Isolation and Identification of endophytic bacteria from *Crinum macowanii* bulbs and leaves and the biological activity of their secondary metabolites

By

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GENERAL ABSTRACT

The World Health Organization reports that the resistance of bacteria to already available antibiotics causes 700,000 deaths globally. The resistance of microbes to antimicrobials is a ticking time bomb globally and action has to be taken to halt this catastrophe. New antibiotics need to be developed to ensure bacterial infections are treatable, as it is estimated that 10 million people will die annually by the year 2050 due to antimicrobial resistance.

Cancer accounted for 9.6 million deaths worldwide in the year 2018. The treatment of cancer is problematic, as most cancers are difficult to diagnose during the early stages. Treatment methods such as surgery, radiotherapy and chemotherapy are not effective as the cancer cells acquire resistance to drugs during treatment. There is an urgent need for medication to treat or cure cancer.

Of the over 252 antimicrobial drugs considered to be critical by the WHO, 11% are derived from plants, and 90 of the 121 drugs prescribed for the treatment of cancer are derived from plants. Bioactive compounds from plants have been widely used for the treatment and cure of many diseases and infections, resulting in high volumes of plants being harvested. Unfortunately, this leads to over harvesting, over-use and in the end extinction of the plants. The use of plant tissue culture for growing the plants is not viable, as this method produces low levels of bioactive compounds, requires long growth periods, and it is difficult to recover the bioactive secondary compounds from other plant-derived metabolites. Other ways of obtaining bioactive compounds need to be explored.

Endophytes are microorganisms (fungi and bacteria) residing within plant tissues. Endophytes exchange fragments of their genomic DNA with the host plant and in so doing, both host plants and endophytes can metabolize secondary metabolites from the endophyte or host plant. Host plants and endophytes are able to produce similar secondary metabolites which perform distinct functions, including antibacterial and anticancer properties, as found in this study.

In this study *Crinum macowanii* leaves and bulbs were surface sterilized by approved techniques. A total of 8 bacterial endophytes were isolated from the bulbs, 9 bacterial endophytes were isolated from the leaves and 5 fungal endophytes were isolated from both the leaves and bulbs. These included *Acinetobacter guillouiae*, *Pseudomonas moraviensis*, *Pseudomonas* sp., *Rahnella*

aquatilis, Bacillus cereus, Novosphingobium sp., *Raoultella ornithinolytica*, *Burkholderia tropica*, *Pseudomonas palleroniana, Pseudomonas putida, Bacillus safensis*, *Enterobacter asburiae*, *Pseudomonas cichorii* and *Arthrobacter pascens* as the isolated and identified bacteria endophytes, and *Filobasidium magnum, Alternaria alternata, Penicillium chrysogenum*, *Alternaria tenuissima* and *Penicillium* sp. were the isolated and characterized fungi endophytes. The microbial diversity demonstrates that *C. macowanii* harbours different species of endophytes. The endophytes isolated not only show the microbial diversity of the endophytes but also possible diverse bioactive compounds which could be produced by the isolated endophytes, this led to testing of the biological activity of the different crude extracts (plants and endophytes).

The antibacterial test was performed using the broth microdilution method, and we found that the crude endophyte extracts of *Raoultella ornithinolytica, Pseudomonas moraviensis*, *Pseudomonas* sp., *Rahnella aquatilis, Burkholderia tropica, Pseudomonas palleroniana, Enterobacter asburiae*, *Pseudomonas cichorii* and *Arthrobacter pascens* displayed noteworthy antibacterial activity. The results are deemed noteworthy as <0.1 mg/ml concentration of a crude sample ideal concentration for anti-infective bioassays, or crude samples with a concentration of 1.00 mg/ml and less than 0.100 mg/ml or less than 0.625 mg/ml are considered to be effective or *in vitro* antibacterial activity of crude extracts. It was observed in this study that the Gram-positive bacterial species were more susceptible to the antibacterial compounds in the crude extracts of endophytes isolated from the bulbs than the Gram-negative bacteria, whereas the Gram-negative bacterial species were more susceptible to the antibacterial compounds in the crude extracts of endophytes isolated from the leaves than the Gram-positive ones. The crude endophyte extracts could be manufactured as antibacterial agents.

The anticancer activity test was performed using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay against U87MG Glioblastoma cells and A549 Lung carcinoma cells. *Pseudomonas putida* and *Bacillus safensis* crude extracts exhibited a 47% and 50% cell reduction, respectively, against lung carcinoma cells at a concentration of 100 µg/ml. An increased cell viability of >50% was observed in most of the crude extracts, indicating no activity on the cancer cell lines. Most cancer cells, including the UMG87 glioblastoma and A549 lung carcinoma cells, experience hypoxia, where high aerobic glycolysis and acid resistance occurred; these metabolic abnormalities allow cancer cells to survive by propagation and proliferation. When a low dose of chemotherapy is administered, tumour tissue tends to regrow, causing tumour repopulation. This indicates that high dosages should be used for the treatment of cancer cells.

Metabolite profiling of crude plant extracts and crude endophyte (bacteria and fungi) profiling were performed using liquid chromatography coupled to quadrupole time-of-flight with tandem mass spectrometry (LC-Q-TOF-MS). Alkaloids lycorine, crinamine, crinine, crinamidine and powelline were identified in all crude plant (bulbs and leaves) extracts, as well as the crude endophytes (bacteria and fungi) extracts tested. These are true alkaloids of the Amaryllidaceae family, which are only produced by members of this plant family. The isolated endophytes were able to synthesize some secondary metabolites similar to the host plants, as they imitate the chemistry of the host plant. This is achieved by host plants and endophytes inducing metabolism and sharing parts of their metabolic pathways. Other secondary metabolites such as isoquinoline, piperidine, ascopyrone P, vernodalol, maculosin and hodgkinsine were identified in the crude endophyte extracts, and these have reported biological activities. The identified secondary metabolites have been reported to possess either antibacterial activity and/or anticancer activity, which explains the biological activities observed in this study. An array of different compounds with vast applications were identified from the endophyte crude extracts, and further exploration and testing of biological activities should be done. The identification of bioactive compounds from endophytes as observed in this study would help to prevent the over-harvesting and extinction of medicinal plants to obtain bioactive compounds. In doing this, medicinal plants are being conserved and revenue will be generated by the bioprospecting of endophytes metabolites to obtain compounds possessing biological activities.

Overall, the extraction and identification of bioactive secondary metabolites from endophytes appears promising as a feasible approach in the preserving and conserving of medicinal plants. The research in this area predicts that ethnopharmacology research into the bioprospecting of endophytes for their bioactive secondary metabolites can assist in conserving and preserving medicinal plants and generating revenue. Issues including overexploitation and extinction of medicinal plants, antibiotic resistance and of the unavailability of safe cancer drugs could be solved while developing a unique approach to drug solutions using endophytes.

Keywords: *Crinum macowanii*, Endophytes, Antibacterial, Anticancer, LC-Q-TOF-MS, Untargeted analysis

DECLARATION

I, Tendani Edith Sebola, hereby declare that this study entitled “Isolation and Identification of endophytic bacteria from *Crinum macowanii* bulbs and leaves and the biological activity of their secondary metabolites”, is my work conducted under the supervision of Prof Ezekiel Green. This study represents my own work and has not previously been submitted by me or any other person to another institution to obtain a research diploma or degree.

TENDANI EDITH SEBOLA

DEDICATION

I dedicate this work to the Lord God almighty, the giver of life, my strong tower, my refuge, and strength. My dad Mr. M.S Sebola, my mom Mrs. M.J Sebola, my sister Dr U.B Sebola and my brother Mr. M Sebola for their love, support, and prayers.

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RESEARCH OUTPUTS

Journal articles

- **Sebola TE,** Uche-Okereafor NC, Tapfuma KI, Mekuto L, Green E, Mavumengwana V. (2019). Evaluating antibacterial and anticancer activity of crude extracts of bacterial endophytes from Crinum macowanii Baker bulbs. MicrobiologyOpen. 8(12),1-10.

- **Tendani E. Sebola**, Nkemdinma C. Uche-Okereafor, Lukhanyo Mekuto, Maya Mellisa Makatini, Ezekiel Green, and Vuyo Mavumengwana. Antibacterial, anticancer activity and untargeted secondary metabolite profiling of crude bacterial endophytes extracts from *Crinum macowanii* Baker leaves. International Journal of Microbiology. **Accepted for publication.**

- **Tendani E. Sebola**, Nkemdinma C. Uche-Okereafor, Lukhanyo Mekuto, Maya Mellisa Makatini, Ezekiel Green, and Vuyo Mavumengwana. Data on identification of fungal endophytes isolated from *Crinum macowanii* and the antibacterial and secondary metabolite profiling of the crude extracts. (**Prepared for submission to Data in brief**).

Conference presentations

- **Sebola, T. E,** Uche-Okereafor, N., Green, E., and Mavumengwana, V., (2020). Isolation and characterization of fungal endophytes isolated from *Crinum macowanii* Baker and the anticancer activity of their crude extracts. Women in Science Technology Engineering and Mathematics (STEM) conference, East London, South Africa, 17th -20th February 2020.

- **Sebola, T. E.,** Uche-Okereafor, N., Tapfuma K. I., Green, E., and Mavumengwana, V. (2018). Anticancer potentials of secondary metabolites from *Arthrobacter pascens* an endophyte isolated from *Crinum macowanii* leaves obtained from Walter Sisulu botanical garden. BIO Africa Convention, Durban, South Africa, 27th – 29th August 2018.

- **Sebola, T.E.,** Green, E. And Mavumengwana, V. (2017). Isolation and identification of endophytic bacteria from *Crinum macowanii* bulbs and leaves. Women in Science Technology Engineering and Mathematics (STEM) conference, Washington DC, United States of America, 24th -27th October 2017.

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LIST OF ABBREVIATIONS

AAs	Amaryllidaceae alkaloids
ACC	1-aminocyclopropane-1-carboxylic acid
BBB	Blood brain barrier
CNS	Central nervous system
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
HPLC	High profile liquid chromatography
GC	Gas chromatography
IAA	Indole-3-acetic acid
ITS	Internal transcribed spacer
LC	Liquid chromatography
LC-MS	Liquid chromatography–mass spectrometry
LC-Q-TOF-MS	Liquid chromatography quadrupole time-of-flight mass spectrometry
MS	Coupled with mass spectrometry
MS/MS	Molecular mass combined with tandem mass spectrometry
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4 sulfophenyl)-2H-tetrazolium)
MIC	Minimum inhibitory concentration
NA	Nutrient Agar
NaClO	Sodium Hypochlorite
NCBI	National Center for Biotechnology Information
NDP	National Development Plan
NHI	National Health Insurance
NMR	Nuclear magnetic resonance
NSCLC	Non-small cell lung cancer
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDA	Potato Dextrose agar

PVDF	Polyvinylidene fluoride
rDNA	Recombinant Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
SCLC	Small cell lung cancer
sdH_2O	Sterile distilled water
SDGs	Sustainable Development Goals
TMZ	Temozolomide
UN	United Nations
UHPLC	Ultra-High-Performance Liquid Chromatography
WHO	World Health Organization

LIST OF UNITS/SYMBOLS

ºC	Degree Celsius
µL	Microliters
µg/ml	Micrograms per milliliter
h	Hour
g	Gram
g/L	Grams per liter
L	Liters
mg	Milligrams
mg/ml	Milligrams per milliliters
min	Minutes
ml	Milliliters
m/z	Mass-to-charge ratio
pH	Potential of Hydrogen
rcf	Relative centrifugal force
rt	Retention time
v/v	Volume per volume
w/v	Weight per volume
x g	Gravitational force

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THESIS OUTLINE

This thesis describes studies on the Isolation and identification of endophytes from *Crinum macowanii* Baker and evaluating the crude extracts (plants and endophytes) for antibacterial and anticancer activity as well as untargeted secondary metabolite profiling of the crude extracts (plants and endophytes). The experiments described herein were conducted in the Department of Biotechnology and Food Technology, University of Johannesburg (South Africa).

The thesis consists of six chapters; a brief description of each chapter is outlined below:

Chapter One: General introduction

This chapter introduces the research subject and provides further relevant background information. The chapter also highlights the justification, hypothesis, aims and objectives of the study.

Chapter Two: Literature Review

This chapter gives a detailed review on medicinal plants and their endophytes. The importance of endophytes and the botanical description, phytochemistry and ethnobotany of *C. macowanii* and its related plant species and techniques used in this study are discussed.

Chapter Three: Isolation and identification of microflora and/or mycoflora from *Crinum macowanii* Baker

This chapter describes the isolation, morphological and molecular identification of endophytic bacteria and fungi from *C. macowanii* bulbs and leaves.

Chapter Four: Evaluating the antibacterial and anticancer activity of crude plant extracts and crude endophytic extracts

This chapter presents the study of the biological activity of the crude extracts and secondary metabolites. Antibacterial tests were carried out on pathogenic bacteria strains and anticancer assays on two cancer cell lines to investigate the activity of the different crude extracts and the secondary metabolites from the endophytic bacteria.

Chapter Five: Untargeted secondary metabolite profiling of crude plant extracts and crude endophyte (bacteria and fungi) extracts using liquid chromatography coupled to a quadrupole time-of-flight with tandem mass spectrometry (LC-Q-TOF-MS)

In this chapter, the phytochemical analysis of the crude extracts and secondary metabolites is described. Although the chemical profile of the plant is known, the interest was to explore the screening using LC-Q-TOF-MS.

Chapter Six: General discussion and conclusion

This is the final chapter in the thesis, which draws a connection between the experimental chapters of the study and gives a brief review of the analytical and experimental work done, and a general of the conclusion is drawn from the study. Recommendations for future work are drawn from the outcomes of the study.

CHAPTER ONE:
GENERAL INTRODUCTION*

* This chapter introduces the research subject and further provides relevant background information. The chapter also highlights the problem statement and justification, aim and objectives of the study.

1.0 BACKGROUND

People have been using plants for centuries for practical applications which include food, shelter, tools and medicine (Reid *et al*., 2009). The wide use of plants globally has been due to easy access, affordability and the cultural significance they may possess (Brusotti *et al.*, 2014; Fennell *et al.*, 2004). People have been using plants as medicine for 60,000 years to maintain and increase physical, mental and spiritual health and to treat specific conditions, infection, diseases and ailments (Rajbhandary, 2014; Yuan *et al*., 2016; Akinyemi *et al*., 2018). Such information on plant usage and herbal preparations would be passed on from one generation to the next through wall paintings or verbally as a form of traditional medicine (Adefolaju, 2011).

The medicaments used in traditional medicine are mostly derived from natural products, including medicinal plants (Yuan *et al*., 2016). Plants naturally produce biologically active chemical compounds known as phytochemicals or secondary metabolites (Saxena *et al.*, 2013). These secondary metabolites include tannins, glycosides, terpenoids alkaloids and phenols, and have been reported to possess pharmacological properties such as antimicrobial and anticancer activities (Tiwari *et al.*, 2011; Saxena *et al.*, 2013; Akinyemi *et al*., 2018).

Akinyemi *et al*. (2018) reported that 11% of drugs considered to be essential by the World Health Organization (WHO) are of plant origin, while many others are synthetic drugs derived from natural precursors. Some of these drugs obtained from plants include morphine from *Papaver omniferum*, cocaine from *Erythroxylum coca*, quinine and quinidine from *Cinchona* spp and atropine from *Atropa belladonna* (Salim *et al.*, 2008; Sebola *et al*., 2016; Yuan *et al*., 2016). Such discoveries and the production of herbal drugs gave birth to modern medicine used today (Pal and Shukla, 2003). Generally medicinal plants often grow and reproduce slowly and only grow in specific habitats, and with their high demand and extensive use they can be over harvested, which can lead to extinction (van Wyk and Prinsloo, 2018). Obtaining bioactive compounds from plants presents a number of challenges, including the over-harvesting of plants; different growing environments and environmental conditions tend to produce different yields, which makes reproducibility difficult, and synthesis of the bioactive compounds is challenging due to their complex structures and side effects (Venugopalan and Srivastava, 2015). Therefore, other ways of obtaining the bioactive compounds or similar bioactive compounds should be investigated.

Endophytes are microorganisms, and include bacteria, fungi, protists and archaea present in plants. They do not cause any disease to the plant and their associations with plants can be commensal or mutualistic (Tymon and Inglis, 2009; Arora and Ramawat, 2017).

Due to the genetic cross talk and horizontal gene transfer between the host and endophytes, endophytes are able to produce the same secondary metabolites as the host plant (Rodriguez *et al*., 2009; Kusari *et al*., 2012; Kumara *et al*., 2014). Tidke *et al*. (2017) reported that bioactive secondary metabolites such as alkaloids, flavonoids, phenolic acids, quinones and steroids have been isolated from endophytes. These bioactive metabolites from endophytes could be alternative sources of therapeutic compounds which could help eradicate problematic infections affecting the human population such as cancer and antibiotic resistance, and therefore have potential applications in medicine (Jalgaonwala and Mahajan 2011; Menpara and Chanda 2013; Tidke *et al*., 2017).

1.1 STATEMENT OF THE PROBLEM AND JUSTIFICATION

The World Health Organization (WHO) has pronounced antibiotic resistance to be a "global public health concern", causing an estimated 700,000 estimated deaths each year globally (Aslam *et al*., 2018; Bloom *et al*., 2018). Over 10 million deaths per year have been estimated will occur globally by 2050 due to antimicrobial resistance (African Union Centres for Disease Control and Prevention, 2018). Ventola (2015) reports that causes of antibiotic resistance include overuse, inappropriate prescribing, extensive agricultural use, regulatory barriers and the availability of few new antibiotics. Enzymatic barrier, membrane barrier, efflux and membrane permeability mutations enable microorganisms to acquire resistance to drugs currently used as therapeutic agents (Davin-Regli and Pages, 2015). This presents a concern, as new multi-resistant strains of microorganism are emerging. There is a need to develop and discover new drugs to combat antimicrobial resistance (Rajbhandary, 2014).

Chen *et al*. (2013) reported that the WHO has elucidated that cancer is one of the top ten leading causes of mortality worldwide. Global cancer statistics indicate that 7.6 million death from cancer have been reported and it is predicted to increase to 27 million new cancer cases and 17.5 million cancer deaths by 2050 world-wide (Santos and Ferraris, 2012). Despite the available treatment methods such as surgery, chemotherapy, and/or radiation therapy, treatment of the disease is very difficult due to treatment side effects, and high cost of the drugs (Kumar *et al*., 2014). The removal of the tumor by surgery has been an effective therapy method as the tumor is removed and there is no tissue damage (Abbas and Rehman, 2018).Chemotherapy drugs such as mitomycin C, floxuridine and epirubicin have shown great effectiveness against cancer cells as they are targeted therapies which attack cancer cells and having less damage to normal cells (Abbas and Rehman,

2018; Meegan and O'Boyle, 2019). Strategies such as microbial biotransformation, microbial co-culture, genome mining, and other molecular tools can help in the search for novel anticancer drugs (Abdalla and Mcgaw, 2018).

Bioprospecting, the search for medicinal drugs from biological resources, can help in the search for novel secondary metabolites, such as focusing on endophytic microorganisms isolated from medicinal plants (Arunachalam and Gayathri, 2010). The South African government has set out public health goals aligned to the Millennium Development Goals 2030, which include combating HIV, TB, malaria and other diseases. Research into the development and innovation capabilities to manufacture active pharmaceutical ingredients, vaccines and biopharmaceuticals will help to achieve these set goals (Department of Science and Technology, 2013).

1.2 HYPOTHESES

It can be hypothesised in this study that microflora and/or mycoflora will be isolated from leaves and bulbs of *C. macowanii*. This is because other *Crinum* species and *C. macowanii* have been reported to inhabit endophytes (Anbukumaran *et al*., 2016; Morare *et al*., 2018). Plant (leaves and bulbs) and endophytes (bacteria and fungi) crude extracts will exhibit antibacterial activity and anticancer activity using microdilution method and MTS assay respectively. The crude extracts will display secondary metabolites present in *C. macowanii* using LC-QTOF- MS.

1.3 AIMS AND OBJECTIVES OF STUDY

1.3.1 Aims

The study is aimed at:

Isolating and identifying culturable microflora and/or mycoflora endophytes from *Crinum macowanii* bulbs and leaves, extracting and characterizing crude extracts from plant material and crude endophytes extracts and further testing the biological activity of the isolated crude extracts on pathogenic bacteria strains, UMG87 glioblastoma cell lines and A549 lung carcinoma cell lines.

1.3.2 Objectives

For the aims stated above to be achieved, these objectives are required;

1. Isolate and identify culturable bacteria and fungi endophytes from the bulbs and leaves.

2. Extract crude endophytes extracts (bacteria and fungi) and crude plant extracts (bulbs and leaves).

3. Identify secondary metabolite from the crude endophyte extracts (bacteria and fungi) and crude plant extracts (bulbs and leaves) with notable biological activity by using liquid chromatography – quadrupole time of flight mass spectrometry (LC-QTOF- MS).

4. Determine the minimum inhibitory concentration of the crude plant extracts and crude endophyte extracts on pathogenic bacterial strains using the micro dilution broth method.

5. Determine the cytotoxicity levels of the crude plant extracts and crude endophyte extracts on U87MG glioblastoma cells and A549 lung carcinoma cells using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay.

References

Abbas, Z., Rehman, S., 2018. An Overview of Cancer Treatment Modalities, in: Neoplasm, Hafiz Naveed Shahzad, IntechOpen. pp. 147–149. https://doi.org/http://dx.doi.org/10.5772/57353

Abdalla, M.A., Mcgaw, L.J., 2018. Bioprospecting of South African Plants as a Unique Resource for Bioactive Endophytic Microbes. Front. Pharmacol. 9, 1–18. https://doi.org/10.3389/fphar.2018.00456

Abdel-Azeem, A.M., Zaki, S.M., Khalil, W.F., Makhlouf, N.A., Farghaly, L.M., 2019. Anti-rheumatoid Activity of Secondary Metabolites Produced by Endophytic Chaetomium globosum, in: Singh, B.P., Rateb, M.E., Rodriguez-Couto, S., Lourdes, M. de, Moraes, T. de, Polizeli, Li, W.-J. (Eds.), Microbial Secondary Metabolites: Recent Developments and Technological Challenges. pp. 18–20. https://doi.org/10.3389/fmicb.2019.00914

Acosta, K., Pigni, N., Oleas, N., Bastida, J., 2014. Identification of the alkaloids of stenomesson aurantiacum (Kunth) herb an amaryllidaceae species from the ecuadorian andes. Pharmacologyonline 3, 178–183.

Adefolaju, T., 2011. of Health Research Enquiries : Enquirie s : Int. J. Heal. Res. 4, 99–106.

Adhikari, P., Pandey, A., Agnihotri, V., Pande, V., 2018. Selection of solvent and extraction method for determination of antimicrobial potential of Taxus wallichiana Zucc. Res. Pharm. 8, 1–9. https://doi.org/10.25081/rip.2018.v8.3487

African Union Centers for Disease Control and Prevention, 2018. Africa cdc framework for

antimicrobial resistance, 2018-2023.

Afroz, S., Oliur Rahman, M., Hassan, M.A., 2018. Taxonomic revision of the genus Crinum L. (Liliaceae) of Bangladesh. Bangladesh J. Plant Taxon. 25, 257–271. https://doi.org/10.3329/bjpt.v25i2.39531

Ahmad, S., 2007. Introduction of Plant Constituents and their Tests. pharamacognosy 10, 1–40.

Ahmad, S., Garg, M., Ali, M., Singh, M., Athar, T., Ansari, S.H., 2009. A phyto-pharmacological overview on Adhatoda zeylanica Medic . syn . A . vasica (Linn .) Nees. Nat. Prod. Radiance 8, 549–554.

Aires, A., 2012. Phenolics in Foods: Extraction, Analysis and Measurements. Intech 72–75. https://doi.org/10.1016/j.colsurfa.2011.12.014

Akinsanya, A.M., Ting, S.A., Sanusi, Om., 2017. Extraction methods and TLC-bioautography for evaluation of antimicrobial activities of endophytic bacteria from medicinal plants. LASU J. Med. Sci. 2, 1–7.

Akinyemi, O., So, O., Ka, J., 2018. Medicinal plants and sustainable human health : a review. Hortic. Int. J. 2, 194–195. https://doi.org/10.15406/hij.2018.02.00051

Ali, J., Rafiq, Q.A., Ratcliffe, E., 2018. Antimicrobial resistance mechanisms and potential synthetic treatments. Futur. Sci. OA 4, 1–10. https://doi.org/10.4155/fsoa-2017-0109

Ali, S., Duan, J., Charles, T.C., Glick, B.R., 2014. A bioinformatics approach to the determination of genes involved in endophytic behavior in Burkholderia spp . J. Theor. Biol. 343, 193–198. https://doi.org/10.1016/j.jtbi.2013.10.007

Almoulah, N.F., Voynikov, Y., Gevrenova, R., Schohn, H., Tzanova, T., Yagi, S., Thomas, J., Mignard, B., Ahmed, A.A.A., Siddig, M.A. El, Spina, R., Laurain-mattar, D., 2017. South African Journal of Botany Antibacterial , antiproliferative and antioxidant activity of leaf extracts of selected Solanaceae species. South African J. Bot. 112, 368–374. https://doi.org/10.1016/j.sajb.2017.06.016

Anbukumaran, A., Ambikapathy, V., Panneerselvam, A., 2016. Isolation and characterization of endophytic fungi from Crinum asiaticum Lin Kodiyakarai , Tamilnadu , India. Der Pharm. Lett. 8, 269–275.

Andreassen, M., Lundt, I., 2006. A new chemical synthesis of Ascopyrone P from 1,5-anhydro-d-fructose. Carbohydr. Res. 341, 1692–1696. https://doi.org/10.1016/j.carres.2006.03.037

Andrews, J.M., 2001. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 1, 5–16.

Andryukov, B., Mikhailov, V., Besednova, N., 2019. The Biotechnological Potential of Secondary

Metabolites from Marine Bacteria. J. Mar. Sci. Eng. 7, 1–16.

Aniszewski, T., 2007a. Definition, Typology and Occurrence of Alkaloids, in: Alkaloids – secrets of life alkaloid chemistry, biological significance, applications and ecological role. Elsevier, Amsterdam, The Netherlands, pp. 40–53.

Aniszewski, T., 2007b. Alkaloids – secrets of life alkaloid chemistry, biological significance, applications and ecological role.

Anjum, N., Chandra, R., 2015a. Endophytic bacteria : Optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J. Pharamaceutical Clin. Res. 8, 233–238.

Anjum, N., Chandra, R., 2015b. Endophytic bacteria : optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J. Pharamaceutical Clin. Res. 8, 233–238.

Antony, M.L., Lee, J., Hahm, E.R., Kim, S.H., Marcus, A.I., Kumari, V., Ji, X., Yang, Z., Vowell, C.L., Wipf, P., Uechi, G.T., Yates, N.A., Romero, G., Sarkar, S.N., Singh, S. V., 2014. Growth arrest by the antitumor steroidal lactone withaferin a in human breast cancer cells is associated with down-regulation and covalent binding at cysteine 303 of β-tubulin. J. Biol. Chem. 289, 1852–1865. https://doi.org/10.1074/jbc.M113.496844

Anuhya, G., Jyostna, V., Aswani, K.Y., Bodaiah, B., Sudhakar, P., 2017. Influence of physico-chemica parameters on secondary metabolite production by marine fungi. Int. J. Curr. Pharm. Res. 9, 112–118. https://doi.org/10.22159/ijcpr.2017v9i5.22152

Aretz, I., Meierhofer, D., 2016. Advantages and pitfalls of mass spectrometry based metabolome profiling in systems biology. Int. J. Mol. Sci. 17, 1–14. https://doi.org/10.3390/ijms17050632

Arora, J., Ramawat, K.G., 2017. Endophytes: Biology and Biotechnology. Endophytes Biol. Biotechnol. 15, 1–24. https://doi.org/10.1007/978-3-319-66541-2

Artun, F.T., Karagoz, A., Ozcan, G., Melikoglu, G., Anil, S., Kultur, S., Sutlupinar, N., 2016. In vitro anticancer and cytotoxic activities of some plant extracts on HeLa and Vero cell lines. J. Balk. Union Oncol. 21, 720–725.

Arunachalam, C., P.Gayathri, 2010. Studies on bioprospecting of endophytic bacteria from the medicinal plant of andrographis paniculata for their antimicrobial activity and antibiotic susceptibility pattern. Int. J. Curr. Pharm. Res. 2, 63–68.

Ashok, P.K., Upadhyaya, K., 2012. Tannins are Astringent. J. Pharmacogn. Phytochem. 1, 45–50.

Asif, H., Studholme, D.J., Khan, A., Aurongzeb, M., Khan, I.A., Kamran Azim, M., 2016. Comparative genomics of an endophytic Pseudomonas putida isolated from mango orchard.

Genet. Mol. Biol. 39, 465–473.

Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., Nisar, M.A., Alvi, R.F., Aslam, M.A., Qamar, M.U., Salamat, M.K.F., Baloch, Z., 2018. Antibiotic resistance: a rundown of a global crisis. Infect. Drug Resist. 11, 1645–1658. https://doi.org/10.2147/IDR.S173867

Asmawi, M.Z., Arafat, O.M., Amirin, S., Eldeen, I.M., 2011. In vivo Antinociceptive Activity of leaf extract of crinum asiaticum and Phytochemical Analysis of the Bioactive Fractions. Int. J. Pharmacol.

Atzori, C., Bruno, A., Chichino, G., Bombardelli, E., Scaglia, M., Ghione, M., 1993. Activity of bilobalide, a sesquiterpene from Ginkgo biloba, on Pneumocystis carinii. Antimicrob. Agents Chemother. 37, 1492–1496. https://doi.org/10.1128/AAC.37.7.1492

Aziz, A., Raju, G.S., Das, A., Ahmed, J., Moghal, M.M.R., 2014. Evaluation of in vitro anthelmintic activity, total phenolic content and cytotoxic activity of crinum latifolium L. (Family: Amaryllidaceae). Adv. Pharm. Bull. 4, 15–19. https://doi.org/10.5681/apb.2014.003

Azwanida, N., 2015. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med. Aromat. Plants 4, 1–6. https://doi.org/10.4172/2167-0412.1000196

Baker, S., Satish, S., 2012. Endophytes : Natural Warehouse of Bioactive Compounds. Drug Invent. Today 4, 548–553.

Bastida, J., Berkov, S., Torras Clavería, L., Pigni, N.B., Andradre, J.P. De, Martínez, V., Codina Mahrer, C., Viladomat Meya, F., 2011. Chemical and biological aspects of Amaryllidaceae alkaloids, Recent Advances in Pharmaceutical Sciences.

Bastida, J., Lavilla, R., Francesc, V., 2006. Chemical and Biological Aspects of Narcissus Alkaloids. Alkaloids Chem. Biol. · 63, 89–92. https://doi.org/10.1016/S1099-4831(06)63003-4

Bedi, A., Adholeya, A., Deshmukh, S.K., 2017. Novel Anticancer Compounds from Endophytic Fungi. Curr. Biotechnol. 6, 1–18. https://doi.org/10.2174/2211550105666160622080 3

Begum, P., Noman, A. Al, Adib, M., Ahsan, M., Islam, S.N., 2017. Antibacterial Activity of Ethnic Vegetables Consumed by the Indigenous People Living in Chittagong Hill Tracts. European J. Med. Plants 18, 1–7. https://doi.org/10.9734/EJMP/2017/32059

Bengal, N., Raj, A.J., Biswakarma, S., Pala, N.A., Shukla, G., Kumar, M., Chakravarty, S., Bussmann, R.W., 2018. Indigenous uses of ethnomedicinal plants among forest-dependent communities of Northern Bengal, India. J. Ethnobiol. Ethnomed. 14, 1–28.

https://doi.org/10.1186/s13002-018-0208-9

Bhandurge, P., Rajarajeshwari, N., Ganapaty, S., Pattanshetti, S., 2013. The Gnidia genus: A review. Asian J. Biomed. Pharm. Sci. 3, 1–31.

Bhawani, S.A., Sulaiman, O., Hashim, R., Mohamad, M.N., 2010. Thin-Layer Chromatographic Analysis of Steroids : A Review. Trop. J. Pharm. Res. 9, 301–313.

Bick, I.R.C., 1996. Alkaloids from Australian Flora, in: Pelletier, S.W. (Ed.), Alkaloids" Chemical and Biological Perspectives. Elsevier, pp. 94–116.

Bladt, T.T., Frisvad, J.C., Knudsen, P.B., Larsen, T.O., 2013. Anticancer and Antifungal Compounds from Aspergillus, Penicillium and Other Filamentous Fungi. Molecules 18, 11338–11376. https://doi.org/10.3390/molecules180911338

Blaženovi, I., Kind, T., Ji, J., Fiehn, O., 2018. Software Tools and Approaches for Compound Identification of LC-MS / MS Data in Metabolomics. Metabolites 8, 1–23. https://doi.org/10.3390/metabo8020031

Bloom, D.E., Black, S., Salisbury, D., Rappuoli, R., 2018. Antimicrobial resistance and the role of vaccines. Proc. Natl. Acad. Sci. 115, 12868–12871. https://doi.org/10.1073/pnas.1717157115

Blumenthal, M., 2009. African Natural Plant Products : A Foreword to the Science and Challenges, in: Juliani, H.R., Simon, J., Ho, C.-T. (Eds.), African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality. ACS Pulbication series, Rutgers, The State University of New Jersey, pp. 157–166.

Boopathy, N.S., Kathiresan, K., 2010. Anticancer Drugs from Marine Flora : An Overview. J. ofOncology 2010, 1–18. https://doi.org/10.1155/2010/214186

Botanical Society of South Africa, 2007. The stately Crinum lilies. wildflower Conserv. 26.

Bozkurt, B., Emir, A., Kaya, G.İ., Önür, M.A., Berkov, S., Bastida, J., Ünver Somer, N., 2017. Alkaloid profiling of galanthus woronowii losinsk. By GC-MS and evaluation of its biological activity. Marmara Pharm. J. 21, 915–920. https://doi.org/10.12991/mpj.2017.12

Brader, G., Stephane, C., Birgit, M., Mitter, B., Trognitz, F., Sessitsch, A., 2014. Metabolic potential of endophytic bacteria. Curr. Opin. Biotechnol. 30–37. https://doi.org/10.1016/j.copbio.2013.09.012

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global Cancer Statistics 2018 : Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA. Cancer J. Clin. 98, 394–424. https://doi.org/10.3322/caac.21492

Brueton, V.J., 2013. Trade in commonly used medicinal bulbs: value and ecological implications.

