THE CYTOTOXIC EFFCTS, ANTI-INFLAMMATORY, ANTIOXIDANT, ANTIBACTERIAL, AND ANTIDIABETIC PROPERTIES OF EIGHT SELECTED SOUTH AFRICAN PLANTS FOR MEDICINAL PURPOSES

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A thesis submitted in the partial fulfilment of the requirements of the degree of Doctor of Philosophy in the Department of Physiology,
School of Laboratory Medicine and Medical Sciences,
University of KwaZulu-Natal

2020

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An investigation into anti-inflammatory, antioxidant, antimicrobial, antidiabetic properties, and cytotoxicity effects of selected South African plants for medicinal purposes.

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Printed in Durban, South Africa 2020

LIST OF PAPERS

I **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T. (2019). A review on selected African medicinal plants with effectiveness in the management of type II diabetes mellitus. *Acta Scientific Pharmaceutical Sciences*, **8** (3): 47-54.

II **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T**.** (2019). The *in vitro* evaluation of some South African plant extracts for minimum inhibition concentration and minimum bactericidal concentration against selected bacterial strains. *International Journal of Scientific and Research Publications*, **9** (7): 996-1004.

III **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T**.** (2020). The evaluation of cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 and RAW 264.7 cells. *International Journal of Scientific and Research Publications*, **10** (2): 207-220.

IV **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T**.** (2020). Estimation of the preliminary screening of phytochemicals, antioxidant, and alpha-amylase inhibitory activity of the selected South African plant extracts for medicinal purposes. *International Journal of Pharma and Bio Sciences* (**in pres**).

CONFERENCE AND MEETING PRESENTATIONS

I **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T. (2020). "The evaluation of cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 and RAW 264.7 cells." *Presented at the 4th International conference on Traditional Medicine Pharmacy and Pharmaceutical Sciences, 13 – 14 December 2019, Bangkok, Thailand*.

II **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T. (2019). "The *in vitro* evaluation of some South African plant extracts for minimum inhibition concentration and minimum bactericidal concentration against selected bacterial strains." *Presented at the 1st Vaal University of Technology, 03 – 05 April 2019, Vanderbijlpark, Gauteng, South Africa*.

DECLARATION

I **Bongani Alphouse Nkala** declare that:

i. The research reported in this thesis, except where otherwise indicated, and is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain other person's data, pictures, graphs or information, unless specifically acknowledged as being source from other person.

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	18 March 2020	**Durban**
Mr. Bongani A. Nkala	Date	Place

	19 March 2020	**Durban**
Supervisor: Dr. Hlengiwe P. Mbongwa	Date	Place

	19 March 2020	**Johannesburg**
Co-supervisor: Dr. Tozama Qwebani-Ogunleye	Date	Place

ACKNOWLEDGEMENTS

I give glory to God Almighty for his loving care, support, and guidance in my journey. To my beloved mother **Mrs Thandi Geneveva Nkala** who has schooled me about life and generally installed God in me. Her constant support in all walks of life and career will never be forgotten. It is absolutely crucial to express my sincere gratitude to **Dr. Hlengiwe P. Mbongwa** and **Dr. Tozama Qwebani-Ogunleye** for mentoring, supervisory role and most of all in ensuring that all resources needed for the research are made available. Furthermore, their intellectual contribution and support in all forms to ensure that this degree is attainable will never be forgotten.

Friends and family are thanked for their support, more especially **Ms. Zinhle Rejoice Gasa, Mr Stanley Makgere, Mr Mduduzi Khoza, Dr Nkosana Jafta,** and **Ms. Nolwazi Nduli,** and my dearest sister, **Mrs Thembelihle Magwaza** for being there for me in troubled moments and also during my celebration in achieving milestones along the journey. To my special friend, **Mr. David Imoh Akpan** your moral support in my hardship won't go unnoticed.

It is imperative to convey sincere gratitude to **Prof Vivienne Russel** and **Mr. Preen Nadioo** for their proofreading this thesis. **Mr. Thulani Vuyo Mannie** and **Mr. Tshepiso Lentswe** are thanked for their assistance with sample collection. A special thanks to the College Health Science Scholarship Grant at the University of KwaZulu-Natal, Institute of Traditional Knowledge and Traditional Medicine at the Vaal University of Technology, the National Research Foundation and Thuthuka grant for financial support received towards this study.

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LIST OF ABBREVIATIONS

ABTS	2,2'-azinobis (-ethylbenzthiazoline-6-sulphonic acid
ATM	African traditional medicine
BHT	Butylated hydroxyoluene
CO_2	Carbon dioxide
CV	Crystal violet
DCM	Dichloromethane
DM	Diabetes mellitus
DMEM	Dulbecco's modified eagle's minimum
DNA	Deoxyribonucleic acid
DNSA	3,5-dintro salicylic acid
DPP-4	Dipeptidyl peptidase – 4
DPPH	2,2-diphenyl-1-picryhdrazyl
EDTA	Ethylenediaminetetraacetic acid
FBS	Foetal bovine serum
$FeCl_3$	Ferric chloride
GAE	Gallic acid equivalent
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HCL	Hydrochloride acid
IC	Inhibition concentration
IDDM	Insulin-depend diabetes mellitus
LC	Lethal concentration
LPS	Lipopolysaccharide
MBC	Minimal bactericidal concentration
MDR	Multi-drug resistance
MeOH	Methanol
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistance *Staphylococcus aureus*
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
NIDDM	Non-insulin-dependent diabetes mellitus
NO	Nitric oxide

NSAIDs	Non-steroidal anti-inflammatory drugs
SEM	Standard error
T2DM	Type II diabetes mellitus
TFC	Total flavonoids content
TPC	Total phenolic content

ABSTRACT

People from the Southern African region have been using the fauna and flora of the region in their homes for millennia to treat all sorts of ailments and complaints with great success. This knowledge transfer was done through 'apprenticeships' and oral communication. Certain communities consider medicinal plants to be safer than drugs and that they can treat more than one ailment. This study investigated cytotoxic effect, antimicrobial, anti-inflammatory, antioxidant, and antidiabetic properties of eight selected South African plants for medicinal purposes. Plant species were collected from the Walter Sisulu National Botanical-Gardens and were extracted with 90% methanol (1 g/10 ml) and concentrate to 10 mg/ml. Antimicrobial activities were determined by the microplate dilution method to establish the ability of the plant extracts to inhibit or kill pathogenic organisms with minimal inhibitory concentrations and minimum bactericidal concentration. Cytotoxicity effects were determined with Alamar blue and crystal violet cell viability assays, against C2C12 and RAW 264.7 cells. Anti-inflammatory effects were identified with stimulated lipopolysaccharide RAW 264.7 cells, and nitric oxide inhibition was measured with Griess reagent assay. The estimation of preliminary phytochemical, antioxidant (DPPH and ABTS radical scavenging), and alpha-amylase inhibition were determined with standard methods. The plant extracts inhibition and bactericidal effects were observed against all bacteria, namely: *Lippia javanica* (0.25±0.00 to 1.13±0.29 mg/ml); *Ziziphus mucronata* leaf (0.44±0.00 to 1.00±0.00 mg/ml); *Erythrina lysistemon* (0.44±0.00 to 1.08±0.00 mg/ml) *and Schkuhria pinnata* (0.5±0.00 to 1.34±0.00 mg/ml). All plant extracts exhibited flavonoids, phenols, terpenoids, and coumarins. The antioxidant inhibition was observed above 80% for *Schkuhria pinnata, Lippia javanica, Clerodendrum myricoides,* and *Erythrina lysistemon*. Also, these plant species exhibited an alpha-amylase inhibitory effect of 80%. The IC_{50} values were > 1000 µg/ml. All plant extracts demonstrated some degree of an anti-inflammatory effect. However, *Clerondendrum myricoides* (35% - 89%), *Lippia javanica* (26% - 77%), *Erythrina lysistemon* (23% - 76%), *Schkuhria pinnata* (27% - 65%), and *Vernonia oligocephala* (16% - 58%) with IC_{50} value >1000 µg/ml, exhibited a marked anti-inflammatory effect. Therefore, the presence of phenolic, flavonoids, anti-inflammatory, antioxidants, and α-amylase properties are potential solutions towards the management of diabetes and other chronic inflammatory diseases.

Keywords: medicinal plants, antimicrobial, cytotoxicity, anti-inflammatory, antidiabetic, antioxidant

CHAPTER ONE: INTRODUCTION

1.1 Introduction

The use of medicinal plants dates back to ancient times for the treatment of various ailments (Petrovska, 2012; Yuan *et al*., 2016). Medicinal plants play an important role as a source of natural ingredients for preserving human health (Sofowora *et al*., 2013). Additionally, medicinal plants have been evaluated for various purposes such as the production of flavours, fertilizers and fragrances (Dar *et al*., 2017). Researchers have recently focused on the evaluation of medicinal plants' ability to treat and manage various aliments (Anand *et al*., 2019; Street & Prinsloo, 2013). The search for plant with medicinal properties has given way to some ethnobotanical studies that have documented indigenous plant species, the mode of preparation, and uses by endemic communities in some parts of Uganda (Tabuti *et al*., 2010; Tugume *et al*., 2016).

Although medicinal plants have been used for centuries, their safety and efficacy were not reported in earlier literature (Mahomoodally, 2013; Petrovska, 2012; Ekor, 2014). A medicinal plant consists of secondary metabolites that contribute to therapeutic properties, such as antioxidant, anti-microbial, antidiabetic, anti-inflammatory, anti-cancer, immune-modulatory and reno-protection effects (Londonkar *et al*., 2013; Tungmunnithum *et al*., 2018). Recently, more researchers have been investigating the respective therapeutic and phytochemical properties of medicinal plants (Wintola & Afolayan, 2015; Egamberdieva, 2016). Eight plant species were selected in this study for their use in African traditional medicine (ATM) as shown in **Table 1**. These selected plants were justified in a review and their medicinal uses clearly articulated (Nkala *et al*., 2019). There is a need to evaluate plants with antidiabetic and anti-inflammatory activities for the treatment and management of diabetes mellitus and other cardiovascular diseases.

Table 1: Medicinal uses, photochemistry, and biological activities of plants of interest

FAMILY NAME	BOTANICAL NAME	MEDICINAL USE	BIOLOGICAL ACTIVITY
Euclea crispa	Ebenaceae	Microbial infections and cough	Antimicrobial activity and antifungal
Euclea natalensis	Ebenaceae	Blood system disorders, digestive system disorders, genitourinary system disorders, infections and infestations	Antibacterial, antifungal, antiviral, antidiabetic, antioxidant, anti-plasmodia, larvicidal, antischistosomal, and molluscicidal
Schkuhria pinnata	Asteraceae	Stomach problems, mastitis and eye infections.	Antibacterial, anti-inflammatory and anti-diarrhea
Ziziphus mucronata	Rhamnaceae	Skin infections, wounds, body pains, infertility in women; boils, sores and swellings.	Anthelmintic and antimicrobial activities
Lippia javanica	Verbenaceae	Cold, cough, fever, malaria, wounds, diarrhea, chest pains, bronchitis, and asthma.	Antimicrobial activity, anticancer, anti-amoebic, antidiabetic, antimalarial, antimicrobial, antioxidant, anti-plasmodia and pesticide effects
Vernonia oligocephala	Asteraceae	Stomachic and bitter tonic	Antimalarial and antiviral
Clerodendrum myricoides	Lamiaceae	Gonorrhea, rabies, measles, glandular TB, colic, eye disease, malaria, body swellings, wound dressings and hemorrhoids.	Antimicrobial activity and antifungal.
Erythrina lysistemon	Fabaceae	Wounds, analgesic and anti-inflammatory.	Antioxidant properties, anti-plasmodia, anti-inflammatory, antibacterial and fungicidal

1.2 Background to the study

Medicinal plants are also referred to as medicinal herbs; they play a pivotal role in ATM, and were used in ancient times. Plants possess secondary metabolites such as phytochemicals with a biological activity used for the treatment and management of diabetes mellitus (DM) and cardiovascular diseases.

- African traditional medicine (ATM) is referred to as knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses.
- Phytochemicals are chemicals that are derived from plants through primary or secondary metabolites.
- Cardiovascular diseases generally refer to conditions that involve narrowed or blocked blood vessels that can lead to a heart attack, chest pain (angina) or stroke.
- Diabetes mellitus is commonly known as diabetes. It is a metabolic disorder characterised by a high blood sugar level over a prolonged period.

There are three types of diabetes.

1) Type 1 diabetes results from the pancreas' failure to produce enough insulin due to the loss of beta cells. It is referred to as insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes.
2) Type 2 diabetes begins with insulin resistance, which is a condition in which cells fail to respond to insulin properly. This is referred to as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes.
3) Gestational diabet**es** occurs **during** pregnancy when a pregnant woman without a previous history of diabetes develops high blood sugar levels.

1.3 Problem statement

From 1980 to 2014 the global prevalence of DM jumped with a staggering 314 million cases (108 million to 422 million) according to a study done by the World Health Organisation (WHO, 2016). DM is a metabolic disorder characterised by hyperglycaemia due to a defect in insulin production and/or resistance by the cells (Rajalakshmi *et al*., 2009). It was estimated that DM would increase from 4% in 1995 to 5.4% in 2025 (Bears *et al*., 2004). In South Africa, the prevalence of DM in adults is on the rise. In the short space of nine years, DM prevalence increased from 5.5% in 2000 to 9% in 2009 (Bertram *et al*., 2013; Pheiffer *et al*., 2018).

On average, in 2009 a diabetic patient paid R9,000 more for hospitalisation costs than non-diabetic patients as reported in a study, namely, R27,000/2,250 USD and R18,000/1,500 USD, respectively (Manyema *et al*., 2015; Ncube-Zulu & Danckwerts, 2004). This is the global context where the total health expenditure is projected to be between 1.1 to 2 billion USD in 2030 (Zhang *et al*., 2010). Poor management of DM can lead to neurological, cardiovascular, retinal, and renal complications (Rahmatullah *et al*., 2012).

Plants used for medicinal properties in the fight against DM have been shown to alleviate one or more symptoms (Rahmatullah *et al*., 2012). A total cure for the disease still eludes the medical fraternity though (Rahmatullah *et al*., 2012). Medicinal plants have been shown to be effective in delaying complications of diseases and rectifying metabolic abnormalities *(*Shukia *et al*., 2000). The use of plants for medicinal purposes has the advantage of having few or no side effects *(*Shukia *et al*., 2000).

1.4 Aims

This study aimed at investigating the cytotoxicity effects, anti-inflammatory, antioxidant, antimicrobial, and antidiabetic properties of selected South African plants for medicinal purposes. Essentially, plant species that demonstrate one of the following properties, namely, antidiabetic, antioxidants and anti-inflammatory, could be useful towards the treatment and management of DM and other cardiovascular diseases. Furthermore, plant species should be none toxic to be fit for human use.

1.5 Objectives

There were three objectives in this study.

(i) To evaluate eight selected South African plant extracts for minimum inhibitory concentration and minimum bactericidal concertation against selected four bacterial strains.

(ii) To evaluate the cytotoxicity and anti-inflammatory effects of eight selected South African medicinal plants against C2C12 cells and RAW 264.7 cells.

(iii) To determine whether the preliminary screening of phytochemicals, antioxidants and alpha-amylase inhibitory activities of the selected eight South African plant extracts could be pertinent for medicinal purposes.

1.6 Research questions

1. Do the selected plants possess either bactericidal or bacteriostatic actions?
2. How many of the selected plant species possess anti-inflammatory effects?
3. Do the selected plants possess any cytotoxicity effects against C2C12 cells and RAW 2647?
4. How many phytochemicals can be found in the selected plant species?
5. Which plant species consists of the highest total phenolic content and total flavonoid content?
6. Do the selected plant species possess either antioxidant or alpha-amylase inhibition effects?

1.7 Significance of the study

ATM has been used since ancient times for the treatment of various ailments (Atanasov *et al*., 2015). ATM has been reported to be used by a lot of people in Africa as primary healthcare (Abdullahi, 2011). Communities strongly believe that ATM is much safer compared to conventional drugs (Sofowora *et al*., 2013; Deutschländer *et al*., 2009). However, very few medicinal plants have been scientifically validated for their safety and efficacy (Mahomoodally, 2013). Recently, more researchers have developed an interest in the scientific validity of ATM, and this has been triggered by drug resistance that has been a growing problem with conventional drugs *(*Zhang *et al*., 2018; Mahomoodally, 2013; Mills *et al*., 2005). Essentially, a search for alternative medicine is imperative. Medicinal plants have been shown to contribute meaningfully to the pharmacology industry toward drug discovery (Atanasov *et al*., 2015; Leonti & Casu, 2013).

Developments have focused on drug discovery due to secondary metabolites found in medicinal plants that can be used for the treatment and management of malaria, cancer, diabetes, cardiovascular diseases, and neurological affliction (Pan *et al*., 2013; Ginsburg & Deharo, 2011). Interestingly, the selected plant species have been reported for their use in ATM by traditional healers, but their safety and efficiency are lacking (Nkala *et al*., 2019).

This study aimed to make a significant contribution toward the body of knowledge in terms of the ability of the selected medicinal plants possessing the following properties: antioxidant, anti-inflammatory, antidiabetic, and antimicrobial capabilities. Importantly, plant species must not be toxic to ensure their safety. A medicinal plant that possesses these bioactive serves as a good candidate for the treatment and management of diabetes and other cardiovascular diseases (Gothai *et al*., 2016; Choudhury *et al*., 2017).

1.8 Research methodology

1.8.1 Sample collection and extraction

Plant species (n=8) were collected from the Walter Sisulu National Botanical Gardens in South Africa in February 2017. The voucher specimen number is held at the Walter Sisulu National Botanical Gardens herbarium. The plant material was air-dried in a well-ventilated room. After drying, the plants were grounded into a powder and stored away from light at room temperature.

1.8.2 The determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The microplate dilution method was used to determine the ability of plant extracts to inhibit or kill pathogenic organisms with minimal inhibitory concentrations (MICs) and minimal bactericidal concentration (MBC), which was developed by Eloff (1998).

1.8.3 The evaluation of the cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 cells and RAW 264.7 cells.

The cytotoxicity effect of plant extracts was assessed against C2C12 cells and RAW 2647 cells using Alamar Blue cell viability assay (Rampersad, 2012; Zachari *et al*., 2013), and crystal violet cell viability assay (Feoktisova *et al*., 2016, Śliwka *et al*., 2016). Furthermore, anti-inflammatory effects were determined by the ability of the plant extracts to inhibit the production of nitric oxide (NO) and assessed using Griess assay (Promega), against RAW 264.7 cells, which was previously described by Lim *et al*., (2018).

1.8.4 The estimation of the preliminary screening of phytochemicals, phenolic content, flavonoid content, antioxidants and alpha-amylase inhibitory activities of the selected South African plant extracts for medicinal purposes.

A phytochemical analysis was conducted with standard methods. The following phytochemicals were analysed: tannins, saponins, flavonoids, quinones, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins, betacynanin, anthocyanin, and steroids (Rajesh *et al*., 2014; Mujeeb *et al*., 2014). The total phenol content was determined by Folin Ciocalteu, which was previously described by Škerget *et al*., (2005). Essentially, the flavonoids total content was analysed with a method that was previously described by Pranoothi *et al*., (2014). The antioxidant activity was analysed using DPPH radical-scavenging assay as described by Blois (1958). Furthermore, the DPPH results were confirmed with ABTS radical scavenging

assay which was described by Re *et al*., (1999). The ability of medicinal plants to inhibit alpha-amylase was analysed as described by Saha and Verma (2012).

1.8.5 Data analysis

The assay was performed in triplicates and repeated three times. All data were expressed as mean and standard deviation using MS Excel 2013 and ANOVA GraphPad Prism 5. Linear regression analysis was used to calculate the IC_{50} values.

1.9. Limitations of the research

The aim of the study was to undertake an investigation into cytotoxicity effects, anti-inflammatory, antioxidant, antimicrobial and antidiabetic properties of selected South African plants for medicinal purposes using *in vitro* testing. The study did not cover in *vivo* testing due to time constraints. The work that was done was deemed adequate for a PhD level.

1.10. Organisation of the research dissertation

This dissertation is organised as follows.

- Chapter one**:** Introduction

This chapter introduces a laboratory study on the medicinal plants used to treat and manage diabetes and other cardiovascular diseases. In this introductory chapter, the rationale for this study is explained and an overview of the dissertation is provided. The chapter starts by presenting the context within which this study was conducted as well as the researcher's background. It then proceeds to explain the rationale and objectives of the study. The theoretical background used in this study is presented. Finally, an overview of how the study was conducted is provided.

- Chapter two: Literature review (paper I)

This chapter presents a review article titled: A review on selected African medicinal plants with effective in the management of type II diabetes mellitus. It was published in ACTA *Scientific Pharmaceutical Sciences*, **3**(8), 2019.

- Chapter three: Objective 1 (paper II)

In this chapter the first objective is listed. A published paper is included. It is titled: he *in vitro* evaluation of some South African plant extracts for minimum inhibitory concentration and minimum bactericidal

concentration against selected bacterial strains, and was published in the *International Journal of Scientific and Research Publications*, **9** (7), 2019.

- Chapter four: Objective 2 (paper III)

This chapter presents findings from the second objective. A manuscript was published in February 2020. Its title is: The evaluations of cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 cells and RAW 264.7 cells. The manuscript was published in the *International Journal of Scientific and Research Publications*, **10**(2). 2020.

- Chapter five: Objective 3 (paper IV)

This chapter presents the third and final objective of this study as well as a manuscript that has been submitted to a journal for consideration. This chapter focuses on the estimation of the preliminary screening of phytochemicals, phenolic content, flavonoid content, antioxidants and alpha-amylase inhibitory activities of the selected South African plant extracts for medicinal purpose. The manuscript was accepted by *International Journal of Pharma and Bio Sciences* (in press).

- Chapter six: Conclusion and recommendations

In this chapter the conclusions that were derived from the findings of this study on the use of traditional medicine for the treatment and management of diabetes and other cardiovascular diseases are presented. The conclusions are based on the plant biological activities analysed in this study. The implications of these findings and the resultant recommendations are explained.

1.11. Summary

South Africa is home to thousands of plant species, and each species has unique and beneficial bioactive compounds (Street & Prinsloo, 2013). The use of medicinal plants in ATM practice is due to affordability as compared to conventional drugs; most importantly ATM has been reported to have less side effects (Mahomoodally, 2013). Furthermore, it is imperative to validate medicinal plant safety and efficiency in order to contribute data on their safety for human use within ATM (Ngcobo *et al*., 2011). This study highlights *in vitro* biological activity determined from the selected medicinal plants. The following activities were validated: antimicrobial, phytochemicals, total phenolic content, total flavonoid content, antioxidant, and alpha-amylases. In addition, the cytotoxicity of medical plants was validated together with anti-inflammatory capabilities. Interesting, plant species exhibited good antioxidant, anti-inflammatory and antidiabetic properties thus have the potential to be used in the treatment and management of diabetes. Chapter two covers a review article published in *ACTA Scientific Pharmaceutical Sciences.* The manuscript focuses on selected South African plants with effectiveness in the management of Type II diabetes mellitus.

References

ABDULLAHI, A. A. 2011. Trends and challenges of traditional medicine in Africa. *AJTCAM,* **8**:115-123.

ANAND, U., JACOBO-HERRERA, N., ALTEMIMI, A. & LAKHSSASSI, N. 2019. A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. *Metabolites,* **9**(258): 1 - 14

ATANASOV, A. G., WALTENBERGER, B., PFERSCHY-WENZIG, E.-M., LINDER, T., WAWROSCH, C., UHRIN, P., TEMML, V., WANG, L., SCHWAIGER, S., HEISS, E. H., ROLLINGER, J. M., SCHUSTER, D., BREUSS, J. M., BOCHKOV, V., MIHOVILOVIC, M. D., KOPP, B., BAUER, R., DIRSCH, V. M. & STUPPNER, H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnology Advances,* **33**:1582-1614.

BEARS, M. A., HAN, T., SCHNECK, M. E., BAREX, S., JACOBEN, C. & ADAMS, A. J. 2004. Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy. *Investigative Ophthanology and Visual Science,* **45**:3259-3265.

BERTRAM, M. Y., JASWAL, A. V., VAN WYK, V. P., VEVITT, N. S. & HOFMAN, K. J. 2013. The non-fetal disease burden caused by type 2 diabetes in South Africa, 2009. *Global Health Action* **6** (6): 202-212.

BLOIS, M. S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature,* **181**:1199-1200.

CHOUDHURY, H., PANDEY, M., HUA, C. K., MUN, C. S., JING, J. K., KONG, L., ERN, L. Y., ASHRAF, N. A., KIT, S. W., YEE, T. S., PICHIKA, M. R., GORAIN, B. & KESHARWANI, P. 2017. Update on natural compounds in the remedy of diabetes mellitus: a systematic review. *Journal of Traditional and Complementary Medicine,* **8** (3): 361-376.

DAR, R. A., SHAHNAWAZ, M. & QAZI, P. H. 2017. A general overview of medicinal plants: a review. *The Journal of Phytopharmacology* **6** (6): 349-351.

DEUTSCHLÄNDER, M. S., LALL, N. & VAN DE VENTER, M. 2009. Plant species used in the treatment of diabetes by South African traditional healers: an inventory. *Pharmaceutical Biology,* **47**(4): 348-365.

EGAMBERDIEVA, D. 2016. Phytochemical and pharmacological properties of medicinal plants from Uzbekistan: a review. *Journal of Medicinally Active Plants* **5** (2): 59-75.

EKOR, M. 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology,* **4** (177): 1 - 10.

ELOFF, J. N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts from bacteria. *Planta Medica,* **64** (8): 711-713.

FEOKTISOVA, M., GESERICK, P. & LEVERKUS, M. 2016. Crystal Violet assay for determining viability of cultured cells. Cold Spring Harbor Protocols, **2016**, https://doi.org/10.1101/pdb.prot087379.

GINSBURG, H. & DEHARO, E. 2011. A call for using natural compounds in the development of new antimalarial treatments - an introduction. *Malaria Journal,* **10** (1): S1-S1.

GOTHAI, S., GANESAN, P., PARK, S.-Y., FAKURAZI, S., CHOI, D.-K. & ARULSELVAN, P. 2016. Natural phyto-bioactive compounds for the treatment of Type 2 diabetes: inflammation as a target. *Nutrients,* **8**(461): 1 - 28.

LEONTI, M. & CASU, L. 2013. Traditional medicines and globalization: current and future perspectives in ethnopharmacology. *Frontiers in Pharmacology,* **4**(92): 1- 13.

LIM, Y., PARK, J. W., KWON, O. K., LEE, J. W., LEE, H. S., LEE, S., CHOI, S., LI, W., JIN, H., HAN, S. B. & AHN, K. S. 2018. Anti-inflammatory effects of a methanolic extract of Castanea seguinii Dode in LPS-induced RAW264.7 macrophage cells. *International Journal of Molecular Medicine,* **41**(1): 391-398.

LONDONKAR, R. L., MADIRE KATTEGOUGA, U., SHIVSHARANAPPA, K. & HANCHINALMATH, J. V. 2013. Phytochemical screening and in vitro antimicrobial activity of *Typha angustifolia* Linn leaves extract against pathogenic gram-negative microorganisms. *Journal of Pharmacy Research,* **6**(2): 280-283.

MAHOMOODALLY, M. F. 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evid Based Complement Alternat Med,* **2013**: 1-14 https://doi.org/10.1155/2013/617459.

MANYEMA, M., VEERMAN, J. L., CHOLA, L., TUGENDHAFT, A., LABADARIOS, D. & HOFMAN, K. 2015. Decreasing the burden of Type 2 diabetes in South Africa: the impact of taxing sugar-sweetened beverages. *PloS one,* **10**(11):1-17.

MILLS, E., COOPER, C., SEELY, D. & KANFER, I. 2005. African herbal medicines in the treatment of HIV: hypoxis and sutherlandia. An overview of evidence and pharmacology. *Nutrition Journal,* **4**(19): 1-6.

MUJEEB, F., BAJPAI, P. & PATHAK, N. 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *BioMed ResearchIinternational,* **2014**: 1-11.

NCUBE-ZULU, T. & DANCKWERTS, M. P. 2004. Comparative hospitalization cost and length of stay between patients with and without diabetes in a large tertiary hospital in Johannesburg, South Africa. *International Journal of Diabetes in Developing Countries* **34**:156-162.

NGCOBO, M., NKALA, B., MOODLEY, I. & GQALENI, N. 2011. Recommendations for the development of regulatory guidelines for registration of traditional medicines in South Africa. *AJTCAM,* **9**(1):59-66.

NKALA, B. A., MBONGWA, H. P. & QWEBANI-OGUNLEYE, T. 2019. A review on selected African medicinal plants with effectiveness in the management of Type II diabetes mellitus. *Acta Scientific Pharmaceutical Sciences,* **3** (8):47-54.

PAN, S.-Y., ZHOU, S.-F., GAO, S.-H., YU, Z.-L., ZHANG, S.-F., TANG, M.-K., SUN, J.-N., MA, D.-L., HAN, Y.-F., FONG, W.-F. & KO, K.-M. 2013. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern Therapeutics. *Evidence-based Complementary and Alternative Medicine: eCAM,* **2013**: 1 - 25 http://dx.doi.org/10.1155/2013/627375.

PETROVSKA, B. B. 2012. Historical review of medicinal plants' usage. *Pharmacognosy Reviews,* **6**(11): 1-5.

PHEIFFER, C., PILLAY-VAN WYK, V., JOUBERT, J. D., LEVITT, N., NGLAZI, M. D. & BRADSHAW, D. 2018. The prevalence of type 2 diabetes in South Africa: a systematic review protocol. *BMJ open,* **8** (9): 1- 4.

PRANOOTHI, E. K., NARENDRA, K., JOSHI, D. S. D. S., SWATHI, J., SOWJANYA, K. M., RATHNAKARREDDI, K. V. N., EMMUEL, S., PADMAVATHI, C. H. & SATYA, A. K. 2014. Studies on qualitative, quantitative, phytochemical analysis and screening of *in vitro* biological activities of *Leuca indica* (L) *VAR.* Nagalapuramiana. *International Journal of Herbal Medicine* **2**(3): 30-36.

RAHMATULLAH, M., AZAM, M. N. K., KHATUN, Z., SERAJ, S., ISLAM, F., RAHMAN, M. A., JAHAN, S. & AZIZ, M. S. 2012. Medicinal plants used for the treatment of diabetes by the Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh. *AJTCAM,* **9**(3):380-385.

RAJALAKSHMI, M., ELIZA, J., PRIYA, C. E., NIRMALA, A. & DAISY, P. 2009. Antidiabetic properties of *Tinospora cordifolia* stem extracts on streptozotocin-induced diabetic rats. *African Journal of Pharmacy and Pharmacology,* **3**(5):171-180.

RAJESH, K. D., VASANTHA, S., RAJESH, N. V. & PANNEERSELVAM, A. 2014. Qualitative and quantitative phytochemical analysis in four pteridophytes. *International Journal of Pharmaceutical Sciences Review and Research,* **27**(72): 408-412.

RAMPERSAD, S. N. 2012. Multiple applications of Alamar Blue as an indicator of metabolic function and cellular health in cell viability bioassays. *Sensors (Basel, Switzerland),* **12**(9):12347-12360.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. & RICE-EVANS, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radical Biology & Medicine* **26** (9-10):1231- 1237.

SAHA, S. & VERMA, R. 2012. Inhibitory potential of traditional herbs on alpha-amylase activity. *Pharm Biol,* **50**(3):326-31.

SHUKIA, R., SHARMA, S. B., PURI, D., PRABHU, K. M. & MURTHY, P. S. 2000. Medicinal plants for the treatment of diabetes mellitus. *Indian Journal of Clinical Biochemistry,* **15**(1):169-177.

ŠKERGET, M., KOTNIK, P., HADOLIN, M., HRAŠ, A., R., SIMONIČ, M. & KNEZ, Z. 2005. Phenols, proanthocyanidins, flavones, and flavonols in some plant materials and their antioxidant activities. *Food Chemistry* **89** (2):191-198.

ŚLIWKA, L., WIKTORSKA, K., SUCHOCKI, P., MILCZAREK, M., MIELCZAREK, S., LUBELSKA, K., CIERPIAŁ, T., ŁYŻWA, P., KIEŁBASIŃSKI, P., JAROMIN, A., FLIS, A. & CHILMONCZYK, Z. 2016. The comparison of MTT and CVS assays for the assessment of anticancer agent interactions. *PLOS ONE,* **11**(5): 1-17.

SOFOWORA, A., OGUNBODEDE, E. & ONAYADE, A. 2013. The role and place of medicinal plants in the strategies for disease prevention. *AJTCAM,* **10**(5):210-229.

STREET, R. A. & PRINSLOO, G. 2013. Commercially important medicinal plants of South Africa: a review. *Journal of Chemistry,* **2013**: 1-16 http://dx.doi.org/10.1155/2013/205048 .

TABUTI, J. R., KUKUNDA, C. B. & WAAKO, P. J. 2010. Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda. *J Ethnopharmacol,* **127**(1): 130-6.

TUGUME, P., KAKUDIDI, E. K., BUYINZA, M., NAMAALWA, J., KAMATENESI, M., MUCUNGUZI, P. & KALEMA, J. 2016. Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda. *Journal of Ethnobiology and Ethnomedicine,* **12**(5): 1 - 28.

TUNGMUNNITHUM, D., THONGBOONYOU, A., PHOLBOON, A. & YANGSABAI, A. 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines (Basel),* **5**(93): 1 - 16.

WHO 2016. *Global report on diabetes.* Geneva, Switzerland: WHO Press.

WINTOLA, O. A. & AFOLAYAN, A. J. 2015. The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of *Hydnora africana*Thunb. used as antidysenteric in Eastern Cape Province, South Africa. *BMC Complement Altern Med,* **15**(307): 1-12.

YUAN, H., MA, Q., YE, L. & PIAO, G. 2016. The traditional medicine and modern medicine from natural products. *Molecules,* **21**(5):1-18.

ZACHARI, M. A., CHONDROU, P. S., POULILIOU, S. E., MITRAKAS, A. G., ABATZOGLOU, I., ZOIS, C. E. & KOUKOURAKIS, M. I. 2013. Evaluation of the alamarblue assay for adherent cell irradiation experiments. *Dose Response,***12** (2):246-258.

ZHANG, P., ZHANG, X., BROWN, J., VISTISEN, D., SICREE, R., SHAW, J. & NICHOLS, G. 2010. Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes Res Clin Pract,* **87**(3):293-301.

ZHANG, Y., XU, G., ZHANG, S., WANG, D., SARAVANA PRABHA, P. & ZUO, Z. 2018. Antitumor research on artemisinin and its bioactive derivatives. *Natural Products and Bioprospecting,* **8**:303-319.

CHAPTER TWO: LITERATURE REVIEW (PAPER I)

In this chapter a review of related literature on the selected South African plants, and their use in treatment and management of diabetes type II, within African traditional medicine, are presented and discussed. The selected medicinal plants are *Euclea crispa* (leaf), *Euclea natalensis* (leaf), *Schkuhria pinnata* (leaf), *Ziziphus mucronata* (leaf), *Ziziphus mucronata* (fruits), *Lippia javanica* (leaf), *Vernonia oligocephala* (leaf), *Clerodendrum myricoides* (leaf), and *Erythrina lysistemon* (leaf).

A literature review for this chapter was published as a journal article. The details are as follows.

Paper I **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T. (2019). A review on selected African medicinal plants with effectiveness in the management of type II diabetes mellitus. *Acta Scientific Pharmaceutical Sciences*, **8** (3): 47-54.

