Inhibition of biofilm formation of foodborne pathogens by selected South African medicinal plants

Chinelo Rosemary Erhabor

18200894

Submitted in fulfilment of the requirements for the degree

Magister Scientiae: Veterinary Science

Supervisor: Prof Lyndy J. McGaw

Phytomedicine Programme

Department of Paraclinical Sciences

Faculty of Veterinary Sciences

University of Pretoria

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

August 2019

Declaration

1. I understand what plagiarism is and am aware of the University's policy in this regard.
2. I declare that this dissertation is my own original work. Where other people's work has been used (either from a printed source, Internet or any other source), this has been properly acknowledged and referenced in accordance with departmental requirements.
3. I have not used work previously produced by another student or any other person to hand in as my own.
4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

SIGNATURE

....

Acknowledgements

I would love to express my profound gratitude to Prof Lyndy J. McGaw my amicable supervisor and mentor, for all she did to assist me in accomplishing this journey of mine. I admire your kind words, your strength and your beautiful smiles that encourages one. May the Almighty God give you the strength to keep up this good work you are doing. I also extend my appreciation to Prof J.N. Eloff for always listening and for all his assistance.

To my hero, Dr J.O Erhabor (my husband), I say a very big thank you for your optimism, support, love, assistance and for trusting in me. I thank Prof M. Aderogba for his time and assistance and to Sanah Nkadimeng I really love and appreciate you. A big thanks to every member and students of the Phytomedicine Family who in one way or the other assisted me along the course of my study.

Special thanks to my parents Mr. and Mrs. Sly Nwabue, my siblings for their love, prayers and understanding and to my lovely in-laws. Many thanks to my Winners Family, and other brothers and sisters too numerous to mention.

And I wouldn't forget the gift of love the Lord gave to me, my dear lovely, beautiful Princesses (Micah and Bernice), I love you girls.

And most of all, I thank the God Almighty for his strength, mercy, protection and all his grace. I am nothing without you Oh Lord.

And I wish to say, a Life without Christ is full of crisis. Jesus is Lord, Amen.

Publications

Published:

Erhabor, C.R., Erhabor, J.O., and McGaw, L.J. 2019. The potential of South African medicinal plants against microbial biofilm and quorum sensing of foodborne pathogens: A review. South African Journal of Botany 126, 214-231.

Manuscripts in Preparation:

Erhabor, C.R., Erhabor, J.O., Nkadimeng, S.M. and McGaw, L.J. *In vitro* antibiofilm, antioxidant and cytotoxicity activities of leaf extracts of six South African medicinal plants with potential against selected foodborne pathogens. Prepared for submission to Trends in Food Science and Technology.

Erhabor, J.O., **Erhabor, C.R**. and McGaw, L.J. Evaluation of the antibacterial activity and antibiofilm formation of foodborne pathogens of three *Combretum* species. Prepared for submission to South African Journal of Botany.

Erhabor, C.R., Aderogba, M.A., Erhabor, J.O., Nkadimeng, S.M. and McGaw, L.J. 2019. *In vitro* bioactivity of the fractions and a compound isolated from *Combretum elaeagnoides* leaf extract against selected foodborne pathogens. Prepared for submission to International Journal of Food Microbiology.

Conferences

Erhabor, C. R., Erhabor, J.O. and McGaw, L.J. Evaluation of the antibacterial activity and antibiofilm formation of foodborne pathogens of three *Combretum* species. Faculty Day, Faculty of Veterinary Sciences, Onderstepoort Campus, Pretoria, South Africa. August 2019. Poster presentation.

Abstract

Microbial biofilm and quorum sensing are related traits employed by microorganisms to improve their survival and virulence. They have been increasingly implicated in the food processing and the medical industries where they cause surface and food surface contamination. In this thesis, the available literature regarding the value of South African plants as potential sources of anti-biofilm and quorum quenching bioactive secondary metabolites was surveyed. The literature survey also covered antimicrobial activity investigations of medicinal plants against foodborne pathogens. The survey revealed that a total of thirty plant species belonging to nineteen families have anti-biofilm and quorum quenching capacity against foodborne pathogens. The survey served to summarize present knowledge and to provide a basis for further investigation of South African medicinal plants with known anti-biofilm and quorum quenching potential.

In subsequent research, the antimicrobial, anti-biofilm, antioxidant and cytotoxicity activities of nine South African medicinal plants was evaluated. The plants (*Combretum elaeagnoides, Combretum molle, Combretum oxystachyum, Carpobrotus edulis, Vachellia rehmanniana, Vachellia xanthophloea, Kigelia africana, Elephantorrhiza elephantina* and *Ochna pretoriensis*) were investigated. The selection of plant species was based on their known antimicrobial activity, chemotaxonomic relationships to plant species with antibacterial activity, availability and/or the existence of traditional uses against foodborne diseases.

The serial microdilution technique and the crystal violet assay were used to assess the antimicrobial and anti-biofilm potential of the acetone and methanol extracts, fractions and isolated compound. The antioxidant activity of the extracts, fractions and isolated compound was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays. The tetrazolium dye cell viability assay was used to ascertain the cytotoxicity of the samples. The methanol extract of *C. elaeagnoides* was

fractionated into five fractions (hexane, dichloromethane, ethyl acetate, butanol and water) via solvent-solvent fractionation. Column chromatography making use of silica gel as stationary phase combined with gradient elution of increasing polarity using hexane and ethyl acetate was used to isolate a bioactive compound via bioassay-guided fractionation. The isolated compound was identified as quercetin 3-*O*-rhamnoside using mass spectrometry (MS) and nuclear magnetic resonance (NMR).

The extracts of the nine plants were active against all selected test organisms with minimum inhibitory concentration (MIC) values ranging from <0.02 to 2.5 mg/ml. The acetone extracts of *Vachellia xanthophloea, Combretum molle*, and *Carpobrotus edulis* had excellent MIC activity of <0.02 mg/mL against *Enterobacter cloacae*. The dichloromethane fraction of *Combretum elaeagnoides* had the best MIC value of 0.03 mg/mL against *Escherichia coli* (ATCC 25922). The result of the minimum bactericidal concentration (MBC) investigation showed that the acetone extracts of *C. elaeagnoides* and *C. molle* had the best activity against *E. coli* (ATCC 25922) with no growth at 0.31 mg/mL, and acetone extracts of *C. elaeagnoides* and *V. xanthophloea* against *Staphylococcus aureus* at 0.63 mg/mL respectively. The acetone extracts of *C. molle* and *V. xanthophloea* had the best MBC activity against the clinical isolate of *E. coli* (clinical isolate), *Campylobacter jejuni*, and *E. cloacae* with 100% inhibition at 0.08, 0.31 and 0.02 mg/mL respectively. The acetone extract of *C. edulis* also inhibited (100%) *E. cloacae* at 0.02 mg/mL. The acetone extract of *C. molle* exhibited 100% inhibition against *Salmonella* Typhimurium, *Stenotrophomonas maltophilia* and *Klebsiella pneumoniae* at 0.04, 0.16 and 0.08 mg/mL respectively. The acetone extract of *V. xanthophloea* also exhibited 100% inhibition against *Salmonella* Typhimurium and *S.* Enteriditis at 0.08 mg/mL. Of all the extracts, fractions and the isolated compound, the acetone extract of *C. molle* had the best MBC against the tested pathogens.

Most of the extracts, fractions and the isolated compound, quercetin 3-*O*-rhamnoside, selectively reduced biofilm growth by at least 50% against the foodborne pathogens. The acetone extract of *C. molle* had the greatest anti-biofilm activity against *S.* Typhimurium. The antioxidant assay revealed that the methanol extract of V. *xanthophloea* had very good activity against the radical scavenging DPPH while the acetone extract of C. *edulis* had excellent activity against the electron reducing ABTS with IC_{50} values of 0.14±0.11μg/mL and 0.01±0.02 μg/mL respectively. Most of the fractions had good antioxidant activity against DPPH ($IC_{50=}$ 0.01 to >100 μg/mL) and ABTS ($IC_{50=}$ 0.01±0.007 to 20.00±1.79 μg/mL) radicals. The butanol fraction of *C. elaeagnoides* and quercetin 3-*O*-rhamnoside had excellent antioxidant activity with the same IC_{50} value of 0.01 μg/mL. The cytotoxicity assay revealed that most of the extracts were relatively safe to cells except for the acetone extract of *C. molle* (0.01 mg/mL) and the methanol extract of *O. pretoriensis* (0.02 mg/ml). Therefore the good antimicrobial activity of *C. molle* may be largely owing to non-specific toxicity.

In summary, the study has established that the leaf extracts of the nine South African medicinal plants as well as the fractions and isolated compound from *C. elaeagnoides* have antimicrobial, antibiofilm and antioxidant potential and are generally relatively non-cytotoxic. Further work is needed to explore the quorum quenching, synergistic effects and possible mechanisms of action of the plants.

Table of Contents

Declaration 2
Acknowledgements 3
Publications 4
Conferences 5
Abstract 6
Table of Contents 9
List of Figures 12
List of Tables 13
List of Abbreviations 15
Chapter One: General Introduction 17
1. Introduction 17
1.1. Background 17
1.2. Hypothesis 18
1.3. Justification 19
1.4. Aim 19
1.5. Objectives 19
Chapter Two: Literature review 21
2. Introduction 21
2.1. Medicinal plants and antimicrobial activities 22
2.2. Foodborne pathogens and their public health importance 23
2.3. Resistance of foodborne pathogens, quorum sensing and biofilm formation 25
2.4. Medicinal plants: an alternative to fighting microbial resistance 29
2.5. Indigenous and naturalized South African medicinal plants with anti-biofilm and quorum quenching activities 30
2.6. Future perspectives and conclusion 47
Chapter Three: In vitro antibiofilm and antioxidant activities and cytotoxicity of nine South African medicinal plants 49
3. Introduction 49
3.1. Botanical descriptions and biological activity of plant species used in the study 51
3.2. Materials and methods 56
3.2.1. Selection of plant species 56
3.2.2. Extraction of plant material 57

3.2.3. Test bacterial strains 58
3.2.4. Culture of bacterial inoculum 58
3.2.5 Bioassay for antibacterial activities 59
3.2.5.1. Minimum inhibitory concentration and minimum bactericidal concentration 59
3.2.5.2. Total activity 60
3.2.6. Anti-biofilm assay 60
3.2.6.1. Inhibition of biofilm formation 60
3.2.6.2. Crystal violet staining (CVS) assay 61
3.2.7. Antioxidant activity 61
3.2.7.1 Assay for free radical scavenging of DPPH radical 61
3.2.7.2 The 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) electron reduction assay 62
3.2.8. Cytotoxicity 63
3.3. Results and discussion 64
3.3.1. Percentage yields of the different plant extracts 64
3.3.2. Antimicrobial effects (MIC and MBC) of the extracts of the nine selected plants 66
3.3.3. Evaluation of the total activity of the seventeen extracts of the nine plants 72
3.3.4. Evaluation of the antibiofilm (ABF) potential of nine selected plant species 75
3.3.5. Evaluation of the antioxidant potential of the nine selected plant leaf extracts 78
3.4. Conclusions 83
Chapter Four: Isolation of antibacterial compound from *Combretum elaeagnoides* 84
4.1. Introduction 84
4.2. Materials and methods 85
4.2.1. Extraction and fractionation of *C. elaeagnoides* leaf extract 85
4.2.2. Isolation of bioactive compound from the ethyl acetate fraction of *C. elaeagnoides* leaf extract 86
4.2.2.1. Column chromatography 86
4.2.2.2. Bulking of fractions 87
4.2.2.3. Purification of subfraction 1 on Sephadex LH-20 column 87
4.2.2.4. Spectroscopic characterization of isolated compound 88
4.2.3. Antibacterial activity 88
4.2.3.1. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the fractions and compound isolated from *C. elaeagnoides* 88
4.2.3.2. Determination of anti-biofilm activity 89

4.2.4. Antioxidant activity 90
4.2.4.1. Assay for free radical scavenging (DPPH) activity 90
4.2.4.2. The ABTS radical scavenging assay 91
4.2.5. Cytotoxicity 91
4.2.6. Statistical analysis 91
4.3. Results and discussion 91
4.3.1. Percentage yield of fractions and isolated compound 91
4.3.2. Characterization of isolated compound 95
4.3.3. Minimum inhibitory concentration and minimum bactericidal concentration activities of fractions and isolated compound from *C. elaeagnoides* 98
4.3.4. The antibiofilm potential of the fractions and isolated compound from *C. elaeagnoides* 102
4.3.5. Antioxidant evaluation 106
4.3.6. Cytotoxicity and selectivity index values of the fractions and isolated compound from *C. elaeagnoides* leaf extract 108
4.4. Conclusions 110
Chapter Five: General conclusions and recommendations 111
5. General conclusions 111
5.1. Antibacterial and antibiofilm screening of the crude leaf extracts of the selected medicinal plants 111
5.2. Evaluation of the antioxidant potential of the nine selected plant extracts 112
5.3. Cytotoxicity of the crude leaf extracts of the selected nine plants 112
5.4. *In vitro* bioactivity of the fractions and compound isolated from *Combretum elaeagnoides* leaf extract against selected foodborne pathogens 113
5.5. Conclusions and recommendations for future work 113
References 114
Appendix 1 132

List of Figures

Chapter Four

Figure 4.1. TLC plate of the fractions of *C. elaeagnoides* 93

Figure 4.2a, b and c. TLC plate of bulked fractions 1, 2 and 3 94

Figure 4.3. TLC plate of sub-fraction of bulked fraction 1 (from column chromatography) 95

Figure 4.4. Structure of Compound 1 98

List of Tables

Chapter Two

Table 2.1. Native and naturalized medicinal plants of South Africa with anti-biofilm (anti-BF) and quorum quenching (anti-QS) activities 32

Chapter Three

Table 3.1. Selected plants and their herbarium accession numbers 57

Table 3.2. The mass in grams (g) and percentage yield (%) of the selected nine plant species 65

Table 3.3. Minimum inhibitory concentrations of the nine selected plants in mg/mL 68

Table 3.4. Minimum bactericidal concentration of the nine selected plants in mg/ml against the test organisms 70

Table 3.5. Total activity of the extracts of the nine selected medicinal plants in mL/g 73

Table 3.6. The effect of the methanol and acetone extracts of the nine selected plants on biofilm formation 76

Table 3.7. Antioxidant potential of the nine selected South African medicinal plant against the tested foodborne pathogens 78

Table 3.8. Cytotoxicity of the seventeen leaf extracts of the nine selected plants 80

Table 3.9. Selectivity index values of selected nine plant leaf extracts against Vero African monkey kidney cells 81

Chapter Four

Table 4.1. Percentage yield of the different fractions and compounds 92

Table 4.2. The ^{13}C NMR Spectra data of Compound 1 97

Table 4.3. Minimum inhibitory concentrations(MIC) of the fractions and isolated compound from *C. elaeagnoides* leaf extract 100

Table 4.4. Minimum bactericidal concentration (MBC) of the fractions and isolated

compound from *C. elaeagnoides* leaf extract 101

Table 4.5. Total antibacterial activity in mL/g of fractions and isolated compound from *C. elaeagnoides* 102

Table 4.6. Effect of fractions and isolated compound from *C. elaeagnoides* leaf extract on the growth and development of biofilm of selected foodborne pathogens 105

Table 4.7. Antioxidant activity of the fractions and isolated compound from *C. elaeagnoides* expressed as mean IC_{50} (µg/ml) 107

Table 4.8. Cytotoxicity (LC_{50}) and selectivity index values (LC_{50}/MIC) of the fractions and isolated compound from *C. elaeagnoides* leaf extract 109

List of abbreviations

ABF	Anti-biofilm
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ADHD	Attention deficit-hyperactivity disorder
AHL	Acyl homoserine lactones
ATCC	American Type Culture Collection
CABI	Centre for Agriculture and Bioscience International
CDC	Centre for Disease Control
CFU	Colony-forming unit
CPF	Ciprofloxacin
CVS	Crystal violet staining
DMSO	Dimethyl sulfoxide/ $(CH_3)_2SO$
DNA	Deoxyribonucleic acid
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
EPS	Extracellular polymeric substance
FAO	Food and Agricultural Organization of the United Nations
ICT	Infection Control Today
IBD	Inflammatory bowel disease
INT	*p*-iodonitrotetrazolium violet)
LC50	Lethal Concentration
LNBG	Lowveld National Botanical Garden
MBC	Minimum bactericidal concentration
MeOH	Methanol
MEM	Minimal Essential Medium
MHA	Mueller-Hinton Agar

MHB	Mueller-Hinton Broth
MIC	Minimum inhibitory concentration
MTT	3-(4, 5- dimethylthiazolyl-2)-2.5-diphenyltetrazolium bromide
NA	not applicable
NICD	National Institution for Communicable Diseases
NIH	National Institute for Health
NMR	Nuclear Magnetic Resonance
PBS	Phosphate-buffered saline
QS	Quorum sensing
SANBI	South African National Botanical Institution
SEM	Standard error of mean
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TLC	Thin Layer Chromatography
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
WHO	World Health Organization

Chapter One: General Introduction

1. Introduction

1.1. Background and motivation of study

Over the years, foodborne disease has become a global burden. The prevalence of foodborne diseases differs from one country to the other. Reports show that, of every ten persons in the world, at least one falls ill due to the consumption of food contaminated by foodborne pathogens (WHO, 2015). Unfortunately, Africa has the highest burden of foodborne diseases, followed by Southeast Asia, while Europe is reported to have the lowest prevalence of foodborne diseases (WHO, 2015).

Despite this massive burden of foodborne diseases, many countries within the African continent do not consider food safety to be a significant concern. In South Africa, like in many other African countries, there is limited capacity to predict and trace foodborne diseases despite many occurrences of such diseases, especially among school children (Niehaus et al., 2011; NICD, 2014a, b; Korsten et al., 2016). It is worrisome to note that foodborne diseases are expected to increase in developing countries due to the consumption of contaminated food (Uyttendaele et al., 2016), migration and increasing income. Other contributors to the increasing emergence of foodborne diseases include poor sanitation, under-reporting of suspected foodborne cases, dearth of infrastructure, poor practices in food establishments and poor knowledge of hygiene (Kibret and Abera, 2012).

Migration and increase in per capita income with changing food habits has been strongly linked to increased consumption of animal products, vegetables, fruits and other products from plants. Following this high demand for food, are intensive and potentially unhealthy methods in animal production (unregulated antibiotic use for increased productivity leading to the proliferation of resistant pathogens) and plant cultivation or propagation, including animal manure use

contaminated with microbes, and unregulated use of herbicides causing emergence of resistant pathogens. Also, via global trade comes an increased vulnerability to food contamination and spread of foodborne pathogens following the movement of processed products (Foley et al., 2008).

Foodborne pathogens can be classified as parasites, bacteria, fungi or viruses that cause illness or food poisoning and in severe cases, death. In 2015, the WHO investigated the burden of foodborne diseases caused by 31 agents including bacteria, parasites, viruses, toxins and chemicals, reporting that every year, 600 million people fall ill globally after eating contaminated food. According to the report, 420 000 people die, particularly more susceptible sectors of the population, such as children under the age of 5 years. This is acknowledged by the WHO as a serious public health concern, and the highest incidence and death rates were recorded as being within the African and South-East Asian regions of the world. The report further revealed that 91 million people fall ill and 137 000 die every year from foodborne diseases within the African region, with diarrhea said to be accountable for 70% of the foodborne diseases in the continent. The significance of foodborne pathogens, resulting from their ability to form biofilms, has led to significant economic losses in various nations (JamunaBai and Rai 2011). Biofilms are formed by the increasing growth of microorganisms on a surface which changes and damages the phenotype of the affected systems, thereby leading to contamination or infections (Samaranayake 2002). The use of natural plant products has increasingly become a recognised treatment for several ailments, and this is one of the wealthiest growing sectors in the agribusiness industry (Makunga *et al*., 2008). In the current study, extracts of nine South African plants were evaluated for their antibacterial, anti-biofilm and antioxidant potential as well as their safety via cytotoxicity.

1.2. Hypothesis

- Some medicinal plants used traditionally in the control of foodborne pathogens may have bioactive compounds
- The mechanism of action of the selected plants may be different from known pathways of antibiotic action

1.3. Justification

- Adherence of foodborne pathogens to food products or processing surfaces results in severe public health risk and substantial economic losses
- Foodborne pathogens have become resistant to antibiotics due to their unregulated or improper use among other reasons
- Biofilm formation and quorum sensing are responsible for many chronic microbial infections, and plants with activity against biofilm formation and quorum sensing can be useful in fighting infections

1.4. Aim

The aim of the study was to determine the antimicrobial, anti-biofilm and antioxidant potential as well as the toxicological effects of selected South African medicinal plants against specific foodborne pathogens. An additional aim was to isolate and characterize bioactive compound(s) from one of the most active plant species.

1.5. Objectives

- To provide an extensive literature review on the potential of South African medicinal plants with activity against microbial biofilm formation as well as quorum sensing of foodborne pathogens

- To determine the anti-biofilm, minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of nine medicinal plants selected against nominated foodborne pathogens
- To determine the antioxidant potential and cytotoxicity of the plant extracts
- To isolate, characterize and test bioactive compound(s) from one of the selected plants for antimicrobial, anti-biofilm, antioxidant and cytotoxic activities

The objectives of the study are addressed in Chapters Two to Four. Chapter Five deals with general conclusions and recommendations for future work.

Chapter Two: Literature Review

Preface

This literature review was submitted in a modified version to the South African Journal of Botany (impact factor 1.504) as a review paper and was accepted. The reference is as follows:

Erhabor, C.R., Erhabor, J.O., McGaw, L.J. 2019. The potential of South African medicinal plants against microbial biofilm and quorum sensing of foodborne pathogens: A review. *In Press: South African Journal of Botany*.

2. Introduction

Globally, it is recognized that traditional medicinal remedies have potential to provide significant benefits to human and animal health, as well as to impact on national income. Traditional remedies have generated great interest worldwide as a striking alternative to synthetic drugs. This is due to the high cost of research for development of chemical drugs and lack of technological facilities, especially in developing countries. Importantly, using natural remedies in developed countries as an alternative or complementary remedy for treating different diseases has also gained global attention.

In South Africa, a large percentage of the population (80%) rely on local medicines, majorly from plants, to manage their diseases and meet their health care needs (Street and Prinsloo, 2013). Plants are known to have a myriad of bioactive secondary metabolites with a broad spectrum of activities, making them a potential source of new antibiotics (Abdalla and Matasyoh, 2014). In scientific terms, plant secondary metabolites have attracted much interest in discovery and industrial development of drugs.

Quorum sensing and formation of microbial biofilms are two linked traits that have been developed by microorganisms to enhance their survival and increase virulence. They may cause severe infections in humans as their occurrence makes it difficult to treat commonly encountered infections, with a high possibility of recurrent infections. Microbial biofilms and quorum sensing have also been increasingly implicated in the food processing and medical industry where they have caused food and surface contamination (Sandasi et al., 2011). This has made foodborne pathogens resistant to antibiotics. Moreover, most infectious diseases are caused by bacteria which multiply within quorum sensing (QS) facilitated biofilms. Efforts to disrupt these biofilms have enabled the identification of biologically active molecules produced by prokaryotes and eukaryotes (Kalia, 2013). As a result of evolving resistance, some commonly used control methods have become ineffective. In this study, the potential of South African medicinal plants with anti-biofilm and quorum quenching abilities are highlighted as a basis for their further investigation as well as to promote investigation of new plants with chemotaxonomic relationships to active plants.

2.1 Medicinal plants and antimicrobial activities

Medicinal plants form an integral part of the world healthcare system, providing affordable, accessible and relatively cheap herbal drugs with cultural implications. Their wide acceptance has led to sourcing for new, affordable and efficacious novel medicines to treat microbial diseases. Infectious diseases caused by bacteria, fungi and viruses are globally of major concern, especially in sub-Saharan Africa (Istúriz and Carbon, 2000). These concerns stem from the resistance posed by these microorganisms, toxicity and high cost of available drugs thus limiting their potency and use.

However, due to the alarming rate of illnesses caused by antibiotic-resistant microorganisms, awarenes in the use of medicinal plants as another source to conventional medicine has greatly

increased in recent years (Dehghan et al., 2014). Some medicinal plants and their constituents are promising sources of antimicrobial agents. Thus, studies on the anti-infective activities of medicinal plants has become a progressive trend. No doubt, with advances in laboratory techniques, reinvigorated interest in the field and scientific validation of the folk/local use, there exists a possibility to incorporate traditional medicines into the treatment regimens of conventional healthcare systems (van Vuuren, 2008). A recent review (van Vuuren and Holl, 2017) discussed the important approaches used by researchers in screening or validating medicinal plants for anti-infective activities. The foremost approach is examining the ethnobotanical use of the plant with a follow up test on the relevant pathogen(s) based on the folk use. Another approach, though a relatively new area, is by evaluating pathogen(s) which may act as a trigger for other diseases. Additionally, chemotaxonomy, synergistic studies of the plants with known antibiotics and prevention/inhibition of cell-to-cell communication (biofilm formation, metabolic activity and quorum sensing) are other approaches that have been adopted in determining antimicrobial activity or efficacy of plants.

2.2 Foodborne pathogens and their public health importance

A common determinant of healthcare is the intake of safe food. As a result of urbanization and increase in per capita income with changing food habits, there has been a surge in the consumption of animal products, vegetables, fruits and other products from plants. Following this high demand for food, are sometimes intensive and unhealthy methods of animal production (unregulated use of antibiotics for increased productivity leading to proliferation of resistant pathogens) and plant cultivation or propagation, for example animal manure used may be contaminated with microbes, and unregulated use of herbicides may cause emergence of resistant pathogens. Also, with global trade comes an increased risk of food contamination and spread of foodborne pathogens due to the movement of processed products (Foley et al., 2008).

These foodborne pathogens can comprise parasites, bacteria, fungi or viruses, which affect both humans and animals through consumption of undercooked food, poultry products, juice, unpasteurized milk, fruits, and vegetables as well as through contact of food with contaminated hands. This causes illness or food poisoning and, in severe cases, death. It has been reported that *Escherichia coli, Staphylococcus aureus*, various serovars of *Salmonella enterica*, *Salmonella typhimurium*, *Listeria monocytogenes, Clostridium botulinum, Vibrio vulnificus* and *Vibrio parahaemolyticus* are the most common bacteria causing foodborne diseases (Busani et al., 2006; Hoque et al., 2007; Gerner, 2008). Other pathogens include *Penicillium expansum*, *Bacillus cereus* and *Pseudomonas aeruginosa*. In 2015, the WHO reported the burden of foodborne diseases caused by 31 agents, including bacteria, parasites, viruses, toxins and chemicals. The report posited that every year, 600 million or about 1 in 10 persons in the world, fall ill after eating contaminated food. According to the report, 420 000 people die annually, with children under the age of 5 years being particularly susceptible. Acknowledged by the WHO as a serious public health concern, the highest incidence and death rates were within the African and South-East Asian regions of the world. The report further revealed that 91 million people fall ill and 137 000 die every year from foodborne diseases within the African region with diarrhea said to be a factor in 70% of foodborne diseases in the region.

Foodborne diseases can result in immediate symptoms, such as nausea, diarrhea and vomiting (food poisoning), but can also cause long-term illnesses, for example cancer, liver and kidney failure, or brain and neural disorders. Such diseases may be very serious in children, antenatal women, the very old and those with weak immune systems. Some foodborne pathogens like *Bacillus* spp., *Campylobacter jejuni, Pseudomonas* spp., *Salmonella* spp. and *Yersinia enterocolitica* have been identified to form biofilms leading to bacterial resistance to drugs (Bridier et al., 2015).