University of the Witwatersrand.

Brusotti, G., Cesari, I., Dentamaro, A., Caccialanza, G., Massolini, G., 2014. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. J. Pharm. Biomed. Anal. 87, 218–228. https://doi.org/10.1016/j.jpba.2013.03.007

Cain, C.C., Lee, D., Iii, R.H.W., Henry, A.T., Jr, E.J.C., Wani, M.C., Wall, M.E., Oberlies, N.H., Iii, J.O.F., 2003. Synergistic Antimicrobial Activity of Metabolites Produced by a Nonobligate Bacterial Predator. Antimicrob. Agents Chemother. 47, 2113–2117. https://doi.org/10.1128/AAC.47.7.2113

Calvo, A.M., Wilson, R.A., Bok, J.W., Keller, N.P., 2002. Relationship between Secondary Metabolism and Fungal Development. Microbiol. Mol. Biol. Rev. 66, 447–459. https://doi.org/10.1128/MMBR.66.3.447

Cancer Council Australia, 2018. Understanding Lung Cancer A guide for people with cancer, their families and friends.

Cao, Z., Yang, P., Zhou, Q., 2013. Multiple biological functions and pharmacological effects of lycorine. Sci. China Chem. 56, 1382–1391. https://doi.org/10.1007/s11426-013-4967-9

Cardoso, J.C., de Oliveira, M.E.B.S., Cardoso, F. de C.I., 2019. Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. Hortic. Bras. 37, 124–132. https://doi.org/10.1590/s0102-053620190201

Changwa, R., Abia, W., Msagati, T., Nyoni, H., Ndleve, K., Njobeh, P., 2018. Multi-Mycotoxin Occurrence in Dairy Cattle Feeds from the Gauteng Province of South Africa : A Pilot Study Using UHPLC-QTOF-MS / MS. Toxicon 10, 1–21. https://doi.org/10.3390/toxins10070294

Cheesman, L., Nair, J.J., van Staden, J., 2012. Antibacterial activity of crinane alkaloids from Boophone disticha (Amaryllidaceae). J. Ethnopharmacol. 140, 405–408. https://doi.org/10.1016/j.jep.2012.01.037

Chen, S.L., Yu, H., Luo, H.M., Wu, Q., Li, C.F., Steinmetz, A., 2016. Conservation and sustainable use of medicinal plants : problems , progress , and prospects. Chin. Med. 11, 1–10. https://doi.org/10.1186/s13020-016-0108-7

Chen, Y., Yuan, Q., Shan, L., Lin, M., Cheng, D., Li, C., 2013. Antitumor activity of bacterial exopolysaccharides from the endophyte Bacillus amyloliquefaciens sp . isolated from Ophiopogon japonicus 1787–1792. https://doi.org/10.3892/ol.2013.1284

Chikezie, P.., Ibegbulem, C.., Mbagwu, F.., 2015. Medicinal & Aromatic Plants Medicinal Potentials and Toxicity Concerns of Bioactive Principles. Med. Aromat. Plants 4, 1–15.

https://doi.org/10.4172/2167-0412.1000202

Chrapusta, E., Kaminski, A., Duchnik, K., Bober, B., Adamski, M., Bialczyk, J., 2017. Mycosporine-Like Amino Acids: Potential health and beauty ingredients. Mar. Drugs 15, 1–29. https://doi.org/10.3390/md15100326

Chutulo, E.C., Chalannavar, R.K., 2018. Endophytic Mycoflora and Their Bioactive Compounds from Azadirachta Indica :A Comprehensive Review. J. Funct. Foods 4, 1–12. https://doi.org/10.3390/jof4020042

Çitoğlu, G.S., Acıkara, Ö.B., 2012. Column Chromatography for Terpenoids and Flavonoids, in: Dhanarasu, S. (Ed.), Chromatography and its applications. InTech, Rijeka, Croatia, pp. 13–52.

Cos, P., Vlietinck, A.J., Berghe, D. Vanden, Maes, L., 2006. Anti-infective potential of natural products : How to develop a stronger in vitro ' proof-of-concept .' J. Ethnopharmacol. 106, 290–302. https://doi.org/10.1016/j.jep.2006.04.003

Coussens, N.P., Braisted, J.C., Peryea, T., Sittampalam, G.S., Simeonov, A., Hall, M.D., 2017. Small-Molecule Screens : A Gateway to Cancer Therapeutic Agents with Case Studies of Food and Drug Administration – Approved Drugs. Pharmacol. Rev. 479–496.

Crouch, N.R., Pohl, T.L., Mulholland, D.A., Ndlovu, E., 2005. Alkaloids from three ethnomedicinal Haemanthus species: H. albiflos, H. deformis and H. pauculifolius (Amaryllidaceae). South African J. Bot. 71, 49–52. https://doi.org/10.1016/S0254-6299(15)30148-4

da Costa Souza, P.N., Grigoletto, T.L.B., de Moraes, L.A.B., Abreu, L.M., Guimarães, L.H.S., Santos, C., Galvão, L.R., Cardoso, P.G., 2016. Production and chemical characterization of pigments in filamentous fungi. Microbiol. (United Kingdom) 162, 12–22. https://doi.org/10.1099/mic.0.000168

Dar, R.A., Shahnawaz, M., Qazi, P.H., 2017. General overview of medicinal plants : A review. J. Phytopharm. 6, 349–351.

Davies, J., 2007. In the beginning there was streptomycin, in: arya, d.p. (ed.), aminoglycoside antibiotics From Chemical Biology to Drug Discovery. John Wiley & Sons, Inc, Hoboken, New Jersey., pp. 1–9.

Davies, J., Davies, D., 2010. Origins and Evolution of Antibiotic Resistance. Microbiol. Mol. Biol. Rev. 74, 417–433. https://doi.org/10.1128/MMBR.00016-10

Davin-Regli, A., Pages, J.-M., 2015. Enterobacter aerogenes and Enterobacter cloacae ; Versatile bacterial pathogens confronting antibiotic treatment. Front. Microbiol. 6, 1–10.

https://doi.org/10.3389/fmicb.2015.00392

De Andrade, J.P., Pigni, N.B., Torras-Claveria, L., Guo, Y., Berkov, S., Reyes-Chilpa, R., El Amrani, A., Zuanazzi, J.A.S., Codina, C., Viladomat, F., Bastida, J., 2012. Alkaloids from the Hippeastrum genus: Chemistry and biological activity. Rev. Latinoam. Quim. 40, 83–98.

De Mot, R., Ghequire, M.G.K., 2014. Ribosomally encoded antibacterial proteins and peptides from Pseudomonas. Fed. Eur. Microbiol. Soc. 38, 523–568. https://doi.org/10.1111/1574-6976.12079

Dela Cruz, C.S., Tanoue, L.T., Matthay, R.A., 2011. Lung Cancer: Epidemiology, Etiology, and Prevention. Clin. Chest Med. 32, 605–644. https://doi.org/10.1016/j.ccm.2011.09.001

Delcour, A.H., 2009. NIH Public Access. Biochim. Biophys. Acta 1794, 808–816. https://doi.org/10.1016/j.bbapap.2008.11.005.Outer

Demain, A.L., Fang, A., 2000. The Natural Functions of Secondary Metabolites, in: Scheper, T. (Ed.), Advances in Biochemical Engineering / Biotechnology. Springer, pp. 1–33. https://doi.org/10.1016/j.jbiotec.2007.09.006

Denicolaï, E., Baeza-kallee, N., Tchoghandjian, A., Carré, M., Colin, C., Jiglaire, C.J., Mercurio, S., Beclin, C., Figarella-branger, D., 2014. Proscillaridin A is cytotoxic for glioblastoma cell lines and controls tumor xenograft growth in vivo. Oncotarget 5, 10934–10948.

Department of Agriculture Forestry and Fisheries Republic of South Africa, 2013. Medicinal plants of South Africa. Pretoria, South Africa.

Department of Science and Technology, S.A., 2013. The Bio-economy Strategy is an initiative of the Department of Science and Technology, South Africa.

Devi, P., Rodrigues, C., Naik, C.G., 2012. Isolation and Characterization of Antibacterial Compound from a Mangrove-Endophytic Fungus , Penicillium chrysogenum MTCC 5108. Indian J. Microbiol. 52, 617–623. https://doi.org/10.1007/s12088-012-0277-8

Dias, D. a., Urban, S., Roessner, U., 2012. A Historical Overview of Natural Products in Drug Discovery. Metabolites 2, 303–336. https://doi.org/10.3390/metabo2020303

Doty, S.L., 2017. Functional Importance of the Plant Endophytic Microbiome : Implications for Agriculture , Forestry , and Bioenergy, in: Doty, S.L. (Ed.), Functional Importance of the Plant Microbiome. Springer International Publishing, Cham, Switzerland, pp. 1–5. https://doi.org/10.1007/978-3-319-65897-1

Doughari, J.H., 2012. Phytochemicals : Extraction Methods , Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, in: Phytochemicals - A Global Perspective of Their Role in Nutrition and Health. pp. 1–33.

Duan, L., Guo, L., Wang, L., Yin, Q., Zhang, C.M., Zheng, Y.G., Liu, E.H., 2018. Application of metabolomics in toxicity evaluation of traditional Chinese medicines. Chin. Med. 13, 1–14. https://doi.org/10.1186/s13020-018-0218-5

Dwivedi, G.R., Sisodia, B.S., Shikha, 2019. Secondary Metabolites: Metabolomics for Secondary Metabolites, in: Gupta, V.K., Pandey, A. (Eds.), New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier, Amsterdam, Netherlands, pp. 337–339. https://doi.org/10.1016/b978-0-444-63504-4.00022-0

Dzotam, J.K., Simo, I.K., Bitchagno, G., Celik, I., Sandjo, L.P., Tane, P., Kuete, V., 2018. In vitro antibacterial and antibiotic modifying activity of crude extract, fractions and 3',4',7-trihydroxyflavone from Myristica fragrans Houtt against MDR Gram-negative enteric bacteria. BMC Complement. Altern. Med. 18, 1–9. https://doi.org/10.1186/s12906-018-2084-1

Egan, J.M., Kaur, A., Raja, H.A., Kellogg, J.J., Oberlies, N.H., Cech, N.B., 2016. Antimicrobial fungal endophytes from the botanical medicine goldenseal (Hydrastis canadensis). Phytochem. Lett. 17, 219–225. https://doi.org/10.1016/j.phytol.2016.07.031

Ek-Ramos, M.J., Gomez-Flores, R., Orozco-Flores, A.A., Rodríguez-Padilla, C., González-Ochoa, G., Tamez-Guerra, P., 2019. Bioactive Products From Plant-Endophytic Gram-Positive Bacteria. Front. Microbiol. 10, 1–12. https://doi.org/10.3389/fmicb.2019.00463

El-Desouky, S.K., Gamal-Eldeen, A.M., 2009. Cytotoxic and anti-inflammatory activities of some constituents from the floral buds of Syringa patula. Pharm. Biol. 47, 872–877. https://doi.org/10.1080/13880200902950785

El-Gendy, M.M.A., Hawas, U.W., Jaspars, M., 2008. Novel bioactive metabolites from a marine derived bacterium Nocardia sp. ALAA 2000. J. Antibiot. (Tokyo). 61, 379–386. https://doi.org/10.1038/ja.2008.53

El-Hendawy, H.., Osman, M.., Sorour, N.., 2003. Characterization of Two Antagonistic Strains of Rahnella aquatilis Isolated from Soil in Egypt. Folia Microbiol. 48, 799–804. https://doi.org/10.1007/BF02931517

Elgorashi, E.E., 2000. Alkaloids from three south african Crinum species. University of Natal Pietermaritzburg.

Elgorashi, Esameldin E., Drewes, S.E., Morris, C., Van Staden, J., 2003. Variation among three Crinum species in alkaloid content. Biochem. Syst. Ecol. 31, 601–615. https://doi.org/10.1016/S0305-1978(02)00222-3

Elgorashi, E.E., Drewes, S.E., van Staden, J., 2002. Organ-to-organ and seasonal variation in

alkaloids from Crinum macowanii. Fitoterapia 73, 490–495. https://doi.org/10.1016/S0367-326X(02)00164-8

Elgorashi, E E, Zschocke, S., Staden, J. Van, 2003. The anti-inflammatory and antibacterial activities of Amaryllidaceae alkaloids. South African J. Bot. 69, 448–449. https://doi.org/10.1016/S0254-6299(15)30329-X

Eljounaidi, K., Kyu, S., Bae, H., 2016. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases – Review and future prospects. Biol. Control 103, 62–68. https://doi.org/10.1016/j.biocontrol.2016.07.013

Ellul, S., Wake, M., Clifford, S.A., Lange, K., Würtz, P., Juonala, M., Dwyer, T., Carlin, J.B., Burgner, D.P., Saffery, R., 2019. Body composition: population epidemiology and concordance in Australian children aged 11–12 years and their parents. BMJ Open 9, 106–117. https://doi.org/10.1136/bmjopen-2018-023698

Erasto, P., Grierson, D.S., Afolayan, A.J., 2006. Bioactive sesquiterpene lactones from the leaves of Vernonia amygdalina. J. Ethnopharmacol. 106, 117–120. https://doi.org/10.1016/j.jep.2005.12.016

Fabricant, D.S., Farnsworth, N.R., 2001. The Value of Plants Used in Traditional Medicine for Drug Discovery. Environ. Health Perspect. 109, 69–75.

Fan, C., Zheng, W., Fu, X., Li, X., Wong, Y., Chen, T., 2014. Enhancement of auranofin-induced lung cancer cell apoptosis by selenocystine , a natural inhibitor of TrxR1 in vitro and in vivo. Nat. Publ. Gr. 5, 2–11. https://doi.org/10.1038/cddis.2014.132

Farias, K.J.S., Machado, P.R.L., de Almeida Júnior, R.F., Lopes da Fonseca, B.A., 2019. Brefeldin A and Cytochalasin B reduce dengue virus replication in cell cultures but do not protect mice against viral challenge. Access Microbiol. 1, 1–8. https://doi.org/10.1099/acmi.0.000041

Fathalla, A.M., Samy, A.M.A.E.-A., Metwaly, A.B., Ramadan, E.M., 2015. Characterization of Antifungal Metabolites from Antagonistic Fluorescent Pseudomonads. J. Appl. Plant Prot. 4, 13–21. https://doi.org/10.21608/japp.2015.7593

Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., Kadri, A., 2017. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. Food Sci. Technol. 37, 483–492. https://doi.org/10.1590/1678-457x.23516

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicology. J. Ethnopharmacol. 94, 205–217.

https://doi.org/10.1016/j.jep.2004.05.012

Fennell, C.W., van Staden, J., 2001. Crinum species in traditional and modern medicine. J. Ethnopharmacol. 78, 15–26.

Ferdous, U.., Shishir, M.., Khan, S.., Hoq, M.., 2018. Bacillus spp .: Attractive Sources of Anti-cancer and Anti-proliferative Biomolecules. Microb. Bioact. 1, 033–045. https://doi.org/10.25163/microbbioacts.11005B0408130818

Fiołka, M.J., Grzywnowicz, K., Rzymowska, J., Lewtak, K., Szewczyk, R., Mendyk, E., Keller, R., 2015. Antitumour and apoptotic effects of a novel Tris-peptide complex obtained after isolation of Raoultella ornithinolytica extracellular metabolites. J. Appl. Microbiol. 118, 1357–1369. https://doi.org/10.1111/jam.12806

Fiolka, M.J., Lewtak, K., Rzymowska, J., Grzywnowicz, K., Hulas-Stasiak, M., Sofińska-Chmiel, W., Skrzypiec, K., 2013. Antifungal and anticancer effects of a polysaccharide-protein complex from the gut bacterium Raoultella ornithinolytica isolated from the earthworm Dendrobaena veneta. Pathog. Dis. 69, 49–61. https://doi.org/10.1111/2049-632X.12056

Fomum, S.W., Nsahlai, I. V., 2017. In vitro nematicidal activity of plant species possessing alkaloids and tannins. Cogent Food Agric. 3, 1–12. https://doi.org/10.1080/23311932.2017.1334295

Fouda, A.H., Hassan, S.E., Eid, A.M., Ewais, E.E., 2015. Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss .). Ann. Agric. Sci. 60, 95–104. https://doi.org/10.1016/j.aoas.2015.04.001

Garcia-Bustos, J.F., Pezzi, N., Mendez, E., 1985. Structure and Mode of Action of Microcin 7 , an Antibacterial Peptide Produced by Escherichia coli. Antimicrob. Agents Chemother. 27, 791–797.

Gavhane, Y., Shete, A., Bhagat, A., Shinde, V., Bhong, K., Khairnar, G., Yadav, A., 2011. Solid Tumors : Facts, Challenges and Solutions. Int. J. Pharma Sci. Res. 2, 1–12.

Ghisalberti, E.L., Pennacchio, M., Alexander, E., 1998. Survey of secondary plant metabolites with cardiovascular activity. Pharm. Biol. 36, 237–279. https://doi.org/10.1076/phbi.36.4.237.4583

Ghosal, S., Saini, K.S., Razdan, S., 1985. Crinum alkaloids: their chemistry and biology. Phytochemistry 24, 2141–2156.

Glushakova, A.M., Kachalkin, A. V, 2017. Endophytic Yeasts in Malus domestica and Pyrus communis Fruits under Anthropogenic Impact. Microbiology 86, 128–135. https://doi.org/10.1134/S0026261716060102

Gouda, S., Das, G., Sen, S.K., Shin, H., Patra, J.K., 2016. Endophytes : A Treasure House of Bioactive Compounds of Medicinal Importance. Front. Microbiol. 7, 1–8. https://doi.org/10.3389/fmicb.2016.01538

Grech, A., Baldacchino, A., Tufignio, M., 2009. Secondary Metabolites and their Exploitation in Medicine. Maltese Fam. Dr. 18, 8–13.

Greenwell, M., Rahman, P.K.S.M., 2015. Europe PMC Funders Group Medicinal Plants : Their Use in Anticancer Treatment. Int. J. Pharm. Sci. Res. 6, 4103–4112. https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12.Medicinal

Griffiths, S., van Dyk, S., Malan, S.F., van Zyl, R.L., 2004. Antimalarial Compounds From Crinum bulbispermum. North West University.

Gross, H., Loper, J.E., 2009. Genomics of secondary metabolite production by Pseudomonas spp Genomics of secondary metabolite production by Pseudomonas spp †. Nat. Prod. Rep. 26, 1408–1446. https://doi.org/10.1039/b817075b

Guerriero, G., Berni, R., Muñoz-Sanchez, J.A., Apone, F., Abdel-Salam, E.M., Qahtan, A.A., Alatar, A.A., Cantini, C., Cai, G., Hausman, J.F., Siddiqui, K.S., Hernández-Sotomayor, S.M.T., Faisal, M., 2018. Production of plant secondary metabolites: Examples, tips and suggestions for biotechnologists. Genes (Basel). 9, 1–22. https://doi.org/10.3390/genes9060309

Gunatilaka, A.A.L., 2006. Natural Products from Plant-associated Microorganisms: Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence. Journak Nat. Prod. 69, 509–526. https://doi.org/10.1021/np058128n.Natural

Guo, Y., 2015. Research on the Alkaloids of Amaryllidaceae Plants : Genera Lycoris and Hippeastrum. Universitat de Barcelona.

Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol. Aspects Med. 27, 1–93. https://doi.org/10.1016/j.mam.2005.07.008

Gutierrez-Orozco, F., Failla, M.L., 2013. Biological Activities and Bioavailability of Mangosteen Xanthones: A Critical Review of the Current Evidence. Nutr. 2013, 5, 3163–3183. https://doi.org/10.3390/nu5083163

Handayani, D., Rivai, H., Mulyana, R., Suharti, N., Rasyid, R., Hertiani, T., 2018. Antimicrobial and Cytotoxic Activities of Endophytic Fungi Isolated from Antimicrobial and Cytotoxic Activities of Endophytic Fungi Isolated from Mangrove Plant Sonneratia alba Sm. J Appl Pharm Sci 8, 49–53. https://doi.org/10.7324/JAPS.2018.8207

Hannah, M.A.C., Krishnakumari, S., 2015. Quantitative estimation of plant metabolites in the hot

aqueous seed extract of watermelon (Citrullus vulgaris Schrad .). J. Med. Plants Stud. 3, 107–111.

Hartmann, C., Wesseling, P., 2019. Glioblastoma: Pathology and Genetics, in: Boffetta, P., Hainaut, P. (Eds.), Encyclopedia of Cancer. Elsevier, pp. 165–178. https://doi.org/10.1016/b978-0-12-801238-3.65070-5

He, M., Qu, C., Gao, O., Hu, X., Hong, X., 2015a. Biological and pharmacological activities of amaryllidaceae alkaloids. RSC Adv. 5, 16562–16574. https://doi.org/10.1039/c4ra14666b

He, M., Qu, C., Gao, O., Hu, X., Hong, X., 2015b. Biological and pharmacological activities of amaryllidaceae alkaloids. RSC Adv. 5, 16562–16574. https://doi.org/10.1039/c4ra14666b

He, Y., Cheng, P., Wang, W., Yan, S., Tang, Q., Liu, D., Xie, H., 2018. Rapid Investigation and Screening of Bioactive Components in Simo Decoction via LC-Q-TOF-MS and UF-HPLC-MD Methods. Molecules 23, 1–18. https://doi.org/10.3390/molecules23071792

Hemmati, S., Seradj, H., 2016. Justicidin B: A promising bioactive lignan. Molecules 21, 1–20. https://doi.org/10.3390/molecules21070820

Hilton-Taylor, C., 1996. Red data list of southern African plants. 1. corrections and additions. Bothalia 26, 177–182. https://doi.org/10.4102/abc.v26i2.705

Ho, Y.-N., Huanga, C.-C., 2015. Draft Genome Sequence of Burkholderia cenocepacia Strain 869T2, a Plant-Beneficial Endophytic Bacterium. Genome Announc. 3, 1–2. https://doi.org/10.1128/genomeA.01327-15

Hoffmann, D., 1951. Hoffmann_David_-_Medical_herbalism.pdf.

Hoffmann, T., Krug, D., Huttel, S., Muller, R., 2014. Improving Natural Products Identification through Targeted LC-MS/ MS in an Untargeted Secondary Metabolomics Work flow. Anal. Chem. 86, 10780−10788. https://doi.org/10.1021/ac502805w

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., Armand, R., 2015. The Application of Medicinal Plants in Traditional and Modern Medicine : A Review of Thymus vulgaris. Int. J. Clin. Med. 6, 635–642.

Hounsome, N., Hounsome, B., Tomos, D., Edwards-Jones, G., 2008. Plant Metabolites and Nutritional Quality of Vegetables. J. Food Sci. 73, 48–65. https://doi.org/10.1111/j.1750-3841.2008.00716.x

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.., 1996. Zulu medicinal plants: an inventory. University of Natal Press, Pietermaritzburg., Pietermaritzburg.

Hutf, A.J., Grady, J.O., 1996. Review Drug chirality : a consideration of the significance of the stereochemistry of antimicrobial agents. J. Antimicrob. Chemother. 37, 7–32.

Iannello, C., Bastida, J., Bonvicini, F., Antognoni, F., Gentilomi, G.A., Poli, F., 2014. Chemical composition, and in vitro antibacterial and antifungal activity of an alkaloid extract from Crinum angustum Steud. Nat. Prod. Res. https://doi.org/10.1080/14786419.2013.877903

Iannello, D. ss. C., 2014. Pharmacological screening and biotechnological production of alkaloids from tissues and cells cultured by plants of the amaryllidaceae family. alma mater studiorum - università di bologna dottorato.

Ibraheem, O., Maimako, R.., 2014. Evaluation of Alkaloids and Cardiac Glycosides Contents of Ricinus communis Linn. (Castor) Whole Plant Parts and Determination of their Biological Properties. Int. J. Toxicol. Pharmacol. Res. 6, 34–42.

Ibrahim, M., Kaushik, N., Sowemimo, A., Chhipa, H., Koekemoer, T., Van De Venter, M., Odukoya, O.A., 2017. Antifungal and antiproliferative activities of endophytic fungi isolated from the leaves of markhamia tomentosa. Pharm. Biol. 55, 590–595. https://doi.org/10.1080/13880209.2016.1263671

Idikio, H.A., 2011. Human cancer classification: A systems biology-based model integrating morphology, cancer stem cells, proteomics, and genomics. J. Cancer 2, 107–115. https://doi.org/10.7150/jca.2.107

Idle, J.R., Gonzalez, F.J., 2007. Metabolomics. Cell Metab. 6, 348–352. https://doi.org/10.1016/j.cmet.2007.10.005

Ionkova, I., 2007. Biotechnological Approaches for the Production of Lignans. Pharamacognosy Reviwes 1, 57–68.

Ismaiel, A.A., Ahmed, A.S., Hassan, I.A., El-Sayed, E.-S.R., El-Din, A.-Z.A.K., 2017. Production of paclitaxel with anticancer activity by two local fungal endophytes , Aspergillus fumigatus and Alternaria tenuissima. Appl. Microbiol. Biotechnol. 101, 5831–5846. https://doi.org/10.1007/s00253-017-8354-x

Jain, A., Choubev, S., Singour, P.K., Rajak, H., Pawar, R.S., 2011. Sida cordifolia (Linn) - An overview. J. Appl. Pharm. Sci. 1, 23–31.

Jain, C., Khatana, S., Vijayvergia, R., 2019. Bioactivity of secondary metabolites of various plants: A review. Int. J. Pharm. Sci. Res. 10, 494–504. https://doi.org/10.13040/IJPSR.0975-8232.10(2).494-04

Jain, P., Pundir, R.K., 2017. Potential Role of Endophytes in Sustainable Agriculture-Recent Developments and Future Prospects, in: Sustainable Development and Biodiversity Endophytes: Biology and Biotechnology. pp. 153–154.

Jalgaonwala, R.E., Mahajan, R.T., 2011. Evaluation of hydrolytic enzyme activities of endophytes

from some indigenous medicinal plants. J. Agric. Technol. 2011 7, 1733–1741.

James, J.T., Dubery, I.A., 2009. Pentacyclic Triterpenoids from the Medicinal Herb, Centella asiatica (L.) Urban. Molecules 14, 3922–3941. https://doi.org/10.3390/molecules14103922

Jasim, B., Joseph, A.A., John, C.J., Mathew, J., Radhakrishnan, E.K., 2014. Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of Zingiber officinale. 3 Biotech 4, 197–204. https://doi.org/10.1007/s13205-013-0143-3

Jayakrishnan, T., Benjamin, S., 2016. Purpureocillium lilacinum Strain BP13 Produces Flavoglaucin. Electron. J. Biol. 12, 472–476.

Jayaprasad, B., Sharavanan, P., 2015. Phytochemical, In Vitro Antioxidant and Antimicrobial Activities in Aqueous and Methanol Extracts Of Chloroxylon Swietenia Bark. Res. J. Pharm. Biol. Chem. Sci. 6, 1416–1424.

Jazieh, A.R., Al-Saggabi, A.H., McClung, M., Carlson, R., Schnipper, L.E., Eniu, A., Blauvelt, B., Zafar, Y., Kerr, D., 2018. Facing the Global Challenges of Access to Cancer Medication. J. Glob. Oncol. 4, 1–7. https://doi.org/10.1200/jgo.17.00205

Jia, M., Chen, L., Xin, H., Zheng, C., Rahman, K., Han, T., Qin, L.-P., 2016. A Friendly Relationship between Endophytic Fungi and Medicinal Plants : A Systematic Review. Front. Microbiol. 7, 1–14. https://doi.org/10.3389/fmicb.2016.00906

Jiang, B., 2017. ScienceDirect Aerobic glycolysis and high level of lactate in cancer metabolism and microenvironment. Genes Dis. 4, 25–27. https://doi.org/10.1016/j.gendis.2017.02.003

Jin, Z., Yao, G., 2019. Natural Product Reports Amaryllidaceae and Sceletium alkaloids. Nat. Prod. Rep. 1–27. https://doi.org/10.1039/c8np00055g

Joshi, S.., Bhadauria, S.., Jadon, G., Diwaker, A.., 2012. Introduction to Neoplasm : "Tumor Classification" A ReviewArticle. Int. J. Adv. Res. Pharm. Bio Sci. 2, 227–263.

Joshi, S.R., Nongkhlaw, F.M.W., 2015. L-Asparaginase and antioxidant activity of endophytic bacteria associated with ethnomedicinal plants. Indian J. Biotechnol. 14, 59–64.

Jouda, J., Kusari, S., Lamshöft, M., Mouafo, F., Douala, C., Wandji, J., Spiteller, M., 2014. Fitoterapia Penialidins A – C with strong antibacterial activities from Penicillium sp ., an endophytic fungus harboring leaves of Garcinia nobilis. Fitoterapia 98, 209–214. https://doi.org/10.1016/j.fitote.2014.08.011

Kabera, J.N., Semana, E., Mussa, A.R., He, X., 2014a. Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. J. Pharm. Pharmacol. 2, 377–392. https://doi.org/10.1016/0300-9084(96)82199-7

Kabera, J.N., Semana, E., Mussa, A.R., He Xin, 2014b. Plant Secondary Metabolites:

Biosynthesis, Classification, Function and Pharmacological Properties. J. Pharm. Pharmacol. 2, 377–392.

Kadhim, M.J., Sosa, A.A., Hameed, I.H., 2016. Evaluation of anti-bacterial activity and bioactive chemical analysis of Ocimum basilicum using Fourier transform infrared (FT-IR) and gas chromatography- mass spectrometry (GC-MS) techniques. J. Pharmacogn. Phyther. 8, 127–146. https://doi.org/10.5897/JPP2015.0366

Kamal, S.A., Hamza, L.F., Hameed, I.H., 2015. Antibacterial activity of secondary metabolites isolated from Alternaria alternata. African J. Biotechnol. 14, 2972–2994. https://doi.org/10.5897/AJB2015.14906

KAMBOJ, A., RATHOUR, A., KAUR, M., 2013. Bufadienolides and their medicinal utility: a review. Int. J. Pharm. Pharm. Sci. 5, 20–27.

Kandel, S.L., Joubert, P.M., Doty, S.L., 2017. Bacterial Endophyte Colonization and Distribution within Plants. Microorganisms 5, 1–26. https://doi.org/10.3390/microorganisms5040077

Kapadia, G.J., Shukla, Y.N., Basak, S.P., Sokolosk, E.A., Fales, H.M., 1980. The melosatins-a novel class of alkaloids from melochza tomentosa. Tetrahedron 36, 2441–2447.

Katoch, D., Kumar, D., Sharma, U., Kumar, N., Padwad, Y.S., Lal, B., Singh, B., 2013. Zephgrabetaine: A New Betaine-type Amaryllidaceae Alkaloid from Zephyranthes grandiflora. Nat. Prod. Commun. 8, 161–164. https://doi.org/10.1177/1934578X1300800206

Kaul, S., Gupta, S., Ahmed, M., Dhar, M.K., 2012. Endophytic fungi from medicinal plants : a treasure hunt for bioactive metabolites. Phytochem. Lett. 1–19. https://doi.org/10.1007/s11101-012-9260-6

Kaya, G.I., Uzun, K., Bozkurt, B., Onur, M.A., Somer, N.U., Glatzel, D., Fürst, R., 2017. Chemical characterization and biological activity of an endemic Amaryllidaceae species: Galanthus cilicicus. South African J. Bot. 108, 256–260. https://doi.org/10.1016/j.sajb.2016.11.008

Khalifa, M.F., Attia, E.Z., Fahim, J.R., Kamel, M.S., 2018. An overview on the chemical and biological aspects of lycorine alkaloid. J. Adv. Biomed. Pharm. Sci. 1, 41–49.

Khan, A.L., Gilani, S.A., Waqas, M., Al-hosni, K., Al-khiziri, S., Kim, Y., Ali, L., Kang, S., Asaf, S., Shahzad, R., Hussain, J., Lee, I., Al-harrasi, A., 2017. Endophytes from medicinal plants and their potential for producing indole acetic acid , improving seed germination and mitigating oxidative stress *#. J. Zhejiang Univ. B (Biomedicine Biotechnol. 18, 125–137.

Khare, E., Mishra, J., Arora, N.K., 2018. Multifaceted interactions between endophytes and plant: Developments and Prospects. Front. Microbiol. 9, 1–12. https://doi.org/10.3389/fmicb.2018.02732

Kharwar, R.N., Mishra, A., Gond, S.K., Stierle, A., Stierle, D., 2011. Anticancer compounds derived from fungal endophytes : their importance andfuture challenges. Nat. Prod. Rep. 28, 1208–1228. https://doi.org/10.1039/c1np00008j

Khazaei, S., Abdul Hamid, R., Ramachandran, V., Mohd Esa, N., Pandurangan, A.K., Danazadeh, F., Ismail, P., 2017. Cytotoxicity and Proapoptotic Effects of Allium atroviolaceum Flower Extract by Modulating Cell Cycle Arrest and Caspase-Dependent and p53 -Independent Pathway in Breast Cancer Cell Lines. Evidence-Based Complement. Altern. Med. 1–16. https://doi.org/10.1155/2017/1468957

Khowala, S., Verma, D., Banik, S.P., 2008. Biomolecules : (introduction , structure & function) Carbohydrates, in: Structure and Function of Biomolecules. pp. 1–5.

Kintzios, S.E., Barberaki, M.G., 2004. Plants that fight cancer. CRC PRESS, Washington, D.C.

Koolen, H.H.F., Soares, E.R., Silva, F.M.A. da, Souza, R.A. de A. e A.D.L. de, 2012. An antimicrobial alkaloid and other metabolites produced by penicillium sp. an endophytic fungus isolated from Mauritia flexuosa L. f. Quim. Nova 35, 771–774.

Körver-Keularts, I.M.L.W., Wang, P., Waterval, H.W.A.H., Kluijtmans, L.A.J., Wevers, R.A., Langhans, C.D., Scott, C., Habets, D.D.J., Bierau, J., 2018. Fast and accurate quantitative organic acid analysis with LC-QTOF/MS facilitates screening of patients for inborn errors of metabolism. J. Inherit. Metab. Dis. 41, 415–424. https://doi.org/10.1007/s10545-017-0129-0

Kresge, N., Simoni, R.D., Hill, R.L., 2010. Glycobiology & Carbohydrates. J. Biol. Chem. 285, 1–5.