ACTA SCIENTIFIC PHARMACEUTICAL SCIENCES (ISSn: 2581-5423)

Volume 3 Issue 8 August 2019

Review Article

A Review on Selected African Medicinal Plants with Effectiveness in the Management of Type II Diabetes Mellitus

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Received: April 18, 2019; **Published:** July 11, 2019

DOI: 10.31080/ASPS.2019.03.0339

Abstract

Type II diabetes mellitus is a metabolic disorder characterised by hyperglycaemia due to a defect in insulin production and/or resistance by the cells. Bioactive compounds with antidiabetic, antioxidant and anti-inflammatory properties are potential solutions towards the management of type II diabetes mellitus. Bioactive compounds, which naturally occur in medicinal plants, such as antioxidants, play a vital role in scavenging free radicals. Free radicals are naturally formed in the body and play a pivotal role in many normal cellular processes. However, at high concentrations, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer, neurodegenerative disorders, cardiovascular diseases, diabetes and other chronic health conditions. Therefore, it is anticipated that increased knowledge of plants with hypoglycaemic, antioxidant and anti-inflammatory activity will not only impact, but also revolutionize phytomedicine industrial and pharmaceutical processes. This review will look into the investigation of plants used for the treatment and management of diabetes type II.

Keywords: Antidiabetic; Antioxidant; Anti-Inflammatory; Hypoglycaemic and Natural Products

Introduction

Type II diabetes mellitus (T2DM) is a metabolic disorder characterised by hyperglycaemia due to a defect in insulin production and/or resistance by the cells. In the past decades (34 years from 1980 to 2014), has witness global prevalence of T2DM jumped a staggering 314 million cases from 108 million to 422 million according to a study done by the World Health Organisation [1]. Bears., *et al.* [2] estimated that T2DM would increase from 4.0% in 1995 up to 5.4% in the year 2025. In South Africa, the prevalence of T2DM in adults is on the rise too. In the short space of 9 years T2DM prevalence increased from 5.5% in

2000, to 9.0% in the year 2009 [3].

The effects of T2DM is further observed with the economic devastation experienced by the suffers of diabetes whom on average R9, 000 higher hospitalisation costs as compared to the non-diabetic in in 2009 as reported by Ncube-Zulu and Danckewert [4], in their study that reported R27, 000 (2,250 USD) per hospitalisation compared to R18, 000 (1,500 USD) for non- diabetic patients. This is the global context where the total health expenditure is projected to be between 1.1 to 2.0 billion USD in

2030 [5]. Moreover, the current conventional drug therapies are not completely effective in the T2DM management, exacerbating the problem is the various associated adverse effects of these therapies currently in usage [6].

The search for alternative therapies to address such challenges is therefore imperative [7]. Plants used for their medicinal properties in fight against T2DM have shown to be able to alleviate one or more symptoms. A total cure for the disease still eludes the medical fraternity [8]. Medicinal plants have also showed to be effective in delaying complications of the diseases and rectifying metabolic abnormalities. The use of plants for medicinal purposes has the advantage of having none or few side effects [9].

Natural products continue to play a significant role in drug discovery and development. Plants are recognised as a useful source of highly biological active drugs for the treatment of various ailments. Thus, promising plants should continue to be fractionated and evaluated for their efficacy and toxicity potential; especially in the local context. South Africa boasts a rich fauna and flora kingdom and much success has already been reported in human and animal practise involving the tapping into the potential of using plants for their medicinal properties [10,11]. These include the treatment of infections, anti-helminthic activity, anti- malarial activity, anti-diarrhoeal activity, anti-cancer activity and antidiabetic potential [12,13].

According to the World Wild Fund (WWF), South Africa's Western Cape is more botanically diverse than the richest tropical rainforest in South America, including the Amazon. It contains 3.0% of the world's plant species and 20% of Africa's. Of the more than
8,500 fynbos species, nearly 6,000 of them are endemic [14]. Very few of these plants have been explored for their phytochemicals and pharmacological potential so the chances of finding plants with medicinal purposes definitely exists [15]. Furthermore, bioactive compounds with anti-inflammatory, antioxidant and antidiabetic properties are potential solutions towards the management of T2DM.

Medicinal plants

Medicinal plants serve as important sources of pharmaceutical, cosmetic and traditional medicine [16]. However, their use in these industries has declined since the discovery of synthetic drugs and their production at a larger scale. Interestingly, despite this decline it is important to note that, half of these synthetic drugs are of natural origin are synthesized using chemical characteristics and structure derived from their natural counterparts, thereby, highlighting the importance of medicinal plant's role in drug discovery [17]. Medicinal plants play an important role in providing promising solutions for the treatment and management of certain diseases [18]. The use of medicinal plants in drug discovery by the international community is expected to increase, as more literature, focusing on their safety and efficacy, becomes available [18].

While the global community tries to come to terms with the use of plants for medicinal use. The use of traditional medicine practices has been entrenched in cultural practice throughout developing countries [19]. WHO [20] estimated that 75% of South Africans rely on traditional medicine as the primary treatment for their healthcare needs. The use of medicinal plants in the South African traditional medicine context can be traced back thousands of years through cave drawings [21]. The harvesting of these plants has led to the establishment of shops and ultimately growth of commercial trades, which include healing, therapeutic and spiritual purposes. The informal trade of these plants are boosted as the adoption of the use of traditional medicines in urban settings continues [22]. Liu and Wang [23] have estimated that approximately 85,0000 plant species are used globally. Essentially, in the United States represents 35% of this market, followed closely by Europe that encompasses Germany, France, UK, Italy and Scandinavia, with 33%. Africa contributes less than 1% to the market (USD 520 million) [24].

Herbal medicine in the global market from African

Only a few of these plants have been used for herbal medicine in Africa make their way onto the global market. These plant species include *Agathosma betulina* (Buchu), *Aloe ferox* (Cape aloe), *Aspalathus linearis* (herbal tea), *Herpagophytum procumbens* (devil's claw), *Hypoxis hemercollidea* (African potato), *Merwilla natalensis* (Scilla natalensins planch), *Pelargonium sidoides* (Umckaloabo), *Sclerocarya birrea* (marula), *Siphonochilus aethiopicus* (wild ginger) and *Sutherlandia frutescens* (cancer bush) [15]. Interestingly, these species contain among other things antioxidant, anti-inflammatory and antidiabetic properties; of which are essential constitute for the treatment and management of diabetics and other cardiovascular disease. In a review by Street and Prinsloo [15] detailed on phytochemicals and bioactivity of these commercially use medicinal plants. It is evident that certain plants possess antidiabetic, antioxidant and anti-inflammatory properties; of which is the key towards the treatment and management of certain diseases.

Inflammation has been defined as a major factor for the progression of various chronic diseases/disorders, including diabetes, cancer, cardiovascular diseases etc. [25]. The production of free radical from different biological and environmental sources are due to an imbalance of natural antioxidants which further leads to various inflammatory associated diseases [26]. It has been observed that natural compound based antioxidant and anti-inflammatory have gain momentum for the preventive role in protecting against the generation of free radicals and therefore natural based antioxidants and anti-inflammatory are one of the more valuable therapeutic agents to reduce the illnesses triggered by oxidative stress [27].

The use of plants is motivated by their affordability, availability and most importantly by the remedial beliefs that certain communities have. Essentially, the following factors need to be taken into considerations when dealing with plants such as the right time for harvesting, collection and use, does not address critical concerns such as safety, efficacy and the mode of action; which is not thoroughly understood.

Aetiology of type II diabetic mellitus

T2DM is one of the top six leading causes of diseases in the world and it accounts for death of approximately 1.6 million people in 2015 [1]. T2DM results from the inability of the pancreas to produce insulin or the inability of the body's metabolic system to use the insulin produced. Various pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas in insulin deficiency to abnormalities that result in resistance to insulin action [28]. The cause of diabetes is not thoroughly understood, however, it is believed that it is associated with various factors such as – genetics bad dietary habits, obesity and the adoption of a sedentary lifestyle. The disease is noticeable from the first stage, which is an increase in glucose or hyperglycaemia. This is due to the body being unable to metabolise sugar and other dietary substance like lipids and proteins and will lead to disorders affecting almost all of the body [29]. Diabetic patients are prone to develop chronic illness resulting in neurological, cardiovascular, retinal and renal complications, which may lead to premature death [30].

The management of type II diabetes mellitus

Daily management of T2DM

Daily management of T2DM involves, among other things, the control of blood glucose levels, which should be between 4.0 to 6.0 mmol/L [28]. Primarily lifestyle interventions are used for T2DM management, but if these lifestyle interventions fail to control blood glucose levels then it becomes crucial to consider pharmacological treatment. Essentially, the following therapies should be considered namely: - taking insulin injections, administering oral medication, following a healthy diet, being physically active, controlling blood pressure levels (i.e. should be kept below 130/80mmHg) and controlling cholesterol levels (i.e. 5 mmol/L or less) [31]. In addition to diabetic medication, one should consider interventions such as making healthy choices, being physical active, controlling blood pressure and controlling cholesterol levels [20].

Drug treatment

The initial and standard drug that is used for the treatment of type 2 diabetes in adults is metformin. In some instances, metformin is contraindicated or not tolerated. The following drugs are considered namely: - a dipeptidyl peptidase-4 (DPP-4) inhibitor or pioglitazone or a sulfonylurea [32]. It is imperative to exercise caution when prescribing pioglitazone, when a person is at high risk of the adverse effects of the drug. The reason being, pioglitazone is associated with an increased risk of heart failure, bladder cancer and bone fracture [33]. Metformin can also be used in conjunction with insulin injection or added, especially when glycaemic targets are not met. In order to control glycaemic bolus insulin can be used, since it is rapid-acting analogues [34].

The management of T2DM is a global concern and it has been observed that available therapy is not being tolerated by patients due to side effects mentioned here above. The side effects can of serious concern such as liver problems, lactic acidosis and diarrhoea [35]. Interestingly, the available therapy is mainly responsible for the improvement insulin sensitivity, increasing insulin production and decrease the amount of glucose in the blood.

However, side effects are not satisfactory and it is imperative to investigate on natural compounds for the treatment of T2DM, of which is believed to have lesser adverse effects [36].

Alternative treatment

The conventional drugs that are used for the management of diabetes have been observed to have some adverse effects. Therefore, it is vital to investigate alternative therapies that have less or no side effects and which are effective in the management of diabetes. Some medicinal plants have been demonstrated to have lesser side effects and have ability to treat various diseases [37]. Moreover, natural bioactive compounds isolated from medicinal plants that have anti-hyperglycaemic activities have been confirmed experimentally [38]. Bioactive compounds that are associated with antidiabetic effects are glycosides, alkaloids, citric acid, malic acid, polyterpenes, cyanhidric acid, essential oils, allicine, nerolidol, pectins, terpenoids, flavonoids, carotenoids, sterols, triperpenes and protein (bixine) [12]. Medicinal plants have been used in ancient times throughout the world for the management of T2DM [39].

It has been noted that, the use of medical plants in the developing countries has gained momentum, whereby many people do not have access to conventional antidiabetic therapy. Essentially, it is not only about the affordability of a convectional drug, but the cost of transport to the nearest health care facilities plays a role in making convectional drug unaffordable among other issues [40]. The developed countries have also shown interest in the investigation of antidiabetic herbal remedies as an alternative therapy. It is believed that they are motivated by factors such as adverse reactions, high secondary failure rates and cost of conventional drugs [41]. Furthermore, WHO has endorsed the use of medicinal plants for the management of T2DM and further recommended more research on the evaluation of hypoglycaemic/ hyperglycaemic properties of diverse plant species [42].

The mechanism of action is not thoroughly understood, however, natural plants have been observed to restore pancreatic tissue by causing an increase in insulin output or decrease in the intestinal absorption of glucose. Essentially, treatment with plants has been observed to protect β-cells and smoothing out fluctuation in glucose levels. The other possible mechanism of action that has been reported is the inhibition of α-glucosidase and α-amylase, the effects on glucose uptake and glucose transports, the enhancement of insulin secretion and pancreatic β-cell proliferation, the inhibition of protein tyrosine phosphate 1B activity and antioxidant activity have been studied in depth [43].

For the purpose of the broader study, plants will be investigated for antidiabetic, antioxidant and anti-inflammatory properties. Essentially, the purpose of this review reflects on the selected African medicinal plants that has been reported by healers and herbalist to be used in the treatment of diabetes. It is imperative to note that not all of these plants are scientifically validated for their use in the traditional medicine practices. However, few of these plants have been reported for their phytochemistry, bioactivity; and most importantly for their use in the treatment and management of diabetes.

Lippia javanica (The lemon bush tea/or fever tea)

Xhosa people use it to disinfect meat that has been contaminated by anthrax. It has been reported to be used in various traditional medicine such as have further reported this plant to be used for the treatment of various ailments including diabetes, fever, cough, bronchitis and influenza. *L. javanica consists* of the following phytochemicals alkaloids, sterols, terpenoids, flavonoids, tannins and saponins. It is important to note that nothing has been reported on bioactivity of this plant in literature [44,45].

Euclea natalensis (Natal guarri)

It has variety of traditional remedies such as to treat worms, stomach disorders, toothache, headache, chest complaints pleurisy, urinary tract infections, venereal diseases, schistosomiasis, dysmenorrhoea and for scrofulous swellings, abnormal growths on skin and leprosy. Furthermore, Deutschländer., *et al*. [12] reported that this plant is used by Venda herbalist for the treatment of diabetes. The phytochemicals and bioactivity of this plant was reported on the review by Deutschländer., *et al*. [12].

Schkuhria pinnata (Dwarf marigold)

S. pinnata have been used as a bactericide in open wounds, to treat acne, malaria and inflammation, and as a blood purifier and diuretic. In addition to this, this plant is used by traditional healers and herbalist in Ga-Rankuwa, Gauteng to treat diabetes. The phytochemicals and activity of *S. pinnata* has not been reported on in literature [12,46].

Clerodendrum myricoides (Blue cat's whiskers)

It has numerous medicinal uses ranging from the treatment of snakebites, reduce bodily swellings, relief indigestion, to treat colds, chest pains and headaches, as well as being applied to bleeding gums. In addition to this, *C. myricoides* has been reported

to be used for the treatment of impotence. Impotence is defined as one the manifestations of autonomic neuropathy in diabetes. Nothing has been reported on phytochemicals in C. *myricodes*, except on its family (Verbernaceae) reported to have various compounds such as anthraquinines, terpenes, steroidal saponins, alkaloids and flavonoids. Furthermore, nothing has been reported on *C. myricodes* bioactivity except for Clerodedrum genus [47].

Euclea crispa (Blue guarri)

It is used for stomach disorders, measles, coughs and constipation. It has been further reported to be taken orally as a remedy for diabetes and also prevents rheumatisms and epilepsy [47]. According to our knowledge nothing has been reported on *E. crispa* phytochemicals or bioactivity in literature.

Ziziphus mucronata (Buffalo thorn)

It has various medicinal uses per each region and the most common one is to treat boils, swollen glands, wounds and sores. The decoction of *Z. mucronata* has been reported to be used in the treatment of diabetes. The *invivo* studies conducted have confirmed antidiabetic activity of *Z. mucronata*. Furthermore, phytochemicals such as alkaloids was reported in this plant [12,48].

Vernonia oligocephala (Bicoloured-leaf vernonia)

The medicinal use for this plant is used to relief of stomach ache reliever which is taken a tonic. The phytochemicals that has been identified from V. *oligocephala are* flavonoids, glycoside, polyphenols, saponins and steroids. In Eastern and West Cape Province this plant has been reported to be used for treatment of diabetes by traditional healers [49].

Erythrina lysistemon (Coral tree)

The bark is used to treat sores, wounds, abscesses and arthritis. Furthermore, the leaves infusions are used as ear drops to relieve earache, and decoctions of the roots are applied to sprains. *E. lysistemon* poses the following phytochemicals flavones, isoflavones, isoflavanones, alkaloids, pterocarpans phenolic acids. Essentially, the bioactivity of these phytochemicals have been validated. Nothing has been reported for the treatment of diabetes for this plant, however, it is promising plant due to its phytochemicals identified [50].

Conclusion

Type II diabetes mellitus (T2DM) is a major endocrine disorder and its growth or prevalence is ascribed to a number of factors that include but are not limited to obesity, social structure, hormonal imbalance, and hereditary. The use of medicinal plants has been observed since the ancient time for the treatment of various diseases. Essentially, medicinal plants have been noted to have lesser side effects; hence, the need to explore rich and potential plants with antidiabetic activity became necessary. The poor management of the diabetes can lead to neurological, cardiovascular, retinal and renal complications. Medical plants with antidiabetic, antioxidant and anti-inflammatory activities are essential for the management of diabetics and other related cardiovascular diseases. However, from our review, it is apparent that plants possess essential compounds to fight diseases such antidiabetic, antioxidant and ant-inflammatory properties. Certain medical plants have shown to have hypoglycaemic effects and can be used for the treatment of type of secondary complications of type II diabetes mellitus. Researchers have reported on the ability of plant to treat and manage T2DM, however, it is imperative to understand the exact mechanism of action of medicinal plants with antidiabetic and insulin mimetic activity.

Antioxidants have been demonstrated for their ability to scavenge free radical and this have served a key element towards the prevention and treatment of disease associated with oxidants or free radicals. It is important to note that antioxidants are derived from food and medicinal plants; of which more research have been focusing on them for various nutritional function and health benefits. The availability of antioxidants varies from plants to plants that is why it is imperative to investigate plants broadly in search for potential plants species. The ability of medicinal plants to possess anti-inflammatory properties have been explored.

T2DM has been observed to be a global concern, of which affecting large population. It is associated with decreased insulin production or resistance towards its action. Medicinal plants ability to treat diabetes patients, both insulin dependent and non- insulin dependent diabetes has been widely reported. Recent developments have justified the role of medicinal plants for the management of diabetes, however, it would be unwarranted to assure that all plants can be blindly used in diabetic patients.

Medicinal plants with promising antidiabetic, antioxidant and anti-inflammatory effect will need to be further validated for toxic effects, herb-drug interaction etc. In our future studies, selected plants will be evaluated for their cytotoxic effect, antidiabetic, antioxidant and anti-inflammatory properties and moreover, the minimal inhibitory concentration and minimal bactericidal concentration will also be studied. Therefore, it is anticipated that increased knowledge of plants with hypoglycaemic, antioxidant and anti-inflammatory activity will not only impact, but also revolutionize phytomedicine industrial and pharmaceutical processes.

Conflict of Interests

The authors declare that they do not have any conflict with respect to the publication of this paper.

Acknowledgements

We thank the College Health Sciences Scholarship Grant at the University of KwaZulu-Natal, Institute of Traditional Knowledge and Traditional Medicine at the Vaal University of Technology, the National Research Foundation and Thuthuka grant for financial support received towards this study.

Bibliography

1. WHO. Global report on diabetes. Geneva, Switzerland, WHO Press (2016).

2. Bears MA., *et al*. "Local multifocal oscillatory potential abnormolaities in diabetes and early diabetic retinopathy". *Investigative Ophthanology and Visual Science* 45 (2004): 3259-3265.

3. Bertram MY., *et al*. "The non-fetal disease burden caused by type 2 diabetes in South Africa, 2009". *Global Health Action* 6 (2013): 202-212.

4. Ncube-Zulu T and Danckwerts MP. "Comparative hospitalization cost and length of stay between patients with and without diabetes in a large tertiary hospital in Johannesburg, South Africa". *International Journal of Diabetes in Developing Countries* 34 (2014): 156-162.

5. Zhang, P., *et al*. "Global healthcare expenditure on diabetes for 2010 and 2030". *Diabetes Research and Clinical Practice* 87 (2010): 293-301.

6. Asha B., *et al*. "Evaluation of antihyperglycemic activity of Zingiber Officinale (Ginger) in albino rats". *Journal of Chemical and Pharmaceutical Research* 3.1 (2011): 452-456.

7. Maruthupandian A., *et al*. "Ethnomedicinal plants used for the treatment of diabetes and juandice by Palliyar tribals in Sirum- alai hills, Western Ghats, Tamil Nadu, India". *Indian Journal of Natural Products and Resources* 2 (2011): 493-497.

8. Rahmatullah M., *et al*. "Medicinal plants used for treatment of diabetes by the marakh sect of the garo tribe living in my- mensingh district, Bangladesh*". African Journal of Complemen- tary and Alternative Medicine* 9 (2012): 380-385.

9. Shukla R., *et al*. "Medicinal plants for treatment of diabetes mellitus". *Indian Journal of Clinical Biochemistry* 15 (2000): 169-177.

10. Ngcobo M., *et al*. "Recommendations for the development of regulatory guidelines for registration of traditional medicines in South Africa". *African Journal of Traditional and Complemen- tary Alternative Medicine* 9 (2012): 59-66.

11. Newman DJ and Cragg GM. "Natural Products as Sources of New Drugs from 1981 to 2014". *Journal of Natural Products* 79 (2016): 629-661.

12. Deutschlander MS., *et al*. "Plant species used in the treatment of diabetes by South African traditional healers: An inventory". *Pharmaceutical Biology* 47.4 (2009): 348-365.

13. Samie A., *et al*. "*In vitro* cytotoxicity and antimicrobial activities, against clinical isolates of *Campylobacter* species and *Entomoeba histolytica*, of local medicinal plants from the Venda region, in South Africa". *Annals of tropical Medicine and Parasi- tology* 103 (2009): 159-170.

14. WWF. "Fynbos, South Africa" (2017).

15. Street RA and Prinsloo G. "Commercially Important Medicinal Plants of South Africa: A Review". *Journal of Chemistry* (2013): 1-17.

16. Kamsu-Foguem B and Foguem C. "Adverse drug reactions in some African herbal medicine: literature review and stake- holder's interview". *Integrative Medicine research* 3.3 (2014): 126-132.

17. Atanasov A., *et al*. "Discovery and resupply of pharmacologically active plant derived natural products: A review". *Biotech- nology Advances* 33.8 (2015): 1582-1614.

18. Fokunang CN., *et al*. "Traditional medicine: Past, Present and Future Research and Development Prospects and Integration in the national Health System of Cameroon". 8.3 (2011): 284-

295.

19. Makunga NP., *et al*. "Current perspectives on an emerging formal natural products sector in South Africa". *Ethnopharmacol- ogy* 119.3 (2008): 365-375.

20. WHO. WHO Traditional Medicine Strategy 2014-2023 (2013).

21. Digby A. "Self-medication and the Trade in Medicine within a Multi-Ethnic Context: A Case Study of South Africa from the Mid-Nineteenth to Mid- Twentieth Centuries". *Social History of Medicine* 1.3 (2005): 439-457.

22. Cocks M., *et al*. Born-trees and worn trees (2006).

23. Liu Y and Wang M.-W. "Botanical drugs: challenges and opportunities contribution to Linnaeus Memorial Symposium 2007". *Life Sciences* 82 (2008): 445-449.

24. De Kock C. Trends in the Natural Products Sector (2004).

25. Arulselvan P., *et al*. "Role of Antioxidants and Natural Products in Inflammation". *Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity* (2016): 1-15.

26. Krishnaih D., *et al*. "A review of the antioxidant potential of medicinal plant species". *Food and Bioproducts Processing* 89 (2011).

27. Olaokun OO., *et al*. "Phytochemical Screening, Antioxidant, Anti-inflammatory, and Glucose Utilization Activities of Three South African Plants Used Traditionally to Treat Diseases". *Bi- ology and Medicine Journal* 9 (2017): 1-7.

28. Association AD. "Diagnosis and Classification of Diabetes Mellitus". *Diabetes Care* 31.1 (2008).

29. Gilani AH and Rahman AU. "Trends in ethnopharmacology". *Journal of Ethnopharmacology* 100 (2005): 43-49.

30. Yokazawa T and Nakagawa T. "Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage". *Food and Chemical Toxicology* 42.6 (2004): 975-981.

31. Harper W., *et al*. "Pharmacologic Management of Type 2 Diabetes". *Canadian Journal of Diabetes* 37 (2013): S61-S68.

32. Pathak R and Bridgeman MB. "Dipeptidyl Peptidase-4 (DPP-4) Inhibitors In the Management of Diabetes". *Drug Class Review* 35 (2010): 509-513.

33. Valli VE., *et al*. "Pathology of canine bladder and urethral cancer and correlation with tumour progression and survival". *Journal of Comparative Pathology* 113.2 (1995): 113-130.

34. Johnson JA and Bowker SL. "Intensive glycaemic control and cancer risk in type 2 diabetes: a meta-analysis of major trials". *Diabelogia* 54.1 (2011): 25-31.

35. Rajalakshmi M., *et al*. "Antidiabetic properties of *Tinospora cordifolia* stem extracts on streptozotocin-induced diabetic rats". *African Journal of Pharmacy and Pharmacology* 3 (2009): 171-180.

36. Mamun-or-Rashid ANM., *et al*. "A review on medical plants with antidiabetic activity". *Journal of Pharmacognosy and Pho- tochemistry* 3 (2014): 149-159.

37. Kadali VN., *et al*. "Antidiabetic plants present in west Godavari District of Andhra Pradesh India - A short review". *Internation- al Journal of Pharma Sciences and Research* 7.2 (2016): 72-76.

38. Kavitha KN and Dattatri AN. "Experimental evaluation of antidiabetic activity of Swertia chirata - Aqueous extract". *Journal of Public Health Research* 1.2 (2013): 71-75.

39. Jung M., *et al*. "Antidiabetic agents from medicinal plants". *Cur- rent Medicinal Chemistry* 13.10 (2006): 1203-1218.

40. Achaya D and Shrivasta K. Indigenous Herbal Medicine. Jaipur, Indian, Aavishkar Distributors (2008).

41. Gurib-Fakim A. "Medicinal plants: traditions of yesterday and drugs of tomorrow". *Molecular Aspects of Medicine* 27 (2006): 1-93.

42. Dirks JH. "The drumbeat of renal failure: symbiosis of prevention and renal replacement therapy". *Blood Purification* 22 (2004): 6-8.

43. Rios L., *et al*. "Natural products for the treatment of type 2 Dia- betes mellitus". *Planta Medical Journal* 81 (2015): 975-994.

44. York T. An ethnopharmacological study of plants used for treating respiratory infections in rural Maputal and. KwaDlangez- wa, South Africa, University of Zululand. Masters (2012).

45. Arika WM., *et al*. "In Vivo Safety of Aqueous Leaf Extract of *Lip- pia javanica* in Mice Models". *Biochemistry and Physiology* 5.1 (2016): 1-9.

46. Bussmann RW., *et al*. "Peruvian plants canchalagua (*Schkuhria pinnata* (Lam.) Kuntze), hercampuri (*Gentianella alborosea* (Gilg.) Fabris), and corpus way (*Gentianella bicolor* (Wedd.) J. Pringle) prove to be effective in the treatment of acne". *Arnal- doa* 15 (2008): 149-152.

47. Raimondo D., *et al*. "Red List of South African plants". Strelitzia 25. Pretoria, South African National Biodiversity Institute (2009).

48. Ibrahim MA and Islama S. "Effects of butanol fraction of *Ziziphus mucronata* root ethanol extract on glucose homeostasis, serum insulin and other diabetes-related parameters in a murine model for type 2 diabetes". *Pharmaceutical Biology* 55.1 (2017): 416-422.

49. Amusan., *et al*. "Some Swazi phytomedicines and their con- stituents". *African Journal of Biotechnology* 6.3 (2007): 267-
272.

50. Farag MA., *et al*. "Metabolomics driven analysis of *Erythrina lysistemon* cell suspension culture in response to methyl jasmonate elicitation". *Journal of Advanced Research* 7 (2016):
681-689.

Volume 3 Issue 8 August 2019

CHAPTER THREE: OBJECTIVE ONE (PAPER II)

In this chapter an *in vitro* evaluation of some South African plant extracts for minimum inhibition concentration and minimum bactericidal concentration against selected bacterial strains is presented and discussed. The ability of eight selected South African plants was evaluated for their antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*.

The results of the *in vitro* evaluation were published in a journal.

Paper II **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T**.** (2019). The *in vitro* evaluation of some South African plant extracts for minimum inhibition concentration and minimum bactericidal concentration against selected bacterial strains. *International Journal of Scientific and Research Publications*, **9** (7): 996-1004

The *in vitro* evaluation of some South African plant extracts for minimum inhibition concentration and minimum bactericidal concentration against selected bacterial strains

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DOI: 10.29322/IJSRP.9.07.2019.p91132
http://dx.doi.org/10.29322/IJSRP.9.07.2019.p91132

ABSTRACT
Bacterial infections are one of the world's most pressing public health problems. The major challenge in anti-bacterial treatment is the development of antibiotic resistant bacterial strains. This then reduces the number of drugs available for treating bacterial infections. In this study, antimicrobial activity of nine South African crude plant extracts were investigated for the first time against *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*). The minimum inhibitory concentrations of the crude plant extracts were determined. Four plant extracts showed good inhibition effects against all four bacteria used which are *Lippia javanica* (0.25±0.00 to 1.13±0.29 mg/ml); *Ziziphus mucronata* leaf (0.44±0.00 to 1.00±0.00 mg/ml); *Erythrina lysistemon* (0.44±0.00 to 1.08±0.00 mg/ml) and *Schkuhria pinnata* (0.5±0.00 to 1.34±0.00 mg/ml). Similarly, gentamycin (positive control) had inhibitory effects ranged from 0.92±0.29 to 2.00±0.00 mg/ml against all four bacteria used. For the bactericidal effects, four plant extracts that showed bactericidal effects against three bacteria used (*E.coli*, *S. aureus and* E. *faecalis*). The plant extracts are Lippa *javanica* (0.25±0.00 to 1.00±0.00 mg/ml); *Ziziphus mucronata* leaf (0.25±0.00 to 2.00±0.00 mg/ml); *Erythrina lysistemon* (0.5±0.00 to 2.00±0.00 mg/ml) and *Schkuhria pinnata* (0.5±0.00 to 2.00±0.00 mg/ml. Furthermore, none of the plant extract showed bactericidal against *P. aeruginosa*. The results of this study suggests that four plants that demonstrated inhibition effect and bactericidal effects which supports their used in the traditional medicine treatment of various ailments.

Keywords: bacterial infections, antimicrobial activities, pathogens, inhibition, bactericidal.

1.0 INTRODUCTION

Bacterial infections are caused by pathogenic microorganisms that have the ability to cause diseases in humans (Alberts *et al*.,2002; Ryan *et al*., 2014). Not all bacteria are pathogenic, the ones that are pathogenic are responsible for causing diseases in humans. The most prominent pathogenic microorganism is Mycobacterium *tuberculosis* which is responsible for the highest tuberculosis burden in humans; and accounts for about 2 million deaths per year, mostly in sub-Saharan Africa (Chan *et al*., 2013; Ryan *et al*., 2014). Another globally important genera of pathogens are *Streptococcus* and *Pseudomonas* which are responsible for diseases such as pneumonia. Genera such as *Shigella*, *Campylobacter and Salmonella are* mainly responsible for foodborne illnesses. In addition to this, they can further cause infections such as tetanus, typhoid fever, diphtheria, syphilis and leprosy (Ben-Noun, 2009; Chan *et al*., 2013).

The increase of antimicrobial resistance is a global concern. It is characterised by multidrug-resistance which is mainly caused by gram-negative bacteria (Exner *et al*., 2017). This is due to genetic mutations and can also be triggered by misuse of antimicrobial drug prescriptions (Mwambete, 2009). Gram-negative strains that contribute towards multidrug-resistance have been reported and these include, Klebsiella *pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp and *Escherichia coli (*Exner *et al*., 2017). Although more attention has been focused on multi-drug resistance (MDR) associated with gram- negative bacteria, and gram-positive antimicrobial resistance are also a serious concern (Doemberg *et al*., 2017). For example, methicillin-resistant *Staphylococcus aureus* (MRSA) is a typical example that has raised increased global concern as a cause of community-acquired and healthcare-associated infection. Gram-positive organisms including bacteria of the genera *Staphylococcus*, *Streptococcus* and *Enterococcus*, are among the most common bacteria causes of clinical infection (Johnson *et al*., 2005; Klevens *et al*., 2007). The substantial increase in the drug resistance of antibiotics in recent years poses an ever-increasing therapeutic challenges.

The organisms play a pivotal role in developing resistance to antibiotics. However, patients are also partly responsible for antibiotic resistance due to overuse, misuse, and/or lack of adherence to the prescription, failure to complete the course which has led to ever-increasing levels of resistance to antibiotic treatment (Franco *et al*., 2009; Prestinaci *et al*., 2016). Drug resistance has become particularly problematic in recent times because of the slow pace at which novel antibiotics are being discovered while antibiotic use continues to rise (Li and Webster, 2018). Due to the prevalence of microbial resistant strains, there is a need to focus on the plant species that show antimicrobial activities. The identification of plant species with antimicrobial properties is of great importance for therapeutic treatment of patients (Artizzu *et al.*, 1995; Izoo *et al.*, 1995; Eloff, 1998; Selvamohan *et al*.,2012).

It is important to evaluate the ability of plant extracts to inhibit the growth of known pathogenic microorganisms. The general method used by most investigators is agar diffusion assay for the determination of microbial activities (Nascimento *et al*., 2000; Al-Hussainin and Mahasneh, 2009). For the interest of this study, a sensitive and quick microplate method previously described by Eloff (1998) was used. This method is useful when investigating extracts with unknown components. Furthermore, this technique addresses shortfalls that have been observed on the agar diffusion; such as microbial effects may be inhibited by extrinsic factors or contamination (Marsh and Goode, 1994). Moreover, this method clearly distinguishes between bactericidal and bacteriostatic effects and it can determine minimum inhibitory concentration (Eloff, 1998). In addition, the minimum bactericidal concentration can be defined as the lowest concentration that can kill any visible bacterial growth (Sen and Batra,2012).

2.0 METHODS AND MATERIALS

2.1 PLANT COLLECTION AND EXTRACTION

Plant species (n=9) were collected from Walter Sisulu National Botanical Gardens in South Africa, in February 2017 (**see Table 1 here below**). The voucher specimen is held at Walter Sisulu National Botanical Gardens herbarium. The plant material was air-dried in a well ventilated room. After drying, the plants were ground into fine power and stored away from light at room temperature.

Table 1: Eight plant species with accession numbers and voucher specimen numbers use in this study.

NUMBER	NAME	FAMILY	PART	ACCESSION NUMBER	VOUCHER OF SPECIMEN COLLECTED	
					DATE	NUMBER
1	*Euclea crispa*	Ebenaceae	Leaf	24/1982	11/10/1982	24, Behr, C.M
2	*Euclea natalensis*	Ebenaceae	Leaf	178/1987	10/6/1987	479; Steel, B.S
3	*Schkuhria pinnata*	Asteraceae	Leaf	N/A	N/A	N/A
4	*Ziziphus mucronata*	Rhamnaceae	Leaf	36/1982	15/10/1982	39; Behr, C.M
5	*Ziziphus mucronata*	Rhamnaceae	Fruit	36/1982	15/10/1982	39; Behr, C.M
6	*Lippia javanica*	Verbenaceae	Leaf	16/2014	22/1/2014	28; Kondlo, M
7	*Vernonia oligocephala*	Asteraceae	Leaf	268/2013	12/05/2013	29; Hankey, A.J
8	*Clerodendrum myricoides*	Lamiaceae	Leaf	11/1987	2/2/1987	367, Steel, B.S
9	*Erythrina lysistemon*	Fabaceae	Leaf	21/1982	7/10/1982	22; Behr, C.M

2.2 Preparation of crude extracts for biological assays

The ground plant extracts (leaves and fruit) were extracted with 90% methanol (1 g/10 ml) in clean honey jars and vigorously shaken for 3 hours. The crude extracts were filtered through Whatman No.1 filter paper and dried at room temperature under a stream of cold air. The crude extracts were reconstituted in methanol, dichloromethane and acetone at a concentration of 10 mg/ml for all assays.

2.3 Bacterial strains

The antimicrobial activities of the plant extracts were tested against gram-negative bacteria [*Escherichia coli* (NCTC10538) and *Pseudomonas aeruginosa* (ATCC27853)] and gram-positive bacteria [*Staphylococcus aureus (*NCTC10538) and Enterococcus *faecalis* (ATCC29212)]. These bacterial strains were donated by the Pearson Institute of Higher Education, South Africa and the University of Pretoria Paraclinical Department. The bacterial strains were continuously maintained in nutrient agar and Mueller-Hinton agar sub-cultured weekly in order to ensure that fresh cultures were used each time for an inoculum preparation.

