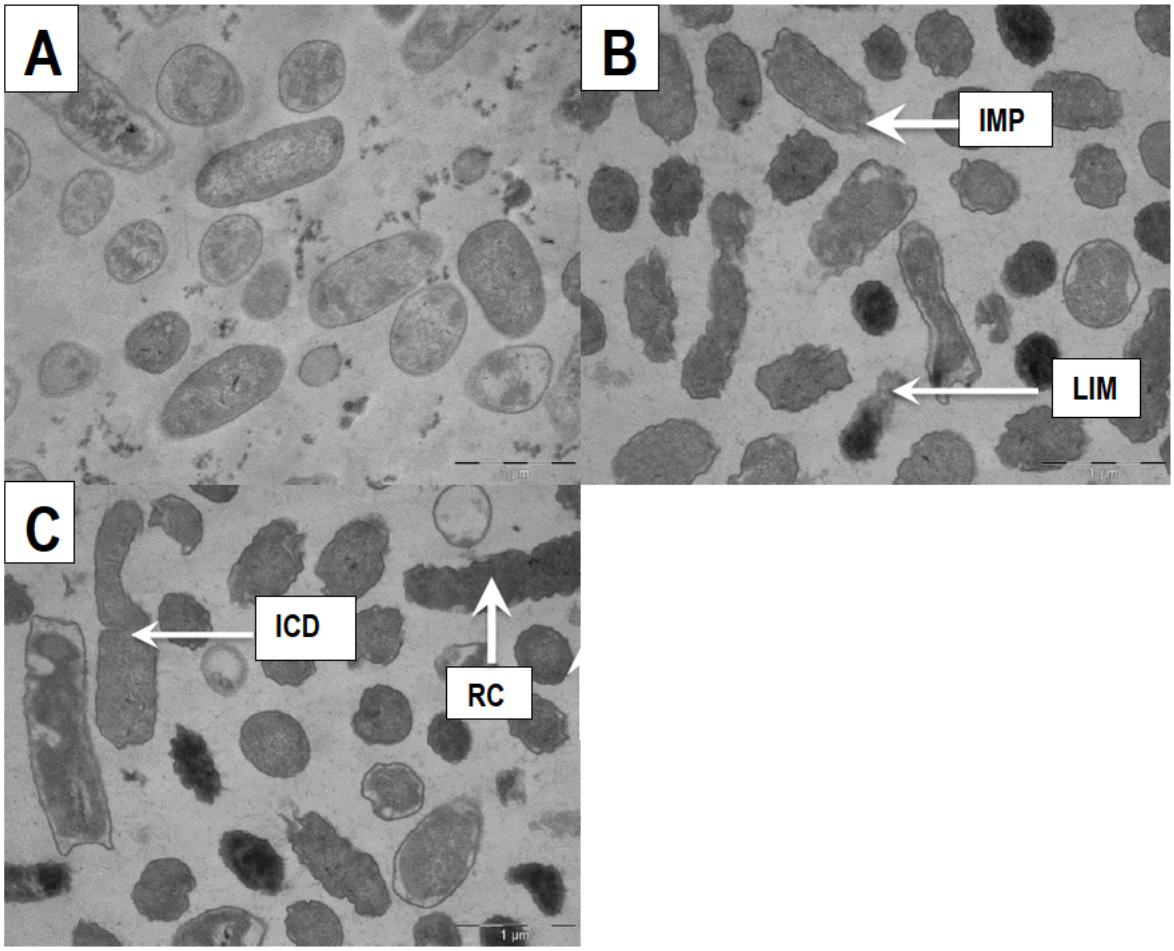



**Figure 2.5**: (A) Control cells (untreated) and (B) morphological changes of *B. cereus* after treatment with *B. macowanii*. Increased membrane permeability (IMP) that resulted in shrinkage of the cell; loss of intracellular material (LIM); incomplete cell division (ICD); and roughness of the cell (RC).

## 2.4. Conclusion

*B. macowanii* showed antibacterial and antifungal activity, with an increase in microbial resistance and a decrease in the effectiveness of the drugs that are currently available, the results obtained indicated that the plant has the potential to be used as an antimicrobial agent. The TLC bioautography indicated that the antimicrobial activity of the extracts of *B. macowanii* might be attributed to flavonoids, alkaloids and other antimicrobial compounds, due to one or more clear zones detected on the TLC

chromatogram. Further analysis using a solvent such as diethylamine to achieve an ideal separation of compounds and direct isolation of antimicrobial compounds, is recommended.

**2.5. References**.


1. Andrews, J.M., 2001. Determination of minimum inhibitory concentrations. *Journal of antimicrobial Chemotherapy*, *48*(suppl 1), pp.5-16.
2. Ata, A., and Andersh, B. J., 2008. *Buxus* steroidal alkaloids: chemistry and biology. The Alkaloids: Chemistry and Biology. 66: 191-213.
3. Ata, A., Iverson, C. D., Kalhari, K. S., Akhter, S., Betteridge, J., Meshkatalsadat, M. H., Sener, B., 2010. Triterpenoidal alkaloids from *Buxus hyrcana* and their enzyme inhibitory, anti-fungal and anti-leishmanial activities. Phytochemistry. 71(14): 1780-1786.
4. Ata, A., Naz, S., Choudhary, M. I., Sener, B., & Turkoz, S., 2002. NewTriterpenoidal Alkaloids from *Buxus sempervirens*. Zeitschrift für Naturforschung C. 57(1-2): 21-28.
5. Atta-ur-Rahman, Parveen, S., Khalid, A., Farooq, A., Choudhary, M.I., 2001. Acetyl and butyrylcholinesterase-inhibiting triterpenoid alkaloids from *Buxus papillosa*. Phytochemistry. 58: 963-968.
6. Babar, Z.U., Ata A., Meshkatalsadat, M.H., 2006. New bioactive steroidal alkaloids from *Buxus hyrcana.* Steroids. 71: 1045–1051.
7. Baris, O., Gulluce, M., Sahin, F., Ozer, H., Kilic, H., Ozkan, H., Sokmen, M., and Ozbek T., 2006. Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii* Afan. (Asteraceae). Turkish Journal of Biology. 30(2): 65-73.
8. Bodeker, G., and Graz, B., 2013. 25 – Traditional Medicine. Hunter's Tropical Medicine and Emerging Infectious Disease (Ninth Edition): 197-202.

9. Brouwer, N., Liu, Q., Harrington, D., Kohen, J., Vemulpad, S., Jamie, J., Randall, M., Randall, D., 2005. An ethnopharmacological study of medicinal plants in New South Wales. Molecules. 10: 1252–1262.

10. Crouch, N.R., Bangani, V., Mulholland, D.A., 1999. Homoisoflavanones from three South African *Scilla* species. Phytochemistry. 51 (7): 943-946.

11. Cos, P., Vlietinck, A. J., Berghe, D. V., Maes, L., 2006. Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. Journal of Ethnopharmacology. 106(3): 290-302.

12. Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Department of pharmacology, University of Pretoria, South Africa.

13. Esterhuizen, L.L., Meyer, R., and Dubery, I.A., 2006. Antimicrobial compounds from *Coleonema album* (Rutaceae). Journal of Biosciences. 61 (7-8): 489-498.

14. Fargana, A., Zerin, N., Kabir, M.D.S., 2014. Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. Asian Pacific Journal of Tropical Diseases. 4: 920-923.

15. Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology. 94: 205-17.

16. Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine. 27(1): 1-93.

17. He, M., Wu, T., Pan, S., Xu, X., 2014. Antimicrobial mechanism of flavonoids against *Escherichia coli* ATCC25922 by model membrane study. Applied Surface Science. 305: 515–521.

18. Joshi, S., Bisht, G.S., Rawat, D.S., Kumar, A., Kumar, R., Maiti, R., Pasha, S., 2010. Interaction studies of novel cell selective antimicrobial peptides with model membranes and *E. coli* ATCC 11775, Biochimica et Biophysica Acta (BBA)-Biomembranes, 1798: 1864–1875.
19. Kimberlin, D. W., and Whitley, R. J., 1996. Antiviral resistance: mechanisms, clinical significance, and future implications. Journal of Antimicrobial Chemotherapy. 37(3): 403-421.
20. Kubmarawa, D., Ajoku, G. A., Enwerem, N. M., Okorie, D. A. 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. African Journal of Biotechnology. 6(14): 1690–1696
21. Loru, F., Duval, D., Aumelas, A., Akeb, F., Guedon, D., Guedj, R., 2000. Four steroidal alkaloids from the leaves of *Buxus sempervirens*. Phytochemistry. 54: 951-957.
22. Lv, F., Liang, H., Yuang, Q., Li, C., 2011. *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four-related microorganisms. Food Research International. 44: 3057–3064.
23. Mabona, U., and Van Vuuren, S.F., 2013. Southern African medicinal plants used to treat skin diseases. South African Journal of Botany. 87: 175–193
24. Manimozhi, D.M., Sankaranarayanan, S., Sampathkumar, G., 2012. Evaluating the antibacterial activity of flavonoids extracted from *Ficus benghalensis*. International Journal of Pharmaceutical and Biological Research. 3(1): 7-18.
25. Masoko, P., 2013. Ethnobotanical study of some selected medicinal plants used by traditional healers in Limpopo Province (South Africa). *A*merican Journal of Research Communication. *1*(8): 15-17.
26. Matochko, W. L., James, A., Lam, C. W., Kozera, D. J., Ata, A., Gengan, R. M. 2010. Triterpenoidal alkaloids from *Buxus natalensis* and their acetylcholinesterase inhibitory activity. Journal of Natural Products. 73(11): 1858-1862.