Kuhn, A., Yu, S., Giffhorn, F., 2006. Catabolism of 1,5-Anhydro-D-Fructose in Sinorhizobium morelense S-30.7.5: Discovery, Characterization, and Overexpression of a New 1,5-Anhydro-D-Fructose Reductase and Its Application in Sugar Analysis and Rare Sugar Synthesis. Appl. Environ. Microbiol. 72, 1248–1257. https://doi.org/10.1128/AEM.72.2.1248

Kuklinsky-Sobral, J., Arajo, W.L., Mendes, R., Pizzirani-Kleiner, A.A., Azevedo, J.L., 2005. Isolation and characterization of endophytic bacteria from soybean (Glycine max) grown in soil treated with glyphosate herbicide. Plant Soil 273, 91–99. https://doi.org/10.1007/s11104-004-6894-1

Kumar, A., Gupta, Nitika, Kumar, Swadesh, Gupta, Nikhil, Kumar, Santosh, Yadav, V., Prakash, A., Gurjar, H., 2015. Metabolites in plants and its classification. WORLD J. Pharm. Pharm. Sci. 4, 287–305.

Kumar, M.L.V., Thippeswamy, B., Kuppust, I.J., Virupaksha, J.H., 2014. Evaluation of analgesic

activities oF Bacillus. Int. J. Chem. Sci. 12, 272–280.

Kumar, S., Pandey, A.K., 2013. Chemistry and Biological Activities of Flavonoids : An Overview Chemistry and Biological Activities of Flavonoids : An Overview. Sci. J. 1–15. https://doi.org/10.1155/2013/162750

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33, 1870–4. https://doi.org/10.1093/molbev/msw054

Kumar, V., Rai, S., Gaur, P., 2014. Endophytic Fungi : Novel Sources of Anticancer Molecules. https://doi.org/10.1007/978-81-322-1575-2

Kumar, V., Roy, S., 2017. Aquaculture Drugs : Sources , Active Ingredients , Pharmaceutic Preparations and Methods of Administration. J. Aquac. Res. Dev. 8, 1–13. https://doi.org/10.4172/2155-9546.1000510

Kumar, V.M.., Thippeswamy, B., Shivakumar, C.., 2013. Evaluation of Antimicrobial Activity of Bacillus cereus And Bacillus pumillus Metaboites Against Human Pathogens. Int. J. Curr. Pharm. Rev. Res. 4, 47–60.

Kumara, P.M., Shweta, S., Vasanthakumari, M.M., Sachin, N., Manjunatha, B.L., Jadhav, S.S., Ravikanth, G., Ganeshaiah, K.N., Shaanker, R.U., 2014. Endophytes and Plant Secondary Metabolite Synthesis: Molecular and Evolutionary Perspective, in: Advances in Endophytic Research. V.C. Verma and A.C. Gange, pp. 177–190. https://doi.org/10.1007/978-81-322-1575-2

Kusari, S., Hertweck, C., Spiteller, M., 2012. Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chem. Biol. 19, 792–798. https://doi.org/10.1016/j.chembiol.2012.06.004

Kwembeya, E.G., Bjorå, C.S., Stedje, B., Nordal, I., 2007. Phylogenetic relationships in the genus Crinum (Amaryllidaceae) with emphasis on tropical African species: evidence from trnL-Fand nucleat ITS DNA sequence data. Taxon 56, 801–810.

Kwembeya, E.G., Stedje, B., 2007. A numerical taxonomy of the pan species of Crinum based on morphological characters. Nord. J. Bot. 25, 137–144. https://doi.org/10.1111/j.0107-055X.2007.00031.x

Le, A. V., Parks, S.E., Nguyen, M.H., Roach, P.D., 2018. Effect of solvents and extraction methods on recovery of bioactive compounds from defatted gac (Momordica cochinchinensis spreng.) seeds. Separations 5, 1–13. https://doi.org/10.3390/separations5030039

Li, A.H.L., Willaman, J.J., 1968. Distribution of Alkaloids in Angiosperm Phylogeny. Econ. Bot.

22, 239–252.

Lí, Y., Chen, Y., 1990. Diterpenoids from rabdosia pseudo-irrorata. J. Nat. Prod. 53, 841–844. https://doi.org/10.1021/np50070a011

Liew, S.Y., Mukhtar, M.R., Hadi, A.H.A., Awang, K., Mustafa, M.R., Zaima, K., Morita, H., Litaudon, M., 2012. Naucline, a new indole alkaloid from the bark of Nauclea officinalis. Molecules 17, 4028–4036. https://doi.org/10.3390/molecules17044028

Lin, L., Lee, L., Huang, C., Chen, C., Song, J., Shiao, Y., Liu, H., 2019. Effects of boschnaloside from Boschniakia rossica on dysglycemia and islet dysfunction in severely diabetic mice through modulating the action of glucagon-like peptide-1. Phytomedicine 62, 1–11. https://doi.org/10.1016/j.phymed.2019.152946

Lloyd, M., Dapar, G., Demayo, C.G., Senarath, W.T.P.S.K., 2018. Antimicrobial and Cellular Metabolic Inhibitory Properties of the Ethanolic Extract From the Bark of "Lunas-Bagon" (Lunasia Sp.). Int. J. Pharm. Sci. Res. 9, 88–97. https://doi.org/10.13040/IJPSR.0975-8232.9(1).88-97

López-lázaro, M., 2009. Distribution and Biological Activities of the Flavonoid Luteolin. Mini-Reviews Med. Chem. 9, 31–59.

Louw, C. a M., Regnier, T.J.C., Korsten, L., 2002. Medicinal bulbous plants of South Africa and their traditional relevance in the control of infectious diseases. J. Ethnopharmacol. 82, 147–154. https://doi.org/10.1016/S0378-8741(02)00184-8

Ludwig-Muller, J., 2015. Plants and endophytes : equal partners in secondary metabolite production ? Biotechnol. Lett. 37, 1325–1334. https://doi.org/10.1007/s10529-015-1814-4

Mager, D.L., 2006. Bacteria and cancer: Cause, coincidence or cure? A review. J. Transl. Med. 4, 1–18. https://doi.org/10.1186/1479-5876-4-14

Mahady, G., 2005. Medicinal plants for the prevention and treatment of bacterial infections. Curr. Pharm. Des. 11, 2405–2427.

Mahomoodally, M.F., 2013. Traditional Medicines in Africa : An Appraisal of Ten Potent African Medicinal Plants. Evidence-Based Complement. Altern. Med. 2013, 1–14.

Maloney, K.N., Macmillan, J.B., Kauffman, C.A., Jensen, P.R., Dipasquale, A.G., Rheingold, A.L., Fenical, W., 2009. NIH Public Access. Org. Lett. 11, 1–7. https://doi.org/10.1021/ol901997k.Lodopyridone

Mandal, S.M., Sharma, S., Pinnaka, A.K., Kumari, A., Korpole, S., 2013. Isolation and characterization of diverse antimicrobial lipopeptides produced by Citrobacter and Enterobacter. BMC Microbiol. 2013, 13, 1–9.

Manfredi, K.P., Vallurupalli, V., Demidova, M., Kindscher, K., Pannell, L.K., 2001. Isolation of an anti-HIV diprenylated bibenzyl from Glycyrrhiza lepidota. Phytochemistry 58, 153–157.

Mariıa Jesus Duran-Pena, Ares, J.M.B., Collado, I.G., Hern´andez-Gal´an, R., 2014. Biologically active diterpenes containing a gem- dimethylcyclopropane subunit: an intriguing source of PKC modulators. Nat. Prod. Rep. 1–14. https://doi.org/10.1039/c4np00008k

Maroyi, A., 2016a. A review of ethnoboatany, therapeutic value, phytochemistry and pharmacology of Crinum macowanii Baker_ A highly traded bulbous plant in Southern Africa. J. Ethnopharmacol. 194, 595–608. https://doi.org/10.1016/j.jep.2016.10.046

Maroyi, A., 2016b. Ethnobotanical, phytochemical and pharmacological properties of crinum bulbispermum (Burm f) milne-redh and schweick (Amaryllidaceae). Trop. J. Pharm. Res. 15, 2497–2506. https://doi.org/10.4314/tjpr.v15i11.27

Maroyi, A., 2014. The genus Warburgia: A review of its traditional uses and pharmacology. Pharm. Biol. 52, 378–391. https://doi.org/10.3109/13880209.2013.837935

Martín-garcía, J., Espiga, E., Pando, V., Diez, J.J., 2011. Factors Influencing Endophytic Communities in Poplar Plantations. Silva Fenn. 45, 169–180.

Martinez-Klimova, E., Rodríguez-Peña, K., Sánchez, S., 2017. Endophytes as sources of antibiotics. Biochem. Pharmacol. 134, 1–17. https://doi.org/10.1016/j.bcp.2016.10.010

Matthijs, S., Vander Wauven, C., Cornu, B., Ye, L., Cornelis, P., Thomas, C., Ongena, M., 2014. Antimicrobial properties of producing the antibiotic mupirocin. Res. Microbiol. 165, 695–704. https://doi.org/10.1016/j.resmic.2014.09.009

Mazid, M., Khan, T.., Mohammad, F., 2011. Role of secondary metabolites in defense mechanisms of plants. Biol. Med. 3, 232–249.

McCauley, J., Zivanovic, A., Skropeta, D., 2013. Bioassays for anticancer activities. Methods Mol. Biol. 1055, 191–205.

McKee, T., McKee, J.R., 2008. Amino Acids, Peptides, and Proteins, in: Biochemistry The molecular basis of life fifth edition. Oxford University Press, New York, USA, pp. 1–56.

Meegan, M.J., O'Boyle, N.M., 2019. Special issue " anticancer drugs". Pharmaceuticals 12, 1–6. https://doi.org/10.3390/ph12030134

Meerow, A.W., Lehmiller, D.J., Clayton, J.R., 2003. Phylogeny and biogeography of Crinum L. (Amaryllidaceae) inferred from nuclear and limited plastid non-coding DNA sequences. Bot. J. Linn. Soc. 141, 349–363. https://doi.org/10.1046/j.1095-8339.2003.00142.x

Mendoza, N., Silva, E.M.E., 2018. Introduction to Phytochemicals: Secondary Metabolites from Plants with Active Principles for Pharmacological Importance, in: Asao, T., Asaduzzaman,

M. (Eds.), Phytochemicals - Source of Antioxidants and Role in Disease Prevention. IntechOpen, pp. 25–47. https://doi.org/http://dx.doi.org/10.5772/57353

Menpara, D., Chanda, S., 2013. Endophytic Bacteria- Unexplored Reservoir of Antimicrobials for Combating Microbial Pathogens, in: Méndez-Vilas, A. (Ed.), Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education. pp. 1095–1103.

Mercado-Blanco, J., Lugtenberg, B.J.J., 2014. Biotechnological Applications of Bacterial Endophytes. Curr. Biotechnol. 3, 60–75. https://doi.org/10.2174/22115501113026660038

Michelsen, C.F., Jensen, H., Venditto, V.J., Hennessy, R.C., Stougaard, P., 2015. Bioactivities by a crude extract from the Greenlandic Pseudomonas sp . In5 involves the nonribosomal peptides , nunamycin and nunapeptin. PeerJ 1476, 1–16. https://doi.org/10.7717/peerj.1476

Migas, P., Krauze-Baranowska, M., 2015. The significance of arbutin and its derivatives in therapy and cosmetics. Phytochem. Lett. 13, 35–40. https://doi.org/10.1016/j.phytol.2015.05.015

Millar, A.A., Smith, M.A., Kunst, L., 2000. All fatty acids are not equal : discrimination in plant membrane lipids. Trends Plant Sci. 5, 95–101.

Mitscher, L.A., Raghav Rao, G.S., Khanna, I., Veysoglu, T., Drake, S., 1983. Antimicrobial agents from higher plants: Prenylated flavonoids and other phenols from Glycyrrhiza lepidota. Phytochemistry 22, 573–576. https://doi.org/10.1016/0031-9422(83)83049-0

Moco, S., Bino, R.J., Vorst, O., Verhoeven, H.A., Groot, J. de, Beek, T.A. van, Vervoort, J., de Vos, C.H.R., 2006. A Liquid Chromatography-Mass Spectrometry-Based Metabolome Database for Tomato. Plant Physiol. 141, 1205–1218. https://doi.org/10.1104/pp.106.078428.once

Mohamed, R., Avis, T., Tsopmo, A., 2015. Distribution of antimicrobial lipopeptides in Bacillus and Pseudomonas spp ., two genera with antagonistic effects against plant pathogens. Carleton University Ottawa, Ontario.

Monakisi, C.M., 2007. Knowledge and Use of Tradition Medicinal Plants By the Setswan a - Speaking Community of Kimberley , Northern Cape of South Africa. October. University of Stellenbosch.

Morales, A., 2008. A simple way to preserve fungal cultures. Ithaca, New York.

Moran-palacio, E.F., Tortoledo-ortiz, O., Yañez-farias, G.A., Stephens-camacho, N.A., Soñanez-organis, J.G., Ochoa-López, L.M., Rosas-Rodríguez, J.A., 2014. Determination of Amino Acids in Medicinal Plants from Southern Sonora , Mexico. Trop. J. Pharm. Res. 13, 601–606.

Morare, R.. ., Serepa-Dlamini, M.H., Ubomba-Jaswa, E., Mavumengwana, V., 2017. Screening of anti-microbial activity of secondary metabolites extracted from Crinum macowanii and its

associated bacterial endophytes. Univrersity of Johannesburg.

Morare, R.F., Ubomba-Jaswa, E., Serepa-Dlamini, M.H., 2018. Isolation and identification of bacterial endophytes from Crinum macowanii Baker. African J. Biotechnol. 17, 1040–1047. https://doi.org/10.5897/AJB2017.16350

Mousa, W.K., Raizada, M.N., 2013. The Diversity of Anti-Microbial Secondary Metabolites Produced by Fungal Endophytes: An Interdisciplinary Perspective. Front. Microbiol. 4, 1–18. https://doi.org/10.3389/fmicb.2013.00065

Muema, S.M., 2013. Phytochemical and anthelmintic study of the root bark of teclea trichocarpa, engl. (rutaceae). University of Nairobi.

Mugwagwa, A.T., Gadaga, L.L., Pote, W., Tagwireyi, D., 2015. Antiamnesic Effects of a Hydroethanolic Extract of Crinum macowanii on Scopolamine-Induced Memory Impairment in Mice. J. Neurodegener. Dis. 1–9.

Mukherjee, A., Das, R., Pal, A., Paul, A.K., 2017. Antibacterial Activity of Endophytic Bacillus safensis Isolated from Ophioglossum reticulatum L . Microbiol. Res. J. Int. 18, 1–12. https://doi.org/10.9734/MRJI/2017/31923

Mustafa, M., JamalulaAzizi, A., IIIzam, E., Nazirah, A., Sharifa, S., Abbas, S., 2016. Lung Cancer: Risk Factors, Management, And Prognosis. IOSR J. Dent. Med. Sci. 15, 94–101. https://doi.org/10.9790/0853-15100494101

Muzzamal, H., Sarwar, R., Sajid, I., Hasnain, S., 2012. Isolation , Identification and Screening of Endophytic Bacteria Antagonistic to Biofilm Formers. Pak. J. Zool. 44, 249–257.

Nair, D.N., Padmavathy, S., 2014. Impact of Endophytic Microorganisms on Plants , Environment and Humans. Sci. World J. 2014, 1–11.

Nair, J.J., Bastida, J., Codina, C., Viladomat, F., van Staden, J., 2013. Alkaloids of the South African Amaryllidaceae : A Review. Nat. Prod. Commun. 8, 1335–1350. https://doi.org/10.1002/chin.201430280

Nair, J.J., Machocho, A.K., Campbell, W.E., Brun, R., Viladomat, F., Codina, C., Bastida, J., 2000. Alkaloids from Crinum macowanii. Phytochemistry 54, 945–950. https://doi.org/10.1016/S0031-9422(00)00128-X

Nair, Jerald J., Staden, J. Van, 2014. Traditional usage , phytochemistry and pharmacology of the South African medicinal plant Boophone disticha (L . f .) Herb . J. Ethnopharmacol. J. 151, 12–26. https://doi.org/10.1016/j.jep.2013.10.053

Nair, Jerald James, Staden, J. Van, 2014. Traditional usage, phytochemistry and pharmacology of the South African medicinal plant Boophone disticha (L.f.) Herb. (Amaryllidaceae). J.

Ethnopharmacol. 151, 12–26. https://doi.org/10.1016/j.jep.2013.10.053

Nair, J.J., Staden, J. Van, Bonnet, S.L., Wilhelm, A., 2017. Antibacterial Properties of the Family Amaryllidaceae: Evaluation of Plant Extracts in vitro. Nat. Prod. Commun. 12, 1145–1151. https://doi.org/10.1177/1934578X1701200735

Nair, J.J., van Staden, J., 2018. Insight to the antifungal properties of Amaryllidaceae constituents. Phytomedicine 1–8. https://doi.org/10.1016/j.phymed.2018.11.013

Nair, J.J., van Staden, J., 2013. Pharmacological and toxicological insights to the South African Amaryllidaceae. Food Chem. Toxicol. J. 62, 262–275. https://doi.org/10.1016/j.fct.2013.08.042

Nakanishi, K., 2005. Terpene trilactones from Gingko biloba : From ancient times to the 21st century. Bioorg. Med. Chem. 13, 4987–5000. https://doi.org/10.1016/j.bmc.2005.06.014

Nalini, M.S., Prakash, H.S., 2017. Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Lett. Appl. Microbiol. 64, 261–270. https://doi.org/10.1111/lam.12718

Narsing Rao, M.P., Xiao, M., Li, W.J., 2017. Fungal and bacterial pigments: Secondary metabolites with wide applications. Front. Microbiol. 8, 1–13. https://doi.org/10.3389/fmicb.2017.01113

National Cancer Registry South Africa, 2016. 2016 National Cancer Registry SA Statistics.

National Cancer Registry South Africa, 2015. 2015 National Cancer Registry South Africa Statistics. https://doi.org/10.16309/j.cnki.issn.1007-1776.2003.03.004

National Cancer Registry South Africa, 2014. 2014 National Cancer Registry SA Statisctics. https://doi.org/10.1192/bjp.112.483.211-a

National Cancer Registry South Africa, 2013. 2013 National Cancer Registry SA Statistics.

Navi, S.S., Bandyopadhyay, R., Hall, A.J., Bramel-Cox, P.J., 1999. A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain. Andhra Pradesh, India.

Naz, S., Gallart-Ayala, H., Reinke, S.N., Mathon, C., Blankley, R., Chaleckis, R., Wheelock, C.E., 2017. Development of a Liquid Chromatography-High Resolution Mass Spectrometry Metabolomics Method with High Specificity for Metabolite Identification Using All Ion Fragmentation Acquisition. Anal. Chem. 89, 7933–7942. https://doi.org/10.1021/acs.analchem.7b00925

Nazemi, M., Moradi, Y., Rezvani, G.F., Ahmaditaba, M.A., Gozari, M., Salari, Z., 2017. Antimicrobial activities of semi polar-nonpolar and polar secondary metabolites of sponge Dysidea pallescens from Hengam Island, Persian Gulf. Iran. J. Fish. Sci. 15, 200–209.

Ncube, E.N., Dubery, I.A., Steenkamp, P.A., Madala, N.E., 2016. Manipulation of secondary

metabolite synthesis in Centella asiatica cells : a metabolomics study. University of Johannesburg.

Ncube, N.S., Afolayan, A.J., Okoh, A.I., 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J. Biotechnol. 7, 1797–1806. https://doi.org/10.5897/AJB07.613

Ndhlala, A.R., Ncube, B., Okem, A., Mulaudzi, R.B., Staden, J. Van, 2013. Toxicology of some important medicinal plants in southern Africa. Food Chem. Toxicol. J. 62, 609–621. https://doi.org/10.1016/j.fct.2013.09.027

Neal, R.D., Robbé, I.J., Lewis, M., Williamson, I., Hanson, J., 2015. The complexity and dif fi culty of diagnosing lung cancer : fi ndings from a national primary-care study in Wales. Prim. Health Care Res. Dev. 16, 436–449. https://doi.org/10.1017/S1463423614000516

Nebula, M., Harisankar, H.S., Chandramohanakumar, N., 2013. Metabolites and bioactivities of Rhizophoraceae mangroves. Nat. Products Bioprospect. 3, 207–232. https://doi.org/10.1007/s13659-013-0012-0

Negi, J.S., Negi, P.S., Pant, G.J., Rawat, M.S.M., Negi, S.K., 2013. Naturally occurring saponins : Chemistry and biology. J. Poisonous Med. Plant Res. Vol. 1, 1–6.

Neu, A.K., Månsson, M., Gram, L., Prol-García, M.J., 2014. Toxicity of Bioactive and Probiotic Marine Bacteria and Their Secondary Metabolites in Artemia sp . and Caenorhabditis elegans as Eukaryotic Model Organisms. Appl. Environ. Microbiol. 80, 146–153. https://doi.org/10.1128/AEM.02717-13

Ngamau, C.N., Matiru, V.N., Tani, A., Muthuri, C.W., 2012. Isolation and identification of endophytic bacteria of bananas (Musa spp .) in Kenya and their potential as biofertilizers for sustainable banana production. African J. Microbiol. Res. 6, 1–10.

Ngo, T. Van, Scarlett, C.J., Bowyer, M.C., Ngo, P.D., Vuong, Q. Van, 2017. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. J. Food Qual. 1–8. https://doi.org/10.1155/2017/9305047

Ngoma, L., Mogatlanyane, K., Babalola, O.O., 2014. Screening of Endophytic Bacteria towards the Development of Cottage Industry : An in Vitro Study. J. Hum. Ecol. 47, 45–63.

Nicoletti, R., Fiorentino, A., 2015. Plant Bioactive Metabolites and Drugs Produced by Endophytic Fungi of Spermatophyta. Agriculture 5, 918–970. https://doi.org/10.3390/agriculture5040918

Nii-Trebi, N.I., 2017. Emerging and Neglected Infectious Diseases : Insights , Advances , and Challenges. Hindawi BioMed Res. Int. 2017, 1–15.

Nithya, G., Panneerselvam, P., Nepolean, R., Selvakumar, N., Jeyavalli, A., 2012. Synthesis &

biological evaluation of some novel schiff bases of isatin derivatives. Int. J. Pharm. Ther. 3, 243–246.

Noch, E.K., Ramakrishna, R., Magge, R., 2018. Challenges in the Treatment of Glioblastoma: Multisystem Mechanisms of Therapeutic Resistance. World Neurosurg. 116, 505–517. https://doi.org/10.1016/j.wneu.2018.04.022

Nongkhlaw, W.M.F., Joshi, S.R., 2015. Original Article Investigation on the bioactivity of culturable endophytic and epiphytic bacteria associated with ethnomedicinal plants. J. Infect. Dev. Ctries. 9, 954–961. https://doi.org/10.3855/jidc.4967

Notten, A., 2013. Crinum macowanii [WWW Document]. Kirstenbosch Natl. Bot. Gard. URL http://www.plantzafrica.com/plantcd/crinummacowanii.htm). (accessed 5.20.07).

Nyamai, D., Arika, W., Ogola, P., Njagi, E., Ngugi, M., 2016. Medicinally Important Phytochemicals : An Untapped Research Avenue. J. Pharmacogn. Phytochem. 4, 35–49.

Ohsaki, A., Ozawa, M., Komiyama, K., Kishida, A., Isobe, T., 2012. Natural Product Communications The Cytotoxic Activity of Diterpenoids from Isodon species. Nat. Prod. Commun. 7, 977–978. https://doi.org/10.1177/1934578X1200700803

Okuda, T., Ito, H., 2011. Tannins of Constant Structure in Medicinal and Food Plants—Hydrolyzable Tannins and Polyphenols Related to Tannins 16, 2191–2217. https://doi.org/10.3390/molecules16032191

Olufunke, M.D., 2012. Developments in Phytochemistry, in: Vallisuta, P.O. (Ed.), Drug Discovery Research in Pharmacognosy. InTech Published, pp. 12–23.

Omar, S.H., 2013. Ginkgolides and Neuroprotective Effects. Nat. Prod. 3697–3741. https://doi.org/10.1007/978-3-642-22144-6

Onyegeme-Okerenta, B.M., Chinedu, S.N., Okafor, U.A., Okochi, V.I., 2009. Antibacterial Activity of Culture Extracts of Penicillium chrysogenum PCL501: Effects of Carbon Sources. Online J. Heal. Allied Sci. 8, 1–7.

Oren, A., Gunde-Cimerman, N., 2007. Mycosporines and mycosporine-like amino acids : UV protectants or multipurpose secondary metabolites ? FEMS Microbiol. Lett. 269, 1–10. https://doi.org/10.1111/j.1574-6968.2007.00650.x

Pagare, Saurabh, Bhatia, M., Tripathi, N., Pagare, Sonal, Bansal, Y.K., 2016. Secondary metabolites of plants and their role : Overview. Curr. Trends Biotechnol. Pharm. 9, 293–304.

Pal, S., Shukla, Y., 2003. Herbal medicine: current status and the future. Asian Pacific J. Cancer Prev. 4, 281–288.

Pang, B., Zhu, Y., Lu, L., Gu, F., Chen, H., 2016. The applications and features of liquid

chromatography-mass spectrometry in the analysis of traditional Chinese medicine. Evidence-based Complement. Altern. Med. 2016, 1–7. https://doi.org/10.1155/2016/3837270

Paolillo, M., Boselli, C., Sergio, S., 2018. Glioblastoma under Siege: An Overview of Current Therapeutic Strategies. Brain Sci. 8, 1–13. https://doi.org/10.3390/brainsci8010015

Patil, R.H., Patil, M.P., Maheshwari, V.L., 2016. Bioactive Secondary Metabolites From Endophytic Fungi: A Review of Biotechnological Production and Their Potential Applications, in: Atta-ur-Rahman (Ed.) (Ed.), Studies in Natural Products Chemistry. Elsevier, pp. 189–205. https://doi.org/10.1016/B978-0-444-63601-0.00005-3

Paul, N.C., Deng, J.X., Shin, K.S., Yu, S.H., 2012. Molecular and Morphological Characterization of Endophytic Heterobasidion araucariae from Roots of Capsicum annuum L . in Korea. Mycology 40, 85–90.

Pereira, A.S.P., Haan, H. Den, Peña-García, J., Moreno, M.M., Pérez-Sánchez, H., Apostolides, Z., 2019. Exploring African Medicinal Plants for Potential Anti-Diabetic Compounds with the DIA-DB Inverse Virtual Screening Web Server. Molecules 24, 1–32. https://doi.org/10.3390/molecules24102002

Persson, K.G., Burger, A.P., Mugabo, P., Green, I., 2007. In vivo effects of Crinum macowanii on the Rat Cardiovascular System. University of the Western Cape.

Pettit, G.R., Ducki, S., Tan, R., Gardella, R.S., Mcmahon, J.B., Boyd, M.R., Pettit, G.R., Blumberg, P.M., Lewin, N.E., Doubek, D.L., Tackett, L.P., Williams, M.D., 2002. Isolation and Structure of Pedilstatin from a Republic of Maldives Pedilanthus. J. Nat. Prod. 65, 1262–1265. https://doi.org/10.1021/np020115b

Phillipson, J.D., Hemingway, S.R., 1974. Angustine and related alkaloids from species of mitragyna, nauclea, uncarza, and strychnos. Phytochemistry 13, 973–978.

Piboonprai, K., Khumkhrong, P., Khongkow, M., Yata, T., Ruangrungsi, N., Chansriniyom, C., Iempridee, T., 2018. Anticancer activity of arborinine from Glycosmis parva leaf extract in human cervical cancer cells. Biochem. Biophys. Res. Commun. 500, 866–872. https://doi.org/10.1016/j.bbrc.2018.04.175

Picardo, S.L., Coburn, B., Hansen, A.R., 2019. The microbiome and cancer for clinicians. Crit. Rev. Oncol. Hematol. 141, 1–12. https://doi.org/10.1016/j.critrevonc.2019.06.004

Pierce, G.B., Wallace, C., 1971. Differentiation of malignant to benign cells. Cancer Rese 31, 127–134. https://doi.org/10.1016/0022-5193(71)90020-8

Pitt, J.I., Hocking, A.., 2009. Fungi and Food Spoilage, third. ed. Springer Dordrecht Heidelberg London, London New York.

Podolak, I., Galanty, A., Sobolewska, D., 2010. Saponins as cytotoxic agents: A review. Phytochem. Rev. 9, 425–474. https://doi.org/10.1007/s11101-010-9183-z

Pombeiro-Sponchiado, S.R., Sousa, G.S., Andrade, J.C.R., Lisboa, H.F., Gonçalves, R.C.R., 2017. Production of Melanin Pigment by Fungi and Its Biotechnological Applications, in: Miroslav Blumenberg, IntechOpen. pp. 47–60. https://doi.org/http://dx.doi.org/10.5772/57353

Pop, C., Vlase, L., Tamas, M., 2009. Natural resources containing arbutin. Determination of arbutin in the leaves of Bergenia crassifolia (L.) Fritsch. acclimated in Romania. Not. Bot. Horti Agrobot. Cluj-Napoca 37, 129–132.

Powers-Fletcher, M., Kendall, B., Griffin, A., Hanson, K., 2016. Filamentous fungi. Microbiol. Spectr. 4, 1–29. https://doi.org/10.1128/microbiolspec.DMIH2-0002-2015.Correspondence

Prabavathy, D., Nachiyar, C.V., 2014. Cytotoxicity of Ethyl Acetate Extract of Endophytic Aspergillus fumigatus on A549 Cell Lines. Biosci. Biotechnol. Res. ASIA, 11, 797–802.

Purwestri, Y.A., Kartikasari, N., Putri, S.G., Wilson, W., Sembiring, L., 2016. Metabolic profiling of endophytic bacteria from Purwoceng (Pimpinella pruatjan Molkend) root and antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. AIP Conf. Proc. 1744, 1–7. https://doi.org/10.1063/1.4953537

Pusztahelyi, T., Holb, I.J., Pócsi, I., 2015. Secondary metabolites in fungus-plant interactions. Front. Plant Sci. 6, 1–23. https://doi.org/10.3389/fpls.2015.00573

Qin, S., Xing, K., Jiang, J., Xu, L., 2011. Biodiversity , bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl. Microbiol. Biotechnol. 89, 457–473. https://doi.org/10.1007/s00253-010-2923-6

Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 56, 81–87. https://doi.org/10.1016/S0378-8741(96)01515-2

Raghu, J., 2012. Endophytic bacterial association and plant secondary metabolite production. University of Agricultural Sciences, Bangalore.

Raimondo, D., 2011. The Red List of South African plants – A global first. S. Afr. J. Sci. 107, 1–2. https://doi.org/10.4102/sajs.v107i3/4.653

Rajbhandary, S., 2014. Healing Traditions of the Himalayas 21–36. https://doi.org/10.1007/978-81-322-1925-5

Ramesh, V., Arivudainambi, U., Rajendran, A., 2017. The molecular phylogeny and taxonomy of endophytic fungal species from the leaves of Vitex negundo L . Stud. Fungi 2, 26–37. https://doi.org/10.5943/stif/

Ramírez-Bahena, Martha-Helena Carmen, T., Peix, A., 2014. Bacterial Endophytes Inhabiting

Potato Plants (Solanum tuberosum L.), in: Mandhatri, P.G. and S. (Ed.), Agricultural Research Updates. Nova Science Publishers, New York, pp. 3–31.

Rana, A., Singh, H.P., Dhyani, D., 2012. Comparative Estimation of Major Iridoid Glucosides from Different Parts of Incarvillea emodi. ISRN Chromatogr. 2012, 1–4. https://doi.org/10.5402/2012/783752

Rangjaroen, C., Sungthong, R., RerkaSem, B., Teaumroong, N., NoiSangiam, R., SaiSamorn, L., 2017. Untapped Endophytic Colonization and Plant Growth-Promoting Potential of the Genus Novosphingobium to Optimize Rice Cultivation. Microbes and Enviroments 32, 84–87. https://doi.org/10.1264/jsme2.ME16112

Raviglione, M., Maher, D., 2017. Ending infectious diseases in the era of the Sustainable Development Goals. Porto Biomed. J. 2, 140–142. https://doi.org/10.1016/j.pbj.2017.08.001

Refaat, J, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2013. Crinum; an endless source of bioactive principles: a review. part iv: non-alkaloidal constituents. Int. J. Pharm. Sci. Res. 3, 1883–1890.

Refaat, John, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2013. Crinum; An endless source of bioactive principles: a review, part V. Biological profile. Int. J. Pharm. Sci. Res. 4, 1239–1252.

Refaat, John, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012. Crinum ; an endless source of bioactive principles : a review , part ii . crinum alkaloids : crinine- type alkaloids. Int. J. Pharma Sci. Res. 3, 3091–3100.

Refaat, J, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012. Crinum; an endless source of bioactive principles: a review. part iii; crinum alkaloids: belladine-, galanthamine-, lycorenine-, tazettine-type alkaloids and other minor types. Int. J. Pharm. Sci. Res. 3, 3630–3638.

Reid, S., Wishingrad, V., McCabe, S., 2009. Plant Uses : California, The University of California Santa Cruz Arboretum.

Rhoden, S.A., Garcia, A., Santos e Silva, M.C., Azevedo, J.L., Pamphile, J.A., 2015. Phylogenetic analysis of endophytic bacterial isolates from leaves of the medicinal plant Trichilia elegans A . Juss . (Meliaceae). Genet. Mol. Res. 14, 1515–1525.

Rinner, U., Dank, C., Hudlicky, T., 2016. Galanthamine. Targets Heterocycl. Syst. 20, 283–315.

Roder, C., Thomson, M.J., 2015. Auranofin : Repurposing an Old Drug for a Golden New Age 13–20. https://doi.org/10.1007/s40268-015-0083-y

Rodriguez, R.J., White, J.J.F., Arnold, A.E., Redman, R.S., 2009. Fungal endophytes : diversity and functional roles. New Phytol. 182, 314–330.