2.4 Determination of minimal inhibitory concentration (MIC)

Minimal inhibitory concentrations (MIC's) were determined using the microplate dilution method developed by Eloff (1998). Methanol (MeOH) crude plant extracts were re-dissolved in 50% distilled water and 50% solvent, namely MeOH, dichloromethane (DCM) and acetone (AC) to a concentration of 8 mg/ml. One hundred microliters (100 µl) of dissolved plant extract and a positive control, gentamicin (Sigma-Aldrich, Cat No, 1405-41-0) were serially diluted with distilled water in a 96 well plate. Bacterial suspension (100 µl), standardised to McFarland standard No. 0.5, was added to each well. The following concentrations of plant extracts were used in these experiments: 2, 1, 0.5, 0.25, 0.125, 0.062, 0.031 and 0.015 mg/ml respectively. Sealed plates were incubated at 37°C for 24 hours. To indicate growth, 40 µl of p-iodonitrotetrazolium violet (INT) (Sigma-Aldrich, Cat NO. 18377), was dissolved in distilled water and added to the microplate wells were incubated at 37°C for 30-45 minutes. The presence of bacterial growth was observed as a pink/red colour, and clear wells indicated the inhibition of bacterial growth by the plant extract. The extract dissolving solvents (MeOH, DCM and AC) were used as negative controls and gentamicin served as a positive control (50 µg/ml). The assay was performed in quadruplicates and repeated three times.

2.5 Determination of minimal bactericidal concentration (MBC)

Minimal bactericidal concentration (MBC) was recorded as the lowest concentration of the crude plant extract that killed 100% of the test organisms. MBC was determined by adding 50 µl aliquots of the serial dilution which did not show any visible growth after incubation in the MIC assay, and 50 µl of INT, to 100 µl of Muller-Hinton broth. The plates were incubated at 37°C for 24 hours. The presence of bacterial growth was indicated by a pink/red colour, and clear wells indicated the inhibition of bacterial growth by the plant extract. The lowest concentration indicating inhibition of growth was recorded as the MBC.

2.6 STATISTICAL ANALYSIS

The assay was performed in quadruplicate and repeated three times. All data was expressed as mean and standard deviation using S Excel 2013 and ANOVA GraphPad Prism 5.

3.0 RESULTS

Antimicrobial effects of eight plant species were evaluated in terms of their ability to inhibit growth of the selected bacterial strains. Eight plant parts were screened and found to be active against one or more of bacterial strains used (**see Table 2**). There was no significant difference observed between the solvents used in reconstitution of the extracts. For the results interpretation, an inhibition effect below 1 mg/ml was considered to be a good activity effect and anything above 1 was considered as moderate activity. Essentially above 2 mg/ml was not considered active effect in this study.

Table 2: Minimal inhibitory concentration of eight plant species expressed in mean and standard deviation.

Plant Species	Plant part	Solvent	*E. coli* (mg/ml)	*P. aeruginosa* (mg/ml)	*S. aureus* (mg/ml)	*E. faecalis* (mg/ml)
			Mean± SDV	Mean± SDV	Mean± SDV	Mean± SDV
Euclea crispa	Leaf	MeOH	1.75±0.00	2.00±0.00	1.67±0.00	1.63±0.00
		DCM	1.75±0.00	2.00±0.00	1.60±0.58	1.44±0.00
		EA	1.28±0.00	1.50±0.71	1.34±0.00	1.38±0.00
Euclea natalensis	Leaf	MeOH	1.25±0.00	1.00±0.00	1.33±0.00	1.12±0.00
		DCM	1.75±0.00	2.00±0.00	1.60±0.58	1.55±0.58
		EA	1.38±0.00	2.00±0.00	1.41±0.00	1.43±0.00
Schkuhria pinnata	leaf	MeOH	1.00±0.00	1.25±0.00	1.25±0.58	1.34±0.00
		DCM	1.00±0.00	1.25±0.00	1.10±0.00	1.13±0.58
		EA	0.50±0.00	0.63±0.00	0.74±0.58	0.94±0.00
Ziziphus mucronata	leaf	MeOH	0.50±0.00	0.63±0.00	0.82±0.58	0.83±0.00
		DCM	1.00±0.00	0.88±0.00	0.84±0.00	0.82±0.00
		EA	0.50±0.00	0.44±0.00	0.61±0.58	0.67±0.00
Ziziphus mucronata	fruit	MeOH	1.00±0.00	0.50±0.00	1.15±0.00	0.98±0.00
		DCM	1.75±0.00	2.00±0.00	1.60±0.58	1.55±0.58
		EA	1.50±0.58	2.00±0.00	1.31±0.00	1.06±0.00
Lippia javanica	leaf	MeOH	0.25±0.00	0.50±0.87	0.53±0.00	0.49±0.00
		DCM	0.50±0.00	1.13±0.29	1.11±0.58	1.03±0.29
		EA	0.50±0.00	0.50±0.29	0.43±0.00	0.50±0.87
Vernonia oligocephala	leaf	MeOH	1.06±0.87	1.00±0.00	1.26±0.00	1.26±0.58
		DCM	1.25±0.00	1.00±0.00	1.25±0.58	1.34±0.00
		EA	1.75±0.00	2.00±0.00	1.67±0.00	1.63±0.00
Clerodendrum myricoides	leaf	MeOH	1.75±0.00	2.00±0.00	1.52±0.58	1.49±0.58
		DCM	1.25±0.00	1.00±0.00	1.17±0.58	1.17±0.58
		EA	1.75±0.00	2.00±0.00	1.67±0.00	1.63±0.00
Erythrina lysistemon	leaf	MeOH	1.00±0.00	0.88±0.00	0.91±0.58	1.08±0.00
		DCM	0.50±0.00	1.00±0.00	1.00±0.58	1.09±0.58
		EA	0.50±0.00	0.44±0.00	0.50±1.01	0.52±0.00
Gentamacin		MeOH	1.75±0.00	2.00±0.00	1.31±0.14	1.19±0.00
		DCM	1.19±0.00	2.00±0.00	1.01±0.29	0.92±0.29
		EA	1.19±0.00	2.00±0.00	1.21±0.58	1.14±0.00

MeOH: methanol; DCM: dichloromethane; EA: ethanol; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. ureus*: *Staphylococcus aureus*; *and E. faecalis: Enterococcus faecalis.*

3.1 Determination of minimal inhibitory concentration (MIC)

Table two lists the antimicrobial activity of all plant extracts. The following four plant extracts showed good antimicrobial activity against all four bacteria used which are *Lippia javanica (*0.25±0.00 to 1.13±0.29mg/ml); *Ziziphus mucronata* leaf (0.44±0.00 to 1.00±0.00 mg/ml); *Erythrina lysistemon* (0.44±0.00 to 1.08±0.00 mg/ml) and *Schkuhria pinnata* (0.5±0.00 to 1.34±0.00 mg/ml). Furthermore, it can be seen that the other five plant extracts showed good inhibition effects against three bacterial straight which were below 1.75±0.00 mg/ml, except for *P. aeruginosa* (2.00±0.00 mg/ml). This suggests *P. aeruginosa* is resistance against plant extracts used. Similarly, gentamycin (positive control) had inhibitory effects ranged from 0.92±0.29 to 2.00±0.00 mg/ml against all four bacteria used.

3.2 Determination of minimal bactericidal concentration (MBC)

Table 3 represents minimum bactericidal effects of nine plant extracts against all four bacteria used. Interestingly, the four plant extracts that showed inhibition effects (**see Table 3**) have demonstrated bactericidal effects against three bacteria used *(E.coli*, *S.aureus* and *E. faecalis*). The plant extracts are *Lippa javanica* (0.25±0.00 to 1.00±0.00 mg/ml); *Ziziphus mucronata* leaf (0.25±0.00 to 2.00±0.00 mg/ml); *Erythrina lysistemon* (0.5±0.00 to 2.00±0.00 mg/ml) and *Schkuhria pinnata* (0.5±0.00 to 2.00±0.00 mg/ml. Moreover, the bactericidal effects for other four plant extracts was observed to be 1.67±0.58 against *E.coli*, *S. aureus* and *E. faecalis*. Essentially, no bactericidal effect was observed for *Euclea crispa*. None of the plant extracts used showed bactericidal effects against *P. aeruginosa*. This indicates that *P. aeruginosa* is residence against plant extracts tested against.

Table 3: Minimal bactericidal concentration of nine plant extracts expressed in mean and standard deviation.

Plant Species	Plant part	Solvent	*E. coli* (mg/ml)	*P. aeruginosa* (mg/ml)	*S. aureus* (mg/ml)	*E. faecalis* (mg/ml)
			Mean± SDV	Mean± SDV	Mean± SDV	Mean± SDV
Euclea crispa	Leaf	MeOH	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
		DCM	2.00±0.00	2.00±0.00	1.67±0.58	1.00±0.00
		EA	1.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
Euclea natalensis	Leaf	MeOH	1.00±0.00	2.00±0.00	2.00±0.00	0.50±0.00
		DCM	2.00±0.00	2.00±0.00	1.67±0.58	1.00±0.00
		EA	1.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
Schkuhria pinnata	Leaf	MeOH	1.00±0.00	2.00±0.00	0.25±0.00	2.00±0.00
		DCM	0.50±0.00	2.00±0.00	1.00±0.00	1.67±0.58
		EA	2.00±0.00	1.00±0.00	1.00±0.00	2.00±0.00
Ziziphus mucronata	Leaf	MeOH	0.50±0.00	1.00±0.00	0.25±0.00	1.00±0.00
		DCM	2.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00
		EA	0.50±0.00	1.00±0.00	1.00±0.00	0.50±0.00
Ziziphus mucronata	Fruit	MeOH	0.50±0.00	2.00±0.00	2.00±0.00	0.50±0.00
		DCM	2.00±0.00	2.00±0.00	1.67±0.58	1.67±0.58
		EA	0.50±0.00	1.00±0.00	2.00±0.00	2.00±0.00
Lippia javanica	Leaf	MeOH	0.25±0.00	0.50±0.00	0.25±0.00	0.50±0.00
		DCM	1.00±0.00	2.00±0.00	0.71±0.51	1.00±0.00
		EA	1.00±0.00	0.25±0.00	0.75±1.08	0.50±0.00
Vernonia oligocephala	Leaf	MeOH	1.00±0.00	2.00±0.00	1.67±0.58	2.00±0.00
		DCM	1.00±0.00	0.50±0.00	1.33±0.58	1.00±0.00
		EA	2.00±0.00	2.00±0.00	1.67±0.58	2.00±0.00
Clerodendrum myricoides	Leaf	MeOH	2.00±0.00	2.00±0.00	1.00±0.00	2.00±0.00
		DCM	1.00±0.00	2.00±0.00	1.50±0.87	1.67±0.58
		EA	2.00±0.00	0.50±0.00	0.42±0.14	2.00±0.00
Erythrina lysistemon	Leaf	MeOH	1.00±0.00	1.00±0.00	0.50±0.43	2.00±0.00
		DCM	2.00±0.00	2.00±0.00	1.42±1.01	0.67±0.29
		EA	1.00±0.00	0.25±0.00	0.19±0.11	0.50±0.00
Gentamacin		MeOH	2.00±0.00	2.00±0.00	0.25±0.33	1.00±0.00
		DCM	0.06±0.00	2.00±0.00	0.83±1.01	0.50±0.43
		EA	0.06±0.00	0.25±0.00	2.00±0.00	0.50±0.00

MeOH: methanol; DCM: dichloromethane; EA: ethanol; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *and E. faecalis: Enterococcus faecalis.*

4.0 DISCUSSION

The plant crude extracts were screened for their minimum inhibitory concentration and minimum bactericidal concentration using eight different concentrations; against four pathogenic organisms. The findings of this research have shown that all plant extracts demonstrated inhibitory effects against pathogenic organisms tested against. However, only four plant extracts showed good inhibition effects against all three bacteria sued which are *Lippia javanica*, *Ziziphus mucronata leaf*, *Erythrina lysistemon* and *Schkuhria pinnata*. Similarly, positive control had inhibitory effects were comparable to those plant extracts gave good inhibition effects in all four pathogenic organisms used.

For the bactericidal effects, the four plant extracts that demonstrated good inhibition effects which are mentioned above also demonstrated good bactericidal effects only against three bacteria used. It was observed that *P. aeruginosa* did not demonstrate bactericidal effect against plant extract used.

The antimicrobial effects of plant extracts that showed inhibitory effects in our experiments are in agreement with their known medicinal use. For example, *Lippia javanica has* been reported to be used by Xhosa people to disinfect meat that has been contaminated by anthrax. In addition to that, it has been reported to be used for fever, cough, bronchitis and influenza (Van Wyk *et al*., 2009; Van Wyk, 2011). *Ziziphus mucronata* has been reported to have various medicinal used per each region and the most common one is to treat boils, swollen glands, wounds and sores (Palmer and Pitman 1972). Interestingly, *Erythrina lysistemon* bark is used to treat sores, wounds, abscesses and arthritis. Furthermore, the leaves infusions are used as ear drops to relieve earache, and decoctions of the roots are applied to sprains (Van Wyk *et al*., 1997). Lastly, *Schkuhria pinnate* have been used as a bactericide in open wounds, to treat acne, malaria and inflammation, and as a blood purifier and diuretic (Bussmann *et al*., 2008).

In this study, four microorganisms were used as to assess the ability of plant extracts to inhibit their growth. These were gram-negative [*E. coli and P. aeruginosa*] and gram-positive [*S. aureus* and *E. faecalis*]. It was observed that plant extracts demonstrated a degree of inhibition towards both gram-negative and gram-positive bacteria. Generally, gram-negative bacteria are resistance to most of antibiotics, mainly lipophilic and amphiphilic. This is mainly due to outer membrane in gram negative bacteria, which excludes certain drugs and antibiotics from penetrating the cell (Miller, 2016; Mia-Prochnow, 2016). In contracts to gram positive bacteria do not have outer membrane, so they are more susceptible to antibiotics (Verma, 2012; Mai-Prochnow, 2016).

In our findings *P. aeruginosa* was observed to be resistance towards most plant extracts tested. This was having been previously reported in literature that *Ps. aeruginosa* has the ability to develop resistance to antibiotics rather rapidly over several generations. Essentially, resistance strains make it difficult to treat once in a host, such as a human or other animal, is infected (Griffin *et al*., 2004). The sensitive organisms (*E.coli*) demonstrated to be sensitive in our findings and this is due to the fact that *E. coli* is well characterised, widely available and sensitive to antibiotics (Livermore *et al*., 2008).

5.0 DISCUSSION

To this effect, the findings suggest that there is a great need for further development of antimicrobial agents that may contribute to the improvement of future chemotherapeutic agents from medicinal plants. It was seen that *Lippia javanica*, *Ziziphus mucronata* leaf, *Erythrina lysistemon* and *Schkuhria pinnata* showed a good inhibitory and bactericidal effects against bacteria used. In four pathogenic organisms used, only *P. eruginosa demonstrated* some degree on resistance is certain plant extracts used. Interestingly, *E.coli* was observed to be sensitive strain in almost all plant extracts tested followed by *S. aureus*. The plants contain diverse class of compounds which possess biologically active agents. The plant extracts have exhibited excellent antimicrobial activities, with minimum inhibitory concentrations comparable to those of standard drugs. The future work involves identification of the active compounds and characterization.

6.0 CONFLICT OF INTERESTS

The authors declare that they do not have any conflict with respect to the publication of this paper.

7.0 ACKNOWLEDGMENTS

We thank the College Health Sciences Scholarship Grant at the University of KwaZulu-Natal, Institute of Traditional Knowledge and Traditional Medicine at the Vaal University of Technology, the National Research Foundation and Thuthuka grant for financial support received towards this study. Mr Tshepiso Lentswe is thanked for assisting with sample collection. Furthermore, we express our sincere gratitude to Prof Vivienne Russel for proofreading this manuscript.

8.0REFERENCES

Alekshun, M. N., Levy, S.B. (2007). "Molecular mechanisms of antibacterial multidrug resistance." *Cell*. **128**: 1037–1050.

Al-Hussaini, R., Mahasneh, A.M. (2009). "Microbial growth and quorum sensing antagonist activities of herbal plants extracts." *Molecules*. **14**: 3425-3435.

Artizzu, N., Bonsignore, L., Cottiglia, F., Loy, G. (1995). "Studies of the diuretic and antimicrobial activity of *Cynodon dactylon* essential oil." *Fitoterapia*. **66**: 174-175.

Barker, K. F. (1999). "Antibiotic resistance: a current perspective." *Journal Clinical Pharmacology*. **48**: 109-124.

Ben-Noun, L. (2009). "Infectious disease that afflicted people of Jericho in ancient times: Medical Research from the Viewpoint of Contemporary Perspective. Ben-Gurion University of the Negev. (1st ed.) Publisher: B.N. Publication House, Israel.

Bussmann, D., Sharon, D., Diaz, D.P., Barocio, Y (2008). "Peruvian plants canchalagua (*Schkuhria pinnata* (Lam.) Kuntze), hercampuri (*Gentianella alborosea* (Gilg.) Fabris), and corpus way (*Gentianella bicolor* (Wedd.) J. Pringle)." *Arnaldoe*. **15**(1): 149-152.

Chan, G.J., Lee, A.C.C., Baqui, A.H., Tan, J., Black, R.E. (2013). "Risk of Early-Onset Neonatal Infection with Maternal Infection or Colonization: A Global Systematic Review and Meta-Analysis." *PLOS Medicine*. **10**(8): 1-20.

Dahal, R. H., Chaudhary, D.K. (2018). "Microbial Infections and Antimicrobial Resistance in Nepal: Current Trends and Recommendations." *The Open Microbiology Journal*. **12**: 230-242.

Davies, J., Davies, D. (2010). "Origins and Evolution of Antibiotic Resistance." *Microbiology and molecular reviews*. **74**(3): 417–433.

Doernberg, S. B., Lodise, T.P., Thaden J.T., Munita, J.M., Cosgrove, S.E., Arias, C.A., Boucher, H.W., Corey, G.R., Lowy, F.D Murray, B., Miller, L.G., Holland, T.L., Gram-Positive Committee of the Antibacterial Resistance Leadership Group (ARLG). (2017). "Gram-positive bacterial infections: research priorities, accomplishments, and future directions of the antibacterial resistance leadership group." *Clinical Infectious Diseases* **64**(1): S24-S29.

Eloff, J. N. (1998). "A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts from Bacteria." *Planta Medica* **64**: 711-713.

Exner, M., Bhattacharya, S., Christiansen, B., Gebel, J., Goroncy-Bermes, P., Hartemann, P., Heeg, P., Ilschner, C., Kramer, A., Larson, E., Merkens, W., Mielke, M., Oltmanns, P., Ross, B., Rotter, M., Schmithausen, R.M., Sonntag, H-G., and Trautmann, M. (2017). "Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria?" *GMS Hygiene and Infection Control*. **12**(1-24).

Franco, B. E. M., M.A., Rodríguez, M.A.S.; Wertheimer, A.I. (2009). "The determinants of the antibiotic resistance process." *Infection and Drug Resistance*. **2**: 1-11.

Griffin, A. S., West, S.A., Buckling, A. (2004). "Cooperation and competition in pathogenic bacteria." *Nature*. **430**(7003): 1024-7.

Izzo, A. A., di Carlo, G., Biscardi, D., de Fusco, Mascolo, N., Borrelli, F., Capasso, F., Fasulo, M.P., Autore, G. (1995). "Biological screening of Italian medicinal plants for antibacterial activity." *Phytotherapy Research*. **9**(4): 281-286.

Johnson, A. P., Pearson, A., Duckworth, G. (2005). "Surveillance and epidemiology of MRSA bacteraemia in the UK." *Journal of Antimicrobial Chemotherapy*. **56**(3): 455–462.

Klevens, R. M., Morrison, M.A., Nadle, J., Petit, S., Gershman, K., Ray, S., Harrison, L.H., Lynfield, R., Dumyati, G., Townes, J.M., Craig, A.S., Zell E.R., Fosheim, G.E., McDougal, L.K., Carey, R.B., Fridkin, S.K., for the Active Bacterial Core surveillance (ABCs) MRSA Investigators (2007). "Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States." *The Journal of the American Medical Association*. **298**(15): 1763–1771.

Li, B., Webster, T.J. (2018). "Bacteria Antibiotic Resistance: New Challenges and Opportunities for Implant-Associated Orthopaedic Infections." *Journal of Orthopaedic Research*. **36**(1): 22-32.

Li, X. Z., Plésiat, P., Nikaidoc, H. (2015). "The Challenge of Efflux-Mediated Antibiotic Resistance in Gram-negative Bacteria." *Clinical Microbiology Reviews*. **28**(2): 337-418.

Livermore, D. M., Hope, R., Brick, G., Lillie, M., Reynolds, R. (2008). "Non-susceptibility Trends among Enterobacteriaceae from Bacteraemia in the UK and Ireland, 2001–06." *Journal of Antimicrobial Chemotherapy*. **62** (Suppl 2): 41-54.

Luh, K. T., Hsueh, P.R., Teng, L.J., Pan, H.J., Chen, Y.C., Lu, J.J., Wu, J.J., Ho, S.W. (2000). "Quinupristin-Dalfopristin Resistance among Gram-Positive bacteria in Taiwan." *Antimicrobial agents and chemotherapy*. **44**(12): 3374-3380.

Mai-Prochnow, A., Clauson, M., Hong, J., Murphy, A.B. (2016). "Gram-positive and Gram-negative bacteria differ in their sensitivity to cold plasma." *Nature*. **6**(38610): 1-11.

Marsh, J., Goode, J. (1994). Antimicrobial peptides Ciba Foundation Symposium. Chichester, John Wiley & Sons. 186.

Miller, S. I. (2016). "Antibiotic resistance and regulation of the Gram-negative bacterial outer membrane barrier by host innate immune molecules." *American Society for Microbiology*. **7**(5): 1-3.

Mwambete, K. D. (2009). "The *in vitro* antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia*: a Tanzania medicinal plant." *African Health Sciences*. **9**(1): 34-39.

Nascimento, G. F., Locatelli, J., Freitas, P.C., Silva, G.L. (2000). "Antibacterial activity of plant extracts and phytochemical on antibiotic resistant bacteria." *Brazilian Journal of Microbiology*. **31**: 247-256.

Palmer, E., Pitman, N. (1972). Trees of southern Africa, vol. 2. Cape Town., Publishers: Balkema, Amsterdam.

Prestinaci, F., Pezzotti, P., Pantosti, A. (2016). "Antimicrobial resistance: a global multifaceted phenomenon." *Pathogens and Global Health*. **109**(7): 309-318.

Ryan, K. J., Ray, C.G., Ahmad, N., Drew, W.L., Lagunoff, M., Pottinger, P., Reller, L.B., Sterling, C.R. (2014). Pathogenesis of Bacterial Infections". Sherris Medical Microbiology (6th ed.). New York, McGraw Hill Education.

Schulz, G. E. (1993). "Bacterial porins: Structure and function." *Current Opinion in Structural Biology*. **5**: 701-707.

Selvamohan, T., Ramadas, V., Kishore, S.S.S. (2012). "Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria" *Advances in Applied Science Research*. **3**(5): 3374-3381.

Sen, A., Batra, A. (2012). "Evaluation of antileishmanial activity of South Indian medicinal plants against *Leishmania donovani*." *Experimental Parasitology*. **105**(5): 1287-1293.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S.M., Kamal, M.A. (2015). "Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment." *Saudi Journal of Biological Sciences*. **22**: 90-101.

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

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N. (2009). Medicinal plants of South Africa. Pretoria, Briza.

Van Wyk, B.-E. (2011). "The potential of South African plants in the development of new medicinal products." *South African Journal of Botany*. **77**: 812-829.

Verma, P. (2012). "Antibiotic sensitivity treatment for Gram-positive bacteria isolated from pus sample." *Bulletin of Environment, Pharmacology and Life Sciences*. **1** (10): 06-06.

CHAPTER FOUR: OBJECTIVE TWO (PAPER III)

In this chapter the evaluation of cytotoxicity and the anti-inflammatory effects of selected South African medicinal plant extracts against C2C12 cells and RAW 264.7 cells are presented and discussed. The manuscript was published in February 2020 in the *International Journal of Scientific and Research***.**

Paper III **Nkala BA**., Mbongwa HP., Qwebani-Ogunleye T**.** (2020). The evaluation of cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 and RAW 264.7 cells. *International Journal of Scientific and Research Publications*, **10** (2): 207 – 220.

THE EVALUATION OF CYTOTOXICITY AND ANTI-INFLAMMATORY EFFECTS OF SELECTED SOUTH AFRICAN MEDICINAL PLANTS AGAINST C2C12 CELLS AND RAW 264.7 CELLS

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ABSTRACT

Medicinal plants are used in traditional medicine throughout the world. In addition to this, certain communities consider medicinal plants to be safer than drugs and able to treat more than one ailment. This study aimed to evaluate the cytotoxicity and anti-inflammatory effects of *Euclea crispa* (leaf)*, Eulea natalensis* (leaf)*, Schkuhria pinnata* (leaf)*, Ziziphus mucronata* (leaf), *Ziziphus mucronata* (fruits), *Lippia javanica* (leaf)*, Vernonia oligocephala* (leaf)*, Clerodendrum myricoides* (leaf)*,* and *Erythrina lysistemon* (leaf) in C2C12, and RAW 264.7 cells. Plants were extracted with 90% methanol (1 g/10 ml) and diluted in distilled water to give a final concentration of 10 mg/ml. C2C12, and RAW 264.7 cells were treated for 24 h with various concentrations of plant extracts (10 - 1000 µg/ml). Cytotoxicity was evaluated with Alamar Blue and crystal violet cell viability assays. RAW 264.7 cells were stimulated with lipopolysaccharide (LPS) to produce nitric oxide (NO). Thereafter, the anti-inflammatory effect of the plant extracts was assessed by their ability to inhibit NO production, using the Griess reagent assay. None of the plants extracts demonstrated cytotoxic effects at the concentrations used against RAW 264.7 cells with LC_{50} value >1000 µg/ml. However, a degree of cytotoxicity in all plant extracts against C2C12 cells in higher concentrations was observed with LC_{50} <1000 µg/ml. All plant extracts demonstrated some degree of anti-inflammatory effect. However, plant extracts exhibited marked anti-inflammatory activities. These were *Clerondendrum myricoides* (35% - 89%), *Lippia javanica* (26% - 77%), *Erythrina lysistemon* (23% - 76%), *Schkuhria pinnata* (27% - 65%), and *Vernonia oligocephala* (16% - 58%) with IC_{50} value >1000 µg/ml. The present findings suggest that these plants' extracts may serve as a promising therapeutic agent for inflammatory diseases and authenticates their use in traditional medicine.

Keywords: Cytotoxicity, Cell viability, Medicinal plants, anti-inflammatory, inhibition.

1. Introduction

Medicinal plants are widely utilized in traditional medicine throughout the world (Deutschländer *et al*., 2009; Yuan *et al*., 2016). Essentially, certain communities consider medicinal plants to be safer than drugs, and able to treat more than one ailment (Pan *et al*., 2013; Sofowora *et al*., 2013). The selected South African plants have been reported for the treatment of numerous ailments by the traditional healers. The plants of interest for this study were *Euclea crispa* (leaf), *Eulea natalensis* (leaf), *Schkuhria pinnata* (leaf), *Ziziphus mucronata* (leaf), *Ziziphus mucronata* (fruits), *Lippia javanica* (leaf), *Vernonia oligocephala* (leaf), *Clerodendrum myricoides* (leaf), and *Erythrina lysistemon* (leaf) (Nkala *et al*., 2019a). The present study seeks to validate the usefulness of these medicinal plants by traditional healers.

Essentially, *Euclea crispa* (leaf) has been reported to be used to treat stomach disorders, measles, coughs, constipation, diabetes, rheumatism, and epilepsy (Raimondo *et al*., 2009). Deutschländer *et al*., (2009) described the use of *Eulea natalensis in* a variety of traditional remedies for worms, stomach disorders, toothache, headache, chest complaints, pleurisy, urinary tract infections, venereal diseases, schistosomiasis, dysmenorrhoea, scrofulous swellings, abnormal growths on skin, leprosy, and diabetes (Maroyi, 2017). *Schkuhria pinnata* has been reported to be useful as a bactericide in open wounds, to treat acne, malaria, inflammation, as a blood purifier, diuretic, and treatment of diabetes (Bussmann *et al*., 2008; Deutschländer *et al*., 2009). *Ziziphus mucronata* has been used for the treatment of boils, swollen glands, wounds, sores, and diabetes (Deutschländer *et al*., 2009; Ibrahim and Islama, 2017). Interestingly, *Lippia javanica* has been used to disinfect meat that has been contaminated by anthrax (Van Wyk, 2011). In traditional medicine, *Lippia javanica* has been used for the treatment of diabetes, fever, cough, bronchitis, and influenza (York, 2012; Arika *et al.,* 2016). *Vernonia oligocephala* has been used for the relief of stomach ache, and the treatment of diabetes (Amusan *et al*., 2007). *Clerodendrum myricoides* has been reported to be used for snakebites, to reduce bodily swellings, relieve indigestion, to treat colds, chest pains, headaches, as well as being applied to bleeding gums, and to treat impotence (Raimondo *et al*., 2009). *Erythrina lysistemon* has been reported to be used for the treatment of sores, wounds, abscesses, arthritis, and to relieve earache (Farag *et al.*, 2016).

Essentially, medicinal plants needed to be validated for safety, to ensure that they are not cytotoxic. The cytotoxicity profiling of these plant species plays an important role to support their use in the medicinal plants' practice. The cell-based assay is often the preferred method of screening for cytotoxicity in various cell lines, including C2C12 cells, and RAW 264.7 cells (Kaur and Dufour, 2012).

The C2C12 cells are a murine myoblast cell line, derived from satellite cells (Yaffe and Saxel, 1977). Essentially, myoblast becomes myocyte during myogenesis to form muscle fibers in skeletal muscles (Hyejin *et al*., 2017). C2C12 cells are mononucleated, fusiform structures which progressively fuse to form plurinucleate syncytia that further differentiate in culture to acquire the morpho-functional features of the muscle cells (Yaffe and Saxel, 1977; Burattini *et al*., 2009; Girgis *et al*., 2013). These cells are well-established mouse myoblast cells used widely as an *in vitro* model of skeletal muscle (Burattini *et al*, 2009; Morissette *et al*, 2009; Girgis *et al*., 2013; Hyejin *et al*, 2017; Musso *et al*., 2019). Furthermore, C2C12 cells have been used to assess the cytotoxicity effects of medicinal plants (van Huyssteen *et al*., 2011; Beseni *et al.*, 2019), and also have been used for glucose regulation as to access the ability of medicinal plants to regulate glucose blood levels (Harbilas *et al.*, 2009; Javad *et al*., 2011; Padmanabha and Kaiser, 2011; Beseni *et al.*, 2019).

The RAW 264.7 cells are commonly used as a model of mouse macrophages for the study of cellular responses to microbes and their products (Berghaus *et al*., 2010). Hence, they have been described as an appropriate model of macrophages, and ultimately capable of performing pinocytosis and phagocytosis (Taciak *et al*., 2018). The cells can increase nitric oxide (NO) production when stimulated with lipopolysaccharide (LPS), and this enhances phagocytosis (Fuentes *et al*., 2014). RAW 264.7 cells has been widely used in medicinal plant's research with particular focus on cytotoxicity effects and anti-inflammatory effects (Soromou *et al.*, 2012; Razali e*t al*, 2014; Lee *et al*., 2017; Soonthornsit *et al.*, 2017; Kamtchueng *et al*., 2017; Kudumela *et al*., 2018; Ayupova *et al*., 2019). The ability of plant extracts to inhibit macrophage functions by decreasing the production of inflammatory mediators such as NO, prostaglandins, and cytokines has been observed (Jo *et al*., 2010). The potential of plant extracts to inhibit NO production in tissue culture medium has been reported (Lee *et al*., 2010). This

study aimed to evaluate the anti-inflammatory effects of the plant extracts in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Besides, the cytotoxicity effects of the plant extract against C2C12 cells, and RAW 264.7 cells was evaluated.

2. Materials and methods

2.1 Collection and extraction

Plant species (n=9) were collected from Walter Sisulu National Botanical Gardens, South Africa, in February 2017. The plant species collected has previously described in this theses in chapter 3, section 2.1 (**Table 1**). The voucher specimens are held at Walter Sisulu National Botanical Gardens herbarium. The plant material was air-dried in a well-ventilated room. After drying, the plants were ground into a powder and stored away from light at room temperature.

Table 1: Accession numbers and voucher specimen numbers of the nine plant species used in this study.

NAME	FAMILY	PART	ACCESSION NUMBER	VOUCHER OF SPECIMEN COLLECTED	
				DATE	NUMBER
Euclea crispa	Ebenaceae	Leaf	24/1982	11/10/1982	24, Behr, C.M
Euclea natalensis	Ebenaceae	Leaf	178/1987	10/6/1987	479; Steel, B.S
Schkuhria pinnata	Asteraceae	Leaf	N/A	N/A	N/A
Ziziphus mucronata	Rhamnaceae	Leaf	36/1982	15/10/1982	39; Behr, C.M
Ziziphus mucronata	Rhamnaceae	Fruits	36/1982	15/10/1982	39; Behr, C.M
Lippia javanica	Verbenaceae	Leaf	16/2014	22/1/2014	28; Kondlo, M
Vernonia oligocephala	Asteraceae	Leaf	268/2013	12/05/2013	29; Hankey, A.J
Clerodendrum myricoides	Lamiaceae	Leaf	11/1987	2/2/1987	367, Steel, B.S
Erythrina lysistemon	Fabaceae	Leaf	21/1982	7/10/1982	22; Behr, C.M

2.2 Preparation of crude extracts for cytotoxicity assays

The ground plant extracts (leaves, and fruits) were extracted with 90% methanol (1 g/10 ml) and vigorously shaken for 3 h. The crude extracts were filtered through Whatman No.1 filter paper and dried at room temperature under a stream of cold air. The crude extracts were reconstituted in distilled water at a concentration of 10 mg/ml for all assays.

2.3 Cell cultures

2.3.1 C2C12 (ATCC CRL – 1772)

The C2C12 (ATCC CRL-1772) cell line is derived from mouse skeletal muscle; myoblasts originally derived from satellite cells from the thigh muscle of a two-month-old female C3H mouse donor 70 h after a crush injury (Yaffe and Saxel, 1997). The cells were donated by the Department of Biotechnology at Vaal University of Technology, South Africa. The cells were cultured in 75 cm^2 tissue culture flasks in Dulbecco's Modified Eagle's Minimum (DMEM) containing L-glutamine and supplemented with 1.0 mM Penicillin/Streptomycin and 10% heated foetal bovine serum (FBS). Thereafter, flasks were incubated at 37^oC in a humidified atmosphere of 5% CO_2. The medium was changed every second day until 80-90% confluent growth was reached. Thereafter, cells were trypsinised with 0.25% trypsin EDTA. Essentially, cell viability was monitored with Trypan Blue and microscopically analysed using Countess II. The total concentration of cells was 1.16 x 10^6 cells/ml, of which 95% were viable (1.10 x 10^6 cells/ml). Cells (5 x 10^4 cells/ml) were seeded into 96-well plates and cultured overnight in a humidified atmosphere of 5% CO_2 before treatment with various plant extract concentrations.

2.3.2 RAW 264.7 (ATTCC – TIB71)

The RAW 264.7 (ATTCC – TIB71) macrophage cell lines are monocyte/macrophage-like cells, originating from Abelson leukaemia virus-transformed cell line derived from BALB/c mice (Fuentes *et al*., 2014). These cells were also donated by the Department of Biotechnology at Vaal University of Technology, South Africa. The RAW 264.7 cells were cultured in 75 cm^2 tissue culture flasks in Dulbecco's Modified Eagle's Medium (DMEM) containing L-glutamine and supplemented with 1.0 mM Penicillin/Streptomycin and 10% heated foetal bovine serum (FBS). Thereafter the flask was incubated at 37^oC in a humidified atmosphere of 5% CO_2. The medium was changed every second day until 80-90% confluent growth was reached. Thereafter, cells

were trypsinised with 0.25% trypsin EDTA. Essentially, cell viability was monitored with Trypan Blue and microscopically analysed using Countess II. The total concentration was 2.40 x 10^6 cells/ml, of which 98% were viable (2.40 x 10^6 cells/ml). Cells (5 x 10^4 cells/ml) were seeded into 96-well plates and cultured overnight in a humidified atmosphere of 5% CO_2 before treatment with various concentrations of plant extract.