27. Meshkatalsadat, M.H., Mollataghi, A., Ata, A., 2006. New triterpenoidal alkaloids from *Buxus hyrcana.* Zeitschrift Fuer Naturforschung B. 61: 201-206.

28. Meyer, J.J.M. and Afolayan, A.J., 1995. Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). *Journal of Ethnopharmacology*, *47*(2):109-111.

29. Mmushi, T., Masoko, P., Mde, L., Mokgotho, M., Mampuru, L. J., Howard, R., 2010. Antimycobacterial evaluation of fifteen medicinal plants in South Africa. African Journal of Traditional, Complementary and Alternative Medicines. 7(1): 34-39.

30. Motsei, M.L., Lindsey, K.L., van Staden, J., Jager, A.K., 2003. Screening of traditionally used South African plants for antifungal activity against *Candida albicans.* Journal of Ethnopharmacology. 86: 235-241.

31. Moosavy, M. H., Basti, A. A., Misaghi, A., Salehi, T. Z., Abbasifar, R., Mousavi, H. A. E., 2008. Effect of *Zataria multiflora Boiss*, essential oil and nisin on *Salmonella typhimurium* and *Staphylococcus aureus* in a food model system and on the bacterial cell membranes. Food Research International. 41: 1050–1057.

32. Nostro, A., Germano, M.P., D'Angelo, V., Marino, A., Cannatelli, M.A., 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in applied Microbiology. 30(1): 379-384.

33. O'Bryan, C.A., Crandall, P.G., Ricke, S.C., 2008. Organic poultry pathogen control from farm to fork. Foodborne Pathogen and Diseases. 5: 709–720.

34. Pooley, E., 1993. The complete guide to trees of Natal, Zululand and Transkei. Durban: Natal Flora Publication Trust.

35. Runyoro, D.K.B., Ngassapa, O.B., Matee, M.I.N., Joseph, C.C., Moshi, M.J., 2006. Medicinal plants used by Tanzanian traditional healers in the management of *Candida* infections. Journal of Ethnopharmacology. 106: 158-165.

36. Shai, L.J., Magano, S.R., Lebelo, S.L., Mogale, A.M., 2011. Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. Journal of Medicinal Plant Research. 5(13): 2863-2867.

37. Wanger, A., Mills, K., Nelson, P.W. and Rex, J.H., 1995. Comparison of Etest and National Committee for Clinical Laboratory Standards broth macrodilution method for antifungal susceptibility testing: enhanced ability to detect amphotericin B-resistant Candida isolates. *Antimicrobial Agents and Chemotherapy*, *39*(11): 2520-2522.

38. Watanakunakorn, C., 1981. The antibacterial action of vancomycin. Review of Infectious Diseases. 3 (supplementary): 210-215.

39. Zaidan, M.R., Noor Rain, A., Badrul, A.R., Adlin, A., Norazah, A. and Zakiah, I., 2005. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop biomed*, *22*(2):165-70.

# CHAPTER 3

# THE *IN VITRO* CYTOTOXICITY OF *BUXUS MACOWANII*, *SCILLA SP.*, *POLYGALA MYRTIFOLIA* AND *XANTHOCERCIS ZAMBESIACA*

## 3.1. Introduction

Most indigenous medicinal plants in Africa are still widely used in unprocessed form, without any scientific validations for the safety and efficacy thereof (Fennell et al., 2004; Ndhlala et al., 2013). There are approximately 1 000 known poisonous plant species, containing 750 poisonous substances, which occur in 150 000 plant secondary metabolites (Wink and Van Wyk, 2008). When traditional medicine is used for the treatment of ailments, misidentification, incorrect preparation and incorrect dosage may result in poisoning (Fennel et al., 2004). Poisoning is common in South Africa, and it is estimated that approximately 8 000 to 20 000 deaths occur from poisoning on an annual basis. The forensic database for Johannesburg revealed that, from 1991 to 1995, 43% of poisoning resulted from the usage of traditional medicine (Thomson, 2000). The current rate of mortality is expected to be higher moreover; most of the cases remain unrecorded, as the use of traditional medicine is usually kept secret (Stewart and Steenkamp, 2000; Thomson, 2000).

Plants that are used as traditional medicine have and are still presumed to be safe to use in any way, as they have been used for the treatment of different ailments for a long time. However, research indicates that most plants that are used as traditional medicine or food are potentially toxic, mutagenic and carcinogenic (Kassie et al., 1996; De Sa Ferrira and Ferrao Vargas, 1999). The toxicity of plants is attributed to the concept that plants produce compounds or substances that prevent infections by bacteria and viruses but at the same time these substances can be toxic to humans, even when consumed in small quantities (Wink and Van Wyk, 2008). Secondary metabolites, such as alkaloids, saponins, terpenes, phenolic compounds and many more of these compounds that have human health benefits, can also be poisonous.

Saponins are known to have hypolipidemic and anticancer activity; however, they can cause toxic effects such as haemolysis and cattle poisoning (Kar, 2007). Alkaloids are known to have antibacterial, anticancer, antimalarial, antiasthma and antiarrhythmia activity, but higher doses can cause death, cardiac or respiratory arrest, mutations, and even cancer (Wink, 2009; Kuete and Efferth, 2010; Wansi et al., 2013). Phenolic compounds such as hydroquinone have shown mutagenic potency, while natural phenolics have been reported to have antimicrobial, antioxidant, antiviral, anti-inflammatory and vasodilatory effects (Roza et al., 2003; Chen et al., 2010). With the forementioned compounds present in most of the plants that are used in traditional medicine, it is unsafe to assume that these plants can be used for the treatment of diseases without regulatory measures.

*Punica granatum L.* has been used to enhance fertility, but if used without any regulations, it can be toxic, causing disturbances in vision, spasms, nausea, vomiting, gastroenteritis, convulsions and death (Wink and Van Wyk, 2008; Lee et al., 2010). The leaves of the *Aloe ferox* can be used as a laxative and emetic; for the relief of arthritis, sinusitis, conjunctivitis and opthalmia for healing skin and wounds; and in the treatment of sexually transmitted infections. However, the plant can also cause intestinal bleeding, enhanced menstrual and uterus bleeding, kidney disturbance, and hypertrophy of intestinal tissues (Kambizi et al., 2007; Wink and Van Wyk, 2008).

Plants such as *B. macowanii*, *P. myrtifolia*, *Scilla sp.* and *X. zambesiaca* are often used for the treatment of different ailments, but information about their toxic effects on human cells, mode of administration, and administration dosages is scarce. It is against this background that the cytotoxicity of extracts from these plants was studied. In this chapter, the cytotoxicity of *B. macowanii, P. myrtifolia, Scilla sp. and X. zambesiaca* against WI-38 (normal fetal lung fibroblast cells) is presented using the Sulforhodamine cytotoxicity assay.

## 3.2. Methods

**3.2.1.** Extracts of *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* were prepared in the same manner described under 2.2.2 in Chapter 2 (see page 29).

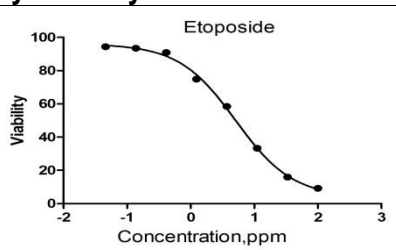
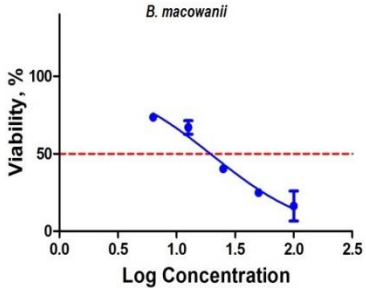
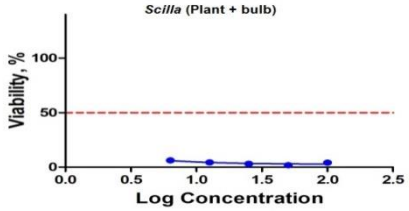
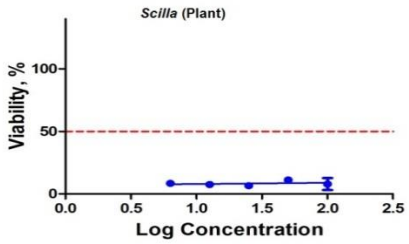
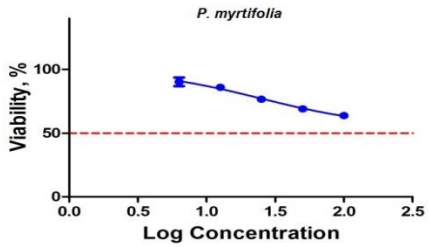
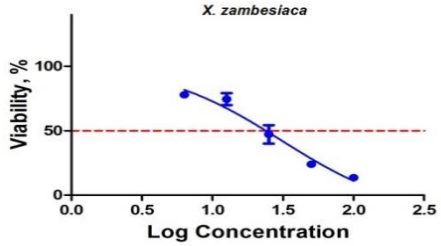
### 3.2.2. The Sulforhodamine cytotoxicity assay