Rønsted, N., Symonds, M.R.E., Birkholm, T., Christensen, S.B., Meerow, A.W., Molander, M., Mølgaard, P., Petersen, G., Rasmussen, N., Staden, J. van, Stafford, G.I., Jäger, A.K., 2012. Can phylogeny predict chemical diversity and potential medicinal activity of plants ? A case study of Amaryllidaceae. BMC Evol. Biol. This 12. https://doi.org/10.1186/1471-2148-12-182

Rosière, R., Berghmans, T., De Vuyst, P., Amighi, K., Wauthoz, N., 2019. The position of inhaled chemotherapy in the care of patients with lung tumors: Clinical feasibility and indications according to recent pharmaceutical progresses. Cancers (Basel). 11, 1–13. https://doi.org/10.3390/cancers11030329

Roy, M., Liang, L., Xiao, X., Feng, P., Ye, M., Liu, J., 2018. Lycorine: A prospective natural lead for anticancer drug discovery. Biomed. Pharmacother. 107, 615–624. https://doi.org/10.1016/j.biopha.2018.07.147

Rustan, A.C., Drevon, C.A., 2005. Fatty Acids : Structures and Properties. Encycl. Life Scienecs. https://doi.org/10.1038/npg.els.0003894

Saad, H.-E., El-Sharkawy, S., Shier, W.T., 1995. Biological Activities of Pyrrolidinoindoline Alkaloids from Calycodendron milnei. Planta Med. 61, 313–316. https://doi.org/10.1055/s-2006-958090

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–25. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Salim, A., Chin, Y., Kinghorn, A., 2008. Drug Discovery from Plants A.A. Bioact. Mol. Med. Plants 1–25. https://doi.org/10.1007/978-3-540-74603-4

Santos, S.N., Ferraris, F.K., 2012. Endophytic fungi from Combretum leprosum with potential anticancer and antifungal activity. Symbiosis 109–117. https://doi.org/10.1007/s13199-012-0218-7

Santoyo, G., Moreno-Hagelsieb, G., Carmen Orozco-Mosqueda, del M., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183, 92–99. https://doi.org/10.1016/j.micres.2015.11.008

Sardul Singh, S., Suneel, K., Aharwal, R.P., 2014. Isolation and identification of endophytic fungi from Ricinus communis linn . And their antibacterial activity. Int. J. Res. Pharm. Chem. 4, 611–618.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Latha, L.Y., 2011. Extraction , Isolation and Characterization of Bioactive Compounds from Plants ' Extracts. African J. Tradit.

Complement. Altern. Med. · 8, 1–10. https://doi.org/10.4314/ajtcam.v8i1.60483

Sasidharan, S., Yoga Latha, L., Kwan, Y.P., Lachumy, S.J., 1993. Screening Methods in the Study of Fungicidal Property of Medicinal Plants. Fungic. Plant Anim. Dis. 107-.

Sauerschnig, C., Doppler, M., Bueschl, C., Schuhmacher, R., 2018. Methanol Generates Numerous Artifacts during Sample Extraction and Storage of Extracts in Metabolomics Research. Metabolites 8, 1–19. https://doi.org/10.3390/metabo8010001

Sawaya, A.C.H.F., Abreu, I.N., Andreazza, L., Eberlin, M.N., Mazzafera, P., 2010. Pilocarpine and related alkaloids in pilocarpus vahl (rutaceae), in: N. M. Cassiano (Ed.), Alkaloids: Properties, Applications. Nova Science Publishers, Inc, pp. 63–80.

Saxena, M., Saxena, J., Nema, R., Singh, D., Gupta, A., 2013. Phytochemistry of medicinal plants. J. Phamacognosy Phytochem. 1, 168–182. https://doi.org/10.1016/0300-9084(96)82199-7

Scholz, M., Gatzek, S., Sterling, A., Selbig, O., Fiehn, J., 2004. Metabolite fingerprinting : detecting biological features by independent component analysis. Bioinformatics 20, 2447–2454. https://doi.org/10.1093/bioinformatics/bth270

Schwikkard, S., van Heerden, F.R., 2002. Antimalarial activity of plant metabolites. Nat. Prod. Rep. 19, 675–692. https://doi.org/10.1039/b008980j

Sebola, T.E., Ndinteh, D.T., Mavumengwana, V., 2016a. A contribution to the phytochemical and antibacterial characteristics of Crinum macowanii bulbs extracts. University of Johannessburg.

Sebola, T.E., Ndinteh, D.T., Niemann, N., Mavumengwana, V., 2016b. Metal Analysis, Phytochemical and Antibacterial Investigation of Crinum Macowanii Bulb, in: International Conference on Advances in Science, Engineering, Technology and Natural Resources. https://doi.org/10.15242/iae.iae1116435

Sebola, T.E., Okereafor, N.C.U., Tapfuma, K.I., Mekuto, L., Green, E., Mavumengwana, V., 2019. Evaluating antibacterial and anticancer activity of crude extracts of bacterial endophytes from Crinum macowanii Baker bulbs. MicrobiologyOpen. 8, 1–10. https://doi.org/10.1002/mbo3.914

Seca, A.M.L., Pinto, D.C.G.., 2018. Plant Secondary Metabolites as Anticancer Agents : Successes in Clinical Trials and Therapeutic Application. Int. J. Mol. Sci. 19, 1–22. https://doi.org/10.3390/ijms19010263

Selim, M.H., Elshikh, H.H., Saad, M.M., Mostafa, E.E., Mahmoud, M.A., 2016. Purification and characterization of a novel thermo stable L-methioninase from Streptomyces sp. DMMMH4 and its evaluation for anticancer activity. J. Appl. Pharm. Sci. 6, 53–60.

https://doi.org/10.7324/JAPS.2016.60708

Selim, M.H., Karm Eldin, E.-Z., Saad, M.M., Mostafa, E.-S.E., Shetia, Y.H., Anise, A.A.H., 2015. Purification, Characterization of L-Methioninase from Candida tropicalis , and Its Application as an Anticancer . Biotechnol. Res. Int. 2015, 1–10. https://doi.org/10.1155/2015/173140

Selvamohan, T., Shibila, V.R.S., Kishore, S., 2012. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Adv. Appl. Sci. Res. 3, 3374–3381.

Sen, A., Batra, A., 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant:melia azedarach l. Int. J. Curr. Pharm. Res. 4, 67–73.

Senyuva, H.Z., Gilbert, J., 2007. Rapid Analysis of Crude Fungal Extracts for Secondary Metabolites by LC / TOF-MS – A New Approach to Fungal Characterization Application. USA.

Shahidullah, M., Al-Mujahidee, M., Nasir Uddin, S.M., Shahadat Hossan, M., Hanif, A., Bari, S., Rahmatullah, M., 2009. Medicinal plants of the Santal tribe residing in Rajshahi district, Bangladesh. Am. J. Sustain. Agric. 3, 220–226.

Shakya, A.K., 2016. Medicinal plants : Future source of new drugs. Int. J. Herb. Med. 4, 59–64.

Shanmugam, G., Dubey, A., Ponpandian, L.N., Rim, S.O., 2018. Genomic Insights into Nematicidal Activity of a Bacterial Endophyte , Raoultella ornithinolytica MG against Pine Wilt Nematode. Plant Pathol. J. 34, 250–255.

Shiva Kameshwari, M.., B, M., Saraswathi.K.J, T., 2015. Isolation and identification of endophytic fungi from urginea indica , a medicinal plant from diverse regions of south india . Int. J. Latest Res. Sci. Technol. 4, 75–80.

Shrestha, P., Adhikari, S., Lamichhane, B., Shrestha, B.G., 2015. Phytochemical Screening of the Medicinal Plants of Nepal. J. Environ. Sci. Toxicol. Food Technol. 1, 11–17.

Singh, A., 2008. Phytochemicals of Gentianaceae : A Review of Pharmacological Properties. Int. J. Pharm. Sci. Nanotechnol. 1, 33–36.

Singh, S.K., 2015. Role of Endophytes in Pharmaceutical Industry. J. Microbiol. Biotechnol. 4, 6–7.

Sinha, T., 2018. Tumors: Benign and Malignant. Cancer Ther. & Oncol. Int. J. 10, 1–3. https://doi.org/10.19080/CTOIJ.2018.10.555790

Skibiński, R., Trawiński, J., 2017. Application of an untargeted chemometric strategy in the impurity profiling of pharmaceuticals: An example of amisulpride. J. Chromatogr. Sci. 55, 309–315. https://doi.org/10.1093/chromsci/bmw186

Sobiczewski, P., 2008. Bacterial diseases of plants : Epidemiology , diagnostics and control. Zemdirbyste-Agriculture 95, 151–157.

Solis, M.J.L., Yurkov, A., Cruz, T.E. dela, Unterseher, M., 2015. Leaf-inhabiting endophytic yeasts are abundant but unevenly distributed in three Ficus species from botanical garden greenhouses in Germany. Mycol. Prog. 14, 1–10. https://doi.org/10.1007/s11557-014-1019-6

Soltis, P.S., Soltis, D.E., 2003. Applying the Bootstrap in Phylogeny Reconstruction. Stat. Sci. 18, 256–267.

South African National Department of Health, 2017. National Cancer Strategic Framework 2017-2022. Pretoria, South Africa.

Stankovic, N., Senerovic, L., Ilic-Tomic, T., Vasiljevic, B., Nikodinovic-Runic, J., 2014. Properties and applications of undecylprodigiosin and other bacterial prodigiosins. Appl. Microbiol. Biotechnol. 98, 3841–3858. https://doi.org/10.1007/s00253-014-5590-1

Statistics South Africa, 2015. Millenium Development Goals: Country report 2015. Pretoria, South Africa.

Stierle, A.C., Cardellina, J.H., Strobel, G.A., 1988. Maculosin, a host-specific phytotoxin for spotted knapweed from Alternaria alternata. Proc. Natl. Acad. Sci. 85, 8008–8011. https://doi.org/10.1073/pnas.85.21.8008

Stolker, A.A.M., Niesing, W., Fuchs, R., Vreeken, R.J., Niessen, W.M.A., Brinkman, U.A.T., 2004. Liquid chromatography with triple-quadrupole and quadrupole-time-of-flight mass spectrometry for the determination of micro-constituents - A comparison. Anal. Bioanal. Chem. 378, 1754–1761. https://doi.org/10.1007/s00216-003-2485-x

Strobel, G., 2018. The Emergence of Endophytic Microbes and Their Biological Promise. J. Fungi 4, 1–9. https://doi.org/10.3390/jof4020057

Strobel, G., Daisy, B., 2003. Bioprospecting for Microbial Endophytes and Their Natural Products. Microbiol. Mol. Biol. Rev. 67, 491–502. https://doi.org/10.1128/MMBR.67.4.491

Strobel, G.A., 2003. Endophytes as sources of bioactive products. Microbes Infect. 5, 535–544.

Sudha, V., Govindaraj, R., Baskar, K., Al-dhabi, N.A., Duraipandiyan, V., 2016. Biological properties of Endophytic Fungi. BRAZILIAN Arch. Biol. Technol. AN 59, 1–7.

Sun, B., Chen, A., Gao, W., Zhou, Y., Liu, H., 2013. Endophytic fungi associated with the medicinal plant , Achyranthes bidentata Blume (Amaranthaceae). African J. Microbiol. Res. 7, 1357–1365. https://doi.org/10.5897/AJMR12.1461

Sun, X., Guo, L., 2012. Endophytic fungal diversity : review of traditional and molecular

techniques. Mycology 31, 65–76.

Szarc Vel Szic, K., Op De Beeck, K., Ratman, D., Wouters, A., Beck, I.M., Declerck, K., Heyninck, K., Fransen, E., Bracke, M., De Bosscher, K., Lardon, F., Van Camp, G., Vanden Berghe, W., 2014. Pharmacological levels of Withaferin A (Withania somnifera) trigger clinically relevant anticancer effects specific to triple negative breast cancer cells. PLoS One 9, 1–17. https://doi.org/10.1371/journal.pone.0087850

Takos, A.M., Rook, F., 2013. Towards a Molecular Understanding of the Biosynthesis of Amaryllidaceae Alkaloids in Support of Their Expanding Medical Use. Int. J. Mol. Sci. 14, 11713–11741. https://doi.org/10.3390/ijms140611713

Tallini, L.R., Torras-Claveria, L., Borges, W. de S., Kaiser, M., Viladomat, F., Zuanazzi, J.A.S., Bastida, J., 2018. N-oxide alkaloids from crinum amabile (amaryllidaceae). Molecules 23, 1–11. https://doi.org/10.3390/molecules23061277

Tamošiune, I., Stanien, G., Haimi, P., Stanys, V., Rugienius, R., Baniulis*, D., 2018. Endophytic Bacillus and Pseudomonas spp . Modulate Apple Shoot Growth , Cellular Redox Balance , and Protein Expression Under in Vitro Conditions. Front. Plant Sci. 9, 1–14. https://doi.org/10.3389/fpls.2018.00889

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6 : Molecular Evolutionary Genetics Analysis Version 6 . 0 30, 2725–2729. https://doi.org/10.1093/molbev/mst197

Tan, J., Zheng, M., Duan, S., Zeng, Y., Zhang, Z., Cui, Q., Zhang, J., Hong, T., Bai, J., Du, S., 2018. Chemical Profiling and Screening of the Marker Components in the Fruit of Cassia fistula by HPLC and UHPLC/LTQ-Orbitrap MSn with Chemometrics. Molecules 23, 1–18. https://doi.org/10.3390/molecules23071501

Taneja, N., Sethi, S., Tahlan, A.K., Kumar, Y., 2019. Introductory Chapter: Stepping into the Post-Antibiotic Era—Challenges and Solutions, in: Antimicrobial Resistance - A Global Threat, Yashwant Kumar, IntechOpen. p. 13. https://doi.org/http://dx.doi.org/10.5772/57353

Tapfuma, K.I., Mekuto, L., Mellisa, M., Mavumengwana, V., 2019a. The LC-QTOF-MS / MS analysis data of detected metabolites from the crude extract of Datura stramonium leaves. Data Br. 25, 1–4. https://doi.org/10.1016/j.dib.2019.104094

Tapfuma, K.I., Uche-Okereafor, N., Sebola, T.E., Hussan, R., Mekuto, L., Makatini, M.M., Green, E., Mavumengwana, V., 2019b. Cytotoxic activity of crude extracts from Datura stramonium's fungal endophytes against A549 lung carcinoma and UMG87 glioblastoma cell lines and LC-QTOF-MS/MS based metabolite profiling. BMC Complement. Altern. Med.

19, 1–12. https://doi.org/10.1186/s12906-019-2752-9

Taylor, J.L.S., Elgorashi, E.E., Maes, A., Gorp, U. Van, Kimpe, N. De, Staden, J. Van, Verschaeve, L., 2003. Investigating the Safety of Plants Used in South African Traditional Medicine : Testing for Genotoxicity in the Micronucleus And Alkaline Comet Assays. Environ. Mol. Mutagen. 154, 144–154. https://doi.org/10.1002/em.10184

Tenorio-Salgado, S., Tinoco, R., Vazquez-Duhalt, R., Caballero-Mellado, J., Perez-Rueda, E., 2013. Identification of volatile compounds produced by the bacterium Burkholderia tropica that inhibit the growth of fungal pathogens. Bioengineered 4, 236–243.

Terhonen, E., Blumenstein, K., Kovalchuk, A., Asiegbu, F.O., 2019. Forest Tree Microbiomes and Associated Fungal Endophytes : Functional Roles and Impact on Forest Health. Forests 10, 1–33. https://doi.org/10.3390/f10010042

Thermo Scientific, 2013. Methods Optimization in Accelerated Solvent Extraction. https://doi.org/10.1080/09638288.2016.1189610

Thomas, L. V., Yu, S., Ingram, R.E., Refdahl, C., Elsser, D., Delves-Broughton, J., 2002. Ascopyrone P, a novel antibacterial derived from fungi. J. Appl. Microbiol. 93, 697–705. https://doi.org/10.1046/j.1365-2672.2002.01751.x

Tidke, S.A., Kumar, R.K.., Ramakrishna, D., Kiran, S., Kosturkova, G., Gokare, R.A., 2017. Current Understanding of Endophytes : Their Relevance , Importance , and Industrial Potentials. J. Biotechnol. Biochem. 3, 43–59. https://doi.org/10.9790/264X-03034359

Tiwari, P., Kumar, B., Mandeep, K., Kaur, G., Kaur, H., 2011. Phytochemical screening and Extraction: A Review. Int. Pharm. Sci. 1, 98–106.

Tiwaskar, M., Manohar, T., 2017. Rational for Antibiotics – Guidelines, in: Medicine Update 2017. pp. 6–15.

Torras-Claveria, L., Tallini, L., Viladomat, F., Bastida, J., 2017. Research in natural products : Amaryllidaceae ornamental plants as sources of bioactive compounds. Recent Adv. Pharm. Sci. VII 69–82.

Tram, N.T.N., Titorenkova, T. V., Bankova, S. V., Handjieva, N.V., Popov, S.S., 2002. Crinum L. Amaryllidaceae. Fitoterapia 73, 183–208.

Truong, D.-H., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H., Nguyen, H.C., 2019. Evaluation of the Use of Different Solvents for Phytochemical Constituents , Antioxidants , and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. J. Food Qual. 1–9.

Tsoukalas, N., Aravantinou-Fatorou, E., Baxevanos, P., Tolia, M., Tsapakidis, K., Galanopoulos, M., Liontos, M., Kyrgias, G., 2018. Advanced small cell lung cancer (SCLC): new challenges

and new expectations. Ann. Transl. Med. 6, 1–8. https://doi.org/10.21037/atm.2018.03.31

Tugizimana, F., Piater, L., Dubery, I., 2013. Plant metabolomics: A new frontier in phytochemical analysis. S. Afr. J. Sci. 109, 1–11. https://doi.org/10.1590/sajs.2013/20120005

Tymon, L., Inglis, D., 2009. What is an Endophyte? https://doi.org/10.2307/2246769

Ullah, A., Mushtaq, H., Ali, U., Ali, E., Mubeen, S., 2018. Screening , isolation , biochemical and plant growth promoting characterization of endophytic bacteria. Microbiol. Curr. Res. 2, 61–68.

Uzma, F., Mohan, C.D., Hashem, A., Konappa, N.M., Rangappa, S., Kamath, P. V., Singh, B.P., Mudili, V., Gupta, V.K., Siddaiah, C.N., Chowdappa, S., Alqarawi, A.A., Abd_Allah, E.F., 2018. Endophytic Fungi — Alternative Sources of Cytotoxic Compounds : A Review. Front. Pharmacol. 9, 1–37. https://doi.org/10.3389/fphar.2018.00309

van Goietsenoven, G., Andolfi, A., Lallemand, B., Cimmino, A., Lamoral-theys, D., Gras, T., Abou-donia, A., Dubois, J., Lefranc, F., Kornienko, A., Kiss, R., Evidente, A., 2010. Amaryllidaceae Alkaloids Belonging to Different Structural Subgroups Display Activity against Apoptosis-Resistant Cancer Cells †. J. Nat. Prod. 73, 1223–1227.

Van Wyk, A.S., Prinsloo, G., 2018. Medicinal plant harvesting , sustainability and cultivation in South Africa. Biol. Conserv. 227, 335–342. https://doi.org/10.1016/j.biocon.2018.09.018

Venieraki, A., Dimou, M., Katinakis, P., 2017. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. Hell. Plant Prot. J. 10, 51–66. https://doi.org/10.1515/hppj-2017-0006

Ventola, C.L., 2015. The Antibiotic Resistance Crisis Part 1 : Causes and Threats 40, 277–283.

Venugopalan, A., Srivastava, S., 2015. Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol. Adv. 33, 873–887. https://doi.org/10.1016/j.biotechadv.2015.07.004

Verdoorn, I.C., 1973. The genus Crinum in southern Africa. Bothalia 11, 27–52.

Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S., Klaassen, C.H.W., Perrone, G., Seifert, K.A., Varga, J., Yaguchi, T., Samson, R.A., 2014. Identification and nomenclature of the genus Penicillium. Stud. Mycol. 78, 343–371. https://doi.org/10.1016/j.simyco.2014.09.001

Volkmann, M., Gorbushina, A.A., 2006. A broadly applicable method for extraction and characterization of mycosporines and mycosporine-like amino acids of terrestrial, marine and freshwater origin. FEMS Microbiol. Lett. 255, 286–295. https://doi.org/10.1111/j.1574-6968.2006.00088.x

Wade, L.. jnr, 2010. Amino acids, peptides, and proteins, in: Organic Chemistry 7th Edition. pp.

1153–1199.

Wang, H., Li, Y., Huang, Y., Zhao, C., Cheung, H., 2018. Chemical Profiling of Lobelia chinensis with High-Performance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry (HPLC/Q-TOF MS) Reveals Absence of Lobeline in the Herb. Mol. Ther. Oncolytics 23, 1–13. https://doi.org/10.3390/molecules23123258

Wang, J., Yue, Y. De, Tang, F., Sun, J., 2012. Screening and analysis of the potential bioactive components in rabbit plasma after oral administration of hot-water extracts from leaves of Bambusa textilis mcclure. Molecules 17, 8872–8885. https://doi.org/10.3390/molecules17088872

Wang, P., Yuan, H.-H., Zhang, X., Li, Y.-P., Shang, L.-Q., Yin, Z., 2014. Novel lycorine derivatives as anticancer agents: synthesis and in vitro biological evaluation. Molecules 19, 2469–2480. https://doi.org/10.3390/molecules19022469

Want, E.J., 2018. LC-MS Untargeted Analysis, in: Theodoridis, G.A., Gika, H.G., Wilson, I.D. (Eds.), Metabolic Profiling Methods and Protocols. Humana Press, New York, NY, U.S.A, pp. 99–116.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. Medicinal and Poisonous Plants, second. ed, Nature. E & S.Livingstone Ltd, London. https://doi.org/10.1038/196609b0

Widyawati, S.P., Budianta, T.D.W., Kusuma, F.A., Wijaya, E.L., 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia less leaves extracts. Int. J. Pharmacogn. Phytochem. Res. 6, 850–855.

Wietz, M., Månsson, M., Bowman, J.S., Blom, N., Ng, Y., Gram, L., 2012. Wide Distribution of Closely Related , Antibiotic-Producing Arthrobacter Strains throughout the Arctic Ocean. Appl. Environ. Microbiol. 78, 2039–2042. https://doi.org/10.1128/AEM.07096-11

Williams, V.L., Raimondo, D., Crouch, N.R., Cunningham, A.B., Scott-Shaw, C.R., Lötter, M., Ngwenya, A.M., Brueton, V.., 2016. Crinum macowanii Baker. National Assessment: Red List of South African Plants version 2017. [WWW Document]. South African Natl. Biodivers. Inst. Red List South African Plants. URL http://redlist.sanbi.org.www57.cpt1.host-h.net/species.php?species=2087-19 (accessed 9.18.19).

Williams, V.L., Victor, J.E., Crouch, N.R., 2013. Red Listed medicinal plants of South Africa: Status, trends, and assessment challenges. South African J. Bot. 86, 23–35. https://doi.org/10.1016/j.sajb.2013.01.006

Wina, E., Susana, I.W.R., Tangendjaja, B., 2010. Biological Activity of Tannins from Acacia

mangium Bark Extracted by Different Solvents. Media Peternak. 33, 103–107. https://doi.org/10.5398/medpet.2010.33.2.103

World Health Organization, 2019. Don't let tobacco take your breath away.

World Health Organization, 2002. WHO Traditional Medicine Strategy 2002-2005, World Health Organisation Geneva. https://doi.org/WHO/EDM/TRM/2002.1

Wu, W., Han, X., Wu, C., Wei, G., Zheng, G., Li, Y., Yang, Y., Yang, L., He, D., Zhao, Y., Cai, Z., 2018. Vernodalol mediates antitumor effects in acute promyelocytic leukemia cells. Oncol. Lett. 15, 2227–2235. https://doi.org/10.3892/ol.2017.7544

Xiao, J.F., Zhou, B., Ressom, H.W., 2012. Metabolite identification and quantitation in LC-MS/MS-based metabolomics. TrAC - Trends Anal. Chem. 32, 1–14. https://doi.org/10.1016/j.trac.2011.08.009

Xie, G., Ni, Y., Su, M., Zhang, Y., Zhao, A., Gao, X., Liu, Z., Long, X., Jia, W., 2008. Application of Ultra-Performance LC-TOF MS Metabolite Profiling Techniques to the Analysis of Medicinal Panax Herbs. Metabolomics 4, 248–260.

Xie, J., Wu, Y., Zhang, T., Zhang, M., Zhu, W., Gullen, E.A., Wang, Z.-J., Cheng, Y.-C., Zhang, Y.-X., 2017. New and bioactive natural products from an endophyte of Panax notoginseng †. R. Soc. Chem. Adv. 7, 38100–38109. https://doi.org/10.1039/c7ra07060h

Xu, J., 2010. Secondary Metabolites from the Endophytic Fungus Pestalotiopsis sp. JCM2A4 and its Microbe-Host Relationship with the Mangrove Plant Rhizophora mucronata. Heinrich-Heine-Universität Düssedldorf.

Xu, Z., Chang, L., 2017. Amaryllidaceae, in: Identication and Control of Common Weeds: Volume 3. Springer Nature, Gateway East, Singapore, pp. 877–886.

Xue, J.H., Xu, L.X., Jiang, Z.H., Wei, X., 2012. Quinazoline Alkaloids from Streptomyces michiganensis. Chem. Nat. Compd. 48, 839–841. https://doi.org/10.1007/s10600-012-0397-8

Yadav, R.N.S., Agarwala, M., 2011. Phytochemical analysis of some medicinal plants. J. Phytol. 3, 10–14. https://doi.org/10.1021/np800144q

Yaish, M.W., 2016. Draft Genome Sequence of Endophytic Bacterium Enterobacter asburiae PDA134 , Isolated from Date Palm (Phoenix dactylifera L .) Roots. Genome Announc. 4, 1–2. https://doi.org/10.1128/genomeA.00848-16.Copyright

Yakandawala, D.M.D., Samarakoon, T.M., 2006. An Empirical Study on the Taxonomy of Crinum Zeylanicum (L.) L. and Crinum Latifolium L. (Amaryllidaceae) Occurring in Sri Lanka. Ceylon J. Sci. (Biological Sci. 35, 53–72.

Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., Wang, Q., 2018. Response of Plant Secondary Metabolites to Environmental Factors. Molecules 23, 1–26. https://doi.org/10.3390/molecules23040762

Yoshioka, T., Wada, T., Uchida, N., Maki, H., Yoshida, H., Ide, N., Kasai, H., Hojo, K., Shono, K., Maekawa, R., Yagi, S., Hoffman, R.M., Sugita, K., 1998. Anticancer efficacy in vivo and in vitro, synergy with 5-fluorouracil, and safety of recombinant methioninase. Cancer Res. 58, 2583–2587.

Yuan, H., Ma, Q., Ye, L., Piao, G., 2016. The Traditional Medicine and Modern Medicine from Natural Products. Molecules 21, 2–18. https://doi.org/10.3390/molecules21050559

Yusnawan, E., 2013. The Effectiveness of Polar and Non Polar Fractions of Ageratum Conyzoides L . To Control Peanut Rust Disease and Phytochemical Screenings of Secondary Metabolites. J. Hama dan Penyakit Tumbuh. Trop. 13, 159–166.

Zanders, E.D., Svensson, F., Bailey, D.S., 2019. Therapy for glioblastoma: is it working? Drug Discov. Today 24, 1193–1201. https://doi.org/10.1016/j.drudis.2019.03.008

Zeng, W., Li, W., Han, H., Tao, Y., Yang, L., Wang, Z., Chen, K., 2014. Microbial Biotransformation of Gentiopicroside by the Endophytic Fungus Penicillium crustosum 2T01Y01. Appl. Environ. Microbiol. 80, 184–192. https://doi.org/10.1128/AEM.02309-13

Zhang, J., Chen, Z., Huang, X., Shi, W., Zhang, R., Chen, M., Huang, H., Wu, L., 2019. Insights on the Multifunctional Activities of Magnolol. Biomed Res. Int. 2019, 1–15. https://doi.org/10.1155/2019/1847130

Zhang, M., Das, C., Vasquez, H., Aguilera, D., Zage, P.E., Gopalakrishnan, V., Wolff, J.E., 2006. Predicting Tumor Cell Repopulation After Response : Mathematical Modeling of Cancer Cell Growth. Anticancer Res. 26, 2933–2936.

Zhao, J., Ma, J., 2016. Phytochemicals and Biological Activities of the Genus Cinnamomum. J. Pharmacogn. Phytochem. 4, 27–34.

Zheng, M., 2016. Classification and Pathology of Lung Cancer. Surg. Oncol. Clin. N. Am. 25, 447–468. https://doi.org/10.1016/j.soc.2016.02.003

Zhou, Yunfei, Zhou, Yan, Shingu, T., Feng, L., Chen, Z., Ogasawara, M., Keating, M.J., Kondo, S., Huang, P., 2011. Metabolic Alterations in Highly Tumorigenic Glioblastoma. J. Biol. Chem. 286, 32843–32853. https://doi.org/10.1074/jbc.M111.260935

Zhu, Z., Schultz, A.W., Wang, J., Johnson, C.H., Yannone, S.M., Patti, G.J., Siuzdak, G., 2013. Liquid chromatography quadrupole time-of-flight mass spectrometry characterization of metabolites guided by the METLIN database. Nat. Protoc. 8, 451–460.

https://doi.org/10.1038/nprot.2013.004

Zinniel, D.K., Lambrecht, P., Harris, N.B., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C.A., Arunakumari, A., Barletta, R.G., Vidaver, A.K., 2002. Isolation and Characterization of Endophytic Colonizing Bacteria from Agronomic Crops and Prairie Plants †. Appl. Environ. Microbiol. 68, 2198–2208. https://doi.org/10.1128/AEM.68.5.2198

Zonyane, S., Makunga, N.P., Vuuren, S.F. Van, 2013. The antimicrobial interactions of Agathosma crenulata, Dodonaea viscosa and Eucalyptus globulus combination and their chemical profiling. Stellenbosch University.

Zubair, M.S., Anam, S., Lallo, S., 2016. Cytotoxic activity and phytochemical standardization of Lunasia amara Blanco wood extract. Asian Pac. J. Trop. Biomed. 6, 962–966. https://doi.org/10.1016/j.apjtb.2016.04.014

CHAPTER TWO:
LITERATURE REVIEW*

*This chapter gives a detailed review on plants and secondary metabolites, medicinal plants and their potential endophytes. It also discusses the importance of endophytes and the botanical description, phytochemistry and ethnobotany of *C. macowanii and* its related plant species and techniques used in this study.

2.0 BRIEF

This chapter discusses the literature survey of plants and medicinal plants, their importance, endophytes isolated from them and the secondary metabolites they produce. It covers threats to medicinal plants leading to their extinction, and possible solutions to the problem. It also discusses our plant of study, *Crinum macowanii*, its phytochemicals (natural plant products) and their pharmacological activities and those of its closely related species, as well as the botanical description of the plant and its traditional medicinal uses and the different techniques used in this study.

2.1 Antibiotic resistance: a global public health concern

The discovery of antibiotics such as salvarsan, prontosil and penicillin changed the history of infectious diseases and stimulated the discovery of newer and novel antibiotics (Tiwaskar and Manohar, 2017; Taneja *et al.*, 2019). The discovery of major classes of antibiotics such as tetracyclines, methicillin, gentamicin gave rise to the golden era of antibiotics discovery but unfortunately due to the massive uptake of existing antibiotics and the absence of newer drugs being discovered, infectious microbes have become resistant to already available antibiotics (Zaman *et al.*, 2017; Aslam *et al.*, 2018; Taneja *et al.*, 2019). The emerging of new infectious diseases, spreading and persistence of multidrug-resistant (MDR) bacteria or 'superbugs' poses a worldwide threat to humans, animals and environmental health (Aslam *et al*., 2018). The World Health Organization (WHO) has declared antibiotic resistance to be a "global public health concern", claiming at least 700,000 annual deaths globally (Aslam *et al.*, 2018; Bloom *et al.*, 2018). Ali *et al*. (2018) reported that novel therapies ought to be discovered to combat antimicrobial resistance.

2.1.1 Urgent need for drug discovery and development

Efforts are currently underway to discover natural solutions to treating infections caused by resistant microbes (Sen and Batra, 2012), and medicinal plants are regarded a natural and renewable target in this regard (Selvamohan *et al.*, 2012). The United Nations (UN) through the Sustainable Development Goals (SDGs) plans to end epidemics such as AIDS, tuberculosis, malaria and infectious disease by the year 2030 (Raviglione and Maher, 2017). There is therefore an imminent need to discover and develop new drugs to combat antimicrobial resistance (Rajbhandary, 2014). Mager (2006) reported that bacterial infection constitutes about 15% of cancers worldwide and therefore is a serious health concern. Bacterial species are found in tumour

cells and play a role in the development and progression of cancers and also influence the course of disease (Picardo *et al*., 2019).

2.2 Cancer, *the leading cause of deaths globally*

A tumour is the uncontrollable growth of cells and can be solid or fluid filled (Sinha, 2018). Solid tumours are characterized as being benign (not cancerous), or malignant (cancerous) and there are more than 100 different types of cancer which are named according to the type of cell that is initially affected (Gavhane *et al.*, 2011; Idikio, 2011; Joshi *et al.*, 2012). The South African National Department of Health (2017) has described cancer as the second foremost cause of death globally, with growing numbers of people dying of cancer-related causes. Bray *et al.* (2018) reported that WHO has ranked cancer as the first leading cause of deaths globally on people below the age of 70 years, with an estimate of 18.1 million new cancer cases and 9.6 million deaths from cancer in the year 2018 worldwide. Sinha (2018) reported that benign tumours can be differentiated from malignant tumours by growth rate, ability to invade locally, ability to spread at distance, cellular appearance and treatments, although this list is not exhaustive.

2.2.1 Glioblastomas

Gliomas, or glial neoplasms, are a type of a tumour originating in the parenchyma of the central nervous system (CNS), further divided into glioblastoma, astrocytoma, oligodendroglioma, and ependymoma. Glioblastoma are diffuse astrocytic tumours which account for about 15% of all primary CNS tumours, and over 50% of all gliomas (Hartmann and Wesseling, 2019). Gliomas are common primary central nervous system (CNS) tumours mostly affecting the brain (Paolillo *et al*., 2018). Brain tumours are common cancers affecting mostly children from birth to adolescence, and their symptoms, such as seizures, morning headaches, lethargy or clumsiness, make it difficult to diagnosis, as these symptoms are not specific to brain cancer (Gavhane *et al*., 2011).