2.4 Cell viability assays

2.4.1 Alamar Blue cell viability assay

Cytotoxicity was quantified using the Alamar Blue cell viability assay (Thermo Fisher), as previously described by Al-Nasiry *et al* (2007). C2C12 cells and RAW 264.7 cells were seeded with a density of 5 x 10^4 cells/ml in 96-well plates and incubated in a humidified atmosphere of 5% CO_2. After 24 h of incubation, cells were rinsed twice with phosphate-buffered saline (Lonza), followed by the addition of 200 µl of plant extracts in varying concentrations (10, 50, 100, 250, 500, 1000 µg/ml, respectively). This was done in triplicates and the experiment was repeated three times. The plant extracts, which were dissolved in distilled water were incubated for 24 h in a humidified atmosphere of 5% CO_2 together with the positive control (hydrogen peroxide) and negative control (media). After the incubation period, 30 µl of Alamar Blue was added to each well, thereafter plates were shaken and incubated for 4 h in the dark. Cell viability was analysed at 570 nm and 600 nm with an Epoch 2 microplate reader (BioTek). Hydrogen peroxide (H_2O_2) was used as a positive control. The percentage of viable cells was calculated according to the equation below:

$$\text{Percentage viability} = \frac{\text{(Sample absorbance)}}{\text{(Positive control absorbance)}} \times 100$$

2.4.2 Crystal violet cell viability assay

Crystal violet (CV) cell viability assay is widely used for cytotoxicity and cell viability studies with adherent cell cultures (Feoktistova *et al*., 2016). Essentially, CV is a triarylmethane dye that can bind to ribose type molecules such as DNA in nuclei. Interestingly, dead cells detach from cell culture plates during washing steps, and only viable cells remain attached to the dish

(Feoktistova *et al*., 2016). For this experiment, C2C12 cells and RAW 264.7 cells were seeded in 96-well plates and incubated in a humidified atmosphere of 5% CO_2 for 24 h. After 24 h of incubation, cells were rinsed twice with phosphate-buffered saline (Lonza), followed by treatment with 200 µl of plant extract at varying concentrations (10, 50, 100, 250, 500, 1000 µg/ml respectively). This was done in triplicates and repeated three times. The plant extracts, which were dissolved in distilled water, were incubated for 24 h in a humidified atmosphere of 5% CO_2 together with the positive control (hydrogen peroxide), untreated cells and negative control (media). After the incubation period, cells were washed twice with phosphate-buffered saline (Lonza). After washing, 50 µl of crystal violet staining was added to all wells and plates were shaken for 20 min with Micro shake, ELISA Plate Shaker. Thereafter, plates were washed under running water and left to stand overnight to drain excess water before reading. The cell biomass was suspended in 70% ethanol and shaken for 20 minutes before analysis of cell viability at 570 nm and 600nm using an Epoch 2 microplate reader (BioTek). Hydrogen peroxide (H_2O_2) was used as positive control. The percentage of viable cells was calculated according to the equation here below:

$$\text{Percentage viability} = \frac{\text{(Sample absorbance)}}{\text{(Positive control absorbance)}} \times 100$$

2.5 Measurement of inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells.

Nitric oxide (NO) released from RAW 264.7 cells was assessed using the Griess assay (Promega) as previously described by Lim *et al*. (2018). RAW 264.7 cells were stimulated with 3 µl of lipopolysaccharide (LPS: *Escherichia coli*, serotype 011: B4, Sigma), and cells were seeded in 96- well culture plate at a density of 5 x 10^4 cells/well. The cells were incubated for 24 h under a humidified atmosphere of 5% CO_2 before treatment with various concentrations of plant extract (10, 50, 100, 250, 500, 1000 µg/ml respectively). This was done in triplicates and repeated three times and further incubated for 24 h under a humidified atmosphere of 5% CO_2 before the addition of 20 µl Griess reagent. After the incubation period, 50 µl of supernatant from the test culture was mixed with 50 µl of Griess reagent [1% sulfanilamide, 0.1% N-1(1-naphtyl)-ethylenediamine diehydrochloride, 2.5% phosphoric acid] followed by incubation for 10 minutes

at room temperature. The optical density at 540 nm was measured with a microplate reader (BioTek). The results were expressed as inhibition of NO production compared to the control (LPS) using the equation below.

$$\text{Percentage NO inhibition} = \frac{\text{(Sample absorbance)}}{\text{(Positive LPS Control absorbance)}} \times 100$$

3. Statistical analysis

All data were expressed as mean and standard deviation using MS Excel 2013 and ANOVA GraphPad Prism 5. Two-way repeated-measures analysis of variance (ANOVA), followed by Bonferroni posthoc test was used to analyse the data. Values were considered to be significantly different from the control if $p < 0.0001$.

4. Results

4.1 Alamar Blue cell viability

The LC_{50} (µg/ml) was determined after treating the cells with plant extracts (10 – 1000 µg/ml) for 24 h (**Table 1**). The plant extracts exhibited LC_{50} value of <1000 µg/ml for all plant extracts against C2C12 cells. Interestingly, the plant extracts exhibited a different LC_{50} value of >1000 µg/ml for RAW264.7 cells. Plant extracts demonstrated cytotoxicity effects in higher concentrations for only C2C12 cells (**Fig 1**) and no cytotoxicity effect was observed for RAW264.7 cells (**Fig 2**). The untreated cells were used to establish significant difference against samples and it was observed, ($F_{(50, 198)} = 41.80$, $p<0.0001$; two-way ANOVA) for C2C12 and RAW264.7 cells were ($F_{(50, 198)} = 99.02$, $p<0.0001$; two-way ANOVA) (**Fig 1 and Fig 2**). A dose-response was observed whereby a decrease of cell viability with the increase of concentration was noted. The plant extracts were compared with the positive control (H_2O_2) and a significant difference was observed, ($F_{(50, 198)} = 41.80$, $p <0.0001$). In addition to this, untreated cells were compared with all plant extracts in all concentrations, and all plant extracts shown significant difference ($F (50, 198) = 41.80$, $p <0.0001$); except *Erythrina lysistemon* (L) was not significantly different with untreated cells at 10 µg/ml.

Table 1: The lethal concentration (LC_{50}) in µg/ml and R^2 of Alamar Blue cell viability after treating with C2C12 cells, and RAW 264.7 cells with plant extracts (10 – 1000 µg/ml).

Plant species	**Parts**	**Cells**			
		C2C12		**RAW264.7**	
		LC_{50} (µg/ml)	**R^2**	**LC_{50} (µg/ml)**	**R^2**
Euclea crispa	Leaf	566.502	0.9167	2276.466	0.8581
Euclea natalensis	Leaf	454.497	0.9172	3814.954	0.8742
Schkuhria pinnata	Leaf	206.079	0.9797	2458.681	0.9538
Ziziphus mucronata	Leaf	150.210	0.9420	1491.555	0.9780
Ziziphus mucronata	Fruits	251.699	0.9534	2582.656	0.9456
Lippia pinnata	Leaf	185.906	0.9744	2477.176	0.9302
Vernonia oligocephala	Leaf	192.524	0.9709	210.502	0.9167
Clerodendrum myricoides	Leaf	508.834	0.9503	636.916	0.9167
Erythrina lysistemon	Leaf	773.427	0.9643	1213.327	0.9215
H_2O_2		4.382		360.604	

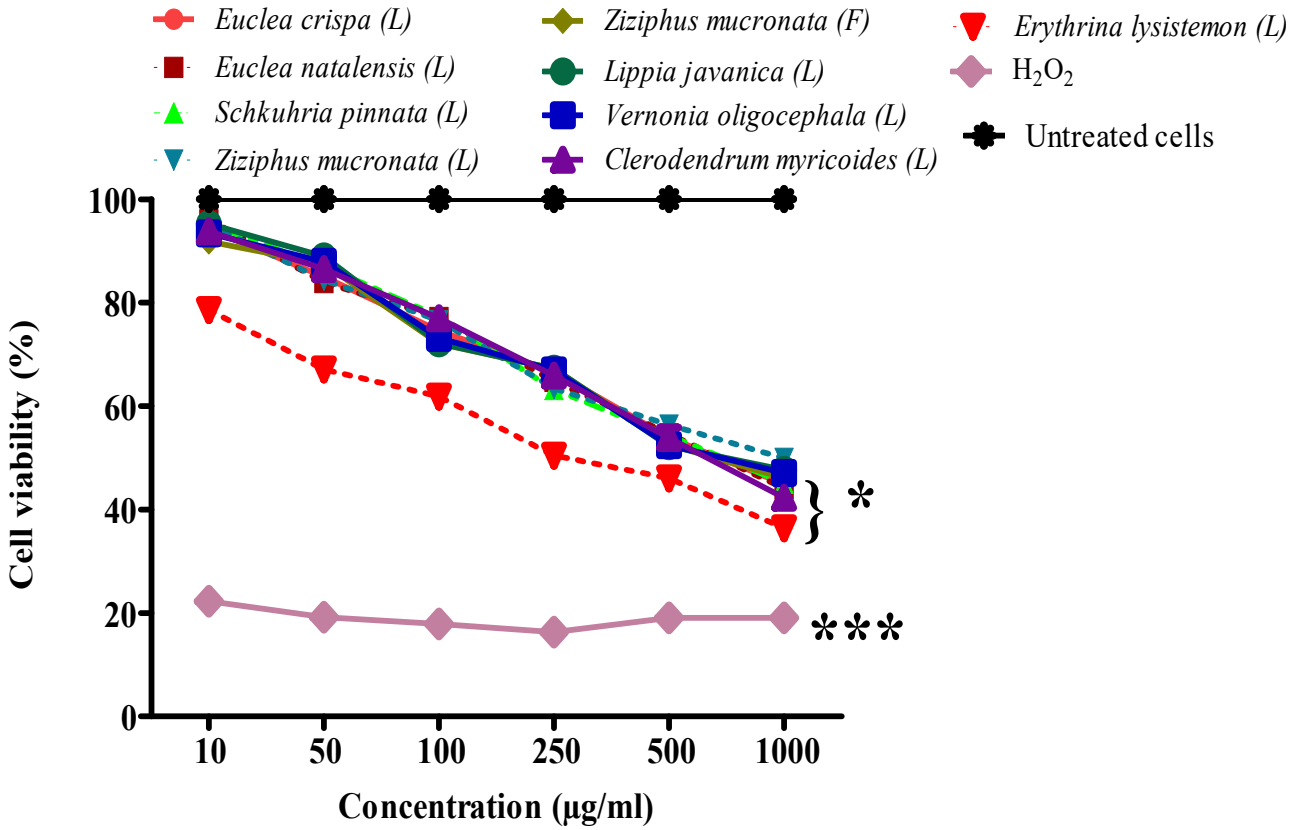

Figure 1: Cell viability was evaluated with the Alamar Blue assay. C2C12 cells were treated with various plant extracts (10 – 1000 µg/ml) for 24 h. The data are presented as mean ± S.D of triplicates experiments with similar results. (Significant treatment effect, $F_{(50, 198)} = 41.80$, $p<0.0001$; two-way ANOVA). * There is a significant difference at 10, 50, and 100 ug/ml for most plant extracts ($p < 0.0001$, Bonferroni posthoc test), except *Euclea natalensis* at 10 µg/ml. *** H_2O_2 differ from untreated cells ($p < 0.0001$, Bonferroni posthoc test).

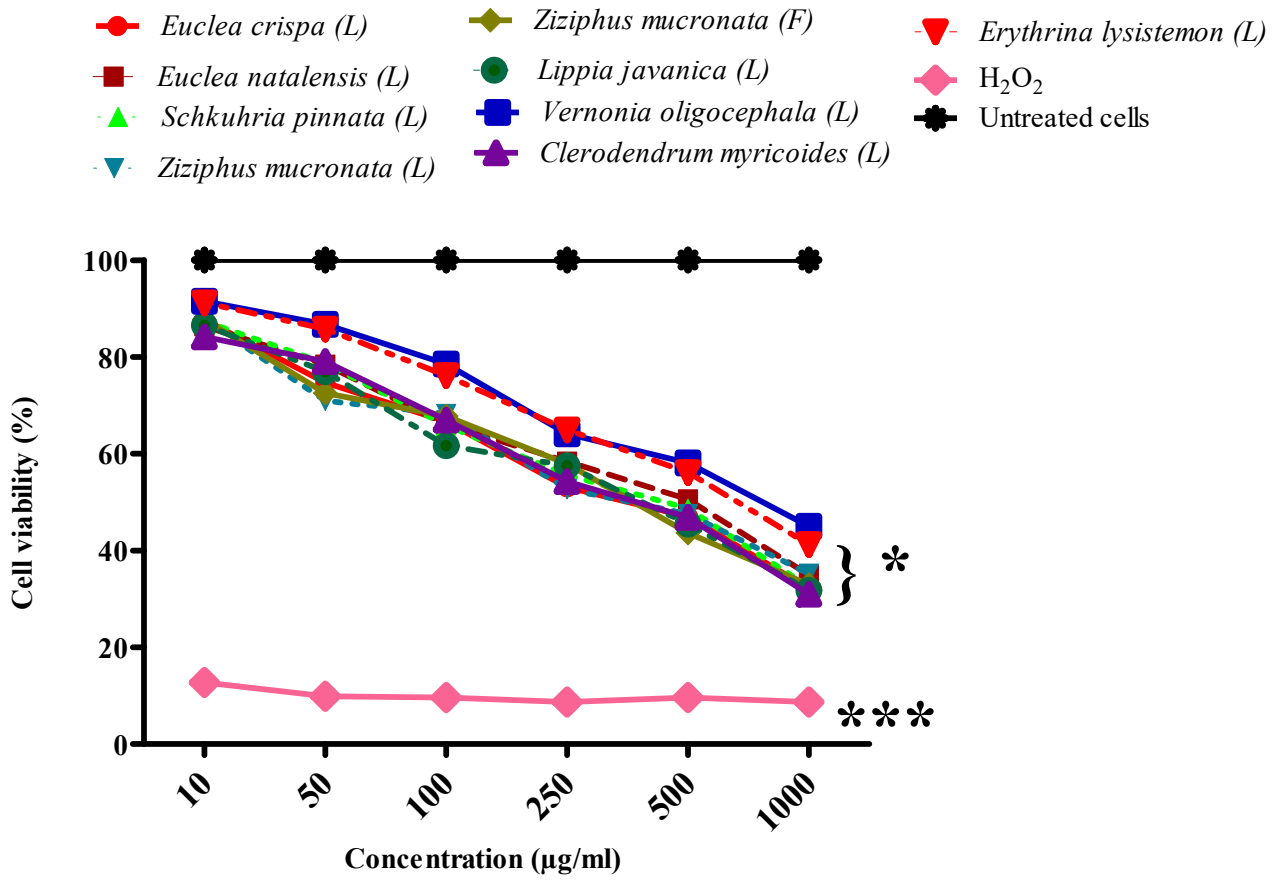

Figure 2: Cell viability was evaluated with the Alamar Blue assay. RAW 264.7 macrophages were treated with various plant extracts (10 – 1000 µg/ml) for 24 h. The data are presented as mean ± S.D of triplicate experiments with similar results. (Significant treatment effect, $F_{(50, 198)} = 99.02$, $p<0.0001$; two-way ANOVA). * All plant extracts were significantly different from untreated cells at all concentrations ($p < 0.0001$, Bonferroni posttest). *** Significant difference between H_2O_2 differ from untreated cells ($p < 0.0001$, Bonferroni posttest).

5.1 Crystal violet cell viability

The LC_{50} (µg/ml) was obtained after treating the cells with plant extracts (10 – 1000 µg/ml) after 24 h (**Table 2**). The crystal violet cell viability assay was used to complement the Alamar Blue cell viability assay. The cytotoxicity was observed in all plant extracts in higher concentrations with LC_{50} values >700 µg/ml against C2C12 cells (**Fig 3**). Similarly, no cytotoxicity was observed for plant extracts against RAW264.7 cells (**Fig 4**) with LC_{50} values <800 µg/ml in all plant extracts. A dose-response was observed whereby a decrease of cell viability with the increase of concentration and cytotoxicity effect was observed in higher concentrations against C2C12 cells (**Fig 3**). None of the plant extracts demonstrated cytotoxicity effects in all plant extracts tested against RAW 264.7 cells (**Fig 4**). The untreated cells were used to establish significant difference against samples and was observed, ($F_{(50, 198)} = 25.82$, $p<0.0001$; two-way ANOVA) for C2C12 and RAW 264.7 was ($F_{(50, 198)} = 99.21$; $p<0.0001$; two-way ANOVA). A dose-response was observed whereby a decrease of cell viability with the increase of concentration was noted.

Table 2: The lethal concentration (LC_{50}) in µg/ml and R^2 of crystal violet cell viability after treating C2C12 and RAW 264.7 cells with plant extracts (10 – 1000 µg/ml).

Plant species	**Parts**	**Cells**			
		C2C12		**RAW264.7**	
		LC_{50} (µg/ml)	**R^2**	**LC_{50} (µg/ml)**	**R^2**
Euclea crispa	Leaf	416.535	0.8756	764.374	0.8936
Euclea natalensis	Leaf	649.733	0.9557	844.167	0.9654
Schkuhria pinnata	Leaf	145.619	0.9803	314.539	0.9234
Ziziphus mucronata	Leaf	133.374	0.9439	448.896	0.9187
Ziziphus mucronata	Fruits	164.421	0.9654	775.017	0.8732
Lippia pinnata	Leaf	410.436	0.9585	2115.634	0.9233
Vernonia oligocephala	Leaf	211.676	0.9453	2754.673	0.8878
Clerodendrum myricoides	Leaf	537.150	0.9726	1545.962	0.9598
Erythrina lysistemon	Leaf	591.764	0.9787	866.625	0.9148
H_2O_2		4.382		435.076	

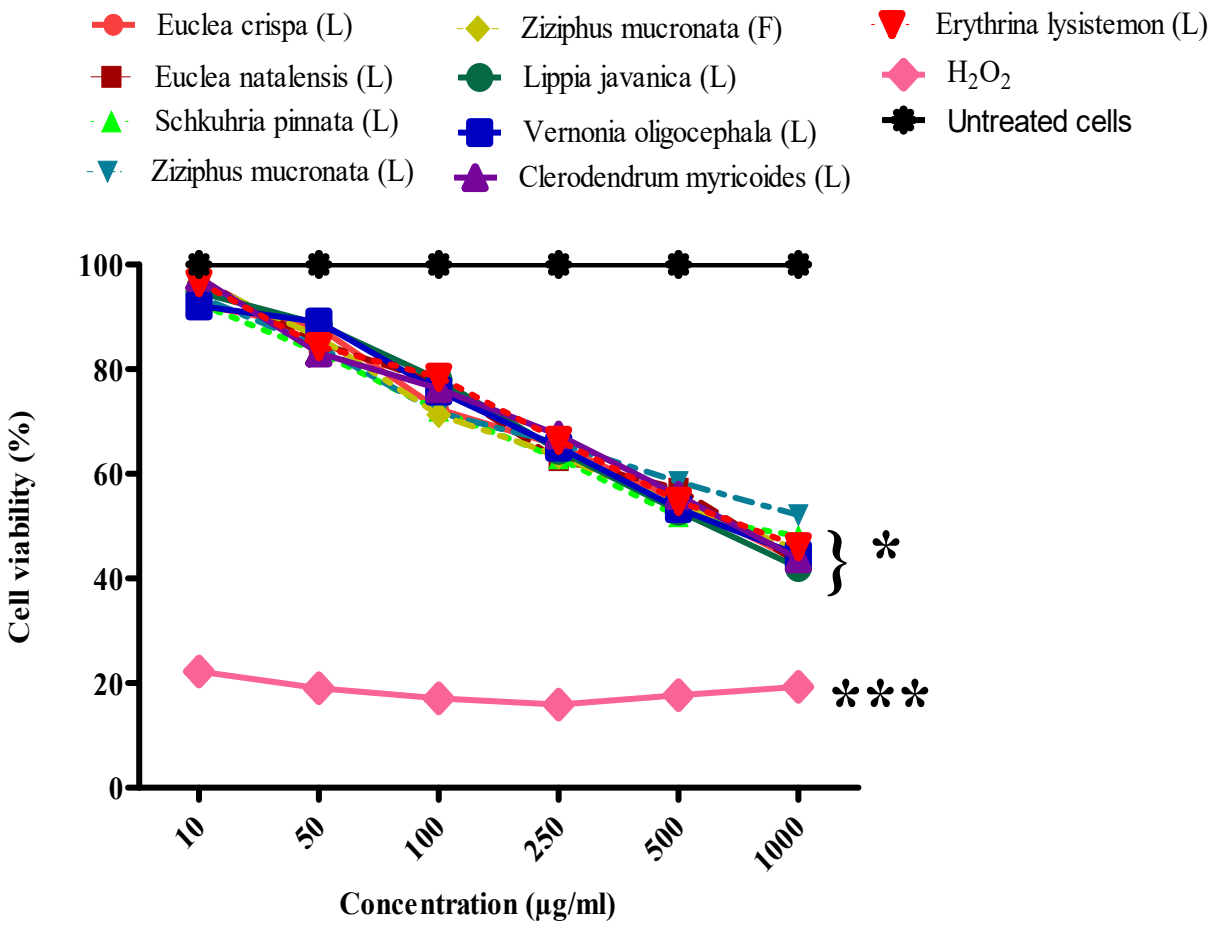

Figure 3: Cell viability was evaluated with the crystal violet assay. C2C12 cells were treated with various plant extracts (10 – 1000 µg/ml) for 24 h. The data are presented as mean ± S.D of triplicates results. (Significant treatment effect, $F_{(50,\ 198)} = 25.82$, $p<0.0001$; two-way ANOVA). * All plant extracts were significantly different from untreated cells at all concentrations ($p < 0.0001$, Bonferroni posttest), except *Euclea natalensis*, *Lippia javanica*, *Clerodebdrum myricoides*, and *Erythrina lysistemon* at 10 µg/ml. *** Significant difference between H_2O_2 and all concentrations of plant extracts ($p < 0.0001$, Bonferroni posttest).

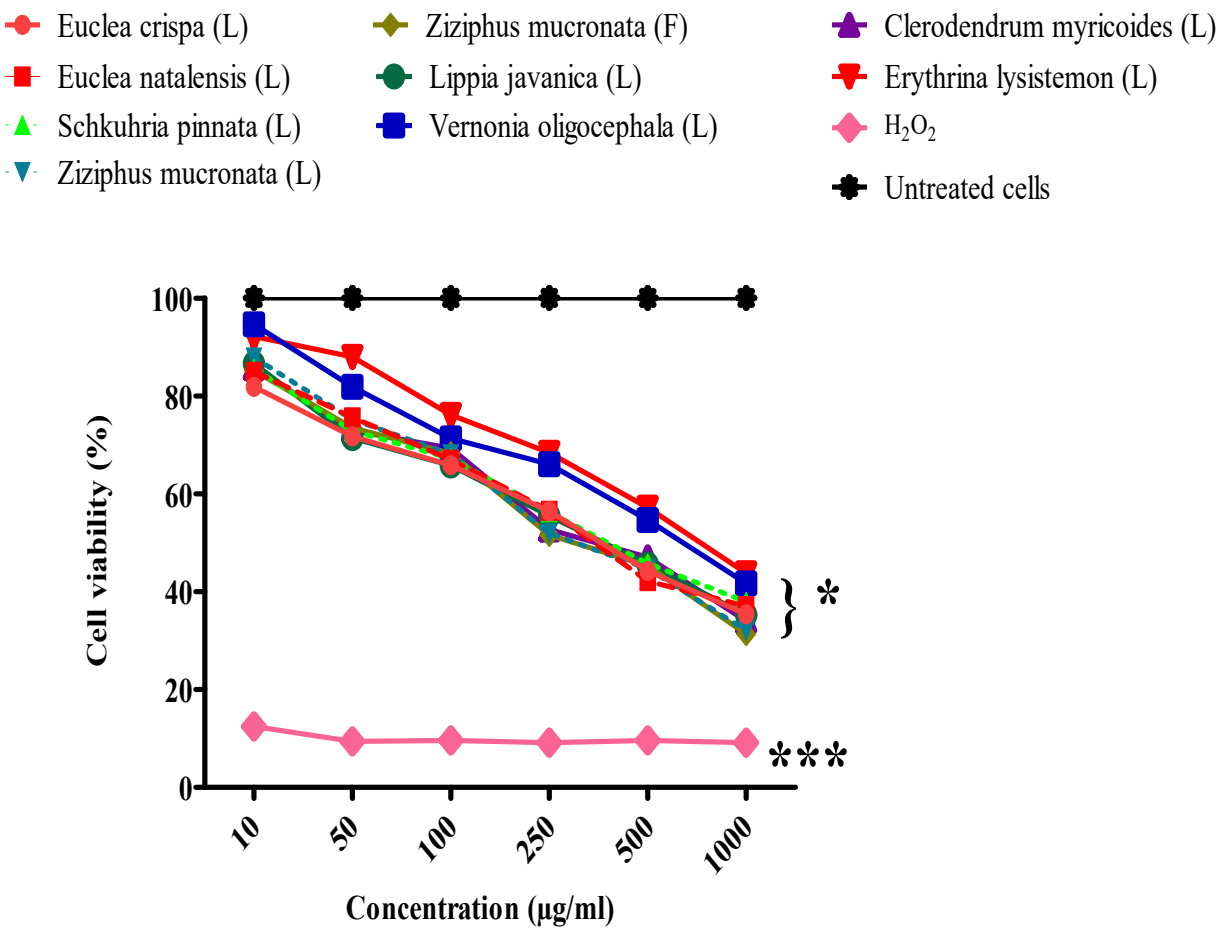

Figure 4: Cell viability was evaluated with the crystal violet assay. RAW 264.7 cells were treated with various plant extracts (10 – 1000 µg/ml) for 24 h. The data are presented as mean ± S.D of triplicate results. (Significant treatment effect, $F_{(50, 198)} = 99.21$, $p<0.0001$; two-way ANOVA). * All plant extracts were significantly different from untreated cells at all concentrations ($p < 0.0001$, Bonferroni posttest). *** Significant difference between H_2O_2 and all concentrations of plant extracts ($p < 0.0001$, Bonferroni posttest).

5.2 Inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells.

The concentration in µg/ml at which 50% inhibition of NO production was achieved in inhibition concentration (IC_{50}) was obtained after treating RAW 264.7 cells with plant extracts (10 – 1000 µg/ml) for 24 h (**Table 3**). All plant extracts exhibited IC_{50} values >1000 µg/ml, except for *Schkuhria pinnata*, *Ziziphus mucronata* (fruits), *Lippia pinnata*, *Clerodendrum myricoides*, and

Erythrina lysistemon. The anti-inflammatory effect of plant extracts was evaluated after RAW 264.7 cells were stimulated with LPS to produce NO (**Fig 5**). Plant extracts exhibited various degrees of inhibition of NO production in a dose-dependent manner. Interestingly, the following plant extracts demonstrated a degree of NO inhibition effects. *Euclea crispa* (17%- 25%), and *Eucela natalensis* (4% - 23%) caused 50% inhibition of NO production at 100, 250, and 500 µg/ml. Similar effects were observed for *Ziziphus mucronanta* (L) (3% - 25%), and *Zisiphus mucronota* (fruits) (3% - 26%) at 100, and 250 µg/ml, respectively. In addition to this, five other plant extracts exhibited a good inhibition of NO production at higher concentrations (250 – 1000 µg/ml), these were *Clerondendrum myricoides* (35% - 89%), *Lippia javanica* (26% - 77%), *Erythrina lysistemon* (23% - 76%), *Schkuhria pinnata* (27% - 65%), and *Vernonia oligocephala* (16% - 58%).

Table 3: The concentration of plant extracts that caused 50% inhibition of NO production (IC_{50}) in LPS-stimulated RAW 264.7 cells.

Plant species	**Parts**	**IC_{50} (µg/ml)**	**R^2**
Euclea crispa	Leaf	1242.366	0.9878
Euclea natalensis	Leaf	1588.573	0.9533
Schkuhria pinnata	Leaf	348.859	0.9484
Ziziphus mucronata	Leaf	11949.000	0.9612
Ziziphus mucronata	Fruits	499.600	0.9371
Lippia pinnata	Leaf	177.902	0.9487
Vernonia oligocephala	Leaf	2634.965	0.9483
Clerodendrum myricoides	Leaf	707.335	0.9858
Erythrina lysistemon	Leaf	264.287	0.9506

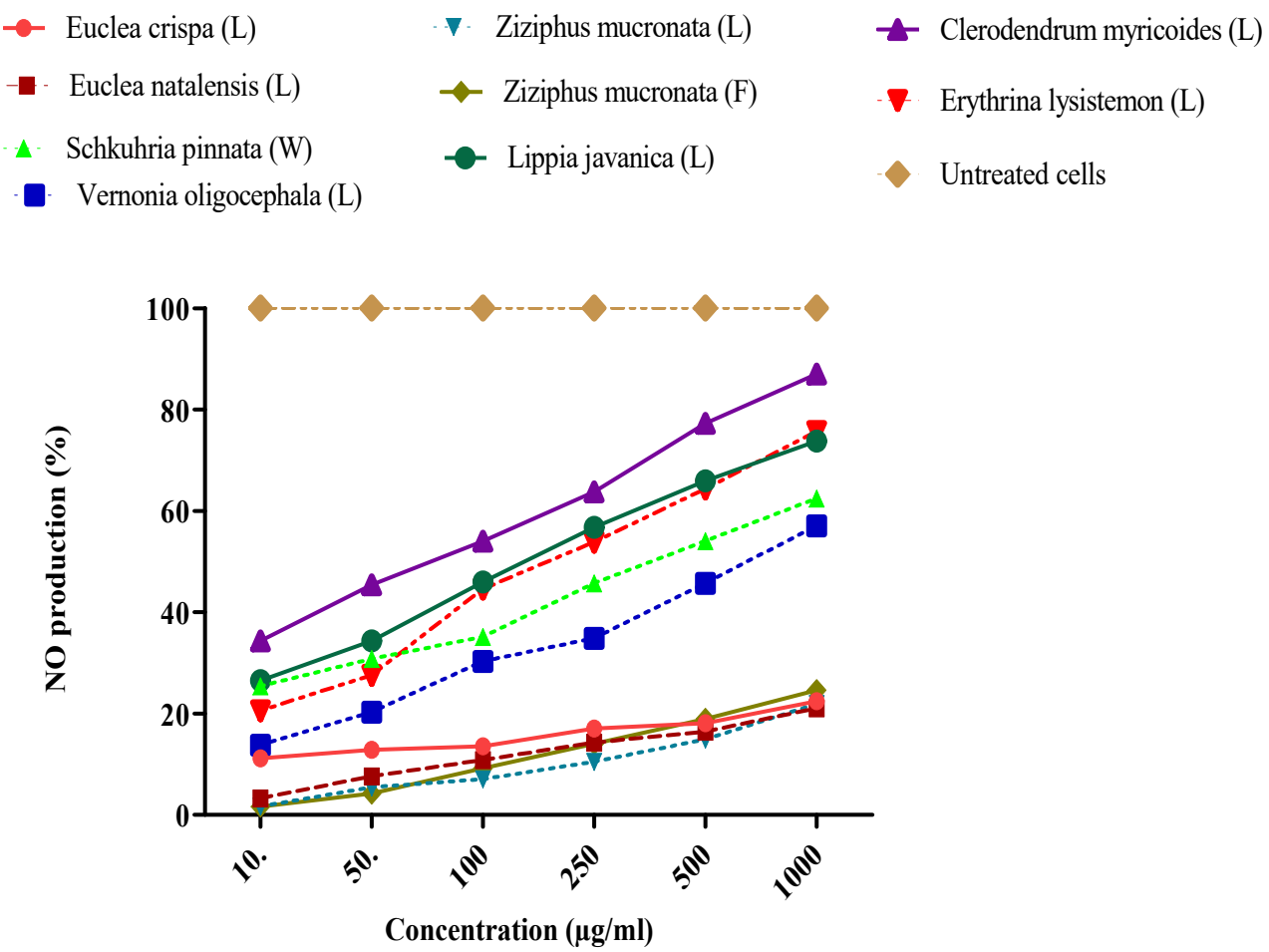

Figure 5: The effect of nine plant extracts on the production of NO in LPS-stimulated RAW 264.7 cells. Cells were treated with various plant extracts (10 – 1000 µg/ml) and stimulated with LPS (3 µl) for 24h. NO production was measured in the cultured cell supernatant by Griess reagent. The results are expressed in percentage inhibition of NO production. The data are presented as mean ± S.D of triplicates results. (Significant treatment effect, $F_{(45, 180)} = 50.57$, $p<0.0001$; two-way ANOVA). * All plant extracts significantly different from untreated cells at all concentrations ($p < 0.0001$, Bonferroni posttest), except *Euclea crispa* at 500 and 1000 µg/ml, *Euclea natalensis* at 100, 250, and 500 µg/ml, and *Ziziphus mucronata* (L) and *Ziziphus mucronata* (F) at 100 µg/ml, and 250 µg/ml the significant difference between control, and all concentrations of plant extracts ($p < 0.0001$, Bonferroni posttest).

5. Discussion

The purpose of this study was to evaluate the cytotoxicity and anti-inflammatory effects of *Euclea crispa* (leaf), *Eulea natalensis* (leaf), *Schkuhria pinnata* (leaf), *Ziziphus mucronata* (leaf), *Ziziphus mucronata* (fruits), *Lippia javanica* (leaf), *Vernonia oligocephala* (leaf), *Clerodendrum myricoides* (leaf), and *Erythrina lysistemon* (leaf) against C2C12 cells, and RAW 264.7 cells (**Fig 1 to Fig 4**). The cytotoxicity effect was observed in higher concentrations for all plant extracts against C2C12 cells, and exhibited LC_{50} value of <1000 µg/ml. In contracts, no cytotoxicity was observed in all plant extracts against RAW 264.7 cells, and LC_{50} value of > 1000 µg/ml. All plant extracts demonstrated some degree of anti-inflammatory effect (**Fig 5**). However, five plant extracts exhibited marked anti-inflammatory activities. These plants *Clerondendrum myricoides* (35% - 89%), *Lippia javanica* (26% - 77%), *Erythrina lysistemon* (23% - 76%), *Schkuhria pinnata* (27% - 65%), and *Vernonia oligocephala* (16% - 58%).

The findings of this study have shown that all plant extracts exhibited a decrease in cell viability against of C2C12 cells, and this was observed only at the highest concentration of 1000 µg/ml. The results can be interpreted that these plant extracts only shown a decrease in cell viability at the highest concentration, but it does not mean that they are toxic to the cells. None of the plant extracts exhibited cytotoxicity effects against RAW 264 cells in all concentrations used. The cell viability was observed to have a dose-response where cell viability decreases with an increase in concentration. Essentially, Alamar Blue cell viability assay was noticeable to agree with crystal violet cell viability assay. Seven plant extracts did not show any cytotoxicity effects even in the high concentrations (1000 µg/ml) against RAW 264.7 cells.

The results of this study were noticed to be least toxic when compared with other researchers. *Euclea crispa* was observed with LC_{50} value of 566.502 µg/ml in this study. In other studies, the toxicity of *Euclea crispa* was observed against breast cancer cells in *Combretum molle* (Rademana., *et al* 2017). The IC_{50} value of *Euclea crispa* extract was reported as low as 45.7 µg/ml and as high as 167.2 µg/ml. The cytotoxicity of *Euclea natalensins* was observed in higher concentrations with LC_{50} value of 454.497 µg/ml. Similarly, cytotoxicity was reported on *Euclea natalensis* in another study where plant extracts were treated with Chang liver cells was reported

cytotoxicity as low as 131.3 µg/ml and as high as 108.9 µg/ml (Ojewole, 2004). The cytotoxicity of *Schkuhria pinnata* with LC_{50} value of 206.079 µg/ml against C2C12 cells and no toxicity was observed against RAW 264.7 cells with LC_{50} value of 2458.681 µg/ml. In contracts, Kudumela., *et al* (2018) described *S. pinnata* as most toxic in plant extracts against Vero cells using MTT assay with LC_{50} <25.0 µg/ml. Furthermore, studies are required to confirm the toxicity of *S. pinnata*, hence both methods used in both occasions are sensitive enough to detect cytotoxicity on plant extracts in cells (Hamid *et al*., 2004), since no agreement on the outcomes in both studies.