2.3 Resistance of foodborne pathogens, quorum sensing and biofilm formation.

Antibiotic resistance is one of the greatest public health threats experienced by both humans and animals. Combating this threat is a priority that deserves attention from stakeholders/sectors globally (CDC, 2018). Antibiotic resistance in foodborne pathogens has assumed an enduring public health threat with trends based on the tracked data analyzed by the Center for Disease Control and Prevention (CDC) (ICT, 2014).

Foodborne pathogens have become significant problems in the food industry following their strong ability to adhere to, produce and accumulate extracellular matrix composed of one or more polymeric substances (protein, extracellular DNA, humic substances and polysaccharide) and other molecules involved in cell-to-cell communication (Flemming and Wingender, 2010). This has led to their resistance to drugs, which results in food spoilage despite the availability of modern food preservation techniques. In recent times, communication between bacterial cells has received attention to elucidate the role of quorum signals in enhancing attachment as well as growth of pathogenic bacteria in food substances (JamunaBai and Rai, 2011). Again, several biochemical processes including proteolytic as well as lipolytic activities associated with food deterioration are said to be regulated by quorum sensing (Ragaert et al., 2007). Identification of quorum signals in spoiled food products is a novel approach to prevent the food spoilage process.

Quorum sensing is a system of chemical communication that boosts the survival of bacteria, and allows resident bacteria to undertake specialized roles which are vital for intra- and inter-bacterial gene regulation, and to keep bacterial colonies intact. Quorum sensing plays a critical role in regulating diverse cellular functions in bacteria, including bioluminescence, virulence gene expression, mating, sporulation and biofilm formation leading to antibiotic resistance. This involves numerous processes and molecules, such as specific signaling molecules called auto-inducers that have the ability to bind to and subsequently activate receptors that transduce the quorum sensing signal into intracellular secondary messenger responses,

almost similar to the process of ligand – receptor interaction (Raffa et al., 2005; Li and Nair 2012).

One popular auto-inducer that has been researched intensively in Gram-negative bacteria is N-acyl homoserine lactone (AHL) (Fuqua et al., 2001). AHL is produced by LuxI auto-inducer homologues and binds particularly to LuxR receptor protein to initiate the expression of specific genes, including virulence factor production in *Pseudomonas aeruginosa* (LasI/R) (Parker and Sperandio, 2001). It is worthy to note that Gram-positive bacteria use oligopeptides to communicate (Bacha et al., 2016). Equally, following the implication of quorum-sensing signaling molecules in food spoilage, an approach was established to break the complex communication system of these microorganisms. This approach, known as quorum quenching (anti-quorum sensing), can interrupt or prevent microbial communication instead of eliminating the microorganism (El-Hamid, 2016). It interrupts the signal molecules that are produced and secreted by the microorganisms via quorum sensing inhibitors such as AHL- lactonase, AHL-acylase and paraoxonase enzymes (Adak et al., 2011). The mechanism of quorum quenching is to bind to the relevant signal receptor and control gene expression in the microbial metabolism. This invariably blocks the signal molecules from the receptors and the resultant cumulative behavior cannot be regulated by the signaling mechanism. Targeting of the quorum sensing mechanism is a new strategy aimed at preventing the pathogen and spoilage microorganisms and biofilm matrix produced by them which could be found in food products. Quorum sensing activities can only occur when a specific cell density is attained by bacteria (biofilm formation). These activities remain inactive if performed by a bacterium but if carried out by a community of bacteria it then becomes active.

Adherence of these foodborne pathogens to the food product or the processing surfaces results in severe public health risks and huge economic losses (Chmmielewski and Frank, 2003). Bacterial migration or settlement of microorganisms on non-living material (biofilm formation) have

a detrimental value in medicine (medical devices, artificial organ contaminations, pathological human tissues and organs) and in some economic fields such as growth on the wall of fermentation processes, contamination of food processing equipment, contamination of water pipelines, industrial piping, ventilation etc. Bacterial biofilms are by nature pathogenic and often cause nosocomial infections. Biofilms have great significance to public health and food industries, as biofilms can render inhabitants of such films resistant to antimicrobial agents and cleaning (Choisy, 2011). Moreover, the effectivity of antimicrobial agents against biofilms cannot be exclusively determined via standard microdilution testing. This is because these tests rely upon the response of planktonic (suspended) form of the organism rather than the biofilm (surface-associated) formed by the organisms. Therefore, susceptibility of the organisms against the antimicrobial agents should include susceptibility determination directly against biofilm formed by the respective organisms, preferably under conditions that simulate conditions in vivo.

A report by the National Institute of Health (NIH) revealed that biofilm formation is responsible for 65% and 80% of all chronic and microbial infections respectively. Hence, microbial activity is the most common cause of food spoilage (Christensen et al., 2003). This has made it necessary to investigate the adhesion processes of the microorganism and surface contents. In order to reach this objective, increasing attention is being paid to the physiology and genetics of the initial stages of adhesion and the description of the regulatory networks allowing the developmental processes necessary for the structural development of biofilms and the usage of plants with anti-biofilm activities. This would help find non-toxic surface treatments that can detach the microorganism from the location they usually contaminate, especially in in-dwelling devices.

A biofilm is a thin layer of microorganisms adhering to an organic or inorganic surface of a structure, together with the secretion of polymers (Saunders, 2003). It also comprises any cross feeding group of microorganisms in which cells attach to each other and frequently also to a surface. These adherent cells become entrenched within a slimy matrix which is composed of

extracellular polymeric substances (EPS). The cells inside the biofilm produce the EPS contents which are most commonly a polymeric conglomeration of extracellular polysaccharides, lipids, proteins and DNA. This establishes the functional and structural integrity of the biofilms. These EPS are considered the fundamental component that determines the physiochemical properties of a biofilm. The adhering surface can be living (tooth enamel, heart valves, the lungs or middle ear) or non-living, and its moisture content makes it a good site for biofilm formation as biofilms thrive on wet or moist surfaces (Hall-Stoodley et al., 2004; Lopez et al.,2010; Aggarwal et al., 2016). The interactions and associations of bacterial communities (biofilm formation), enable bacteria to colonize a surface, thereby altering their phenotype and making conditions suitable for them to survive and multiply. This process (biofilm formation) in turn encourages microbial development (Rodney, 2001). At this stage, they send several exterior and interior signals to effectively produce a new morphological structure that is adapted to endurance in a variety of harsh environments (O'Toole et al., 2000). This in most cases leads to resistant spore formation, especially in Gram-negative bacteria. Bacterial invasion on non-living (abiotic) materials and biofilm formation can include one microbial species or many microbial species and can form on a surface. Mixed-species biofilms are predominant in most environments and are infectious in nature. This is especially true for the food industries as biofilms can render organisms on such films resistant to antimicrobial agents and washing (Chmielewski and Frank, 2003; Jamal et al., 2018). Henceforth, the activities of microorganisms can be regarded as the most common cause of food spoilage (Christensen et al., 2003). Accordingly, contaminated foods are a huge problem in health care, with serious acute diseases often resulting from poisoned food . Regular preservatives can often produce unpleasant by-products, so finding an alternative, efficient natural antimicrobial agent(s) to partially (synergistically) or wholly replace them has become essential (Nazir, 2017).

2.4 Medicinal plants: an alternative to fighting microbial resistance

The rapid spread of foodborne pathogens and emergence of antibiotic resistant microbial strains coupled with the alarming failure of available chemotherapeutics has led to the search for alternatives including medicinal plants. A large number of studies, though not exhaustive, has shown the antimicrobial potential of South African traditional medicinal plants (Eloff, 1999, 2001; McGaw et al., 2002; Mahlo et al., 2010; Koh et al., 2013; Nair and Van Staden 2014, Cock and van Vuuren, 2015; Hussain et al., 2016; Motlhatlego et al, 2018). Generally, natural products have been identified to play a highly interesting role in interfering with the microbial cell to-cell communication processes of quorum sensing (Koh et al., 2013). The effects of plant extracts, fractions and plant-derived compounds on bacterial cell-to-cell communication and biofilm formation are yet to be fully explored. Targeting this communication system of microorganisms becomes vital as it is thought that many bacteria make use of chemical signaling systems to control cellular behaviors which are triggered by the local bacterial population density for effective colonization and manipulation of host organisms (Blackwell and Fuqua, 2011). For microbial biofilms, methods such as physical, chemical and biological techniques have been adopted (Kumar and Anand, 1998; Steinberg, 2005). The common physical methods include scraping, brushing, heat and mechanical scrubbing, as well as high-pressure spraying (Dreeszen, 2003) while common chemical biocides like detergents, disinfectants and preservatives are also used. Bacteriocins and enzymes make up the common bio-control agents used mainly in the food industry, though on a small scale. The use of these biological agents faces limitations such as availability and cost (Kumar and Anand, 1998). Although there are control agents for preventing or eradicating microbial biofilms, most of these agents may become ineffective due to increased resistance conferred by the microbial sessile cells. This has led to much interest in seeking alternatives from natural sources like plants. These new biological agents from plants are

expected to either prevent or inhibit cell adhesion, biomass formation and metabolic activities, or disrupt the phenotypic features of the organism.

2.5 Indigenous and naturalized South African medicinal plants with anti-biofilm and quorum quenching activities

Over the years, medicinal plants have been shown to have exciting therapeutic value worldwide. This has been corroborated by the World Health Organization, which estimates that 70-80% of the world population still uses traditional remedies (WHO, 2008; Arunkumar et al., 2009) with over 35 000 plant species used to treat severe ailments in many human cultures (Koshy et al., 2001). These plants include medicinal plants which form an essential part of the African culture due to their long history of use and probable safety (Bacha et al., 2016; Gidey et al., 2009). No doubt, African traditional medicine is the most ancient medicinal system, as Africa is culturally known as as the Cradle of Mankind (Gurib-Fakim, 2006). South Africa, an African country with a range of climatic conditions, possesses an uncommon and diverse botanical heritage with over 30 000 plant species, of which 3 000 species are used for therapeutic purposes. The plants are richly diversified and are mostly endemic (Van Wyk et al., 1997; Mulholland, 2005). Many South Africans depend on medicinal plants for their health care needs, including patients who use conventional drugs (Ahmed et al., 2012). South Africa is home to nearly 10% of the vascular plant species diversity of the world, a possible source of undiscovered bioactive metabolites with activity against a variety of bacterial and fungal pathogens (Shai et al., 2008). In 2002, Van Wyk in his review of South African literature reported a wide array of research activity where traditional medicinal practices were used to treat diverse infections/diseases including skin diseases, urinary tract infections, tuberculosis and gastrointestinal infections/disorders. Available scientific reports have alluded to the use of medicinal plants as a valuable source of treatment in virtually all cultures worldwide. This is due to the presence of important antimicrobial principles, immune

boosting properties and general maintenance of health, thus enabling prevention and cure for a myriad of diseases and disorders of human and animal origin (Baquar, 1995, Nair and Van Staden 2014, Mahima et al.,2012; Rahal et al., 2014b). Many South African plants have been reported to have medicinal properties such as antimicrobial activities (Adamu et al., 2012; Sharma and Lall, 2014; Mthethwa et al., 2014; Aro et al., 2015; Elisha et al., 2017; Ramadwa et al., 2017; Van Vuuren and Holl 2017, Omokhua et al., 2018), anti-diabetic (Afolayan and Sunmonu, 2011; Olaokun et al., 2013; Georgekutty, 2015; Hafsa et al., 2016; Olaokun et al., 2017), anti-inflammatory and antioxidant (Otang et al., 2012; Dzoyem and Eloff, 2015; Adebayo et al., 2015, Ondua et al.,2019) and anti-parasitic properties (Aremu et al., 2010; Maphosa and Masika, 2012; Ademola and Eloff, 2010; Mokoka et al., 2014). Table 2.1 presents a list of South African plants (indigenous and naturalized) with potential against microbial biofilms and quorum sensing.

Table 2.1. Native and naturalized medicinal plants of South Africa with anti-biofilm (anti-BF) and quorum quenching (anti-QS) activities

Plants species (Family)	Uses and biological activities	Plant part tested	Tested strains	Scientific validation (anti-BF and anti-QS activities)
Agathosma betulina (P.J.Bergius) Pillans (Rutaceae)	The essential oils and extracts of the leaves are used as flavours for teas, candy and a liquor known as buchu brandy. It has diuretic and properties	Leaves	*Pseudomonas aeruginosa*	≥80% inhibition of pyocyanin and LasB Elastase production in *P.aeruginosa* showed the plant has anti-QS activity (Cosa and Chenia, 2014)
Agave sisalana Perrine. (Agavaceae)	The sap is used as binding agent in some powder used as poultices on wounds. It is used for the treatment of diarrhea, dysentery, indigestion, flatulence, constipation, jaundice and has antiseptic, diaphoretic, diuretic and laxative properties. Also used in textiles industry, and other handicrafts (Lust, 1983; Chopra et al., 1986; Chevallier, 1996; Anonymous	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Reduction of biofilm biomass formation of *P. aeruginosa* isolate 2 by 87.5% at 0.3mg/ml. Las A protease activity of *P. aeruginosa* isolate 1 was inhibited (65.2%) at 10.4 mg/ml (Al-Refi, 2016)

	1995; Duke and Ayensu 1983.)			
Artemisia absinthium L. (Asteraceae)	Used in beverages and wine. It is used for dyspepsia, as a bitter to counteract poor appetite and against various infectious diseases, Crohn's disease, and IgA nephropathy (Krebs et al., 2010)	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Inhibition (47.8%) of biofilm formation of *P. aeruginosa* isolate 2 as well as minimal reduction (47.8%) of pyocyanin production of *P. aeruginosa* isolate 1. Reduction (52.4%) of LasA protease activity of *P. aeruginosa* isolate 1 was reported (Al-Refi, 2016)
Aspalathus linearis (Burm.f.) R.Dahlgren (Fabaceae)	Used as milk substitute for infants prone to colic and to treat eczema (Van Wyk 1997). It also has antispasmodic, antioxidant and antifungal activities and commonly used in cosmetics (Sandasi et al., 2010)	Leaves	*Listeria monocytogenes* (ATCC 19111) and clinical strain (CI001)	Dichloromethane/methanol extracts had anti-BF activity by inhibiting listerial cell attachment by 55% and 70% respectively (Sandasi et al., 2010).
Boswellia serrata Triana & Planch. (Burseraceae)	It is used to treat inflammatory diseases, such as arthritis, osteoarthritis,	Leaves	*Pseudomonas aeruginosa* ATCC 35032	≥90% reduction of *P. aeruginosa* biofilm formation

	and IBD, and is also effective in preventing lipid oxidation, also used as incense in religious and cultural ceremonies (Kimmatkar et al., 2003)			
Centella asiatica (L.) Urb. (Apiaceae)	Used to treat leprosy, wounds, cancer, fever, syphilis and applied as diuretic and purgative (Van Wyk et al., 1997).	Leaves	*Chromobacterium violaceum* (ATCC 12472), *C. violaceum* (ATCC31532), a mini-Tn5 mutant *C. violaceum* (CV026), and *Pseudomonas aeruginosa* (PAO1)	Anti-BF activity (decreased biofilm formation by > 80%) against *P. aeruginosa* PAO1 at 400 µg/ml. At 80 µg/disc, ethanolic extract had anti-QS activity revealing pigmentless zone (20 mm) indicating violacein inhibition around the disc against *C. violaceum* CV026 while the ethyl acetate fraction at 100µg/ml had > 50% inhibition and at 400 µg/ml completely inhibited the violacein production of *C. violaceum* (ATCC12472). AHL (N-acyl homoserine lactone)-mediated violacein production in *C. violaceum* AT31532 was

				also inhibited at 100 – 400 µg/ml. The anti-QS activity was also reflected in the inhibition of elastolytic and proteolytic activities at 200 ug/ml by > 50% inhibition and complete inhibition at 400 ug/ml was observed against *P. aeruginosa* PAO1. Complete inhibition of swarming motility in *P. aeruginosa* PAO1 at 50 ug/ml was also observed (Vasavi et al., 2014)
Cinnamomum zeylanicum Blume (Lauraceae)	To remedy respiratory, digestive and gynaecological ailments (Ranasingh et al., 2003).	Sticks	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Inhibition of *P. aeruginosa* isolate 2 biofilm biomass formation and reduction (44.3%) of pyocyanin production of *P. aeruginosa* isolate 1 at 7.8 mg/ml. Slight reduction (22.9%) of LasA protease activity was also reported(Al-Refi 2016)
Cuminum cyminum L. (Apiaceae)	Used for treatment of diarrhea, colic, bowel spasms,	Groundseed	Clinical isolates of *P. aeruginosa* 1, 2, *S.*	Reduction of *P. aeruginosa* isolate 2 biofilm biomass

	and gas. Also used to relieve bloating by increasing urine flow as a diuretic; to start menstruation and as an aphrodisiac. Used to boost immune system, to treat rheumatism, used as a flavoring component and as fragrance in cosmetics amongst others (Goreja, 2003).		*aureus* and *C. albicans*	formation by 46.1%. Slightly reduced pyocyanin production in *P. aeruginosa* isolate 1 by 32.4% inhibited motility of both strains of *P. aeruginosa* at 10.4 mg/ml respectively. (Al-Refi, 2016)
Echinacea angustifolia DC. (Asteraceae)	Used against acid indigestion, chronic fatigue syndrome, attention deficit-hyperactivity disorder (ADHD), gum disease, migraine and pain, diphtheria, dizziness, genital herpes, syphilis, malaria, typhoid, rattlesnake bite, rheumatism, septicemia, influenza, tonsillitis, urinary tract infections and vaginal yeast infection (Nordqvist, 2017)	Ground plant material (leaves)	*Listeria monocytogenes* ATCC 19111	Inhibition (77%) of cell adhesion and a reduction in listeria metabolic activity (Sandasi et al., 2011)

Eucalyptus globulus Labill. (Myrtaceae)	Used as herbal tea, bio-pesticide, flavouring, and in perfumery. Also has antimicrobial properties (Yang et al., 2004).	Essential oil	*Acinetobacter baumannii*	Regulates production of violacein pigment in *A. baumanni* (Myszka et al., 2016)
Foeniculum vulgare Mill. var. *vulgare* (Apiaceae)	Mature fruit and essential oil used to flavouring food products such as liqueurs, bread, pastries, pickles and cheese. They also form constituents of pharmaceutical and cosmetic products (Piccaglia and Marotti, 2001). Herbal drugs and essential oils from the plant have hepatoprotective effect (Ozbek et al., 2003), as well as antispasmodic effects (Reynolds, 1982). They are also known for their diuretic, anti-inflammatory, analgesic and antioxidant activities (Choi and Hwang, 2004).	Aerial parts	*P. aeruginosa* PaO1	Inhibited formation of biofilm by *P. aeruginosa* PaO1 (Artini et al., 2018)

Glycyrrhiza glabra L. (Fabaceae)	Used to treat arthritis, mouth ulcers and liver detoxification (Walters, et al., 2006). Anti-*Helicobacter pylori* and antibacterial activities of *G. glabra* flavonoids reported (Taga, et al., 2003; Riedel et al ., 2006) Plant is also used as a flavoring agent for [, candies and sweets. The root is used as a mouth freshener (Assadourian, 2005)	Rhizome	Clinical strains of *Acinetobacter baumannii* (C1-4, ATCC 17978 and 19606) and *A. nosocomialis* M2	Active fraction at 0.5 mg/ml significantly reduced (20-70%) surface motility of the clinical strains. Biofilm index of *A. baumannii* was significantly reduced (30 -70%) at 2 mg/ml. The active fraction also exhibited anti-QS activity by decreasing (56-86%) the production of AHLs at 0.5 mg/ml against *A.nosocomialis* M2 and *A. baumannii*(clinical isolates) (Bhargava et al., 2015)
Hibiscus sabdariffa L. (Malvaceae)	Used to treat high blood pressure, gastrointestinal disorders, diaphoresis, and anuria. It has aphrodisiac activity and can be used as tea (Orisakwe et al., 2004; Lans, 2006; Seujany et al., 2013)	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Reduced *P. aeruginosa* isolate 2 biofilm biomass formation (84.6%) at 0.01 mg/ml. *P. aeruginosa* isolate 1 pyocyanin production was reduced by 47.8% at 0.3 mg/ml while the motility of *P. aeruginosa* isolate 1 and 2 at 0.08 and 0.1 mg/ml was inhibited (Al-Refi, 2016).

Kigelia africana (Lam.) Benth. (Bignoniaceae)	Boiled chopped bark and fruit taken orally for blood cleansing and pelvic pains during pregnancy. The back is peeled from both the east and west side of the tree and chopped with *Acalypha vilacaulus* root and boiled; this is taken to induce lactation by a new mother following delivery (De Wet and Ngubane 2014)	Fruit	*Pseudomonas aeruginosa*	≥80% inhibition of pyocyanin and LasB Elastase production of *P. aeruginosa* (Cosa and Chenia, 2014)
Lawsonia inermis L. (Lythraceae)	For the treatment of burn wound infections (Muhammad and Muhammad, 2005)	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Inhibition (62%) of *P. aeruginosa* isolate 2 biofilm formation at 0.1mg/ml. Anti-QS activity showed reduction (39.2%) of pyocyanin production in *P. aeruginosa* isolate 1 (Al-Refi, 2016)
Marrubium vulgare L. (Lamiaceae)	Used against ear and eye problems, worms, colds, whooping cough and non-productive coughs of bronchitis and tuberculosis.	Leaves	*Pseudomonas aeruginosa*	Ethanol extract inhibited biofilm biomass formation by 53% at 0.6 mg/ml. The anti-QS activity revealed a reduction in *P. aeruginosa*

	Also, to treat bites from rabid dogs, respiratory ailments, catarrh and sore throats, and as a laxative, sedative and a cure for poisoning (Tyler, 1993; Newall et al., 1996; Moerman, 1998). Leaves and young stems are antiseptic, antispasmodic, diuretic, stimulant, expectorant diaphoretic, anti-diabetic, and are used as emmenagogues, cholagogues and tonics (Chiej, 1984; Bown, 1995)			isolate 2 pyocyanin production (signaling factor) by 42.6% and inhibited by 48% *P. aeruginosa* isolate 1 Las A protease activity (Al-Refi, 2016).
Mentha longifolia (L.) Huds. (Lamiaceae)	Used for the treatment of cough, cold, asthma and other respiratory ailment (Van Wyk, 1997).	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Ethanolic extract reduced biofilm biomass formation of *P. aeruginosa* isolate 2 by 81.2% at 0.3 mg/ml. Anti-QS activity by decreasing pyocyanin production by *P. aeruginosa* isolate 1 (32.4%). A reduction in Las A protease activity of *P. aeruginosa* isolate 1 by 66.8%

				at 5.2 mg/ml was also reported (Al-Refi 2016)
Pelargonium x hortorum L.H. Bailey. (Geraniaceae)	Used in treating fever, dysentery, diarrhea, wounds, respiratory tract infections, gastroenteritis, liver complaints, haemorrhage and kidney and bladder disorders (Bakker et al., 2004).	Leaves, flower and stalks	*P. aeruginosa* (isolate 1 and 2), *S. aureus* and *C. albicans* (Clinical isolates).	Anti-BF activity was by reduction of *P. aeruginosa* isolate 2 biofilm biomass formation by 85.7% at 0.005 mg/ml. Anti-QS activity showed 59.8% reduction of Las A protease activity. Also, *P. aeruginosa* isolate 2 motility at 0.02 mg/ml was significantly inhibited (Al-Refi, 2016)
Pelargonium sidoides DC. (Geraniaceae)	Infusion of the tubers are used to treat diarrhea, dysentery and bronchitis in children (Van Wyk et al., 1997)		*P. aeruginosa*	The ethanolic extract inhibited violacein production by ≥80% at ≥250 µL (Cosa and Chenia, 2014)
Portulaca oleracea L. (Portulacaceae)	It is edible and a good remedy for headaches, inflammation of the eyes and other organs. It can be used for burning of the stomach, erysipelas, disorders of the bladder, numbness of the	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Biofilm biomass formation of P. aeruginosa isolate 2 was inhibited (66.6%) at 1.3mg/ml. Las A protease activity of *P. aeruginosa* was reduced (20.2%) at 10.4 mg/ml (Al-Refi, 2016).

	teeth, excessive sexual desire, burning fevers, worms, dysentery, hemorrhoids and bites (Iranshahy et al., 2017).			
Punica granatum L. (Lythraceae)	Fruit rind is astringent. Whole fruit act as laxative. The plant has antifungal, CNS depressant, diuretic, and hypothermic activities. It also has excellent activity against giardiasis (Yogeeta et al., 2007).	Peels	*Pseudomonas aeruginosa* 2 isolates, *S. aureus* and *C. albicans*	The ethanolic extract showed a reduction in *P. aeruginosa* biofilm biomass formation by 61% at 0.005 and 0.5 mg/ml. Inhibition of *P. aeruginosa* isolate 1 and 2 motility at 0.04 and 0.005 mg/ml and the reduction of Las A protease activity by 58.3% was observed (Al-Refi, 2016)
Salvia officinalis L. (Lamiaceae)	Used internally for mild dyspepsia, heartburn, flatulence and against excessive sweating. The leaves support gastrointestinal function and used for the symptomatic treatment of mouth, gum and throat inflammation and to	Leaves and flowers	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Anti-BF activity revealed 40% reduction in *P. aeruginosa* biofilm formation while 44.3% reduction of pyocyanin production was reported (Al-Refi, 2016)

	treat mild skin infections. It also helps against mental and physical exhaustion (Felter et al., 1898; Lasser et al., 1958; Briese et al., 2007)			
Sutherlandia frutescens (L.) R.Br. (Fabaceae)	Decoctions used to wash wounds and eyes and bring down fever. The leaf and stem infusions are used to treat cancers, fever, diabetes, rheumatism, stomach, kidney and liver ailments (Van Wyk, 2002; Thring and Weitz, 2006)	Dried plant material	*P. aeruginosa* ATCC 35032	Inhibited (≥80%) violacein production at 250µL and reduced (≥90%) of *P. aeruginosa* biofilm biomass formation. It also reduced (80%) pyocyanin production and 80% LasB Elastase activity (Cosa and Chenia, 2014)
Tamarix aphylla (L.) Karst. (Tamaricaceae)	Seed extracts are used in the treatment of mild digestive disorders, diarrhea, flatulence, dyspepsia, to improve liver function and morning sickness; also considered to be carminative, eupeptic and antispasmodic (Sahoo et al., 2014). It has anti-diabetic (Dhandapani, et	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Inhibition of *P. aeruginosa* isolate 2 biofilm biomass formation. Anti-QS was by reduction (63%) of Las A protease activity (Al-Refi, 2016)

	al., 2002), antioxidant, anti-bacterial and anti-fungal properties (Romagnoli et al., 2010)			
Terminalia catappa L. (Combretaceae)		Leaves	*Chromobacterium violaceum*, *Pseudomonas aeruginosa*	Inhibition of violacein production by *C. violaceum* and inhibition of biofilm biomass maturation (Taganna, et al., 2011)
Terminalia chebula Retz. (Combretaceae)	Dried fruit used as an antitussive, cardiotonic, homeostatic, diuretic and laxative (Tewari et al., 2017)	Fruit	*Burkholderia cepacia*	Reduction of *B. cepacia* biofilm biomass formation (Huber et at., 2003)
Thymus vulgaris L. (Lamiaceae)	Used as disinfectants, flavoring agent. It can be applied as vaporizers against different human pathogenic Gram-positive and Gram-negative bacteria and yeasts (Varga et al., 2015).	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Reduction of Las A protease activity in *P. aeruginosa* isolate 1 and reduced (60%) biofilm formation at 0.6mg/ml, (Al-Refi., 2016)
Triticum aestivum L. (Poaceae)	This plant is an important human food crop. Young stems are used to treat biliousness and intoxication.	Straw	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Slight reduction (20%) in *P. aeruginosa* isolate 2 biofilm formation, and reduction (44.3%) of pyocyanin

	Ash is used to remove skin blemishes, while the fruit is antipyretic and sedative. The seed promotes female fertility (Duke and Ayensu, 1985). The light grain is antihydrotic, for the treatment of night sweats and spontaneous sweating (Yeung, 1985)			production in *P. aeruginosa* isolate 1 (Al- Refi, 2016)
Urtica dioica L. (Urticaceae)	Used as tea to treat kidney, gastrointestinal and urinary tract infections, locomotors system, skin, cardiovascular system, haemorrhage, influenza, rheumatism, and gout (Vogl et al., 2013)	Leaves	Clinical isolates of *P. aeruginosa* 1, 2, *S. aureus* and *C. albicans*	Anti-QS activity was by reduction (44.3%) of pyocyanin production in *P. aeruginosa* isolate 1 and decreased activity (33%) of Las A protease.
Vachellia karroo (Hayne) Banfi & Galasso (Fabaceae)	Used medicinally to treat diarrhea, colds, dysentery, conjunctivitis and haemorrhage (Van Wyk et al., 1997; Van Wyk and Gericke, 2000)	Leaves	Pathogenic strain of *Listeria monocytogenes* (LMG21263)	Anti-BF activity of the isolated compound (β-sitosterol) from *V. karroo* showed decreased cell aggregation of listeria cells (inhibited listeria biofilm formation) (Nyila, et al., 2011)
Warburgia salutaris	Used as food, fuel, tannin, and the inner bark to treat	Leaves	*P. aeruginosa*	≥80% inhibition of pyocyanin and LasB Elastase production

(G.Bertol.) Chiov. (Canellaceae)	malaria, colds, chest complaints, coughs, diarrhea, muscle pains, stomach-ache and general body pains (Orwa et al., 2009)		in *P. aeruginosa* (Cosa and Chenia, 2014)

2.6 Future perspectives and conclusion

Following the upsurge in drug resistance shown by many pathogenic microbial organisms worldwide, it is essential to improve drug discovery efforts to identify bioactive metabolites against clinically relevant bacterial and fungal strains. In view of this, testing methods should go beyond dealing with the planktonic stage of the microorganisms but focus more on the activities of the microbial community (quorum sensing and biofilm formation). It is also important that in determining the potential of South African medicinal plants in antimicrobial drug discovery, in vivo bioassays as well as more relevant in vitro bioassays should be considered.