Glioblastomas are aggressive cancers, resistant to treatment, with a poor prognosis and an average patient survival of 15 months. Only 27% of patients survive more than 2 years (Zanders *et al*., 2019). Hartmann and Wesseling (2019) reported that even with treatment methods such as surgery, radiotherapy, and chemotherapy, most glioblastoma patients die within 1–2 years of diagnosis. Challenges such as the poor brain penetration of anticancer drugs due to the blood brain barrier (BBB), and the emergence of acquired resistance to commonly used drugs such as temozolomide (TMZ) complicate treatment (Noch *et al*., 2018; Zanderse *et al*., 2019). In South Africa, brain cancer seems to be more prevalent in males than females. The following were recorded with

reference to brain cancer diagnosis, in females 0.38%, 0.47%, 0.48% and 0.44% were recorded for the year 2013,2014,2015 and 2016 respectively, whereas in males 0.57%, 0.65%, 0.61% and 0.66% were recorded for the year 2013,2014,2015 and 2016 respectively (National Cancer Registry South Africa, 2013;National Cancer Registry South Africa, 2014;National Cancer Registry South Africa, 2015;National Cancer Registry South Africa, 2016). With such increasing diagnosis annually, a synergistical approach is needed for effectively treating glioblastoma by attacking the tumour from several directions (Noch *et al*., 2018).

2.2.2 Lung carcinoma

Lung cancer, or lung carcinoma, is a malignant lung tumour categorized by uncontrolled cell growth in the lung tissues (Mustafa *et al*., 2016), and it is a leading cause of cancer deaths globally (Dela Cruz *et al*., 2011). Lung cancer can be classified as either non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC) (Zheng, 2016; Cancer Council Australia, 2018). Clinical manifestations of lung cancer include a persistent new cough or a change in an ongoing cough, breathlessness, chest and/or shoulder pain and coughing or spitting up blood (Cancer Council Australia, 2018). The World Health Organization reports that tobacco smoking is the primary cause for lung cancer and (World Health Organization, 2019) and Dela Cruz *et al*., (2011) further attributes that an increase in lung cancer deaths globally is due to the accumulated use of tobacco worldwide. Lung cancer is more common in males than females (Mustafa *et al*., 2016); this is evident in South Africa where the following diagnosed statistics were recorded in males 4.91%, 4.87%, 4.67% and 4.84% in the year 2013,2014,2015 and 2016 respectively while in females 2.52%, 2.48%, 2.4% and 2.5% were recorded in the year 2013,2014,2015 and 2016 respectively (National Cancer Registry South Africa, 2013;National Cancer Registry South Africa, 2014;National Cancer Registry South Africa, 2015;National Cancer Registry South Africa, 2016). Lung cancer has been deemed to be one of the most prevalent cancers in the developed world and has a very poor survival rate, as most patients are diagnosed at an advanced stage (Stage IV) at which the cancer has spread to other parts of the body and curative treatment is impossible. It is one of the most difficult cancers to diagnose or detect at an early stage as the clinical manifestations are non-specific (Neal *et al*., 2015; Cancer Council Australia, 2018). Currently prescribed chemotherapeutic drugs paclitaxel, docetaxel, gemcitabine, vinorelbine and ifosfamid have limited success (Tsoukalas *et al*., 2018). They are administered intravenously, leading to toxicities throughout the body and therefore, the use of pulmonary delivery is advisable to combat this problem (Rosière *et al*., 2019). Kumar *et al.* (2014) and Jazieh *et al.* (2018) emphasised that

discovery and development of chemotherapeutic agents is vital in the treatment of cancer, as current therapies are ineffective, costly and have side effects, and Aggarwal *et al*., 2016, highlighted the need for identifying new targets for therapeutic agents .

2.3 Plants and their importance to human health

People have been using plants for food, shelter, tools and medicine, in the form of wildflowers, edible bulbs, and carefully groomed grasslands since their existence (Reid and Wishingrad, 2009). Plants have been used as medicine to treat and reduce diseases, and their usage as medicines dates back 60,000 years (Yuan *et al*., 2016). The Department of Agriculture Forestry and Fisheries, Republic of South Africa (2013), describes medicinal plants as plants used for medicinal and therapeutic purposes due to the presence of certain bioactive scaffolds within them. Medicinal plants have been used since time immemorial to heal and cure diseases and to improve the health and wellbeing of people and animals (Hosseinzadeh *et al.*, 2015; Shakya, 2016). The long history of use of medicinal plants has prompted their development and recognition in developing countries (Mahomoodally, 2013; Dar *et al*., 2017).

2.3.1 Medicinal plants and their significance

South Africa is home to a variety of medicinal plants, with 24 000 taxa available. About 3 000 of these plant species are currently utilized as medicine, and 350 are used as traditional herbal medicine (Abdalla and Mcgaw, 2018). Of the over 252 drugs considered to be critical by the WHO, 11% are derived from plants, and many others are built on template compounds isolated from plants (Kumar and Roy, 2017; Akinyemi *et al*., 2018). Begum *et al.* (2017) reported that an estimate of about 25% of prescribed drugs worldwide are derived from plants, and 121 such active compounds are currently used to date (Table 2.1). The discovery of medicinal plants' usage for treatment of diseases has led to bioactive secondary metabolites from plants being used in medicines, either in their original state or in a modified form (Salim *et al.*, 2008), and thus the use of herbal medicines for healing purposes and the production of herbal drugs gave birth to modern medicine used today (Pal and Shukla, 2003).

Table 2.1 Drugs derived from plants, with their clinical uses

Plant source	Pharmaceutical drugs	Drug Action or clinical use
Taxus brevifolia	Taxol	Antitumour
Gimkgo biloba L	Ginkgolide B	Cerebra infarction

Ranunculus ternatus hunb.	Ternatolide	Anti-tuberculosis
Papaver somniferum	Morphine	Analgesic
Atropa belladonna L.	Atropine	Anticholinergic

Adapted from (Fabricant and Farnsworth, 2001; Yuan *et al.*, 2016)

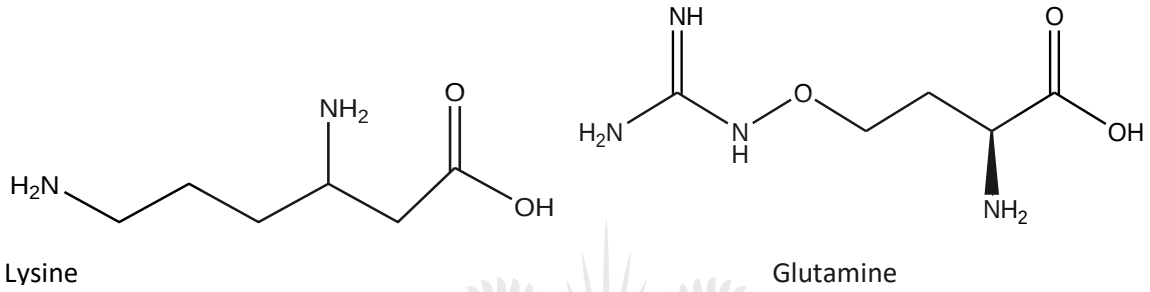
2.3.2 Natural plant products (Phytochemicals)

Plants naturally produce chemical compounds which are biologically active, known as phytochemicals (Saxena *et al.*,2013; Kadhim *et al.*, 2016). Phytochemicals protect plants from disease, damage and environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack, and also contribute to a plant's colour, aroma and flavour (Saxena *et al.*, 2013; Jayaprasad and Sharavanan, 2015). Phytochemicals are found in different plant parts, including the roots, stems, leaves, flowers, fruits or seeds and bulbs, and factors such as the nature of the plant material, processing, storing and growing conditions effect the accumulation levels of phytochemicals (Tiwari *et al.*, 2011; Saxena *et al.*, 2013). Phytochemicals are produced through primary or secondary metabolism by plants, resulting in primary and secondary metabolites (Hoffmann, 1951). Primary metabolites are compounds responsible for the growth, development and metabolism of the plant and are essential for the survival of the plant. Examples include proteins, nucleotides, common fatty acids, chlorophyll and sugars (Ibraheem and Maimako, 2014; Hannah and Krishnakumari, 2015). Secondary metabolites, on the other hand, are known to be products of primary metabolism and are not directly involved in the metabolic activities of the plant but rather serve specific functions such as attracting pollinators or in defence against herbivores. Their examples include tannins, alkaloids, flavonoids, steroids, saponins and cardiac glycosides (Hoffmann,1951; Hannah and Krishnakumari, 2015).

2.3.2.1 Common primary metabolites

2.3.2.1.1 Amino acids

Amino acids are molecules containing a central carbon atom to which an amino group, a carboxylate group, a hydrogen atom, and an R (side chain) group are attached, and appear as colourless crystals which are soluble in water and insoluble in ether and other organics (McKee and McKee, 2008; Wade, 2010). Amino acids are chemical units or building blocks, and when 50 or more are joined together they form proteins (Hounsome *et al.*, 2008). Biological functions of amino acids include acting as chemical messengers in a variety of biochemical pathways, acting as metabolic intermediates and maintaining intestinal integrity (McKee and McKee, 2008; Moran-Palacio *et al.*, 2014). Common amino acids such as lysine and glutamate are show in Figure 2.1.

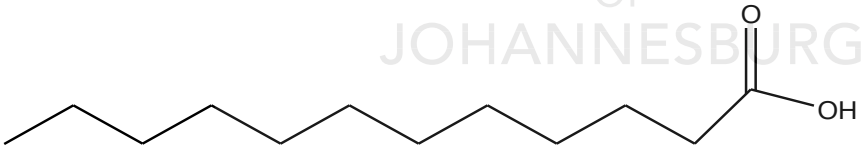


Lysine Glutamine

Figure 2.1: Structures of common amino acids adopted from Sebola et al., 2019

2.3.2.1.2 Fatty acids

Fatty acids are hydrocarbon carbon chains with a carboxyl group at the other end (Millar *et al.*, 2000). Their vital roles are the storage and transport of energy and they are also essential components of all membranes, and function as gene regulators (Rustan and Drevon, 2005). Examples include palmitic acid, lauric acid and capric acid (Figure 2.2).



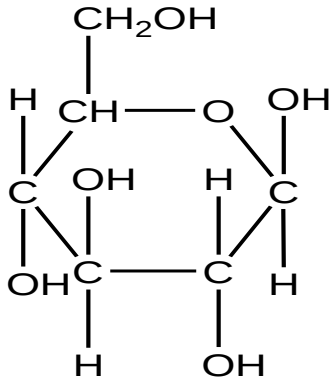
Lauric acid

Figure 2.2: Structures of fatty acids adopted from Sebola *et al*., 2019

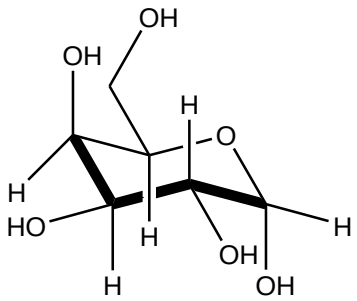
2.3.2.1.3 Carbohydrates

Carbohydrates are biological molecules which occur in living organisms, consisting of carbon (C), hydrogen (H) and oxygen (O) atoms (Ahmad, 2007). Carbohydrates are the first complex organic compounds formed in plants as a result of photosynthesis, and are metabolic precursors to all other organic compounds (Kresge *et al*., 2010). Khowala *et al*. (2008) stated that carbohydrates are the major source of metabolic energy in both animals and plants.

Examples of different carbohydrates groups are shown in Figure 2.3 (Hounsome *et al.*, 2008).



Glucose



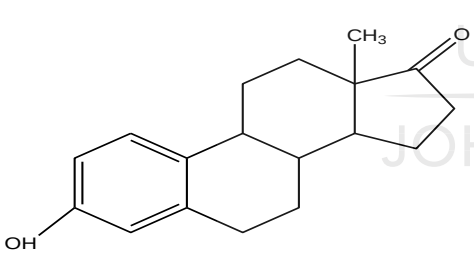
D (+) Galactose

Figure 2.3 Structures of carbohydrates adopted from Sebola *et al*., 2019

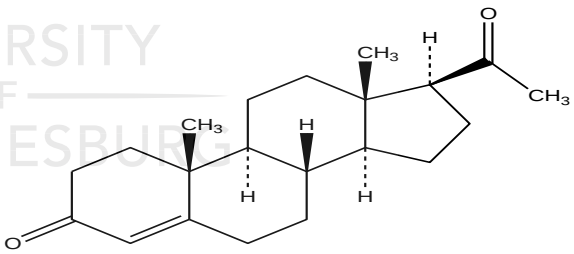
2.3.2.2 Common secondary metabolites

2.3.2.2.1 Steroids

Steroids are organic compounds classified as terpenoid lipids which contain a steroid nucleus (a carbon skeleton with four fused rings), and their different biological actions are determined by various groups attached to the common nucleus (Bhawani *et al.*, 2010; Çitoğlu and Acıkara, 2012). Most steroids function as hormones that control metabolism and assist in the development and appropriate functioning of sexual organs; these include androgens, oestrogens and progestogens (Figure 2.4) (Kintzios and Barberaki, 2004; Bhawani *et al.*, 2010).



Oestrogen



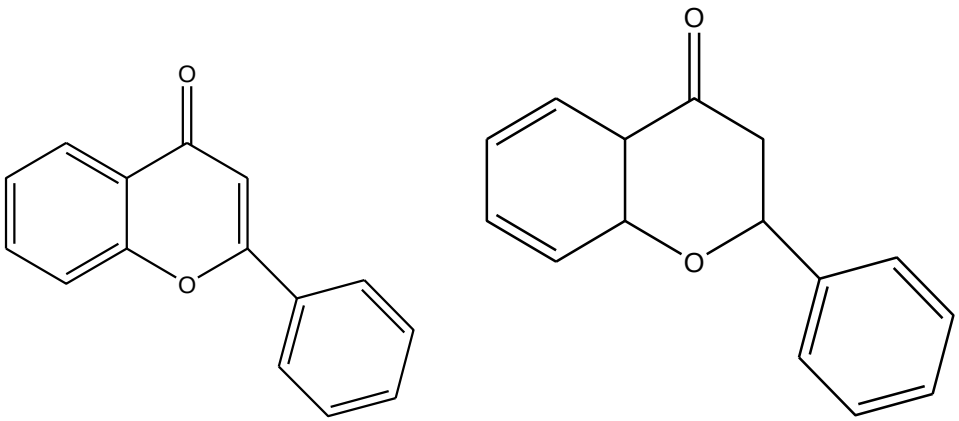
Progesterone

Figure 2.4: Structures of steroids adopted from Sebola *et al*., 2019

2.3.2.2.2 Flavonoids

Flavonoids are polyphenolic compounds made up of fifteen carbons, which consist of two phenyl rings connected by a three-carbon bridge (López-lázaro, 2009; Kumar *et al.*, 2015). High concentrations of flavonoids are found in the epidermis of leaves and the skin of different fruits (Kumar *et al*., 2015). Flavonoids perform different functions in plants, including plant pigmentation for pollen attraction and defence against pathogenic and herbivore attacks (Olufunke, 2012; Kumar and Pandey, 2013). Flavonoids may be divided into six main subclasses, namely,

flavanone, anthocyanin, flavonol, flavanol, isoflavone and flavone (Figure 2.5).

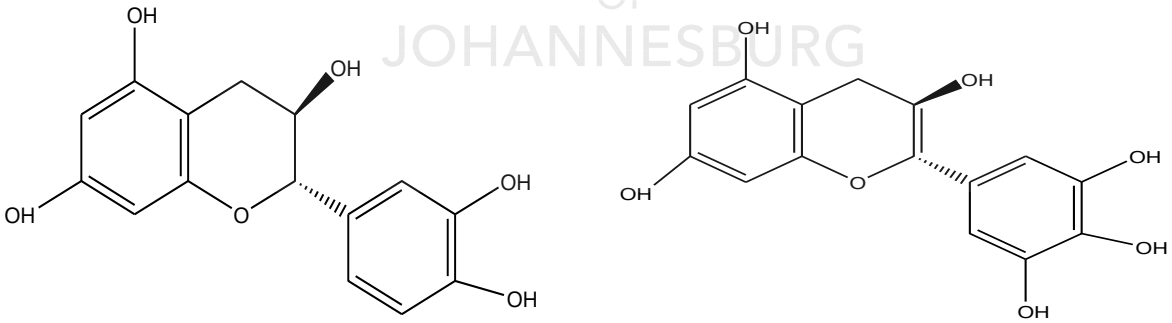


Flavone Flavanone

Figure 2.5: Structures of flavonoids adopted from Sebola *et al*., 2019

2.3.2.2.3 Tannins

Tannins are polyphenolic compounds containing two or three phenolic hydroxyl groups on a phenyl ring, in a molecule of moderately large size (Okuda and Ito, 2011). Tannins function in plant defensive mechanisms by reducing the growth and survivorship of herbivores due to their toxins (Wina, 2010; Mazid *et al*., 2011; Ashok and Upadhyaya, 2012). Tannins are known to possess astringent and haemostatic properties (Figure 2.6). These polyphenolic biomolecules can be assembled into four groups, namely, hydrolysable tannins, condensed tannins, complex tannins and pseudo-tannins (Ahmad, 2007).



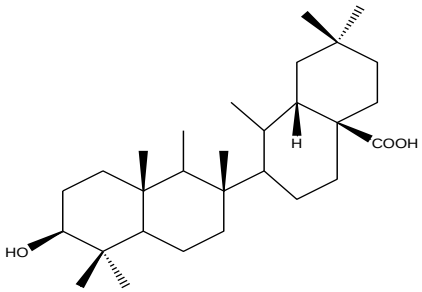
Catechin Gallocatechin

Figure 2.6: Structures of tannins adopted from Sebola *et al*., 2019

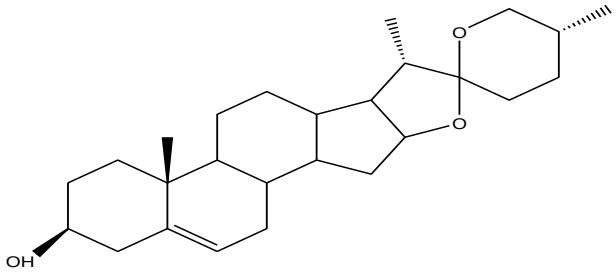
2.3.2.2.4 Saponins

Saponins are glycosylated metabolites and are grouped into three subsets, namely, glycosylated steroids, triterpenoids, and steroid alkaloids (Podolak *et al*., 2010; Saxena *et al*., 2013). Saponins contain both polar and nonpolar domains and are therefore called amphipathic glycosides, where

the aglycon (glycoside-free) is called sapogenin and is nonpolar (Negi *et al.,* 2013). In most plants, saponins are known to assist in plant growth and development (James and Dubery, 2009). Examples of saponins include diosgenin and oleanolic acid (Figure 2.7).



Oleanolic acid

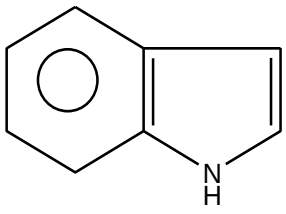


Diosgenin acid

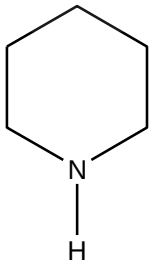
Figure 2.7: Structures of saponins adopted from Sebola *et al*., 2019

2.3.2.2.5 Alkaloids

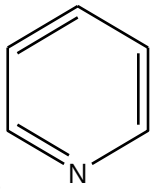
Alkaloids are organic-based nitrogenous compounds which contain heterocyclic nitrogen atoms but are not amino acids, and exist in both plants and animals (Saxena *et al.,* 2013; Kumar and Roy, 2017). Most alkaloids appear as colourless, crystalline material and have a bitter taste (Saxena *et al.,* 2013). Doughari (2012) and Olufunke (2012) reported that alkaloids are toxic and act as defence compounds in plants against herbivores and pathogens. Alkaloids are grouped according to the type of ring structure or the botanical taxa in which the alkaloids are found, such as piperidine alkaloids, steroidal alkaloids and pyridine alkaloids (Kumar *et al*., 2015). Factors such as biological activity, chemical structure and biosynthetic pathways further classify alkaloids into distinct biochemical groups such as indole, isoquinoline, isoxazole, phenethylamine, purine, pyrrolidine and piperidine, quinolizidine and tropane (Figure 2.8) (Saxena *et al.,* 2013).



Indole nucleus



Piperidine nucleus



Pyridine nucleus

Figure 2.8: Structures of alkaloids nucleus adopted from Sebola *et al*., 2019

2.3.3 Importance of secondary metabolites

Secondary metabolites are known to perform vital functions for their host plants, which include protection against predators and microbial pathogens, defence against abiotic stress, as well as performing as pollinator attractors and signalling communication between plants and microbes (Mazid *et al*., 2011; Yang *et al.*, 2018). Plant secondary metabolites are bioactive compounds with pharmacological activities that can possess antioxidative, anticarcinogenic, anti-inflammatory and antimicrobial properties (Kabera *et al.*, 2014; Kumar *et al.*, 2015; Nyamai *et al.*, 2016; Almoulah *et al.*, 2017; Mendoza and Silva, 2018). The World Health Organization (WHO) reports that 80% of the Africa's population depends on traditional medicine in the form of medicinal plants for their primary health care needs, resulting in the over use and over harvesting of medicinal plants (World Health Organization, 2002). With this high demand to obtain secondary metabolites from medicinal plants, commercial exploitation and extinction threaten biodiversity, as medicinal plants need a specific natural environment to grow, which limits their distribution (Ramesh *et al*., 2017; Jain and Pundir, 2017; Bengal *et al*., 2018; van Wyk and Prinsloo, 2018). Biotechnological approaches including the use of tissue culture, micropropagation and synthetic seed technology have been used to limit the over use of medicinal plants (Chen *et al*., 2016). Unfortunately these approaches are not always feasible, as they can produce low levels of bioactive compounds, require long growth periods and there is a difficulty in recovering the bioactive secondary compounds from other plant-derived metabolites such as primary metabolites (Venieraki *et al*., 2017). Strobel (2003) reported that microorganisms, mostly fungi and bacteria that inhabit plants, produce secondary metabolites, making them an ideal substitute for medicinal plants, as they have short generation times, good handling features in bioreactors and high growth rates, resulting in high biomass production (Ludwig-Muller, 2015). It is believed that most endophytes exchange fragments of their genomic DNA with that of the host plant, and this allows endophytes to synthesize some phytochemicals similar to the host plant. The endophytes achieve this by imitating the chemistry of the host plant and producing the same bioactive metabolites (Baker and Satish, 2012; Santos and Ferraris, 2012; Bedi *et al*., 2017; Venieraki *et al*., 2017; Uzma *et al.*, 2018). Currently scientists worldwide are studying endophytes and their secondary metabolites due to their medicinal potential (Strobel, 2003).

2.4 Endophytes: 'the plethora of bioactive secondary metabolites'

Endophytes are microorganisms (bacteria or fungi or actinomycetes) which reside within healthy plant tissues without causing disease symptoms in their host (Paul *et al.*, 2012; Zeng *et al.*, 2014). The association between endophytes and host plants include commensal, parasitic and mutualistic

relationships, and endophytes offer benefits such as promoting plant growth, maintaining the plant's health and ensuring tolerance of host plants to heat and salt, as well as resistance to plant pathogens (Tymon and Inglis, 2009; Khare *et al*., 2018). With over 300,000 plant species present on earth and each plant being able to play host to one or more endophytes, endophytes have been isolated from many different parts of plants, including leaf segments, roots, stem, bark, leaf and buds (Muzzamal *et al.*, 2012; Nair and Padmavathy, 2014). Endophytes gain access to plant tissues through root zones but aerial parts of plants such as flowers, stems, and cotyledons may also be used for entry. Endophytes' entry into plant tissue may also be via germinating radicles, secondary roots, stomates or as a result of leaf damage (Zinniel *et al*., 2002). The plant source, age of plant, type of tissue, season of sampling and environment determine the diversity and community of endophytes (Jasim *et al*., 2014). Joshi and Nongkhlaw (2015) and Eljounaidi *et al.* (2016) report that endophytes inhabit unique biological niches growing in unusual environments, and their isolation and identification is vital for further exploration.

2.4.1 Isolation and identification of endophytes

The type of plant selected for the isolation of endophytes determines the diversity of the endophytes. Various strategies are used for plant selection, including selecting plants from unique environmental settings, plants that have an ethnobotanical history, plants that are endemic and plants growing in areas of great biodiversity (Strobel and Daisy, 2003). Such plants have the potential to harbour endophytes with great biodiversity. The isolation of endophytes includes surface sterilization of the plant material followed by incubation of isolates in appropriate growth media and the identification of culturable endophytes performed by morphological and molecular techniques (Strobel and Daisy, 2003; Anjum and Chandra, 2015; Patil *et al*., 2016; Martinez-Klimova *et al*., 2017; Tidke *et al.*, 2017). Bacterial endophytic genera such as *Burkholderia*, *Klebsiella*, *Pantoea*, *Rahnella* and *Pseudomonas* have been isolated from different plants (Kandel *et al*., 2017), and *Alternaria*, *Aspergillus*, *Penicillium* and *Fusarium* have been isolated as fungal endophytes (Martinez-Klimova *et al*., 2017). Pusztahelyi *et al.* (2015) and Ek-Ramos *et al.* (2019) report that secondary metabolites produced by endophytic microorganisms act as antimicrobial and anticancer agents against human, animal and plant pathogens, and display significant potential in medical and veterinary treatments. Pathways such as the shikimate pathway, mevalonate pathway and polyketide pathway are used by endophytes to produce secondary metabolites (Ahmad, 2007; Mousa and Raizada, 2013; Kumara *et al.*, 2014). Plant secondary metabolites produced by endophytes include paclitaxel from *Taxomyces andreanae*, podophyllotoxin produced by *Trametes hirsute* and huperzine produced by *Shiraia* sp. Slf14 strains were all

produced by endophytic fungi (Kumara *et al.*, 2014; Singh *et al.*, 2019). Bacterial endophytes produced include ecomycins from *Pseudomonas viridiflav*, kakadumycins produced by endophytic streptomycete (NRRL 30566) and pseudomycins, produced by a plant-associated pseudomonad (Strobel and Daisy, 2003). Qin *et al.* (2011) reported that by the end of the year 2002, microbes had produced over 22,000 biologically active compounds. Baker and Satish (2012) predicted that since endophytes produce novel bioactive compounds of pharmaceutical importance, the fermentation of the microbial source would reduce the price of drugs, and therefore the fermentation of endophytes to produce bioactive compounds is important for the sustainable development of bioactive compounds.

2.4.2 Fermentation of culturable endophytes to obtain secondary metabolites

The fermentation of endophytes to produce metabolites is a preferred method as it is cost-effective, continuous and environmentally friendly. Submerged fermentation is the most advantageous method as its produces bioactive metabolites in a shorter space of time, requires less space and has fewer chances of contamination than liquid fermentation (Patil *et al*., 2016). Parameters such as temperature, media composition, pH, aeration, and agitation affect the synthesis of metabolites (Patil *et al*., 2016). Fermentation media used for growing the endophytes, incubation temperature and time and pH levels can influence the biomass and metabolite yield (Patil *et al*., 2016). The fermentation of culturable endophytes for the production of bioactive secondary metabolites can assist in the advancement of drug discovery and development, paving a way for bioprospecting (Martinez-Klimova *et al*., 2017).

2.5 Bioprospecting, the advancement in drug discovery and drug development

With the rise in antimicrobial resistance and diseases such as cancer, the United Nations has set out public health goals which align with the Millennium Development Goals 2030, aimed at halting diseases affecting the human population (Statistics South Africa, 2015). The bioprospecting of natural products from endophytes isolated from medicinal plants could assist in the search for novel bioactive secondary metabolites in the preparation of biopharmaceuticals (Arunachalam & Gayathri 2010; Department of Science and Technology, 2013; Martinez-Klimova *et al*., 2017). The development and innovation capabilities to manufacture active pharmaceutical ingredients, vaccines and biopharmaceuticals will help to achieve the set goals (Department of Science and Technology, 2013). The bioprospecting of endophytes for isolated metabolites could help save the environment as endophytes have been reported to contain similar

bioactive compounds to the host plant (Abdalla and Mcgaw, 2018; van Wyk and Prinsloo, 2018), and in so doing medicinal plants will be conserved and revenue generated (Department of Science and Technology, 2013).

2.6 An overview of the Amaryllidaceae family

The plant under investigation is *Crinum macowanii* Baker, which belongs to the family Amaryllidaceae J. St.-Hil. or Amaryllis, (often called amaryllids). This is a family of herbaceous monocotyledonous flowering plants, with most species being perennial and bulbous, and seldom rhizomatous (Nair and van Staden, 2013; Xu and Chang, 2017). The plant family consists of three subfamilies, namely, Amaryllidoideae, Agapanthoideae and Allioideae (Tallini *et al*., 2018) and *Crinum macowanii* Baker falls under the Amaryllidoideae subfamily.

The Amaryllis family is classified under the order of Asparagales and consists of roughly 1600 species with about 75 genera distributed across the world (Aziz *et al.*, 2014; Iannello, 2014; Guo, 2015; Xu and Chang, 2017). Generally, these plants are found in tropical and warm temperate regions with centres of origin in South America, mostly in the Andean region, South Africa, the Mediterranean region, and Australia (Kwembeya *et al.*, 2007; Iannello, 2014; Tallini *et al.*, 2018). Amaryllidaceae plants adapt to different environments and this allows them to be found in tropical, subtropical regions and temperate zones (Guo, 2015). The botanical characteristics of Amaryllidaceae plants in terms of leaves, flowers, fruits and seeds is summarized in Table 2.2.

Table 2.2: General botanical characteristics of the Amaryllis family

Botanical Forms and Parts	Characteristics
Botanical forms	Herbs and Ornamental flowers
Some typical genera	*Crinum*, *Haemanthus*, *Hippeastrum*, *Narcissus*
Special characteristics	Medium-sized herbs, Bulbs, Reduced stems
Leaves	Fleshly with parallel veins and different shapes
Flowers	Bisexual, symmetrical and consist of six sepals and form a floral tube.
Fruits	A capsule or a berry with dry or fleshy fruit.
Seeds	Small seeds, Testa and Embryo curved

Adopted from Aniszewski, (2007) and Iannello, (2014).

Plants from the Amaryllidaceae family have been utilized as herbal remedies for the treatment of ailments and disorders such as inflammatory, circulatory, and neurological conditions (Khalifa *et al*., 2018). Other species of the Amaryllidaceae family are used for poultices and decoctions for

the treatment of sores and itchy rashes, swelling of the body, urinary tract problems and digestive disorders (Nair and van Staden, 2013; Khalifa *et al.*, 2018). The medicinal value and properties of Amaryllidaceae plant species is attributed to their alkaloids, which are exclusively produced by this family, as seen in Table 2.3 (Bastida *et al*., 2006; De Andrade *et al.*, 2012; Wang *et al.*, 2014; Bozkurt *et al.*, 2017; Kaya *et al.*, 2017; Khalifa *et al.*, 2018; Roy *et al.*, 2018).

Table 2.3: Examples of some *Amaryllidaceae* plants with biological activity of their isolated alkaloids

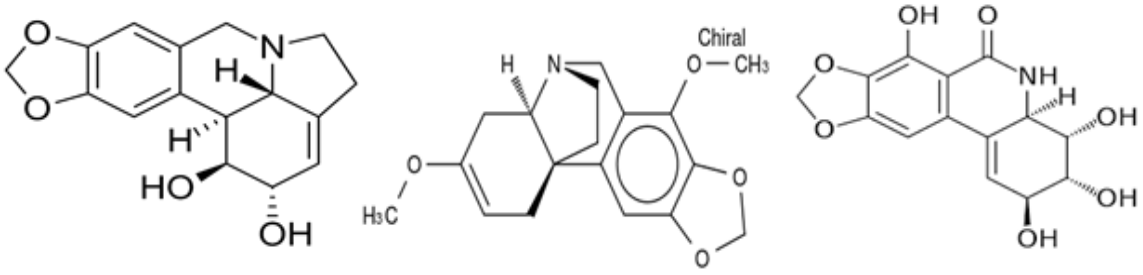
Plant name	Isolated alkaloid	Biological activity
Galanthus woronowii	Galanthamine	Treatment of Alzheimer disease (Rinner *et al*., 2016).
Lycoris radiate	Lycorine	Treatment of tumours (Roy *et al*., 2018)
Crinum bulbispermum	Bulbispermine	Kidney and bladder infections treatment (Louw *et al.*, 2002).
Boophone disticha	Buphanidrine and distichamine	Treatment of wounds, infections and inflammatory conditions (Nair and van Staden, 2014).

2.6.1 Phytochemicals and pharmacology activity of Amaryllidaceae species

Chemical traits, including the presence of chemical compounds such as alkaloids, flavonoids, coumarins and terpenoids, are used to classify plant species as true representatives of the Amaryllidaceae family (Asmawi *et al*., 2011; Fennell and van Staden, 2001). These chemical compounds create a platform for drug discovery as they are responsible for many biological activities (Refaat *et al.*, 2012; Nair *et al.*, 2013). The alkaloids from the Amaryllidaceae family, known as Amaryllidaceae alkaloids (AAs), are categorized into nine skeleton types, norbelladine, lycorine, homolycorine, crinine, haemanthamine, narciclasine, tazettine, montanine and galanthamine (Bastida *et al.*, 2011; Katoch *et al.*, 2013).

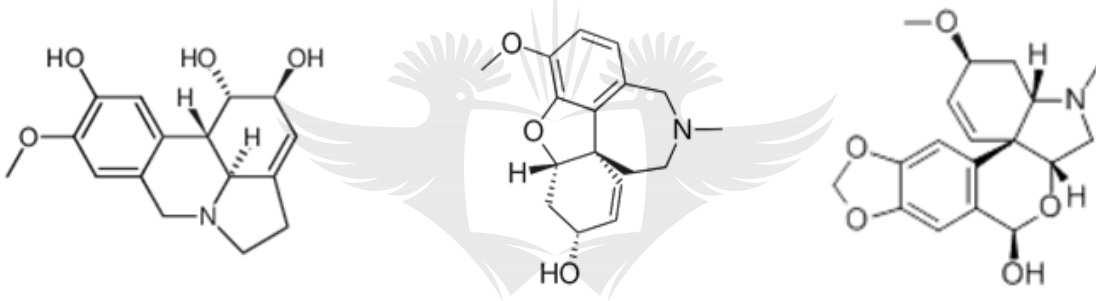
Amaryllidaceae alkaloids have intriguing chemical structures and a vast number of biological properties (Kaya *et al*., 2017) including antimicrobial, anti-inflammatory, cytotoxic, antinociceptive, antidepressant, and cholinesterase inhibitory activities (van Goietsenoven *et al.*, 2010; Bastida *et al.*, 2011; Nair and van Staden, 2013; Ndhlala *et al.*, 2013; He *et al.*, 2015; Khalifa *et al.*, 2018; Jin and Yao, 2019). The biological properties of AAs isolated from different Amaryllidaceae species are indicated in Figure 2.9.