In the present study, *Ziziphus mucronata* did not show any cytotoxicity effects with LC_{50} values of 2582.656 µg/ml against RAW 264.7 cells, however, it was toxic against C2C12 cells with LC_{50} value of 150.210 µg/ml. Previous studies have reported cytotoxicity of *Ziziphus mucronata* with LC_{50} value ranged from 0.10 µg/ml to 0.22 µg/ml against Bovine dermis and Vero cells (Mongalo *et al*., 2018). In other studies, no cytotoxicity was reported for *Z. mucronata* in RAW 264.7 cells with LC_{50} value as low as >50 µg/ml. Furthermore, selective cytotoxicity was reported for *Z. mucronata* against U937 cancer to be >500 µg/ml (Sigidi *et al*., 2016). In the present study, cytotoxicity was observed for *Lippia javanica* with LC_{50} values value of 185.906 µg/ml against C2C12 cells, and interesting no cytotoxicity was observed against RAW 264.7 cells with LC_{50} value of 2477.176 µg/ml. Makhafola *et al*., (2019) confirmed our findings of *L. javanica* on liver cells with reported LC_{50} value >1000 µg/ml, of which is in agreement with RAW 2643.7 cells. The cytotoxicity effects were observed for *Vernonia oligocephala* against both cells with LC_{50} value <250 µg/ml. Furthermore, nothing has been reported in the literature on *V. oligocephala* cytotoxicity. The cytotoxicity effects were observed for *Clerodendrum myricoides* against both cells LC_{50} values <650 µg/ml. In other studies, reported *C. myricoides* cytotoxicity of IC_{50} value below 1 µg/ml against breast cancer cells (Tuasha *et al*., 2019). In contracts to the present study, Kamanja *et al*., (2018), reported cytotoxicity levels showing high LC_{50} <1000 µg/ml in chloroform extracts and lower LC_{50} (>1000 µg/ml) in methanol extracts. Essentially, the toxicity of this plant depends on the solvent used, however, it has been noticeable to be safe for use in traditional medicine space (Kamanja *et al*., 2018). No cytotoxicity was observed for *Erythrina lysistemon* with noticeable LC_{50} values ranged from 773.427 µg/ml to 1213.327 µg/ml. In other studies, cytotoxicity was reported for *E. lysistemon* with IC_{50} value

below 100 µg/ml using MTT against C3A human liver cells (Mukandiwa *et al*., 2012). This plant extract has been observed to have contradiction results and further animal studies can validate its toxicity, which will confirm its medicinal use.

In addition to this, the ability of plant extracts to inhibit NO production by RAW 264.7 cells – stimulated with LPS was assessed (**Fig 5**). All plant extracts exhibited a degree of NO inhibition effects against all concentrations used. Essentially, inhibition of NO production was observed for *Euclea crispa* at 500 and 1000 µg/ml with IC_{50} value of 1242.366 µg/ml, *Euclea natalensis* at 100, 250, and 500 µg/ml with IC_{50} value of 1588.573 µg/ml, *Ziziphus mucronata* (L) with IC_{50} value of 11949.000 µg/ml, and *Ziziphus mucronata* (F) at 100 µg/ml, and 250 µg/ml with IC_{50} value of 499.600 µg/ml. Furthermore, *Clerondendrum myricoides*, *Lippia javanica, Erythrina lysistemon*, *Schkuhria pinnata*, and *Vernonia oligocephala* were observed to inhibit NO production at higher concentrations (100 – 1000 µg/ml) LPS induced RAW 264.7 cells. The IC_{50} values ranged from 707,335, 177.902, 264.287, 348.859, and 2634.965 µg/ml, respectively against RAW 264.7 cells.

Interestingly, the inhibition NO production was observed for *Eucela crispa* which ranged from 17 to 25% and more prominent in higher concentrations (100, 250, and 500 µg/ml), and IC_{50} value was noted to be 124.366 µg/ml. Although, no study in the literature to substantiate these findings, the results validate the use of this plant in traditional medicinal practice. The uses include treatment stomach disorders, measles, coughs, constipation, remedy for diabetes, and also prevents rheumatisms and epilepsy (Raimondo *et al*., 2009; Deutschländer *et al*., (2009). Similarly, *Euclea natalensis* was observed to have a similar inhibition effect as *E.crispa*. The NO inhibition ranged from 4% to 23% which was more effective in higher concentrations (100, 250, and 500 µg/ml), and IC_{50} value of 1588.573 µg/ml was observed. No other studies have been reported for inhibition of NO production by *E. natalensis*. These study results validate *E. natalensis* for conventional medicinal applications. This plant has been used for snakebite cure, hypertension, vomiting, measles, roundworms, stomach problems, toothache, venereal diseases, and injuries (Maroyi, 2017).

Schkuhria pinnata was also observed to be effective at higher concentrations with inhibition of NO production from 27% to 65% at 100 to 1000 µg/ml with IC_{50} value of 348.859 µg/ml. In another study, a similar pattern was reported whereby inhibition was more effective in higher concentrations, which ranged from 64% to 98% respectively (Kudumela *et al*., 2018). A good inhibition of NO production was observed for *Ziziphus mucronata* which ranged from 3% to 26% with IC_{50} value of 11949.000 µg/ml. In contracts, *Z. mucronata* the inhibition of NO production was reported at 150% at IC_{50} value of 50 µg/ml (Sigidi *et al*., 2016).

The inhibition of NO production for *Lippia javanica* was also observed to range from 26% to 77% with IC_{50} value measured at 177.902 µg/ml. Dzoyem and Eloff, (2014) reported on the inhibition of NO production was of *L. javanica* which was reported at 97% for 25 µg/ml with IC_{50} value of 18 µg/ml. The results validate the use of *L. javanica* in traditional medicine uses such as herbal tea and ethnomedicinal applications for (in descending order of importance) colds, cough, fever or malaria, wounds, repelling mosquitos, diarrhea, chest pains, bronchitis, and asthma (Maroyi, 2017).

Essentially, NO inhibition was observed for *Vernonia oligocephala* to be effective in higher concentrations, and ranged from 26% to 58% and IC_{50} value noticeable to be 2634.965 µg/ml. No other studies have been found to substantiate these finding and to the best of our knowledge, these findings complement the use of this plant in traditional medicine practice. The medicinal use includes treatment of abdominal pain, colic, and other complaints as well as to drive away hailstorms. In addition to this, used as a remedy to treat mild forms of diabetes (Amusan *et al*., 2017). The inhibition of NO production ranged from 35% to 89% for *Clerodendrum myricoides* was only observed in higher concentrations (250 – 1000 µg/ml) with IC_{50} value of 707.335 µg/ml. Similarly, inhibition of NO production ranged from 23% to 76% for *Erythrina lysistemon* was only prominent at higher concentrations (250 – 1000 µg/ml) with IC_{50} value of 264.287 µg/ml.

The anti-inflammatory effects may be associated with antioxidant properties. Interestingly, these plant extracts exhibited ROS inhibition activity in high concentrations. It is imperative to further evaluate anti-inflammatory efficacy *in vivo* as to substantiate these findings and to ensure that is

safe for human use. Inflammation has been implicated to be associated with the pathogenesis of conditions such as infections, arthritis, type 2 diabetes mellitus, obesity and cancer (Johnson *et al*., 2012; Maconi *et al*., 2014). Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for pain and inflammation conditions (Yuan *et al*., 2006). Unfortunately, NSAIDs have been reported to be associated with adverse side effects such as gastrointestinal bleeding and suppressed the function of the immune system (Hougee, 2008). They have been increased research on the use of natural-source concerning anti-inflammatory properties because it has been reported to have fewer side effects as opposed to NSAIDs (Maroon *et al*., 2010; Pelkonen *et al*., 2014; Nondo *et al*., 2015). Medicinal plants consist of major natural bioactive compounds that attribute to scavenging ROS such as antioxidants (Singh., *et al* 2016; Engwa, 2018). In this study, it can be seen that plant extracts possess protective effects on cells. The results support the uses of these medicinal plants in African traditional, complementary and alternative medicine practice (Nkala., *et al* 2019a). Essentially, four plant extracts that demonstrated promising anti-inflammatory effects which can be a good candidate for the treatment or management of inflammatory diseases. Even though all plant species in this study demonstrated a degree of cytotoxicity against C2C12 cells in higher concentrations. Similarly, these plants exhibited anti-inflammatory abilities, of which counteract for their cytotoxicity observed against C2C12.

The findings of the current study complement our previous review of the uses of selected medicinal plants by healers (Nkala *et al*., 2019a). To this date, the selected South African plants have been validated for minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) (Nkala *et al*., 2019b), and most importantly, they have been recently confirmed for being none cytotoxicity against RAW 264.7 cells, however, toxicity was observed against C2C12 in higher concentrations. Furthermore, they have been observed to possess anti-inflammatory potential.

6. Conclusion

None of the selected South African plants demonstrated cytotoxicity effects in RAW 264.7 cells. The observed cytotoxicity effects were against C2C12 cells in higher concentrations. Importantly, this will need further validation in animal studies to confirm these findings. Furthermore, the results demonstrated these selected South African plants exhibited a degree of anti-inflammatory

activity in LPS-induced RAW 264.7 cells. Therefore, the findings suggest that *Clerondendrum myricoides, Lippia javanica, Erythrina lysistemon, Schkuhria pinnata, and Vernonia oligocephala* can be a promising therapeutic agent for inflammatory diseases. Further studies are required to evaluate these plant extracts for antioxidants and antidiabetic potential.

7. Conflict of interest

The authors declare that they do not have any conflict concerning the publication of this paper.

8. Acknowledgment

We thank the College Health Sciences Scholarship Grant at the University of KwaZulu-Natal, Institute of Traditional Knowledge and Traditional Medicine at the Vaal University of Technology, the National Research Foundation and Thuthuka grant for the financial support received towards this study. Dr Cornelius Ssemkalu is thanks for allowing us to use his tissue culture laboratory at the Vaal University of Technology. Finally, Mr. Gary Mohlala from Vaal University of Technology tissue culture laboratory is thanks for providing technical assistance towards this work. Furthermore, we express our sincere gratitude to Prof Vivienne Russel for proofreading this manuscript.

9. References

Al-Nasiry. S., Geusens, N., Hanssens, M., Luyten, C., Pijnenborg, R. (2007). “The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells.” *Human Reproduction* **22** (5): 1304–1309.

Amusan, O. O. G., Sukati, N.A., Dlamini, P.S., Sibandze, F.G. (2017). "Some Swazi phytomedicines and their constituents." *African Journal of Biotechnology* **6** (3): 267-272.

Arika, W. M., Ogola P.E., Abdirahman, Y.A., Mawia, A.M., Wambua, F.K., Nyamai, D.W., Kiboi, N.G., Wambani, J.R., Njagi, S.M., Rachuonyo, H.O., Muchori, A.N., Lagat, R.C., Agyirifo, D.S., Ngugi, M.P., Njagi, E.N.M (2016). "*In Vivo* Safety of Aqueous Leaf Extract of *Lippia javanica* in Mice Models." *Biochemistry and Physiology* **5**(1): 1-9.

Ayupova, D., Dobhal, G., Laufersky, G., Nann, T., Goreham, R.V., (2019). "An *In Vitro* Investigation of Cytotoxic Effects of InP/Zns Quantum Dots with Different Surface Chemistries." *Nanomaterials* **22**(9): 1-13.

Berghaus, L. J., James, N., Moore, D.J., Hurley, M.L., Vandenplas, B.P., Fortes, B. P., Wolfert, M. A., Boons, G.J. (2010). "Innate immune responses of primary murine macrophage-lineage cells and RAW 264.7 cells to ligands of Toll-like receptors 2, 3, and 4." *Comparative immunology, microbiology and infectious diseases* **33** (5): 443-454.

Beseni, B. K., Matsebatlela, T. M., Bagla, V. P., Njanje, I., Poopedi, K., Mbazima, V., Mampuru, L., Mokgotho, M. P. (2019). "Potential Antiglycation and Hypoglycaemic Effects of *Toona ciliata* M. Roem. and *Schkuhria pinnata* Lam. Thell. Crude Extracts in Differentiated C2C12 Cells." *Evidence-Based Complementary and Alternative Medicine,* **2019**: 1-12, viewed February 2019, https://doi.org/10.1155/2019/5406862.

Burattini, S., Ferri, P., Battistelli, M., Curci, R., Luchetti, F., Falcieri, E (2009). "C2C12 murine myoblasts as a model of skeletal muscle development: morpho-functional characterization." *European Journal of Histochemistry* **48**: 223–234.

Bussmann, R. W., Sharon, D., Daiz, D.P (2008). "Peruvian plants canchalagua (*Schkuhria pinnata* (Lam.) Kuntze), hercampuri (Gentianella alborosea (Gilg.) Fabris), and corpus way (Gentianella bicolor (Wedd.) J. Pringle) prove to be effective in the treatment of acne." *Arnaldoa* **15** (1): 149-152.

Deutschländer, M. S., Lall, N., van de Venter, M (2009). "Plant species used in the treatment of diabetes by South African traditional healers: An inventory." *Pharmaceutical Biology* **47**(4): 348-365.

Dzoyem, J. P. and Eloff. J.N (2014). "Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa." *Journal of Ethnopharmacology* **160**: 194-201.

Engwa, G. A. (2018). Free Radicals and the Role of Plant Phytochemicals as Antioxidants Against Oxidative Stress-Related Diseases. Chapter 4: Phytochemicals - Source of Antioxidants and Role in Disease Prevention. Viewed on 24th November 2019, http://dx.doi.org/10.5772/intechopen.76719.

Farag, M. A., Mekky, H., El-Masry, S (2016). "Metabolomics driven analysis of *Erythrina lysistemon* cell suspension culture in response to methyl jasmonate elicitation." *Journal of Advanced Research* **7**: 681-689.

Feoktisova, M., Geserick, P., Leverkus, M. (2016). Crystal violet assay for cultured cells., Cold Spring Protocols.

Fuentes, A.L., Mills, L., Vapenik, J., Sigola, L. (2014). "Lipopolysaccharide-mediated enhancement of zymosan phagocytosis by RAW 264.7 macrophages is independent of opsonins, laminarin, mannan, and complement receptor 3." *Journal of Surgical Research* **189**(2): 304-312.

Girgis, C. M., Clifton-Bligh, R.J., Mokbel, N., Cheng, K. and Gunton, J.E (2013). "Vitamin D signaling regulates proliferation, differentiation, and myotube size in C2C12 skeletal muscle cells." *Endocrinology* **155**: 347–357

Hamid, R., Rotshteyn, Y., Rabadi, L., Parikh, R., Bullock, P. (2004). "Comparison of Alamar Blue and MTT assays for high throughput screening." *Toxicology in vitro: an international journal published in association with BIBRA* **18**: 703-710.3

Harbilas, D., Martineau, L. C., Harris, C. S., Adeyiwola-Spoor, D. C., Saleem, A., Lambert, J., Caves, D., Johns, T., Prentki, M., Cuerrier, A., Arnason, J. T., Bennett, S. A., Haddad, P. (2009). "Evaluation of the antidiabetic potential of selected medicinal plant extracts from the Canadian boreal forest used to treat symptoms of diabetes: part II." *Canadian Journal of Physiology and Pharmacology* **87**(6): 479-492.

Hougee, S. (2008). Plant- derived modulators of inflammation and cartilage metabolism The Netherlands, Utrecht University. PhD.

Hyejin, L., Sang-Jin, L., Gyu-Un, B., Nam-In, B., Jae-Ha, R. (2017). "Canadine from Corydalis turtschaninovii Stimulates Myoblast Differentiation and Protects against Myotube Atrophy." *International Journal of Molecular Sciences* **18**:1-13.

Ibrahim, M. A. and Islama, S. (2017). "Effects of butanol fraction of *Ziziphus mucronata* root ethanol extract on glucose homeostasis, serum insulin and other diabetes-related parameters in a murine model for type 2 diabetes." *Pharmaceutical Biology* **55**(1): 416-422.

Javad, M., Vakili, T., Hadinedoushan, H., Ali, K. (2011). "C2C12 cell line is a good model to explore the effects of herbal extracts on muscular GLUT4 metabolism." *Clinical Biochemistry* **44**(13): S332 – S336.

Jo, W.-S., Choi, Y.J., Kim, H.J., Nam, B.H., Lee, G.A., Seo, S.Y., Lee, S.W., Jeong, M.H. (2010). "Methanolic extract of Asterina pectinifera inhibits LPS-induced inflammatory mediators in murine macrophage." *Toxicology Research* **26**(1): 37-46.

Johnson, A. R., Milner, J.J., Makowski, L (2012). "The inflammation highway: metabolism accelerates inflammatory traffic in obesity " *Immunological Review* **249**(1): 218-238.

Kamanja, I. T., Mbaria, J.M., Gathumbi, P.K., Mbaabu, M., John, D.K., Kiama, S.G. (2018). "Cytotoxicity of selected medicinal plants extracts using the brine shrimp lethality assay from Samburu county, Kenya." *The Journal of Medical Research* **4**(5): 249-255.

Kamtchueng, M. O., Balyan, R., Mouokeu, R. S., Tume, C., Banerjee, C., Singh, C.A., Oumar, M., Kuiate, J. R., (2017). "Anti-Inflammatory Activity of Methanol Extract and Fractions from Alchemilla kiwuensis Engl. on LPS Activated Macrophages." *International Journal of Pharmacognosy and Phytochemical Research* **9**(4): 473-481.

Kaur, G. and Dufour, J.M. (2012). "Cell lines: Valuable tools or useless artifacts." *Spermatogenesis* **2**(1): 1-5.

Kudumela, R. G., McGaw, L.J., Masoko, P (2018). "Antibacterial interactions, anti-inflammatory and cytotoxic effects of four medicinal plant species." *BMC Complementary and Alternative Medicine* **18**(199): 1-7.

Lee, C. J., Chen, L.G., Liang, W.L., Wanga, C.C (2010). "Anti-inflammatory effects of Punica granatum Linne *in vitro* and *in vivo*." *Food Chemistry Journal* **118**: 315-322.

Lee, S. C., Kwon, Y.W., Park, J.Y., Park, S., Lee, J.H., Park, S.G. (2017). "Antioxidant and Anti-Inflammatory Effects of Herbal Formula SC-E3 in Lipopolysaccharide-Stimulated RAW 264.7 Macrophages." *Evidence-based Complementary and Alternative Medicine* 1-13

Lim, Y., Park, J.W., Kwon, O.K., Lee, J.W., Lee, H.S., Lee, S., Choi, S., Li, W., Jin, H., Han, S.B., Ahn, K.S. (2018). "Anti-inflammatory effects of a methanolic extract of Castanea seguinii Dode in LPS-induced RAW264.7 macrophage cells." *International Journal of Molecular Medicine* **41**(1): 391-398.

Maconi, G., Furfaro, F., Scieurti, R., Bezzi, C., Ardizzone, S., de Franchis, R (2014). "Glucose intolerance and diabetes mellitus in ulcerative colitis: Pathogenetic and therapeutic implications." *World Journal of Gastroenterology* **20** (13): 3507-3515.

Makhafola, M. A., Middleton, L., Olivier, M. T., Olaokun, O. O. (2019). "Cytotoxic and Antibacterial Activity of Selected Medicinal Plants used in South African Traditional Medicine." *Asian Journal of Chemistry* **31**(11): 2623-2627.

Maroon, J. C., Bost, J.W., Maroon, A (2010). "Natural anti-inflammatory agents for pain relief." *Surgical Neurology International* **1**(80): 1-16.

Maroyi, A. (2017). "Review of Ethnomedicinal Uses, Phytochemistry and Pharmacological Properties of Euclea natalensis A.DC." *Molecules* **22**(12): 1-16.

Mongalo, N. I., Dikhoba, P. M., Soyingbe, S. O., Makhafola, T. J. (2018). "Antifungal, anti-oxidant activity and cytotoxicity of South African medicinal plants against mycotoxigenic fungi." *Heliyon* **4**(11): 1-23.

Morissette, M. R., Cook, S.A., Buranasombati, C., Rosenberg, M.A., Rosenzweig, A (2009). "Myostatin inhibits IGF-I-induced myotube hypertrophy through Akt." *American Journal of Physiology - Cell Physiology* **297**: 1124–1132.

Mukandiwa, L., McGawa, L., Eloff, J.N., Naidoo, V (2012). "Extracts of four plant species used traditionally to treat myiasis influence pupation rate, pupal mass and adult blowfly emergence of *Lucilia cuprina* and *Chrysomya marginalis* (Diptera: Calliphoridae)." *Journal of Ethnopharmacology* **143**(3): 812-818.

Musso, F., Lincor, D., Vasconsuelo, A., Pronsato, L., Faraoni, B., Milanesi, L. (2019). "Adverse Effects in Skeletal Muscle Following the Medicinal Use of *Nicotiana glauca*." *Biological and Pharmaceutical Bulletin* **42**(5): 671–679.

Nkala, B. A., Mbongwa, H.P., Qwebani-Ogunleye, T (2019a). "A Review on Selected African Medicinal Plants with Effectiveness in the Management of Type II Diabetes Mellitus." *Acta Scientific Pharmaceutical Sciences* **3** (8): 2581-5423.

Nkala, B. A., Mbongwa, H.P., Qwebani-Ogunleye, T (2019b). "The *in vitro* evaluation of some South African plant extracts for minimum inhibition concentration and minimum bactericidal concentration against selected bacterial strains." *International Journal of Scientific and Research Publications* **9**(7): 995-1004.

Nondo, R. S. O., Moshi, M.J., Erasto, P., Zofou, D., Njouendou, A.J., Wanji, S., Ngemenya, M.N., Kidukuli, A.W., Masimba, P.J., Titanji, V.P.K (2015). "Evaluation of the cytotoxic

activity of extracts from medicinal plants used for the treatment of malaria in Kagera and Lindi regions, Tanzania." *Journal of Applied Pharmaceutical Science* **5**(4): 007-012.

Ojewole, O. J. A. (2004). "Indigenous plants and schistosomiasis control in south africa: molluscicidal activity of some Zulu medicinal plants." *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **3**: 8–22.

Padmanabha, R.A., and Kaiser, J. (2011). "Pharmacological evaluation of herbal extracts for their *in vitro* hypoglycaemic activity." *International Journal of Phytopharmacology* **2**(1): 15-21.

Pan, S. Y., Zhou, S.F., Gao, S.H., Yu, Z.L., Zhang, S.F., Tang, M.K., Sun, J.N., Ma, D.L., Han, D.F., Fong, W.F., Ko, K.M (2013). "New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics." *Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine* 1-25.

Pelkonen, O., Xu, Q., Fan, T. P. (2014). "Why is Research on Herbal Medicinal Products Important and How Can We Improve Its Quality*?" Journal of Traditional and Complementary Medicine* **4**(1): 1-7.

Rademana, S., Anantharajub, P.G., Rao V., Madhunapantulab, S., Lalla, N (2017). " The anti-proliferative and antioxidant activity of four indigenous South African plants." *African Journal of Traditional, Complementary and Alternative Medicines* **16**(1): 13-23.

Raimondo, D., Von Staden, L., Foden, W., Victor, J.E., Helme, N.A., Turner, R.C., Kamundi, D.A. and Manyama, P.A (2009). " Red List of South African plants. Strelitzia 25. Pretoria, South African National Biodiversity Institute."

Razali, F. N., Ismail, A., Abidin, N., Zainal, S., Adawiyah, S. (2014). "Stimulatory effects of polysaccharide fraction from *Solanum nigrum* on RAW 264.7 murine macrophage cells." *PLoS One* **9**(10): e108988-e108988.

Sigidi, M. T., Anokwuru, C. P., Zininga, T., Tshisikhawe, M. P., Shonhai, A., Ramaite, I. D.I., Traoré, A. N., Potgieter, N. (2016). "Comparative *in vitro* cytotoxic, anti-inflammatory and anti-microbiological activities of two indigenous Venda medicinal plants." *Translational Medicine Communications,* **201***6:* 1-9.

Singh, A., Singh, S., Prasad, M.S. (2016). "Role of Medicinal Plants for Health Perspective: Special Reference to Antioxidant Potential." *Journal of Chemical Biology and Therapeutics* **01**(02): 1-5.

Sofowora, A., Ogunbodede, E., Onayade, A (2013). " The role and place of medicinal plants in the strategies for disease prevention." *African Journal of Traditional, Complementary and Alternative Medicines* **10**(5): 210-229.

Soonthornsit, N., Pitaksutheepong, C., Hemstapat, W., Utaisincharoen, P., Pitaksuteepong, T. (2017). "*In Vitro* Anti-Inflammatory Activity of *Morus alba* L. Stem Extract in LPS-Stimulated RAW 264.7 Cells." *Evidence-Based Complementary and Alternative Medicine,* **2017***:* 1-8.

Soromou, L. W., Zhang, Z., Li, R., Chen, N., Guo, W., Huo, M., Guan, S., Lu, J., Deng, X. (2012). "Regulation of Inflammatory Cytokines in Lipopolysaccharide-Stimulated RAW 264.7 Murine Macrophage by 7-O-Methyl-naringenin." *Molecules* **17**(3): 3574-3585

Taciak, B., Białasek, M., Braniewska, A., Sas, Z., Sawicka, P., Kiraga, Ł., Rygiel, T., Król, M. (2018). "Evaluation of phenotypic and functional stability of RAW 264.7 cell line through serial passages." *PLoS One* **13**(6): e0198943.

Tuasha, N., Seifu, D., Gadisa, E, Petros, B., Stina, O. (2019). "Cytotoxicity of selected Ethiopian medicinal plants used in traditional breast cancer treatment against breast-derived cell lines." *Journal of Medicinal Plants Research* **13**(9): 188-198.

van Huyssteen, M., Milne, P.J., Campbell, E.E., van de Venter, M. (2011). "Antidiabetic and cytotoxicity screening of five medicinal plants used by traditional practitioners in the Nelson

Mandela Metropole, South African." *African Journal of Traditional, Complementary and Alternative Medicines* **8**(2): 150-158.

Van Wyk, B.-E. (2011). "The potential of South African plants in the development of new medicinal products." South African Journal of Botany. **77**: 812-829

Yaffe, D., and Saxel, O (1977). "Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle." *Nature* **270**: 725-727.

York, T. (2012). An ethnopharmacological study of plants used for treating respiratory infections in rural Maputal and. KwaDlangezwa, University of Zululand. Masters.

Yuan, G., Wahlqvist, M.L., He, G., Yang, M., Li, D (2006). "Natural products and anti-inflammatory activity." *Asia Pacific Journal of Clinical Nutrition* **15**(2): 143-152.

Yuan, H., Ma, Q. Ye, L., Piao, G. (2016). "The Traditional Medicine and Modern Medicine from Natural Products." *Molecules* **21**(559): 1-18.

CHAPTER FIVE: OBJECTIVE THREE (PAPER IV)

In this chapter, the evaluation of the preliminary screening of phytochemicals, antioxidant, and alpha-amylase inhibitory activities of the selected South African plant extracts for medicinal purposes, are presented and discussed. The manuscript has been accepted for the publication in the *International Journal of Pharma and Bio Science* for consideration.

Paper IV Estimation of preliminary screening of phytochemicals, total phenolic content, total flavonoid content, antioxidant, and alpha-amylase inhibitory activities of the selected South African plant extract for medicinal purposes. "*International Journal of Pharma and Bio Sciences*" (in press).

ESTIMATION OF THE PRELIMINARY SCREENING OF PHYTOCHEMICALS, ANTIOXIDANT AND ALPHA-AMYLASE INHIBITORY ACTIVITIES OF THE SELECTED SOUTH AFRICAN PLANT EXTRACTS FOR MEDICINAL PURPOSES.

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ABSTRACT

Medicinal plants serve as important sources of pharmaceutical, cosmetic and traditional medicine. Natural products continue to play a significant role in drug discovery and development. Bioactive compounds, which naturally occur in medicinal plants, such as antioxidants, play a vital role in scavenging free radicals. The purpose of this study was to evaluate preliminary phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha (α)-amylase potential of selected South African plant extracts.

In a qualitative analysis the phytochemical compounds such as tannins, saponins, flavonoids, quinones, phenols, terpenoids, alkaloids, glycosides, cardio glycosides, coumarins, betacyanin, anthocyanin, and steroids were screened and quantified. In addition to this, antioxidants were analysed using DPPH and ABTS radical scavenging assays. Out of thirteen phytochemicals analysed, ten phytochemicals were present. The antioxidant inhibition was observed in four plant species whose scavenging activity was above 80% and these plants are *Schubria pinnata, Lippia javanica, Clerodendrum myricoides, Erythrina lysistemon,* respectively. Also, these plant species exhibited an alpha-amylase inhibitory effect of 80%. The IC_{50} values were > 1000 µg/ml. These findings confirm the presence of phenolic, flavonoids, antioxidants, and α-amylase properties, and are potential solutions towards the management of diabetes and other chronic inflammatory diseases.

Keywords: phytochemicals, phenolic, flavonoid, antioxidants, alpha-amylase, medicinal plants

1. INTRODUCTION

Phytochemicals are chemicals produced by plants either through primary or secondary metabolism (Kennedy & Wightman, 2011). In general, phytochemicals play an important role in protecting plant growth or act as a defense against competitors, pathogens, or predators (Saxena *et al*., 2013). Importantly, phytochemicals have been reported to protect humans against diseases (Roa, 2003). The protective role of secondary metabolites extends to antioxidants free radical-scavenging, UV light-absorbing, antimicrobial, and anti-proliferative agents (Saxena *et al*., 2013; Wink, 2003; Kurutas, 2016). Antioxidants have also been defined as chemical substances that protect the body cells from injury by free radicals (Lobo *et al*., 2010). As delineated by Knight (1995) and Lobo (2010), free radicals contribute to more than one hundred disorders in humans, including atherosclerosis, arthritis, ischemia-reperfusion injury of many tissues, central nervous system injury, gastritis, and cancer. Furthermore, the protective effects from antioxidants have been observed to be useful towards the prevention of lipids damage, protein, and DNA using preventing radicals initiation, breaking chain propagation or suppressing formation (Kurutas, 2016). Moreover, these antioxidants can prevent deteriorating diseases such as cancer, and also slow down the ageing process (Bahiense *et al*., 2017; Kada *et al*., 2017). These metabolites are essentially used in traditional medicine to treat and manage certain diseases (Boadu & Asase, 2017).

Phytochemicals extracted from plants are classified into three major groups: terpenoids, phenolic metabolites, and alkaloids (Harborne *et al*., 1999). Approximately 10, 000 phytochemicals have been identified thus far (Zhang *et al*., 2015). The well described phytochemicals are alkaloids, tannins, flavonoids, saponins, glycosides, and other phenolic compounds, due to their biological activities screened from medicinal plants thus far (Dluya *et al*., 2017; Ali, 2011; Bossou *et al*., 2013; Mladenka *et al*., 2018; Sayhan *et al*., 2017; War *et al*., 2012).

Very few of these medicinal plants have been explored for their phytochemicals, and pharmacological potential so the chance of finding plants with medicinal uses exists (Mgbeahuruike *et al*., 2017; Street & Prinsloo, 2013). Furthermore, bioactive compounds with anti-inflammatory, antioxidant and antidiabetic properties are potential solutions towards the management of diabetes, and other chronic inflammatory diseases (Huang *et al*., 2016). The free

radicals are naturally formed in the body, and play a pivotal role in many normal cellular processes (Diplock *et al*., 1998; Valko *et al*., 2007). However, at high concentrations, the free radicals can be hazardous to the body and may damage all major components of cells, including DNA, proteins, and cell membranes (Phaniendra *et al*., 2015). The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer, neurodegenerative disorders, cardiovascular diseases, diabetes, and other chronic health conditions (Halliwell, 1994; Diplock *et al*., 1998; Valko *et al*., 2007). Africa is host to a large percentage of the global floral diversity (Aslan *et al*., 2013). Only a few of these plants have been explored for their pharmacological potential; most of these plants therefore remain untapped, especially in the southern African region (Street & Prinsloo, 2013). Therefore, the purpose of this study was to evaluate preliminary phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha (α)-amylase potential on selected South African plant extracts.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade: quercetin, gallic acid, ascorbic acid, aluminium chloride, sodium carbonate, sulphuric acid, ferric chloride, Fehling's A and B, Rutin, folin Cocteau reagent, dragendorff reagent 2,2-diphenyl-1-picryhydrazyl (DPPH), and 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS).

2.2 Plant collection and extraction

Plant species (n=9) were collected from the Walter Sisulu National Botanical Gardens in South Africa in February 2017. The plant species collected has been described in this thesis in Chapter 3, section 2.1 (**Table 1**). The voucher specimen number is held at the Walter Sisulu National Botanical Gardens herbarium. The plant material was air-dried in a well-ventilated room. After drying, the plants were grounded into a powder and stored away from light at room temperature.

Table 1: Accession numbers and voucher specimen numbers of the nine plant species used in this study.

NAME	FAMILY	PART	ACCESSION NUMBER	VOUCHER OF SPECIMEN COLLECTED	
				DATE	NUMBER
Euclea crispa	Ebenaceae	Leaf	24/1982	11/10/1982	24, Behr, C.M
Euclea natalensis	Ebenaceae	Leaf	178/1987	10/6/1987	479; Steel, B.S
Schkuhria pinnata	Asteraceae	Leaf	N/A	N/A	N/A
Ziziphus mucronata	Rhamnaceae	Leaf	36/1982	15/10/1982	39; Behr, C.M
Ziziphus mucronata	Rhamnaceae	Fruits	36/1982	15/10/1982	39; Behr, C.M
Lippia javanica	Verbenaceae	Leaf	16/2014	22/1/2014	28; Kondlo, M
Vernonia oligocephala	Asteraceae	Leaf	268/2013	12/05/2013	29; Hankey, A.J
Clerodendrum myricoides	Lamiaceae	Leaf	11/1987	2/2/1987	367, Steel, B.S
Erythrina lysistemon	Fabaceae	Leaf	21/1982	7/10/1982	22; Behr, C.M

2.3 Preparation of plant extracts

The grounded plant extracts (leaves and fruits) were extracted with 90% methanol (1 g/10 ml), and vigorously shaken for 3 hours. The crude extracts were filtered through Whatman No.1 filter paper and dried at room temperature under a stream of cold air. The crude extracts were reconstituted and concentrated at 10 mg/ml.

2.4 Qualitative phytochemical tests

2.4.1 Test for alkaloids

A few drops of concentrated hydrochloric acid (HCl) were added to 2 ml of each plant extract solution in a test tube. The dragendorff reagent was then added to the solution. The formation of orange precipitation indicated the presence of alkaloids.

2.4.2 Test for cardiac glycosides

Plant extract (0.25 g) was dissolved into 1 ml methanol (MeOH) in a test tube. Two ml of glacial acetic acid and a few drops of 5% ferric chloride were added. In addition to this, 1 ml of concentrated sulphuric acid (H_2SO_4) was carefully added on the side of the test tube. The formation of the brown ring at interface indicated the presence of cardiac glycosides.

2.4.3 Test for flavonoids

Plant extract (0.75 g) was dissolved in 3 ml of MeOH in a test tube. Four ml of 1 N sodium hydroxide (NaOH) was added into the test tube. The formation of dark yellow colour was observed which indicated the presence of flavonoids.

2.4.4 Test for phenols

Plant extract (0.25 g) was dissolved in 1 ml MeOH into a test tube. Two ml of distilled water was added into the test tube followed by 0.5 ml of sodium carbonate (Na_2CO_3) and 0.5 ml Folin Ciocalteuau's reagent. The formation of a blue/green colour indicated the presence of phenols.

2.4.5 Test for saponins

Plant extract (0.5 g) was dissolved into 2 ml of boiling water in a test tube. This was allowed to cool, and shaken well to mix thoroughly. The presence of foam indicated the presence of saponins.

2.4.6 Test for steroids

Plant extract (0.25 g) was dissolved into 1 ml MeOH in a test tube. Two ml of chloroform was added followed by 1 ml of H_2SO_4. The formation of the reddish-brown ring at interface indicates the presence of steroids.