The Sulforhodamine B (SRB) assay was conducted to evaluate the toxicity of the extracts of *B. macowanii, Scilla sp., P.myrtifolia and X. zambesiaca* against the WI-38 (normal human fetal lung fibroblast) cell line, as described by Itharat et al. (2004). The method is based on the ability of the protein dye Sulforhodamine B to bind electrostatically to (Potential hydrogen) pH dependent protein-basic amino acid residues of trichloroacetic acid-fixed cells. The WI-38 cell line from the European Collection of Cell Culture (ECACC) were maintained at 37ºC, 5% carbon dioxide $CO_2$, 95% air and 100% relative humidity as a monolayer cell culture in Eagle's Minimal Essential Medium (EMEM), supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 50 µg/ml gentamicin. For screening processes, the cells (21-50 passages) were inoculated in 96-well microtiter plates at a plating density of 10 000 cells/wells, and incubated for 24 hours. After 24 hours, the cells were treated with the plant extracts at five different concentrations, ranging from 6.25 to 100 µg/ml (five x two-fold serial dilutions), and were incubated for another 48 hours (Skehan et al., 1990). The untreated cells served as a control, the blank contained the medium without cells, and etoposide was used as a standard at concentrations ranging from 100 µg/ml to 0.05 µg/ml (eight x three-fold serial dilutions) (Wellington and Kolesnicova, 2012). After 48-hours incubation, viable cells were fixed to the bottom of each well with 50% cold trichloroacetic acid, washed, dried and dyed by SRB. The protein-bound dye was removed with 10 mM Tris base, and the optical density determined at a wavelength of 540 nm, using a multiwell spectrophotometer. The $IC_{50}$ was considered as the 50% cell growth inhibition, and it was determined by non-linear regression. GraphPad Prism software was used to perform data analysis and create the graphs.

### 3.3. Results and discussion

The bulb, roots and entire plant extracts of *Scilla sp.* showed toxicity against the human fetal lung fibroblast cells, with an $IC_{50}$ less than 6.25 µg/ml, even at lower concentrations. The extracts decreased the viability of the normal fetal fibroblast cell (Table 3.1.). These results correlate with the results published by Sparg et al. (2000), who confirmed that *Scilla natalensis,* which belongs to the *Scilla* genus, was cytotoxic, and should be used with caution as herbal medicine. The toxicity can be attributed to the presence of steroids, cardiac glycosides and alkaloids (Van Wyk et al., 1997). According to Council for Scientific and Industrial Research (CSIR) criteria, the extracts of *B. macowanii* showed moderately hazardousness against the WI-38 cell line, as they showed an $IC_{50}$ of less than 30 µg/ml and greater than 5 µg/ml. As indicated by the graph in Table 3.1, results confirmed that the extracts of *B. macowanii* had lower toxicity at lower concentrations, but proved to be toxic at higher concentrations, as the cell viability increased to 50% at 20.4 µg/ml. A number of articles have reported that plants belonging to the *Buxus* genus have an abundance of steroidal alkaloids (Loru et al., 2000; Atta-ur-Rahman et al., 2001). The steroidal alkaloids found in *B. macowanii* were indicated to have various biological properties such as anti-Human Immune Virus (HIV), antimalarial, anti-Tuberculosis (TB) properties, to mention only a few (Babar et al., 2006; Ata and Andersh, 2008). Despite the fact that alkaloids can be associated with the aforementioned good biological properties, it has been reported that alkaloids can be toxic to humans and animals; therefore caution must be taken in consumption (Allgaier and Franz, 2015). *X. zambesiaca* showed moderately hazardousness at a concentration of 22.96 µg/ml, whereas *P. myrtifolia* exhibited low toxicity against the WI-38 cell line, as the cell viability did not decrease as the concentration of the extract increased, therefore, it can be suggested that *P. myrtifolia* is safer to use.

**Table 3.1:** The $IC_{50}$ of plant extracts of *B.macowanii, Scilla sp., P.myrtifolia* and *X.zambesiaca*

| Plant extract | $IC_{50}$ (µg/ml) | Cytotoxicity |
|---|---|---|
| Etoposide (standard) | 5.1 |  |
| *Buxus macowanii* | 20.4 |  |
| *Scilla sp. (bulb and roots)* | <6.25 |  |
| *Scilla sp. (entire plant)* | <6.25 |  |
| *P. myrtfolia* | 22.96 |  |
| *X. zambesiaca* | >100 |  |

### 3.4. Conclusion

This chapter investigated the toxicity of different plant extracts of *B. macowanii*, *P. myrtifolia*, *Scilla sp.* and *X. zambesiaca*. With many people relying on plants as traditional medicine for their health care, toxicity to humans is one of the major factors that must be taken into consideration. The above-mentioned plant extracts are usually used in traditional medicine to treat ailments without hospital supervision; therefore, it was necessary to highlight or investigate their toxicity to human cells. Although plants such as *Scilla, B. macowanii* and *X. zambesiaca* have been highlighted as toxic to WI-38, it does not mean their use should be prohibited. Rather, care should be taken in their consumption. Structure-related activity studies and chemical-modification experiments that will reduce toxicity and maintain activity of the plant extracts can be employed, confirming that these plants are the best candidates in manufacturing of new alternative drugs.

### 3.5. References


1. Allgaier, C., and Franz, S., 2015. Risk assessment on the use of herbal medicinal products containing pyrrolizidine alkaloids. Regulatory Toxicology and Pharmacology. 73: 494-500.
2. Ata, A., Andersh, B.J., 2008. The Alkaloids, vol. 66. Elsevier Science Publishers, San Diego, CA: 191–213.
3. Atta-ur-Rahman, Parveen, S., Khalid, A., Farooq, A., Choudhary, M.I., 2001. Acetyl and butyrylcholinesterase-inhibiting triterpenoid alkaloids from *Buxus papillosa.* Phytochemistry. 58: 963-968.
4. Babar, Z.U., Ata, A., Meshkatalsadat, M.H., 2006. New bioactive steroidal alkaloids from *Buxus hyrcana*. Steroids. 71: 1045–1051
5. Chen, H., Yao, J., Wang, F., Zhou, Y., Chen, K., Zhuang, R., 2010. Toxicity of three phenolic compounds and their mixtures on the gram-positive bacteria *Bacillus subtilis* in the aquatic environment. Science of the total Environment. 408(5): 1043-9.
6. De Sã Ferrira, I.C.F., Ferrão Vargas, V.M., 1999. Mutagenicity of medicinal plant extracts in salmonella/microsome assay. Phytotherapy Research. 13: 397–400.
7. Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology. 94:205–217.
8. Itharat, A., Houghton, P.J., Eno-Amooquaye, E., Burke, P.J., Sampson, J., Raman, A., 2004. In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. Journal of Ethnopharmacology. 90: 33–38.
9. Kambizi, K., Goosen, B.M., Taylor, M.B., Afolayan, A.J., 2007. Anti-viral effects of aqueous extracts of *Aloe ferox* and *Withania somnifera* on herpes simplex virus type 1 in cell culture. South African Journal of Science. 103 (9-10): 359-360.

10. Kar, A., 2007. Pharmaocgnosy and Pharmacobiotechnology (Revised-Expanded Second Edition). New Age International Limited Publishers New Delhi: 332-600.
11. Kassie, F., Parzefall, W., Musk, S., Johnson, I., Lamprecht, G., Sontag, G., Knasmueller, S., 1996. Genotoxic effects of crude juices from Brassica vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. Chemico-Biological Interactions. 102: 1 -16.
12. Kuete, V., and Efferth, T., 2010. Cameroonian medicinal plants: pharmacology and derived natural products. Front Pharmacology. 1: 123.
13. Lee, C.J., Chen, L.G., Liang, W.L. and Wang, C.C., 2010. Anti-inflammatory effects of *Punica granatum Linne in vitro* and *in vivo*. Food chemistry. 118(2): 315-322
14. Loru, F., Duval, D., Aumelas, A., Akeb, F., Guedon, A., Guedj, R., 2000. Four steroidal alkaloids from the leaves *Buxus sempervirens.* Phytochemistry. 54: 951-957.
15. Ndhlala, A.R., Ncube, B., Okem, A., Mulaudzi, R.B. and Van Staden, J., 2013. Toxicology of some important medicinal plants in southern Africa. *Food and chemical toxicology*, *62*: 609-621.
16. Roza, L., De Vogel, N. and Van Delft, J.H.M., 2003. Lack of clastogenic effects in cultured human lymphocytes treated with hydroquinone. Food and chemical toxicology. 41(10):1299-1305.
17. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S. and Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, *82*(13): 1107-1112.
18. Sparg, S.G., van Staden, J., Jager, A.K. 2000. Efficiency of tradition-ally used South African plants against schistosomiasis. Journal of Ethnopharmacology. 73: 209-214.
19. Stewart, M.J., Steenkamp, V., 2000. Toxicology of African herbal remedies. South African Ethnobotany. 1: 32–33.
20. Thomson, S.A., 2000. South African government genocide and ethnopiracy. The Gaia Research Institute, 12 April 2002.