1. Antiparasitic **2. Antibacterial** **3.Antifungal**



Lycorine Buphanidrine Narciclasine

4. Antiviral **5. Acetylcholinesterase inhibition** **6. Antitumoural**



Pseudolycorine Galanthamine Pretazettine

Figure 2.9: The biological properties of Amaryllidaceae alkaloids (AAs)
Adopted from Bastida *et al.*, (2011), Cheesman *et al*., (2012), Nair and van Staden, (2018).

Over 500 different alkaloids have been isolated from various species of the Amaryllidoideae sub family (Takos and Rook, 2013; Rønsted *et al.*, 2012) and the majority of these alkaloids have biological properties such as anti-cancer, antiviral, analgesic, antibacterial, antifungal, anti-inflammatory, antimalarial, antiparasitic and effects on the central nervous system (CNS), for example, hallucinogenic effects, mental disorders and age-related dementia (Maroyi, 2016; Torras-Claveria *et al.*, 2017; Khalifa *et al.*, 2018; Jin and Yao, 2019). The Amaryllidoideae subfamily has about 70 genera, which include *Galanthus* L., *Habranthus* Herb, *Narcissus* L., *Crinum* L, *Vagaria* Herb. and *Boophone* Herb (Takos and Rook, 2013; Khalifa *et al.*, 2018). *Crinum* L. is an endemic genus to southern Africa and is well-studied due to its wide diversity and history of traditional use

(Louw *et al*., 2002 ; Shahidullah *et al.*, 2009; Takos and Rook, 2013; Torras-Claveria *et al.*, 2017). As it yields a vast array of compounds it is an interesting genus to study.

2.7 *Crinum macowanii* Baker

The genus *Crinum* L. derives its name from the Greek word 'Krinos' which means trailing hair or comet-tail, since most species have long trailing petals (Yakandawala and Samarakoon, 2006). Afroz *et al*. (2018) reported that this is a pantropical genus which has over 112 species occurring in Americas, Africa, and southern Asia to Australia, with Africa having the most diverse species (Verdoorn, 1973; Kwembeya *et al.*, 2007). Over 23 of the 112 species of *Crinum* are recognized in southern Africa, and biogeographical studies indicate that the genus originates from southern Africa (Meerow *et al.*, 2003; Afroz *et al*., 2018). Some species of this Crinum genus include *C. amabile*, *C. bracteatum*, *C. bulbispermum*, *C. jagus*, *C. kirkii*, *C. asiaticum* and *C. macowanii* (Fennell and van Staden, 2001). Maroyi (2016) reported that *C. macowanii* has about 32 ethnomedicinal uses and has numerous isoquinoline alkaloids that have different biological activities; unfortunately, this has led to over-collecting and over harvesting, resulting in a population decline of the plant.

Crinum macowanii Baker was first described by Baker (1878) (Brueton, 2013), and the sobriquet 'macowanii' is in honour of Peter MacOwan (1830–1909), who extensively collected the plant in South Africa (Maroyi, 2016). It is distributed in central, east and southern Africa (Watt and Breyer-Brandwijk, 1962; Maroyi, 2016) and is native to Angola, Malawi, Mozambique, Zambia, Zimbabwe, South Africa, Botswana, Namibia, Swaziland, Kenya, Uganda, Tanzania, Democratic Republic of Congo (DRC) and Congo (Notten, 2013). *Crinum macowanii* has about 28 vernacular names, with South Africa having the highest at 13 (Maroyi, 2016). The number of vernacular names indicates an active use and interaction with *Crinum macowanii*, as people hardly name plant species that they do not use (Maroyi, 2014). Some of the vernacular names used in South Africa are Cape coast lily, common vlei-lily, river crinum, river lily and sabie crinum (English), rivierlelie and sabielelie (Afrikaans), intelezi (Xhosa), umduze (Zulu/ Ndebele), whilst in Namibia and Kenya it is referred to as Grosse Omurambalillie (German) and gûtûngûrû kla ngoma (Kikuyu), respectively (Notten, 2013; Maroyi, 2016).

2.7.1 Plant Description

Crinum macowanii Baker (Figure 2.10) grows in savannah regions and grasslands and is mostly found beside rivers and along the coast. It can grow well in various soil types, with frequent growth observed in black cotton soils (Watt and Breyer-Brandwijk, 1962; Persson *et al.*, 2007). It grows

extensively in summer rainfall regions although field conditions can slow down the reproduction rate of the plant (Botanical Society of South Africa, 2007; Persson *et al.*, 2007).

This plant develops a deciduous bulb, with fleshy roots and bright green to bluish green leaves and large white lilies with dark pink stripes (Botanical Society of South Africa, 2007). The leaves are green or glaucous and are slightly to very strongly undulate and spread to the ground and are 80 cm long, or longer and 2–16 cm broad (Brueton, 2013). The flower produces about 20 to 80 small seeds that appear as smooth, pale green to silvery and fleshy and the flowers have a heavy scent and are normally 4–20 cm with a pedicel up to 6.5 cm long. This plant flowers throughout the year while the highest flowering and fruiting frequency occurs between October and January (Brueton, 2013; Notten, 2013). The bulbs are normally 6–25 cm in diameter and are unexpectedly shaped into a small short or long neck and stretched fibres are observed when the bulbs are pulled apart; unfortunately *Crinum macowanii* does not have defining bulb characteristics and the bulb looks the same as that of other Crinum species (Brueton, 2013). *C. macowanii* can be distinguished from other Crinum species such as *Crinum moorei* and *Crinum bulbispermum* by the appearance of its large bell-shaped flowers with black anthers while *Crinum moorei* and *Crinum bulbispermum* have light grey and pale brown anthers, respectively (Elgorashi, 2000), and also by its broad leaves and longer flower pedicels (Kwembeya and Stedje, 2007). The fruit appears green to a fading dull yellow colour. The fruit is a capsule with 3–6 seeds which is smooth, with a pale green to silvery, colour and is fleshy (Notten, 2013).

Figure 2.10: Morphology of *C. macowanii*, flowers, leaves and seeds and bulbs (Elgorashi, 2000)

2.7.2 Plant Distribution

C. macowanii is found in areas with large seasonal variation and grows in various soils including gravelly soil, shale or sandy flats (Notten, 2013). Its habitats include grassland with water supply and it is distributed in places such as roadsides and abandoned cultivations (Maroyi, 2016). *Crinum macowanii* has been recorded in most provinces of South Africa, most frequently along the eastern coast and border of South Africa from the Eastern Cape to Limpopo but it is absent in the south-western Cape (Elgorashi, 2000; Brueton, 2013), while elsewhere in Africa it occurs in east, central and southern Africa (Maroyi, 2016). The geographical distribution of *Crinum macowanii* is shown in Figure 2.11.

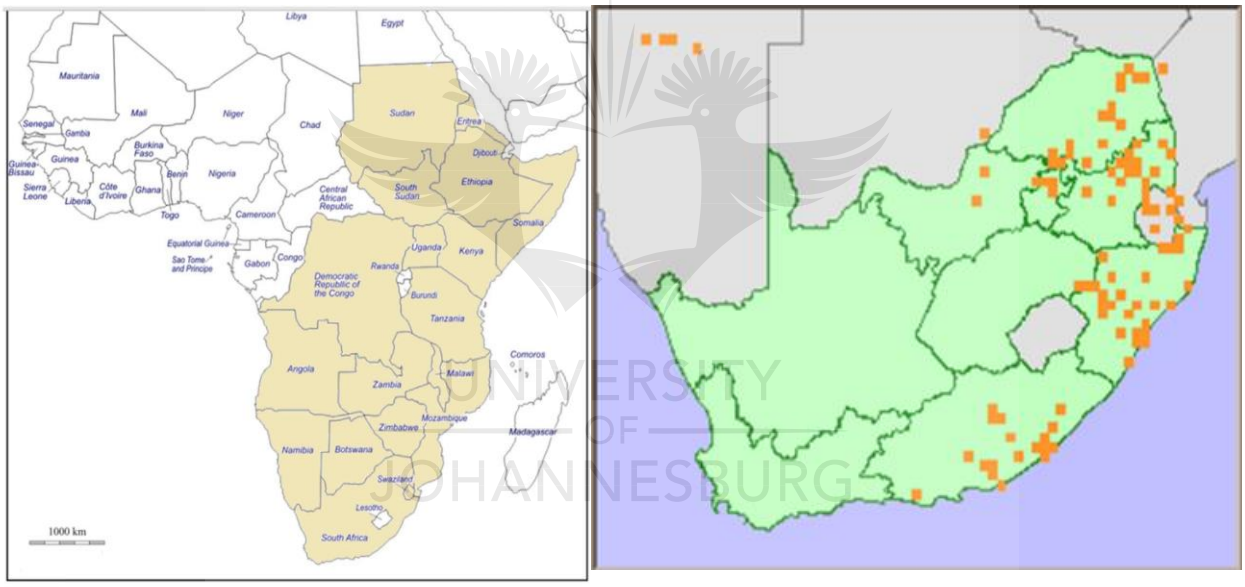


Figure 2.11: The geographical distribution map of *Crinum macowanii* in Africa and South Africa. Distribution map obtained from Brueton, (2013), Notten, (2013) and Maroyi, (2016).

2.7.3 Ethnopharmacological uses of *C. macowanii*

The *Crinum macowanii* has an extensive range of traditional medicinal applications as seen in Table 2.4, these include the treatment of sexually transmitted diseases and backache, urinary rheumatic diseases and tuberculosis. It is also used for the cleansing of internal organs, the skin and blood (Fennell and van Staden, 2001; Kwon *et al.*, 2011; Louw *et al.*, 2002; Mugwagwa *et al.*, 2015) and is used to increase lactation (in human and animal mothers) (Nair *et al.*, 2013; Nair and van Staden, 2013). The bulbs and leaves are used to treat infected sores, boils, fever and pus

diseases (Rabe and van Staden, 1997). The bulb is used on a regular basis during pregnancy for facilitate easy delivery (Ndhlala *et al.*, 2013) and it is also used to treat amenorrhea and pregnancy-related problems (Crouch *et al*., 2005). The bulb is also utilized to treat kidney and bladder diseases and rheumatic fever (Taylor *et al.*, 2003; Griffiths *et al.*, 2004).

Table 2.4: Traditional medicinal uses of *C. macowanii*

Conditions to be treated/ Use	Plant part (s) used	Traditional applications
Circulatory System		Decoctions of the bulbs is used by the Zulu people for the treatment of heart disease (Persson *et al*., 2007).
1. Heart disease	Bulb	
Digestive System		An infusion of the bulbs is used as emetic for humans and the plant is known to treat stomach diseases (Elgorashi *et al.*, 2003).
1. Stomach diseases	Bulb	
Genitourinary System		The Zulu people ingest the decoctions of the bulb orally for the treatment of kidney and bladder disease. The leaves are used as remedy for the bladder and kidneys. The overall plant is known to be used as an aphrodisiac (Elgorashi *et al.*, 2003).
1. Kidney and bladder disease	Bulbs and leaves Whole plant	
2. Aphrodisiac		
Infections		The Zulu people ingest the decoctions of the bulb orally for the treatment of tuberculosis. In Zimbabwe, powdered bulb is taken in porridge for the treatment of venereal diseases. The bulbs and leaves are used as remedy for fever and scrofula (Nair *et al.*, 2000).
1. Tuberculosis	Bulb	
2. Venereal diseases	Bulb	
3. Fever and scrofula	Bulbs and leaves	
Sensory System		The plant is known to be used for the treatment of eye diseases (Nair *et al.*, 2000).
1. Eye diseases	Whole plant	
Skin/Subcutaneous		The bulbs and leaves are used as a remedy for skin problems such as boils, sores and acne. The plant is used for the treatment
1. Boils, sores and acne	Bulb and leaves	
2. Itchy rashes and swelling of the body	Whole plant	

of itchy rashes and swelling of the body (Elgorashi *et al.,* 2003).

2.7.4 Phytochemistry and pharmacological activity of *C. macowanii*

C. macowanii is known to contain alkaloids, as the *Amaryllidaceae* family has a documented prodigious alkaloid output. Chemical compounds such as flavonoids, coumarins, alkaloids, terpenoids and tannins have been isolated from *Crinum macowanii* (Asmawi *et al.,* 2011; Fomum and Nsahlai, 2017; Pereira *et al.,* 2019). Lycorine, crinine, and powerline have been described as three principle alkaloids of *Crinum macowanii* (Persson *et al*., 2007). Elgorashi *et al*. (2002), reported the isolation of alkaloids lycorine, 1-O-acetyllycorine, crinine, powelline, crinamine, crinamidine, 3-O-acetylhamayne, 1-epideacetylbowdensine and cherylline, which were isolated from the bulb, root, leaf and flower during different seasons. The alkaloids macowine, cherylline, krepowine, buphanidrine, undulatine, 1-epideacetylbowdensine and 4a-dehydroxycrinamabine have also been isolated from *C. macowanii* (Tram *et al.,* 2002; Nair *et al.,* 2000; Refaat *et al.,* 2012; Nair *et al.,* 2013). Compounds such as oleic acid, campesterol, andrographolide and tyramine have been reported to be present in *C. macowanii* plants from either the bulb, leaves or the whole plant (Refaat *et al.,* 2013; Sebola *et al*., 2016).

2.7.5 Pharmacological activities of phytochemicals isolated from *C. macowanii*

Maroyi (2016) reported that biological activities such as antibacterial, antifungal, antiviral, antiplasmodial and effects on the central nervous system have been displayed by either crude extracts from *C. macowanii* or pure compounds obtained from the plant, as displayed in Table 2.5. Fennell and van Staden (2001) reported that extracts of *C. macowanii* displayed antifungal activity against *Candida albicans*. Cao *et al*. (2013) reported that lycorine possesses multiple biological activities, including anti-tumour, anti-virus, anti-bacterial, anti-inflammation, anti-malarial properties, and control of duration of menses. Alkaloids vittatine and crinamine have been shown to be active against *Bacillus subtilis* and *Staphylococcus aureus* (Fennell and van Staden, 2001). Extensive research has been done on alkaloids isolated from most C*rinum* species including *C. macowanii*, however, the presence of other chemical compounds such as flavonoids, tannins and coumarins has not been thoroughly investigated (Refaat *et al.,* 2013). The pharmacological activity of *C. macowanii* with regards to classes of other compounds besides alkaloids is lacking, and this lack of information hinders the conservation and transformation of the medicinal plant into a commodity of high value (Fennell and van Staden, 2001). Mugwagwa *et al.* (2015) reported

that extracts from *C. macowanii* could be used in the treatment of cognitive disorders such as Alzheimer's disease.

Table 2.5: Biological activities of alkaloids isolated from of *C. macowanii*

Biological activity	Compound	Plant part	Reference (s)
Antimalarial and antiparasitic	Krepowine	Bulbs	(Nair *et al*., 2013)
Treatment of Alzheimer's disease	Galanthamine	Bulbs	(Nair and van Staden, 2013)
Anticancer activity	Pancratistatin and narciclasine	Bulbs	(Nair and van Staden, 2013)
Antifungal activity	Vittatine and lycorine	Bulbs	(Nair and van Staden, 2013; Roy *et al*., 2018)
Antibacterial	Bulbispermine	Whole plant	(Nair *et al*., 2013)
Antiparasitic activity	Crinamine	Whole plant	(Nair and van Staden, 2013; Iannello *et al.*, 2014)
Anti-inflammatory	Haemanthidine	Bulbs	(Nair and van Staden, 2013)

2.7.6 *C. macowanii* ranking on the red list of South African plants

The Red List of South African plants is a scientific criterion used for assessing the risk of extinction of species. This lists plant taxa as either threatened, near threatened, data deficient or rare, based on the International Union for the Conservation of Nature (IUCN) (Hilton-Taylor, 1996; Raimondo, 2011). According to the red listed medicinal plants of South Africa, *C. macowanii* has been classified as of least concern, meaning it is at a low risk of extinction since it is distributed over a large area (Williams *et al*., 2016), however *Crinum* spp. are at risk of over-exploitation due to their over harvesting for medicinal purposes. The similarities of the different *Crinum* species make it difficult to distinguish one species from the other and therefore careful consideration has to be taken. Williams *et al*. (2013) reported that *Crinum macowanii* was declining due to its occurrence in muti markets across South Africa, prompting research into the conservation and sustainability of such medicinal plants.

2.7.7 Endophytes isolated from *C. macowanii*

Endophytes isolated from *Crinum* species such as *Crinum asiaticum* are *Alternaria sp, Alternaria tenuis, Chaetomium globosum* and *Chrysosporium tropicum*, which are all fungal endophytes (Anbukumaran *et al*., 2016). Morare *et al*. (2018) reported the isolation of bacterial endophytes from *C. macowanii* bulbs, namely, *Staphylococcus* species C2, *Staphylococcus species* C3, *Bacillus* species C4, *Acinetobacter* species C5 and *Staphylococcus* species C6. This leaves fungal

endophytes and bacterial endophytes from the leaves unexplored and therefore such investigations ought to be performed.

2.8 Metabolome and metabolomics of secondary metabolites

Metabolome is a collection of all the primary and secondary metabolites in plants, and its constituents influence the overall performance of the plant by performing physiological roles (Idle and Gonzalez, 2007; Tugizimana *et al*., 2013) and so the identification, quantification and measurement of the metabolites present is necessary. The qualitative and quantitative analysis of all metabolites present within a biological system under specific conditions yields the metabolomics (Aretz and Meierhofer, 2016; Duan *et al.*, 2018; Ellul *et al.*, 2019). The isolation and characterization of bioactive secondary metabolites help in distinguishing between new and already recognized bioactive secondary metabolites, which in turn assists in the development and discovery of new drug leads (Chikezie *et al*., 2015). Different techniques are used for the analysis of metabolomics, including metabolite profiling, metabolite fingerprinting and metabolite footprinting (Tugizimana *et al*., 2013).

2.8.1 Metabolite profiling, metabolite fingerprinting and metabolite footprinting

Metabolite profiling is done to identify detected metabolites from a large group of metabolites belonging to a specific metabolic pathway or a class of compounds, whereas metabolite fingerprinting detects intracellular metabolites obtained from crude samples or simple cellular extracts from spectra of total compositions, and the metabolites are neither quantified nor identified, while metabolite footprinting measures metabolites secreted intracellularly from an organism into its extracellular medium, and is mostly used in microbial metabolomics (Scholz *et al.*, 2004; Tugizimana *et al.*, 2013; Ncube *et al.*, 2016). These strategies used for metabolomic analysis help in the identification of vital compounds, and different analytical techniques are used to achieve this (Hounsome *et al*., 2008). Techniques such as nuclear magnetic resonance (NMR), liquid chromatography (LC) or gas chromatography (GC) coupled with mass spectrometry (MS) are used for metabolite profiling (Xie *et al.*, 2008; Chikezie *et al*., 2015).

2.8.1.1 Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS)

Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) is an analytical technique used to screen, detect and identify crude extracts of biological sample for the characterization of secondary metabolites (Zhu *et al.*, 2013; Jayakrishnan and Benjamin, 2016),

LC-Q-TOF-MS is able to quantify target metabolites and enables the screening of untargeted metabolites from available databases (Skibiński and Trawiński, 2017; Körver-Keularts *et al.*, 2018). LC-Q-TOF-MS can provide accurate molecular mass combined with tandem mass spectrometry (MS/MS) spectra UV spectra, retention time and main fragment information, and chemical compounds are arranged in peaks (Xie *et al.*, 2008; Stolker *et al*., 2004; Wang *et al.*, 2012; Tan *et al.*, 2018). Pang *et al*. (2016) and Wang *et al*. (2018) stated that the high sensitivity and high mass accuracy of LC-Q-TOF-MS assist in giving a detailed chemical profile of possibly unknown compounds in a crude extract, making it an ideal technique to use for the fast detection and identification of secondary metabolites in crude extracts, with potential in drug development and discovery (Senyuva and Gilbert, 2007; Xie *et al.*, 2008; He *et al.*, 2018).

2.9 Summary

From the literature reviewed so far, antibiotic resistance and cancer are affecting the human population, and result in large numbers of deaths globally. South Africa is implementing South Africa's National Development Plan 2030 (NDP) and the National Health Insurance (NHI), which will promote the prevention of cancer and antimicrobial resistance to ensure a healthy population. This will be achieved by research aimed at discovering and developing new drugs as indicated by the Department of Science and Technology DRAFT White Paper on Science, Technology and Innovation. It is evident that *C. macowanii* is widely used for its medicinal purposes to treat a variety of ailments and has the potential to contribute to the health and wellbeing of those who use it, as it possesses a wide array of chemical scaffolds (sometimes complex) known to possess efficacious bioactivities in *in vitro* experiments, and it harbours endophytes which could produce valuable secondary metabolites. The isolation and characterization of endophytes is important for further studies. It is vital to perform *in vitro* assays (minimum inhibitory concentration), through microdilution methods and cytotoxicity screening, to determine bioactive crude extracts from endophytes, to establish the biological properties of the crude endophytes extracts and assist in the development of anticancer and/or antibacterial drugs. Analytical techniques (metabolite profiling, footprinting and fingerprinting) can be used to identify and characterize bioactive metabolites. Chapters 3 to 5 describe the isolation of endophytes from *C. macowanii*, solvent extraction and concentration of crude endophyte extracts and their antibacterial and cytotoxicity evaluation, and metabolite profiling and fingerprinting of the bioactive extracts.

References

Abbas, Z., Rehman, S., 2018. An Overview of Cancer Treatment Modalities, in: Neoplasm, Hafiz Naveed Shahzad, IntechOpen. pp. 147–149. https://doi.org/http://dx.doi.org/10.5772/57353

Abdalla, M.A., Mcgaw, L.J., 2018. Bioprospecting of South African Plants as a Unique Resource for Bioactive Endophytic Microbes. Front. Pharmacol. 9, 1–18. https://doi.org/10.3389/fphar.2018.00456

Abdel-Azeem, A.M., Zaki, S.M., Khalil, W.F., Makhlouf, N.A., Farghaly, L.M., 2019. Anti-rheumatoid Activity of Secondary Metabolites Produced by Endophytic Chaetomium globosum, in: Singh, B.P., Rateb, M.E., Rodriguez-Couto, S., Lourdes, M. de, Moraes, T. de, Polizeli, Li, W.-J. (Eds.), Microbial Secondary Metabolites: Recent Developments and Technological Challenges. pp. 18–20. https://doi.org/10.3389/fmicb.2019.00914

Acosta, K., Pigni, N., Oleas, N., Bastida, J., 2014. Identification of the alkaloids of stenomesson aurantiacum (Kunth) herb an amaryllidaceae species from the ecuadorian andes. Pharmacologyonline 3, 178–183.

Adefolaju, T., 2011. of Health Research Enquiries : Enquirie s : Int. J. Heal. Res. 4, 99–106.

Adhikari, P., Pandey, A., Agnihotri, V., Pande, V., 2018. Selection of solvent and extraction method for determination of antimicrobial potential of Taxus wallichiana Zucc. Res. Pharm. 8, 1–9. https://doi.org/10.25081/rip.2018.v8.3487

African Union Centers for Disease Control and Prevention, 2018. Africa cdc framework for antimicrobial resistance, 2018-2023.

Afroz, S., Oliur Rahman, M., Hassan, M.A., 2018. Taxonomic revision of the genus Crinum L. (Liliaceae) of Bangladesh. Bangladesh J. Plant Taxon. 25, 257–271. https://doi.org/10.3329/bjpt.v25i2.39531

Ahmad, S., 2007. Introduction of Plant Constituents and their Tests. pharamacognosy 10, 1–40.

Ahmad, S., Garg, M., Ali, M., Singh, M., Athar, T., Ansari, S.H., 2009. A phyto-pharmacological overview on Adhatoda zeylanica Medic . syn . A . vasica (Linn .) Nees. Nat. Prod. Radiance 8, 549–554.

Aires, A., 2012. Phenolics in Foods: Extraction, Analysis and Measurements. Intech 72–75. https://doi.org/10.1016/j.colsurfa.2011.12.014

Akinsanya, A.M., Ting, S.A., Sanusi, Om., 2017. Extraction methods and TLC-bioautography for evaluation of antimicrobial activities of endophytic bacteria from medicinal plants. LASU J. Med. Sci. 2, 1–7.

Akinyemi, O., So, O., Ka, J., 2018. Medicinal plants and sustainable human health : a review. Hortic. Int. J. 2, 194–195. https://doi.org/10.15406/hij.2018.02.00051

Ali, J., Rafiq, Q.A., Ratcliffe, E., 2018. Antimicrobial resistance mechanisms and potential synthetic treatments. Futur. Sci. OA 4, 1–10. https://doi.org/10.4155/fsoa-2017-0109

Ali, S., Duan, J., Charles, T.C., Glick, B.R., 2014. A bioinformatics approach to the determination of genes involved in endophytic behavior in Burkholderia spp . J. Theor. Biol. 343, 193–198. https://doi.org/10.1016/j.jtbi.2013.10.007

Almoulah, N.F., Voynikov, Y., Gevrenova, R., Schohn, H., Tzanova, T., Yagi, S., Thomas, J., Mignard, B., Ahmed, A.A.A., Siddig, M.A. El, Spina, R., Laurain-mattar, D., 2017. South African Journal of Botany Antibacterial , antiproliferative and antioxidant activity of leaf extracts of selected Solanaceae species. South African J. Bot. 112, 368–374. https://doi.org/10.1016/j.sajb.2017.06.016

Anbukumaran, A., Ambikapathy, V., Panneerselvam, A., 2016. Isolation and characterization of endophytic fungi from Crinum asiaticum Lin Kodiyakarai , Tamilnadu , India. Der Pharm. Lett. 8, 269–275.

Andreassen, M., Lundt, I., 2006. A new chemical synthesis of Ascopyrone P from 1,5-anhydro-d-fructose. Carbohydr. Res. 341, 1692–1696. https://doi.org/10.1016/j.carres.2006.03.037

Andrews, J.M., 2001. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 1, 5–16.

Andryukov, B., Mikhailov, V., Besednova, N., 2019. The Biotechnological Potential of Secondary Metabolites from Marine Bacteria. J. Mar. Sci. Eng. 7, 1–16.

Aniszewski, T., 2007a. Definition, Typology and Occurrence of Alkaloids, in: Alkaloids – secrets of life alkaloid chemistry, biological significance, applications and ecological role. Elsevier, Amsterdam, The Netherlands, pp. 40–53.

Aniszewski, T., 2007b. Alkaloids – secrets of life alkaloid chemistry, biological significance, applications and ecological role.

Anjum, N., Chandra, R., 2015a. Endophytic bacteria : Optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J. Pharamaceutical Clin. Res. 8, 233–238.

Anjum, N., Chandra, R., 2015b. Endophytic bacteria : optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J. Pharamaceutical Clin. Res. 8, 233–238.

Antony, M.L., Lee, J., Hahm, E.R., Kim, S.H., Marcus, A.I., Kumari, V., Ji, X., Yang, Z., Vowell, C.L., Wipf, P., Uechi, G.T., Yates, N.A., Romero, G., Sarkar, S.N., Singh, S. V., 2014. Growth arrest by the antitumor steroidal lactone withaferin a in human breast cancer cells is associated with down-regulation and covalent binding at cysteine 303 of β-tubulin. J. Biol. Chem. 289, 1852–1865. https://doi.org/10.1074/jbc.M113.496844

Anuhya, G., Jyostna, V., Aswani, K.Y., Bodaiah, B., Sudhakar, P., 2017. Influence of physico-chemica parameters on secondary metabolite production by marine fungi. Int. J. Curr. Pharm. Res. 9, 112–118. https://doi.org/10.22159/ijcpr.2017v9i5.22152

Aretz, I., Meierhofer, D., 2016. Advantages and pitfalls of mass spectrometry based metabolome profiling in systems biology. Int. J. Mol. Sci. 17, 1–14. https://doi.org/10.3390/ijms17050632

Arora, J., Ramawat, K.G., 2017. Endophytes: Biology and Biotechnology. Endophytes Biol. Biotechnol. 15, 1–24. https://doi.org/10.1007/978-3-319-66541-2

Artun, F.T., Karagoz, A., Ozcan, G., Melikoglu, G., Anil, S., Kultur, S., Sutlupinar, N., 2016. In vitro anticancer and cytotoxic activities of some plant extracts on HeLa and Vero cell lines. J. Balk. Union Oncol. 21, 720–725.

Arunachalam, C., P.Gayathri, 2010. Studies on bioprospecting of endophytic bacteria from the medicinal plant of andrographis paniculata for their antimicrobial activity and antibiotic susceptibility pattern. Int. J. Curr. Pharm. Res. 2, 63–68.

Ashok, P.K., Upadhyaya, K., 2012. Tannins are Astringent. J. Pharmacogn. Phytochem. 1, 45–50.

Asif, H., Studholme, D.J., Khan, A., Aurongzeb, M., Khan, I.A., Kamran Azim, M., 2016. Comparative genomics of an endophytic Pseudomonas putida isolated from mango orchard. Genet. Mol. Biol. 39, 465–473.

Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., Nisar, M.A., Alvi, R.F., Aslam, M.A., Qamar, M.U., Salamat, M.K.F., Baloch, Z., 2018. Antibiotic resistance: a rundown of a global crisis. Infect. Drug Resist. 11, 1645–1658. https://doi.org/10.2147/IDR.S173867

Asmawi, M.Z., Arafat, O.M., Amirin, S., Eldeen, I.M., 2011. In vivo Antinociceptive Activity of leaf extract of crinum asiaticum and Phytochemical Analysis of the Bioactive Fractions. Int. J. Pharmacol.

Atzori, C., Bruno, A., Chichino, G., Bombardelli, E., Scaglia, M., Ghione, M., 1993. Activity of bilobalide, a sesquiterpene from Ginkgo biloba, on Pneumocystis carinii. Antimicrob. Agents Chemother. 37, 1492–1496. https://doi.org/10.1128/AAC.37.7.1492

Aziz, A., Raju, G.S., Das, A., Ahmed, J., Moghal, M.M.R., 2014. Evaluation of in vitro anthelmintic activity, total phenolic content and cytotoxic activity of crinum latifolium L. (Family: Amaryllidaceae). Adv. Pharm. Bull. 4, 15–19. https://doi.org/10.5681/apb.2014.003

Azwanida, N., 2015. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med. Aromat. Plants 4, 1–6. https://doi.org/10.4172/2167-0412.1000196

Baker, S., Satish, S., 2012. Endophytes : Natural Warehouse of Bioactive Compounds. Drug Invent. Today 4, 548–553.

Bastida, J., Berkov, S., Torras Clavería, L., Pigni, N.B., Andradre, J.P. De, Martínez, V., Codina Mahrer, C., Viladomat Meya, F., 2011. Chemical and biological aspects of Amaryllidaceae alkaloids, Recent Advances in Pharmaceutical Sciences.

Bastida, J., Lavilla, R., Francesc, V., 2006. Chemical and Biological Aspects of Narcissus Alkaloids. Alkaloids Chem. Biol. · 63, 89–92. https://doi.org/10.1016/S1099-4831(06)63003-4

Bedi, A., Adholeya, A., Deshmukh, S.K., 2017. Novel Anticancer Compounds from Endophytic Fungi. Curr. Biotechnol. 6, 1–18. https://doi.org/10.2174/2211550105666160622080 3

Begum, P., Noman, A. Al, Adib, M., Ahsan, M., Islam, S.N., 2017. Antibacterial Activity of Ethnic Vegetables Consumed by the Indigenous People Living in Chittagong Hill Tracts. European J. Med. Plants 18, 1–7. https://doi.org/10.9734/EJMP/2017/32059

Bengal, N., Raj, A.J., Biswakarma, S., Pala, N.A., Shukla, G., Kumar, M., Chakravarty, S., Bussmann, R.W., 2018. Indigenous uses of ethnomedicinal plants among forest-dependent communities of Northern Bengal, India. J. Ethnobiol. Ethnomed. 14, 1–28. https://doi.org/10.1186/s13002-018-0208-9

Bhandurge, P., Rajarajeshwari, N., Ganapaty, S., Pattanshetti, S., 2013. The Gnidia genus: A review. Asian J. Biomed. Pharm. Sci. 3, 1–31.

Bhawani, S.A., Sulaiman, O., Hashim, R., Mohamad, M.N., 2010. Thin-Layer Chromatographic Analysis of Steroids : A Review. Trop. J. Pharm. Res. 9, 301–313.

Bick, I.R.C., 1996. Alkaloids from Australian Flora, in: Pelletier, S.W. (Ed.), Alkaloids" Chemical and Biological Perspectives. Elsevier, pp. 94–116.

Bladt, T.T., Frisvad, J.C., Knudsen, P.B., Larsen, T.O., 2013. Anticancer and Antifungal Compounds from Aspergillus, Penicillium and Other Filamentous Fungi. Molecules 18, 11338–11376. https://doi.org/10.3390/molecules180911338

Blaženovi, I., Kind, T., Ji, J., Fiehn, O., 2018. Software Tools and Approaches for Compound Identification of LC-MS / MS Data in Metabolomics. Metabolites 8, 1–23. https://doi.org/10.3390/metabo8020031

Bloom, D.E., Black, S., Salisbury, D., Rappuoli, R., 2018. Antimicrobial resistance and the role of vaccines. Proc. Natl. Acad. Sci. 115, 12868–12871. https://doi.org/10.1073/pnas.1717157115

Blumenthal, M., 2009. African Natural Plant Products : A Foreword to the Science and Challenges, in: Juliani, H.R., Simon, J., Ho, C.-T. (Eds.), African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality. ACS Pulbication series, Rutgers, The State University of New Jersey, pp. 157–166.

Boopathy, N.S., Kathiresan, K., 2010. Anticancer Drugs from Marine Flora : An Overview. J. ofOncology 2010, 1–18. https://doi.org/10.1155/2010/214186

Botanical Society of South Africa, 2007. The stately Crinum lilies. wildflower Conserv. 26.