Quorum sensing inhibition or interfering with the communication route of the causative pathogen offers new hope for mitigating and treating infectious diseases (Bacha et al., 2016). It is imperative to identify potential substances (such as secondary metabolites) that could restrict the communication systems of pathogens by interfering with the auto-inducer molecules or their expression to control microbial diseases. Again, it is also necessary to find new leads that can prevent/inhibit microbial cell adhesion, viability, metabolic activity and biofilm formation. This will ultimately ensure food security which is the constant availability of acceptable world food supplies of basic foodstuffs to sustain a steady growth of food consumption to offset instabilities in production and prices (FAO, 2006). Food security occurs when people have ready economic and physical access to safe, adequate and nutritious food to meet their dietary needs and food preferences (FAO, 2003). Food safety is a shared responsibility, as reported by the WHO (2015). The findings of the report emphasize the importance and indeed the global threat posed by foodborne diseases and support the need for the food industry and governments as well as individuals to ensure that food is safe as a means of preventing foodborne diseases (WHO, 2015). Alternative and complementary approaches to combating foodborne pathogens are needed and bioactive compounds or extracts derived from plants may offer one solution to the problem. Most antibacterial investigations of plants to date have focused on analyzing activity of extracts and

purified plant compounds against planktonic forms of bacteria. A major component of bacterial ability to avoid antibiotic action is by forming protective, resistant biofilms. With its vast plant diversity, South Africa has much to offer in terms of research on discovery of extract preparations or isolated plant constituents with significant efficacy against bacterial quorum sensing and biofilm development.

Chapter Three: In vitro antibiofilm and antioxidant activities and cytotoxicity of nine South African medicinal plants

3. Introduction

The problem of bacterial resistance to antimicrobial agents and biofilm formation by pathogens dates back to the mass production of penicillin in 1945 (Shaughnessy, 2007). Today, antibacterial resistance has become a foremost concern in medicine all over the world, with resistance to several groups of antibiotics being reported. Microbial resistance to antibiotics can be characterized into two forms: innate resistance, which indicates certain microorganisms are inherently resistant to many antibiotics, and acquired resistance, which can result from a number of varied mechanisms like mutation and horizontal gene transfer (Tenover, 2006).

Bacteria often act as a group despite being unicellular organisms. This explains the formation of biofilm, where individual bacterial cells perform different activities. There is a threat of bacterial communities representing a progression in the impact between unicellular and non-specialized organisms and multicellular organisms that operate in specialized ways by maintaining their population density (McLean et al., 1997). Some of the organisms forming biofilms are associated with food spoilage, for example *Staphylococcus* spp., *Bacillus* spp., *Yersinia enterocolitica, Pseudomonas* spp., *Salmonella* spp., *E. coli* and *Campylobacter jejuni* amongst others (Bridier et al., 2014).

The adherence of these foodborne pathogens to food products or food processing surfaces results in severe public health risks and huge economic losses (Chmielewski and Frank, 2003). Bacterial migration or settlement of non-living material (biofilm formation) has detrimental effects in medicine and in some economic fields such as growth on the wall of fermentation processes,

contamination of food processing equipment, contamination of water pipelines, industrial piping, ventilation, medical devices and artificial organ contamination, as well as pathological human tissues and organs. Bacterial biofilms are generally pathogenic and often cause nosocomial infections. Biofilms have great significance for public health and food industries, as biofilms can render inhabitants on such films resistant to antimicrobial agents and cleaning because they have adapted to exist as adherent populations. Sessile (adherent population) bacteria seem to be protected in these antagonistic environments by growing as colonies surrounded by an extracellular matrix of carbohydrate or exopolysaccharide. The sensitivity of biofilms to antibiotics and other antimicrobial agents cannot be ascertained using a model microdilution technique/testing. This is probably because these tests rely on the response of the planktonic, or suspended, form of the organism rather than the biofilm, or surface-associated, stage of the organisms. Susceptibility should rather be determined directly against biofilm-associated organisms under conditions simulating the in vivo situation (Costerton et al., 1999; Costerton et al., 1995; Rosser et al 1987; Mah and O'Toole 2001).

The United States of America (USA) National Institutes of Health (NIH) report that among all chronic and microbial infections, 65% and 80% respectively are caused by biofilm formation (Jamal et al., 2018). Hence, microbial activity is probably the commonest cause of food spoilage (Christensen et al., 2003). These have made it necessary to investigate the adhesion processes of the microorganism and surface contents. In order to reach this objective, increasing attention is being paid to the physiology and genetics of the initial stages of adhesion and the description of the regulatory networks allowing the developmental processes necessary for the structural development of biofilms and the usage of plants with anti-biofilm activities. This would help find non-toxic surface treatments that can detach the microorganism from the location they usually contaminate, especially in in-dwelling devices. This has led to the search for microbiologically active medicinal plants with useful mechanisms of action. Natural products are known to

significantly interfere with microbial cell-to-cell communication routes that can induce biofilm formation (Koh et al., 2013).

In this study, nine South African plants were selected following a comprehensive literature review based on earlier reported antibacterial activities, ethnopharmacological use, availability and chemotaxonomic similarities to other plant species used customarily for the management of foodborne diseases such as diarrhea. Extracts of these plants were screened for antibacterial and anti-biofilm activity against a range of bacteria implicated in causing foodborne disease. The antioxidant and level safety of the extracts of the plants were also assessed for detection of additional positive attributes.

3.1 Botanical descriptions and reported biological activities of plant species used in this study

Combretum elaeagnoides Klotzsch (*Combretum stevensonii* Excell)

Combretum elaeagnoides belongs to the family Combretaceae. It is generally called large-fruit jesse-bush and oleaster bushwillow in English and Vaaljesse-boswilg in Afrikaans. It is a small deciduous tree. The opposite leaves are rarely 3-whorled, narrowly elliptic, generally hairless with minute silvery scales on both surfaces. Flowers are in short, dense axillary spikes and creamy-white. It produces flowers when the tree is leafless. Fruits are 4-winged, 2-3.5 cm long (Hyde et al., 2019). *C. elaeagnoides*, like other Combretaceae species, are broadly distributed within the tropics of Africa, South America and Asia. No pharmacological activity had been reported on this species at the time of this study.

*Combretum oxystachyum (*Welw). ex. M.A Lawson

Combretum oxystachyum, commonly known as the bottlebush combretum, also belongs to the family Combretaceae. It is a scrambling shrub of 1-3 m in height with bark peeling in stringy strips. The leaves are longitudinally folded, ovate to lanceolate, and the upper surface is dull green with dense to scattered white hair. The flower is dense, with erect spikes, large and very striking with crimson to red-brown stamens. The fruit is typically five winged (Curtis and Mannheimer, 2005). No pharmacological information on this species could be obtained at the time of compilation of this report.

***Combretum molle* R.Br. ex G.Don**

Combretum molle, commonly known as velvet bushwillow (English), baster rooibos (Afrikaans), umbondwe-omhlope (Zulu), moduba (Tswane), mokgwethe (North Sotho), also belongs to the family Combretaceae. It is commonly distributed in six of the nine provinces of South Africa, namely Gauteng, the Free State, Limpopo, KwaZulu-Natal, Mpumalanga and North West. It is a medium-sized evergreen deciduous tree with a rounded crown which can grow up to 13 m in height. It has grey bark when young but this may become grey-brown or nearly black when the tree is older. The simple leaves are opposite, compactly covered by velvety hairs when juvenile and smoother when developed. Young leaves are striking with light pink or orange colour. It flowers between September and November. The flowers occur in dense axillary spikes with a greenish yellow colour. Their strong scent is attractive to bees and other insects. The fruit is 4-winged, about 20 mm in diameter and light green with a reddish tinge, turning red-brown when dry (Thabo, 2011).

C. molle is used for the treatment of abdominal pain, fever, and convulsions, hookworm, snake bite, leprosy, dysentery, chest complaints, headaches, diarrhea and infertility in women. It is also used to stop bleeding after childbirth, to fatten babies and as a dressing for wounds (Mabogo,

1990, Bessong et al., 2005). It has also been reported to have the following pharmacological activities: analgesic, anti-inflammatory, anthelmintic and anti-schistosomal effects (McGaw et al., 2001, Ojewole 2008), antibacterial activity (Geyid et al., 2005, Njume et al., 2011), antifungal activity (Baba-Moussa et al., 1999, Masoko et al., 2007; Mogashoa, 2017) and antitrypanosomal activity (Kloos et al., 1987, Atindehou et al., 2004, Ademola and Eloff, 2010).

***Carpobrotus edulis* (L.) N.E.Br.**

Carpobrotus edulis is widely distributed in South Africa and belongs to the family Aizoaceae. *C. edulis* is usually called sour fig or Cape fig in English, Kaapsevy, hottentotsvy in Afrikaans, ikhambi-lamabulawo and umgongozi in Zulu. It is a robust, trailing perennial growing flat on the ground. It roots at the nodes to form dense mats. The soft horizontal stems bend up at the growing point. The succulent leaves are crowded laterally on the stem which is 60-130 x 10-12 mm, sharply 3-angled and triangular in cross-section, yellowish to grass-green in colour and becoming reddish when fully mature.

It is used traditionally to treat diarrhea, dysentery and stomach cramps, eczema, wounds, toothache, earache and serves as a valuable source of natural antioxidants (Malan and Notten, 2006). *C. edulis* has been reported to show anti-inflammatory and antioxidant activities (Omoruyi et al., 2012; Ondua et al., 2019), antibacterial effects (van der Watt and Pretorius 2001) and antifungal potential (Henley-Smith 2011).

***Vachellia rehmanniana* (Schinz) Kyal. & Boatwr.**

Vachellia rehmanniana is a small, flat-crowned tree with young branches compactly shielded with golden, furry hairs that become grey and peel off to expose a powdery, rusty-red bark with striking grey-green, velvety leaves. The spines are long and straight, white with a reddish-brown tip. The

flowers are white balls, clustered at the ends of the young branches. The fruit is a straight, flat greyish-brown pod (Glenice Ebedes, 2018). *V. rehmanniana* belonged to the Fabaceae family and was previously called *Acacia rehmanniana.* No traditional use and pharmacological activity on this species were found.

Vachellia xanthophloea (Benth.) P.J.H. Hurter

Vachellia xanthophloea, formerly called *Acacia xanthophloea* belongs to the family Fabaceae. *V. xanthophloea* is usually called the fever tree in English, Koorsboom (Afrikaans), More o Mossetha (Tswana), Muunga-gwena (Venda), Umhlosinga (Zulu) and nkelenga (Tsonga). It is a striking, tall, deciduous, fast growing tree with distinctive greeny-yellow, smooth and powdery bark. It bears fragrant, yellow flowers from August to November and is widely distributed across South Africa. It is used as medicine to treat diabetes, high cholesterol, cancer, pharyngitis, gingivitis and mouth sores. The bark is additionally employed to treat fevers and eye complaints. The bark decoction is used to treat indigestion in Kenya while in Tanzania it is used to treat sickle-cell anaemia. The Zulu people of South Africa use the powdered bark as an emetic to treat malaria. The wood is used in the building industry as it is heavy, hard and a useful general-purpose timber; however it should first be well-seasoned to avoid cracking (Exotichealingherbs, 2012).

V. xanthophloea possesses antibacterial, antioxidant and anti-carcinogenic activities (Katerere and Eloff, 2004; Exotichealingherbs, 2012).

Kigelia africana (Lam.) Benth.

Kigelia africana belongs to the family Bignoniaceae, commonly known as cucumber or the sausage tree. It is a tree that grows up to 20 m (66 feet) tall with spreading branches. It grows a

sausage-shaped fruit up to 2 feet in length and weighs about 15 lbs. In Malawi, during famine the roasted seeds are eaten as food. The baked fruits are used to ferment beer, and boiled ones yield a red dye. It is majorly used by traditional healers to treat several skin ailments like fungal infections, boils, psoriasis and eczema. It is also internally usedtreat dysentery, ringworm, tape-worm, post-partum haemorrhage, malaria, diabetes, pneumonia and toothache (Gill, 1992). The fruits are used as as purgatives and to increase the flow of milk in lactating women. The boiled chopped bark and fruit are taken orally for blood cleansing and pelvic pains during pregnancy (De Wet and Ngubane, 2014). *K. africana* possesses antibacterial activity and antioxidant properties (Sandasi et al., 2014, Hussain et al., 2016).

Elephantorrhiza elephantina **(Burch.) Skeels**

E. elephantina of the family Fabaceae is popularly known as the elephant's root or eland's wattle, and is a subshrub in the mimosoid clade of legumes. The plants occur widely and in several areas of southern Africa. In Botswana, the anus of children with bloody diarrhea is wiped with the rhizome or root powder (Hedberg and Staugard, 1989). The root, aerial parts, and bulb are used for diarrhea, heartwater, coughing and pneumonia (Van der Merwe et al., 2001). The rhizome, roots, leaves and stem are used to treat diverse human and animal ailments (Maroyi, 2017). It is also used in Mozambique, South Africa and Swaziland to treat diarrhea (Van Wyk et al., 2009)

E. elephantina possesses anthelmintic, anti-inflammatory and anti-nociceptive properties (Maphosa et al., 2009), antibacterial and antifungal activities (Aaku et al., 1998), anti-antioxidant (Mpofu et al., 2014), antiplasmodial (Clarkson et al., 2004), antirickettsial and antibabesial effects (Naidoo et al., 2005).

Ochna pretoriensis **E. Phillips**

Ochna pretoriensis belongs to the family Ochnaceae. It is commonly known as Magalies plane in English and Magalies-rooihout in Afrikaans. It is a multi-branched evergreen shrub that grow to about 2 m tall. The stems have rough brown bark, which as it grows older flakes in thin, small, longitudinal strips. The main stem is pale grey to white and is generally covered in white lenticels. It has a distinguishing yellow flower colour and fragrance which appeals to birds and insects. *Ochna pretoriensis* is extensively distributed. It can be found within the northern provinces of South Africa and is a very common tree in the area around Pretoria. It is also found growing in Gaborone, Botswana. It may grow on rocky outcrops on mainly north-facing slopes, at altitudes between 500 and 1520 m (Grunyuza et al., 2008). Studies have shown that *O. pretoriensis* possesses antibacterial activity (Makhafola et al., 2012), but no reports of traditional uses could be found.

3.2 Materials and methods

3.2.1 Collection of plant species

The leaves of the plants were sourced from the Pretoria National Botanical Garden (SANBI), Pretoria and the Lowveld National Botanical Gardens (LNBG) in Nelspruit. The plant samples were appropriately cleaned and dried in a well-ventilated room at room temperature for two to four weeks. Herbarium voucher specimens were prepared and deposited at the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria, South Africa (Table 3.1). The dried plant material was milled to fine powder and kept in glass jars in the dark until required.

Table 3.1. Selected plants and their herbarium accession numbers

Plant species	Family	Accession numbers
Carpobrotus edulis (L.) N.E.Br.	Aizoaceae	PRU 125173
Kigelia africana (Lam.) Benth.	Bignoniaceae	PRU 125172
Combretum elaeagnoides Klotzsch	Combretaceae	PRU 123603
Combretum molle R. Br. ex G. Don	Combretaceae	PRU 125175
Combretum oxystachyum (Welw). ex. M.A Lawson	Combretaceae	PRU 123602
Elephantorrhiza elephantina (Burch.) Skeels	Fabaceae	PRU 125174
Vachellia rehmanniana (Schinz) Kyal. & Boatwr.	Fabaceae	PRU 125170
Vachellia xanthophloea (Benth.) P.J.H. Hurter	Fabaceae	PRU 125171
Ochna pretoriensis E. Phillips	Ochnaceae	PRU 125176

3.2.2 Extraction of plant material

The finely ground plant material was extracted following standard methods as described by Eloff (1998a). In brief, the various powdered plant materials were extracted separately with two solvents, namely acetone (100%) and 80% methanol in a ratio of 1:10 (w: v) of plant material to solvent. The mixtures were soaked at 25°C for 48 h and filtered via Whatman No 1 filter paper. The supernatant was poured into weighed labelled glass vials. The extraction process was repeated twice to fully extract plant material. The solvent was evaporated in a BUCHI Rotavapor (Labotech (PTY) Ltd. under reduced pressure at 40°C until left with a residue which was thereafter dried under cold air and used to calculate the percentage yield using the formula

Percentage yield = (Mass of extract / Mass of powdered plant material) X 100

3.2.3 Test bacterial strains

Gram-positive and Gram-negative ATCC bacterial strains and clinical bacterial strains obtained from the collection of the Phytomedicine research laboratory in the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria were used in this study. The bacterial strains included:

- *Salmonella* Typhimurium ATCC 39183
- *Salmonella* Enteritidis ATCC 13076
- *Escherichia coli* 1 ATCC 25922
- *Escherichia coli* 2 *(*Clinical isolate)
- *Staphylococcus aureus* ATCC 29213
- *Campylobacter jejuni* ATCC 33560
- *Sterotrophonas maltophilia* (Clinical isolate)
- *Klebsiella pneumoniae* (Clinical isolate)
- *Enterobacter cloacae* (Clinical isolate)

Besides these bacterial strains representing Gram-positive and Gram-negative categories, they belong to the ecologically rich, hence potential contaminants, and common residents of the human body including skin (*S. aureus*), food and gastrointestinal tract (*E. coli*). The commencement of the pathogenicity processes in certain bacterial strains like *S. aureus* (that produces enterotoxin) depend on the density of the cell-to cell signaling (QS) molecules that can lead to the formation of biofilm, which subsequently is the probable target of plant extracts. Consequently, the inclusion of the aforementioned bacterial strains is rational and justifiable.

3.2.4 Culture of bacterial inoculum

The bacteria were grown on Mueller Hinton agar (MHA) (Fluka, Spain) at 4°C. The bacterial cultures were cultured in Mueller Hinton broth (MHB) for at least 12-16 h at an incubation temperature of 37 °C. MHA and MHB were used for the assessment of the minimum inhibitory concentration tests, while Tryptic Soy agar (TSA) and Tryptic Soy broth (TSB) were used in the minimum bactericidal concentration and biofilm assays respectively.

3.2.5 Bioassay for antibacterial activities

3.2.5.1 Assessment of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) is the least concentration of the extracts at which the microorganism does not show visible growth or revealed inhibition of growth. This was determined following the method of Eloff (1998b). The extracts were tested at a starting concentration of 2.5 mg/ml (from a stock concentration of 10 mg/ml) in a 96-well microtitre plate and serially diluted two-fold to 0.02 mg/ml. Following this, 100 μL of the overnight bacterial culture at a known inoculum (standardized by measuring the absorbance of the diluted culture at 560 nm compared to Mcfarland No. 1 standard) was added to each well. The positive control, gentamicin, with a serially diluted concentration range between 0.5 to 0.004 mg/ml, was used as the reference drug for the assay. The microtitre plates were covered with lids and parafilm and incubated overnight for at least 18 h at 37°C. As an indicator of bacterial growth, 40 μl *p*-iodonitrotetrazolium chloride (INT) (Sigma, 0.2 mg/ml) dissolved in sterile distilled water was added to the wells and incubated at 37°C for 1h. The MIC values were recorded as the least concentration of the extract that inhibited the growth of the bacteria, as indicated by a marked reduction in colour formation. The INT turns to a red-pink formazan where bacterial growth is not inhibited. The assays was repeated three times.

The minimum bactericidal concentration (MBC) was assessed by adding aliquots of 50 μL of the preparations from the concentrations which did not show any bacterial growth after incubation during the MIC assay to 150 μL of freshly prepared TSB and spread on a TSA plate. These preparations were incubated at 37°C for 24h. The least concentration of extract with no bacterial growth (100% inhibition), was taken as the MBC value. This was done using the method by Cohen et al. (1998) with slight modification.

3.2.5.2 Total activity

The total activity which indicates the volume to which active compounds in one gram of plant material can be diluted and still inhibit the growth of the tested organisms was also determined using the formula below (Eloff, 2004).

Total activity= quantity of extract residue from 1g of plant material in mg / MIC in mg/ml.

3.2.6 Anti-biofilm assay

3.2.6.1 Inhibition of biofilm formation

The inhibition of biofilm biomass formation was evaluated via the modified protocol described by O'Toole and Kotler (1998), Mohsenipour et al. (2015) and Sandasi et al. (2010) with slight modification. Biofilms were allowed to form for 24 h (T24). Biofilm formation was attained by pipetting 100 μl of the respective culture (OD_{590} = 0.02 or 1.0 x 10^6 CFU/ml) into a sterile flat bottomed 96-well microtitre plate and sterilely sealed with a sealing tape. One hundred microliter (100 μl) of the sample (at a final concentration of 1 mg/ml from a stock of 2 mg/ml) and the respective controls were transferred to the wells of the sterile plate and incubated for 24 h at 37°C without shaking. Suitable control wells were included in the plate: negative control (culture+ media

(TSB)), positive control (culture + TSB + antibiotic), sample control (sample + TSB), antibiotic control (antibiotic + TSB) and media control (TSB) for each test batch. After incubation, the modified crystal violet staining (CVS) assay (Sandasi et al., 2010) was performed to assess the biofilm biomass.

3.2.6.2. Crystal violet staining (CVS) assay

In the CVS assay, first, the wells were carefully emptied and washed at least three times with sterile distilled water to remove any unattached cells. The plates were then air-dried and 150 µl of 96% methanol added to the wells for 15-20 minutes to fix the adherent cells. The plates were emptied, and the adhered cells stained with 100 µl of 0.1% crystal violet solution for 20 min at room temperature while covered with the microtitre lids. The plates were washed at least five times with tap water to rinse off any excess stain. Afterwards, the biofilm biomass was evaluated semi-quantitatively by re-solubilizing the crystal violet stain bound to the adherent cells with 150 µl of 100 % ethanol. The absorbance of the plates after careful shaking was read at 590 nm using a microplate reader (Epoch™ Microplate Spectrophotometer). The mean absorbance (OD_{590nm}) of the sample was determined and results expressed as percentage inhibition using the equation below.

$$\% \text{ Inhibition} = \frac{(\text{ODnegative control} - \text{ODmedia control}) - (\text{ODsample} - \text{ODsample control})}{(\text{ODnegative control} - \text{ODmedia control})} \times 100$$

3.2.7 Antioxidant activity

3.2.7.1 Assay for free radical scavenging of DPPH radical

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was done according to the method described by Gyamfi et al. (1999) with slight modifications. First, the optical density (OD) of the DPPH solution was calibrated at 517 nm to between 0.9 and 1.00. Thereafter, the DPPH solution (160

µL) was added to 40 µL of each crude extract at serially diluted concentrations (3.125–400 µg/mL). The mixture was incubated for 30 min in the dark, and absorbance was then measured at 517 nm using a microplate reader (Epoch, Biotek). Positive controls were ascorbic acid (Vitamin C) and Trolox. Higher free radical scavenging activity was reflected by low absorbance values.

The percentage scavenging activity was calculated using the formula as follows:

% DPPH scavenging activity = [(A0 − As)/A0] × 100

Where A0 is absorbance of control (DPPH solution without sample), and As is absorbance of tested sample (DPPH plus sample). Linear and non-linear regression curves where necessary of percentage scavenging activity against the actual or logarithm of concentrations was used to determine the 50% inhibitory concentration (IC_{50}) values of the extracts. Each test was done three times and results are presented as mean± standard error of mean (SEM).

3.2.7.2 The 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) electron reduction assay

The protocol outlined by Re et al. (1999) was employed in determining the ability of the extracts of the selected plants to inhibit the ABTS radical. A stock solution of the ABTS radical was initially prepared by dissolving the powdered ABTS (0.0720 g) in 20 ml of methanol and mixing with separately prepared potassium persulfate (0.0132 g) dissolved in 20 ml of methanol and left at room temperature in the dark for 12–16 h. The working solution was obtained by calibrating the stock solution to obtain an optical density (OD) of 0.70 ± 0.02 at 734 nm. The ABTS working solution (160 µL) was mixed with the samples (40 µL) at serially diluted concentrations (3.125–400 µg/mL), and the absorbance was measured after 7 min at 734 nm using a microplate reader (Epoch, BioTek).

The percentage of scavenging activity was calculated using the formula:

% scavenging activity= {(AO- AS)/AO} X 100

The 50% inhibitory concentration (IC_{50}) values of samples were determined using linear and non-linear regression curves where necessary of the percentage of scavenging activity against the actual or logarithm of concentrations. Ascorbic acid and trolox were used as positive controls. Each test was done three times and results presented as mean ± standard error of mean (SEM).