21. Van Wyk, B., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza, Pretoria.
22. Wansi, J.D., Devkota, K.P., Tshikalange, E., Kuete, V., 2013. Alkaloids from medicinal plants of Africa. Medicinal plants research in Africa. 14: 557-605.
23. Wink, M., Van Wyk, B.-E., 2008. Mind-Altering and Poisonous Plants of the World. Briza Publications, Pretoria.
24. Wink, M., 2009. Mode of action and toxicology of plant toxins and poisonous plants. Wirbeltierforschung in der Kulturlandschaft. 421: 93-12.

# CHAPTER 4

# PHYTOCHEMICAL CONTENT OF THE EXTRACTS OF *B. MACOWANII, P. MYRTIFOLIA, SCILLA SP.* AND *X. ZAMBESIACA*

## 4.1. Introduction

Plants produce a large number of bioactive compounds of selective and survival importance. These bioactive compounds possess important therapeutic properties for use in the treatment and management of diseases by producing physiological actions in the human body (Mitscher et al., 1987; Reayi and Arya, 2005; Edeoga et al., 2005; Akinpelu et al., 2008). Recently, there is great interest in medicinal plants, as the pharmaceutical industry considers them as a great option in the provision of a large number of leads that are essential in the production of new drugs (Soejarto, 1999).

The most important bioactive compounds in plants are alkaloids, tannins, saponins, flavonoids, steroids and phenolic compounds (Hill, 1952; Kubmarawa, 2007). Plants containing such bioactive compounds are usually used by traditional healers, and are known to have antimicrobial, anthelmintic, antiviral, antifungal, anti-inflammatory and antioxidant activity (McGaw et al., 2000). Alkaloids made up of ammonia compounds, containing a nitrogen base, are the largest group of secondary metabolites (Doughari, 2012). There are more than 12 000 alkaloids that exist or are present in approximately 12% of plant species. In combination with vegetable acids, alkaloids are usually present in the seeds and roots of plants. Alkaloids make up most of the drugs used pharmacologically as anaesthetics and central nervous system (CNS) stimulants (Madziga et al., 2010).

Other important chemical compounds are polyphenols that are responsible for the pigmentation and protection of plants from herbivores and pathogens. Polyphenolics are used in the control of human pathogenic infections, and as antioxidants and anticancer agents (Kar, 2007). Secondary metabolites,

such as flavonoids, are an important group of polyphenols. They are made of C15 aromatic compounds, and traces of them are usually found in many plant flora (Doughari, 2012). They have been recognised as having a protective effect against microbial invasion in plants, which could explain their positive effect in protecting humans from microbial invasion (Horborne and William, 2000; Doughari, 2012). Isolated flavonoids are known to possess biological activities such as antifungal and antibacterial activities (Sathiamoorthy et al., 2007). Terpenes are another important class of secondary compounds that are made from isoprene units. They can occur as monoterpenes, diterpenes, triterpenes, tetraterpenes and sesquiterpenes. Terpenes have important biological activities, such as antimicrobial activities, against *Candida* species (Alves et al., 2013). Other secondary metabolites with antimicrobial activity include phenols and steroids; further indicating the potential of plants and their phytochemicals in the treatment of infectious diseases.

The contribution of plants in the fight against diseases is indicated by the fact that an estimated 25% of prescribed drugs world-wide are manufactured from plants (Gurib-Fakim, 2006). Examples of antimicrobial drugs that were manufactured from plants include the fluoroquinolone and quinolone antibiotics that are used to control malaria, which were derived from alkaloid quinine that was isolated from a fever tree called *Cinchona succirubra* (Demain and Sanchez, 2009). Arteether is an antimalarial drug that was derived from artemisinin, which was isolated from a plant called *Artemisia annua*(Graul, 2001). Secondary metabolites have contributed significantly to the control of infections, the development of the pharmaceutical world, and an increase in the life expectancy of people world-wide (Lederberg, 2000).

The screening of natural products is of vital importance, as it assists in the discovery of novel active compounds, and the efficacy and safety of their use (Shihabudeen et al., 2010; Ncube et al., 2008). The study of phytochemicals also assists in establishing the authenticity of plants, especially when the

plants are not easily identifiable, and documentation of such information can be useful even in the future. Hence, in this chapter, a phytochemical study of the methanol extracts of *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* was conducted using Thin Layer Chromatography (TLC) fingerprinting, Gas Chromatography Mass Spectrometry (GCMS) and standard phytochemical screening methods.

## 4.2. Methods

### 4.2.1. Extract preparation

The extracts of *B. macowanii, P. myrtifolia, Scilla* sp and *X. zambesiaca* were prepared in the same manner as described under 2.2.2 in Chapter 2 (see page 29).

### 4.2.2. Phytochemical analysis of the plant extract

The extracts were subjected to phytochemical screening tests for tannins, flavonoids, alkaloids, saponins, steroids and phenols (Kubmarawa et al., 2007) using standard methods.

#### 4.2.2.1. Detection of tannins and phenols

The dried powder of the plant extract was boiled in 20 ml of water in a test tube, whereafter it was filtered. A few drops of 0.1% ferric chloride were added, and observed for a brownish-green or a blue-black colouration (Sofowara, 1993; Ayoola et al., 2006).

#### 4.2.2.2. Detection of steroids

The plant extract was dissolved in 3 ml of $CHCl_3$ and filtered. Concentrated $H_2SO_4$ was added to the filtrate to form a lower layer. A reddish-brown colour was taken as a positive indication of a steroid ring (Igwe et al., 2007).

#### 4.2.2.3. Detection of flavonoids

The flavonoids were tested using *The Alkaline Reagent Test,* where the extracts were subjected to a few drops of sodium hydroxide solution. The formation of an intense yellow colour, which becomes colourless when a dilute acid is added to it, indicates the presence of flavonoids (Tiwari et al., 2011).

#### 4.2.2.4. Detection of alkaloids

Alkaloids were detected using the Dragendroff's test, where the extracts were individually dissolved in water and filtered. The filtrates were treated with Dragendroff's reagent (a solution of potassium Bismuth Iodide). The formation of a red or yellow precipitate indicates the presence of alkaloids (Doughari, 2012).

#### 4.2.2.5. Detection of saponins

The extracts were dissolved in distilled water, and made up to 20mL. The suspension was shaken in a graduated cylinder for 15 minutes. A two-centimetre layer of foam indicated the presence of saponins (Kokate, 1999; Ayoola et al., 2006).

#### 4.2.2.6. Test for carbohydrates

One ml of the Fehling's reagent A and B were mixed; the solution was shaken, and then boiled for 1 minute. An equal volume of the test solution was added into the mixture and heated in a boiling water bath for 5 – 10 minutes. The formation of a red precipitate indicated the presence of carbohydrates (Boxi et al., 2010).

#### 4.2.2.7. Test for terpenes (Salkowski test)

The extracts were mixed in 2 ml of chloroform, and 3 ml of concentrated $H_2S0_4$ was carefully added to the mixture to form a layer. A reddish-brown colouration of the interface indicated positive results for the presence of terpenoids (Edeoga et al., 2005).

#### 4.2.2.8. Test for glycosides (Keller-kiliani test)

The filtrate of the extracts was mixed with 1ml of a mixture glacial acetic acid, ferric chloride and concentrated $H_2SO_4$**.** A green-blue colour indicated the presence of glycosides (Parekh and Chanda, 2007).

### 4.2.3. TLC fingerprinting

Chemical components of plant extracts were separated or eluted using aluminium-backed,thin layer chromatography plates and solvent systems Ethyl acetate/Methanol/Water (40:5.4:5)(polar/neutral), Chloroform/Ethylacetate/Formic acid (5:4:1) (intermediate polarity/acidic), Benzene/Ethanol/Ammonium hydroxide (18:2:0.2) (Kotze et al., 2002) (non-polar/basic), and Toluene/Acetone/Ethanol (28.5:57:14.5) because they are usually used to isolate compounds that show antimicrobial properties (Loru et al., 2000). The separated chemical compounds were detected by spraying acidified vanillin (0.1g vanillin: 28ml methanol: 1ml Sulfuric acid) on the TLC plates where after it was heated at 110 ºC in an incubator to allow colour development (Suleiman et al., 2010). The plates were viewed under ultraviolet (UV) light at a wavelength of 354 nm.