2.4.7 Test for tannins

Plant extract (0.25 g) was dissolved into 1 ml MeOH in a test tube. Two ml of 10% ferric chloride ($FeCl_3$) was added into the test tube. The formation of dark blue or greenish-grey colouring indicated the presence of tannins.

2.4.8 Test for anthocyanin and betacyanin

Plant extract (0.5 g) was dissolved into 2 ml MeOH in a test tube. One ml of 2 N NaOH was added and heated for 5 min at 100°C. The formation of bluish-green colour indicated the presence of anthocyanin; the formation of yellow colour indicated the presence of betacyanin.

2.4.9 Test for coumarins

Plant extract (0.25 g) was dissolved into 1 ml MeOH in a test tube. The test tube was covered with filter paper which was moistened with 1 ml NaOH. The test tube was placed in boiling water for a few minutes. Then the filter paper was removed, and examined under UV light for yellow fluorescence to indicate the presence of coumarins.

2.4.10 Test for carbohydrates (Fehling's Test)

Plant extract (0.5 g) was dissolved in 2 ml of methanol in a test tube. One ml of Fehling's A and 1 ml of Fehling's B were added into the test tube. Test tubes were placed in a boiling water bath for a few minutes. The test tube content was mixed as it started to boil. Colour change and precipitate formation were observed. Formation of a yellow or brownish-red precipitate indicated the presence of reducing sugars.

2.4.11 Test for quinones

Pant extract (0.25 g) was dissolved in 1 ml MeOH in a test tube. One ml of concentrated H_2SO_4 was added into the test tube. The formation of red colour indicated the presence of quinones.

2.4.12 Test for terpenoids

Plant extract (0.25 g) was dissolved into 1 ml MeOH into a test tube. Two ml of chloroform was added into the test tube, and followed by 1.5 ml concentrated H_2SO_4 was carefully added. The formation of reddish-brown colour at the interface indicates the presence of terpenoids.

2.5 Determination of total phenolic content

The total phenolic content was evaluated by Folin Ciocalteu's method. One ml of plant extract (50, 150, 300, 450 µg/ml), and standard gallic acid (50, 100, 150, 300, 400, 450 µg/ml), were placed in test tube, and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent were

mixed and shaken. After 5 min, 1.5 ml of 20% of sodium carbonate (Na_2CO_3) was added and made up to 10 ml with distilled water. This was incubated for 2 h at room temperature, and ab intense blue colour developed. After incubation, absorbance was measured at 750 nm with a spectrophotometer (Anthos Zenyth 200 UV/Vis spectrophotometer). The experiment was performed in triplicates. The blank was done using reagent blank with solvent. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of plant extracts were expressed as mg of gallic acid equivalent weight (GAE)/100 g of dry weight (Samidha *et al*., 2014; Aryal *et al*., 2019).

2.6 Determination of total flavonoid content

The total flavonoid content was evaluated with the aluminium chloride colourimetric assay. One ml of plant extract (50, 150, 300, 450 µg/ml), and 1 ml of standard quercetin solution (50, 100, 150, 300, 400, 450 µg/ml), were added into test tubes and followed by 4 ml of distilled water, and 0.3 ml of 5% sodium nitrite solution. After 5 min, 0.3 ml of 10 % aluminium chloride was added. At the 6^{th} min, 2 ml of 1 M sodium hydroxide was added. The final volume was adjusted to 10 ml with distilled water and mixed well. The formation of orange yellowish colour was observed. The absorbance was measured at 510 nm with Athos Zenyth 200 UV/Vis spectrophotometer. The plant experiment was performed in triplicates. The blank was done using distilled water. Quercetin was used as a standard. The calibration curve was plotted using standard quercetin. The data of total flavonoids of plant extracts were expressed as mg of quercetin equivalents to 100 g of plant dry mass (Aryal *et al*., 2019, Chandra *et al*., 2014).

2.7 *In vitro* antioxidant assay - 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical-scavenging activity was carried out using the methods previously described by Alara *et al*., (2017). Essentially 0.3 mM of DPPH was prepared fresh in an ethanoic solution, and 10 mg of each plant extract in various concentrations (10, 30, 40 and 50 mg/ml) was dissolved in 2 ml of methanol. Thereafter, 0.1 ml of prepared plant extract was added into a test tube containing 1.9 ml of DPPH radical solution. The mixtures were vortexed thoroughly and incubated in room temperature in the dark for 30 min. The absorbance was measured at 515 nm using Anthos Zenyth 200 UV/Vis spectrophotometer. Methanol was used as a blank. Ascorbic

acid (Sigma-Aldrich, Johannesburg, South Africa) was used as a standard drug. The percentage of DPPH inhibition was evaluated using an equation that was previously described by Omede *et al*., (2018).

$$(\%)\ \text{inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

The extracts with a scavenging activity higher than 80% were considered active. For extracts that had a scavenging activity ranged between 50% and below 80% inhibition were considered moderate, and inactive were extract with <50% inhibition, and those inactive were rejected.

2.8 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity assay

The ability of selected plant extracts to scavenge free radical activities was determined by ABTS radical decolourisation assay. The assay was previously described by Re *et al*., (1999), and was further modified for this study. Briefly, ABTS+ cation radical was produced by the reaction between 7 mM ABTS in water, and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12 – 16 h before use. ABTS+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 mM. After the addition of 5 µl plant extract to obtain 3.995 ml of diluted ABTS+ solution, the absorbance was measured at 30 min after the initial mixing. The methanol solvent blank was run in each assay. The ABTS scavenging capacity of the extract was compared to butylated hydroxytoluene (BHT). The percentage inhibition of absorbance at 734 mM was calculated using the formula below.

$$\text{Scavenging capacity } (\%) = \frac{100 - [\text{OD sample} - \text{OD sample blank}] \times 100\%}{\text{OD control} - \text{OD control blank}}$$

Where OD either represents optical density or absorbance.

2.9 Alpha-amylase (α-amylase) inhibition assay

Different concentrations (50, 150, 250, 350, 450 µg/ml) of each plant extract (500 µl) were incubated with 500 µl of α-amylase enzyme solution (2 units/ml) that was obtained by dissolving 0.001 g of α-amylase in 100 ml of 0.02 M sodium phosphate buffer pH 6.9 with 6.7 mM sodium chloride) at room temperature (32℃) for approximately 10 min. After the incubation period this was followed by the addition of 500 µl of 1% starch solution (1 g of potato starch was dissolved in 100 ml of boiling distilled water and stirred for 15 min) and incubated at room temperature (32℃) for a further 10 min. After the incubation period, 1 ml of 3,5-dintro salicylic acid (DNSA) reagent was obtained by dissolving 1 g of DNSA in 50 ml of distilled water and added to 30 g of sodium potassium tartrate in small lots, until the solution turned milky yellow. In the mixture above, 20 ml of 2 N sodium hydroxide was added and it turned transparent orange-yellow colour and this was made up to a mark of 100 ml in a volumetric flask. The stop reagent added in a solution and it was wrapped with foil and kept in the dark. and was incubated in a hot water bath (85℃) for 5 min. The reaction was observed after the 5 min incubation time; the colour changed to orange-red and was removed from the water bath and cooled to room temperature.

The mixture was further diluted with 5 ml distilled water. The different plant extract concentrations were performed in triplicates. Individual blank was performed by replacing an enzyme with a buffer. Controls were performed by replacing plant extracts with solvent. Acarbose™ (500 µg/ml) was used as positive controls in the following concentrations (100, 200, 300, 400 and 500 µg/ml). Absorbance was measured at 540 nm with Anthos Zenyth 200 UV/Vis spectrophotometer. The percentage inhibition of α-amylase was analysed using the equation below.

$$\%\text{inhibition} = \frac{(A_{540\ \text{Control}} - B_{540\ \text{Extract}})}{A_{540\ \text{Control}}} \times 100$$

3. STATISTICAL ANALYSIS

The experimental results were expressed as mean ± standard deviation of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} values. Significance difference (**$p < 0.05$) and (***$p<0.0001$) was determined using student "t" test by one-way ANOVA, Tukey's multiple comparison test, and Dunnet test in Prism version 5.0.

4. RESULTS

The purpose of this study was to evaluate preliminary phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha (α)-amylase potential on selected South Africa plant extracts. Thirteen phytochemicals were screened using standardised methods (**Table 1**). Furthermore, the determination of total phenol content and total flavonoid content was conducted in this study (**Fig. 1, Fig. 2,** and **Table 2**). In addition, the ability of plant extracts to scavenging free radicals was evaluated using DPPH radical scavenging assay (**Fig. 3 1 and Fig. 4**), and ABTS radical scavenging assay (**Fig. 5**). Finally, the ability of the plant extracts to inhibit α-amylase (**Fig. 6**) was also evaluated.

4.1 The qualitative results of phytochemicals

The qualitative phytochemicals investigations showed the presence of phytochemicals in various concentrations. The results are summarised in Table 2 below.

Table 2: Qualitative analysis of phytochemicals results of nine plant extracts

PLANT EXTRACTS	PART	[A]ALK	[B]CAR	[C]FLA	[D]PHE	[E]SAP	[F]STE	[G]TA	[H]ANT	[I]BEC	[J]COU	[K]CRB	[M]QUI	[N]TEP
Euclea crispa	L	-	-	+	+	+	-	+	+	-	+	-	+	+
Euclea natalensis	L	-	-	+	+	+	-	+	+	-	+	+	+	+
Schkuhria pinnata	L	+	-	+	+	-	-	-	-	+	+	-	+	+
Ziziphus mucronata	L	+	-	+	+	+	-	+	+	+	+	+	+	+
Ziziphus mucronata	F	+	-	+	+	+	-	+	+	+	+	+	+	+
Lippia javanica	L	+	+	+	+	+	+	+	-	+	+	-	-	+
Vernonia oligocephala	L	+	-	+	+	+	+	+	+	-	+	-	-	+
Clerodendrum myricoides	L	+	+	+	+	+	+	+	+	-	+	-	-	+
Erythrina lysistemon	L	+	+	+	+	-	+	+	+	-	+	-	+	+

L: Leaf; F: fruit; [a]ALK: Alkaloids; [b]CAR: Cardiac glycosides; [c]FLA: flavonoids; [d]PHE: phenols; [e]SAP: saponins; [f]STE: steroids; [g]TA: tannins; [h]ANT: anthocyanin; [i]BEC: Bectacynin; [j]COU: coumarins; [k]CRB: carbohydrates; [m]QUI: quinones; [m]TEP: terpenoids; +: present and -: absent.

5.2 The determination of total phenolic content

The gallic acid solution of concentration (50 – 450 µg/ml) conformed to Beer's Law at 750 nm with a regression coefficient (R^2) = 0.9928. The plot has a slope (m) = 0.00061 and intercept = 0.03671. The equation of standard curve is y = 0.00061x + 0.03671 (**Table 3** and **Fig. 1**).

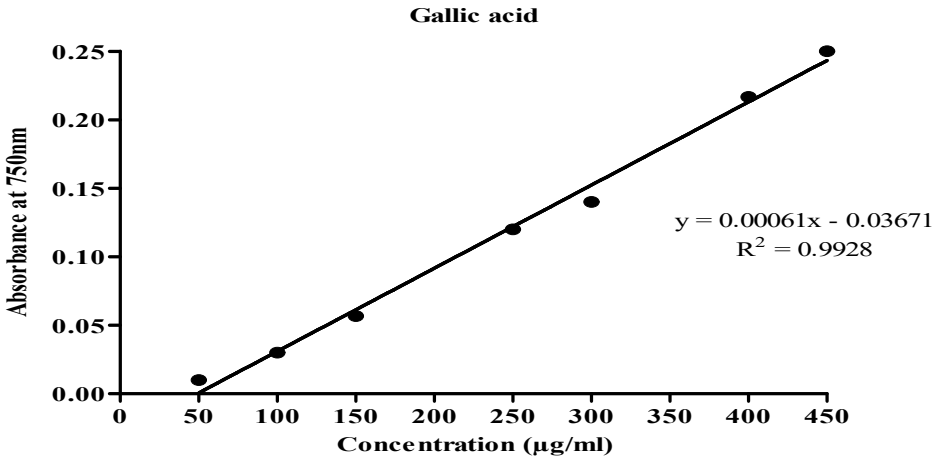

Fig. 1: Total phenolic content for standard gallic acid. R^2 value represented mean data of n=3.

4.2 The determination of total flavonoids

The quercetin solution of concentration (100 – 500 mg/ml) conformed to Beer's Law at 510 nm with a regression coefficient (R^2) = 0.9586. The plot has a slope (m) = 0.0001839 and intercept = 0.01839. The equation of standard curve is y = 0.0001839 x + 0.01839 (**Table 3** and **Fig 2**).

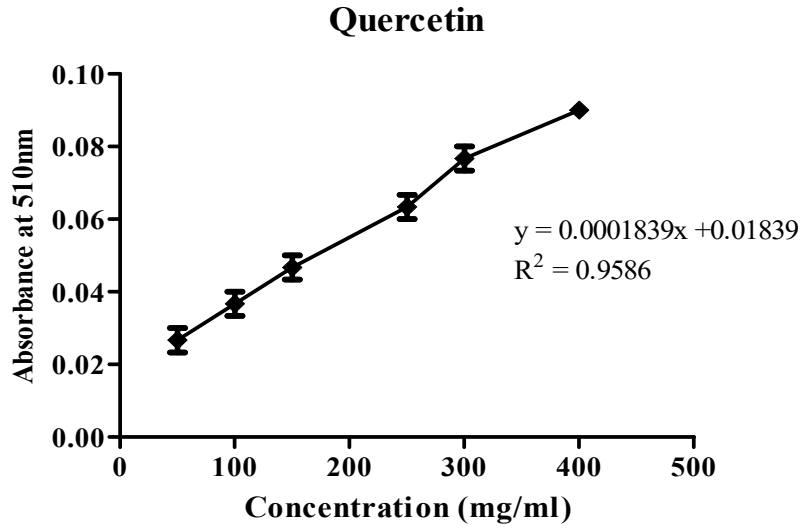

Fig. 2: Total flavonoids content for standard quercetin. R2 value represents the mean data set of (n=3).

Table 3: Total phenolic content and total flavonoids content of the selected South African plant extracts

Plant species	Plant parts	Concentrations (µg/ml)	TPC (mg GAE/g) *	TFC (mg of quercetin equivalent/g dry material)
Euclea crispa	Leaf	50	36,60± 0.94	4,60 ± 0.94
		100	46,19± 1.41	10,90 ± 0.94
		150	57,79± 0.94	32,95 ± 0.94
		250	70,39± 1.41	36,43 ± 1.41
		300	71,99± 2.36	38,80 ± 1.41
		400	82,58± 0.94	49,26 ± 0.94
		450	93,18± 1.41	61,05 ± 2.36
Euclea natalensis	Leaf	50	13,51± 0.94	6,18 ± 0.94
		100	36,67± 0.94	12,81 ± 1.41
		150	61,75± 1.41	16,75 ± 1.41
		250	72,26± 0.94	32,50 ± 1.41
		300	82,77± 0.94	48,24 ± 1.41
		400	93,28± 1.41	64,33 ± 1.89
		450	93,80± 1.89	79,74 ± 1.41
Schkuhria pinnata	Leaf	50	1,93± 0.47	3,70 ± 1.89
		100	7,72± 0.47	18,39 ± 0.94
		150	21,23± 1.41	34,09 ± 1.41
		250	38,60± 0.47	49,11 ± 0.94
		300	44,39± 0.94	64,81 ± 1.41
		400	50,18± 1.89	80,17 ± 1.41
		450	55,96± 1.41	82,34 ± 1.41

Plant species	Plant parts	Concentrations (µg/ml)	TPC (mg GAE/g) *	TFC (mg of quercetin equivalent/g dry material)
Ziziphus mucronata	Leaf	50	7,72± 0.47	2,24 ± 0.94
		100	19,30± 1.41	3,70 ± 1.89
		150	30,88± 1.89	4,60 ± 0.94
		250	36,67± 0.94	5.39 ± 0.94
		300	47,25± 0.94	26,45 ± 1.41
		400	57,83± 1.41	47,17 ± 1.41
		450	68,40± 0.94	67,56 ± 0.94
Ziziphus mucronata	Fruits	50	13,51± 1.41	2,58 ± 1.41
		100	25,09± 1.41	3,36 ± 1.41
		150	46,32± 0.94	4,60 ± 0.94
		250	55,97± 1.41	7,75 ± 0.94
		300	66,54± 0.94	34,02 ± 1.41
		400	77,12± 1.41	60,29 ± 1.89
		450	87,70± 1.41	65,56 ± 0.94
Lippia javanica	Leaf	50	1,93± 0.47	3,03 ± 0.94
		100	5,79± 0.94	4,94 ± 1.41
		150	7,72± 0.47	20,87 ± 1.41
		250	21,23± 1.41	36,81 ± 1.41
		300	34,74± 0.94	53,08 ± 1.89
		400	48,25± 0.94	68,69 ± 1.41
		450	61,76± 1.41	74,62 ± 1.41

Plant species	Plant parts	Concentrations (µg/ml)	TPC (mg GAE/g) *	TFC (mg of quercetin equivalent/g dry material)
Vernonia oligocephala	Leaf	50	1,93± 0.47	2,91 ± 1.89
		100	3,86± 0.94	23,48 ± 1.89
		150	5,79± 0.47	44,06 ± 1.89
		250	5,72± 0.94	64,30 ± 1.41
		300	23,44± 1.41	74,88 ± 1.41
		400	39,16± 1.41	79,88 ± 1.41
		450	54,88± 0.94	85,45 ± 1.41
Clerodendrum myricoides	Leaf	50	3,86± 0.94	2,58 ± 1.41
		100	7,72± 0.94	3,70 ± 1.89
		150	11,58± 1.41	19,06 ± 1.89
		250	19,30± 1.41	34,09 ± 1.41
		300	35,02± 1.41	49,78 ± 1.89
		400	50,74± 1.89	64,14 ± 0.47
		450	66,46± 0.94	80,51 ± 1.89
Erythrina lysistemon	Leaf	50	17,37± 0.94	6,51 ± 1.41
		100	42,46± 1.89	7,30 ± 1.41
		150	52,91± 1.89	23,48 ± 2.36
		250	63,37± 1.41	38,66 ± 1.89
		300	73,82± 0.47	54,17 ± 1.89
		400	84,28± 2.83	70,02 ± 2.36
		450	94,74± 0.94	75,87 ± 1.41

*all values are expressed as mean ± standard error means (SEM) (n=3), TPC = Total phenolic content, and TFC = Total flavonoids content.

4.3 *In vitro* antioxidant assay - 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical

The determination of antioxidant activity from nine plant extracts was evaluated using DPPH radical scavenging activity. The standard curve of ascorbic acid was measured and shown in Fig. 3. The results are expressed in percentage of inhibition; of which active extract was above 50% and anything below 50% was considered inactive and rejected. Four active plant extracts are summarised (**Fig. 4**) below. One-way ANOVA was used to establish statistical significance between plant extracts and concentrations used. A degree of significance was observed with one or two concentrations used. The inhibition effects that were observed were: *Schkuhria pinnata* showed 95% inhibition (50 mg/ml); *Lippia javanica* showed 95% inhibition (50 mg/ml); *Clerodendrum myricoides* showed 93% inhibition (50 mg/ml); and *Erythrina lysistemon* showed 92% inhibition (50 mg/ml), respectively. A significant difference between groups was observed **(P<0.005) between *S. pinnata* vs *C. myricoides*, *S. pinnata* vs *E, lysistemon*, and *L. javanica* vs *E. lysistemon.* It can be further seen that the inhibition had a dose-respond.

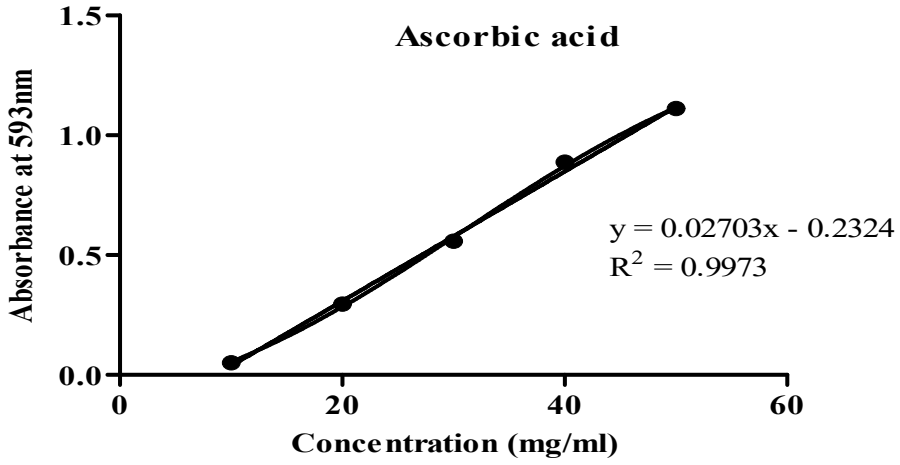

Fig. 3: *In vitro* antioxidant (DPPH) assay for standard Ascorbic acid. R^2 values represented mean data set of n=3.

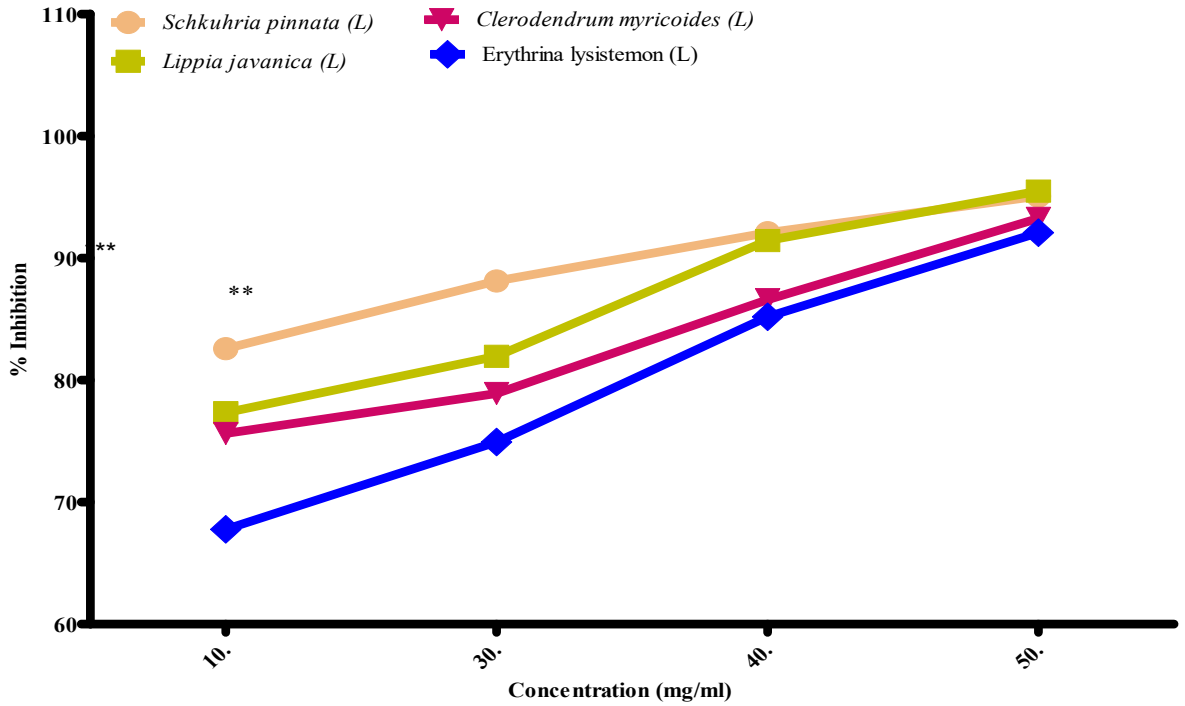

Fig. 4: DPPH scavenging activities for *Schkuhria pinnata* (IC_{50} = 38.244 mg/ml), *Lippia javanica* (IC_{50} = 34.966 mg/ml), *Erythrina lysistemon* (IC_{50} = 37.828 mg/ml), and *Clerodendrum myricoides* (IC_{50} = 38.939 mg/ml). L: Leaf, and F: fruit. (Significant, **p<0.005).

4.4 ABTS radical scavenging activity assay

The four plant extracts that demonstrated good scavenging activity against DPPH radical assay were further validated with ABTS and similar results were observed (**Fig. 5**). The inhibition effects and IC_{50} value were observed *Clerodendrum myricoides* showed 88% inhibition (0.04 mg/ml). The IC_{50} value of 7.148 mg/ml for *Erythrina lysistemon* showed 86% inhibition (0.04 mg/ml), The IC_{50} value of 5.353 mg/ml *Schkuhria pinnata* showed 85% inhibition (0.04 mg/ml). The IC_{50} value of 0.944 mg/ml and *Lippia javanica* showed 83% inhibition (0.04 mg/ml), and IC_{50} value of 0.759 mg/ml. The standard inhibitor BHT showed 97% (0.04 mg/ml). The significant difference between plant extracts and BHT was observed to be **($p<0.05$).

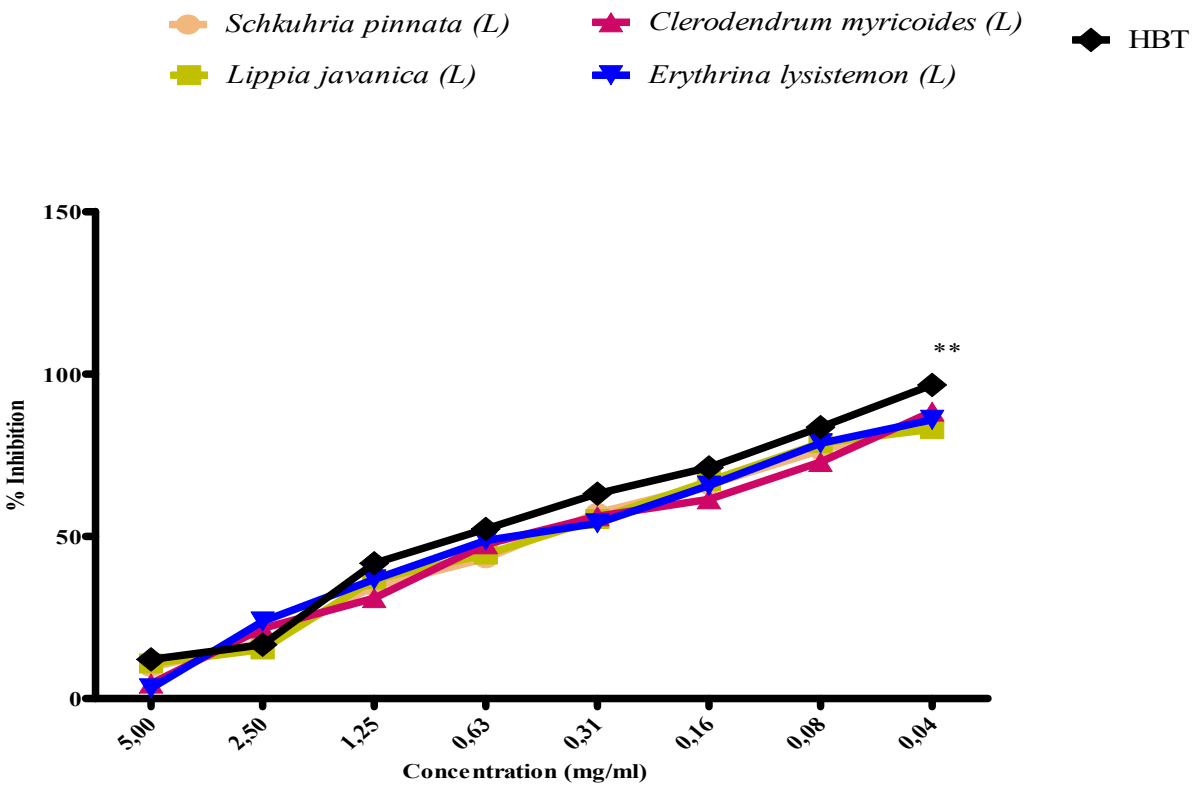

Fig. 5: ABTS scavenging activities for *Schkuhria pinnata* (IC_{50} = 0.944 mg/ml), *Lippia javanica* (IC_{50} = 0.759 mg/ml), *Erythrina lysistemon* (IC_{50} = 5.353 mg/ml), and *Clerodendrum myricoides* (IC_{50} = 7.148 mg/ml). L: Leaf, and F: fruit. (Significant between groups and control (HBT (IC_{50} = 2.763 mg/ml)), **($p<0.005$).

4.5 *In vitro* inhibitory α-amylase assay

Nine selected plant extracts were used to assess the ability of α-amylase inhibitory effects and were compared with a known inhibitor (Acarbose^{TM}). A degree of α-amylase was observed in all plant extracts which ranged from 9% to 98%. However, only four plant extracts demonstrated a good inhibition effect, and similar results were observed for Acarbose^{TM}. Essentially, plant extracts were further quantified by chromogenic DNSA method and good inhibition effects was observed and lower IC_{50} values for *Schkuhria pinnata* (IC_{50} = 52.673 µg/ml), and *Clerodendrum myricoides* (IC_{50} = 322.287 µg/ml) showed 98% inhibition (450 µg/ml), *Erythrina lysistemon* (IC_{50} = 205.089 µg/ml) showed 97% inhibition (450 µg/ml), and *Lippia javanica* (IC_{50} = 247.708 µg/ml) showed 96% inhibition (450 µg/ml). The standard inhibitor Acarbose^{TM} (IC_{50} = 291.395 µg/ml) showed 87% (450 µg/ml). The significant difference was observed between groups **($p<0.05$) and between groups and control *** ($p<0.0001$).

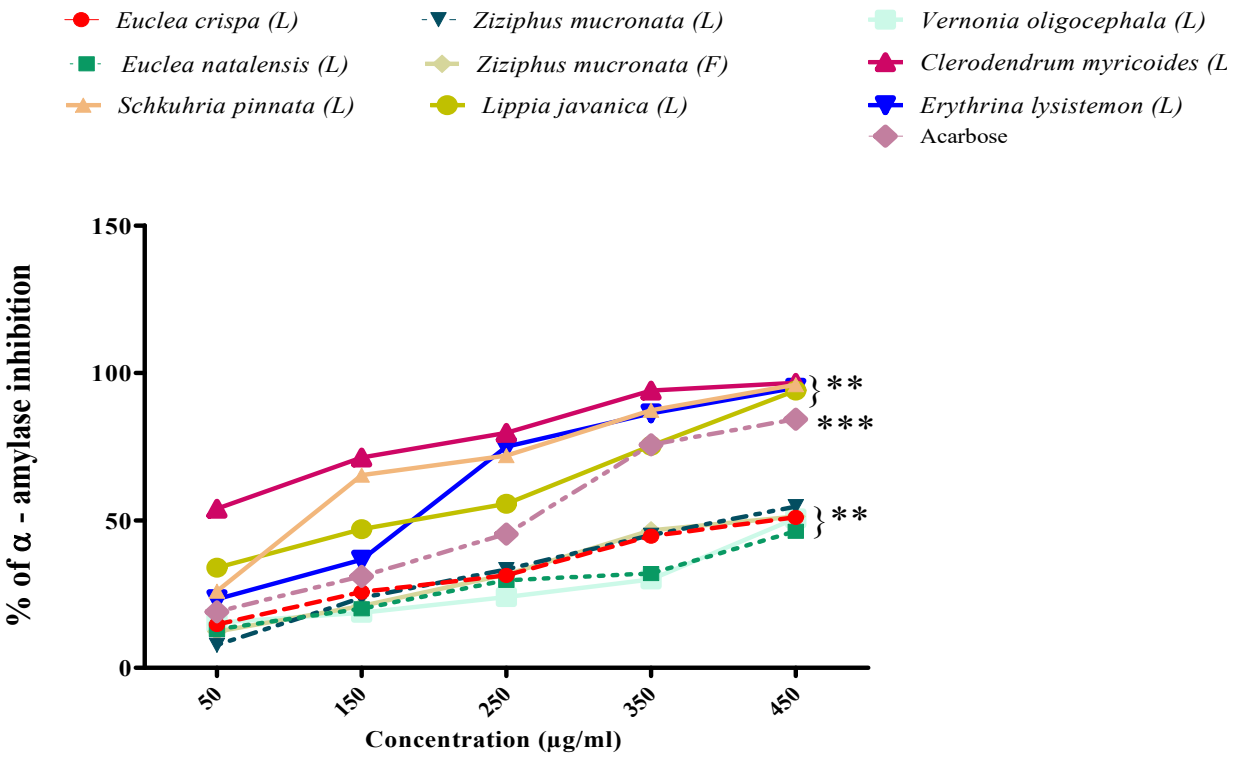

Fig. 6: α-amylase inhibition activity of Acarbose™ and nine selected plant extracts. L: Leaf, and F: fruit. The four plant extract exhibited good α-amylase inhibition and lower IC_{50} values were measured for *Schkuhria pinnata* (IC_{50} = 52.673 µg/ml), and *Clerodendrum myricoides* (IC_{50} = 322.287 µg/ml) showed 98% inhibition (450 µg/ml), *Erythrina lysistemon* (IC_{50} = 205.089 µg/ml) showed 97% inhibition (450 µg/ml), and *Lippia javanica* (IC_{50} = 247.708 µg/ml) showed 96% inhibition (450 µg/ml). The standard inhibitor Acarbose™ (IC_{50} = 291.395 µg/ml) showed 87% (450 µg/ml). The (Significant between groups was observed to be**$p<0.05$, and for control vs groups to be ***$p<0.0001$).

5. DISCUSSION

This study aimed to evaluate preliminary phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha (α)-amylase potential on selected South Africa plant extracts. In the quantitative analysis, the phytochemicals that were analysed ranged from 7 to 10 out of 13 tested phytochemicals identified. The total phenolic content was determined and results were shown (**Fig. 1** and **Table 1**). For total flavonoid content the results were shown (**Fig. 2** and **Table 2**). Four plant extracts demonstrated scavenging activities above 80%, namely, *Schkuhria pinnata*, *Lippia javanica*, *Clerodendrum myricoides*, and *Erythrina lysistemon* (**Fig. 4** and **Fig. 5**). Similarly, these plant extracts also exhibited α-amylase inhibition effects of up to 98% (**Fig. 6**).

5.1 Total phenolic content, total flavonoid content and qualitative of phytochemicals

In this study it was observed that plant extracts exhibited total phenolic content, however, *Erythrina lysistemon* (94.71 ± 0.94 GAE/g), *Euclea crispa* (93.18 ±1.41 GAE/g), *Euclea natalensis* (93.18 ± 1.89 GAE/g), *Ziziphus mucronata* (87.7 ± 87.7 GAE/g) exhibited high total phenolic in high concentration (450 µg/ml) (**Table 3**). The total flavonoid content was also observed to have relatively high results. Interestingly, no similar plant extracts exhibited similar results as the total phenolic content. However, the following plants exhibited high total flavonoid content in higher concentrations (450 µg/ml). The plant extracts were *Vernonia oligocephala* (85.45 ± 1.41 mgG/g), *Schkuhria pinnata* (82.34 ± 1.41 mgG/g), and *Clerodendrum myricoides* (80.51 ±1.89 mgG/g) (Table 3).

The qualitative phytochemicals investigations conducted on selected plant extracts showed the presence of various phytochemicals constituents. All plant extracts indicated the presence of flavonoids, phenolics, coumarins, and terpenoids. Alkaloids were not present in *Euclea crispa*, and *Euclea natalensis*. Cardiac glycosides were present in *Lippia javanica*, *Clerodendrum myricoides*, and *Erythrina lysistemon*. Saponins were present in *Euclea crispa*, *Euclea natalensis*, *Vernonia oligocephala, Lippia javanica*, and *Clerodendrum myricoides*. Steroids were present in *Lippia javanica, Veronia oligocephala*, *Clerodenrum myricoides*, and *Erythrina lysistemon*. Tannins were present in all the plant extracts, except *Schkuhria pinnata*. Bectaynin was present in *Schkuhria pinnnata*, *Ziziphus mucronata* (leaf and fruits), and *Lippia javanica*. Anthocynin were absent in *Schkuhria pinnnata*, and *Lippia javanica*. Carbohydrates were present in *Euclea natalensis* and Ziziphus mucronata (leaf and fruits). Quinones were not present in *Lippa javanica*, *Vernonia oligocephala* and *Clerodendrum*

myricoides. The phytochemicals identified in this study have been extensively studied for their uses as described in the literature.