3.2.8. Cytotoxicity

The 3-(4, 5-dimethylthiazolyl-2)-2.5-diphenyltetrazolium bromide (MTT) colorimetric assay was used to measure effects on cell proliferation and cytotoxic level of the plant extracts using the method described by Mosmann (1983) with slight modification (McGaw et al., 2007). MTT is a yellow water-soluble dye which is reduced by living cells to a purple formazan product. The intensity of the colour (measured spectrophotometrically) of the MTT formazan converted by metabolically active cells is proportional to the number of live cells present. The extracts were tested for cytotoxicity against Vero African green monkey kidney cells. The cells of a sub-confluent culture were harvested and centrifuged at 200 × g for 5 min and re-suspended in growth medium. Minimal Essential Medium (MEM, Whitehead Scientific) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Gibco, Sigma-Aldrich) was used as growth medium. A total of ten thousand cells were seeded in each well (in 100 µL of MEM) of columns 2 to 12 of a sterile 96-well microtitre plate (Whitehead Scientific). A 100 µL aliquot of the growth medium (MEM) was added to wells of columns 1 and used as blank. The plates were incubated for 24 h at 37°C in a 5% CO_2 incubator. The MEM was aspirated from the cells using a suction pump and replaced with 200 µL of the test extracts at a range of concentrations (0.0075 mg/ml to 0.1 mg/ml) and the plates were incubated at 37°C in a 5% CO_2 incubator for a further 48 h. Each dilution was tested in quadruplicate. Untreated cells and positive control (doxorubicin chloride, Pfizer Laboratories) were also included.

After incubation, the MEM containing test substance was removed, and the cells were washed with PBS, and fresh MEM (100 µL) added to each well. Then, 30 µL MTT (stock solution of 5 mg/ml in PBS) was added to each well, and the plates were re-incubated for another 4 h at 37°C. After removing the MEM and MTT, the MTT formazan crystals were dissolved by adding 50 µL of DMSO to each well. The plates were shaken gently until the MTT solution dissolved for about 2 min. The amount of MTT reduction was measured immediately by reading the absorbance using a microplate reader at a wavelength of 570 nm with a reference wavelength of 630 nm. The wells in column 1, which earlier contained medium and MTT but without cells were used to blank the plate reader. The LC_{50} values were calculated using linear regression as the concentration of test compound resulting in a 50% reduction of absorbance (implying killing 50% of the cells) compared to untreated cells.

3.3 Results and discussion

3.3.1 Percentage yields of the different plant extracts

Acetone (100%) and 80% methanol were used to extract leaf material of the plants to produce the extracts. Methanol (80%) and acetone were selected because they have been used to extract various polarities of antimicrobial metabolites from plants, with 80% methanol being more polar (Cowan, 1999). Eloff (1998a) stated that acetone can extract both polar and non-polar compounds in plants, and has less toxicity to microorganisms in bioassays.

The acetone extract of *V. xanthophloea* had the highest percentage yield of 42.4 % while the 80% methanol extract of *C. elaeagnoides* had the most abundant yield in grams (84.92 g) amongst the extracts (Table 3.1). The acetone extract of *Ochna pretoriensis* yield in percentage and grams was not determined due to insufficient powdered sample, so only the 80% methanol extract was prepared. Percentage yield =

$$\frac{\text{mass of extract residue}}{\text{mass of dry plant sample}} \times 100$$

Table 3.2. The mass in grams (g) and percentage yield (%) of the selected nine plant species

Plant species	Mass of extracts (g)		Percentage yield of extracts (%)	
	80% methanol	**Acetone**	**80% methanol**	**acetone**
C. elaeagnoides	84.92	2.16	18.38	14.4
C. oxystachyum	5.29	7.22	13.23	18.05
C. molle	8.17	3.09	27.23	10.30
C. edulis	4.56	6.00	16.29	21.43
V. rehmanniana	6.17	29.53	6.17	29.53
V. xanthophloea	5.81	8.48	29.05	42.40
K. africana	3.79	11.16	9.48	27.90
E. elephantina	1.40	2.08	3.33	4.94
O. pretoriensis	2.66	-	35.47	-

- = Not determined due to insufficient sample

3.3.2. Antimicrobial effects (MIC and MBC) of the extracts of the nine selected plants

The leaf extracts of the nine selected plants were active against all the tested microorganisms with MIC values ranging between <0.02 to 2.5 mg/ml. The best MIC value of <0.02 mg/ml was obtained against *E. cloacae* by the acetone extracts of *C. molle, V. xanthophloea* and *C. edulis* while the methanol extract of *V. xanthophloea* (MIC = 2.5 mg/ml) had the weakest inhibitory activity against *S.* Typhimurium (Table 3.3).

The methanol and acetone extracts of *C. elaeagnoides* had the best inhibitory activity against *E. coli1* (MIC =0.04 and 0.08 mg/ml respectively). The acetone extract of *C. molle* had the strongest inhibitory activity against *S. aureus* (MIC= 0.08 mg/ml). The acetone extracts of *V. xanthophloea* and *C. molle* were the only extracts with very strong inhibitory activity (MIC = 0.03 mg/ml) against *S.* Typhimurium. The methanol and acetone extracts of *V. xanthophloea* also had very good inhibitory activity against *S.* Enteritidis (MIC = 0.26 and 0.03 mg/ml). Interestingly, the methanol and acetone extracts of *C. molle* had the best inhibitory activity against *C. jejuni* (MIC = 0.21 and 0.09 mg/ml) and *K. pneumoniae* (MIC = 0.11 and 0.03 mg/ml) while the acetone extract particularly had the best inhibitory activity against S. *maltophilia* (MIC = 0.05 mg/ml). The acetone extracts of *K. africana, C. molle, C. edulis* and *V. xanthophloea* were all active against *E. coli*2 and had the same MIC values of 0.03 mg/ml. The acetone extracts of *C. edulis*, *V. xanthophloea* and *C. molle* had similar excellent activity against *E. cloacae* (MIC < 0.02 mg/ml).

All the test organisms were more susceptible to the acetone extract of *C. molle* compared to the other plant extracts with MIC values between < 0.02 and 0.47 mg/mL. Of the nine test organisms, five (*C. jejuni*, *S. maltophilia, K. pneumoniae, E. coli*2 (clinical isolate and *E. cloacae)* were most susceptible to both extracts of *C. molle* with the MIC value ranging between < 0.02 and 0.63 mg/mL. The findings of Mogashoa (2017) on the inhibitory activity of the acetone extract of *C. molle* against *E. coli* ATCC 25922 and *S. aureus* (MIC = 0.26 and 0.17 mg/mL respectively) corroborates the findings of this study. MBC activity was not assessed on

those samples with MIC value of 2.5 mg/ml. The acetone extracts of *C. molle*, *C. edulis* and *V. xanthophloea* had the best MBC value (0.02 mg/ml) against *E. cloacae* with complete (100%) inhibition (Table 3.4).

Table 3.3. Minimum inhibitory concentrations of the nine selected plants in mg/ml

Plant spp.	Test organisms									
	E. coli1		***S. aureus***		***S.* Typhimurium**		***S.* Enteritidis**		***C. jejuni***	
	MeOH	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**
Ce	0.04±0.00	0.08±0.00	0.26±0.05	0.16±0.00	0.42±0.11	0.26±0.05	0.84±0.21	1.25±0.00	0.26±0.05	0.16±0.00
Co	0.84±0.21	0.21±0.05	0.52±0.11	0.52±0.00	1.04±0.21	0.52±0.11	0.42±0.11	0.52±0.11	2.08±0.42	1.67±0.42
Cm	0.47±0.16	0.12±0.04	0.52±0.11	0.08±0.00	0.52±0.11	0.03±0.01	0.52±0.11	0.12±0.04	0.21±0.05	0.09±0.04
Ee	0.26±0.05	0.42±0.11	0.42±0.11	0.52±0.11	1.04±0.21	0.52±0.11	0.42±0.11	0.63±0.00	1.25±0.00	1.04±0.21
Op	0.62±0.31	ND	0.62±0.31	ND	0.26±0.05	ND	0.52±0.11	ND	0 .84±0.21	ND
Ka	0.52±0.11	1.88±0.62	1.04±0.21	0.84±0.21	0.42±0.11	0.26±0.05	0.42±0.11	0.84±0.21	0.84±0.21	0.84±0.21
Vr	0.52±0.11	0.21±0.05	0.26±0.05	0.21±0.05	0.26±0.05	0.26±0.05	0.42±0.11	0.52±0.11	1.04±0.21	0.52±0.11
Vx	0.26±0.05	0.12±0.04	0.26±0.05	0.12±0.04	2.5±0.00	0.03±0.01	0.26±0.05	0.03±0.01	0.84±0.21	0.12±0.04
Ced	0.52±0.11	0.52±0.11	0.26±0.05	0.47±0.16	0.84±0.21	0.47±0.16	0.84±0.21	0.84±0.21	1.15±0.68	0.84±0.21
Control	<0.004		< 0.004		0.008		0.008		0.06	

Table 3.3 Contd.

Plant spp.	*S. maltophilia*		*K. pneumoniae*		*E. coli2*		*E. cloacae*	
	MeOH	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**
Ce	0.84±0.21	0.42±0.11	0.42±0.11	0.21±0.05	0.63±0.00	0.31±0.00	0.63±0.00	0.42±0.11
Co	0.42±0.11	1.67±0.42	0.52±0.11	0.42±0.11	0.84±0.21	0.63±0.00	0.84±0.21	0.63±0.00
Cm	0.63±0.00	0.05±0.01	0.11±0.03	0.03±0.01	0.42±0.11	0.03±0.01	0.42±0.11	< 0.02±0.0
Ee	1.04±0.21	0.42±0.11	0.26±0.05	0.42±0.11	0.84±0.21	0.26±0.05	0.31±0.00	0.63±0.00
Op	0.52±0.11	ND	1.04±0.21	ND	1.25±0.00	ND	1.25±0.00	ND
Ka	0.84±0.21	0.21±0.05	0.31±0.00	0.21±0.05	0.42±0.11	0.03±0.01	0.31±0.00	0.03±0.01
Vr	1.25±0.00	1.25±0.00	0.52±0.11	1.04±0.21	0.21±0.05	1.04±0.21	0.31±0.00	0.16±0.00
Vx	0.52±0.11	0.12±0.04	0.13±0.04	0.12±0.04	0.42±0.11	0.03±0.01	0.31±0.00	<0.02±0.00
Ced	0.52±0.11	0.12±0.04	0.63±0.00	0.03±0.01	1.04±0.21	0.03±0.01	0.52±0.11	<0.02±0.00
Gentamicin	0.004		< 0.004		0.004		0.004	

Ce (*Combretum elaeagnoides*), Co (*Combretum oxystachyum*), Cm (*Combretum molle*), Ee (*Elephantorrhiza elephantine*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis),* ND (not determined), MeOH (methanol)

Table 3.4. Minimum bactericidal concentration of the nine selected plants in mg/ml against the test organisms.

Plant species	Test organisms									
	E. coli 1		*S. aureus*		*S.* Typhimurium		*S.* Enteritidis		*C. jejuni*	
	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone
C. elaeagnoides	0.63	0.31	2.5	0.63	>2.5	1.25	>2.5	>2.5	1.25	0.63
C. oxystachyum	2.5	1.25	1.25	2.5	>2.5	2.5	>2.5	2.5	ND	ND
C. molle	1.25	0.31	2.5	1.25	2.5	0.04	>2.5	0.31	2.5	0.31
E. elephantina	2.5	2.5	2.5	2.5	>2.5	>2.5	2.5	2.5	>2.5	>2.5
O. pretoriensis	2.5	ND	2.5	ND	2.5	ND	>2.5	ND	>2.5	ND
K. africana	2.5	ND	>2.5	2.5	2.5	1.25	>2.5	2.5	2.5	2.5
V. rehmanniana	2.5	2.5	2.5	1.25	2.5	>2.5	>2.5	>2.5	2.5	2.5
V. xanthophloea	2.5	0.63	1.25	0.63	2.5	0.08	2.5	0.08	>2.5	0.31
C. edulis	2.5	1.25	1.25	1.25	>2.5	1.25	>2.5	2.5	>2.5	2.5
Gentamicin		<0.004	<0.004		0.016		0.016		0.250	

Table 3.4 contd.

Plant species	*S. maltophilia*		*K. pneumoniae*		*E. coli 2*		*E. cloacae*	
	MeOH	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**
C. elaeagnoides	>2.5	2.5	1.25	0.63	2.5	1.25	2.5	2.5
C. oxystachyum	1.25	ND	2.5	1.5	>2.5	2.5	0.63	2.5
C. molle	2.5	0.16	2.5	0.08	1.25	0.08	2.5	0.02
E. elephantine	1.04	2.5	1.25	1.25	>.5	1.25	2.5	1.5
O. pretoriensis	2.5	ND	>2.5	ND	2.5	ND	>2.5	ND
K. africana	2.5	0.63	1.25	1.25	2.5	0.08	1.25	0.08
V. rehmanniana	ND	ND	2.5	>2.5	1.25	>2.5	2.5	2.5
V. xanthophloea	2.5	0.31	0.63	0.31	2.5	0.08	2.5	0.02
C. edulis	0.31	2.5	0.31	>2.5	2.5	>2.5	< 0.02	0.02
Control	0.008		0.008		0.008		0.008	

ND: Not determined

3.3.3 Evaluation of the total activity of the seventeen extracts of the nine plants

Total activity is the volume at which antibacterial compounds present in 1 g of the different plant samples can be diluted and still inhibit bacterial growth. The results of the total activity are presented in Table 3.5. Extracts with low MIC values against the test organisms with correspondingly high yield of extract had the highest total activity. The acetone extract of *V. xanthophloea* had the highest total activity of 42,800 mL/g, followed by the acetone extract of *C. molle* (21,429 mL/g) against *E. cloacae*. This implies that when 1 g of *C. xanthophloea* and *C. molle* are diluted with 42,800 mL and 21,429 mL of acetone respectively, the extracts will still be active against *E. cloacae*.

Table 3.5. Total activity of the extracts of the nine selected medicinal plants in ml/g

Plant species	Test organisms									
	***E. coli* 1**		***S. aureus***		***S.* Typhimurium**		***S.* Enteriditis**		***C. jejuni***	
	MeOH	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**
Ce	4595.00	2297.50	706.93	1148.75	437.62	706.93	218.81	147.04	706.93	1148.75
Co	157.44	859.52	254.33	347.12	127.16	347.12	318.88	347.12	63.58	108.08
Cm	581.55	858.33	526.63	429.17	131.47	3433.33	526.63	858.33	1301.57	1144.44
Ee	127.96	117.69	79.21	95.06	31.99	95.06	79.21	78.46	26.62	47.53
Op	525.77	-	525.77	-	1253.77	-	626.88	-	388.07	-
Ka	182.21	148.40	91.11	296.81	225.60	1162.5	225.60	296.81	112.80	296.81
Vr	118.63	1406.19	237.31	1406.19	237.31	1135.77	146.90	567.88	59.33	567.88
Vx	1117.31	3566.67	1117.30	3566.67	116.20	14266.67	1117.31	14266.67	345.83	3566.67
Ced	173.26	227.97	626.38	455.94	193.88	455.94	193.88	227.97	141.62	227.97

Table 3.5 Contd.

Plant species	*S. maltophilia*		*K. pneumoniae*		*E. coli* 2		*E. cloacae*	
	MeOH	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**
Ce	218.81	437.62	437.62	706.92	291.75	592.90	391.06	291.75
Co	314.88	108.08	314.88	401.11	209.92	286.51	281.38	384.04
Cm	433.86	1761.67	2484.82	3433.33	650.79	3433.33	581.55	10300
Ee	31.99	117.69	127.96	117.69	39.61	190.12	107.32	78.46
Op	626.88	-	313.44	-	260.78	-	260.78	-
Ka	112.80	1162.50	305.65	1162.5	225.60	9300.00	3158.33	9300.00
Vr	49.36	236.24	118.63	2271.54	237.31	283.94	385.63	1845.63
Vx	556.65	3566.67	2234.62	3292.31	691.67	14266.67	937.10	42800.00
Ced	313.19	1785.75	258.51	7143.00	156.60	7143	313.19	21429.00

Ce (*Combretum elaeagnoides*), Co (*Combretum oxystachyum*), Cm (*Combretum molle*), Ee (*Elephantorrhiza elephantine*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis),* - (Not determined), MeOH (methanol).

3.3.4 Evaluation of the antibiofilm (ABF) potential of nine selected plant species

The effects of the leaf extracts of the nine plants on the growth and development of biofilms formed by the tested foodborne pathogens are presented in Table 3.6. The seventeen extracts had selective antibiofilm (ABF) activity against the foodborne pathogens. The acetone extract of *C. oxystachyum* displayed the strongest inhibitory (252%) activity against *E coli* 1. The acetone extracts of *C. edulis* and *V. rehmanniana* were observed to have ABF activity (> 50% inhibition) against the majority (6 organisms) of the nine tested organisms. All other extracts with greater than 50% inhibition were considered to have good ABF activity. It was observed in this study, like elsewhere (Ofek et al. 2003; Sandasi et al. 2008), that some extracts enhanced the formation of biofilm by the organisms, depicted as 0% inhibition (Table 3.6). This can be attributed to the presence of metabolites or production of conditioning films for microbial adhesion that can enhance the growth and development of biofilms (Ofek et al. 2003; Sandasi et al. 2008, 2010).

Table 3.6. The effect of the methanol and acetone extracts of the nine selected plants on biofilm formation

Sample (extracts)	Percentage inhibition of biofilm (%)								
	Ec1	**Sa**	**St**	**Se**	**Cj**	**Sm**	**Kp**	**Ec2**	**Ent**
Ce (MeOH)	0.000	0.000	0.000	0.000	229.287	0.000	20.344	50.354	0.000
Ce (acetone)	54.304	65.486	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Co (MeOH)	0.000	0.000	129.127	0.000	220.744	0.000	0.000	80.998	0.000
Co (acetone)	252.007	63.116	0.000	0.000	93.896	0.000	0.000	271.353	0.000
Cm (MeOH)	238.385	72.805	0.000	0.000	83.302	0.000	0.000	159.457	72.483
Cm (acetone)	0.000	0.000	246.992	0.000	0.000	0.000	149.369	129.182	0.000
Ee (MeOH)	145.750	78.289	0.000	0.000	94.056	61.619	0.000	170.610	0.000
Ee (acetone)	0.000	90.414	199.456	0.000	14.736	0.000	199.443	0.000	0.000
OP (MeOH)	0.000	0.000	75.206	0.000	73.799	0.000	84.914	63.416	0.000
Ka (MeOH)	0.000	101.431	96.339	0.000	0.000	0.000	8.050	61.270	53.484
Ka (acetone)	0.000	0.000	59.992	0.000	0.000	0.000	71.838	0.000	0.000
Vr (MeOH)	0.000	69.823	126.996	0.000	99.000	0.000	90.521	69.188	55.842
Vr (acetone)	0.000	60.866	96.339	0.000	0.000	0.000	0.000	57.237	95.190
Vx (MeOH)	0.000	0.000	62.762	77.126	96.849	0.000	72.418	122.883	142.422
Vx (acetone)	0.000	0.000	231.992	0.000	0.000	62.208	101.221	115.683	0.000

Ced (MeOH)	0.000	0.000	0.000	0.000	0.000	66.720	66.653	108.751	0.000
Ced (acetone)	0.000	83.210	116.352	0.000	88.620	0.000	197.258	188.354	14.699
CPF	99.570	54.971	78.970	-	92.946	74.042	94.920	90.993	51.870

Ce (*Combretum elaeagnoides*), Co (*Combretum oxystachyum*), Cm (*Combretum molle*), Ee (*Elephantorrhiza elephantine*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis),* - (Not determined), MeOH (methanol), CPF= ciprofloxacin, *Ec 1, 2 (E. coli), Sa (S. aureus), St (S.* Typhimurium*), Se (S.* Enteriditis*), Cj (C. jejuni), Sm (S. maltophilia), Kp (K. pneumoniae), Ent (E. cloacae).*

3.3.5 Evaluation of the antioxidant potential of the nine selected plant leaf extracts

The results of the DPPH and ABTS radical scavenging activity of the nine selected plant leaf crude extracts are presented in Table 3.7. The methanol extract of *C. xanthophloea* and the acetone extract of *C. edulis* had the best antioxidant activity against the DPPH and ABTS radicals with IC_{50} values of 0.14 ± 0.19 µg/ml and 0.01 ± 0.02 µg/ml respectively when compared to the other extracts and controls. The controls (Vitamin C and Trolox) had IC_{50} values of 0.39 ± 0.23 and 2.64± 0.27 against DPPH, and 0.36± 0.35 and 0. 74± 0.47 against the ABTS radical.

Table 3.7. Antioxidant potential of the nine selected South African medicinal plant against the tested foodborne pathogens

Plant species	DPPH (IC_{50} in µg/mL)		ABTS (IC_{50} in µg/mL)	
	MeOH	Acetone	MeOH	Acetone
C. elaeagnoides	3.28± 0.31	18.76±0.25	4.41±0.53	2.17±0.03
C. oxystachyum	6.37±0.20	16.44±1.15	5.71±1.23	0.80±0.17
C. molle	2.37±0.05	5.53±0.07	5.89±0.02	0.70±0.61
E. elephantina	7.65±0.12	>100	3.80±1.29	20.63±1.37
O. pretoreinsis	10.91±1.47	-	10.44±0.91	-
K. africana	5.47±0.08	>100	>100	>100
V. rehmanniana	11.90±0.67	>100	>100	>100
V. xanthophleoa	0.14± 0.11	4.99±0.09	2.83±0.05	0.20±0.13
C. edulis	11.36±0.22	0.25±0.49	2.96±1.97	0.01±0.02
Vitamin C	0.39 ± 0.23		0.36± 0.25	
Trolox	2.64± 0.27		0. 74± 0.47	

- = Not determined , MeOH= methanol, values =mean± standard deviation

3.3.6 Cytotoxicity evaluation of the crude extracts of the nine selected plants against Vero African green monkey kidney cells

The cytotoxic effect of the nine plant extracts expressed in LC_{50} and selectivity index values are reported in Tables 3.8 and 3.9, respectively. The acetone extract of *E. elephantina* had the highest LC_{50} value of 3.69 mg/ml amongst the extracts while the acetone extract of *C. molle* had the lowest LC_{50} value of 0.01 mg/mL. The acetone extract of *C. elaeagnoides* had the highest selectivity index value of 36.78 against *E. coli1*. Interestingly, except the acetone extract of *C. molle*, all other extracts may be described as relatively safe to cells (LC_{50}> 0.02 mg/mL). According to Zirihi et al. (2002), extracts with LC_{50} values greater than 0.02 mg/mL are relatively safe or have weak cytotoxicity. The safety level of the extracts was also measured using the selectivity index values. In a previous report by Makhafola et al. (2012), extracts with selectivity index (SI) values greater than one were regarded to have the potential to offer safer therapy. Of the seventeen leaf extracts from the selected nine plants, the acetone extracts of *C. edulis and C. xanthophloea* with SI values of 17.07 and 14.96 had the best potential to offer the safest therapy against *E. cloacae* (Table 3.9). In other words, the aforementioned extracts were more toxic to the bacterial cells than they were to the Vero cells.

Table 3.8. Cytotoxicity of the seventeen leaf extracts of the nine selected plants

Plant species	LC_{50} (mg/mL)	
	MeOH	**Acetone**
C. elaeagnoides	0.0249 ± 0.0094	2.9424 ± 0.5138
C. oxystachyum	1.5151 ± 0.2569	0.0653 ± 0.0258
C. molle	0.0860 ± 0.0376	0.0100 ± 0.0081
E. elephantina	0.0760 ± 0.0439	3.6945 ± 0.1149
O. pretoriensis	0.0205 ± 0.0086	-
K. africana	0.0884 ± 0.0612	0.0251 ± 0.0143
V. rehmanniana	0.1166 ± 0,0375	0.0538 ± 0.0094
V. xanthophloea	0.0762 ± 0,0312	0.1496 ± 0.0029
C. edulis	0.1411 ± 0.0644	0.1707 ± 0.0454
Doxorubicin	0.0054 ± 0.0011	

NA (Not applicable), MeOH= methanol, values = mean± standard deviation

Table 3.9. Selectivity index values of selected nine plant leaf extracts against Vero African monkey kidney cells

Plant samples	Selectivity index (LC_{50}/MIC)									
	E. coli 1		*S. aureus*		*S.* Typhimurium		*S.* Enteritidis		*C. jejuni*	
	MeOH	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**	**MeOH**	**Acetone**
Ce	0.623	**36.780**	0.096	**18.390**	0.059	**11.317**	0.030	**2.354**	0.096	**18.390**
Co	**1.804**	0.311	**2.914**	0.126	**1.457**	0.126	**3.607**	0.126	0.728	0.039
Cm	0.183	0.083	0.165	0.042	0.041	0.333	0.165	0.083	0.410	0.111
Ee	0.292	8.796	0.181	**7.105**	0.073	**7.105**	0.181	**5.864**	0.061	3.552
Op	0.033	-	0.033	-	0.079	-	0.039	-	0.024	-
Ka	0.170	0.013	0.340	0.027	0.210	0.105	0.210	0.027	0.105	0.026
Vr	0.224	0.256	0.448	0.256	0.448	0.207	0.278	0.103	0.112	0.103
Vx	0.293	**1.247**	0.293	0.575	0.030	**4.987**	0.293	**4.987**	0.091	**1.247**
Ced	0.271	0.182	0.543	0.363	0.168	0.363	0.168	0.182	0.123	0.182
Doxorubicin	0.0054		NA		NA		NA		NA	

Table 3.9 Continued

Sample	*S. matophilia*		*K. pneumoniae*		*E. coli* 2		*E. cloacae*	
	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone
Ce	0.030	**7.006**	0.059	**11.317**	0.040	**9.492**	0.053	**4.670**
Co	3.607	0.039	**2.914**	0.155	**1.804**	0.104	**3.224**	0.139
Cm	0.137	0.167	0.782	0.333	0.205	0.333	0.183	1.000
Ee	0.073	**8.796**	0.292	**8.796**	0.090	**14.210**	0.205	**5.864**
Op	0.039	-	0.020	-	0.016	-	0.016	-
Ka	0.105	0.105	0.285	0.105	0.210	0.837	0.285	0.837
Vr	0.093	0.043	0.112	0.052	0.448	0.052	0.376	0.336
Vx	0.147	**1.247**	0.586	**1.247**	0.181m	**4.987**	0.246	**14.960**
Ced	0.271	**1.423**	0.224	**5.690**	0.136	5.690	0.271	**17.070**
Doxorubicin	0.0054		NA		NA		NA	

Ce (*Combretum elaeagnoides*), Co (*Combretum oxystachyum*), Cm (*Combretum molle*), Ee (*Elephantorrhiza elephantina*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis).* Values in bold had best SI values (> 1), NA (not applicable).

3.4 Statistical analysis

The mean and standard error of means, or mean and standard deviations where appropriate, for the different assays, were determined. Percentages were also calculated. The IC_{50} and LC_{50} were determined using linear and non-linear regression curves where necessary. The SPSS 25.0 computer software package was used in all the analysis.

3.5 Conclusion

Most of the plant species presented in this study are reported for the first time to inhibit bacterial biofilm formation. Of the seventeen leaf extracts from the nine South African medicinal plants, the acetone extract of *C. oxystachyum* had the best antibiofilm activity. The study established that the leaf extracts of these nine South African medicinal plants have antimicrobial, antibiofilm and antioxidant potential and are relatively safe in vitro. The pharmacological activities of the different plants were at varying degrees in the different assays used in determining their biological activities. Thus a synergistic approach to determine the activity of the screened leaf extracts of the plants in various combinations with each other as well as with currently used antibiotics is highly recommended for further studies. Following the large yield, after extracting the leaf of *C. elaeagnoides* with 80% methanol and its relatively good biological activities, it was chosen for isolation and characterization of bioactive compounds in further studies.