### 4.2.4. Gas chromatography mass spectrometry (GCMS)

The dried extracts were dissolved in ethyl acetate, samples were then centrifuged and analysed by GCMS using an Agilent 6890N linked to a Mass Detector 5975B. Total ion chromatograms (TIC) and their associated spectra were acquired in full scan mode using the Chemstation software linked to the

GCMS. Peaks and their associated spectra were then searched using the Wiley 375 Mass Spectral Database. Due to the complexity of the chromatograms, there is a certain degree of spectral overlap. To overcome this, Automated Mass Spectral Deconvolution and Identification Software (AMDIS) was used to separate multiple overlapping spectra lying within single chromatographic peaks.

### 4.3. Results and discussion

#### 4.3.1. Phytochemical screening (Standard methods)

The results of the phytochemical screening of *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* using standard methods are indicated in Table 4.1. The presence of secondary metabolites such as alkaloids may suggest that the use of *B. macowanii* might be a good option for the production of antimalarial, anticancer, antibacterial (see Chapter 2) and antihypertensive drugs (Wansi et al., 2013). The presence of tannins may also indicate that *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* can be potential sources of antioxidant, antitumor, antiviral and antimicrobial agents (Haslam, 1996; Kar, 2007). The presence of flavonoids and phenolics also contribute to anti-inflammatory and antioxidant activity (Kar, 2007, Walch et al., 2011). Traces of saponins also indicate that *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* can be considered for the manufacturing of antifungal, anticancer, anti-inflammatory, immune-stimulating and antithrombotic agents (Morrissey and Osbourn, 1999; Lacalle-Dubois and Wagner, 2000). The presence of saponins was also identified in the aqueous extracts of *P. myrtifolia,* which were also found to have antifungal properties (Bruneton, 1995). Terpenes tested positive in all the plant extracts and are known for antimicrobial properties, such as antiprotozoal, anthelmintic and antifungal activities (Doughari, 2012; Rubio et al., 2013). The test for cardiac glycosides was positive only *B. macowanii*, suggesting its potential in the treatment of heart diseases (Doughari, 2012). The medicinal effects of plants can be attributed to the activity of a single compound, or to a synergism of compounds (Eloff et al., 2008).

**Table: 4.1.** Phytochemical content of the plant extracts

| *Plant extract* | Flavonoids | Tannins | Saponins | Phenols | Alkaloids | Steroids | Carbohydrates | Terpenes | Cardiac glycosides |
|---|---|---|---|---|---|---|---|---|---|
| *Scilla sp. (entire plant)* | + | + | + | + | - | + | + | + | - |
| *Scilla (bulb and roots)* | + | + | + | + | - | + | - | + | - |
| *Polygala myrtifolia* (Leaves and twigs) | + | + | + | + | - | - | + | + | - |
| *Buxus macowanii (Leaves and twigs)* | + | + | + | + | + | + | + | + | + |
| *Xanthocercis zambesiaca (Stem)* | + | + | + | + | - | + | + | + | - |

**(+) means presence of secondary metabolites**

**(-) means absence of secondary metabolites**

### 4.3.2. TLC fingerprinting

The phytochemical analysis of the plant extracts using TLC fingerprinting after the elution of the compounds resulted in different colour changes due to their reaction with the spray reagent used (Vanillin/Sulfuric acid) (Figure 4.1). The spray reagent (Vanillin/Sulfuric acid) is usually used to detect terpenes and phenylpropanoids. Plants such as *B. macowanii* and *Scilla sp.* revealed violet spots after being eluted with the solvent system Benzene/Ethanol/Ammonia hydroxide (BEA), Chloroform/Ethyl acetate/Formic acid (CEF) and Toluene/Chloroform/Ethanol (TCE). Diterpenes exhibit a violet colour on the chromatograms when sprayed with Vanillin/Sulfuric acid. Therefore, it can be suggested that diterpenes can also be found in extracts of *B. macowanii* and *Scilla* extracts. Research has shown that diterpenes can inhibit cancer multiplicity, and therefore plant extracts that indicated the presence of diterpenes can be used to manufacture anticancer agents (Martinez et al., 2008). Rf values were not indicated because it was a qualitative evaluation of compounds.

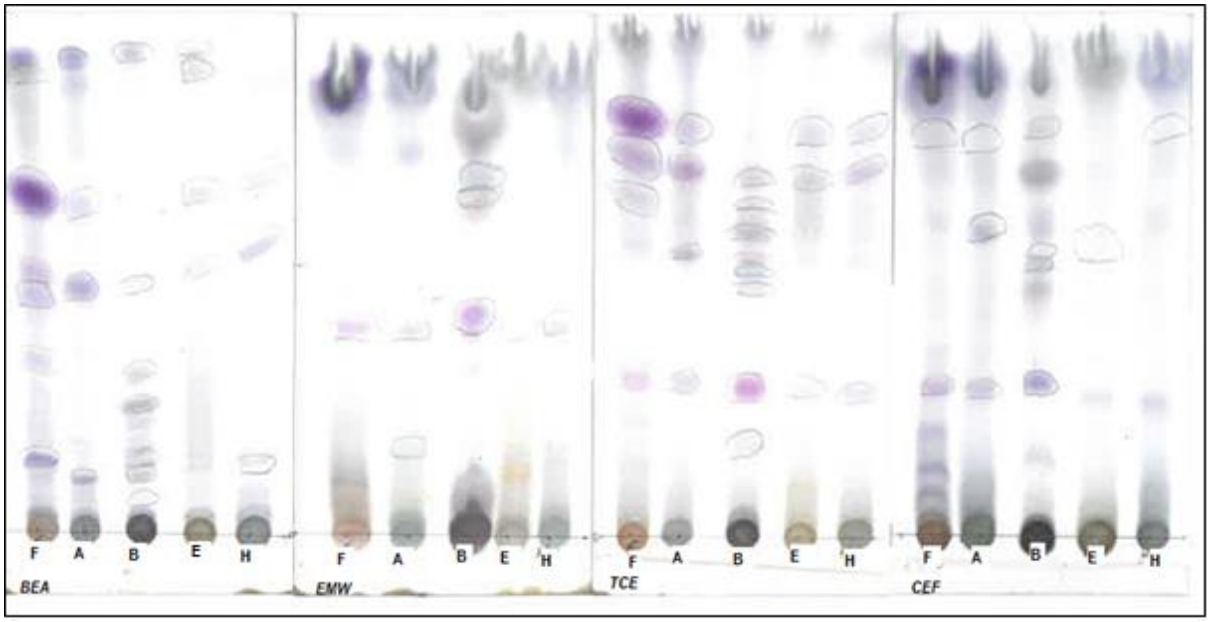



**Figure 4.1:** Vanillin-sprayed TLC chromatograms of the methanol extracts developed in Benzene/Ethanol/Ammonia hydroxide: 90:10:1 (BEA), Ethyl acetate/Methanol/Water: 40:5.4:4 (EMW), Toluene/Chloroform/Ethanol: 28.5:57:14.5 (TCE), and Chloroform/Ethyl acetate/Formic acid: 5:4:1

(CEF). F = *B. macowanii,* A = *Scilla sp.* (entire plant), B = *Scilla sp.* (roots and bulb), E = *P. myrtifolia,* and H = *X. zambesiaca*.

### 4.3.3. Gas chromatography mass spectrometry (GCMS)

GCMS analysis of the plant extracts was conducted using the National Institution of Standard Technology (NIST) software and the Wiley Online Library. The identification of the compounds was based on the molecular weight, peak area and molecular formula, and only prominent peaks were selected for identification. A compound was only regarded as positively identified when the percentage of similarities was more than 90%. See annexure A for total ion chromatograms of suggested compounds detected from plant extracts of *B. macowanii, Scilla sp*, *P. myrtifolia* and *X. zambesiaca*. The GCMS analysis of the leaves and twigs of the extracts of *B. macowanii* showed that neophytadiene was detected at a retention time of 10.92 (as indicated in Table 4.2), and it has been proposed to have some antimicrobial activities (Inoue et al., 2005). Neophytadiene has also been reported for the treatment of headaches, rheumatism and some skin diseases (Suresh et al., 2010). It has been reported that other plants from the genus *Buxus,* such as *B. papillosa,* is used to treat rheumatism and skin disorders (Cordell, 1981). It can be suggested that the presence of neophytadiene may be the reason why *B. macowanii* and *B. papillosa* have the ability to treat such ailments. The antimicrobial activity (see Chapter 2) of *B. macowanii* can also be explained by the presence of neophytadiene in its extracts. Therefore, it suggests that the plant can be an option for use in the manufacturing of antimicrobial drugs and other remedies used to treat headaches, rheumatism and skin conditions.

The GCMS analysis of the plant extracts of *Scilla sp.* indicated a detection of a number of compounds, such as hexadecanoic acid, which was detected at a retention time of 11.8. A study conducted by Joshi-Barve et al., (2007) reported that palmitic acid can cause an increase in levels of biologically active neutrophil chemoattractant, IL-8, from hepatocytes that can potentially cause hepatic

inflammation and liver injury. The findings suggest that the reckless use of *Scilla sp.* can be potentially hazardous. The GCMS analysis of the roots, bulb and plant extracts of *Scilla sp.* indicated the detection of n-hexadecanoic acid at a retention time of 11.8, as indicated in Table 4.2. N-Hexadecanoic acid is a compound that is known to have inflammatory activity; therefore, it can also be considered as potential compound for use in the manufacturing of anti-inflammatory drugs (Aparna et al., 2012).