Flavonoids are naturally found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine (Panche *et al*., 2016; Ali, 2011). Interestingly, flavonoids play an important role in the following industries: nutraceutical, pharmaceutical, medicinal and cosmetic applications (Panche *et al*., 2016). Recent research has shown that flavonoids contribute towards anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Kumar & Pandey, 2013; Ayaz *et al*., 2019).

Phenols are organic compounds and are found in various plant species, and have protective effects against infection and herbivory by an insect (Ali, 2011). The protective effects contribute to anti-inflammatory and antiseptic and can have anti-viral properties (Cianciosi *et al*., 2018; Shahidi & Yeo, 2018). Coumarins are an aromatic organic chemical compound that is a colourless crystalline solid with a sweet odour resembling the scent of vanilla and a bitter taste (Jain & Joshi, 2012). They are naturally found in various plant species and can serve as a defence against predators (Yamane *et al*., 2010). Coumarins have been reported to possess a biological activity and have limited approval for a few medical uses as pharmaceuticals, such as in the treatment of lymphedema (Musa *et al*., 2008). Terpenoids are natural chemical compounds that are present in all living organisms (Pichersky & Raguso, 2018). They are found in abundance mostly in green plants and, particularly, flowering plants, when compared with living organisms (Bergman *et al*., 2019). Essentially, they have a variety of functions such as hormones, components of electron transfer systems, protein modification agents, membrane fluidity determinants, and antioxidants (Tan *et al*., 2018, Bergman *et al*., 2019).

Alkaloids naturally occur in plants, and are more common in certain families of flowering plants (Hussain *et al.*, 2018). The most common alkaloids are morphine, codeine, erogonive, and ephedrine *(Sayhan et al*., 2017; Harborne, 1993). They provide protective effects on plants against predators such as insects (War *et al*., 2012; Furstenberg-Hagg *et al*., 2013). They ave diverse medicinal properties such as relief of pain (morphine) (Shoaib *et al*., 2016; Hussain *et al*., 2018), treatment analgesic (codeine) *(Bhandari et al*., 2011), treatment of arrhythmias (quinidine) (Eyal, 2018), and are useful for blood-vessel constrictors (ergonovine and ephedrine) (Hartmann, 2004). In addition to this, ergonovine is used

to reduce uterine haemorrhage after birth (Weeks, 2015; Chelmow, 2008), Ephedrine has been used often for the relief of discomfort associated with common colds, sinusitis, hay fever, and bronchial asthma (Thacher, 1946). Cardiac glycosides are a class of organic compounds found in various medical plants (Musa *et al*., 2008). Compounds that have been isolated from cardiac glycosides, such as digitoxin, digoxin, and convallotoxin, have been reported to support heart strength and rates of contraction (Grosso *et al*., 2017; Ambrosy *et al*., 2014). Moreover, cardiac glycosides have an additional function, namely, a diuretic effect that stimulates urine production and aids in the removal of fluid from tissues and the circulatory system (Mladenka *et al*., 2018). Essentially, cardiac glycosides are also used as treatments for congestive heart failure, and cardiac arrhythmias (Mashour *et al*., 1998).

Saponins are a class of chemical compounds that are widely found in varies plant species (Faizal & Geelen, 2013). Researchers have investigated numerous properties of including beneficial and detrimental effects on human health, pesticidal, insecticidal, molluscicidal and fungicidal activity, bitterness and sweetness and other industrial applications such as foaming and surface-active agents (Wagner, 2000; Kharkwal *et al*., 2012; Wisetkomolmat *et al*., 2019; Bossou *et al*., 2013). The steroids are biologically active organic compounds, which are found in various plants, animals and fungi (Dembitsky *et al*., 2017). The biological function of steroids is to alter membrane fluidity, and also molecule signalling (Dufourc, 2008). Steroids are hormones that play an important role in alterations in anatomy and physiology during important developmental stages (Zubeldia-Brenner *et al*., 2016).

Tannins are polyphenolics that are produced by plants and are bitter (El Gharras, 2009). The bitterness in tannins serves as a protective mechanism against predators such as insects and grazing animals (War *et al*., 2012). Tannins are also useful in curing leather because of their tendency to contract and astringe tissues by binding with precipitating proteins (Ashok & Upadhyaya, 2012). Anthocyanins are water-soluble and they can have a red, blue or black pigment which is found in plants and vegetables (Archetti *et al*., 2009). Anthocyanin is usually found on the blueberry, raspberry, black rice, and black soybean (Khoo *et al*., 2017). The importance of plants with pigmentations is mainly for signalling, and in attracting in pollinating and dispersal agents and repelling herbivores (Lee, 2005). Anthocyanins are used in the food industry as an additive and possess anti-oxidant properties (Khoo *et al*., 2017). Similarly, bectacyanin are yellow indole-derived pigments found in plants (Khoo *et al*., 2017). They are commonly noticeable in the petals of flowers but may colour the fruit, leaves, stems, and roots of plants

that contain them (Khoo *et al*., 2017; Miguel, 2018) They are used as an additive as food dye; they have also been observed to possess antioxidants (Miguel, 2018).

Carbohydrates are commonly found in organisms and plants (Ainsworth & Bush, 2011). They are among the first organic compounds formed during photosynthesis (Ainsworth & Bush, 2011). Their key function is a source of energy by all cells (Jéquier, 1994). They can be used for the production of cellulose in cell walls, for the synthesis of storage products such as starch (Stein & Granot, 2019). Quinones are secondary metabolites that are found in various plant species (Lu *et al*., 2013). They possess biological activities, such as a purgative effect, and also antibacterial and anti-cancer activities (Koyama *et al*., 2010; Bolton *et al*., 2000). Moreover, quinine has been instrumental in the derivatives of various natural and artificial colouring substances (dyes and pigments) (Yusuf *et al*., 2017).

5.2 *In vitro* antioxidant assay - 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity was determined using DPPH radical scavenging assay and ascorbic acid was used as a standard. The ascorbic acid solution of concentration (10 – 50 mg/ml) conformed to Beer's Law at 593 nm with the regression coefficient (R^2) = 0.9973. The plot has a slope (m) = 0.02703 and intercept = 0.2324. The equation of standard curve is y = 0.02703x – 0.2324 (**Fig. 3**). The results were expressed as a percentage of inhibition and IC_{50} (where 50% concentration of the extract scavenged free radical). The lower the IC_{50} (µg/ml) value the higher the percentage of free radicals. The plant extracts with a scavenging activity higher than 80% were considered active. For extracts that had scavenging activity ranged between 50% and below 80% inhibition were considered moderate and inactive were extract with <50% inhibition, and those inactive were rejected. The antioxidant using DPPH radical scavenging activity revealed that four plant extracts scavenged free radicals namely: *Schkuhria pinnata* (IC_{50} = 38.244 mg/ml), *Lippia javanica* (IC_{50} = 34.966 mg/ml), and *Erythrina lysistemon* (IC_{50} = 37.828 mg/ml) (**Fig. 4**).

5.3 ABTS radical scavenging activity assay

The plant extracts that were considered active from DPPH were further verified with ABTS antioxidant assay. The results were expressed as percentage inhibition and IC_{50} (where 50% concentration of the plant extract scavenged free radical). Essentially, the lower IC_{50} (mg/ml) value the higher the percentage inhibition of free radicals. The antioxidant determined using ABTS radical scavenging activity revealed Schkuhria *pinnata* (IC_{50} = 0.944 mg/ml), *Lippia javanica* (IC_{50} = 0.759 mg/ml), *Erythrina lysistemon* (IC_{50} = 5.353 mg/ml), *Clerodendrum myricoides* (IC_{50} = 7.148 mg/ml), (HBT (IC_{50} = 2.763 mg/ml) (**Fig. 5**). Natural bioactive compounds such as antioxidants normally occur in medicinal plants and they play a pivotal role in scavenging free radicals (Babajide *et al*., 2010; Kalaivani & Mathew, 2010). In this study, four plant species exhibited high antioxidant activities in both DPPH and ABTS radical assays. These plant species can play a pivotal role in the prevention of various degeneration diseases, and these results confirm their use in traditional medicine practice purposes (Rice-evans *et al*., 1995; Xu *et al*., 2017).

5.4 *In vitro* inhibitory α-amylase assay

The plant extracts that were studied demonstrated some degree of α-amylase inhibition, however, a good inhibition was noticeable. *Schkuhria pinnata* (IC_{50} = 52.673 µg/ml), and *Clerodendrum myricoides* (IC_{50} = 322.287 µg/ml) showed 98% inhibition (450 µg/ml), *Erythrina lysistemon* (IC_{50} = 205.089 µg/ml) showed 97% inhibition (450 µg/ml), and *Lippia javanica* (IC_{50} = 247.708 µg/ml) showed 96% inhibition (450 µg/ml). The standard inhibitor Acarbose™ (IC_{50} = 291.395 µg/ml) showed 87% inhibition (450 µg/ml) (**Fig. 6**).

The oxidative damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer, neurodegenerative disorders, cardiovascular diseases, diabetes, and other chronic health conditions (Halliwell, 1994; Diplock *et al*., 1998; Valko *et al*., 2007; Oyedemi *et al*., 2017), and play a significant role in the pathogenesis of metabolic syndrome including diabetes mellitus (DM). The ability of medicinal plants to inhibit α-amylase is an important therapeutic target in the regulation of postprandial increase of blood glucose in diabetic patients (Oyedemi *et al*., 2017). Importantly, the plant species that exhibited good α-amylase inhibition activity can be a candidate for use in the blood glucose regulation in diabetic patients.

6. CONCLUSION

This study has shown the presence of medicinally important properties in plant species studied. The presence of phytochemicals in plant species showed the usefulness of these plant species in traditional medicine practices. Also, certain plant species demonstrated antioxidant potential; and α-amylase inhibition potential was observed. The results suggest that the presence of phenolic, flavonoids, antioxidants, and α-amylase properties are potential solutions towards the management of diabetes and other chronic inflammatory diseases. The results support the use of these plants in the management of various ailments in traditional medicine practices.

7. CONFLICT OF INTEREST

The authors declare that they do not have any conflict concerning the publication of this paper.

8. ACKNOWLEDGEMENT

We thank the College Health Sciences Scholarship Grant at the University of KwaZulu-Natal, Institute of Traditional Knowledge and Traditional Medicine at the Vaal University of Technology, the National Research Foundation and Thuthuka grant for the financial support received towards this study. Dr Ashwell Ndlala is thanks for allowing us to use his chemistry laboratory at Agriculture Research Council, Roodeplaant, Vegetable, and Ornamental Plant Institute. Finally, Ms Nobuhle Mbhele from the Agriculture Research Council, Roodeplaant, Vegetable, and Ornamental Plant Institute is thanked for providing technical assistance towards this work.

9. REFERENCES

AINSWORTH, E. A. & BUSH, D. R. 2011. Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity. *Plant Physiol,* **155**: 64-9.

ALARA, O. R., ABDURAHMAN, N. H., MUDALIP, S. K. A. & OLALERE, O. A. 2017. Effect of drying methods on the free radicals scavenging activity of Vernonia amygdalina growing in Malaysia. *Journal of King Saud University – Science***, 2017**: 1-5, http://dx.doi.org/10.1016/j.jksus.2017.05.018.

ALI, G. 2011. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research,* **5** (31): 6697-6703.

AMBROSY, A. P., BUTLER, J., AHMED, A., VADUGANATHAN, M., VAN VELDHUISEN, D. J., COLUCCI, W. S. & GHEORGHIADE, M. 2014. The use of digoxin in patients with worsening chronic heart failure: reconsidering an old drug to reduce hospital admissions. *J Am Coll Cardiol,* **63**(18): 1823-1832.

ARCHETTI, M., DORING, T. F., HAGEN, S. B., HUGHES, N. M., LEATHER, S. R., LEE, D. W., LEV-YADUN, S., MANETAS, Y., OUGHAM, H. J., SCHABERG, P. G. & THOMAS, H. 2009. Unravelling the evolution of autumn colours: an interdisciplinary approach. *Trends Ecol Evol,* **24**(3):166-173.

ARYAL, S., BANIYA, M. K., DANEKHU, K., KUNWAR, P., GURUNG, R. & KOIRALA, N. 2019. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants* **8**(4): 1-12.

ASHOK, P. K. & UPADHYAYA, K. 2012. Tannins are Astringent. *Journal of Pharmacognosy and Phytochemistry,* **1**(3): 45-50.

ASLAN, C. E., ZAVALETA, E. S., TERSHY, B. & CROLL, D. 2013. Mutualism Disruption Threatens Global Plant Biodiversity: A Systematic Review. *PLOS ONE,* **8**(6)**:** e66993.

AYAZ, M., SADIQ, A., JUNAID, M., ULLAH, F., OVAIS, M., ULLAH, I., AHMED, J. & SHAHID, M. 2019. Flavonoids as Prospective Neuroprotectants and Their Therapeutic Propensity in Aging Associated Neurological Disorders. *Frontiers in Aging Neuroscience,* **11**(115): 1-20.

BABAJIDE, O. J., MABUSELA, W. T., GREEN, I. R., AMEER, F., WEITZ, F. & IWUOHA, E. I. 2010. Phytochemical screening and biological activity studies of five South African indigenous medicinal plants. *Journal of Medicinal Plant Research,* **2**: 1924-1932.

BAHIENSE, B. J., MARQUES, F. M., FIGUEIRA, M. M., VARGAS, T. S., KONDRATYUK, T. P., ENDRINGER, D. C., SCHERER, R. & FRONZA, M. 2017. Potential anti-inflammatory,

antioxidant and antimicrobial activities of Sambucus australis. *Pharmaceutical biology* **55:** 991-997.

BERGMAN, M. E., DAVIS, B. & PHILLIPS, M. A. 2019. Medically Useful Plant Terpenoids: Biosynthesis, Occurrence, and Mechanism of Action. *Molecules,* **24**(21):1-23.

BHANDARI, M., BHANDARI, A. & BHANDARI, A. 2011. Recent updates on codeine. *Pharmaceutical Methods,* **2**(1):3-8.

BOADU, A. A. & ASASE, A. 2017. Documentation of Herbal Medicines Used for the Treatment and Management of Human Diseases by Some Communities in Southern Ghana. *Evidence-Based Complementary and Alternative Medicine***, 2017**: 1-12, https://doi.org/10.1155/2017/3043061 .

BOLTON, J. L., TRUSH, M. A., PENNING, T. M., DRYHURST, G. & MONKS, T. J. 2000. Role of quinones in toxicology. *Chem. Res. Toxicol.,* **13**(3):135-60.

BOSSOU, A. D., MANGELINCKX, S., YEDOMONHAN, H., BOKO, P. M., AKOGBETO, M. C., DE KIMPE, N., AVLESSI, F. & SOHOUNHLOUE, D. C. K. 2013. Chemical composition and insecticidal activity of plant essential oils from Benin against Anopheles gambiae (Giles). *Parasites & Vectors,* **6**(337): 1-17.

CHANDRA, S., KHAN, S., AVULA, B., LATA, H., YANG, M. H., ELSOHLY, M. A. & KHAN, I. A. 2014. Assessment of Total Phenolic and Flavonoid Content, Antioxidant Properties, and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. *Evidence-Based Complementary and Alternative Medicine***, 2014**:1-9, http://dx.doi.org/10.1155/2014/253875.

CHELMOW, D. 2008. Postpartum haemorrhage: prevention. *BMJ clinical evidence,* **12**(1410): 1-38.

CIANCIOSI, D., FORBES-HERNÁNDEZ, T. Y., AFRIN, S., GASPARRINI, M., REBOREDO-RODRIGUEZ, P., MANNA, P. P., ZHANG, J., BRAVO LAMAS, L., MARTÍNEZ FLÓREZ, S., AGUDO TOYOS, P., QUILES, J. L., GIAMPIERI, F. & BATTINO, M. 2018. Phenolic Compounds in Honey and Their Associated Health Benefits: A Review. *Molecules (Basel, Switzerland),* **23**(2322):1-20.

DEMBITSKY, V. M., GLORIOZOVA, T. A. & POROIKOV, V. V. 2017. Biological Activities of Organometalloid (As, At, B, Ge, Si, Se, Te) Steroids. *Journal of Applied Pharmaceutical Science,* **7**(1): 184-202.

DIPLOCK, A. T., CHARLEUX, J. L., GROZIER-WILLI, G., KOK, J., RICE-EVANS, C., ROBERFOID, M., STAHL, W. & VINA-RIBES, J. 1998. Functional Food Science and Defence Against Reactive Oxidative Species. *Journal of Nutrition,* **80**(1):S77-S112.

DLUYA, T., DANIEL, D. & YUSUF, U. 2017. In vitro Antioxidant Activity and Phytochemical Evaluation of Five Medicinal Plants Extract. *The Pharmaceutical and Chemical Journal,* **4**(5):73-82.

DUFOURC, E. J. 2008. Sterols and membrane dynamics. *Journal of chemical biology,* **1**(1-4): 63-77.

EL GHARRAS, H. 2009. Polyphenols: food sources, properties and applications - a review. *International Journal of Food Science & Technology,* **44**:2512-2518.

EYAL, S. 2018. The Fever-Tree: from Malaria to Neurological Diseases. *Toxins,* **10**(12):1-12.

FAIZAL, A. & GEELEN, D. 2013. Saponins and their role in biological processes in plants. *Phytochemistry Reviews,* **12**(4)**,** 877-893.

FURSTENBERG-HAGG, J., ZAGROBELNY, M. & BAK, S. 2013. Plant defence against insect herbivores. *Int J Mol Sci,* **14**(5):10242-97.

GROSSO, F., STOILOV, P., LINGWOOD, C., BROWN, M. & COCHRANE, A. 2017. Suppression of Adenovirus Replication by Cardiotonic Steroids. *Journal of virology,* **91**(3): 1-16.

HALLIWELL, B. 1994. Free radicals, antioxidants, and human disease: curiosity, cause or consequence. *Lancet* **344**(8924):721-724.

HARBORNE, J. B., BAXTER, H. & MOSS, G. P. 1999. *Phytochemical Dictionary. A Handbook of Bioactive Compounds from Plants,* London, Taylor & Francis.

HARBORNE, J. B., BAXTER, H. 1993. *Pyrrolizidine Alkaloids,* Bristol, Phytochemical Dictionary,**1993**: 255-266.

HARTMANN, T. 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. *Planta,* **219**(1):1-4.

HUANG, S.-S., SU, S.-Y., CHANG, J.-S., LIN, H.-J., WU, W.-T., DENG, J.-S. & HUANG, G.-J. 2016. Antioxidants, anti-inflammatory, and antidiabetic effects of the aqueous extracts from Glycine species and its bioactive compounds. *Botanical studies,* **57**(1): 38-38.

HUSSAIN, G., RASUL, A., ANWAR, H., AZIZ, N., RAZZAQ, A., WEI, W., ALI, M., LI, J. & LI, X. 2018. Role of Plant-Derived Alkaloids and Their Mechanism in Neurodegenerative Disorders. *International journal of biological sciences,* **14**(3):341-357.

JAIN , P. K. & JOSHI, H. 2012. Coumarin: Chemical and Pharmacological Profile. *Journal of Applied Pharmaceutical Science,* **02**(6): 236-240.

JÉQUIER, E. 1994. Carbohydrates as a source of energy. *The American Journal of Clinical Nutrition,* **59**(3):682S-685S.

KADA, S., BOURICHE, H., SENATOR, A., DEMIRTAŞ, I., ÖZEN, T., TOPTANCI, B. C., KıZıL, G.

& KıZIL, M. 2017. Protective activity of Hertia cheirifolia extracts against DNA damage, lipid peroxidation and protein oxidation. *Pharmaceutical biology,* **55**(1): 330-337.

KALAIVANI, T. & MATHEW, L. 2010. Free radical scavenging activity from leaves of Acacia nilotica (L) Wild. Ex Delile, and Indian medicinal tree. *Food and Chemical Toxicology,* **48**(1):298-305.

KENNEDY, D. O. & WIGHTMAN, E. L. 2011. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Advances in nutrition (Bethesda, Md.),* **2**(1): 32-50.

KHARKWAL, H., BAHUGUNA, D. P., PANT, M., KHARKWAL, H., KHARKWAL, A. & JOSHI, D. 2012. Foaming glycosides: A review. *IOSR Journal of Pharmacy,* **2**(5): 23-28.

KHOO, H. E., AZLAN, A., TANG, S. T. & LIM, S. M. 2017. Anthocyanidins and anthocyanins: coloured pigments as food, pharmaceutical ingredients, and potential health benefits. *Food & nutrition research,* **61**(1):1-21.

KNIGHT, J. A. 1995. Diseases related to oxygen-derived free radicals. *Annals of clinical and laboratory science,* **25**(2):111-121.

KOYAMA, J., MORITA, I. & YAMORI, T. 2010. Correlation between cytotoxic activities and reduction potentials of heterocyclic quinones. *Molecules (Basel, Switzerland),* **15**(9): 6559-6569.

KUMAR, S. & PANDEY, A. K. 2013. Chemistry and biological activities of flavonoids: an overview. *Scientific World Journal,* **2013:**1-17, http://dx.doi.org/10.1155/2013/162750.

KURUTAS, E. B. 2016. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal,* **15**(1):71-71.

LEE, D. 2005. Plant pigments and their manipulation. Annual Plant Reviews. Davies KM, ed. Oxford/Boca Raton: Blackwell Publishing/CRC Press, Boca Raton. £110 (hardback). *Annals of Botany,* **96**(12): 1332-1333.

LOBO, V., PATIL, A., PHATAK, A. & CHANDRA, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews,* **4**(8):118-126.

LU, J.-J., BAO, J.-L., WU, G.-S., XU, W.-S., HUANG, M.-Q., CHEN, X.-P. & WANG, Y.-T. 2013. Quinones Derived from Plant Secondary Metabolites as Anti-cancer Agents. *Anti-Cancer Agents in Medicinal Chemistry,* **13**(3): 456-463.

MASHOUR, N. H., LIN, G. I. & FRISHMAN, W. H. 1998. Herbal Medicine for the Treatment of Cardiovascular Disease: Clinical Considerations. *Archives of Internal Medicine,* **158**(20): 2225-2234.

MGBEAHURUIKE, E. E., YRJÖNEN, T., VUORELA, H. & HOLM, Y. 2017. Bioactive compounds from medicinal plants: Focus on Piper species. *South African Journal of Botany,* **112**: 54-69.

MIGUEL, M. G. 2018. Betalains in Some Species of the Amaranthaceae Family: A Review. *Antioxidants (Basel, Switzerland),* **7**(4):1-33.

MLADENKA, P., APPLOVA, L., PATOCKA, J., COSTA, V. M., REMIAO, F., POUROVA, J., MLADENKA, A., KARLICKOVA, J., JAHODAR, L., VOPRSALOVA, M., VARNER, K. J., STERBA, M., TOX, O. E. R., RESEARCHERS, C. H. K. & COLLABORATORS 2018. A comprehensive review of cardiovascular toxicity of drugs and related agents. *Med Res Rev,* **38**(4):1332-1403.

MUSA, M. A., COOPERWOOD, J. S. & KHAN, M. O. F. 2008. A review of coumarin derivatives in the pharmacotherapy of breast cancer. *Current medicinal chemistry,* **15**(26):2664-2679.

OMEDE, A., SULEIMAN, M. S., ATANU, F. O., SHENENI, V. D. & JEGEDE, E. R. 2018. Evaluation of Antioxidant and Cytotoxic Properties of Vernonia Amygdalina. *International Journal of Plant Studies,* **1**(1):1-6.

OYEDEMI, S. O., OYEDEMI, B. O., IJEH, II, OHANYEREM, P. E., COOPOOSAMY, R. M. & AIYEGORO, O. A. 2017. Alpha-Amylase Inhibition and Antioxidative Capacity of Some Antidiabetic Plants Used by the Traditional Healers in Southeastern Nigeria. *Scientific World Journal,* **2017:** 1-11**,** https://doi.org/10.1155/2017/3592491.

PANCHE, A. N., DIWAN, A. D. & CHANDRA, S. R. 2016. Flavonoids: an overview. *Journal of nutritional science,* **5(**47):1-14.

PHANIENDRA, A., JESTADI, D. B. & PERIYASAMY, L. 2015. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian journal of clinical biochemistry: IJCB,* **30**(1):11-26.

PICHERSKY, E. & RAGUSO, R. A. 2018. Why do plants produce so many terpenoid compounds? *New Phytol,* **220**(3): 692-702.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. & RICE-EVANS, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radical Biology and Medicine,* **26**(9-10):1231-1237.

RICE-EVANS, C. A., MILLER, N. J., BOLWELL, P. G., BRAMLEY, P. M. & PRIDHAM, J. B. 1995. The Relative Antioxidant Activities of Plant-Derived Polyphenolic Flavonoids. *Free Radical Research,* **22**(4): 375-383.

ROA, N. 2003. Bioactive phytochemicals in Indian foods and their potential in health promotion and

disease prevention. *Asia Pacific Journal of Clinical Nutrition,* **12**(1): 9-22.

SAMIDHA, K., VRUSHALI, K. & VIJAYA, P. 2014. Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha-amylase Inhibitory Activity of Marketed Polyherbal Formulation. *Journal of Applied Pharmaceutical Science,* **4**(9):061-065.

SAXENA, M., SAXENA, J., NEMA, R., SINGH, D. & GUPTA, A. 2013. Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry,* **1**(6):168-182.

SAYHAN, H., BEYAZ, S. L. G. & ÇELIKTAŞ, A. 2017. The Local Anesthetic and Pain Relief Activity of Alkaloids. *Alkaloids - Alternatives in Synthesis, Modification and Application,* ***2017***:1-11, 10.5772/intechopen.69847.

SHAHIDI, F. & YEO, J. 2018. Bioactivities of Phenolics by Focusing on Suppression of Chronic Diseases: A Review. *International journal of molecular sciences,* **19**(6):1-16.

SHOAIB, M., SHAH, S. W., ALI, N., SHAH, I., ULLAH, S., GHIAS, M., TAHIR, M. N., GUL, F., AKHTAR, S., ULLAH, A., AKBAR, W. & ULLAH, A. 2016. Scientific investigation of crude alkaloids from medicinal plants for the management of pain. *BMC Complement Altern Med,* **16**(178):1-8.

STEIN, O. & GRANOT, D. 2019. An Overview of Sucrose Synthases in Plants. *Frontiers in Plant Science,* **10**(95):1-14.

STREET, R. A. & PRINSLOO, G. 2013. Commercially Important Medicinal Plants of South Africa: A Review. *Journal of Chemistry,* **2013:** 1-16.

TAN, B. L., NORHAIZAN, M. E., LIEW, W.-P.-P. & SULAIMAN RAHMAN, H. 2018. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. *Frontiers in Pharmacology,* **9**(1162):1-28.

THACHER, E. A. 1946. Chronic Nasal Obstruction: Its Causes and Treatment. *Journal of the American Medical Association,* **131**: 1039-1045.

VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M. T., MAZUR, M. & TELSER, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology,* **39**(1): 44-84.

WAGNER, H. 2000. Bioactive saponins from plants: An update. *Studies in Natural Products Chemistry,* **21**: 1-10, https://www.researchgate.net/journal/1572-5995_Studies_in_Natural_Products_Chemistry.

WAR, A. R., PAULRAJ, M. G., AHMAD, T., BUHROO, A. A., HUSSAIN, B., IGNACIMUTHU, S. & SHARMA, H. C. 2012. Mechanisms of plant defence against insect herbivores. *Plant*

signalling & behaviour, **7**(10):1306-1320.

WEEKS, A. 2015. The prevention and treatment of postpartum haemorrhage: what do we know, and where do we go to next? *BJOG,* **122**(2):202-210.

WINK, M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry,* **64**(1):3-19.

WISETKOMOLMAT, J., SUPPAKITTPAISARN, P. & SOMMANO, R. S. 2019. Detergent Plants of Northern Thailand: Potential Sources of Natural Saponins. *Resources,* **8**(10):1-14.

XU, D.-P., LI, Y., MENG, X., ZHOU, T., ZHOU, Y., ZHENG, J., ZHANG, J.-J. & LI, H.-B. 2017. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *International journal of molecular sciences,* **18**(1):1-32.

YAMANE, H., KONNO, K., SABELIS, M., TAKABAYASHI, J., SASSA, T. & OIKAWA, H. 2010. Chemical Defence and Toxins of Plants. *Journal of Hospital Infection - J HOSP INFECT,* **4**:339-385.

YUSUF, M., SHABBIR, M. & MOHAMMAD, F. 2017. Natural Colorants: Historical, Processing and Sustainable Prospects. *Natural products and bioprospecting,* **7**(1):123-145.

ZHANG, Y. J., GAN, R. Y., LI, S., ZHOU, Y., LI, A. N., XU, P. D. & LI, H. B. 2015. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules,* **20**(12):21138-21156.

ZUBELDIA-BRENNER, L., ROSELLI, C. E., RECABARREN, S. E., GONZALEZ DENISELLE, M. C. & LARA, H. E. 2016. Developmental and Functional Effects of Steroid Hormones on the Neuroendocrine Axis and Spinal Cord. *Journal of neuroendocrinology,* **28**(7):1-28,10.1111/jne.12401.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

People from the Southern African region have used fauna and flora in their homes for millennia to treat all sorts of ailments and complaints with great success. This knowledge transfer was done through 'apprenticeships' and oral communication. However, the challenge remains that these medicines have not been tested through comprehensive evidence-based scientific or clinical studies. There is a fallacious view that herbal medicines are harmless and free of side effects because they are 'natural'. In recent times, there have been several cases of hepatic injury and even death associated with their use (Popata et al., 2002). Administration of the traditional Zulu remedy impila (*Callilepis laureola*) in high doses results in severe or fatal hepatotoxicity and, in some cases, nephrotoxicity (Popat et al., 2001). Patients poisoned with impila characteristically show severe hypoglycaemia as a precursor to catastrophic hepatocellular necrosis (Seedat & Hitchock, 1971; Frenzel & Teschke, 2016; Bye & Dutton, 1991). Therefore, the aim of this study was to undertake an investigation into cytotoxicity effects, antimicrobial, anti-inflammatory, antioxidant, and alpha-amylase properties of eight selected South African plants for medicinal purposes. There were nine plants extracts of the selected plants.

The research findings of this study in terms of its research objectives are summarised in this chapter. The conclusions are based on the results which were presented in published papers in Chapters three to five. Recommendations are based on the findings. Conclusions are provided. Lastly, the limitations of this research and suggestions for further research are offered.

6.2 A summary of the objectives

The aim of this study was to undertake an investigation into cytotoxicity effects, antimicrobial, anti-inflammatory, antioxidant, and alpha-amylase properties of eight selected South African plants for medicinal purposes.

The plant species that demonstrated one of the following properties: antidiabetic, antioxidants, and anti-inflammatory in order to be useful in the treatment and management of diabetes mellitus (DM) and other cardiovascular diseases.

The objectives of the study were as follows.

(i) To evaluate eight selected South African plant extracts for minimum inhibitory concentration and minimum bactericidal concertation against selected four bacterial strains.

(ii) To evaluate the cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 cells and RAW 264.7 cells.

(iii)To estimate the preliminary screening of phytochemicals, antioxidants, and alpha-amylase inhibitory activities of the selected South African plant extracts for medicinal purposes.

6.3. Conclusions drawn from the research findings

The eight selected South African plant species demonstrated some degree of antimicrobial activities, antioxidant, anti-inflammatory, and α-amylase inhibition effects. In addition to this, cytotoxicity of medicinal plants against C2C12 cells and RAW 264.7 cells was validated. The results suggest that Schkuhria pinnata; Lippia javanica; Clerodendrum myricoides; and Erythrina lysistemon exhibited good antimicrobial, anti-inflammatory, and antioxidant properties amongst the studies of the plant species. Therefore, the presence of phenolic, flavonoids, antioxidants, and α-amylase properties are potential solutions towards the management of diabetes and other chronic inflammatory diseases. Furthermore, the selected plants may serve as a promising therapeutic agent for inflammatory diseases and this authenticates their use in traditional medicine. The conclusions drawn from this study are presented below.

6.3.1 Antimicrobial activities

Antimicrobial activity of the nine selected South African crude plant extracts (*Euclea crispa* (leaf), *Eulea natalensis* (leaf), *Schkuhria pinnata* (leaf), *Ziziphus mucronata* (leaf), *Ziziphus mucronata* (fruits), *Lippia javanica* (leaf), *Vernonia oligocephala* (leaf), *Clerodendrum myricoides* (leaf), and *Erythrina lysistemon* (leaf)), was investigated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*. The minimum inhibitory concentrations of the crude plant extracts were determined. Good inhibition and bactericidal effects against all four bacteria were shown by four plant extracts: *Lippia javanica* (leaf); *Ziziphus mucronata* (leaf); *Erythrina lysistemon;* and *Schkuhria pinnata*. Similarly, gentamycin (positive control) had inhibitory effects. However, none of the plant extracts showed bactericidal against *Pseudomonas aeruginosa*. The results of this study suggest that four plant species that exhibited inhibition effect and bactericidal effects does support their use in the traditional medical treatment of various ailments. Furthermore, these promising plants can be of great importance in the treatment of various ailments.

6.3.2. Cytotoxicity and anti-inflammatory effects

The cytotoxicity and anti-inflammatory effects of plant extracts were validated against C2C12 cells and RAW 264.7 cells. It was observed that none of the plants' extracts exhibited cytotoxic effects at the concentrations used against RAW 264.7 cells. However, a degree of cytotoxicity in all plant extracts against C2C12 cells at higher levels was observed. All plant extracts demonstrated some degree of anti-inflammatory effect. Plant extracts that exhibited marked anti-inflammatory activities were *Clerondendrum myricoides*; *Lippia javanica*; *Erythrina lysistemon*; *Schkuhria pinnata*; and *Vernonia oligocephala*. These indings suggest that these plants' extracts may serve as a promising therapeutic agent for inflammatory diseases and thus authenticate their use in traditional medicine. The plant species that exhibited a certain level of toxicity should be used with caution within ATM practice.

6.3.3 Phytochemicals screening, antioxidants, and alpha-amylase effects

The phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha (α)-amylase effects were evaluated for the selected South African plant extracts. All plant extracts exhibited flavonoids, phenolics, coumarins, and terpenoids. The other seven phytochemicals were not present in all of the plant extracts. Interestingly, all plant extracts showed total phenolic and total flavonoid content; however, total phenolic content was observed to be higher to *Erythrina lysistemon*; *Euclea crispa; Euclea natalensis;* and *Ziziphus mucronata*. The highest total flavonoid was seen for *Vernonia oligocephala*; *Schkuhria pinnata*; and *Clerodendrum myricoides*. The antioxidant and α-amylase inhibition was observed in four plant species, namely: *Schkuhria pinnata*; *Lippia javanica*; *Clerodendrum myricoides*; and *Erythrina lysistemon*. These findings suggest that the presence of phenolic, flavonoids, antioxidants, and α-amylase properties are potential solutions towards the management of diabetes and other chronic inflammatory diseases.

6.4. Recommendations

Medicinal plants possess natural occurring bioactive compounds such as antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties (Gothai et al., 2016). South Africa is rich in plant diversity hence the use of plants in ATM since ancient times; however, a number of plant species lack authentication scientifically (Street & Prinsloo, 2013; Kamsu-Foguem & Foguem, 2014). This finding of this study demonstrated the following in terms of certain plant species:

(i) showed no cytotoxicity effects,
(ii) exhibited antimicrobial properties in all pathogenic organisms used,
(iii) anti-inflammatory properties were evident,
(iv) antioxidant activities were evident,
(v) the presence of phytochemicals, total phenolic, and total flavonoid properties, and
(vi) antidiabetic properties were also observed.

The results of this study substantiate the use of these plants within the ATM system. However, there is a need for recommendations based on the findings.