Chapter Four: Isolation of antibacterial compound from *Combretum elaeagnoides*

4.1. Introduction

Medicinal plants have long been part of African culture. They are used traditionally by more than 80% of the world population for the treatment of various ailments. For example, in South Africa, it is projected that 200 000 traditional medicine healers are consulted by over 60% of the population, especially in the rural areas (Van Wyk et al., 1997). Medicinal plant products have been incorporated in the development of several new drugs due to the presence of different classes of bioactive compounds in the plant extracts. Moreover, there are difficulties in the use of natural products as a source of drugs. This is probably because there is always the need to separate other plant constituents from the active compounds in the extracts using appropriate separation techniques (McRae et al., 2007).

Foodborne pathogens are a significant source of concern in the food industry due to their resistance to antimicrobial agents as a result of their ability to form biofilms. Microbial biofilms are of utmost importance to the food industry, mainly as a source of contamination leading to food spoilage or transmission of microbial diseases (Van Houdt and Michiels, 2010). It has been established that biofilm-forming microorganisms including foodborne pathogens are more resistant to antibiotics, food preservatives, disinfectants, biocides and other antimicrobial agents than their planktonic counterparts (Khiralla and El-Deeb, 2015). There is a need for new and effective alternative antimicrobial and antibiofilm agents for controlling the growth and development of bacterial pathogens, especially from plants. Plants have a considerable ability to produce a myriad of bioactive secondary metabolites like flavonoids, tannins, alkaloids, glycosides, terpenoids, saponins, steroids, quinones and coumarins (Das et al., 2010). These

bioactive molecules are the precursor of plant-derived antimicrobial substances, and many have been described to be extremely efficient in treating bacterial infections ((Srivastava et al., 2014, Fernebro, 2011).

Combretum species are a group of plants belonging to the family Combretaceae. Many of the species are reputed to be used for the treatment of diarrhea, hookworm, fever, dysmenorrhea, infertility in women, leprosy, scorpion and snakebite, abdominal pains, backache, bilharzia, cough, syphilis, toothache and general body weakness (Hutchings et al., 1996). In addition, the use of *Combretum* species in several cultures in folk medicine to treat microbial infections and many inflammatory maladies such as headache, abdominal pains and toothache have been reported (Hutchings et al., 1996; Eloff et al., 2001). Antimicrobial activities of several *Combretum* species have been established and some of the isolated compounds have higher activities than currently used antibiotics in the market like chloramphenicol and ampicillin (Martini and Eloff, 1998; Eloff, 1999b; McGaw et al., 2001; Martini et al., 2004; Masoko and Eloff, 2005, Eloff and McGaw, 2006). Thus, it is essential to investigate other *Combretum* species like *Combretum elaeagnoides* which is expected to have similar biosynthetic pathways for producing its bioactive secondary metabolites. There is little or no information in available literature on the antimicrobial, antibiofilm and antioxidant activity and cytotoxicity of *C. elaeagnoides* extracts. Given the above, this study was designed to evaluate the biological potential of the fractions and to isolate secondary metabolites from *Combretum elaeagnoides* Klotzsch with activity against foodborne pathogens.

4.2. Materials and methods

4.2.1. Extraction and fractionation of *C. elaeagnoides* leaf extract

The plant material was collected in Lowveld National Botanical Gardens (LNBG) Nelspruit in April 2017 and prepared as previously detailed in Chapter 3, section 3.2.2. The dried powdered leaves of *C. elaeagnoides* (462 g) were extracted in 4620 ml of aqueous methanol (80%) soaked for 48 h and filtered through Whatman No 1 filter paper. The extraction process was repeated twice to completely extract the plant material. The filtrate was concentrated using a Rotavapor (Büchi, Germany) under reduced pressure at a temperature of 40°C to produce the crude extract.

Solvent-solvent fractionation was employed to partition the constituents of the crude extract based on polarity. This fractionation technique is one of the frequently used methods for partial purification (group separation, according to polarity) of plant material. The crude extract was suspended in distilled water and was successively partitioned in a separatory funnel with 300 ml each of hexane, dichloromethane (DCM), ethyl acetate (EtOAc), butanol (BuOH) respectively. This yielded four solvent fractions along with the aqueous extract. The yield of each of the fractions was determined by applying the formula

Yield = (Mass of extract/ weight of dry sample) x 100

The fractions obtained were analyzed on thin layer chromatography (TLC) plates where 10 µL of each fraction (a 10 mg/ml aliquot) were loaded on TLC plates in lines of about 2 cm wide and developed using a mixture of chloroform methanol ($CHCl_3$/MeOH = 4:1). The chromatograms obtained were sprayed with vanillin in sulphuric acid to identify the classes of the compounds present in each of the fractions.

4.2.2. Isolation of bioactive compound from the ethyl acetate fraction of *C. elaeagnoides* leaf extract

4.2.2.1. Column chromatography

Biological activity results showed that the ethyl acetate fraction was one of the most active and abundant fractions (see section 4.1.4.1) and was selected for fractionation and isolation of its bioactive constituents. Column chromatography was utilized to separate the compounds in this fraction using silica gel (230-400 mesh) as a stationary phase. The ethyl acetate fraction (27.0 g) was dissolved in a small volume of methanol and adsorbed on silica gel by mixing with a small amount of silica powder (40.0 g). The methanol in the mixture was dried completely using a rotavapor. The silica column was packed using a glass column (100 x 2.5 cm) which was rinsed several times with 100% hexane. Cotton wool was inserted into the column using a glass rod, after which it was packed with a slurry of silica gel (270 g). One hundred mL of hexane was added and allowed to flow out to enable the silica gel to settle. A mixture of hexane and ethyl acetate (7:3) was poured into the column, and the fine powder of the ethyl acetate fraction adsorbed on silica gel was loaded into the column. The polarity of the eluting solvent mixture was increased gradually by increasing the volume of ethyl acetate in the hexane mixture by 10% up to 100% EtOAc. The solvent mixture was then changed to a mixture of ethyl acetate and methanol starting with 10% methanol up to 100% methanol. A total of 89 test tubes (25 ml each) were collected and analyzed using TLC and appropriate eluting solvents.

4.2.2.2. Bulking of fractions

Test tube fractions collected were developed on TLC plates using a chloroform and methanol eluting solvent mixture (9:1). The chromatograms obtained were sprayed with vanillin in sulphuric acid. Fractions with similar TLC profiles were combined to give three subfractions, namely test tube 10-33 (subfraction 1), 67-74 (subfraction 2) and 75-89 (subfraction 3). (Figure 2 a, b, c).

4.2.2.3. Purification of subfraction 1 on Sephadex LH-20 column

Subfraction 1 contained the bioactive compound (see section 4.1.4.1) and was selected for purification and isolation of the bioactive constituent (Figure 4.2 a). A glass column (35 cm x 2 cm) packed with pre-soaked Sephadex LH-20 was flushed several times with 100% methanol to clean the column. A solvent mixture of chloroform/methanol (4:1) was prepared, and 50 ml of the solvent mixture was added to the column while 10 ml was added to the sample (2.29 g). It was then dissolved using the sonicator to free residue from the flask. The sample was then applied onto the column and eluted with chloroform/methanol. The solvent mixture gradient was increased to 3:2; 2:3; 1:4 and finally to 100% methanol. The eluent was collected in 25 ml test tubes and analysed on TLC plates using DCM/MeOH (ratio 1:4). Test tubes 15-20, 107-110 and 114-118 were combined following the similarity of their TLC profile. The test tubes 15-20 showed TLC of a single spot. The contents of the test tubes were combined and allowed to dry under a stream of cold air for about 4 h to give compound **1** (259.7 mg) (Figure 3).

4.2.2.4. Spectroscopic characterization of isolated compound

The structure elucidation of the isolated compound was achieved in collaboration with Prof M.A. Aderogba (a visiting Professor) at the Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria. The Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) data were obtained from a 400 MHz Bruker Spectrometer at the Department of Chemistry, University of Pretoria, South Africa.

4.2.3 Antibacterial activity

4.2.3.1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the fractions and compound isolated from *C. elaeagnoides*

The MIC values of the fractions and isolated compound were determined following the method of Eloff (1998b) as outlined in Chapter 3, section 3.2.6.1. The fractions were tested at a starting concentration of 2.5 mg/ml (from a stock concentration of 10 mg/ml) and the compound at a starting concentration of 2 mg/ml in 96-well microtitre plates and serially diluted two-fold to 0.02 mg/ml and 0.016 mg/ml respectively. The assays were repeated three times in triplicate.

The MBC was determined by adding aliquots of 50 μl from the wells of the concentration which did not show any bacterial growth after incubation during MIC assays, to 150 μl of freshly prepared TSB broth. The preparations were thereafter spread on a TSA agar plate. These preparations were incubated at 37°C for 24h. The least concentration of extract with no bacterial growth determined the MBC value (Reiner et al., 1981).

4.2.3.2. Determination of anti-biofilm activity

The inhibition of biofilm biomass formation was assessed using the modified protocol described in Chapter 3, section 3.2.7.1 (O'Toole and Kotler, 1998, Mohsenipour et al., 2015, Sandasi et al., 2010). Briefly, biofilm was allowed to form at time T24 (24 h) to mark irreversible attachment. Biofilm production was achieved by aliquoting 100 µl of the respective culture (OD_{590} = 0.02 or 1.0 x 10^6 CFU/ml) into a sterile flat bottomed 96-well microtitre plate and sterile sealed with a sealing tape. One hundred microliter (100 µl) of the sample (at a final concentration of 1 mg/ml from a stock of 2 mg/ml) and the respective controls were transferred to the wells of the sterile plate and incubated for 24 h at 37ºC without shaking. Appropriate control wells were included in the plate - negative control (culture + TSB), positive control (culture + TSB + antibiotic), sample control (sample + TSB), antibiotic control (antibiotic + TSB) and media control (TSB) for each test batch. After incubation, the modified crystal violet staining (CVS) assay as described in section 3.2.7.2 (Sandasi et al., 2010) was performed to assess the biofilm biomass or adhered cell biomass. First,

in the CVS assay, the wells were carefully emptied and washed three times with sterile distilled water to remove any unattached cells. The plates were then air-dried and 150 µl of 96% methanol added to the wells for 15-20 min to fix the adherent cells. The plates were emptied, and the adhered cells stained with 100 µl of 0.1 % crystal violet solution for 20 min at room temperature while covered with the microtitre lids. The plates were washed five times with running tap water to remove any excess stain and unabsorbed stain. Thereafter, the biofilm biomass was assessed semi-quantitatively by re-solubilizing the crystal violet stain bound to the adherent cells with 150 µl of 100 % ethanol. The absorbance of the plates was read at 590 nm using a microplate reader (Epoch™ Microplate Spectrophotometer). The mean absorbance (OD_{590nm}) of the sample was determined and results expressed as percentage inhibition using the equation below.

$$\% \text{ Inhibition} = \frac{(\text{ODnegative control} - \text{ODmedia control}) - (\text{ODsample} - \text{ODsample control})}{(\text{ODnegative control} - \text{ODmedia control})} \times 100$$

4.2.4. Antioxidant activity

4.2.4.1. Assay for free radical scavenging (DPPH) activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was done according to the method described in section 3.2.7.1 (Gyamfi et al., 1999). The IC_{50} (50% inhibitory concentration) values of the fractions and isolated compound were determined using linear and non-linear regression curves where appropriate percentages of scavenging activity against the actual or logarithm of concentrations. Each test was done in triplicate and results are presented as mean± standard error of mean (SEM).

4.1.6.2 The ABTS radical scavenging assay

The ABTS assay on the fractions and compound was conducted following the procedure described in Chapter 3, section 3.2.7.2. The IC_{50} (50% inhibitory concentration) values of the

fractions and isolated compound were determined using linear and non-linear regression curves where appropriate percentages of scavenging activity against the actual or logarithm of concentrations. Ascorbic acid and Trolox served as the positive controls. Each test was done thrice and results presented as mean ± standard error of mean (SEM).

4.2.5. Cytotoxicity

The fractions and isolated compound were tested for cellular safety against Vero African green monkey kidney cells, as described in Chapter 3, section 3.2.8.1. The LC_{50} values were calculated using linear regression as the concentration of test compound resulting in a 50% reduction of absorbance (which implies killing 50% of the cells) compared to untreated cells. The experiment was done at three different times with each concentration tested in quadruplicate.

4.2.6. Statistical analysis

The mean and standard error of means or mean and standard deviations where appropriate of the different assays were determined. Percentages were also calculated. The IC_{50} and LC_{50} were determined using linear and non-linear regression curves where necessary. SPSS 25.0 computer software package was used in all the analysis.

4.3. Results and discussion

4.3.1. Percentage yield of fractions and isolated compound

The weight and percentage yield of the initial five fractions and bulked fractions are shown in Table 4.1. Of the fractions, the ethyl acetate fraction had the highest yield of 27.10 g while dichloromethane recorded the lowest yield of 3.98 g. Compound 1 (bulked fraction 1) had the

highest yield of 0.26 g of the three compounds. Compound 1 was obtained as a greenish-yellow to yellow colour while compounds 2 and 3 had a light brownish colour.

Table 4.1. Percentage yield of the different fractions and compounds

Fractions and compounds	Yield in gram (g)	Yield in percentage (%)
Butanol fraction	8.10	9.54
Hexane fraction	6.55	7.71
Dichloromethane fraction	3.98	4.69
Ethyl acetate fraction	27.10	31.91
Water fraction	10.32	12.15
Compound 1	0.26	0.31
Compound 2	0.070	0.08
Compound 3	0.0091	0.011

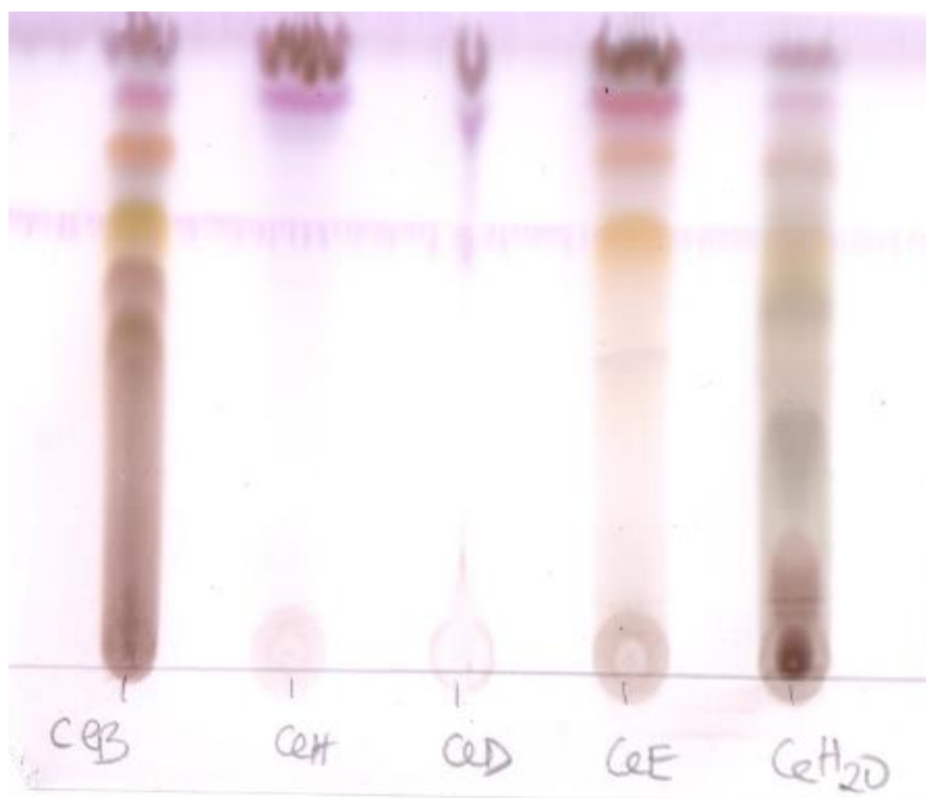


Figure 4.1. Thin Layer Chromatogram (TLC) of the fractions of aqueous methanol extract of *Combretum elaeagnoides* leaf extract

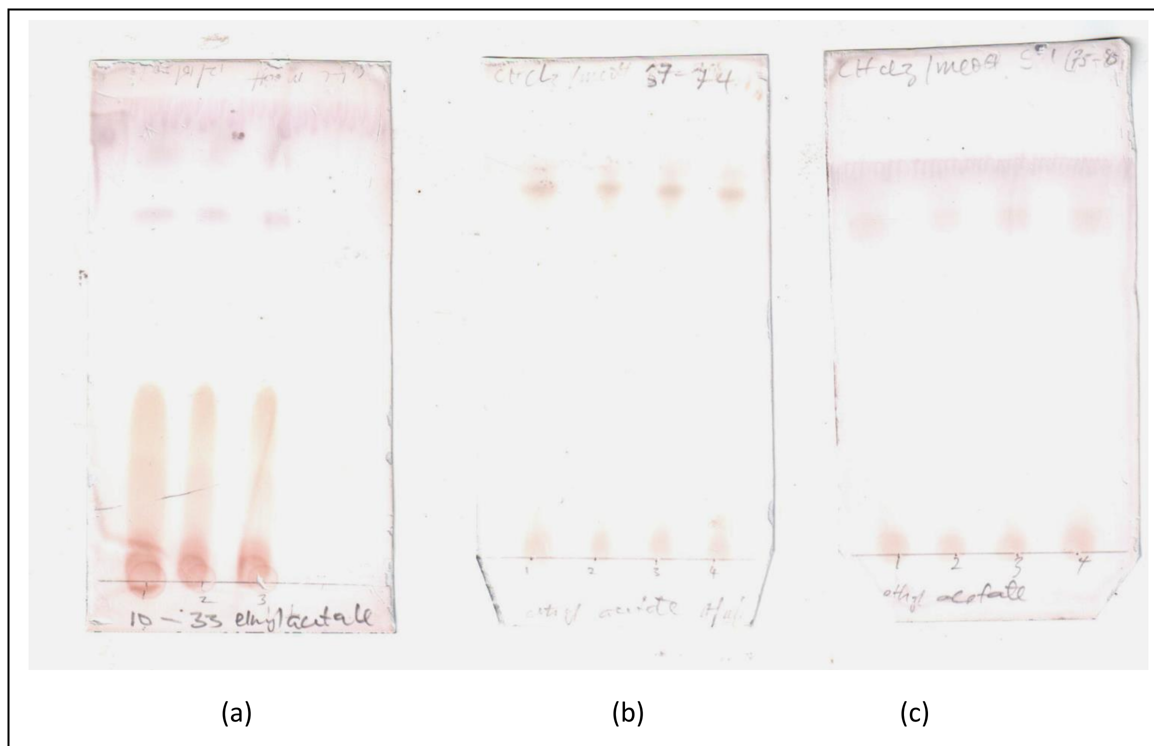


Figure 4.2. TLC of subfraction 1 (a), subfraction 2 (b) and subfraction 3 (c)

CeB (butanol fraction), CeH (hexane fraction), CeD (dichloromethane fraction), CeE (ethyl acetate fraction), CeH20 (water fraction)

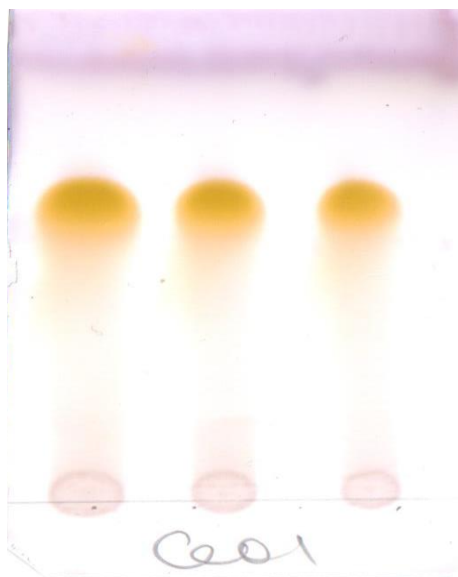


Figure 4.3. Chromatogram of Compound 1 (Ceo) spotted three times

4.3.2. Characterization of isolated compound

The ^{1}H NMR (DMSO-d_6) spectrum of compound **1** (Appendix 1a) showed a splitting pattern of a flavonol glycoside. There was a signal at δ 12.64 ppm that indicated the presence of a chelated 5-OH. The substitution pattern of ring B was established from the multiplicity of its protons signals. There was a doublet signal at δ 7.30 ppm (1H, H-2′, J= 2.4 Hz) indicating a meta orientation aromatic proton. A doublet of doublet signal appeared at δ 7.26 ppm (1H, H-6′, J =8.4 and 2.4 Hz) indicating a doubly coupled (meta and ortho related) aromatic proton. Another ortho coupled aromatic proton signal appeared at δ 6.88 ppm (1H, H-5′, 8.0 Hz). This substitution pattern revealed an ABX pattern for the ring B of the flavonol. The spectrum also showed the presence of two meta related ring A protons at δ 6.40 ppm (1H, H-8, 2.0 Hz) and δ 6.21 ppm (1H, H-6, J= 2.0 Hz). The presence of sugar moiety in the flavonol was evident from the presence of the following signals in the spectrum: anomeric sugar signal at δ 5.25 ppm (1H, d, J= 1.2 Hz, anomeric H-1″), δ 4.97-3.20 ppm (H-2″- H-5″ rhamnosyl Hs) and δ 0.82 ppm, (3H, d, J= 6.0 Hz, rhamnosyl CH_3-6″). The ^{13}C NMR data of this compound are presented in Table 4.2. The ^{1}H NMR (Appendix 1a) and ^{13}C NMR spectra (Appendix 1a, c) as well as the expanded spectra showing the aliphatic

and aromatic regions (Appendix 1b, c) are displayed in the appendix. Compound **1(Ceo1)** was identified as quercetin-3-*O*-rhamnoside (Figure 4.4) from the spectra data, which are in good agreement with the literature (Harbone and Mabry, 1982). Previous work by Osborne and Pegel (1984,1985) showed that four compounds had been isolated from the leaves of *C. elaeagnoides*. The previously isolated compounds, which were triterpenoids, include jessic acid (1 α, *β*-dihydroxyl), its α-L-arabinopyranoside, its methyl ester (methyl jessate) and methyl jessate 1α,11 α -oxide or methyl l α ,11 α -epoxy-3 *β* hydroxy-23-oxo-24-methylenecycloartan-30-oate. From this study, following literature search, the isolated compound-quercetin-3-*O*-rhamnoside is reported here for the first time from the leaf extract of *C. elaeagnoides.* Though the compound-quercetin-3-*O*-rhamnoside had been previously isolated from *Combretum apiculatum* subsp. apiculatum (Aderogba et al., 2012), *Croton penduliflorus* (Aderogba et al., 2013a), *Croton menyharthii* (Aderogba et al., 2013b)*, Anacardium occidentale* (Ajileye et al., 2015), *Euphorbia hirta* (Gopi et al., 2016). The compound exhibited diverse biological activities.

Table 4.2. The ^{13}C NMR Spectra data of Compound 1

Carbon	Compound 1 (DMSO-d_6)	Quercetin-3-*O*-rhamnoside (DMSO-d_6), (Harborne and Mabry, 1982)
2	157.5	156.7
3	134.3	134.6
4	177.9	178.0
5	161.4	161.6
6	98.8	99.0
7	164.3	164.4
8	93.8	93.9
9	156.6	157.5
10	104.2	104.5
1′	121.3	121.4
2′	115.8	115.9
3′	145.3	145.4
4′	148.6	148.7
5′	115.6	116.1
6′	120.9	121.2
1″	101.9	102.2
2″	70.5	70.4
3″	70.2	70.4
4″	71.3	71.7
5″	70.8	70.8
6″	17.6	17.8

CeO1_1H NMR

NMR ^{1}H and ^{13} C analysis

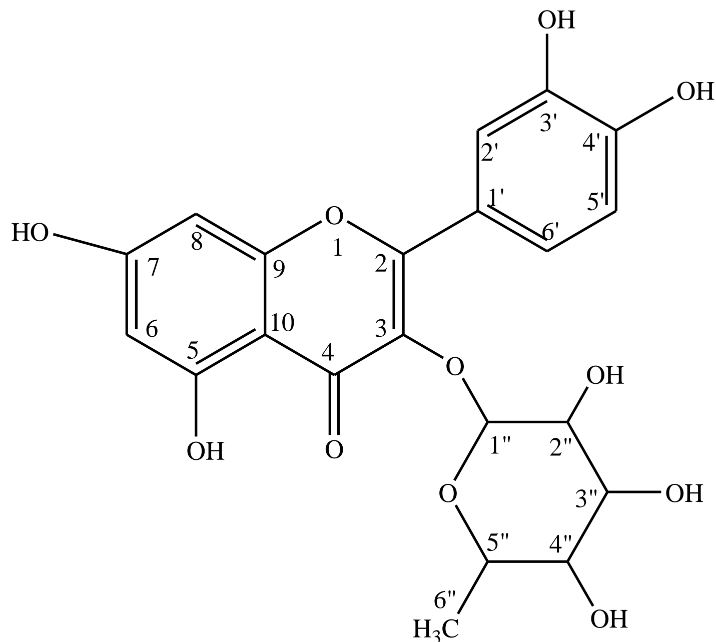


Figure 4.4: **Structure of Compound 1**

4.3.3. Minimum inhibitory concentration and minimum bactericidal concentration activities of fractions and isolated compound from *C. elaeagnoides*

The fractions (butanol, hexane, ethyl acetate, dichloromethane and water) of *C. elaeagnoides* showed excellent to good antibacterial activity. The identified compound (quercetin-3-*O*-rhamnoside) – Compound 1 - generally did not show strong antibacterial activity. A low MIC indicates excellent antibacterial activity. The MIC result indicated that the more polar fractions (ethyl acetate and butanol) had the most potent growth inhibitory activity against the foodborne pathogens. The ethyl acetate fraction generally had the best antibacterial activity against most of the test organisms. The dichloromethane fraction (DCM) at a MIC of 0.03 mg/ml had the best antibacterial activity against *E. coli* 1. The hexane and ethyl acetate fractions showed the same inhibitory action (MIC = 0.63 mg/ml) against *E. coli* 2 (clinical strain), just as the DCM and ethyl

acetate fractions exhibited the same inhibitory action (MIC = 0.1 mg/ml) against *S. aureus.* The ethyl acetate fraction had better antibacterial activity (MIC = 0.1 mg/ml) against *S. aureus* compared to the positive control, gentamicin, which had MIC of 0.12 mg/ml (Table 4.3). The MBC result reveals that the DCM fraction had the best bactericidal activity against *E. coli* 1 (0.31 mg/ml) followed by butanol and ethyl acetate fractions with MBC value of 0.63 mg/ml against the same organism. The dichloromethane and ethyl acetate fractions also had the same MBC value of 0.63 mg/ml against *S. aureus* (Table 4.4). The ethyl acetate fraction had the highest total antibacterial activity (TAA) against all test organisms with a TAA ranging from 506.35- 5316.67 ml/g. The compound (quercetin 3-O-rhamnoside) had the lowest total activity (1.22- 2.45 ml/g) against all test organisms. This result is presented in Table 4.5. It is interesting to note that *E. coli* 1 was the most susceptible organism while *E. coli* 2 and *E. cloacae* were the most resistant to the fractions and isolated compound from *C. elaeagnoides*.