The GCMS analysis of the leave and twig extracts of *P. myrtifolia* indicated the presence of 2-methoxy-4-vinylphenol at a retention time of 6.87. 2M4VP has been reported to inhibit cancer proliferation and to have anti-inflammatory activity (Jeong and Jeong, 2010; Jeong et al., 2011). Anti-inflammatory activity has also been reported in other species in the *Polygala* genus, such as in *P. japonica* and *P. cyparissias* (El Sayah et al., 1999; Kou et al., 2005; Kou et al., 2006). It is safe to say that *P. myrtifolia* can be considered as the best option for use in the manufacturing of anti-inflammatory and anticancer agents, because of the presence of 2M4VP. The GCMS analysis of the stem extracts of *X. zambesiaca* indicated the presence of 2M4VP at a retention time of 6.87. n-Hexadecanoic acid was also detected at a retention time of 11.8. The findings also suggest that *X. zambesiaca* can be considered an option for use in the manufacturing of anticancer and anti-inflammatory agents.

**Table 4.2: The GCMS analysis of five plant extracts**

| Plant extract | Suggested Compounds | Retention time | Molecular formula | Molecular weight |
|---|---|---|---|---|
| ***Buxus macowanii* (Leaves and Twigs)** | Neophytadiene | 10.92 | $C_{20}H_{38}$ | 278.5 |
| ***Scilla sp.* (Whole plant)** | Hexadecanoic acid | 11.8 | $C_{16}H_{32}O_2$ | 256.4 |

| | | | | |
|---|---|---|---|---|
| ***Scilla sp.* (roots and bulbs)** | n-Hexadecanoic acid | 11.8 | $C_{16}H_{32}O_2$ | 256.4 |
| ***Polygala myrtifolia (Leaves and twigs)*** | 2-methoxy-4-vinylphenol | 6.87 | $C_9H_{10}O_2$ | 150.2 |
| ***X. zambesiaca (Stem)*** | 2-methoxy-4-vinylphenol | 6.87 | $C_9H_{10}O_2$ | 150.2 |
| | n-Hexadecanoic acid | 11.8 | $C_{16}H_{32}O_2$ | 256.4 |

### 4.4. Conclusions

The phytochemical screening of *B. macowanii, Scilla sp., P. myrtifolia* and *X. zambesiaca* extracts indicated that they contain secondary metabolites that are of great importance for the pharmaceutical industry. Secondary metabolites, such as alkaloids, flavonoids, polyphenolics, saponins, steroids, and terpenes, are present in most of the tested extracts and biological properties have made researchers to search for new drug leads in them. Alkaloids, flavonoids, polyphenolics, steroids, terpenes, neophytadiene, n-hexadecanoic acid, 2MV4P contain properties that assist with the treatment of diseases, such as antimicrobial, anticancer, anti-inflammatory activities, etc. The phytochemical screening results suggest that *B. macowanii, Scilla sp.*, *P. myrtifolia* and *X. zambesiaca* are plants that can be used to manufacture novel drugs for the treatment of infectious diseases.

## 4.5. References


1. Akinpelu, D.A., Adegboye, M.F., Adeloye, O.A., and Okoh, A.I., 2008. Biocidal activity of partially purified fractions from methanolic extracts of *Garcinia kola (Heckel)* seeds on bacterial isolates. Biological Research. 41: 277-287.
2. Alves, M.J., Ferreira, I.C.F.R., Dias, J., Teixeira, V., Martins, A., Pintado, M., 2013. A review on antifungal activity of mushroom (basidiomycetes) extracts and isolated compounds. Current Topics Medicinal Chemistry. 13: 2648–2659.
3. Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C., Haridas, M., 2012. Anti-inflammatory properties of n-Hexadecanoic acid: Structural evidence and Kinetic assessment. Chemical Biology and Drug Design. 80: 434-439.
4. Ayoola, G.A., Sofidiya, T., Odukoya, O., Coker, H.A.B., 2006. Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. Journal of Pharmaceutical Science & Pharmacy Practice. 8: 133-136.
5. Boxi, M., Ragesh, Y., Raja, Kumar, V., Praveen, B., Mangamma, K., 2010. Extraction, phytochemical screening and *in-vitro* evaluation of anti-oxidant properties of *Commicarpus chinesis* (aqueous leaf extract). International Journal of Pharma Biosciences. 1: 537-547.
6. Bruneton, J., 1995. Terpenoids and steroids. *J. Bruneton. Lavoisier Technique and Documentation, Paris*: 385-621. Cordell, G.A., 1981. Introduction to alkaloids: A Biogenetic Approach. Wiley-Interscience, New York.
7. Cordell, G. A., 1981. Introduction to alkaloids: A biogenetic approach. New York: Wiley.
8. Demain, A.L., and Sanchez, S., 2009. Microbial drug discovery: 80 years of progress. The Journal of Antibiotics. 62: 5–16.
9. Doughari, J.H., 2012. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. ISBN: 978-953-51-0296-0.

10. Edeoga, H.O., Okwu. D.E., and Mbaebie, B.O., 2005. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 4: 685-688.
11. Eloff, J.N., Katerere, D.R., McGraw, L.J., 2008. The biological activity and chemistry of the southern African Combretaceae. Journal of Ethnopharmacol. 119: 686-699.
12. El Sayah, M., Cechinel Filho, V., Pinheiro, T.R., Yunes, R.A., Calixto, J.B., 1999. *In vitro* effect of the extract and the 1, 7-dihydroxy-2, 3- dimethoxy xanthone from *Polygala cyparissias* on the contractions induced by inflammatory mediators and albumin in normal and actively sensitized trachea from guinea pig. Inflammation Research. 48: 218-223.
13. Graul, A.I., 2001. The year's new drugs. Drug News and Perspectives. 14 (1): 12–31.
14. Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine. 27: 1–93.
15. Hill, R.T., 1952. On discontinuous plastic states, with special reference to localized necking in thin sheets. Journal of the Mechanics and Physics of Solids, *1*(1): 19-30.
16. Haslam, E., 1996. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. Journal of Natural Products. 59: 205-215.
17. Harborne, J.B., and Baxter, H., 1999. The handbook of natural flavonoids, Vols 1 and 2. Chichester, UK: John Wiley and Sons.
18. Igwe, C.U., Nwaogu, L.A., and Ujuwandu, C.O., 2007. Assessment of the hepatic effects, phytochemical and proximate composition of *Phyllanthus amarus*; African Journal Biotechnology. 6: 728-731.
19. Inoue, Y., Hada, T., Shiraishi, A., Hirose, K., Hamashima, H., Kobayashi, S., 2005. Biphasic effects of gernylgeraniol, teprenone, and phytol on the growth of *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy. 49: 1770–1774.

20. Jeong, J. B., and Jeong, H. J., 2010. 2-methoxy-4-vinylphenol can induce cell cycle arrest by blocking the hyper-phosphorylation of retinoblastoma protein in benzo[a]pyrene-treated NIH3T3 cells. Biochemical and Biophysical Research Communications. 400: 752–757.

21. Jeong, J.B., Hong, S.C., Jeong, H.J., Koo, J.S., 2011. Anti-inflammatory effect of 2-methoxy-4-vinylphenol via the suppression of NF-kB and MAPK activation, and acetylation of histone H3. Achieves of Pharmacal Research. 34 (12): 2109-2116.

22. Joshi-Barve, S., Barve, S.S., Amancherla, K., Gobejishvili, L., Hill, D., Cave, M., Hote, P., McClain, C.J., 2007. Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. Hepatology. 46(3).

23. Kar, A., 2007. *Pharmaocgnosy and Pharmacobiotechnology* (Revised-Expanded Second Edition). New Age International Limited Publishers New Delhi. pp 332-600.

24. Kokate, C.K., 1999. Practical Pharmacology. 4th edn. Vallabh Prakashan Publication, New Delhi, India.

25. Kotze, M., Eloff, J. N., Houghton, P. J. 2002. Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae). South African Journal of Botany. *68*(1): 62-67.

26. Kou, J., Sun, Y., Lin, Y., Cheng, Z., Zheng, W., Yu, B. and Xu, Q., 2005. Anti-inflammatory activities of aqueous extract from Radix *Ophiopogon japonicus* and its two constituents. *Biological and Pharmaceutical Bulletin*, *28*(7): 1234-1238.

27. Kou, J., Si, M., Dai, G., Lin, Y., Zhu, D., 2006. Anti-inflammatory activity of *Polygala japonica* extract. Fitoterapia. 77: 411-415.

28. Kubmarawa, D., Ajoku, G. A., Enwerem, N. M., Okorie, D. A., 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. African Journal of Biotechnology. 6(14): 1690-1696.