Bozkurt, B., Emir, A., Kaya, G.İ., Önür, M.A., Berkov, S., Bastida, J., Ünver Somer, N., 2017. Alkaloid profiling of galanthus woronowii losinsk. By GC-MS and evaluation of its biological activity. Marmara Pharm. J. 21, 915–920. https://doi.org/10.12991/mpj.2017.12

Brader, G., Stephane, C., Birgit, M., Mitter, B., Trognitz, F., Sessitsch, A., 2014. Metabolic potential of endophytic bacteria. Curr. Opin. Biotechnol. 30–37. https://doi.org/10.1016/j.copbio.2013.09.012

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global Cancer Statistics 2018 : Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA. Cancer J. Clin. 98, 394–424. https://doi.org/10.3322/caac.21492

Brueton, V.J., 2013. Trade in commonly used medicinal bulbs: value and ecological implications. University of the Witwatersrand.

Brusotti, G., Cesari, I., Dentamaro, A., Caccialanza, G., Massolini, G., 2014. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. J. Pharm. Biomed. Anal. 87, 218–228. https://doi.org/10.1016/j.jpba.2013.03.007

Cain, C.C., Lee, D., Iii, R.H.W., Henry, A.T., Jr, E.J.C., Wani, M.C., Wall, M.E., Oberlies, N.H., Iii, J.O.F., 2003. Synergistic Antimicrobial Activity of Metabolites Produced by a Nonobligate Bacterial Predator. Antimicrob. Agents Chemother. 47, 2113–2117. https://doi.org/10.1128/AAC.47.7.2113

Calvo, A.M., Wilson, R.A., Bok, J.W., Keller, N.P., 2002. Relationship between Secondary Metabolism and Fungal Development. Microbiol. Mol. Biol. Rev. 66, 447–459. https://doi.org/10.1128/MMBR.66.3.447

Cancer Council Australia, 2018. Understanding Lung Cancer A guide for people with cancer, their families and friends.

Cao, Z., Yang, P., Zhou, Q., 2013. Multiple biological functions and pharmacological effects of lycorine. Sci. China Chem. 56, 1382–1391. https://doi.org/10.1007/s11426-013-4967-9

Cardoso, J.C., de Oliveira, M.E.B.S., Cardoso, F. de C.I., 2019. Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. Hortic. Bras. 37, 124–132. https://doi.org/10.1590/s0102-053620190201

Changwa, R., Abia, W., Msagati, T., Nyoni, H., Ndleve, K., Njobeh, P., 2018. Multi-Mycotoxin Occurrence in Dairy Cattle Feeds from the Gauteng Province of South Africa : A Pilot Study Using UHPLC-QTOF-MS / MS. Toxicon 10, 1–21. https://doi.org/10.3390/toxins10070294

Cheesman, L., Nair, J.J., van Staden, J., 2012. Antibacterial activity of crinane alkaloids from Boophone disticha (Amaryllidaceae). J. Ethnopharmacol. 140, 405–408. https://doi.org/10.1016/j.jep.2012.01.037

Chen, S.L., Yu, H., Luo, H.M., Wu, Q., Li, C.F., Steinmetz, A., 2016. Conservation and sustainable use of medicinal plants : problems , progress , and prospects. Chin. Med. 11, 1–10. https://doi.org/10.1186/s13020-016-0108-7

Chen, Y., Yuan, Q., Shan, L., Lin, M., Cheng, D., Li, C., 2013. Antitumor activity of bacterial exopolysaccharides from the endophyte Bacillus amyloliquefaciens sp . isolated from Ophiopogon japonicus 1787–1792. https://doi.org/10.3892/ol.2013.1284

Chikezie, P.., Ibegbulem, C.., Mbagwu, F.., 2015. Medicinal & Aromatic Plants Medicinal Potentials and Toxicity Concerns of Bioactive Principles. Med. Aromat. Plants 4, 1–15. https://doi.org/10.4172/2167-0412.1000202

Chrapusta, E., Kaminski, A., Duchnik, K., Bober, B., Adamski, M., Bialczyk, J., 2017. Mycosporine-Like Amino Acids: Potential health and beauty ingredients. Mar. Drugs 15, 1–29. https://doi.org/10.3390/md15100326

Chutulo, E.C., Chalannavar, R.K., 2018. Endophytic Mycoflora and Their Bioactive Compounds from Azadirachta Indica :A Comprehensive Review. J. Funct. Foods 4, 1–12. https://doi.org/10.3390/jof4020042

Çitoğlu, G.S., Acıkara, Ö.B., 2012. Column Chromatography for Terpenoids and Flavonoids, in:

Dhanarasu, S. (Ed.), Chromatography and its applications. InTech, Rijeka, Croatia, pp. 13–52.

Cos, P., Vlietinck, A.J., Berghe, D. Vanden, Maes, L., 2006. Anti-infective potential of natural products : How to develop a stronger in vitro ‘ proof-of-concept .’ J. Ethnopharmacol. 106, 290–302. https://doi.org/10.1016/j.jep.2006.04.003

Coussens, N.P., Braisted, J.C., Peryea, T., Sittampalam, G.S., Simeonov, A., Hall, M.D., 2017. Small-Molecule Screens : A Gateway to Cancer Therapeutic Agents with Case Studies of Food and Drug Administration – Approved Drugs. Pharmacol. Rev. 479–496.

Crouch, N.R., Pohl, T.L., Mulholland, D.A., Ndlovu, E., 2005. Alkaloids from three ethnomedicinal Haemanthus species: H. albiflos, H. deformis and H. pauculifolius (Amaryllidaceae). South African J. Bot. 71, 49–52. https://doi.org/10.1016/S0254-6299(15)30148-4

da Costa Souza, P.N., Grigoletto, T.L.B., de Moraes, L.A.B., Abreu, L.M., Guimarães, L.H.S., Santos, C., Galvão, L.R., Cardoso, P.G., 2016. Production and chemical characterization of pigments in filamentous fungi. Microbiol. (United Kingdom) 162, 12–22. https://doi.org/10.1099/mic.0.000168

Dar, R.A., Shahnawaz, M., Qazi, P.H., 2017. General overview of medicinal plants : A review. J. Phytopharm. 6, 349–351.

Davies, J., 2007. In the beginning there was streptomycin, in: arya, d.p. (ed.), aminoglycoside antibiotics From Chemical Biology to Drug Discovery. John Wiley & Sons, Inc, Hoboken, New Jersey., pp. 1–9.

Davies, J., Davies, D., 2010. Origins and Evolution of Antibiotic Resistance. Microbiol. Mol. Biol. Rev. 74, 417–433. https://doi.org/10.1128/MMBR.00016-10

Davin-Regli, A., Pages, J.-M., 2015. Enterobacter aerogenes and Enterobacter cloacae ; Versatile bacterial pathogens confronting antibiotic treatment. Front. Microbiol. 6, 1–10. https://doi.org/10.3389/fmicb.2015.00392

De Andrade, J.P., Pigni, N.B., Torras-Claveria, L., Guo, Y., Berkov, S., Reyes-Chilpa, R., El Amrani, A., Zuanazzi, J.A.S., Codina, C., Viladomat, F., Bastida, J., 2012. Alkaloids from the Hippeastrum genus: Chemistry and biological activity. Rev. Latinoam. Quim. 40, 83–98.

De Mot, R., Ghequire, M.G.K., 2014. Ribosomally encoded antibacterial proteins and peptides from Pseudomonas. Fed. Eur. Microbiol. Soc. 38, 523–568. https://doi.org/10.1111/1574-6976.12079

Dela Cruz, C.S., Tanoue, L.T., Matthay, R.A., 2011. Lung Cancer: Epidemiology, Etiology, and Prevention. Clin. Chest Med. 32, 605–644. https://doi.org/10.1016/j.ccm.2011.09.001

Delcour, A.H., 2009. NIH Public Access. Biochim. Biophys. Acta 1794, 808–816. https://doi.org/10.1016/j.bbapap.2008.11.005.Outer

Demain, A.L., Fang, A., 2000. The Natural Functions of Secondary Metabolites, in: Scheper, T. (Ed.), Advances in Biochemical Engineering / Biotechnology. Springer, pp. 1–33. https://doi.org/10.1016/j.jbiotec.2007.09.006

Denicolaï, E., Baeza-kallee, N., Tchoghandjian, A., Carré, M., Colin, C., Jiglaire, C.J., Mercurio, S., Beclin, C., Figarella-branger, D., 2014. Proscillaridin A is cytotoxic for glioblastoma cell lines and controls tumor xenograft growth in vivo. Oncotarget 5, 10934–10948.

Department of Agriculture Forestry and Fisheries Republic of South Africa, 2013. Medicinal plants of South Africa. Pretoria, South Africa.

Department of Science and Technology, S.A., 2013. The Bio-economy Strategy is an initiative of the Department of Science and Technology, South Africa.

Devi, P., Rodrigues, C., Naik, C.G., 2012. Isolation and Characterization of Antibacterial Compound from a Mangrove-Endophytic Fungus , Penicillium chrysogenum MTCC 5108. Indian J. Microbiol. 52, 617–623. https://doi.org/10.1007/s12088-012-0277-8

Dias, D. a., Urban, S., Roessner, U., 2012. A Historical Overview of Natural Products in Drug Discovery. Metabolites 2, 303–336. https://doi.org/10.3390/metabo2020303

Doty, S.L., 2017. Functional Importance of the Plant Endophytic Microbiome : Implications for Agriculture , Forestry , and Bioenergy, in: Doty, S.L. (Ed.), Functional Importance of the Plant Microbiome. Springer International Publishing, Cham, Switzerland, pp. 1–5. https://doi.org/10.1007/978-3-319-65897-1

Doughari, J.H., 2012. Phytochemicals : Extraction Methods , Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, in: Phytochemicals - A Global Perspective of Their Role in Nutrition and Health. pp. 1–33.

Duan, L., Guo, L., Wang, L., Yin, Q., Zhang, C.M., Zheng, Y.G., Liu, E.H., 2018. Application of metabolomics in toxicity evaluation of traditional Chinese medicines. Chin. Med. 13, 1–14. https://doi.org/10.1186/s13020-018-0218-5

Dwivedi, G.R., Sisodia, B.S., Shikha, 2019. Secondary Metabolites: Metabolomics for Secondary Metabolites, in: Gupta, V.K., Pandey, A. (Eds.), New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier, Amsterdam, Netherlands, pp. 337–339.

https://doi.org/10.1016/b978-0-444-63504-4.00022-0

Dzotam, J.K., Simo, I.K., Bitchagno, G., Celik, I., Sandjo, L.P., Tane, P., Kuete, V., 2018. In vitro antibacterial and antibiotic modifying activity of crude extract, fractions and 3',4',7-trihydroxyflavone from Myristica fragrans Houtt against MDR Gram-negative enteric bacteria. BMC Complement. Altern. Med. 18, 1–9. https://doi.org/10.1186/s12906-018-2084-1

Egan, J.M., Kaur, A., Raja, H.A., Kellogg, J.J., Oberlies, N.H., Cech, N.B., 2016. Antimicrobial fungal endophytes from the botanical medicine goldenseal (Hydrastis canadensis). Phytochem. Lett. 17, 219–225. https://doi.org/10.1016/j.phytol.2016.07.031

Ek-Ramos, M.J., Gomez-Flores, R., Orozco-Flores, A.A., Rodríguez-Padilla, C., González-Ochoa, G., Tamez-Guerra, P., 2019. Bioactive Products From Plant-Endophytic Gram-Positive Bacteria. Front. Microbiol. 10, 1–12. https://doi.org/10.3389/fmicb.2019.00463

El-Desouky, S.K., Gamal-Eldeen, A.M., 2009. Cytotoxic and anti-inflammatory activities of some constituents from the floral buds of Syringa patula. Pharm. Biol. 47, 872–877. https://doi.org/10.1080/13880200902950785

El-Gendy, M.M.A., Hawas, U.W., Jaspars, M., 2008. Novel bioactive metabolites from a marine derived bacterium Nocardia sp. ALAA 2000. J. Antibiot. (Tokyo). 61, 379–386. https://doi.org/10.1038/ja.2008.53

El-Hendawy, H.., Osman, M.., Sorour, N.., 2003. Characterization of Two Antagonistic Strains of Rahnella aquatilis Isolated from Soil in Egypt. Folia Microbiol. 48, 799–804. https://doi.org/10.1007/BF02931517

Elgorashi, E.E., 2000. Alkaloids from three south african Crinum species. University of Natal Pietermaritzburg.

Elgorashi, Esameldin E., Drewes, S.E., Morris, C., Van Staden, J., 2003. Variation among three Crinum species in alkaloid content. Biochem. Syst. Ecol. 31, 601–615. https://doi.org/10.1016/S0305-1978(02)00222-3

Elgorashi, E.E., Drewes, S.E., van Staden, J., 2002. Organ-to-organ and seasonal variation in alkaloids from Crinum macowanii. Fitoterapia 73, 490–495. https://doi.org/10.1016/S0367-326X(02)00164-8

Elgorashi, E E, Zschocke, S., Staden, J. Van, 2003. The anti-inflammatory and antibacterial activities of Amaryllidaceae alkaloids. South African J. Bot. 69, 448–449. https://doi.org/10.1016/S0254-6299(15)30329-X

Eljounaidi, K., Kyu, S., Bae, H., 2016. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases – Review and future prospects. Biol. Control 103, 62–68. https://doi.org/10.1016/j.biocontrol.2016.07.013

Ellul, S., Wake, M., Clifford, S.A., Lange, K., Würtz, P., Juonala, M., Dwyer, T., Carlin, J.B., Burgner, D.P., Saffery, R., 2019. Body composition: population epidemiology and concordance in Australian children aged 11–12 years and their parents. BMJ Open 9, 106–117. https://doi.org/10.1136/bmjopen-2018-023698

Erasto, P., Grierson, D.S., Afolayan, A.J., 2006. Bioactive sesquiterpene lactones from the leaves of Vernonia amygdalina. J. Ethnopharmacol. 106, 117–120. https://doi.org/10.1016/j.jep.2005.12.016

Fabricant, D.S., Farnsworth, N.R., 2001. The Value of Plants Used in Traditional Medicine for Drug Discovery. Environ. Health Perspect. 109, 69–75.

Fan, C., Zheng, W., Fu, X., Li, X., Wong, Y., Chen, T., 2014. Enhancement of auranofin-induced lung cancer cell apoptosis by selenocystine , a natural inhibitor of TrxR1 in vitro and in vivo. Nat. Publ. Gr. 5, 2–11. https://doi.org/10.1038/cddis.2014.132

Farias, K.J.S., Machado, P.R.L., de Almeida Júnior, R.F., Lopes da Fonseca, B.A., 2019. Brefeldin A and Cytochalasin B reduce dengue virus replication in cell cultures but do not protect mice against viral challenge. Access Microbiol. 1, 1–8. https://doi.org/10.1099/acmi.0.000041

Fathalla, A.M., Samy, A.M.A.E.-A., Metwaly, A.B., Ramadan, E.M., 2015. Characterization of Antifungal Metabolites from Antagonistic Fluorescent Pseudomonads. J. Appl. Plant Prot. 4, 13–21. https://doi.org/10.21608/japp.2015.7593

Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., Kadri, A., 2017. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. Food Sci. Technol. 37, 483–492. https://doi.org/10.1590/1678-457x.23516

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicology. J. Ethnopharmacol. 94, 205–217. https://doi.org/10.1016/j.jep.2004.05.012

Fennell, C.W., van Staden, J., 2001. Crinum species in traditional and modern medicine. J. Ethnopharmacol. 78, 15–26.

Ferdous, U.., Shishir, M.., Khan, S.., Hoq, M.., 2018. Bacillus spp .: Attractive Sources of Anti-

cancer and Anti-proliferative Biomolecules. Microb. Bioact. 1, 033–045. https://doi.org/10.25163/microbbioacts.11005B0408130818

Fiołka, M.J., Grzywnowicz, K., Rzymowska, J., Lewtak, K., Szewczyk, R., Mendyk, E., Keller, R., 2015. Antitumour and apoptotic effects of a novel Tris-peptide complex obtained after isolation of Raoultella ornithinolytica extracellular metabolites. J. Appl. Microbiol. 118, 1357–1369. https://doi.org/10.1111/jam.12806

Fiolka, M.J., Lewtak, K., Rzymowska, J., Grzywnowicz, K., Hulas-Stasiak, M., Sofińska-Chmiel, W., Skrzypiec, K., 2013. Antifungal and anticancer effects of a polysaccharide-protein complex from the gut bacterium Raoultella ornithinolytica isolated from the earthworm Dendrobaena veneta. Pathog. Dis. 69, 49–61. https://doi.org/10.1111/2049-632X.12056

Fomum, S.W., Nsahlai, I. V., 2017. In vitro nematicidal activity of plant species possessing alkaloids and tannins. Cogent Food Agric. 3, 1–12. https://doi.org/10.1080/23311932.2017.1334295

Fouda, A.H., Hassan, S.E., Eid, A.M., Ewais, E.E., 2015. Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss .). Ann. Agric. Sci. 60, 95–104. https://doi.org/10.1016/j.aoas.2015.04.001

Garcia-Bustos, J.F., Pezzi, N., Mendez, E., 1985. Structure and Mode of Action of Microcin 7 , an Antibacterial Peptide Produced by Escherichia coli. Antimicrob. AGENTS Chemother. 27, 791–797.

Gavhane, Y., Shete, A., Bhagat, A., Shinde, V., Bhong, K., Khairnar, G., Yadav, A., 2011. Solid Tumors : Facts, Challenges and Solutions. Int. J. Pharma Sci. Res. 2, 1–12.

Ghisalberti, E.L., Pennacchio, M., Alexander, E., 1998. Survey of secondary plant metabolites with cardiovascular activity. Pharm. Biol. 36, 237–279. https://doi.org/10.1076/phbi.36.4.237.4583

Ghosal, S., Saini, K.S., Razdan, S., 1985. Crinum alkaloids: their chemistry and biology. Phytochemistry 24, 2141–2156.

Glushakova, A.M., Kachalkin, A. V, 2017. Endophytic Yeasts in Malus domestica and Pyrus communis Fruits under Anthropogenic Impact. Microbiology 86, 128–135. https://doi.org/10.1134/S0026261716060102

Gouda, S., Das, G., Sen, S.K., Shin, H., Patra, J.K., 2016. Endophytes : A Treasure House of Bioactive Compounds of Medicinal Importance. Front. Microbiol. 7, 1–8. https://doi.org/10.3389/fmicb.2016.01538

Grech, A., Baldacchino, A., Tufignio, M., 2009. Secondary Metabolites and their Exploitation in Medicine. Maltese Fam. Dr. 18, 8–13.

Greenwell, M., Rahman, P.K.S.M., 2015. Europe PMC Funders Group Medicinal Plants : Their Use in Anticancer Treatment. Int. J. Pharm. Sci. Res. 6, 4103–4112. https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12.Medicinal

Griffiths, S., van Dyk, S., Malan, S.F., van Zyl, R.L., 2004. Antimalarial Compounds From Crinum bulbispermum. North West University.

Gross, H., Loper, J.E., 2009. Genomics of secondary metabolite production by Pseudomonas spp Genomics of secondary metabolite production by Pseudomonas spp †. Nat. Prod. Rep. 26, 1408–1446. https://doi.org/10.1039/b817075b

Guerriero, G., Berni, R., Muñoz-Sanchez, J.A., Apone, F., Abdel-Salam, E.M., Qahtan, A.A., Alatar, A.A., Cantini, C., Cai, G., Hausman, J.F., Siddiqui, K.S., Hernández-Sotomayor, S.M.T., Faisal, M., 2018. Production of plant secondary metabolites: Examples, tips and suggestions for biotechnologists. Genes (Basel). 9, 1–22. https://doi.org/10.3390/genes9060309

Gunatilaka, A.A.L., 2006. Natural Products from Plant-associated Microorganisms: Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence. Journak Nat. Prod. 69, 509–526. https://doi.org/10.1021/np058128n.Natural

Guo, Y., 2015. Research on the Alkaloids of Amaryllidaceae Plants : Genera Lycoris and Hippeastrum. Universitat de Barcelona.

Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol. Aspects Med. 27, 1–93. https://doi.org/10.1016/j.mam.2005.07.008

Gutierrez-Orozco, F., Failla, M.L., 2013. Biological Activities and Bioavailability of Mangosteen Xanthones: A Critical Review of the Current Evidence. Nutr. 2013, 5, 3163–3183. https://doi.org/10.3390/nu5083163

Handayani, D., Rivai, H., Mulyana, R., Suharti, N., Rasyid, R., Hertiani, T., 2018. Antimicrobial and Cytotoxic Activities of Endophytic Fungi Isolated from Antimicrobial and Cytotoxic Activities of Endophytic Fungi Isolated from Mangrove Plant Sonneratia alba Sm. J Appl Pharm Sci 8, 49–53. https://doi.org/10.7324/JAPS.2018.8207

Hannah, M.A.C., Krishnakumari, S., 2015. Quantitative estimation of plant metabolites in the hot aqueous seed extract of watermelon (Citrullus vulgaris Schrad .). J. Med. Plants Stud. 3, 107–111.

Hartmann, C., Wesseling, P., 2019. Glioblastoma: Pathology and Genetics, in: Boffetta, P., Hainaut, P. (Eds.), Encyclopedia of Cancer. Elsevier, pp. 165–178. https://doi.org/10.1016/b978-0-12-801238-3.65070-5

He, M., Qu, C., Gao, O., Hu, X., Hong, X., 2015a. Biological and pharmacological activities of amaryllidaceae alkaloids. RSC Adv. 5, 16562–16574. https://doi.org/10.1039/c4ra14666b

He, M., Qu, C., Gao, O., Hu, X., Hong, X., 2015b. Biological and pharmacological activities of amaryllidaceae alkaloids. RSC Adv. 5, 16562–16574. https://doi.org/10.1039/c4ra14666b

He, Y., Cheng, P., Wang, W., Yan, S., Tang, Q., Liu, D., Xie, H., 2018. Rapid Investigation and Screening of Bioactive Components in Simo Decoction via LC-Q-TOF-MS and UF-HPLC-MD Methods. Molecules 23, 1–18. https://doi.org/10.3390/molecules23071792

Hemmati, S., Seradj, H., 2016. Justicidin B: A promising bioactive lignan. Molecules 21, 1–20. https://doi.org/10.3390/molecules21070820

Hilton-Taylor, C., 1996. Red data list of southern African plants. 1. corrections and additions. Bothalia 26, 177–182. https://doi.org/10.4102/abc.v26i2.705

Ho, Y.-N., Huanga, C.-C., 2015. Draft Genome Sequence of Burkholderia cenocepacia Strain 869T2, a Plant-Beneficial Endophytic Bacterium. Genome Announc. 3, 1–2. https://doi.org/10.1128/genomeA.01327-15

Hoffmann, D., 1951. Hoffmann_David_-_Medical_herbalism.pdf.

Hoffmann, T., Krug, D., Huttel, S., Muller, R., 2014. Improving Natural Products Identification through Targeted LC-MS/ MS in an Untargeted Secondary Metabolomics Work flow. Anal. Chem. 86, 10780−10788. https://doi.org/10.1021/ac502805w

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., Armand, R., 2015. The Application of Medicinal Plants in Traditional and Modern Medicine : A Review of Thymus vulgaris. Int. J. Clin. Med. 6, 635–642.

Hounsome, N., Hounsome, B., Tomos, D., Edwards-Jones, G., 2008. Plant Metabolites and Nutritional Quality of Vegetables. J. Food Sci. 73, 48–65. https://doi.org/10.1111/j.1750-3841.2008.00716.x

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.., 1996. Zulu medicinal plants: an inventory. University of Natal Press, Pietermaritzburg., Pietermaritzburg.

Hutf, A.J., Grady, J.O., 1996. Review Drug chirality : a consideration of the significance of the stereochemistry of antimicrobial agents. J. Antimicrob. Chemother. 37, 7–32.

Iannello, C., Bastida, J., Bonvicini, F., Antognoni, F., Gentilomi, G.A., Poli, F., 2014. Chemical

composition, and in vitro antibacterial and antifungal activity of an alkaloid extract from Crinum angustum Steud. Nat. Prod. Res. https://doi.org/10.1080/14786419.2013.877903

Iannello, D. ss. C., 2014. Pharmacological screening and biotechnological production of alkaloids from tissues and cells cultured by plants of the amaryllidaceae family. alma mater studiorum - università di bologna dottorato.

Ibraheem, O., Maimako, R.., 2014. Evaluation of Alkaloids and Cardiac Glycosides Contents of Ricinus communis Linn. (Castor) Whole Plant Parts and Determination of their Biological Properties. Int. J. Toxicol. Pharmacol. Res. 6, 34–42.

Ibrahim, M., Kaushik, N., Sowemimo, A., Chhipa, H., Koekemoer, T., Van De Venter, M., Odukoya, O.A., 2017. Antifungal and antiproliferative activities of endophytic fungi isolated from the leaves of markhamia tomentosa. Pharm. Biol. 55, 590–595. https://doi.org/10.1080/13880209.2016.1263671

Idikio, H.A., 2011. Human cancer classification: A systems biology-based model integrating morphology, cancer stem cells, proteomics, and genomics. J. Cancer 2, 107–115. https://doi.org/10.7150/jca.2.107

Idle, J.R., Gonzalez, F.J., 2007. Metabolomics. Cell Metab. 6, 348–352. https://doi.org/10.1016/j.cmet.2007.10.005

Ionkova, I., 2007. Biotechnological Approaches for the Production of Lignans. Pharamacognosy Reviwes 1, 57–68.

Ismaiel, A.A., Ahmed, A.S., Hassan, I.A., El-Sayed, E.-S.R., El-Din, A.-Z.A.K., 2017. Production of paclitaxel with anticancer activity by two local fungal endophytes , Aspergillus fumigatus and Alternaria tenuissima. Appl. Microbiol. Biotechnol. 101, 5831–5846. https://doi.org/10.1007/s00253-017-8354-x

Jain, A., Choubev, S., Singour, P.K., Rajak, H., Pawar, R.S., 2011. Sida cordifolia (Linn) - An overview. J. Appl. Pharm. Sci. 1, 23–31.

Jain, C., Khatana, S., Vijayvergia, R., 2019. Bioactivity of secondary metabolites of various plants: A review. Int. J. Pharm. Sci. Res. 10, 494–504. https://doi.org/10.13040/IJPSR.0975-8232.10(2).494-04

Jain, P., Pundir, R.K., 2017. Potential Role of Endophytes in Sustainable Agriculture-Recent Developments and Future Prospects, in: Sustainable Development and Biodiversity Endophytes: Biology and Biotechnology. pp. 153–154.

Jalgaonwala, R.E., Mahajan, R.T., 2011. Evaluation of hydrolytic enzyme activities of endophytes

from some indigenous medicinal plants. J. Agric. Technol. 2011 7, 1733–1741.

James, J.T., Dubery, I.A., 2009. Pentacyclic Triterpenoids from the Medicinal Herb, Centella asiatica (L.) Urban. Molecules 14, 3922–3941. https://doi.org/10.3390/molecules14103922

Jasim, B., Joseph, A.A., John, C.J., Mathew, J., Radhakrishnan, E.K., 2014. Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of Zingiber officinale. 3 Biotech 4, 197–204. https://doi.org/10.1007/s13205-013-0143-3

Jayakrishnan, T., Benjamin, S., 2016. Purpureocillium lilacinum Strain BP13 Produces Flavoglaucin. Electron. J. Biol. 12, 472–476.

Jayaprasad, B., Sharavanan, P., 2015. Phytochemical, In Vitro Antioxidant and Antimicrobial Activities in Aqueous and Methanol Extracts Of Chloroxylon Swietenia Bark. Res. J. Pharm. Biol. Chem. Sci. 6, 1416–1424.

Jazieh, A.R., Al-Saggabi, A.H., McClung, M., Carlson, R., Schnipper, L.E., Eniu, A., Blauvelt, B., Zafar, Y., Kerr, D., 2018. Facing the Global Challenges of Access to Cancer Medication. J. Glob. Oncol. 4, 1–7. https://doi.org/10.1200/jgo.17.00205

Jia, M., Chen, L., Xin, H., Zheng, C., Rahman, K., Han, T., Qin, L.-P., 2016. A Friendly Relationship between Endophytic Fungi and Medicinal Plants : A Systematic Review. Front. Microbiol. 7, 1–14. https://doi.org/10.3389/fmicb.2016.00906

Jiang, B., 2017. ScienceDirect Aerobic glycolysis and high level of lactate in cancer metabolism and microenvironment. Genes Dis. 4, 25–27. https://doi.org/10.1016/j.gendis.2017.02.003

Jin, Z., Yao, G., 2019. Natural Product Reports Amaryllidaceae and Sceletium alkaloids. Nat. Prod. Rep. 1–27. https://doi.org/10.1039/c8np00055g

Joshi, S.., Bhadauria, S.., Jadon, G., Diwaker, A.., 2012. Introduction to Neoplasm : "Tumor Classification" A ReviewArticle. Int. J. Adv. Res. Pharm. Bio Sci. 2, 227–263.

Joshi, S.R., Nongkhlaw, F.M.W., 2015. L-Asparaginase and antioxidant activity of endophytic bacteria associated with ethnomedicinal plants. Indian J. Biotechnol. 14, 59–64.

Jouda, J., Kusari, S., Lamshöft, M., Mouafo, F., Douala, C., Wandji, J., Spiteller, M., 2014. Fitoterapia Penialidins A – C with strong antibacterial activities from Penicillium sp ., an endophytic fungus harboring leaves of Garcinia nobilis. Fitoterapia 98, 209–214. https://doi.org/10.1016/j.fitote.2014.08.011

Kabera, J.N., Semana, E., Mussa, A.R., He, X., 2014a. Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. J. Pharm. Pharmacol. 2, 377–392. https://doi.org/10.1016/0300-9084(96)82199-7

Kabera, J.N., Semana, E., Mussa, A.R., He Xin, 2014b. Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. J. Pharm. Pharmacol. 2, 377–392.

Kadhim, M.J., Sosa, A.A., Hameed, I.H., 2016. Evaluation of anti-bacterial activity and bioactive chemical analysis of Ocimum basilicum using Fourier transform infrared (FT-IR) and gas chromatography- mass spectrometry (GC-MS) techniques. J. Pharmacogn. Phyther. 8, 127–146. https://doi.org/10.5897/JPP2015.0366

Kamal, S.A., Hamza, L.F., Hameed, I.H., 2015. Antibacterial activity of secondary metabolites isolated from Alternaria alternata. African J. Biotechnol. 14, 2972–2994. https://doi.org/10.5897/AJB2015.14906

Kamboj, A., Rathour, A., Kaur, M., 2013. Bufadienolides and their medicinal utility: a review. Int. J. Pharm. Pharm. Sci. 5, 20–27.

Kandel, S.L., Joubert, P.M., Doty, S.L., 2017. Bacterial Endophyte Colonization and Distribution within Plants. Microorganisms 5, 1–26. https://doi.org/10.3390/microorganisms5040077

Kapadia, G.J., Shukla, Y.N., Basak, S.P., Sokolosk, E.A., Fales, H.M., 1980. The melosatins-a novel class of alkaloids from melochza tomentosa. Tetrahedron 36, 2441–2447.

Katoch, D., Kumar, D., Sharma, U., Kumar, N., Padwad, Y.S., Lal, B., Singh, B., 2013. Zephgrabetaine: A New Betaine-type Amaryllidaceae Alkaloid from Zephyranthes grandiflora. Nat. Prod. Commun. 8, 161–164. https://doi.org/10.1177/1934578X1300800206

Kaul, S., Gupta, S., Ahmed, M., Dhar, M.K., 2012. Endophytic fungi from medicinal plants : a treasure hunt for bioactive metabolites. Phytochem. Lett. 1–19. https://doi.org/10.1007/s11101-012-9260-6

Kaya, G.I., Uzun, K., Bozkurt, B., Onur, M.A., Somer, N.U., Glatzel, D., Fürst, R., 2017. Chemical characterization and biological activity of an endemic Amaryllidaceae species: Galanthus cilicicus. South African J. Bot. 108, 256–260. https://doi.org/10.1016/j.sajb.2016.11.008

Khalifa, M.F., Attia, E.Z., Fahim, J.R., Kamel, M.S., 2018. An overview on the chemical and biological aspects of lycorine alkaloid. J. Adv. Biomed. Pharm. Sci. 1, 41–49.

Khan, A.L., Gilani, S.A., Waqas, M., Al-hosni, K., Al-khiziri, S., Kim, Y., Ali, L., Kang, S., Asaf, S., Shahzad, R., Hussain, J., Lee, I., Al-harrasi, A., 2017. Endophytes from medicinal plants and their potential for producing indole acetic acid , improving seed germination and mitigating oxidative stress *#. J. Zhejiang Univ. B (Biomedicine Biotechnol. 18, 125–137.

Khare, E., Mishra, J., Arora, N.K., 2018. Multifaceted interactions between endophytes and plant:

Developments and Prospects. Front. Microbiol. 9, 1–12. https://doi.org/10.3389/fmicb.2018.02732

Kharwar, R.N., Mishra, A., Gond, S.K., Stierle, A., Stierle, D., 2011. Anticancer compounds derived from fungal endophytes : their importance andfuture challenges. Nat. Prod. Rep. 28, 1208–1228. https://doi.org/10.1039/c1np00008j

Khazaei, S., Abdul Hamid, R., Ramachandran, V., Mohd Esa, N., Pandurangan, A.K., Danazadeh, F., Ismail, P., 2017. Cytotoxicity and Proapoptotic Effects of Allium atroviolaceum Flower Extract by Modulating Cell Cycle Arrest and Caspase-Dependent and p53 -Independent Pathway in Breast Cancer Cell Lines. Evidence-Based Complement. Altern. Med. 1–16. https://doi.org/10.1155/2017/1468957

Khowala, S., Verma, D., Banik, S.P., 2008. Biomolecules : (introduction , structure & function) Carbohydrates, in: Structure and Function of Biomolecules. pp. 1–5.

Kintzios, S.E., Barberaki, M.G., 2004. Plants that fight cancer. CRC PRESS, Washington, D.C.

Koolen, H.H.F., Soares, E.R., Silva, F.M.A. da, Souza, R.A. de A. e A.D.L. de, 2012. an antimicrobial alkaloid and other metabolites produced by Penicillium sp. an endophytic fungus isolated from mauritia flexuosa L. f. Quim. Nova 35, 771–774.

Körver-Keularts, I.M.L.W., Wang, P., Waterval, H.W.A.H., Kluijtmans, L.A.J., Wevers, R.A., Langhans, C.D., Scott, C., Habets, D.D.J., Bierau, J., 2018. Fast and accurate quantitative organic acid analysis with LC-QTOF/MS facilitates screening of patients for inborn errors of metabolism. J. Inherit. Metab. Dis. 41, 415–424. https://doi.org/10.1007/s10545-017-0129-0

Kresge, N., Simoni, R.D., Hill, R.L., 2010. Glycobiology & Carbohydrates. J. Biol. Chem. 285, 1–5.