Table 4.3. Minimum inhibitory concentrations (MIC) of the fractions and isolated compound from *C. elaeagnoides* leaf extract

Test Organisms	Fractions and compound from *C. elaeagnoides* MIC (mg/ml)						
	CeB	**CeH**	**CeD**	**CeE**	**CeH20**	**Ceo1***	**Control**
E. coli 1	0.26±0.05	0.09±0.04	0.03±0.01	0.06±0.00	0.52±0.11	2.5±0.00	0.02±0.00
E. coli 2	1.25±0.00	0.63±0.00	2.5±0.00	0.63±0.00	2.5±0.00	2.5±0.00	0.02±0.00
S. aureus	0.31±0.00	0.63±0.00	0.1±0.04	0.1±0.04	0.84±0.21	2.5±0.00	0.12±0.04
S. Typhimurium	0.63±0.00	2.5±0.00	0.31±0.00	0.63±0.00	0.52±0.11	1.25±0.00	0.42±0.11
S. Enteriditis	1.04±0.21	0.63±0.00	0.37±0.16	0.63±0.00	0.84±0.21	2.5±0.00	0.03±0.01
C. jejuni	0.73±0.21	0.42±0.11	0.21±0.05	0.42±0.11	2.5±0.00	2.5±0.00	0.09±0.04
S. maltophilia	0.63±0.00	0.63±0.00	1.25±0.00	0.42±0.11	1.67±0.42	>2.5±0.00	0.02±0.00
K. pneumoniae	0.37±0.11	0.63±0.00	0.63±0.00	0.31±0.00	0.63±0.00	2.5±0.00	0.02±0.00
E. cloacae	1.25±0.00	0.63±0.00	2.5±0.00	0.42±0.11	1.25±0.00	2.5±0.00	0.02±0.00

CeB (*Combretum elaeagnoides* butanol fraction), CeH (*Combretum elaeagnoides* hexane fraction), CeD (*Combretum elaeagnoides* dichloromethane fraction), CeE (*Combretum elaeagnoides* ethyl acetate fraction), CeH20 (*Combretum elaeagnoides* water fraction), Ceo1* (Quercetin-3-*O* rhamnoside), Control (Gentamicin), Na (Not applicable as MIC values were at the highest concentrations).

Table 4.4. Minimum bactericidal concentration (MBC) of the fractions and isolated compound from *C. elaeagnoides* leaf extract

Test organisms	**MBC (mg/ml)**						
	CeB	**CeH**	**CeD**	**CeE**	**CeH20**	**Ceo1***	**Control**
E. coli 1	0.63	0.31	0.08	0.16	2.5	Na	0.02
E. coli 2	>2.5	2.5	Na	2.5	Na	Na	0.02
S. aureus	1.25	2.5	0.63	0.63	2.5	Na	0.12
S. Typhimurium	>2.5	Na	1.25	2.5	2.5	>2.5	<0.43
S. Enteriditis	>2.5	1.25	1.25	2.5	>2.5	Na	0.03
C. jejuni	>2.5	2.5	1.25	1.25	Na	Na	0.09
S. maltophilia	2.5	>2.5	>2.5	2.5	>2.5	Na	0.02
K. pneumoniae	2.5	2.5	2.5	2.5	2.5	Na	0.02
E. cloacae	>2.5	2.5	Na	2.5	>2.5	Na	0.02

CeB (*Combretum elaeagnoides* butanol fraction), CeH (*Combretum elaeagnoides* hexane fraction), CeD (*Combretum elaeagnoides* dichloromethane fraction), CeE (*Combretum elaeagnoides* ethyl acetate fraction), CeH20 (*Combretum elaeagnoides* water fraction), Ceo1* (Quercetin-3-*O* rhamnoside), Control (Gentamicin), Na (Not applicable)

Table 4.5. Total antibacterial activity in mL/g of fractions and isolated compound from *C. elaeagnoides*

Organisms	CeB	CeD	CeH	CeE	CeH20	Ceo1
E. coli1	368.85	1546.67	857.00	5316.67	233.71	1.22
E.coli2	76.30	18.56	122.43	506.35	48.61	1.22
S. aureus	307.68	464.00	122.43	3190.00	144.68	1.22
S. Typhimurium	151.40	73.65	30.85	506.35	233.71	2.45
S. Enteriditis	91.71	73.65	122.43	506.35	144.68	1.22
C. jejuni	130.66	220.95	183.64	759.52	48.61	1.22
S. maltophilia	151.40	37.12	122.43	759.52	72.77	1.22
K. pneumoniae	257.78	73.65	122.43	1029.03	192.90	1.22
E. cloacae	76.30	18.56	122.43	759.52	97.22	1.22

CeB (*Combretum elaeagniodes* butanol fraction), CeH (*Combretum elaeagniodes* hexane fraction), CeD (*Combretum elaeagniodes* dichloromethane fraction), CeE (*Combretum elaeagnides* ethyl acetate fraction), CeH20 (*Combretum elaeagniodes* water fraction), Ceo1* (Quercetin-3-*O* rhamnoside)

4.3.4. The antibiofilm potential of the fractions and isolated compound from *C. elaeagnoides*

The fractions and isolated compound had varying effects on the growth and development of biofilms by the foodborne pathogens. Some of the fractions, as well as the isolated compound, had excellent antibiofilm activity (ABF) against some of the organisms while others enhanced biofilm growth, expressed as 0.00% inhibition (Table 4.5). Two of the fractions (CeB and CeH) exhibited the most active ABF activity against *K. pneumoniae* with percentage inhibition greater than 200 percent (>200%). Interestingly, none of the fractions except the compound had ABF activity against the ATCC isolate of *E. coli* 1. This result is dissimilar to the report by Sandasi et al. (2010) where the ATCC isolate of *Listeria monocytogenes* was more sensitive than the clinical

isolate to the tested samples. Surprisingly, the planktonic cells of *E. coli* 1 (ATCC), which was sensitive to the fractions and compound in the antimicrobial studies (MIC and MBC), was more resistant to the same fractions and compound in the antibiofilm studies. The isolated compound had excellent ABF activity with percentage inhibition value of >150% against *E. coli* 1. It is worthy to note that *S. aureus* and *S.* Typhimurium were the most sensitive organisms, as all the fractions and isolated compound had good antibiofilm activity with percentage inhibition values of >50% (Table 4.6). These results show that the inhibition of biofilm growth and development was more difficult to achieve than combating the planktonic cells of the organism. This assertion was also observed by Sandasi and colleagues in their investigation of the ABF activity of selected herbs, spices and beverages against *Listeria monocytogenes* (Sandasi et al., 2010). However, one factor that has been linked to the ability of foodborne pathogens to form biofilm and express resistance is the presence of an EPS (glycocalyx) that encompasses the biofilm cells as well as the negative charge on the EPS that limits the infiltration of molecules by charge attraction, thereby conferring resistance (Hugo and Russell 2004). Another route through which these pathogens form resistant biofilms includes the deactivation of the antimicrobial agent and efflux pumps that expel drugs from the cells (Hugo and Russell 2004). Also, the slow growth rate in biofilms compared to free-floating cells following reduced nutrient and oxygen supply has been reported as another factor (Costerton et al. 1999; Mah and O'Toole 2001).

It is interesting to note that some of the samples (fractions and isolated compound) enhanced biofilm formation of some organisms in this study. This observation could be as a result of the samples comprising compounds with the capacity to produce conditioning films for microbial adhesion (Sandasi et al., 2010). This enhancement in the growth and development of biofilm correlates with earlier studies that certain metabolites can stimulate the growth of micro-organisms (Ofek et al. 2003; Sandasi et al. 2008). In their study, Sandasi and colleagues demonstrated that specific components of essential oils stimulate the growth and development of

preformed *L. monocytogenes* biofilm (Sandasi et al., 2008). Ofek et al. (2003) showed that plant lectins improve the adsorption of cells onto a surface by acting as receptors of bacterial glycan, thus promoting cell attachment. The enhanced attachment found upon exposure to some of the tested samples can be attributed to the presence of metabolites that support the development of these biofilms.

Table 4.6. Effect of fractions and isolated compound from *C. elaeagnoides* leaf extract on the growth and development of biofilm of selected foodborne pathogens

Test organisms	**Percentage inhibition of biofilm (%)**					
	CeB	**CeH**	**CeD**	**CeE**	**CeH20**	**Ceo1**
E. coli 1	0.00*	0.00*	0.00*	0.00*	0.00*	153.77
E. coli 2	62.52	119.16	0.00*	0.00*	0.00*	269.78
S. aureus	77.39	66.69	77.46	71.93	80.57	67.59
S. Typhimurium	74.01	70.37	52.35	88.02	73.62	69.75
S. enterica	0.00*	0.00*	0.00*	0.00*	63.35	0.00*
C. jejuni	67.24	0.00*	0.00*	52.27	0.00*	119.36
S. maltophilia	0.00*	0.00*	76.15	0.00*	0.00*	0.00*
K. pneumoniae	247.76	237.51	0.00*	91.53	54.17	0.00*
E. cloacae	118.30	0.00*	0.00*	0.00*	66.40	0.00*

***= fractions and compound that enhance biofilm growth (negative inhibition),** CeB (*Combretum elaeagniodes* butanol fraction), CeH (*Combretum elaeagniodes* hexane fraction), CeD (*Combretum elaeagniodes* dichloromethane fraction), CeE (*Combretum elaeagnides* ethyl acetate fraction), CeH20 (*Combretum elaeagniodes* water fraction), Ceo1* (Quercetin-3-*O* rhamnoside)

4.3.5. Antioxidant evaluation

The antioxidant activity of the fractions and isolated compound from *C. elaeagnoides* expressed as IC_{50} against tested foodborne pathogens is presented in Table 4.7. In this study, the fractions and isolated compound (Ceo1) had good to excellent antioxidant activity against the DPPH and ABTS radicals with IC_{50} values ranging between 0.01± 0.31 ug/mL to 7.00± 0.51 and 0.01± 0.07 ug/mL to 20.00± 1.79 ug/mL respectively compared to the positive controls (vitamin C and Trolox) with IC_{50} values of 0.39 ± 0.23 ug/mL and 2.64± 0.27 for DPPH and 0.36± 0.35 ug/mL and 0. 74± 0.47 for ABTS. The water fraction (CeH20) with an IC_{50} value of 0.06 ug/mL had the best DPPH radical scavenging activity amongst the fractions but was less active than the purified compound (IC_{50}=0.01 ug/mL). The fractions, except for the ethyl acetate fraction, were more active against the DPPH radical than the two positive controls (Trolox, IC_{50}=2.64 ug/mL and vitamin C, IC_{50} = 0.39 ug/mL). The butanol fraction (CeB) was the most active fraction against the ABTS radical with IC_{50} value of 0.01± 0.07.

The compound (Ce01), identified as quercetin-3-*0*- rhamnoside, had the best antioxidant activity (IC_{50}= 0.01±0.31 and 0.90± 1.19 ug/mL) against the DPPH and ABTS radicals respectively when compared to the fractions and positive controls (vitamin C and Trolox) (Table 4.7). Quercetin-3-*0*- rhamnoside isolated from the EtOAc fraction of *C. elaeagnoides* was earlier isolated from the EtOAc fraction of *Anacardium occidentale* and assessed for antioxidant capacity. It was found that the Ce01 isolated from *C. elaeagnoides* had better DPPH radical scavenging activity with an IC_{50} value of 0.01±0.31 ug/ than the same compound in a mixture with quercetin-3-*0*- rutinoside isolated from *Anacardium occidentale* with IC_{50} value of 0.96±0.01 ug/mL (Ajileye et al., 2015). In another study, Aderogba and colleagues reported that quercetrin (quercetin-3-*0*- rahmnoside) isolated from *Combretum apiculatum* subsp. *apiculatum* had good antioxidant activity with an EC_{50} value of 11.8±0.32 μM (0.0053 mg/mL) (Aderogba et al., 2012). Their findings corroborate the results of antioxidant capacity of compounds similar to that obtained in this study.

Table 4.7. Antioxidant activity of the fractions and isolated compound from *C. elaeagnoides* expressed as mean IC_{50} (µg/ml)

Samples	**DPPH**	**ABTS**
CeB	0.16±0.02	0.01±0.07
CeH	0.38±0.21	6.28±0.15
CeD	> 100	20.00±1.79
CeE	7.00±0.51	1.44±0.86
CeH20	0.06±0.74	2.81±0.20
Ceo1	0.01±0.31	0.90±1.19
Trolox	2.64±0.27	0. 74±0.47
Vitamin C	0.39±0.23	0.36±0.35

CeB (*Combretum elaeagnoides* butanol fraction), CeH (*Combretum elaeagnoides* hexane fraction), CeD (*Combretum elaeagnoides* dichloromethane fraction), CeE (*Combretum elaeagnoides* ethyl acetate fraction), CeH20 (*Combretum elaeagnoides* water fraction), Ceo1*(quercetin-3-*0*-rhamnoside)

4.3.6. Cytotoxicity and selectivity index values of the fractions and isolated compound from *C. elaeagnoides* leaf extract

The results of the cytotoxicity assay on Vero African monkey kidney cells and the selectivity index values are presented in Table 4.8. The LC_{50} values of the fractions ranged between 22.20 and 50.26 µg/mL while the isolated compound had an LC_{50} value of 28.17 µg/ml. The water fraction (CeH20) had the highest LC_{50} value of 50.26 ug/mL (lowest toxicity), while the dichloromethane fraction (CeD) was more cytotoxic with LC_{50} value of 22.20 ug/mL. The positive control (doxorubicin) with an LC_{50} value of 15.95 ug/mL was the most toxic to the Vero cells. The relative non-toxicity of the fractions and the isolated compound is similar to an earlier report by Zirihi et al. (2005) that plant extracts with $LC_{50} \geq 20$ µg/ml are considered safe. Previous studies have shown that flavonoids generally have low toxicity to animal cells (Havsteen, 2002), for example a lack of cytotoxicity by 2″-*0*-rhamnosylvitexin against Vero kidney and bovine dermis cells (Aderogba et al., 2007). This assertion is supported by the findings in this study where the isolated flavonoid glycoside (quercetin-3-*0*-rhamnoside) was relatively non-toxic.

Selectivity index (SI) was used as another safety parameter (Table 4.8). The CeD fraction had the highest selectivity index value of 0.7333 amongst the fractions against *E. coli*1. The isolated compound had weak selectivity index values against virtually all the tested foodborne organisms. Ce01 had SI values below 1 in all cases. Plant extracts or compounds with SI values greater than one implies that the samples are more toxic to the bacteria than the host cells (Eloff, 2002; Makhafola et al., 2012).

Table 4.8. Cytotoxicity (LC_{50}) and selectivity index values (LC_{50}/MIC) of the fractions and isolated compound from *C. elaeagnoides* leaf extract

Samples	**LC_{50} (mg/mL)**	**Selectivity index**								
		Ec1	**Ec2**	**Sa**	**St**	**Se**	**Cj**	**Sm**	**Kp**	**Entero**
CeB	0.0484±1.4509	0.1862	0.0387	0.1561	0.0768	0.0465	0.0663	0.0768	0.1308	0.0387
CeH	0.0428±0.0035	0.4756	0.0679	0.0679	0.0171	0.0679	0.1019	0.0679	0.0679	0.0679
CeD	0.0222±0.1402	0.7333	0.0089	0.2220	0.0352	0.0352	0.1057	0.0178	0.0352	0.0089
CeE	0.0249±0,1050	0.4155	0.0396	0.2493	0.0396	0.0396	0.0594	0.0594	0.0804	0.0594
CeH20	0.0503±0.0050	0.0967	0.0201	0.0598	0.0967	0.0598	0..0201	0.0301	0.0798	0.0402
Ceo1	0.0282±0.6690	0.0113	0.0113	0.0113	0.0225	0.0113	0.0113	0.0113	0.0113	0.0113
Doxo	0.0160±0.1268	NA	NA	NA	NA	NA	NA	NA	NA	NA

CeB (*Combretum elaeagnoides* butanol fraction), CeH (*Combretum elaeagnoides* hexane fraction), CeD (*Combretum elaeagnoides* dichloromethane fraction), CeE (*Combretum elaeagnoides* ethyl acetate fraction), CeH20 (*Combretum elaeagnoides* water fraction), Ceo1 (Quercetin-3-*0*-rhamnoside), Doxo (Doxorubicin), NA (Not applicable).

4.3 Conclusions

This is the first time that quercetin-3-0-rhamnoside has been isolated and characterized from *C. elaeagnoides* leaf extract. The antimicrobial, antibiofilm, antioxidant and cytotoxicity of the fractions and isolated compound from *C. elaeagnoides* was also investigated for the first time. The study established that the extract fractions prepared using solvents of different polarities and the isolated compound have reasonably strong antimicrobial, antibiofilm and antioxidant properties, though with varying degrees of activity. The solvent fractions and isolated compound were relatively non-cytotoxic based on the findings of this study. The fractions and isolated compound can therefore be potentially useful in managing oxidative stress and diseases caused by foodborne pathogens and other infectious agents. The strong antibiofilm activity of the fractions and particularly the compound indicates that these samples can possibly be developed into promising alternate anti-infectious agents where resistance of the foodborne pathogens has been attributed to biofilm formation by the organisms. The antibiofilm activity of the fractions and isolated compound could be a possible mechanism of action through which the samples combat the foodborne pathogens. The findings of this study lastly support the ethnomedical use of many *Combretum* species in combating inflammation and infectious diseases. Further work, such as combination (synergistic) studies and *in vivo* toxicity and efficacy screening, particularly with the compound (quercetin-3- *O* -rhamnoside), is recommended.

Chapter Five: General conclusions and recommendations

5. General conclusions

The purpose of this study was to evaluate the methanol and acetone extracts of nine South African medicinal plants used for the treatment of ailments associated with foodborne diseases. A literature survey of the potential of South African plants against specific foodborne pathogens was also undertaken. Nine selected ATCC and clinical bacterial strains (*Salmonella* Typhimurium ATCC 39183, *Salmonella* Enteritidis ATCC 13076, *Escherichia coli* 1 ATCC 25922, *Escherichia coli 2 (*clinical isolate), *Staphylococcus aureus* ATCC 29213, *Stenotrophomonas maltophilia* (clinical isolate), *Klebsiella pneumoniae* (clinical isolate), *Enterobacter cloacae* (clinical isolate) and *Campylobacter jejuni* ATCC 33560) known to cause foodborne diseases or food poisoning were investigated. The leaf extracts of the nine selected plants were assessed for their antibacterial, anti-biofilm, antioxidant and cytotoxic activity. An active compound from *Combretum elaeagnoides* was further isolated and characterized. The isolated compound was screened for its antimicrobial, anti-biofilm and antioxidant activity and cytotoxicity.

5.1 Antibacterial and antibiofilm screening of the crude leaf extracts of the selected medicinal plants

The antibacterial activity of the methanol and acetone extracts of the plants was investigated. The broth serial microdilution method was used to determine the MIC activity of the nine plant extracts against the selected strains. The extracts were active against all the tested microorganisms with MIC values ranging from <0.02 to 2.5 mg/ml, but the test strains were more susceptible to the acetone extracts of all the plant extracts. The MBC of the plant extracts with MIC values ranging from <0.02 to 1.25 mg/ml was further investigated to determine at what

concentration the plant extract inhibited 100% bacterial growth. MBC values of 0.02 to 1.25 mg/ml were obtained. The total activity was also assessed as a measure of the antimicrobial potential of the plants. The acetone extract of *V. xanthophloea* had the highest total activity of 42,800 ml/g. The anti-biofilm investigation revealed that some of the plant extracts had very good inhibitory activity and inhibited more than 50% of the test organism biofilm biomass. The ABF activity of the seventeen extracts of the leaf of the nine plants was reported for the first time. The acetone extract of *C. oxystachyum* had the strongest ABF activity (252%) of the seventeen extracts investigated against *E. coli* 1.

5.2 Evaluation of the antioxidant potential of the nine selected plant extracts

The antioxidant activity investigation of the seventeen crude extracts of the nine selected plants showed that the methanol extract of *C. xanthophloea* and the acetone extract of *C. edulis* had the best antioxidant activity against the DPPH and ABTS radicals with IC_{50} values of 0.14± 0.19 µg/mL and 0.01±0.02 µg/mL respectively when compared to the other extracts and positive controls.

5.3 Cytotoxicity of the crude leaf extracts of the selected nine plants

The cytotoxic effect of the nine plant leaf extracts was investigated and expressed in LC_{50} and selectivity index (SI) values. The acetone extract of *E. elephantina* had the highest LC_{50} value of 3.69 mg/mL amongst the extracts, while the acetone extract of *C. molle* had the lowest LC_{50} value of 0.01 mg/mL. The acetone extract of *C. edulis and C. xanthoploea* had SI values of 17 and 15 and could be described to have the best potential to offer the safest therapy against *E. cloacae.*

5.4 *In vitro* bioactivity of the fractions and compound isolated from *Combretum elaeagnoides* leaf extract against selected foodborne pathogens

The methanol extract of *C. elaeagnoides* was fractionated into five fractions (hexane, dichloromethane, ethyl acetate, butanol and water) via solvent-solvent fractionation. Column chromatography with silica gel as the stationary phase and a hexane-ethyl acetate gradient as the mobile phase was used to isolate the active compound in bioassay-guided fractionation. The isolated compound was identified using nuclear magnetic resonance (NMR) and mass spectrometry (MS) as quercetin 3-*O*-rhamnoside. The fractions showed excellent to good antibacterial activity. The dichloromethane fraction of *Combretum elaeagnoides* had the best MIC activity of 0.03 mg/mL against *E. coli* 1 (ATCC 25922).

5.5 Conclusions and recommendations for future work

The results presented in this thesis demonstrate the potential of South African plants against foodborne pathogens. The antibiofilm and antioxidant activity of the crude extracts, fractions and isolated compound from the selected plants could be possible pathways through which the plants could be developed into therapies against the pathogens. Much further work is possible in this study. The exact MIC values for those with MIC values less than 0.02 mg/ml need to be confirmed and more accurately determined. More active compounds need to be isolated and identified from the most active plant extracts to provide possible marker compounds if an active extract or fraction is to be developed for use against foodborne pathogens. The mechanism of action of the active plant preparations (extracts, fractions or isolated compounds) needs to be elucidated to identify how they can be most useful in preventing or treating diseases caused by foodborne pathogens.

References

Aaku, E., Office, M., Dharani, S.P., Majinda, R.R.T., Motswaiedi, M.S. 1998. Chemical and antimicrobial studies on *Elephantorrhiza elephantina*. Fitoterapia 69, 464-465.

Abdalla, M., Matasyoh, J., 2014. Endophytes as Producers of Peptides: An Overview about the Recently Discovered Peptides from Endophytic Microbes. Natural Products and Bioprospecting 4, 257-270.

Adak, S., Upardrasta, L., Kumar, S.J., Soni, R., Bannerjee, R., 2011. Quorum quenching-alternative antimicrobial therapeutics. Science against microbial pathogens: communicating current research and technological advances. Formatex Research Center, Badajoz, 586-593

Adamu, M., Naidoo, V., Eloff, J.N., 2012. Some Southern African plant species used to treat helminth infections in ethnoveterinary medicine have excellent antifungal activities. BMC Complementary and Alternative Medicine 12, 213

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

Ademola, I.O., Eloff, J.N., 2010. *In vitro* anthelmintic activity of *Combretum molle* (R. Br. ex G. Don) (Combretaceae) against *Haemonchus contortus* ova and larvae. Veterinary Parasitology, 169, 198–203

Aderogba, M. A., Kgatle, D. T., McGaw, L. J., Eloff, J. N. 2012. Isolation of antioxidant constituents from *Combretum apiculatum* subsp. *apiculatum*. South African Journal of Botany, *79*, 125-131

Aderogba, M. A., Ndhlala, A. R., Van Staden, J. 2013a. Acetyl cholinesterase inhibitory activity and mutagenic effects of *Croton penduliflorus* leaf extract constituents. South African Journal of Botany 87, 48-51

Aderogba, M., Ndhlala, A., Rengasamy, K., Van Staden, J. 2013b. Antimicrobial and selected in vitro enzyme inhibitory effects of leaf extracts, flavonols and indole alkaloids isolated from *Croton menyharthii*. Molecules 18, 12633-12644

Aderogba, M. A., McGaw, L. J., Ogundaini, A. O., Eloff, J. N. 2007. Antioxidant activity and cytotoxicity study of the flavonol glycosides from *Bauhinia galpinii*. Natural Product Research 21, 591-599

Afolayan, A.J., Sunmonu, T.O., 2011. *Artemisia afra* Jacq. ameliorates oxidative stress in the pancreas of streptozotocin-induced diabetic Wistar rats. Bioscience Biotechnology and Biochemistry 75, 2083–2086

Aggarwal, S., Stewart, P. S., Hozalski, R. M., 2015. Biofilm cohesive strength as a basis for biofilm recalcitrance: are bacterial biofilms overdesigned? Microbiology Insights, 8, (Suppl 2): 29–32, MBI-S31444

Ahmed, A.S., Elgorashi, E.E., Moodley, N., McGaw, L.J., Naidoo, V., Eloff, J.N., 2012. The antimicrobial, antioxidative, anti-inflammatory activity and cytotoxicity of different fractions of four South African *Bauhinia* species used traditionally to treat diarrhoea. Journal of Ethnopharmacology 143, 826-839

Ajileye, O. O., Obuotor, E. M., Akinkunmi, E. O., & Aderogba, M. A. 2015. Isolation and characterization of antioxidant and antimicrobial compounds from *Anacardium occidentale* L. (Anacardiaceae) leaf extract. Journal of King Saud University-Science 27, 244-252

Al-Refi, M.R., 2016. Antimicrobial, anti-biofilm, anti-quorum sensing and synergistic effects of some medicinal plants extracts. Master of Biological Sciences/Medical Technology dissertation. The Islamic University-Gaza. 150p

Aremu, A.O., Ndhlala, A.R., Fawole, O.A., Light, M.E., Finnie, J.F., Van Staden, J., 2010. *In vitro* pharmacological evaluation and phenolic content of ten South African medicinal plants used as anthelmintics. South African Journal of Botany 76, 558–566

Aro, A.O., Dzoyem, J.P., Hlokwe, T.M., Madoroba, E., Eloff J.N., McGaw, L.J., 2015. Some South African Rubiaceae tree leaf extracts have antimycobacterial activity against pathogenic and non-pathogenic *Mycobacterium* species. Phytotherapy Research 29, 1004–1010

Arunkumar, S., Muthuselvam, M., 2009. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. World Journal of Agricultural Sciences 5, 572–576

Artini, M., Patsilinakos, A., Papa, R., Božović, M., Sabatino, M., Garzoli, S., et al., 2018. Antimicrobial and antibiofilm activity and machine learning classification analysis of essential oils from different Mediterranean plants against *Pseudomonas aeruginosa*. Molecules 23, 482

Assadourian, E., 2005. Cigarette production drops. Vital Signs, pp 70-71

Asres, K.; Bucar, F.; Knauder, E.; Yardley, V.; Kendrick, H.; Croft, S.L. 2001. *In vitro* antiprotozoal activity of extract and compounds from the stem bark of *Combretum molle*. Phytotherapy Research 15, 613–617

Atindehou, K.K.; Schmid, C.; Brun, R.; Koné, M.W.; Traore, D. 2004. Antitrypanosomal and antiplasmodial activity of medicinal plants from Côte d'Ivoire. Journal of Ethnopharmacology 90, 221–227

Baba-Moussa, F.; Akpagana, K.; Bouchet, P. 1999. Antifungal activities of seven West African Combretaceae used in traditional medicine. Journal of Ethnopharmacology 66, 335–338

Bacha, K., Tariku, Y., Gebreyesus, F., Zerihun, S., Mohammed, A., Weiland-Bräuer, N., et al., 2016. Antimicrobial and anti-quorum sensing activities of selected medicinal plants of Ethiopia: Implication for development of potent antimicrobial agents. BMC Microbiology 16, 139

Bakker, F.T., Culham, A., Hettiarachi, P., Touloumenidou, T., Gibby, M., 2004. Phylogeny of *Pelargonium* (Geraniaceae) based on DNA sequences from three genomes. Taxon 53, 17-31

Baquar, S.R., 1995. The role of traditional medicine in a rural environment in Kenya. Procedings of the National Workshop on Traditional Medicine, its Practice and the Law, Kenya, November 4-7, 1992, Nairobi, Kenya, pp.: 140-152

Bennett, B. C., Prance, G. T., 2000. Introduced plants in the indigenous pharmacopoeia of Northern South America 1. Economic Botany 1, 90–102

Bessong, P. O., Obi, C. L., Andreola, M. L., Rojas, L. B., Pouysegu, L., Igumbor, E., Marion Meyer, J. J., Quideau, S., Litvak, S., 2005. Evaluation of selected South African medicinal plants for inhibitory properties against human immunodeficiency virus type 1 reverse transcriptase and integrase. Journal of Ethnopharmacology 99, 83–91

Bhargava, N., Singh, S. P., Sharma, A., Sharma, P., Capalash, N., 2015. Attenuation of quorum sensing-mediated virulence of *Acinetobacter baumannii* by *Glycyrrhiza glabra* flavonoids. Future Microbiology 10, 1953-1968

Blackwell, H.E., Fuqua, C., 2011. Introduction to bacterial signals and chemical communication. Chemical Reviews 111, 1-3

Bown, D., 1995. Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London. ISBN 0-7513-020-31

Bridier, A., Sanchez-Vizuete, P., Guilbaud, M., Piard, J.C., Naïtali, M., Briandet, R., 2015. Biofilm-associated persistence of food-borne pathogens. Food Microbiology 45,167–178

Briese. V, Stammwitz. U, Friede, M., Henneickevon Zepelin, H.H., 2007. Black cohosh with or without St. John's wort for symptom-specific climacteric treatment--results of a large-scale, controlled, observational study. Maturitas 57, 405-414

Busani, L., Scavia, G., Luzzi, I., Caprioli, A., 2006. Laboratory surveillance for prevention and control of foodborne zoonoses. Annali dell'Istituto superiore di sanita 42(4), 401-404.