29. Lacalle-Dubois, M.A., Wagner, H., 2000. Bioactive saponins from plants: an update. In: Atta-Ur-Rahman (Ed.), Studies in Natural Products Chemistry Series. 21: 633–687.

30. Lederberg, J., 2000. Infectious history. Science. 288: 287–293.

31. Loru, F., Duval, D., Aumelas, A., Akeb, F., Guedon, D., and Guedj, R., 2000. Four steroidal alkaloids from the leaves of *Buxus sempervirens*. Phytochemistry. 54: 951-957.

32. Madziga, H.A., Sanni, S., and Sandabe, U.K., 2010. Phytochemical and Elemental Analysis of *Acalypha wilkesiana* Leaf. Journal of American Science. 6(11): 510-514.

33. Martinez, M.J.A., Lazaro, R.M., del Olmo, L.M.B., Benito, P.B., 2008. Anti-infectious activity in the Anthemideae tribe. Studies in Natural Products Chemistry. 35: 445-516.

34. McGaw, L.J., Jager, A.K., van Staden, J., 2000. Antibacterial, antihelminthic and antiamoebic activity in South African medicinal plants. Journal of Ethnopharmacol. 72: 247–263.

35. Mitscher, L.A., Drake, S., Gollapudi, S.R., and Okwute, S.K., 1987. A modern look at the folkloric use of anti-infective agents. Journal of Natural Products. 50: 1025-1040.

36. Morrissey, J.P., Osbourn, A.E., 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Micribiology and Molecular Biology Reviews. 63: 708–724.

37. Ncube, B., Finnie, J.F. and Van Staden, J., 2008. Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. *South African Journal of Botany*, *77*(2): 387-396.

38. Parekh, J., and Chanda, S., 2007. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research. 10: 175 – 181.

39. Reayi, A., and Arya, P., 2005. Natural product-like chemical space: Search for chemical dissectors of macromolecular interactions. Current Opinion in Chemical Biology. 9: 240-247.

40. Rubió, L., Motilva, M.J., Romero, M.P., 2013. Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles. Critical Reviews in Food Science and Nutrition. 53: 943–953.

41. Sathiamoorthy, B., Gupta, P., Kumar, M., Chaturvedi, A. K., Shukla, P. K., Maurya, R., 2007. New antifungal flavonoid glycoside from *Vitex negundo*. Bioorganic and Medicinal chemistry Letters. 17(1): 239-242.

42. Shihabudeen, M.S., Priscilla, H.H., and Thirimurugan, D.K., 2010. Antimicrobial activity and phytochemical analysis of Indian folk medicinal plants. International Journal of Pharma Sciences and Research. 1(10): 430-434.

43. Soejarto, D.D., 1999. Biodiversity prospecting and benefit-sharing: perspective from the field. Journal of Ethnopharmacology. 51: 1-15.

44. Sofowara, A., 1993. Medicinal plants and traditional medicines in Africa. Chichester John Wiley & Sons New York: 97-145.

45. Suleiman, M.M., McGaw, L.J., Naidoo, V., Eloff, J.N., 2010. Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. African Journal of Traditional Complementary and Alternative Medicines. 7 (10): 64-67.

46. Suresh, L., Veerabah, R. M., Gnanasingh, S. R., 2010. GC-MS analysis of ethanolic extract of *Zanthoxylum rhetsa (roxb.)* dc spines. Journal Herbal Medicine and Toxicology. 4: 191-192.

47. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H., 2011. Phytochemical screening and extraction: a review. Internationale Pharmaceutica Sciencia. 1(1): 98-106.

48. Walch, S.G., Tinzoh, L.N., Zimmermann, B.F., Stühlinger, W., Lachenmeier, D.W., 2011. Antioxidant capacity and polyphenolic composition as quality indicators for aqueous infusions of *Salvia officinalis L.* (sage tea). Frontiers in Pharmacology. 2: 1–6.

49. Wansi, J.D., Devkota, K.P., Tshikalange, E., Kuete, V., 2013. Alkaloids from the medicinal plants of Africa. Medicinal Plant Research in Africa: 557-605.

# CHAPTER 5

# GENERAL DISCUSSION AND CONCLUSIONS

## 5.1. General discussion

World-wide, medicinal plants are important for the treatment of diseases and are potential sources of novel drugs that can overcome the challenges associated with the drugs that are currently available, such as drug-resistant pathogens, toxicity and inaccessibility. *B. macowanii*, *Scilla species, P. myrtifolia* and *X. zambesiaca* are traditionally used for the treatment of various ailments, such as rheumatism, skin disorders, high sugar concentrations, headaches, respiratory problems and gastrointestinal problems. The above-mentioned traditional uses classify these plants as major contributors to the health care of people who cannot afford or access modern medicine. With all the challenges pertaining to the treatment of infectious diseases and the traditional use of the selected plants, it was necessary to investigate the antimicrobial activity of the selected plants. The results from the study are summarised in Table 5.1 below.

**Table 5.1:** A summary of the results obtained in the study

| **Plant extract** | **Antimicrobial activity** | **Phytochemical screening** | **Cytotoxicity (CSIR Criteria) IC50,µg/ml** |
|---|---|---|---|
| ***B. macowanii (Leaves and Twigs)*** | **+** | Standard methods: Alkaloids, steroids, flavonoids, terpenes, carbohydrates, tannins and cardiac glycosides<br>Thin Layer Chromatography fingerprinting: Diterpenes<br>Gas Chromatrophy Mass Spectrometry: Neophytadiene | Moderate hazard<br>< 30<br>>5 |

| | | | |
|---|---|---|---|
| ***P. myrtifolia (Leaves and Twigs)*** | - | Standard methods: Alkaloids, tannins, taponins, phenols, carbohydrates and terpenes<br>TLC fingerprinting: Negative<br>GCMS: 2-methoxy-4-vinylphenol | Low hazard<br>> 100 |
| ***Scilla sp.* (Whole Plant)** | - | Standard methods: Flavonoids, tannins, phenols, steroids, carbohydrates and terpenes<br>TLC fingerprinting: Diterpenes<br>GCMS: Hexadecanoic acid | High hazard<br><5 |
| ***Scilla sp. (Roots and Bulbs)*** | - | Standard methods: Flavonoids, tannins, saponins, phenols, steroids and terpenes<br>TLC fingerprinting: Diterpenes<br>GCMS: n-hexadecanoic acid | High hazard<br><5 |
| ***X. zambesiaca (Stem)*** | - | Standard method: Flavonoids, tannins, saponins, phenols, steroids, carbohydrates and terpenes<br>TLC fingerprinting: Negative<br>GCMS: 2-methoxy-vinylphenol and n-hexedecanoic acid | Moderate hazard<br>< 30<br>>5 |

(+) Activity

(-) No activivity

The extracts of *Buxus macowanii* showed antimicrobial activity against *S. aureus*, *C. perfringens*, *P. aeriginosa*, *S. epidermidis, C. albicans* and *C. tropicalis* at Minimum Inhibitory Concentration (MIC) of 2.5 mg/ml, and against *E. faecalis* and *E. coli* at an MIC of 1.2mg/ml. *B. macowanii* was the only plant

that showed growth inhibition of all bacterial and fungal species against which it was tested; for this reason, it was selected for further studies. This study is the first to report on the antimicrobial activity of *B. macowanii. P. myrtifolia, Scilla sp and X. zambesiaca* did not show any inhibitory effects against the tested bacterial and fungal species, although *P. myrtifolia* was reported to have antibacterial and antifungal activities (Lall and Meyer, 1999; Motsei et al., 2003). The inactivity of the plant extracts of *X. zambesiaca* and *P. myrtifolia* may be due to the choice of the solvent of extraction and the age of the extracts.

*B. macowanii* was further investigated using the TLC bioautography method discussed in **Chapter 2,** for purposes of identifying and locating the antimicrobial compounds against the most affected bacteria (*E. faecalis*). Clear zones on the chromatogram indicated that *B. macowanii* has antimicrobial compounds. Alkaloid and flavonoid spots were located in close proximity to the antimicrobial spots of the extracts of *B. macowanii*. Therefore, it can be suggested that flavonoids and alkaloids contributed positively to the antimicrobial activity of the extracts of *B. macowanii*. The effect of the extracts of *B. macowanii* on the morphology of bacteria (*B. cereus*) was also studied using the scanning electron microscope and the transmission electron microscope (see **Chapter 2)**. The cells of *B. cereus* indicated abnormal morphological properties after treatment with the extracts of *B. macowanii,* such as a distortion of the cell membrane, leakage of the cytoplasmic contents and separation of the cell membrane from the cell wall. Most plants that belong to the genus Buxus have been reported to contain steroidal alkaloids. Steroidal alkaloids are known to cause cell-membrane disruption, which also leads to cytoplasmic content leakage (Wink and Twardowski, 1992; Wink and Latz-Bruning, 1995).The morphological properties of bacterial cells that were observed under microscopy after treatment with *B. macowanii* can be attributed to the presence of steroidal alkaloids. *B. macowanii* can be considered as the best option to use for the manufacturing of novel antimicrobial drugs that can overcome the challenges associated with the remedies that are currently available.