- Attention should be given to those plant extracts that demonstrated cytotoxicity effects against a cell line. It is recommended that they should be used with caution.
- Interestingly, plant species may show cytotoxicity effects, but on the other hand may exhibit anti-inflammatory effects. This could be due to synergistic interaction since medicinal plants are typically used for the treatment of more than one aliment (Kudumela *et al*., 2018; Naz *et al*., 2017; Sigidi *et al*., 2016). It is recommended that future studies should seek to identify the typical modes of action of these active extracts.
- It is recommended that the biologically active properties observed in these plant extracts should be further verified by *in vivo* studies.

References

BYE, S. N. & DUTTON, M. F. 1991. The inappropriate use of traditional medicines in South Africa. *Journal of Ethnopharmacology,* **34**(2-3): 253-259.

FRENZEL, C. & TESCHKE, R. 2016. Herbal h-epatotoxicity: Clinical characteristics and listing compilation. *International Journal of Molecular Sciences,* **17**(588):1-38.

GOTHAI, S., GANESAN, P., PARK, S.-Y., FAKURAZI, S., CHOI, D.-K. & ARULSELVAN, P. 2016. Natural phyto-bioactive compounds for the treatment of Type 2 diabetes: inflammation as a target. *Nutrients,* **8**(461): 1-28.

KAMSU-FOGUEM, B. & FOGUEM, C. 2014. Adverse drug reactions in some African herbal medicine: literature review and stakeholder's interview. *Integrative Medicine Research,* **3**(3): 126-132.

KUDUMELA, R. G., MCGAW, L. J. & MASOKO, P. 2018. Antibacterial interactions, anti-inflammatory and cytotoxic effects of four medicinal plant species. *BMC Complementary and Alternative Medicine,* **18**(199):1-7.

NAZ, R., AYUB, H., NAWAZ, S., ISLAM, Z. U., YASMIN, T., BANO, A., WAKEEL, A., ZIA, S. & ROBERTS, T. H. 2017. Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. *BMC Complementary and Alternative Medicine,* **17**(1):302-302.

POPAT, A., SHEAR, N., MALKIEWICZ, I., STEWART, M., STEENKAMP, V., THOMSON, S. & NEUMAN, M. 2001. The toxicity of Callilepis laureola, a South African traditional herbal medicine. *Clinical Biochemistry,* **34**(3): 229-36.

POPATA, A., SHEARA, N. H., I., M., THOMSONC, S. & NEUMANA, M. G. 2002. Mechanism of impila (Callilepis laureola)-induced cytotoxicity in Hep G2 cells. *Clinical Biochemistry,* **35**: 57–64.

SEEDAT, Y. K. & HITCHOCK, P. J. 1971. Acute renal failure from Callilepsis laureola. *South Africa Medical Journal,* **45**(30): 832 - 833.

SIGIDI, M. T., ANOKWURU, C. P., ZININGA, T., TSHISIKHAWE, M. P., SHONHAI, A., RAMAITE, I. D. I., TRAORÉ, A. N. & POTGIETER, N. 2016. Comparative *in vitro* cytotoxic, anti-inflammatory and anti-microbiological activities of two indigenous Venda medicinal plants. *Translational Medicine Communications,* **1**(9):1-7.

STREET, R. A. & PRINSLOO, G. 2013. Commercially important medicinal plants of South Africa: a review. *Journal of Chemistry,* **2013,** 1-16.

APPENDIX: TURNTIN REPORT

Chapter I and Chapter SIX

by Bongani Alphouse Nkala

Submission date: 11-Jan-2020 07:16AM (UTC+0200)

Submission ID: 1240840033
File name: Chapter_I_and_Chapter_Six_11.01.2020.pdf (527.33K)
Word count: 5335
Character count: 31563

CHAPTER ONE: INTRODUCTION

1.1. Introduction

The use of medicinal plants date back to ancient times for the treatment of various ailments (Petrovska, 2012, Yuan *et al*., 2016). Medicinal plants play an important role as a source of natural ingredients for preserving human health (Sofowora *et al*., 2013). Additionally, medicinal plants have been evaluated for various purposes such as the production of flavours, fertilizes and fragrances (Dar *et al*., 2017). Recently, researchers have been focusing on the evaluation of medicinal plants' ability to treat and manage various aliments (Anand *et al*., 2019, Street and Prinsloo, 2013). The search for plants medicinal properties has given way to some ethnobotanical studies that have documented indigenous plant species, the mode of preparation, and uses by endemic communities in some parts of Uganda (Tabuti *et al*., 2010, Tugume *et al*., 2016).

Although medical plants have been used for centuries, safety and efficacy were not reported in earlier literature (Mahomoodally, 2013, Petrovska, 2012, Ekor, 2014). The medical plant consists of secondary metabolites that attribute to therapeutic properties, such as antioxidant, anti-microbial, anti-diabetic, anti-inflammatory, anti-cancer, immune-modulatory and reno-protection effects (Londonkar *et al*., 2013, Tungmunnithum *et al*., 2018). Recently, more researchers have been investigating the therapeutic properties and phytochemical properties of medicinal plants (Wintola and Afolayan, 2015, Egamberdieva, 2016). Therefore, there is a need to evaluate plants with antidiabetic and anti-inflammatory activity for the treatment and management of diabetes mellitus and other cardiovascular diseases.

1.2. Background to the study

The medicinal plants are also referred to as medicinal herbs, they play a pivotal role in African traditional medicine (ATM) and has been used in ancient times. Plants possess secondary metabolites such as phytochemicals with potential or biological activity that are used for the treatment and management of diabetes mellitus and other cardiovascular diseases.

- **African traditional medicine**: is referred to as knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses.
- **Phytochemicals**: are chemicals that are derived from plants through primary or secondary metabolites.
- **Cardiovascular diseases**: are generally refers to conditions that involves narrowed or blocked blood vessels that can lead to a heart attack, chest pain (angina) or stroke.
- **Diabetes mellitus**: commonly known as diabetes, is a metabolic disorder characterised by a high blood sugar level over a prolonged period.

There are four types of diabetes:

- **Type 1 diabetes**: results from the pancreases' failure to produce enough insulin due to the loss of beta cells. It is referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes".
- **Type 2 diabetes**: begins with insulin resistance a condition in which cells fail to respond to insulin properly. This is referred to as "non-insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes".
- **Gestational diabetes**: is the third main form, and occurs when pregnant without a previous history of diabetes and develop high blood sugar levels.

1.3. Problem Statement

In the 34 years from 1980 to 2014, the global prevalence of diabetes mellitus (DM) jumped a staggering 314 million cases, from 108 million to 422 million according to World Health Organisation (WHO, 2016). DM is a metabolic disorder characterized by hyperglycaemia due to a defect in insulin production and/or resistance by the cells (Rajalakshmi et al., 2009). It was estimated that DM would increase from 4 percent in 1995 up to 5.4 percent in the year 2025 (Bears *et al*., 2004). In South Africa, the prevalence of DM in adults is on the rise too. In the short space of 9 years, DM prevalence increased from 5.5% in 2000 to 9% in the year 2009 (Bertram *et al*., 2013, Pheiffer *et al*., 2018).

On average, a diabetic patient pays R9,000 more for hospitalisation costs than non-diabetic patients in 2009 according to study which is R27,000 (2,250 USD) per hospitalisation compared to R18,000 (1,500 USD) for non-diabetic patients (Manyema et al., 2015, Ncube-Zulu and Danckwerts, 2004). This is the global context where the total health expenditure is projected to be between 1.1 to 2 billion USD in 2030 (Zhang *et al*., 2010). Poor management of DM can lead to neurological, cardiovascular, retinal and renal complications (Rahmatullah *et al*., 2012).

Plants used for medicinal properties in the fight against DM have shown to be able to alleviate one or more symptoms. A total cure for the disease still eludes the medical fraternity though (Rahmatullah *et al*., 2012). Medicinal plants have also shown to be effective in delaying complications of the diseases and rectifying metabolic abnormalities. The use of plants for medicinal purposes has the advantage of having few or no side effects (Shukia *et al*., 2000).

1.4. Aims

This study is aimed at an investigation into cytotoxicity effects, anti-inflammatory, antioxidant, antimicrobial and antidiabetic properties of the selected South African plants for medicinal purposes. Essentially, plant species that demonstrate one of the following properties namely: antidiabetic, antioxidants and anti-inflammatory will be useful towards the treatment and management of DM and other cardiovascular diseases.

1.5. Objectives

The objectives of the study were:

(i) To evaluate [redacted] inhibitory [redacted] concertation [redacted].

(ii) To evaluate the cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 cells and RAW 264.7 cells.

(iii) To estimate the preliminary screening of phytochemicals, phenolic content, flavonoid content, antioxidants [redacted]-amylase [redacted] the [redacted] South African [redacted] for medicinal purposes.

1.6. Research Questions

1. Does the selected plant species possess either bactericidal or bacteriostatic?
2. How many of the selected plant species possess anti-inflammatory effects?
3. Do the selected plant species possess any cytotoxicity effects against C2C12 cells and RAW 2647?
4. How many phytochemicals can be found in the selected plant species?
5. Which plant species consists of the highest [redacted]?
6. Does [redacted] selected plant species possess either antioxidant or alpha-amylase inhibition effects?

1.7. Significance of the study

African traditional medicine (ATM) has been used in ancient times for the treatment of various ailments (Atanasov *et al*., 2015). The ATM has been reported to be used by a lot of people in Africa as primary healthcare (Abdullahi, 2011). Despite the availability and affordability of ATM, communities strongly believe that ATM is much safer compared to conventional drugs (Sofowora *et al*., 2013, Deutschländer *et al*., 2009). However, very few medicinal plants have been validated for their safety and efficacy to substantiate their use in the ATM system (Mahomoodally, 2013). Recently, more researchers have developed an interest in the authentication of ATM, and this has been triggered by drug resistance that has been a growing problem with conventional drugs (Zhang *et al*., 2018, Mahomoodally, 2013, Mills *et al*., 2005). Essentially a search for alternative medicine is imperative. Medicinal plants have been shown

to contribute meaningfully in the pharmacology industry toward drug discovery (Atanasov *et al*., 2015, Leonti and Casu, 2013).

Developments have been focusing on drug discovery due to secondary metabolites found on medicinal plants that can be used for the treatment and management of malaria, cancer, diabetes, cardiovascular diseases and neurological affliction (Pan *et al*., 2013, Ginsburg and Deharo, 2011). Interestingly, the selected plant species have been reported for their use in ATM by traditional healers, however, safety and efficiency is lacking (Nkala *et al*., 2019). This study aimed to make a significant contribution toward the body of knowledge concerning the ability of the selected medicinal plants to possess the following properties: antioxidant, anti-inflammatory, antidiabetic and antimicrobial capabilities. Importantly, plant species must not be toxic to ensure their safety. The medicinal plant that possesses these bioactive serves as a good candidate for the treatment and management of diabetes and other cardiovascular diseases (Gothai *et al*., 2016, Choudhury *et al*., 2017).

1.8. Research Methodology

1.8.1. Sample

. The voucher specimen number is grounded a powder .

1.8.2 The determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The microplate dilution method was used to determine the ability of plant extracts to inhibit or kill pathogenic organisms with minimal inhibitory concentrations (MIC's) and minimal bactericidal concentration (MBC), which was developed by (Eloff, 1998).

1.8.3 The evaluation of the cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 cells and RAW 264.7 cells.

The cytotoxicity effect of plant extracts was assessed against C2C12 cells and RAW 2647 cells using Alamar Blue cell viability assay (Rampersad, 2012, Zachari *et al*., 2013), and crystal violet cell viability assay (Feoktisova *et al*., 2016, Śliwka *et al*., 2016). Furthermore, the anti-inflammatory effects were determined by the production nitric oxide (NO) was assessed using Griess assay (Promega), against RAW 264.7 cells which was previously described by (Lim *et al*., 2018).

1.8.4 The estimation of the preliminary screening of phytochemicals, phenolic content, flavonoid content, antioxidants -amylase the South African for medicinal purposes.

The phytochemical analysis was conducted with standard methods and the following phytochemicals were analysed: Tannins, Saponins, Flavonoids, Quinones, Phenols, Terpenoids, Alkaloids, Glycosides, Cardio glycosides, Coumarins, Betacynanin, Anthocyanin, and Steroids (Rajesh *et al*., 2014, Mujeeb *et al*., 2014). phenol determined which previously described by (Škerget *et al*., 2005). Essentially, the flavonoids total content was analysed with a method that was previously described by (Pranoothi *et al*., 2014). The antioxidant activity was analysed using DPPH radical-scavenging assay as previously described by (Blois, 1958). Furthermore, the DPPH results were confirmed with ABTS radical scavenging assay which was previously described by (Re *et al*., 1999). The

ability of medicinal plants to inhibit alpha-amylase was analysed as previously described by (Saha and Verma, 2012).

1.8.5 Data

[redacted] triplicates [redacted] were [redacted]. Linear regression analysis was used to calculate the IC_{50} values.

1.9. Limitations of the research

This study is aimed at an investigation into cytotoxicity, anti-inflammatory, antioxidant, antimicrobial and anti-diabetic properties of selected South African plants for medicinal purposes using *in vitro* testing.

1.10. Organisation of Research Dissertation

This dissertation will be organised as follows:

1.10.1. Chapter 1 – Introduction

This chapter introduces a laboratory study on the medicinal plants used to treat and manage diabetes and other cardiovascular diseases. In this introductory chapter, the rationale for this study is explained and an overview of the dissertation is provided. The Chapter starts by presenting the context within which this study was conducted as well as the researcher's background. It then proceeds to explain the rationale and objectives of the study. The theoretical background used in this study. Finally, an overview of how the study was conducted is provided.

1.10.2. Chapter 2 – Literature Review (published paper)

This chapter presents a review article titled "A review on selected African medicinal plants with effective in the management of type II diabetes mellitus." This manuscript was published by *ACTA Scientific Pharmaceutical Sciences*, **3**(8), 2019.

1.10.3. Chapter 3 – Objective 1 (published)

This chapter presents the first objective, and a manuscript was published titled "The *in vitro* evaluation of some South African plant extracts for minimum inhibitory concentration and minimum bactericidal concentration against selected bacterial strains." This manuscript was published by *the* [redacted](7), 2019.

1.10.4. Chapter 4 – Objective 2 (accepted for publication)

This chapter presents findings from the second objective, and a manuscript has been accepted for publication in January of 2020. A manuscript is titled "The evaluations of cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 cells and RAW 264.7 cells". The manuscript was accepted by *the International Journal of Scientific and Research Publications*, 2020.

1.10.5. Chapter 5 – Objective 3 (in press)

This chapter presents the third and final objective of this study, and a manuscript has been submitted to a journal for consideration. This chapter focuses on the estimation of the preliminary screening of phytochemicals, phenolic content, flavonoid content, antioxidants [redacted]-amylase [redacted] the [redacted] South African [redacted] for medicinal purpose.

1.10.6 Chapter 5 – Conclusion and recommendations

In this chapter the conclusions derived from the findings of this study on the use of traditional medicine for the treatment and management of diabetes and other cardiovascular diseases. The conclusions were based on the plant biological activities analysed in this study. The implications of these findings and the resultant recommendations will also be explained.

1.11. Summary

South Africa is home to thousands of plant species, and each species have unique and beneficial bioactive compounds (Street and Prinsloo, 2013). The use of medicinal plants in the African traditional medicine (ATM) practice is due affordability as compared to conventional drugs and most importantly, it has been reported to have lesser side effects (Mahomoodally, 2013). Furthermore, it is imperative to validate medicinal plant safety and efficiency as to contribute with data on their safety for human uses within the ATM (Ngcobo *et al*., 2011).

The present study highlights *in vitro* biological activity determined from the selected medicinal plants and the following activities were validated namely: antimicrobial, phytochemicals, total phenolic content, total flavonoid content, antioxidant, and alpha-amylases. In addition to this, the cytotoxicity of medical plants was validated together with anti-inflammatory capabilities. Interesting, plant species exhibited good antioxidant, anti-inflammatory and anti-diabetic properties are the potential to be used in the treatment and management of diabetes. Chapter two covers a review article published on *ACTA Scientific Pharmaceutical Sciences* and the manuscript focus on selected South African plants with effectiveness in the management of Type II Diabetes Mellitus.

References

ABDULLAHI, A. A. 2011. Trends and challenges of traditional medicine in Africa. *African journal of traditional, complementary, and alternative medicines: AJTCAM,* 8**,** 115-123.

ANAND, U., JACOBO-HERRERA, N., ALTEMIMI, A. & LAKHSSASSI, N. 2019. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites,* 9.

ATANASOV, A. G., WALTENBERGER, B., PFERSCHY-WENZIG, E.-M., LINDER, T., WAWROSCH, C., UHRIN, P., TEMML, V., WANG, L., SCHWAIGER, S., HEISS, E. H., ROLLINGER, J. M., SCHUSTER, D., BREUSS, J. M., BOCHKOV, V., MIHOVILOVIC, M. D., KOPP, B., BAUER, R., DIRSCH, V. M. & STUPPNER, H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology advances,* 33**,** 1582-1614.

BEARS, M. A., HAN, T., SCHNECK, M. E., BAREX, S., JACOBEN, C. & ADAMS, A. J. 2004. Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy. *Investigative Ophthanology and Visual Science* 45, 3259-3265.

BERTRAM, M. Y., JASWAL, A. V., VAN WYK, V. P., VEVITT, N. S. & HOFMAN, K. J. 2013. The non-fetal disease burden caused by type 2 diabetes in South Africa, 2009. *Global Health Action 6,* 6, 202-212.

BLOIS, M. S. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature,* 181, 1199-1200.

CHOUDHURY, H., PANDEY, M., HUA, C. K., MUN, C. S., JING, J. K., KONG, L., ERN, L. Y., ASHRAF, N. A., KIT, S. W., YEE, T. S., PICHIKA, M. R., GORAIN, B. & KESHARWANI, P. 2017. Update on natural compounds in the remedy of diabetes mellitus: A systematic review. *Journal of traditional and complementary medicine,* 8, 361-376.

DAR, R. A., SHAHNAWAZ, M. & QAZI, P. H. 2017. A general overview of medicinal plants: A review. *The Journal of Phytopharmacology* 6, 349-351.

DEUTSCHLÄNDER, M. S., LALL, N. & VAN DE VENTER, M. 2009. Plant species used in the treatment of diabetes by South African traditional healers: An inventory. *Pharmaceutical Biology,* 47, 348-365.

EGAMBERDIEVA, D. 2016. Phytochemical and Pharmacological Properties of Medicinal Plants from Uzbekistan: A Review. *Journal of Medicinally Active Plants* 5, 59-75.

EKOR, M. 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology,* 4, 177-177.

1 [redacted] .

FEOKTISOVA, [redacted]., GESERICK, P. & LEVERKUS, M. 2016. *Crystal violet assay for cultured cells*, Cold Spring Protocols.

GINSBURG, H. & DEHARO, E. 2011. A call for using natural compounds in the development of new antimalarial treatments - an introduction. *Malaria journal,* 10 Suppl 1, S1-S1.

2 [redacted] , 461.

LEONTI, M. & CASU, L. 2013. Traditional medicines and globalization: current and future perspectives in ethnopharmacology. *Frontiers in pharmacology,* 4, 92-92.

LIM, Y., PARK, J. W., KWON, O. K., LEE, J. W., LEE, H. S., LEE, S., CHOI, S., LI, W., JIN, H., HAN, S. B. & AHN, K. S. 2018. Anti-inflammatory effects of a methanolic extract

of Castanea seguinii Dode in LPS-induced RAW264.7 macrophage cells. *International Journal of Molecular Medicine* 41, 391-398.

LONDONKAR, R. L., MADIRE KATTEGOUGA, U., SHIVSHARANAPPA, K. & HANCHINALMATH, J. V. 2013. Phytochemical screening and in vitro antimicrobial activity of Typha angustifolia Linn leaves extract against pathogenic gram-negative microorganisms. *Journal of Pharmacy Research,* 6, 280-283.

MAHOMOODALLY, M. F. 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evid Based Complement Alternat Med,* 2013, 617459.

MANYEMA, M., VEERMAN, J. L., CHOLA, L., TUGENDHAFT, A., LABADARIOS, D. & HOFMAN, K. 2015. Decreasing the Burden of Type 2 Diabetes in South Africa: The Impact of Taxing Sugar-Sweetened Beverages. *PloS one,* 10, e0143050-e0143050.

MILLS, E., COOPER, C., SEELY, D. & KANFER, I. 2005. African herbal medicines in the treatment of HIV: Hypoxis and Sutherlandia. An overview of evidence and pharmacology. *Nutrition Journal,* 4, 19-19.

MUJEEB, F., BAJPAI, P. & PATHAK, N. 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *BioMed research international,* 2014, 497606-497606.

NCUBE-ZULU, T. & DANCKWERTS, M. P. 2004. Comparative hospitalization cost and length of stay between patients with and without diabetes in a large tertiary hospital in Johannesburg, South Africa. *International Journal of Diabetes in Developing Countries* 34, 156-162.

NGCOBO, M., NKALA, B., MOODLEY, I. & GQALENI, N. 2011. Recommendations for the development of regulatory guidelines for registration of traditional medicines in South Africa. *African journal of traditional, complementary, and alternative medicines: AJTCAM,* 9, 59-66.

NKALA, B. A., MBONGWA, H. P. & QWEBANI-OGUNLEYE, T. 2019. [redacted] Selected African [redacted] Effectiveness in the Management of Type II Diabetes Mellitus. *Acta Scientific Pharmaceutical Sciences,* 3, 47-54.

PAN, S.-Y., ZHOU, S.-F., GAO, S.-H., YU, Z.-L., ZHANG, S.-F., TANG, M.-K., SUN, J.-N., MA, D.-L., HAN, Y.-F., FONG, W.-F. & KO, K.-M. 2013. New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. *Evidence-based complementary and alternative medicine : eCAM,* 2013, 627375-627375.

PETROVSKA, B. B. 2012. Historical review of medicinal plants' usage. *Pharmacognosy reviews,* 6, 1-5.

PHEIFFER, C., PILLAY-VAN WYK, V., JOUBERT, J. D., LEVITT, N., NGLAZI, M. D. & BRADSHAW, D. 2018. The prevalence of type 2 diabetes in South Africa: a systematic review protocol. *BMJ open,* 8**,** e021029-e021029.

PRANOOTHI, E. K., NARENDRA, K., JOSHI, D. S. D. S., SWATHI, J., SOWJANYA, K. M., RATHNAKARREDDI, K. V. N., EMMUEL, S., PADMAVATHI, C. H. & SATYA, A. K. 2014. Studies on qualitative, quantitative, phytochemical analysis and screening of in vitro biological activities of Leuca indica (L) VAR. Nagalapuramiana. *International Journal of Herbal Medicine* 2**,** 30-36.

RAHMATULLAH, M., AZAM, M. N. K., KHATUN, Z., SERAJ, S., ISLAM, F., RAHMAN, M. A., JAHAN, S. & AZIZ, M. S. 2012. Medicinal plants used for the treatment of diabetes by the Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh. *African journal of traditional, complementary, and alternative medicines: AJTCAM,* 9**,** 380-385.

RAJALAKSHMI, M., ELIZA, J., PRIYA, C. E., NIRMALA, A. & DAISY, P. 2009. Antidiabetic properties of Tinospora cordifolia stem extracts on streptozotocin-induced diabetic rats. *African Journal of Pharmacy and Pharmacology,* 3**,** 171-180.

RAJESH, K. D., VASANTHA, S., RAJESH, N. V. & PANNEERSELVAM, A. 2014. Qualitative and quantitative phytochemical analysis in four pteridophytes. *International Journal of Pharmaceutical Sciences Review and Research,* 27**,** 408-412.

RAMPERSAD, S. N. 2012. Multiple applications of Alamar Blue as an indicator of metabolic function and cellular health in cell viability bioassays. *Sensors (Basel, Switzerland),* 12**,** 12347-12360.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. & RICE-EVANS, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radical Biology & Medicine* 26**,** 1231-1237.

SAHA, S. & VERMA, R. 2012. Inhibitory potential of traditional herbs on alpha-amylase activity. *Pharm Biol,* 50**,** 326-31.

SHUKIA, R., SHARMA, S. B., PURI, D., PRABHU, K. M. & MURTHY, P. S. 2000. Medicinal plants for the treatment of diabetes mellitus. *Indian journal of clinical biochemistry: IJCB,* 15**,** 169-177.

ŠKERGET, M., KOTNIK, P., HADOLIN, M., HRAŠ, A., R., SIMONIČ, M. & KNEZ, Z. 2005. Phenols, proanthocyanidins, flavones, and flavonols in some plant materials and their antioxidant activities. *Food Chemistry* 89**,** 191-198.

ŚLIWKA, L., WIKTORSKA, K., SUCHOCKI, P., MILCZAREK, M., MIELCZAREK, S., LUBELSKA, K., CIERPIAŁ, T., ŁYŻWA, P., KIEŁBASIŃSKI, P., JAROMIN, A.,

FLIS, A. & CHILMONCZYK, Z. 2016. The Comparison of MTT and CVS Assays for the Assessment of Anticancer Agent Interactions. *PLOS ONE,* 11, e0155772.

SOFOWORA, A., OGUNBODEDE, E. & ONAYADE, A. 2013. The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary, and alternative medicines: AJTCAM,* 10, 210-229.

STREET, R. A. & PRINSLOO, G. 2013. Commercially Important Medicinal Plants of South Africa: A Review. *Journal of Chemistry,* 2013, 1-16.

TABUTI, J. R., KUKUNDA, C. B. & WAAKO, P. J. 2010. Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda. *J Ethnopharmacol,* 127, 130-6.

TUGUME, P., KAKUDIDI, E. K., BUYINZA, M., NAMAALWA, J., KAMATENESI, M., MUCUNGUZI, P. & KALEMA, J. 2016. Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda. *Journal of ethnobiology and ethnomedicine,* 12, 5-5.

2 [redacted] *(Basel),* 5.

WHO 2016. *Global report on diabetes.* , Geneva, Switzerland, WHO Press.

WINTOLA, O. A. & AFOLAYAN, A. J. 2015. The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of Hydnora africanaThunb. used as antidysenteric in Eastern Cape Province, South Africa. *BMC Complement Altern Med,* 15, 307.

YUAN, H., MA, Q., YE, L. & PIAO, G. 2016. The Traditional Medicine and Modern Medicine from Natural Products. *Molecules,* 21.

ZACHARI, M. A., CHONDROU, P. S., POULILIOU, S. E., MITRAKAS, A. G., ABATZOGLOU, I., ZOIS, C. E. & KOUKOURAKIS, M. I. 2013. Evaluation of the Alamar blue assay for adherent cell irradiation experiments. *Dose-response: a publication of International Hormesis Society,* 12, 246-258.

ZHANG, P., ZHANG, X., BROWN, J., VISTISEN, D., SICREE, R., SHAW, J. & NICHOLS, G. 2010. Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes Res Clin Pract,* 87, 293-301.

ZHANG, Y., XU, G., ZHANG, S., WANG, D., SARAVANA PRABHA, P. & ZUO, Z. 2018. Antitumor Research on Artemisinin and Its Bioactive Derivatives. *Natural Products and Bioprospecting,* 8, 303-319.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

5.1. Introduction

People from the Southern African region have been using the fauna and flora of the region in their homes for millennia to treat all sorts of ailments and complaints with great success. This knowledge transfer was done through "apprenticeships" and oral communication. However, the challenge remains that these medicines have not been tested through comprehensive evidence based scientific or clinical study. There is a fallacious view that herbal medicines are harmless and free of side effects because they are "natural". In recent times, there have been several cases of hepatic injury and even death associated with their use (Popata *et al*., 2002). Administration of the traditional Zulu remedy impila (*Callilepis laureola*) in high doses results in severe or fatal hepatotoxicity and, in some cases, nephrotoxicity (Popat *et al*., 2001). Patients poisoned with impila characteristically show severe hypoglycaemia as a precursor to catastrophic hepatocellular necrosis (Seedat and Hitchock, 1971, Frenzel and Teschke, 2016, Bye and Dutton, 1991). Therefore, this study aimed an investigation into cytotoxicity, antimicrobial, anti-inflammatory, antioxidant, and alpha-amylase properties of selected South African plants for medicinal purposes.

This chapter summarises the research findings of this study concerning the research objectives. The conclusions are based on the results which are discussed in Chapters 3 to 5 of this study. The recommendations, centred on the findings and conclusions are provided. Lastly, the limitations of this research and suggestions for further research are offered.

5.2. A summary of the objectives

This study is aimed at an investigation into anti-inflammatory, antioxidant, antimicrobial, phytochemicals, and anti-diabetic properties of selected South African plants for medicinal purposes. Necessarily, plant species that demonstrate one of the following properties, namely: antidiabetic, antioxidants, and anti-inflammatory, will be useful towards the treatment and management of DM and other cardiovascular diseases.

The objectives of the study were:

(iv) To evaluate [redacted] inhibitory [redacted] concertation [redacted].

(v) To evaluate the cytotoxicity and anti-inflammatory effects of selected South African medicinal plants against C2C12 cells and RAW 264.7 cells.

(vi) To estimate the preliminary screening of phytochemicals, phenolic content, flavonoid content, antioxidants, [redacted]-amylase [redacted] the [redacted] South African [redacted] for medicinal purposes.

5.3. Conclusions are drawn from research findings

The selected South African plant species have demonstrated some degree of antimicrobial activities, antioxidant, anti-inflammatory, and α-amylase inhibition effects. In addition to this, cytotoxicity of medicinal plants against C2C12 cells and RAW 264.7 cells were validated. The results of this study suggest that *Schkuhria pinnata>Lippia javanica>Clerodendrum myricoides>Erythrina lysistemon* exhibited good antimicrobial, anti-inflammatory, and antioxidant properties amongst the nine studies plant species.

Therefore, the presence of phenolic, flavonoids, antioxidants, and α-amylase properties are potential solutions towards the management of diabetes and other chronic inflammatory diseases. Furthermore, the selected plant may serve as a promising therapeutic agent for inflammatory diseases and authenticates their use in traditional medicine. Below are the conclusions that could be drawn from this study:

5.3.1. Antimicrobial activities

[redacted] the [redacted] selected [redacted] (*Euclea crispa* (leaf), *Eulea natalensis* (leaf), *Schkuhria pinnata* (leaf), *Ziziphus mucronata* (leaf), *Ziziphus mucronata* (fruits), *Lippia javanica* (leaf), *Vernonia oligocephala* (leaf), *Clerodendrum myricoides* (leaf), and *Erythrina lysistemon* (leaf)), was investigated [redacted] and bactericidal effects [redacted] (leaf)> [redacted], gentamycin ([redacted]. However, [redacted] extracts [redacted] *Pseudomonas* [redacted] suggest [redacted] plant species [redacted] exhibited [redacted] use [redacted] medical [redacted] ailments.

5.3.2. Cytotoxicity and anti-inflammatory effects

The cytotoxicity and anti-inflammatory effects of plant extracts were validated against C2C12 cells and RAW 264.7 cells. It was observed that none of the plants' extracts exhibited cytotoxic effects at the concentrations used against RAW 264.7 cells. However, a degree of cytotoxicity in all plant extracts against C2C12 cells at higher levels was observed. All plant extracts demonstrated some degree of anti-inflammatory effect. However, plant extracts exhibited marked anti-inflammatory activities were *Clerondendrum myricoides*> *Lippia javanica*> *Erythrina lysistemon*> *Schkuhria pinnata*>*Vernonia oligocephala*. The present findings suggest that these plants' extracts may serve as a promising therapeutic agent for inflammatory diseases and authenticates their use in traditional medicine.

5.3.3 Phytochemicals screening, antioxidants, and alpha-amylase effects

The phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha (α)-amylase effects were evaluated for the selected South African plant extracts. All plant extracts exhibited flavonoids, phenolics, coumarins, and terpenoids. The other seven phytochemicals were present, not in all plant extracts. Interestingly, all plant extracts showed total phenolic and total flavonoid content; however, total phenolic content was observed to be higher to *Erythrina lysistemon*> *Euclea crispa* > *Euclea natalensis* >*Ziziphus mucronata*. The highest total flavonoid was seen for *Vernonia oligocephala*>*Schkuhria pinnata*>*Clerodendrum myricoides*. The antioxidant and α-amylase inhibition was observed in four plant species, namely:

Schkuhria pinnata>*Lippia javanica*>*Clerodendrum myricoides*>*Erythrina lysistemon*. These findings suggest that the presence of phenolic, flavonoids, antioxidants, and α-amylase properties are potential solutions towards the management of diabetes and other chronic inflammatory diseases.

5.4. Future Recommendations

The medicinal plant possesses natural occurring bioactive compounds such as antimicrobial, antioxidant, anti-inflammatory, and anti-diabetic properties (Gothai *et al*., 2016). South Africa is rich in plant diversity, and the number of these plants species lack authentication scientifically, however, they have been used in ATM since the ancient times (Street and Prinsloo, 2013, Kamsu-Foguem and Foguem, 2014). This study demonstrated that certain plant species:

1. showed no cytotoxicity effects,
2. exhibited antimicrobial properties in all pathogenic organisms used,
3. anti-inflammatory properties,
4. antioxidant activities,
5. the presence of phytochemicals, total phenolic, and total flavonoid properties, and
6. antidiabetic properties were also observed.

The results of this study substantiate the use of these plants within the ATM system. However, attention should be given to those plant extracts that demonstrated cytotoxicity effects against a cell line, and they should be used with caution. Interestingly, plant species may show cytotoxicity effects, and on the other hand, it may exhibit anti-inflammatory effects. This could be due to synergistic interaction since medicinal plants are typically used for the treatment of more than one aliments (Kudumela *et al.*, 2018, Naz *et al*., 2017, Sigidi *et al*., 2016).

Future studies should seek to identify the typical modes of action of these active extracts. Furthermore, the biologically active properties observed in this plant extracts should be further verified by *in vivo* studies.

References

BYE, S. N. & DUTTON, M. F. 1991. The inappropriate use of traditional medicines in South Africa. *Journal of Ethnopharmacology,* 34**,** 253-259.

FRENZEL, C. & TESCHKE, R. 2016. Herbal Hepatotoxicity: Clinical Characteristics and Listing Compilation. [redacted], 17**,** 588.

[redacted], 461.

KAMSU-FOGUEM, B. & FOGUEM, C. 2014. Adverse drug reactions in some African herbal medicine: literature review and stakeholder's interview. *Integrative Medicine Research,* 3**,** 126-132.

KUDUMELA, R. G., MCGAW, L. J. & MASOKO, P. 2018. Antibacterial interactions, anti-inflammatory and cytotoxic effects of four medicinal plant species. *BMC complementary and alternative medicine,* 18**,** 199-199.

NAZ, R., AYUB, H., NAWAZ, S., ISLAM, Z. U., YASMIN, T., BANO, A., WAKEEL, A., ZIA, S. & ROBERTS, T. H. 2017. Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. *BMC complementary and alternative medicine,* 17**,** 302-302.

POPAT, A., SHEAR, N., MALKIEWICZ, I., STEWART, M., STEENKAMP, V., THOMSON, S. & NEUMAN, M. 2001. The toxicity of Callilepis laureola, a South African traditional herbal medicine. *Clinical biochemistry,* 34**,** 229-36.

POPATA, A., SHEARA, N. H., I., M., THOMSONC, S. & NEUMANA, M. G. 2002. Mechanism of Impila (Callilepis laureola)-induced cytotoxicity in Hep G2 cells. *Clinical Biochemistry* 35**,** 57–64.

SEEDAT, Y. K. & HITCHOCK, P. J. 1971. Acute Renal Failure from Callilepsis laureola. *South Africa Medical Journal,* 45**,** 832 - 833.

SIGIDI, M. T., ANOKWURU, C. P., ZININGA, T., TSHISIKHAWE, M. P., SHONHAI, A., RAMAITE, I. D. I., TRAORÉ, A. N. & POTGIETER, N. 2016. Comparative in vitro cytotoxic, anti-inflammatory and anti-microbiological activities of two indigenous Venda medicinal plants. *Translational Medicine Communications,* 1**,** 9.

STREET, R. A. & PRINSLOO, G. 2013. Commercially Important Medicinal Plants of South Africa: A Review. *Journal of Chemistry,* 2013**,** 1-16.

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