CAB International, 2000. Forestry Compendium Global Module. Wallingford, UK: CAB International

CDC (Centers for Disease Control and Prevention), 2018. Antibiotic/Antimicrobial Resistance (AR/AMR). https://www.cdc.gov/drugresistance/index.html (Accessed 23/02/2019).

Chevallier. A., 1996. The Encyclopedia of Medicinal Plants Dorling Kindersley. London. ISBN 9-780751-303148

Chiej. R., 1984. *Encyclopaedia of Medicinal Plants.* MacDonald, ISBN 0-356-10541-5

Chmielewski, R.A.N., Frank, J.F., 2003. Biofilm formation and control in food processing facilities. Comprehensive Reviews in Food Science and Food Safety 2, 22–32

Choi, E.M., Hwang, J.K., 2004. Antiinflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. Fitoterapia 75, 557–565.

Choisy, C., 2011. Biofilms and public health. Bulletin of the National Academy of Medicine 195, 1105-111

Chopra, R. N., Nayar, S. L., Chopra, I. C. 1986. Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, New Delhi

Christensen, A. B., Riedel, K., Eberl, L., Flodgaard, L. R., Molin, S., Gram, L., Givskov, M., 2003. Quorum-sensing-directed protein expression in *Serratia proteamaculans* B5a. Microbiology 149, 471-483

Clarkson C., Maharaj V.J., Crouch N.R et al. 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalized in South Africa. Journal of Ethnopharmacology 92, 177–191

Cock, I.E., van Vuuren, S., 2015. The potential of selected South African plants with anti-*Klebsiella* activity for the treatment and prevention of ankylosing spondylitis. Inflammopharmacology 2, 21-35

Cosa, S., Chenia, H.Y., 2014. Investigating the potential of ten South African indigenous plants extracts as quorum sensing inhibitors (QSI) of *Pseudomonas aeruginosa* virulence-related factors. Journal of Biotechnology and Biomaterials 3, 5

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. 1995. Microbial biofilms. Annual Reviews in Microbiology 49, 711–745

Costerton, J. W., Stewart, P. S., Greenberg, E. P. 1999. Bacterial biofilms: a common cause of persistent infections. Science 284, 1318-1322

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

Curtis, B. A., & Mannheimer, C. A. 2005. Tree Atlas of Namibia National Botanic Research Institute, Windhoek, Namibia. ISBN 99916-68-06-3

Das, K., Tiwari, R. K. S., Shrivastava, D. K. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research 4, 104-111

De Wet, H and Ngubane, S.C. 2014. Traditional herbal remedies used by women in a rural community in northern Maputa land (South Africa) for the treatment of gynaecology and obstetrics complaints. South African journal of Botany 94, 129-139

Donlan, R. M., 2001. Biofilm formation: a clinically relevant microbiological process. Clinical Infectious Diseases 33, 1387-1392

Dreeszen, P.H., 2003. Biofilm: the key to understanding and controlling bacterial growth in automated drinking water systems, 2nd ed. Edstrom industries Inc. Available from: http://www.edstrom.com/DocLib/4230- DS3100_CompleteBiofilm.pdf [Accessed on 10/03/2010]

Duke. J. A., Ayensu. E. S. 1985. Medicinal Plants of China. Reference Publications, Inc. ISBN 0-917256-20-4

Dzoyem, J.P., Eloff, J.N., 2015. 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

El-Hamid, M.I.A., 2016. A new promising target for plant extracts: inhibition of bacterial quorum sensing. Journal of Molecular Biology and Biotechnology 1, 1-3

Elisha, I.L., Botha, F.S., McGaw, L.J., Eloff, J.N., 2017. The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. BMC Complementary and Alternative Medicine 17, 133

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

Eloff, J.N., 1998b. A sensitive and quick method to determine the minimum inhibitory concentration of plant extracts for bacteria. Plant Medica 60, 1-8

Eloff, J.N., 1999a. The antibacterial activity of 27 southern African members of the Combretaceae. South African Journal of Science 95, 148–152.

Eloff, J.N., 1999b. It is possible to use herbarium specimens to screen for antibacterial components in some plants. Journal of Ethnopharmacology 67, 355–360

Eloff, J.N., 2001. Antibacterial activity of Marula (*Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro) (Anacardiaceae) bark and leaves. Journal of Ethnopharmacology 76, 305–308

Eloff, J. N., Jäger, A. K., Van Staden, J. 2001. The stability and the relationship between anti-inflammatory activity and antibacterial properties of southern African *Combretum* species: research in action. South African Journal of Science 97, 291-293

Eloff, J.N., 2004. Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine 11, 372-371

Exotichealingherbs, 2012. African Mind Healing Herbs (*Acacia xanthophloea*). https://sacredhealingherbs.wordpress.com/author/exotichealingherbs/, accessed 30/1/2019

FAO (Food and Agriculture Organization of the United Nations). 2003. Trade Reforms and Food Security: Conceptualizing the Linkages. Food and Agriculture Organization of the United Nations; Rome, Italy: 313

FAO (Food and Agriculture Organization of the United Nations). 2006. Policy Brief. FAO Agricultural and Development Economics Division: http://www.fao.org/es/esa/ EC-FAO Food Security Programme

Felter, H.W., Lloyd, J.U., 1898. King's American Dispensatory. 18th edition, 3rd Revision. Cincinnati: Ohio Valley Co.

Fernebro, J. 2011. Fighting bacterial infections—future treatment options. Drug Resistance Updates 14, 125-139

Felter, H.W., Lloyd, J.U. 1898. King's American Dispensatory. 18th edition, 3rd Revision. Cincinnati: Ohio Valley Co

Flemming, H.C., Wingender, J. 2010. The biofilm matrix. Nature Reviews Microbiology 8, 623–633

Foley, S., Lynne, A., Nayak, R. 2008. Challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates. Journal of Animal Science*,* 86 (14) suppl, pp. E149-E162.

Fuqua, C., Parsek, M.R., Greenberg, E.P. 2001. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annual Review of Genetics 35, 439–468

Gelfand, M. 1985. *The traditional medical practitioner in Zimbabwe: his principles of practice and pharmacopoeia*. Mambo Press.

Gerner-Smith, P., Whichard, J.M., 2008. Food disease trends and reports Foodborne Pathogens Disease 5, 551-554.

Geyid, A., Abebe, D., Debella, A., Makonnen, Z., Aberra, F., Teka, F., Kebede, T., Urga, K., Yersaw, K., Biza, T., et al. 2005. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. Journal of Ethnopharmacology 97, 421–427

Georgekutty, R., 2015. The anti-diabetic properties of four plants grown in India and KwaZulu-Natal, South Africa, suitable for diabetic management. Ph.D. Thesis, Department of Biochemistry and Microbiology, Faculty of Science and Agriculture, University of Zululand

Gerner-Smith, P., Whichard, J.M., 2008. Food disease trends and reports. Foodborne Pathogens Disease 5, 551-554

Gidey, Y. 2009. Assessment of indigenous knowledge of medicinal plants in Central Zone of Tigray, Northern Ethiopia. African Journal of Plant Science 4, 6–11

Gill LS, Ethnomedical uses of plants in Nigeria, Uniben Press, Benin City, 1992, p. 143

Glenice Ebedes 2018. *Vachellia rehmanniana.* Plantbook http://www.plantbook.co.za/vachellia-rehmanniana/. Accessed 7/1/2019

Gopi, K., Anbarasu, K., Renu, K., Jayanthi, S., Vishwanath, B. S., & Jayaraman, G. (2016). Quercetin-3-O-rhamnoside from *Euphorbia hirta* protects against snake Venom induced toxicity. Biochemica et Biophysica Acta (BBA)-General Subjects 1860, 1528-1540

Goreja, W. G., 2003. Black Seed: Nature’s Miracle Remedy. New York, Amazing Herbs Press

Grunyuza, T. Mothapo, M.A. Mothogoane, M.S. Nonyane, F.M. Rampho, E.T. Ready, J, Sebothoma, P.N. 2008. *Ochna pretoriensis*, plantzAfrica, com. http://pza.sanbi.org/ochna-pretoriensis, accessed 30/1/2019

Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27, 1–93

Gyamfi, M.A., Yonamine, M., Aniya, Y., 1999. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. Gen. Pharmacol.: Vasc. Syst. 32, 661–667

Hafsa, J., Hammi, K.M., Khedher, M.R.B., Smach, M.A., Charfeddine, B., Limem, K., Hatem Majdoub, H., 2016. Inhibition of protein glycation, antioxidant and antiproliferative activities of *Carpobrotus edulis* extracts. Biomedicine and Pharmacotherapy 84, 1496–1503

Hall-Stoodley, L., Costerton, J.W., Stoodley, P., 2004. Bacterial biofilms: from the natural environment to infectious disease. Nature Reviews Microbiology 2, 95–108

Harborne, J. B. and Marby, T.J. (1982). The flavonoids: Advances in research. Hapman and Hall Ltd. U.S.A. Spectrum 65

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

Hoque, M.M., Inatsu, M.B., Juneja, V.K., Kawamoto, S., 2007. Journal of Food Science and Technology 72, 9. Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria, and inactivation of *Listeria monocytogenes* in ground chicken meat with their essential oils. Journal of Food Science and Technology 72, 9-21

Huber, B., Eberl, L., Feucht, W., Polster, J., 2003. Influence of polyphenols on bacterial biofilms formation and quorum-sensing. Zeitschrift fur Naturforschung 58, 879-884

Hussain, T., Fatima, I., Rafay, M., Shabiri, S., Akram, M., Bano, S. 2016. Evaluation of antibacterial and antioxidant activity of leaves, fruit and bark of *Kigelia africana*. Pakistan Journal of Botany 48, 277-283

Hutchings, A., Scott, A.M., Lewis, G., Cunningham, A.M., 1996. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg, South Africa

Hyde, M.A., Wursten, B.T., Ballings, P. & Coates Palgrave, M. (2019). Flora of Zimbabwe: *Combretum elaeagnoides*. https://www.zimbabweflora.co.zw/speciesdata/species.php?species_id=141890, retrieved 5 February 2019

ICT (Infection Control Today), 2014. Antibiotic Resistance in Foodborne pathogen is an ongoing threat. https://www.infectioncontroltoday.com/antibiotics-antimicrobials/antibiotic-resistance-foodborne-pathogens-ongoing-threat-0 (Accessed 22/02/2019)

Iwu, M.M., 1993. Handbook of African Medicinal Plants CRS Press, Boca Raton

Iranshahy, M., Javadi, B., Iranshahi, M., Jahanbakhsh, S.P., Mahyari, S., Hassani, F.V., Karimi, G., 2017. A review of traditional uses, phytochemistry and pharmacology of *Portulaca oleracea* L. Journal of Ethnopharmacology 205, 158-172

Isturiz, R.E, Carbon, C, 2000. Antibiotic resistance in developing countries. Infection Control and Hospital Epidemiology 21, 394-397.

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Asif Nawaz, M., Hussain, T., Ali, M., Rafiq, M., Kamil, M.A., 2018. Bacterial biofilm and associated infections. Journal of the Chinese Medical Association 81, 7-11

Jamuna Bai, A., Rai, V.R., 2011. Bacterial quorum sensing and food industry. Comprehensive Review of Food Science and Food Safety 10, 183–193

Kalia, V.C., 2013. Quorum sensing inhibitors: an overview. Biotechnology Advances 31, 224-245

Katerere D.R., Eloff J.N., 2004. Variation in chemical composition, antibacterial and antioxidant activity of fresh and dried *Acacia* leaf extracts. South African Journal of Botany 70, 303-305

Khiralla, G.M., El-Deeb, B.A., 2015. Antimicrobial and anti-biofilm effects of selenium nanoparticles on some foodborne pathogens. LWT-Food Science and Technology 63, 1001-1007

Kibret M. & Abera B., 2012. The Sanitary Conditions of Food Service Establishments and Food Safety Knowledge and Practices of Food Handlers in Bair Dar Town. Ethiopian Journal of Health Science. p. 30

Kimmatkar, N., Thawani, V., Hingorani, L., Khiyani, R., 2003. Efficacy and tolerability of *Boswellia serrata* extract in treatment of osteoarthritis of knee-a randomized double blind placebo controlled trial. Phytomedicine 10, 3–7

Kloos, H.; Thiongo, F.W.; Ouma, J.H., Butterworth, A.E. 1987.Preliminary evaluation of some wild and cultivated plants for snail control in Machakos District, Kenya. Journal of Tropical Medicine and Hygiene 90, 197–204

Koh, C-L., Sam, C-K., Yin, W-F., Tan, L.Y., Krishnan, T., Chong, Y.M., Kok-Gan Chan, K-G., 2013. Plant-derived natural products as sources of anti-quorum sensing compounds. Sensors. 13, 6217–28

Korsten L. SA Doesn't have capacity to forecast, track a foodborne disease [homepage on the Internet]. EWN; 2016. [Cited 2016 Jun 26]. Available from: http://ewn.co.za/2016/02/03/SA-doesnt-have-adequate-capacity-to-forecast-tract-a-foodborne-disease

Koshy, P., Wirakarnain, S., Sim, K.S., Saravana, K., Hong, S.L., Lee, G.S., Syarifah, N., Rahman, S.A., 2009. Antimicrobial activity of some medicinal plants from Malaysia. American Journal of Applied Sciences 6, 1613–7

Krebs, S., Omer, B., Omer, T.N., Fliser, D., 2010. Wormwood (*Artemisia absinthium*) for poorly responsive early-stage IgA nephropathy: a pilot uncontrolled trial. American Journal of Kidney Disease 56, 1095-1099

Kumar, C.G., Anand, S.K., 1998. Significance of microbial biofilms in food industry: a review. International Journal of Food Microbiology 42, 9–27

Lans, C.A., 2006. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. Journal of Ethnobiology and Ethnomedicine 13, 45

Lassel, M., 1985. Gesundheit und Kraft durch Kräutergold. [Health and Strength with Kräutergold.] Rosenheim: M. Lassel Publications, 124.

Li, Z., Nair, S.K., 2012. Quorum sensing: How bacteria can coordinate activity and synchronize their response to external signals? Protein Science 21, 1403-1417

López, D., Vlamakis, H., Kolter, R., 2010. Biofilms. Cold Spring Harbor Perspectives in Biology. 2, a000398

Luciardi, M.C., Blazquez. M, A., Cartagena. E, Bardon, A., Arena, M.E., 2016. Mandarin essential oils inhibit quorum sensing and virulence 89 factors of *Pseudomonas aeruginosa*. LWT Food Science and Technology 68, 373-380

Luís A, Duarte A, Gominho J, Domingues F, Duarte AP. 2016. Chemical composition, antioxidant, antibacterial and anti-quorum sensing activities of *Eucalyptus globulus* and *Eucalyptus radiata* essential oils. Industrial Crops and Products 2016; 79: 274-282.

Lust. J., 1983. The Herb Book. Bantam books ISBN 0-553-23827-2

Mabogo, D. E. N., 1990. The ethnobotany of the VhaVenda. MSc. dissertation, University of Pretoria, South Africa

Mahima, R.A., Deb, R., Latheef, S.K., Abdul Samad, H., Tiwari, R., Verma, A.K., Kumar, A., Dhama, K., 2012. Immunomodulatory and therapeutic potentials of herbal traditional/indigenous and ethnoveterinary medicines. Pakistan Journal of Biological Sciences 15, 754-774

Mah, T. F. C., O'Toole, G. A. 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiology 9, 34-39

Mahlo, S.M., McGaw, L.J., Eloff, J.N., 2010. Antifungal activity of leaf extracts from South African trees against plant pathogens. Crop Protection 29, 1529-1533

Makunga, N.P., Philander, L.E., Smith, M., 2008. Current preservatives on an emerging formal natural products sector in South Africa. Journal of Ethnopharmacology 119, 365- 367

Makhafola, T.J., Babatunde B.S., Esameldin E. E., Eloff. J.N. 2012. Ochnaflavone and ochnaflavone 7-0- methyl ether two antibacterial bioflavonoids from *Ochna pretoriensis* (Ochnaceae). Natural Product Communications 7, 1601-1604

Malan, C and Notten, A. 2006. *Carpobrotus edulis*. PlantzAfrica, com. http://pza.sanbi.org/carpobrotus-edulis accessed 30/1/2019

Maphosa V., Masika P.J., Moyo B., 2009. Investigation of the anti-inflammatory and anti-nociceptive activities of *Elephantorrhiza elephantina* (Burch.) Skeels root extract in male rats. African Journal of Biotechnology 8, 7068–7072

Maphosa, V., Masika, P., 2012. *In vivo* validation of *Aloe ferox* (Mill). *Elephantorrhiza elephantina* Bruch. Skeels and *Leonotis leonurus* (L) R. BR as potential anthelminthics

and antiprotozoals against mixed infections of gastrointestinal nematodes in goats. Parasitology Research 110, 103–108

Maroyi, A., 2017. Phytochemical and ethnopharmacological review of *Elephantorrhiza goetzei* (Harms) Harms. Asian Pacific Journal of Tropical Medicine 10, 107–113

Martini, N.D., Eloff, J.N., 1998. The preliminary isolation and of several antibacterial compounds from *Combretum erythrophyllum* (Combretaceae). Journal of Ethnopharmacology 62, 255–263

Marwat, S K., Khan, M A., Khan, M A., Fazal-ur-Rehman., Ahmad, M., Zafar, M., and Sultana, S., 2009. *Salvadora persica*, *Tamarix aphylla* and *Zizyphus mauritiana*-Three Woody Plant Species Mentioned in Holy Quran and Ahadith and Their Ethnobotanical Uses in North Western Part (D.I. Khan) of Pakistan. Pakistan Journal of Nutrition 8, 542-547

Masoko, P and Eloff J.N. 2005. The diversity of antifungal compounds in six South African *Terminalia* species (Combretaceae) determined by bioautography. African Journal of Biotechnology 14, 1425- 1431

Masoko, P., Picard, J., Eloff, J.N., 2007.The antifungal activity of twenty-four southern African *Combretum* species (Combretaceae). South African Journal of Botany 73, 173–183.

Mathabe, M. C., Nikolova, R. V., Lall, N., & Nyazema, N. Z., 2006. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. Journal of Ethnopharmacology 105(1-2), 286-293.

McGaw, L.J., Rabe, T., Sparg, S.G., Jager, A.K., Eloff, J.N., van Staden, J., 2001. An investigation on the biological activity of *Combretum* species. Journal of Ethnopharmacology 75, 45–50

McGaw, L.J., Jäger, A.K., Van Staden, J., 2002. Variation in antibacterial activity of *Schotia* species. South African Journal of Botany 68, 41–46

McGaw, L.J., Steenkamp, V and Eloff, J.N. 2007. Evaluation of *Athrixia* bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. Journal of Ethnopharmacology 110, 16- 22

McLean, R. J., Whiteley, M., Stickler, D. J., & Fuqua, W. C. (1997). Evidence of autoinducer activity in naturally occurring biofilms. FEMS Microbiology Letters 154, 259-263.

McRae, J., Yang, Q., Crawford, R and Palombo, E. 2007. Review of the methods used for isolating pharmaceutical lead compounds from traditional medicinal plants. Environmentalist 27, 165- 174

Moerman, D.E. 1998. *Native American Ethnobotany*. Portland, OR: Timber Press.

Mohammedi, Z and Atik, F., 2011. Impact of solvent extraction type on total polyphenols content and biological activity from tamarix aphylla (l.) karst. International Journal of Pharma and Bio Sciences, 6, 609-615.

Mohsenipour, Z. and Hassanshahian, M. 2015. The Effects of *Allium sativum* Extracts on Biofilm Formation and Activities of Six Pathogenic Bacteria. *Jundishapur Journal of Microbiology*. August; 8(8): e18971. DOI: 10.5812/jjm.18971v2

Mokoka, T.A., Peter, X.K., Fouche, G., Moodley, N., Adams, M., Hamburger, M., Kaiser, M., Brun, R., Maharaj, V., Koorbanally, N., 2014. Antileishmanial activity of 12-methoxycarnosic acid from *Salvia repens* Burch. ex Benth. (Lamiaceae). South African Journal of Botany 90, 93–95

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65, 55–63

Motlhatlego, K., Njoya, E.M., Abdalla, M.A., Eloff, J.N., McGaw, L.J., 2018. The potential use of leaf extracts of two *Newtonia* (Fabaceae) species to treat diarrhoea. South African Journal of Botany 116, 25-33

Mpofu S.J., Msagati T.A.M., Krause R.W.M., 2014. Cytotoxicity, phytochemical analysis and antioxidant activity of crude extracts from rhizomes of *Elephantorrhiza elephantina* and *Pentanisia prunelloides*. African Journal of Traditional, Complementary, and Alternative Medicines 11, 34– 52

Mthethwa, N.S., Oyedeji, B.A.O., Obi, L.C., Aiyegoro, O.A., 2014. Anti-staphylococcal, anti-HIV and cytotoxicity studies of four South African medicinal plants and isolation of bioactive compounds from *Cassine transvaalensis* (Burtt. Davy) Codd. BMC Complementary and Alternative Medicine 14, 512

Muhammad, H.S., Muhammad, S. 2005. The use of *Lawsonia inermis* linn. (Henna) in the management of burn wound infections. African Journal of Biotechnology 4 (9), 934-937.

Mulholland, D.A., 2005. The future of ethnopharmacology: a southern African perspective. Journal of Ethnopharmacology 100, 124–126

Myszka, K., Schmidt, M.T., Majcher, M., Juzwa, W., Olkowicz, M., Czaczyk, K., 2016. Inhibition of quorum sensing-related biofilm of *Pseudomonas fluorescens* KM121 by *Thymus vulgare* essential oil and its major bioactive compounds. International Biodeterioration & Biodegradation 114, 252-259

Naidoo V., Zweygarth E., Eloff J.N., Swan G.E., 2005. Identification of anti-babesial activity for four ethnoveterinary plants in vitro. Veterinary Parasitology 130, 9–13

Nair, J., Van Staden, J., 2014. Traditional usage, phytochemistry and pharmacology of the South African medicinal plant *Boophone disticha* (L.f.) Herb. (Amaryllidaceae) Journal of Ethnopharmacology 151, 12-26

Nazir, F., Salim, R., Yousf, N., Bashir, M., Naik, H.R., Hussain, S.Z., 2017. Natural antimicrobials for food preservation. Journal of Pharmacognosy and Phytochemistry 6, 2078-2082.

Newall, C.A., Anderson, L.A., Phillipson, J.D., 1996. *Herbal Medicines: A Guide for Health-Care Professionals.* London: The Pharmaceutical Press

NICD (National Institute for Communicable Diseases). 2014a. Food and water-borne diseases. Commun Dis Communique 3(11):3

NICD (National Institute for Communicable Diseases). 2014b. Food and water-borne diseases. Commun Dis Communique, 13(2):4

Niehaus, A.J., Apalata, T., Coovadia, Y.M., Smith, A.M., Moodley, P., 2011. An outbreak of foodborne salmonellosis in rural KwaZulu-Natal, South Africa. Foodborne Pathogens Disease 8, 693–697

Njume, C., Jide, A.A., Ndip, R.N., 2011. Aqueous and organic solvent-extracts of selected South African medicinal plants possess antimicrobial activity against drug-resistant strains of *Helicobacter pylori*: Inhibitory and bactericidal potential. International Journal of Molecular Sciences 12m 5652–5665

Nordqvist, C., 2017. Benefits, uses, and side effects of echinacea. Medical News Today. MediLexicon, Intl., Retrieved from https://www.medicalnewstoday.com/articles/252684.php.14/01/2019

Nyila, M.A., Leonard, C.M., Hussein, A.A., Lall, N., 2012. Activity of South African medicinal plants against *Listeria monocytogenes* biofilms, and isolation of active compounds from *Acacia karroo*. South African Journal of Botany 78, 220–227

Ofek, I., Hasty, D. L., & Sharon, N. 2003. Anti-adhesion therapy of bacterial diseases: prospects and problems. FEMS Immunology and Medical Microbiology 38, 181-191

Ojewole J.A.O. 2008. Analgesic and anti-inflammatory effects of mollic acid glycosides, a1a-hydroxycycloartenoid saponin extractive from *Combretum molle* R. Br. ex G. Don (Combretaceae) leaf. Phytotherapy Research 22, 30–35

Olaokun, O.O., McGaw, L.J., Eloff, J.N., Naidoo, V., 2013. Evaluation of the inhibition of carbohydrate hydrolyzing enzymes, antioxidant activity and polyphenolic content of extracts of ten African *Ficus* species (Moraceae) used traditionally to treat diabetes. BMC Complementary and Alternative Medicine.13, 94.