Plants such as *B. macowanii, P. myrtifolia, Scilla sp. and X. zambesiaca* are generally used by traditional healers to treat various ailments. However, these traditional healers often do not have sufficient knowledge of the plants' toxicity to human cells. Therefore, it is necessary to ensure that the plants are safe to be used by humans. The cytotoxicity of the methanol extracts of *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* was tested against the WI-38 cell line (Normal human fetal lung fibroblast) using Sulforhodamine cytotoxicity assay (see Chapter 3), the results of which are summarised in Table 5.1. According to CSIR criteria, the extracts of *B. macowanii* and *X. zambesiaca* exhibited moderate hazardous toxicity against the WI-38 cell line with 20.4 µg/ml and 22.96 µg/ml $IC_{50}$ respectively. The bulb, root and entire plant extracts of *Scilla sp.* showed high-hazard toxicity against the WI-38 cell line, with both less than 6.25µg/ml $IC_{50}$. The leave and twig extracts of *P. myrtifolia* showed low-hazard toxicity or no toxicity against the WI-38 cell line, according to the Council for Scientific and Industrial Research (CSIR) criteria. The compounds such as steroids, cardiac glycosides and alkaloids (Van Wyk et al., 1997) found in the plant extracts may have contributed to the toxicity that the extracts exhibited against the WI-38 cell lines. Although plant extracts such as *B. macowanii*, *Scilla sp.* and *X. zambesiaca* exhibited toxicity against the WI-38 cell line, their use in the treatment of ailments should not be prohibited; rather, caution must be applied by regulating their administration dosage. Structure activity studies and chemical modification experiments that will reduce toxicity and maintain the activity of the plant extracts can also be employed, so that the plants can be the best option for consideration in the manufacturing of new drugs.

The methanol extracts of *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* were subjected to a screening of the most important phytochemicals, such as the phenols, flavonoids, alkaloids, terpenes, cardiac glycosides, tannins, saponins and steroids (see Chapter 4). The phytochemical screening was conducted by using the standard methods, TLC and GCMS, the results of which are summarised in

Table 5.1. The leaves and twigs of *B. macowanii* were found to contain all the phytochemicals screened for, and may explain why the extracts showed antimicrobial activity against all the microbial species against which it was tested. The presence of diterpenes in the extracts of *B. macowanii, P. myrtifolia, Scilla sp.* and *X. zambesiaca* was tested using TLC (see Chapter 4). The separated compounds were detected using acidified vanillin. *B. macowanii* and bulb, root and entire plant extracts of *Scilla* sp showed the presence of diterpenes on the chromatograms after being eluted by the solvent systems Benzene/Ethanol/Ammonia hydroxide (BEA), Chloroform/Ethyl acetate/Formic acid (CEF) and Toluene/Chloroform/Ethanol (TCE). GCMS managed to isolate and identify very important compounds from the extracts of *B. macowanni, P. myrtifolia, Scilla sp.* and *X. zambesiaca.* Neophytadiene was detected from the extracts of *B. macowanii.* The compound was reported to have antimicrobial activities, and it can be used to treat headaches, rheumatism and skin disorders (Inoue et al., 2005). N-hexadecanoic acid was also identified in the extracts of *Scilla sp.* and *X. zambesiaca,* which suggests that the extracts may be good options for the manufacturing of anti-inflammatory agents. 2M4VP is another compound that was identified in the extracts of *P. myrtifolia* and *X. zambesiaca*. 2M4VP is known for its anticancer and anti-inflammatory activities. According to the CSIR criteria, *Scilla* sp. extracts were reported to have toxicity against human fetal lung fibroblasts (see Chapter 3), and the phytochemical screening revealed that the extracts contain Palmitic acid (see Chapter 4). Palmitic acid has been reported to be a causative agent of hepatic inflammation and liver injury; therefore, the findings could suggest that palmitic acid contributed to the toxicity potential that *Scilla sp.* extracts exhibited against WI-38 (see Chapter 3). The overall phytochemical screening of the plant extracts indicated that they contain important compounds that can provide leads to the manufacturing of new drugs that can overcome the problems that are associated with the agents that are currently available.

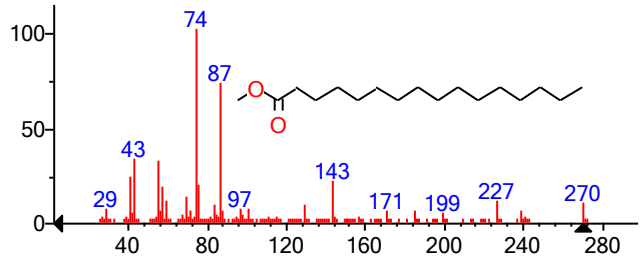
## 5.2. Conclusions and recommendations

These findings suggest that, to date, plants can still be considered as an important source of medicine for the treatment of various diseases. *B. macowanii* excellently showed that it can be the best option to use in the manufacturing of antimicrobial agents. However, isolation and identification of antimicrobial compounds using solvents such as diethylamine must be considered for future studies. Although plant extracts of *P. myrtifolia, Scilla sp.* and *X. zambesiaca* did not show inhibition against the bacterial species against which they were tested, phytochemical screening revealed that these plants possess other important pharmacological properties, based on the compounds that were identified in them.

## 5.3. References

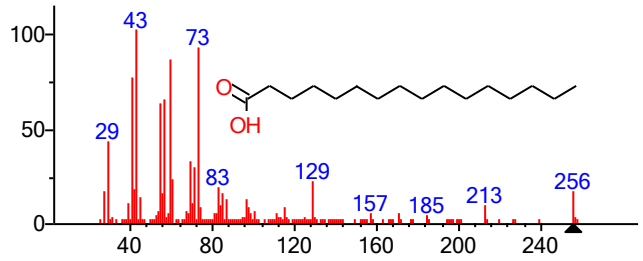

1. Lall, N., and Meyer, J.J., 1999. In vitro inhibition of drug-resistant and drug-sensitive strains of Mycobacterium tuberculosis by ethnobotanically selected South African plants. Journal of Ethnopharmacology. 66: 347-354.
2. Motsei, M.L., Lindsey, K.L., van Staden, J., Jager, A.K., 2003. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. Journal of Ethnopharmacology. 86: 235–241.
3. Inoue, Y., Hada, T., Shiraishi, A., Hirose, K., Hamashima, H., Kobayashi, S., 2005. Biphasic effects of gernylgeraniol, teprenone, and phytol on the growth of *Staphylococcus aureus*, Antimicrobial Agents and Chemotherapy. 49: 1770–1774.
4. Van Wyk, B., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza, Pretoria.
5. Wink, M., and Latz-Bruning, B., 1995. Allelopathic properties of alkaloids and other natural products: possible modes of actions, in: Allelopathy: Organisms, processes and Applications. ACS. Symo.Serv. 582: 117-126.
6. Wink, M., and Twardowski, T., 1992. Allelochemical properties of alkaloids. Effects on plants, bacteria and protein biosynthesis. In: Allelopathy: Basic and Applied Aspects. Chapman and Hall, London: 129-150.

## Annexure A



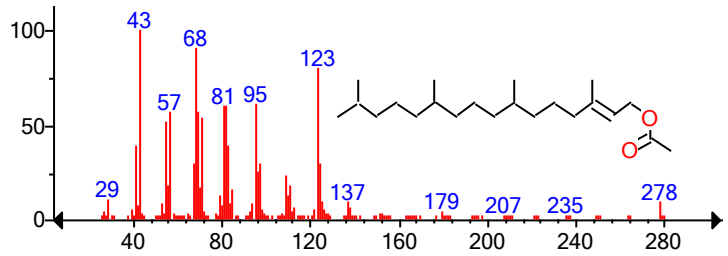

(nistdemo) Hexadecanoic acid, methyl ester

Total ion chromatogram of hexadecanoic acid that was detected in *Scilla sp.*



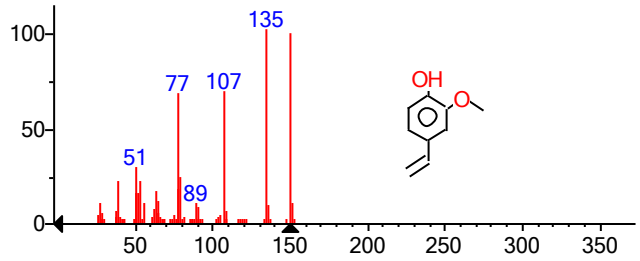

(nistdemo) n-Hexadecanoic acid

Total ion chromatogram of n-hexadecanoic acid detected from extracts of *Scilla sp.* and *X. zambesiaca*




(mainlib) Phytol, acetate

Total ion chromatogram of Phytol, actate or Neophytadiene detected in extracts of *Buxus macowanii*




(mainlib) 2-Methoxy-4-vinylphenol

Total ion chromatogram of 2-Methoxy-4-vinylphenol detected in extracts of *P. myrtifolia* and *X. zambesiaca*

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