Olaokun, O.O., Mkolo, N.M., Mogale, M.A., King, P.H., 2017. Phytochemical screening, antioxidant, anti-inflammatory and glucose utilization activities of three South African plants used traditionally to treat diseases. Biology and Medicine (Aligarh) 9(412), 2.

Olivero, J., Pajaro, N., Stashenko, E., 2011. Anti-quorum sensing activity of essential oils isolated from different species of the genus *Piper*. Vitae 18, 77-82

Omokhua, A.G., Madikizela, B., Aro, A.O., Uyi, O.O., Van Staden, J., McGaw, L.J., 2018. Noxious to ecosystems, but relevant to pharmacology: Four South African alien invasive plants with pharmacological potential. South African Journal of Botany 117, 41-49

Ondua, M., Njoya, E.M., Abdalla, M.A., McGaw, L.J., 2019. Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation. Journal of Ethnopharmacology 234, 27-35

Orisakwe, O.E., Husaini, D.C., Afonne, O.J., 2004. Testicular effects of sub-chronic administration of *Hibiscus sabdariffa* calyx aqueous extract in rats. Reproductive Toxicology 18, 295-298

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Simons A., 2009. Agroforestree Database: a tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/af/treedb/)

Osborne, R., & Pegel, K. H. 1984. Jessic acid and related acid triterpenoids from *Combretum elaeagnoides*. Phytochemistry 23, 635-637

Osborne, R., & Pegel, K. H. 1985. Methyl jessate 1α, 11α-oxide, a further novel triterpenoid ester from *Combretum elaeagnoides*. South African Journal of Chemistry 38, 83-86

Otang, W.M., Scott, G.D., Ndip R.N., 2012. Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. BMC Complementary and Alternative Medicine 12, 43

O'Toole, G. A., Kolter, R. 1998. Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. Molecular Microbiology 30, 295-304

O'Toole, G., Kaplan, H.B., Kolter, R., 2000. Biofilm formation as microbial development. Annual Review of Microbiology 54,49-79

Ozbek, H., Ugras, S., Dulger, H., Bayram, I., Tuncer, I., Ozturk, G., 2003. Hepatoprotective effect of *Foeniculum vulgare* essential oil. Fitoterapia 74, 317–319

Palgrave, K.C., Drummond, R.B., Moll, E.G., Palgrave, M.C. 2003. Trees of Southern Africa 3rd Edition, Struik Publishers, South Africa

Parker, C.T., Sperandio, V. 2009. Cell-to-cell signaling during pathogenesis. Cell Microbiology 11, 363–369

Piccaglia, R., Marotti, M., 2001. Characterization of some Italian types of wild fennel (*Foeniculum vulgare* Mill.), Journal of Agricultural and Food Chemistry, 49, 239-244

PFAF, 2013. Database. Plants for a Future. http://www.pfaf.org/user/plantsearch.aspx

Prasanth Reddy, V., Ravi Vital, K., Varsha, P. V., Satyam, S. 2014. Review on *Thymus vulgaris* traditional uses and pharmacological properties. Medicinal and Aromatic Plants 3, 164

Quattrocchi, U., 2012. CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology. Quattrocchi, U. (Ed). London, UK: CRC Press Inc., 3960p.

Radio 4 Farming Programme. 1995. An article on the potential of *Coriandrum sativum* as an oil crop.25/08/95

Rahal, A., Mahima, A.K., Verma, A., Kumar R., Tiwari, 2014. Phytonutrients and nutraceuticals in vegetables and their multi-dimensional medicinal and health benefits for humans and their companion animals. A review. Journal of Biological Sciences 14, 1-19

Raffa, R.B., Iannuzzo, J.R., Levine, D.R., Saeid, K.K., Schwartz, R.C, et al. 2005 Bacterial communication ("quorum sensing") via ligands and receptors: a novel pharmacologic target for the design of antibiotic drugs. Journal of Pharmacology and Experimental Therapeutics 312, 417-423

Ragaert, P., Devlieghere, F., Debevere, J., 2007. Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. Postharvest Biology and Technology 44, 185–194

Ramadwa, T.E., Elgorashi, E.E., McGaw, L.J., Ahmed, A.S., Eloff, J.N., 2017. Antimicrobial, anti-inflammatory activity and cytotoxicity of *Funtumia africana* leaf extracts, fractions and the isolated methyl ursolate. South African Journal Botany 108, 126–131

Ranasinghe, P., Pigera, S., Premakumara, G. A., Galappaththy, P., Constantine, G. R., Katulanda, P., 2013. Medicinal properties of 'true' cinnamon (*Cinnamomum zeylanicum*): a systematic review. BMC Complementary and Alternative Medicine 13, 275

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical action decolourization assay. Free Radical Biology and Medicine 26, 1231–1237

Reynolds, J. E.,1982. *Martindale: the extra pharmacopoeia*. London, UK; The Pharmaceutical Press.

Riedel, K., Köthe, M., Kramer, B., Saeb, W., Gotschlich, A., Ammendola, A., Eberl, L., 2006. Computer-aided design of agents that inhibit the cep quorum-sensing system of *Burkholderia cenocepacia*. Antimicrobial Agents and Chemotherapy 50, 318-323.

Rodney M., 2001. Donlan; biofilm formation: a clinically relevant microbiological process. Clinical infectious Disease 33,1387-1392

Romagnoli, C., Andreotti, E., Maietti, S., Mahendra, R., Mares, D., 2010. Antifungal activity of essential oil from fruits of Indian *Cuminum cyminum.* Pharmaceutical Biology, 48(7), 834-838.

Rosser, B.T., Taylor, P.A., Cix, P.A., Cluland, R., 1987. Methods for evaluating antibiotics on bacterial biofilms. Antimicrobial Agents Chemotherapy 31, 1502–1506

Samaranayake, L.P. 2002. Essential microbiology for dentistry; with a contribution by B.M. Jones; Forewood by Crispian Scully. Edinburgh, New York Churchill Livingstone

Sandasi, M., Leonard, C. M., & Viljoen, A. M. 2008. The effect of five common essential oil components on *Listeria monocytogenes* biofilms. *Food control*, *19*(11), 1070-1075.

Sandasi, M., Leonard, C.M., Viljoen, A.M., 2010. The *in vitro* anti-biofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes.* Letters in Applied Microbiology 50, 30–35

Sandasi, M., Leonard, C. M., Van Vuuren, S. F., Viljoen, A. M., 2011. Peppermint (*Mentha piperita*) inhibits microbial biofilms in vitro. South African Journal of Botany 77, 80-85

Saunders, 2003. Biofilm. Miller-Keane Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, Seventh Edition., an imprint of Elsevier, Inc. https://medical-dictionary.thefreedictionary.com/biofilm. (Accessed 23 February, 2019)

Seujange, Y., Leelahavanichkul, A., Yisarakun, W., Khawsuk, W., Meepool, A., Phamonleatmongkol, P., Saechau, W., Onlamul, W., Tantiwarattanatikul, P., Oonsook, W., Eiam-Ong. S., 2013. *Hibiscus sabdariffa* Linnaeus aqueous extracts attenuate the progression of renal injury in 5/6 nephrectomy rats. Renal Failure 35, 118-125

Shai, L.J., McGaw, L.J., Masoko, P., Eloff, J.N., 2008. Antifungal and antibacterial activity of seven traditionally used South African plant species active against *Candida albicans.* South African Journal of Botany 74, 677-684

Sharma, R., Lall, N., 2014. Antibacterial, antioxidant activities and cytotoxicity of plants against *Propionibacterium acnes.* South African Journal of Science 110, 1–8

Shaughnessy, A. F. (2007). Penicillin and a series of fortunate events. BMJ Global Health, 334(7602), 1059-1059

Silva, M. I., de Melo, C. T., Vasconcelos, L. F., de Carvalho, A. M., Sousa, F. C., 2012. Bioactivity and potential therapeutic benefits of some medicinal plants from the Caatinga (semi-arid) vegetation of Northeast Brazil: a review of the literature. Revista Brasileira de Farmacognosia 22, 193-207

Simpson, D., 1998. Buchu--South Africa's amazing herbal remedy. Scott Medical Journal 43, 189–9

Srivastava, J., Chandra, H., Nautiyal, A. R., Kalra, S. J. (2014). Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. 3 Biotech 4, 451-460

Steinberg, D., Feldman, M., Ofek, I., Weiss, E.I., 2005. Cranberry high molecular weight constituents promote *Streptococcus sobrinus* desorption from artificial biofilm. International Journal of Antimicrobial Agents 25, 247–251

Street, R.A., Prinsloo, G., 2013. “Commercially Important Medicinal Plants of South Africa: A Review,” Journal of Chemistry,Article ID 205048, 16. https://doi.org/10.1155/2013/205048.

Taga, M, Miller, S., Bassler, B., 2003. Lsr-mediated transport and processing of AI-2 in *Salmonella* Typhimurium. Molecular Microbiology 50, 1411-1427.

Taganna, J., Quanico, J., Perono, R, Amor, E., Rivera, W., 2011. Tannin-rich fraction from *Terminalia catappa* inhibits quorum sensing (QS) in *Chromobacterium violaceum* and the QS-controlled biofilms maturation and LasA staphylolytic activity in *Pseudomonas aeruginosa*. Journal of Ethnopharmacology 134, 865-871

Tenover, F. C. 2006. Mechanisms of antimicrobial resistance in bacteria. The American Journal of Medicine 119, S3-S10

Tewari, D., Mocan, A., Parvanov, E. D., Sah, A. N., Nabavi, S. M., Huminiecki, L., Ma, Z. F., et al., 2017. Ethnopharmacological approaches for therapy of jaundice: Part II. Highly used plant species from Acanthaceae, Euphorbiaceae, Asteraceae, Combretaceae, and Fabaceae families. Frontiers in Pharmacology 8, 519

Thabo Masupa 2011. *Combretum molle*. National Herbarium, Pretoria

Thring, T.S.A., Weitz, F.M., 2006. Medicinal plant use in the Bredasdorp/Elim region of Southern Overberg in the Western Cape province of South Africa. Journal of Ethnopharmacology 103, 261–275

Trentin, D.D.S, Giordani, R.B., Zimmer, K.R., da Silva, A.G., da Silva, M.V., Correia, M.T., Baumvol, I.J., Macedo, A.J., 2011. Potential of medicinal plants from the Brazilian semi-

arid region (Caatinga) against *Staphylococcus epidermidis* planktonic and biofilm lifestyles. Journal of Ethnopharmacology 137, 327-335

Tyler, V., 1993. *The Honest Herbal: A Sensible Guide to the Use of Herbs and Related Remedies,* 3rd ed. New York: Pharmaceutical Products Press.

USDA-ARS, 2014. Germplasm Resources Information Network (GRIN). Online Database. Beltsville, Maryland, USA: National Germplasm Resources Laboratory. https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch.aspx

Uyttendaele, M., Franz, E., Schlüter, O., 2016. Food safety, a global challenge. International Journal of Environmental Research and Public Health 13, 67

Van der Merwe, D., Swan. G.E., Botha C.J., 2001. Use of ethnoveterinary medicinal plants in cattle by Setwswana-speaking people in the Madikwe area of the North West province of South Africa. Journal of South African Veterinary Association 72, 189-196

Van Houdt, R., Michiels, C.W., 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. Journal of Applied Microbiology 109, 1117-1131

Van Vuuren, S., Holl, D., 2017. Antimicrobial natural product research: A review from a South African perspective for the years 2009–2016. Journal of Ethnopharmacology 208, 236-252

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

Van Wyk B-E., 2002. A review of ethnobotanical research in southern Africa. South African Journal of Botany 68, 1–13

Van Wyk, B.-E, Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa, Second Edition. Briza Publications, Pretoria South Africa

Van Wyk, B.-E, Gericke, N., 2000. People's Plants: A Guide to Useful Plants of Southern Africa. Briza Publications, Pretoria

Varga, E., Bardocz, A., Belak, A., Maraz, A., Boros, B., Felinger, A., et al.,2015. Antimicrobial activity and chemical composition of thyme essential oils and the polyphenolic content of different *Thymus* extracts. Thymus, 63(3).

Vasavi, H.S., Arun, A.B., Rekha, P.D., 2016. Anti-quorum sensing activity of flavonoid-rich fraction from *Centella asiatica* L. against *Pseudomonas aeruginosa* PAO1. Journal of Microbiology, Immunology and Infection 49, 8-1

Viju, N., Satheesh, S., Vincent, S., 2013. Antibiofilm activity of coconut (*Cocos nucifera* Linn.) husk fibre extract. Saudi Journal of Biological Sciences 20, 85-91

Vogl, S., Picker, P., Mihaly, B.J., Fakhrudin, N., Atanasov, A.G., Heiss, E.H., Wawrosch, C., Reznicek, G., Dirsch, V.M., Saukel, J., Kopp, B., 2013. Ethnopharmacological in vitro

studies on Austria's folk medicine-An unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. Journal of Ethnopharmacology 149, 750–771

Walters, M., Sircili, M. P., Sperandio, V., 2006. AI-3 synthesis is not dependent on luxS in *Escherichia coli*. Journal of Bacteriology, 188, 5668-5681.

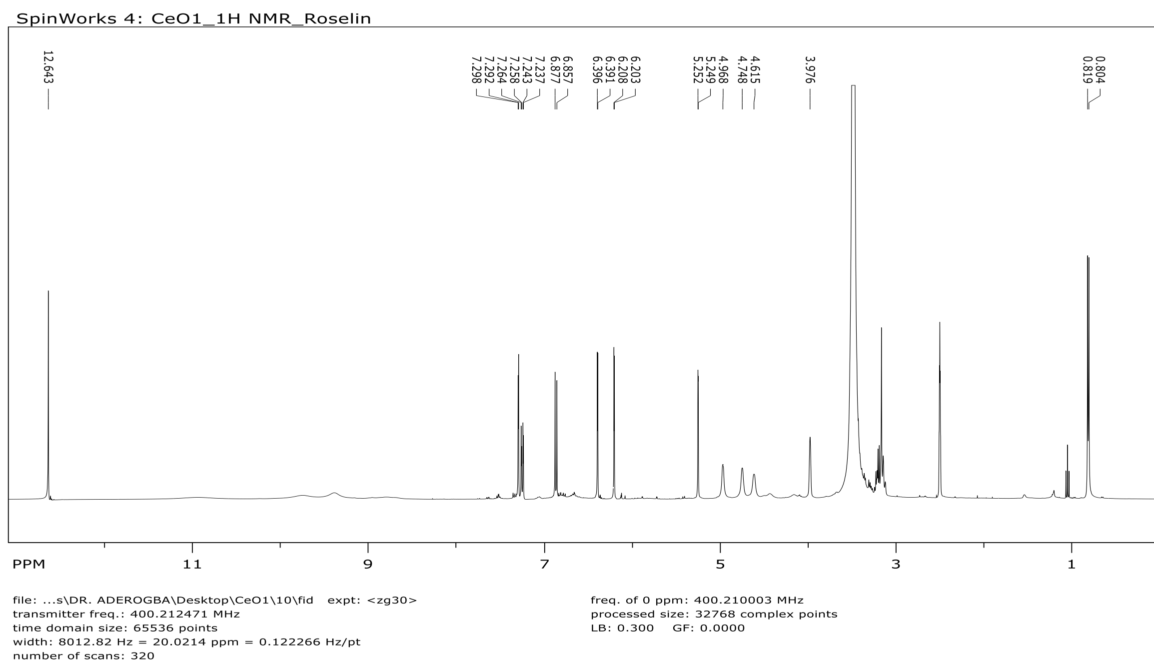
WHO 2015. WHO's first ever global estimates of foodborne diseases find children under 5 account for almost one third of deaths. http://www.who.int/mediacentre/news/releases/2015/foodborne-disease-estimates/en/ (Accessed 4/11/2017).

Yang, Y.C., Choi, H.Y., Choi, W.S., Clark, J.M., Ahn, Y.J., 2004. Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). Journal of Agricultural and Food Chemistry 52, 2507-2511

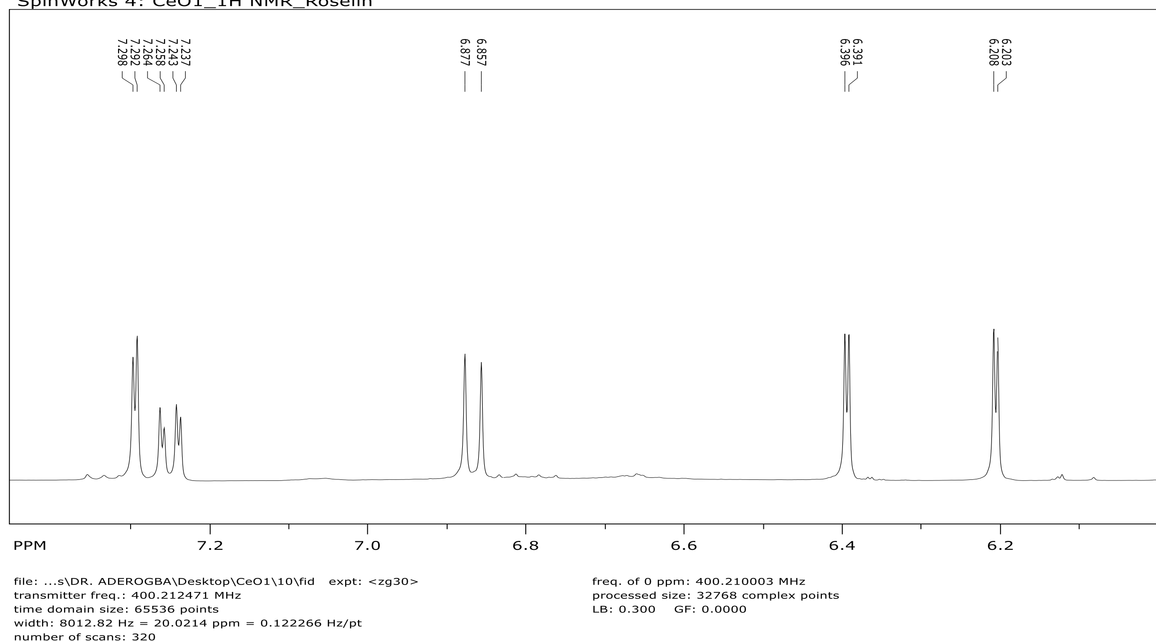
Yeung, H-C., 1985. Handbook of Chinese Herbs and Formulas. Institute of Chinese Medicine, Los Angeles

Yogeeta, S., Ragavender, H. R. B., Devaki, T., 2007. Anti-hepatotoxic effect of *Punica granatum* acetone extract against isoniazid-and rifampicin-induced hepatotoxicity. Pharmaceutical Biology 45, 631-637

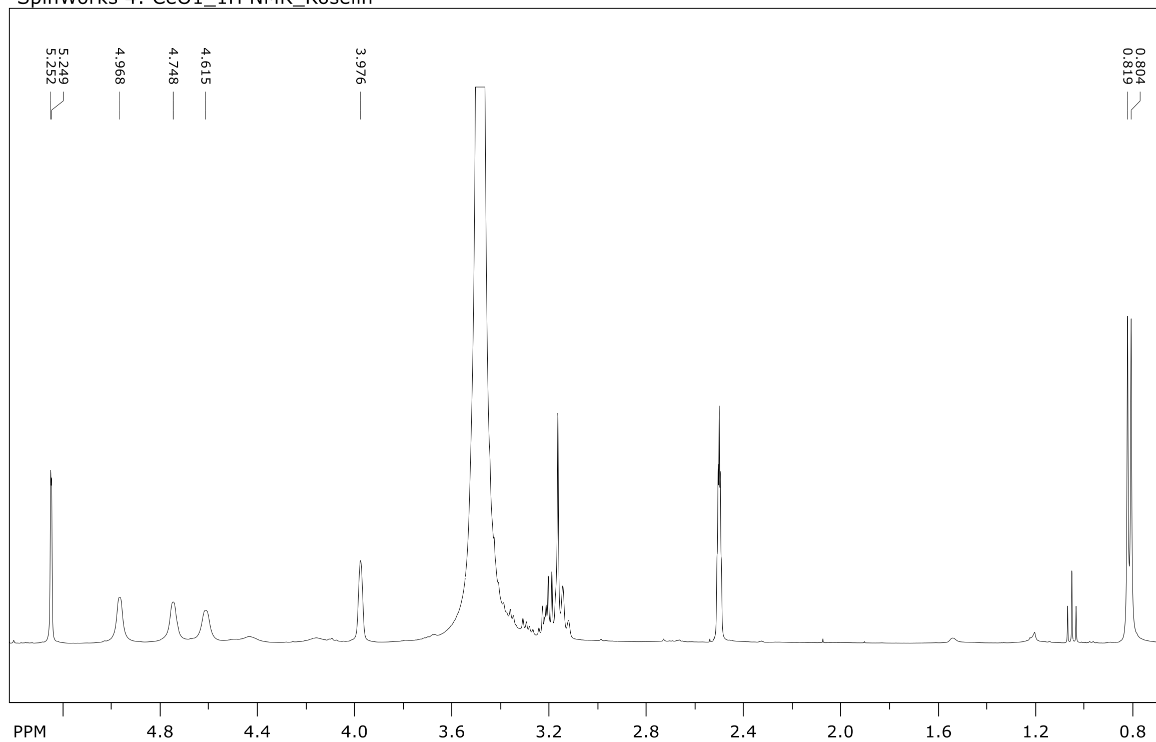
Appendix 1



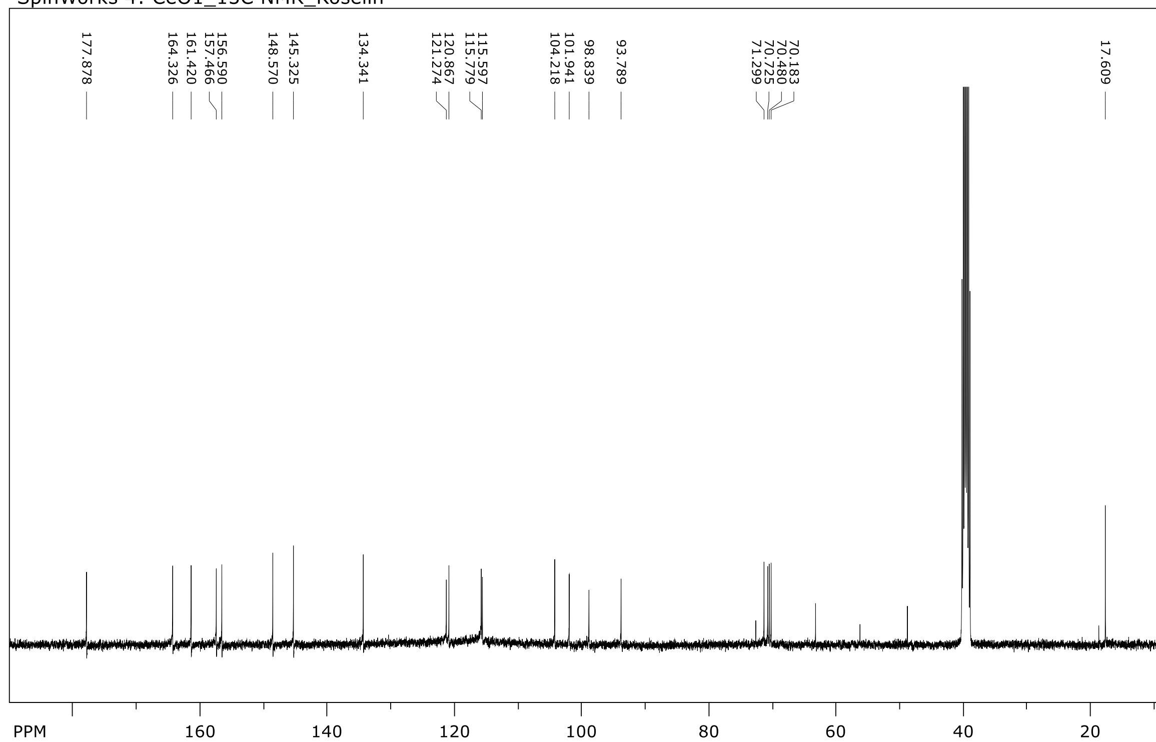
Appendix 1a: The ^{1}H NMR spectrum of compound **1**



Appendix 1b: The expanded ^{1}H NMR spectrum of compound **1** (Aromatic region)



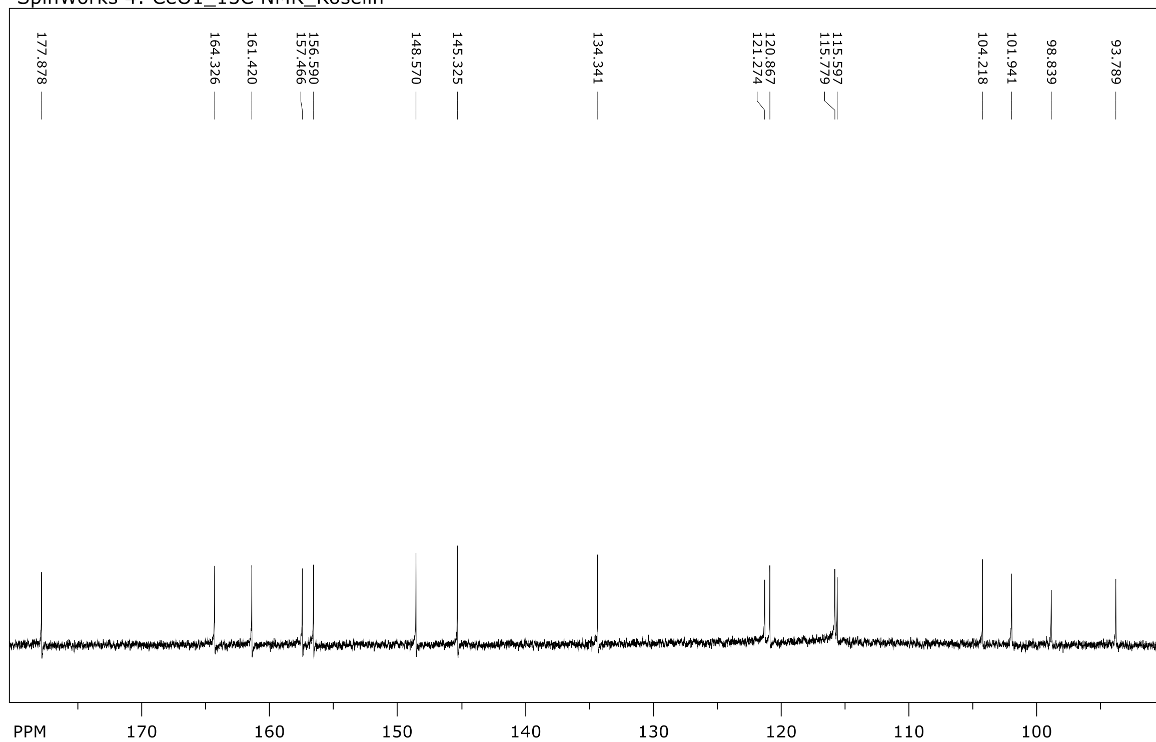
Appendix 1c: The expanded ^1H NMR spectrum of compound **1** (Aliphatic region)



file: ...s\DR. ADEROGBA\Desktop\CeO1\11\fid expt: <zgpg30>
transmitter freq.: 100.642947 MHz
time domain size: 65536 points
width: 24038.46 Hz = 238.8489 ppm = 0.366798 Hz/pt
number of scans: 4000

freq. of 0 ppm: 100.632916 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000

Appendix 1d: The ^{13}C NMR spectrum of compound **1**



Appendix 1e: The expanded ^{13}C NMR spectrum of compound **1**