Kuhn, A., Yu, S., Giffhorn, F., 2006. Catabolism of 1,5-Anhydro-D-Fructose in Sinorhizobium morelense S-30.7.5: Discovery, Characterization, and Overexpression of a New 1,5-Anhydro-D-Fructose Reductase and Its Application in Sugar Analysis and Rare Sugar Synthesis. Appl. Environ. Microbiol. 72, 1248–1257. https://doi.org/10.1128/AEM.72.2.1248

Kuklinsky-Sobral, J., Arajo, W.L., Mendes, R., Pizzirani-Kleiner, A.A., Azevedo, J.L., 2005. Isolation and characterization of endophytic bacteria from soybean (Glycine max) grown in soil treated with glyphosate herbicide. Plant Soil 273, 91–99. https://doi.org/10.1007/s11104-004-6894-1

Kumar, A., Gupta, Nitika, Kumar, Swadesh, Gupta, Nikhil, Kumar, Santosh, Yadav, V., Prakash,

A., Gurjar, H., 2015. Metabolites in plants and its classification. WORLD J. Pharm. Pharm. Sci. 4, 287–305.

Kumar, M.L.V., Thippeswamy, B., Kuppust, I.J., Virupaksha, J.H., 2014. Evaluation of analgesic activities of bacillus. Int. J. Chem. Sci. 12, 272–280.

Kumar, S., Pandey, A.K., 2013. Chemistry and Biological Activities of Flavonoids : An Overview Chemistry and Biological Activities of Flavonoids : An Overview. Sci. J. 1–15. https://doi.org/10.1155/2013/162750

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33, 1870–4. https://doi.org/10.1093/molbev/msw054

Kumar, V., Rai, S., Gaur, P., 2014. Endophytic Fungi : Novel Sources of Anticancer Molecules. https://doi.org/10.1007/978-81-322-1575-2

Kumar, V., Roy, S., 2017. Aquaculture Drugs : Sources , Active Ingredients , Pharmaceutic Preparations and Methods of Administration. J. Aquac. Res. Dev. 8, 1–13. https://doi.org/10.4172/2155-9546.1000510

Kumar, V.M.., Thippeswamy, B., Shivakumar, C.., 2013. Evaluation of Antimicrobial Activity of Bacillus cereus And Bacillus pumillus Metaboites Against Human Pathogens. Int. J. Curr. Pharm. Rev. Res. 4, 47–60.

Kumara, P.M., Shweta, S., Vasanthakumari, M.M., Sachin, N., Manjunatha, B.L., Jadhav, S.S., Ravikanth, G., Ganeshaiah, K.N., Shaanker, R.U., 2014. Endophytes and Plant Secondary Metabolite Synthesis: Molecular and Evolutionary Perspective, in: Advances in Endophytic Research. V.C. Verma and A.C. Gange, pp. 177–190. https://doi.org/10.1007/978-81-322-1575-2

Kusari, S., Hertweck, C., Spiteller, M., 2012. Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chem. Biol. 19, 792–798. https://doi.org/10.1016/j.chembiol.2012.06.004

Kwembeya, E.G., Bjorå, C.S., Stedje, B., Nordal, I., 2007. Phylogenetic relationships in the genus Crinum (Amaryllidaceae) with emphasis on tropical African species: evidence from trnL-Fand nucleat ITS DNA sequence data. Taxon 56, 801–810.

Kwembeya, E.G., Stedje, B., 2007. A numerical taxonomy of the pan species of Crinum based on morphological characters. Nord. J. Bot. 25, 137–144. https://doi.org/10.1111/j.0107-055X.2007.00031.x

Le, A. V., Parks, S.E., Nguyen, M.H., Roach, P.D., 2018. Effect of solvents and extraction methods on recovery of bioactive compounds from defatted gac (Momordica cochinchinensis spreng.) seeds. Separations 5, 1–13. https://doi.org/10.3390/separations5030039

Li, A.H.L., Willaman, J.J., 1968. Distribution of Alkaloids in Angiosperm Phylogeny. Econ. Bot. 22, 239–252.

Lí, Y., Chen, Y., 1990. Diterpenoids from rabdosia pseudo-irrorata. J. Nat. Prod. 53, 841–844. https://doi.org/10.1021/np50070a011

Liew, S.Y., Mukhtar, M.R., Hadi, A.H.A., Awang, K., Mustafa, M.R., Zaima, K., Morita, H., Litaudon, M., 2012. Naucline, a new indole alkaloid from the bark of Nauclea officinalis. Molecules 17, 4028–4036. https://doi.org/10.3390/molecules17044028

Lin, L., Lee, L., Huang, C., Chen, C., Song, J., Shiao, Y., Liu, H., 2019. Effects of boschnaloside from Boschniakia rossica on dysglycemia and islet dysfunction in severely diabetic mice through modulating the action of glucagon-like peptide-1. Phytomedicine 62, 1–11. https://doi.org/10.1016/j.phymed.2019.152946

Lloyd, M., Dapar, G., Demayo, C.G., Senarath, W.T.P.S.K., 2018. Antimicrobial and Cellular Metabolic Inhibitory Properties of the Ethanolic Extract From the Bark of "Lunas-Bagon" (Lunasia Sp.). Int. J. Pharm. Sci. Res. 9, 88–97. https://doi.org/10.13040/IJPSR.0975-8232.9(1).88-97

López-lázaro, M., 2009. Distribution and Biological Activities of the Flavonoid Luteolin. Mini-Reviews Med. Chem. 9, 31–59.

Louw, C. a M., Regnier, T.J.C., Korsten, L., 2002. Medicinal bulbous plants of South Africa and their traditional relevance in the control of infectious diseases. J. Ethnopharmacol. 82, 147–154. https://doi.org/10.1016/S0378-8741(02)00184-8

Ludwig-Muller, J., 2015. Plants and endophytes : equal partners in secondary metabolite production ? Biotechnol. Lett. 37, 1325–1334. https://doi.org/10.1007/s10529-015-1814-4

Mager, D.L., 2006. Bacteria and cancer: Cause, coincidence or cure? A review. J. Transl. Med. 4, 1–18. https://doi.org/10.1186/1479-5876-4-14

Mahady, G., 2005. Medicinal plants for the prevention and treatment of bacterial infections. Curr. Pharm. Des. 11, 2405–2427.

Mahomoodally, M.F., 2013. Traditional Medicines in Africa : An Appraisal of Ten Potent African Medicinal Plants. Evidence-Based Complement. Altern. Med. 2013, 1–14.

Maloney, K.N., Macmillan, J.B., Kauffman, C.A., Jensen, P.R., Dipasquale, A.G., Rheingold,

A.L., Fenical, W., 2009. NIH Public Access. Org. Lett. 11, 1–7. https://doi.org/10.1021/ol901997k.Lodopyridone

Mandal, S.M., Sharma, S., Pinnaka, A.K., Kumari, A., Korpole, S., 2013. Isolation and characterization of diverse antimicrobial lipopeptides produced by Citrobacter and Enterobacter. BMC Microbiol. 2013, 13, 1–9.

Manfredi, K.P., Vallurupalli, V., Demidova, M., Kindscher, K., Pannell, L.K., 2001. Isolation of an anti-HIV diprenylated bibenzyl from Glycyrrhiza lepidota. Phytochemistry 58, 153–157.

Mariıa Jesus Duran-Pena, Ares, J.M.B., Collado, I.G., Hern´andez-Gal´an, R., 2014. Biologically active diterpenes containing a gem- dimethylcyclopropane subunit: an intriguing source of PKC modulators. Nat. Prod. Rep. 1–14. https://doi.org/10.1039/c4np00008k

Maroyi, A., 2016a. A review of ethnoboatany, therapeutic value, phytochemistry and pharmacology of Crinum macowanii Baker_ A highly traded bulbous plant in Southern Africa. J. Ethnopharmacol. 194, 595–608. https://doi.org/10.1016/j.jep.2016.10.046

Maroyi, A., 2016b. Ethnobotanical, phytochemical and pharmacological properties of crinum bulbispermum (Burm f) milne-redh and schweick (Amaryllidaceae). Trop. J. Pharm. Res. 15, 2497–2506. https://doi.org/10.4314/tjpr.v15i11.27

Maroyi, A., 2014. The genus Warburgia: A review of its traditional uses and pharmacology. Pharm. Biol. 52, 378–391. https://doi.org/10.3109/13880209.2013.837935

Martín-garcía, J., Espiga, E., Pando, V., Diez, J.J., 2011. Factors Influencing Endophytic Communities in Poplar Plantations. Silva Fenn. 45, 169–180.

Martinez-Klimova, E., Rodríguez-Peña, K., Sánchez, S., 2017. Endophytes as sources of antibiotics. Biochem. Pharmacol. 134, 1–17. https://doi.org/10.1016/j.bcp.2016.10.010

Matthijs, S., Vander Wauven, C., Cornu, B., Ye, L., Cornelis, P., Thomas, C., Ongena, M., 2014. Antimicrobial properties of producing the antibiotic mupirocin. Res. Microbiol. 165, 695–704. https://doi.org/10.1016/j.resmic.2014.09.009

Mazid, M., Khan, T.., Mohammad, F., 2011. Role of secondary metabolites in defense mechanisms of plants. Biol. Med. 3, 232–249.

McCauley, J., Zivanovic, A., Skropeta, D., 2013. Bioassays for anticancer activities. Methods Mol. Biol. 1055, 191–205.

McKee, T., McKee, J.R., 2008. Amino Acids, Peptides, and Proteins, in: Biochemistry The molecular basis of life fifth edition. Oxford University Press, New York, USA, pp. 1–56.

Meegan, M.J., O'Boyle, N.M., 2019. Special issue " anticancer drugs". Pharmaceuticals 12, 1–6.

https://doi.org/10.3390/ph12030134

Meerow, A.W., Lehmiller, D.J., Clayton, J.R., 2003. Phylogeny and biogeography of Crinum L. (Amaryllidaceae) inferred from nuclear and limited plastid non-coding DNA sequences. Bot. J. Linn. Soc. 141, 349–363. https://doi.org/10.1046/j.1095-8339.2003.00142.x

Mendoza, N., Silva, E.M.E., 2018. Introduction to Phytochemicals: Secondary Metabolites from Plants with Active Principles for Pharmacological Importance, in: Asao, T., Asaduzzaman, M. (Eds.), Phytochemicals - Source of Antioxidants and Role in Disease Prevention. IntechOpen, pp. 25–47. https://doi.org/http://dx.doi.org/10.5772/57353

Menpara, D., Chanda, S., 2013. Endophytic Bacteria- Unexplored Reservoir of Antimicrobials for Combating Microbial Pathogens, in: Méndez-Vilas, A. (Ed.), Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education. pp. 1095–1103.

Mercado-Blanco, J., Lugtenberg, B.J.J., 2014. Biotechnological Applications of Bacterial Endophytes. Curr. Biotechnol. 3, 60–75. https://doi.org/10.2174/22115501113026660038

Michelsen, C.F., Jensen, H., Venditto, V.J., Hennessy, R.C., Stougaard, P., 2015. Bioactivities by a crude extract from the Greenlandic Pseudomonas sp . In5 involves the nonribosomal peptides , nunamycin and nunapeptin. PeerJ 1476, 1–16. https://doi.org/10.7717/peerj.1476

Migas, P., Krauze-Baranowska, M., 2015. The significance of arbutin and its derivatives in therapy and cosmetics. Phytochem. Lett. 13, 35–40. https://doi.org/10.1016/j.phytol.2015.05.015

Millar, A.A., Smith, M.A., Kunst, L., 2000. All fatty acids are not equal : discrimination in plant membrane lipids. Trends Plant Sci. 5, 95–101.

Mitscher, L.A., Raghav Rao, G.S., Khanna, I., Veysoglu, T., Drake, S., 1983. Antimicrobial agents from higher plants: Prenylated flavonoids and other phenols from Glycyrrhiza lepidota. Phytochemistry 22, 573–576. https://doi.org/10.1016/0031-9422(83)83049-0

Moco, S., Bino, R.J., Vorst, O., Verhoeven, H.A., Groot, J. de, Beek, T.A. van, Vervoort, J., de Vos, C.H.R., 2006. A Liquid Chromatography-Mass Spectrometry-Based Metabolome Database for Tomato. Plant Physiol. 141, 1205–1218. https://doi.org/10.1104/pp.106.078428.once

Mohamed, R., Avis, T., Tsopmo, A., 2015. Distribution of antimicrobial lipopeptides in Bacillus and Pseudomonas spp ., two genera with antagonistic effects against plant pathogens. Carleton University Ottawa, Ontario.

Monakisi, C.M., 2007. Knowledge and Use of Tradition Medicinal Plants By the Setswan a - Speaking Community of Kimberley , Northern Cape of South Africa. October. University of

Stellenbosch.

Morales, A., 2008. A simple way to preserve fungal cultures. Ithaca, New York.

Moran-palacio, E.F., Tortoledo-ortiz, O., Yañez-farias, G.A., Stephens-camacho, N.A., Soñanez-organis, J.G., Ochoa-López, L.M., Rosas-Rodríguez, J.A., 2014. Determination of Amino Acids in Medicinal Plants from Southern Sonora , Mexico. Trop. J. Pharm. Res. 13, 601–606.

Morare, R.. ., Serepa-Dlamini, M.H., Ubomba-Jaswa, E., Mavumengwana, V., 2017. Screening of anti-microbial activity of secondary metabolites extracted from Crinum macowanii and its associated bacterial endophytes. Univrersity of Johannesburg.

Morare, R.F., Ubomba-Jaswa, E., Serepa-Dlamini, M.H., 2018. Isolation and identification of bacterial endophytes from Crinum macowanii Baker. African J. Biotechnol. 17, 1040–1047. https://doi.org/10.5897/AJB2017.16350

Mousa, W.K., Raizada, M.N., 2013. The Diversity of Anti-Microbial Secondary Metabolites Produced by Fungal Endophytes: An Interdisciplinary Perspective. Front. Microbiol. 4, 1–18. https://doi.org/10.3389/fmicb.2013.00065

Muema, S.M., 2013. Phytochemical and anthelmintic study of the root bark of teclea trichocarpa, engl. (rutaceae). University of Nairobi.

Mugwagwa, A.T., Gadaga, L.L., Pote, W., Tagwireyi, D., 2015. Antiamnesic Effects of a Hydroethanolic Extract of Crinum macowanii on Scopolamine-Induced Memory Impairment in Mice. J. Neurodegener. Dis. 1–9.

Mukherjee, A., Das, R., Pal, A., Paul, A.K., 2017. Antibacterial Activity of Endophytic Bacillus safensis Isolated from Ophioglossum reticulatum L . Microbiol. Res. J. Int. 18, 1–12. https://doi.org/10.9734/MRJI/2017/31923

Mustafa, M., JamalulaAzizi, A., IIIzam, E., Nazirah, A., Sharifa, S., Abbas, S., 2016. Lung Cancer: Risk Factors, Management, And Prognosis. IOSR J. Dent. Med. Sci. 15, 94–101. https://doi.org/10.9790/0853-15100494101

Muzzamal, H., Sarwar, R., Sajid, I., Hasnain, S., 2012. Isolation , Identification and Screening of Endophytic Bacteria Antagonistic to Biofilm Formers. Pak. J. Zool. 44, 249–257.

Nair, D.N., Padmavathy, S., 2014. Impact of Endophytic Microorganisms on Plants , Environment and Humans. Sci. World J. 2014, 1–11.

Nair, J.J., Bastida, J., Codina, C., Viladomat, F., van Staden, J., 2013. Alkaloids of the South African Amaryllidaceae : A Review. Nat. Prod. Commun. 8, 1335–1350. https://doi.org/10.1002/chin.201430280

Nair, J.J., Machocho, A.K., Campbell, W.E., Brun, R., Viladomat, F., Codina, C., Bastida, J., 2000. Alkaloids from Crinum macowanii. Phytochemistry 54, 945–950. https://doi.org/10.1016/S0031-9422(00)00128-X

Nair, Jerald J., Staden, J. Van, 2014. Traditional usage , phytochemistry and pharmacology of the South African medicinal plant Boophone disticha (L . f .) Herb . J. Ethnopharmacol. J. 151, 12–26. https://doi.org/10.1016/j.jep.2013.10.053

Nair, Jerald James, Staden, J. Van, 2014. Traditional usage, phytochemistry and pharmacology of the South African medicinal plant Boophone disticha (L.f.) Herb. (Amaryllidaceae). J. Ethnopharmacol. 151, 12–26. https://doi.org/10.1016/j.jep.2013.10.053

Nair, J.J., Staden, J. Van, Bonnet, S.L., Wilhelm, A., 2017. Antibacterial Properties of the Family Amaryllidaceae: Evaluation of Plant Extracts in vitro. Nat. Prod. Commun. 12, 1145–1151. https://doi.org/10.1177/1934578X1701200735

Nair, J.J., van Staden, J., 2018. Insight to the antifungal properties of Amaryllidaceae constituents. Phytomedicine 1–8. https://doi.org/10.1016/j.phymed.2018.11.013

Nair, J.J., van Staden, J., 2013. Pharmacological and toxicological insights to the South African Amaryllidaceae. Food Chem. Toxicol. J. 62, 262–275. https://doi.org/10.1016/j.fct.2013.08.042

Nakanishi, K., 2005. Terpene trilactones from Gingko biloba : From ancient times to the 21st century. Bioorg. Med. Chem. 13, 4987–5000. https://doi.org/10.1016/j.bmc.2005.06.014

Nalini, M.S., Prakash, H.S., 2017. Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Lett. Appl. Microbiol. 64, 261–270. https://doi.org/10.1111/lam.12718

Narsing Rao, M.P., Xiao, M., Li, W.J., 2017. Fungal and bacterial pigments: Secondary metabolites with wide applications. Front. Microbiol. 8, 1–13. https://doi.org/10.3389/fmicb.2017.01113

National Cancer Registry South Africa, 2016. 2016 National Cancer Registry SA Statistics.

National Cancer Registry South Africa, 2015. 2015 National Cancer Registry South Africa Statistics. https://doi.org/10.16309/j.cnki.issn.1007-1776.2003.03.004

National Cancer Registry South Africa, 2014. 2014 National Cancer Registry SA Statisctics. https://doi.org/10.1192/bjp.112.483.211-a

National Cancer Registry South Africa, 2013. 2013 National Cancer Registry SA Statistics.

Navi, S.S., Bandyopadhyay, R., Hall, A.J., Bramel-Cox, P.J., 1999. A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain. Andhra Pradesh, India.

Naz, S., Gallart-Ayala, H., Reinke, S.N., Mathon, C., Blankley, R., Chaleckis, R., Wheelock, C.E., 2017. Development of a Liquid Chromatography-High Resolution Mass Spectrometry Metabolomics Method with High Specificity for Metabolite Identification Using All Ion Fragmentation Acquisition. Anal. Chem. 89, 7933–7942. https://doi.org/10.1021/acs.analchem.7b00925

Nazemi, M., Moradi, Y., Rezvani, G.F., Ahmaditaba, M.A., Gozari, M., Salari, Z., 2017. Antimicrobial activities of semi polar-nonpolar and polar secondary metabolites of sponge Dysidea pallescens from Hengam Island, Persian Gulf. Iran. J. Fish. Sci. 15, 200–209.

Ncube, E.N., Dubery, I.A., Steenkamp, P.A., Madala, N.E., 2016. Manipulation of secondary metabolite synthesis in Centella asiatica cells : a metabolomics study. University of Johannesburg.

Ncube, N.S., Afolayan, A.J., Okoh, A.I., 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J. Biotechnol. 7, 1797–1806. https://doi.org/10.5897/AJB07.613

Ndhlala, A.R., Ncube, B., Okem, A., Mulaudzi, R.B., Staden, J. Van, 2013. Toxicology of some important medicinal plants in southern Africa. Food Chem. Toxicol. J. 62, 609–621. https://doi.org/10.1016/j.fct.2013.09.027

Neal, R.D., Robbé, I.J., Lewis, M., Williamson, I., Hanson, J., 2015. The complexity and dif fi culty of diagnosing lung cancer : fi ndings from a national primary-care study in Wales. Prim. Health Care Res. Dev. 16, 436–449. https://doi.org/10.1017/S1463423614000516

Nebula, M., Harisankar, H.S., Chandramohanakumar, N., 2013. Metabolites and bioactivities of Rhizophoraceae mangroves. Nat. Products Bioprospect. 3, 207–232. https://doi.org/10.1007/s13659-013-0012-0

Negi, J.S., Negi, P.S., Pant, G.J., Rawat, M.S.M., Negi, S.K., 2013. Naturally occurring saponins : Chemistry and biology. J. Poisonous Med. Plant Res. Vol. 1, 1–6.

Neu, A.K., Månsson, M., Gram, L., Prol-García, M.J., 2014. Toxicity of Bioactive and Probiotic Marine Bacteria and Their Secondary Metabolites in Artemia sp . and Caenorhabditis elegans as Eukaryotic Model Organisms. Appl. Environ. Microbiol. 80, 146–153. https://doi.org/10.1128/AEM.02717-13

Ngamau, C.N., Matiru, V.N., Tani, A., Muthuri, C.W., 2012. Isolation and identification of endophytic bacteria of bananas (Musa spp .) in Kenya and their potential as biofertilizers for sustainable banana production. African J. Microbiol. Res. 6, 1–10.

Ngo, T. Van, Scarlett, C.J., Bowyer, M.C., Ngo, P.D., Vuong, Q. Van, 2017. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. J. Food Qual. 1–8. https://doi.org/10.1155/2017/9305047

Ngoma, L., Mogatlanyane, K., Babalola, O.O., 2014. Screening of Endophytic Bacteria towards the Development of Cottage Industry : An in Vitro Study. J. Hum. Ecol. 47, 45–63.

Nicoletti, R., Fiorentino, A., 2015. Plant Bioactive Metabolites and Drugs Produced by Endophytic Fungi of Spermatophyta. Agriculture 5, 918–970. https://doi.org/10.3390/agriculture5040918

Nii-Trebi, N.I., 2017. Emerging and Neglected Infectious Diseases : Insights , Advances , and Challenges. Hindawi BioMed Res. Int. 2017, 1–15.

Nithya, G., Panneerselvam, P., Nepolean, R., Selvakumar, N., Jeyavalli, A., 2012. Synthesis & biological evaluation of some novel schiff bases of isatin derivatives. Int. J. Pharm. Ther. 3, 243–246.

Noch, E.K., Ramakrishna, R., Magge, R., 2018. Challenges in the Treatment of Glioblastoma: Multisystem Mechanisms of Therapeutic Resistance. World Neurosurg. 116, 505–517. https://doi.org/10.1016/j.wneu.2018.04.022

Nongkhlaw, W.M.F., Joshi, S.R., 2015. Original Article Investigation on the bioactivity of culturable endophytic and epiphytic bacteria associated with ethnomedicinal plants. J. Infect. Dev. Ctries. 9, 954–961. https://doi.org/10.3855/jidc.4967

Notten, A., 2013. Crinum macowanii [WWW Document]. Kirstenbosch Natl. Bot. Gard. URL http://www.plantzafrica.com/plantcd/crinummacowanii.htm). (accessed 5.20.07).

Nyamai, D., Arika, W., Ogola, P., Njagi, E., Ngugi, M., 2016. Medicinally Important Phytochemicals : An Untapped Research Avenue. J. Pharmacogn. Phytochem. 4, 35–49.

Ohsaki, A., Ozawa, M., Komiyama, K., Kishida, A., Isobe, T., 2012. Natural Product Communications The Cytotoxic Activity of Diterpenoids from Isodon species. Nat. Prod. Commun. 7, 977–978. https://doi.org/10.1177/1934578X1200700803

Okuda, T., Ito, H., 2011. Tannins of Constant Structure in Medicinal and Food Plants—Hydrolyzable Tannins and Polyphenols Related to Tannins 16, 2191–2217. https://doi.org/10.3390/molecules16032191

Olufunke, M.D., 2012. Developments in Phytochemistry, in: Vallisuta, P.O. (Ed.), Drug Discovery Research in Pharmacognosy. InTech Published, pp. 12–23.

Omar, S.H., 2013. Ginkgolides and Neuroprotective Effects. Nat. Prod. 3697–3741. https://doi.org/10.1007/978-3-642-22144-6

Onyegeme-Okerenta, B.M., Chinedu, S.N., Okafor, U.A., Okochi, V.I., 2009. Antibacterial Activity of Culture Extracts of Penicillium chrysogenum PCL501: Effects of Carbon Sources. Online J. Heal. Allied Sci. 8, 1–7.

Oren, A., Gunde-Cimerman, N., 2007. Mycosporines and mycosporine-like amino acids : UV protectants or multipurpose secondary metabolites ? FEMS Microbiol. Lett. 269, 1–10. https://doi.org/10.1111/j.1574-6968.2007.00650.x

Pagare, Saurabh, Bhatia, M., Tripathi, N., Pagare, Sonal, Bansal, Y.K., 2016. Secondary metabolites of plants and their role : Overview. Curr. Trends Biotechnol. Pharm. 9, 293–304.

Pal, S., Shukla, Y., 2003. Herbal medicine: current status and the future. Asian Pacific J. Cancer Prev. 4, 281–288.

Pang, B., Zhu, Y., Lu, L., Gu, F., Chen, H., 2016. The applications and features of liquid chromatography-mass spectrometry in the analysis of traditional Chinese medicine. Evidence-based Complement. Altern. Med. 2016, 1–7. https://doi.org/10.1155/2016/3837270

Paolillo, M., Boselli, C., Sergio, S., 2018. Glioblastoma under Siege: An Overview of Current Therapeutic Strategies. Brain Sci. 8, 1–13. https://doi.org/10.3390/brainsci8010015

Patil, R.H., Patil, M.P., Maheshwari, V.L., 2016. Bioactive Secondary Metabolites From Endophytic Fungi: A Review of Biotechnological Production and Their Potential Applications, in: Atta-ur-Rahman (Ed.) (Ed.), Studies in Natural Products Chemistry. Elsevier, pp. 189–205. https://doi.org/10.1016/B978-0-444-63601-0.00005-3

Paul, N.C., Deng, J.X., Shin, K.S., Yu, S.H., 2012. Molecular and Morphological Characterization of Endophytic Heterobasidion araucariae from Roots of Capsicum annuum L . in Korea. Mycology 40, 85–90.

Pereira, A.S.P., Haan, H. Den, Peña-García, J., Moreno, M.M., Pérez-Sánchez, H., Apostolides, Z., 2019. Exploring African Medicinal Plants for Potential Anti-Diabetic Compounds with the DIA-DB Inverse Virtual Screening Web Server. Molecules 24, 1–32. https://doi.org/10.3390/molecules24102002

Persson, K.G., Burger, A.P., Mugabo, P., Green, I., 2007. In vivo effects of Crinum macowanii on the Rat Cardiovascular System. University of the Western Cape.

Pettit, G.R., Ducki, S., Tan, R., Gardella, R.S., Mcmahon, J.B., Boyd, M.R., Pettit, G.R., Blumberg, P.M., Lewin, N.E., Doubek, D.L., Tackett, L.P., Williams, M.D., 2002. Isolation and Structure of Pedilstatin from a Republic of Maldives Pedilanthus. J. Nat. Prod. 65, 1262–1265. https://doi.org/10.1021/np020115b

Phillipson, J.D., Hemingway, S.R., 1974. Angustine and related alkaloids from species of mitragyna, nauclea, uncarza, and strychnos. Phytochemistry 13, 973–978.

Piboonprai, K., Khumkhrong, P., Khongkow, M., Yata, T., Ruangrungsi, N., Chansriniyom, C., Iempridee, T., 2018. Anticancer activity of arborinine from Glycosmis parva leaf extract in human cervical cancer cells. Biochem. Biophys. Res. Commun. 500, 866–872. https://doi.org/10.1016/j.bbrc.2018.04.175

Picardo, S.L., Coburn, B., Hansen, A.R., 2019. The microbiome and cancer for clinicians. Crit. Rev. Oncol. Hematol. 141, 1–12. https://doi.org/10.1016/j.critrevonc.2019.06.004

Pierce, G.B., Wallace, C., 1971. Differentiation of malignant to benign cells. Cancer Rese 31, 127–134. https://doi.org/10.1016/0022-5193(71)90020-8

Pitt, J.I., Hocking, A.., 2009. Fungi and Food Spoilage, third. ed. Springer Dordrecht Heidelberg London, London New York.

Podolak, I., Galanty, A., Sobolewska, D., 2010. Saponins as cytotoxic agents: A review. Phytochem. Rev. 9, 425–474. https://doi.org/10.1007/s11101-010-9183-z

Pombeiro-Sponchiado, S.R., Sousa, G.S., Andrade, J.C.R., Lisboa, H.F., Gonçalves, R.C.R., 2017. Production of Melanin Pigment by Fungi and Its Biotechnological Applications, in: Miroslav Blumenberg, IntechOpen. pp. 47–60. https://doi.org/http://dx.doi.org/10.5772/57353

Pop, C., Vlase, L., Tamas, M., 2009. Natural resources containing arbutin. Determination of arbutin in the leaves of Bergenia crassifolia (L.) Fritsch. acclimated in Romania. Not. Bot. Horti Agrobot. Cluj-Napoca 37, 129–132.

Powers-Fletcher, M., Kendall, B., Griffin, A., Hanson, K., 2016. Filamentous fungi. Microbiol. Spectr. 4, 1–29. https://doi.org/10.1128/microbiolspec.DMIH2-0002-2015.Correspondence

Prabavathy, D., Nachiyar, C.V., 2014. Cytotoxicity of Ethyl Acetate Extract of Endophytic Aspergillus fumigatus on A549 Cell Lines. Biosci. Biotechnol. Res. ASIA, 11, 797–802.

Purwestri, Y.A., Kartikasari, N., Putri, S.G., Wilson, W., Sembiring, L., 2016. Metabolic profiling of endophytic bacteria from Purwoceng (Pimpinella pruatjan Molkend) root and antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. AIP Conf. Proc. 1744, 1–7. https://doi.org/10.1063/1.4953537

Pusztahelyi, T., Holb, I.J., Pócsi, I., 2015. Secondary metabolites in fungus-plant interactions. Front. Plant Sci. 6, 1–23. https://doi.org/10.3389/fpls.2015.00573

Qin, S., Xing, K., Jiang, J., Xu, L., 2011. Biodiversity , bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl. Microbiol.

Biotechnol. 89, 457–473. https://doi.org/10.1007/s00253-010-2923-6

Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 56, 81–87. https://doi.org/10.1016/S0378-8741(96)01515-2

Raghu, J., 2012. Endophytic bacterial association and plant secondary metabolite production. University of Agricultural Sciences, Bangalore.

Raimondo, D., 2011. The Red List of South African plants – A global first. S. Afr. J. Sci. 107, 1–2. https://doi.org/10.4102/sajs.v107i3/4.653

Rajbhandary, S., 2014. Healing Traditions of the Himalayas 21–36. https://doi.org/10.1007/978-81-322-1925-5

Ramesh, V., Arivudainambi, U., Rajendran, A., 2017. The molecular phylogeny and taxonomy of endophytic fungal species from the leaves of Vitex negundo L . Stud. Fungi 2, 26–37. https://doi.org/10.5943/stif/

Ramírez-Bahena, Martha-Helena Carmen, T., Peix, A., 2014. Bacterial Endophytes Inhabiting Potato Plants (Solanum tuberosum L.), in: Mandhatri, P.G. and S. (Ed.), Agricultural Research Updates. Nova Science Publishers, New York, pp. 3–31.

Rana, A., Singh, H.P., Dhyani, D., 2012. Comparative Estimation of Major Iridoid Glucosides from Different Parts of Incarvillea emodi. ISRN Chromatogr. 2012, 1–4. https://doi.org/10.5402/2012/783752

Rangjaroen, C., Sungthong, R., RerkaSem, B., Teaumroong, N., NoiSangiam, R., SaiSamorn, L., 2017. Untapped Endophytic Colonization and Plant Growth-Promoting Potential of the Genus Novosphingobium to Optimize Rice Cultivation. Microbes and Enviroments 32, 84–87. https://doi.org/10.1264/jsme2.ME16112

Raviglione, M., Maher, D., 2017. Ending infectious diseases in the era of the Sustainable Development Goals. Porto Biomed. J. 2, 140–142. https://doi.org/10.1016/j.pbj.2017.08.001

Refaat, J, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2013. Crinum; an endless source of bioactive principles: a review. part iv: non-alkaloidal constituents. Int. J. Pharm. Sci. Res. 3, 1883–1890.

Refaat, John, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2013. Crinum; An endless source of bioactive principles: a review, part V. Biological profile. Int. J. Pharm. Sci. Res. 4, 1239–1252.

Refaat, John, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012. Crinum ; an endless source of bioactive principles : a review , part ii . crinum alkaloids : crinine- type alkaloids. Int. J.

Pharma Sci. Res. 3, 3091–3100.

Refaat, J, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012. Crinum; an endless source of bioactive principles: a review. part iii; crinum alkaloids: belladine-, galanthamine-, lycorenine-, tazettine-type alkaloids and other minor types. Int. J. Pharm. Sci. Res. 3, 3630–3638.

Reid, S., Wishingrad, V., McCabe, S., 2009. Plant Uses : California, The University of California Santa Cruz Arboretum.

Rhoden, S.A., Garcia, A., Santos e Silva, M.C., Azevedo, J.L., Pamphile, J.A., 2015. Phylogenetic analysis of endophytic bacterial isolates from leaves of the medicinal plant Trichilia elegans A . Juss . (Meliaceae). Genet. Mol. Res. 14, 1515–1525.

Rinner, U., Dank, C., Hudlicky, T., 2016. Galanthamine. Targets Heterocycl. Syst. 20, 283–315.

Roder, C., Thomson, M.J., 2015. Auranofin : Repurposing an Old Drug for a Golden New Age 13–20. https://doi.org/10.1007/s40268-015-0083-y

Rodriguez, R.J., White, J.J.F., Arnold, A.E., Redman, R.S., 2009. Fungal endophytes : diversity and functional roles. New Phytol. 182, 314–330.

Rønsted, N., Symonds, M.R.E., Birkholm, T., Christensen, S.B., Meerow, A.W., Molander, M., Mølgaard, P., Petersen, G., Rasmussen, N., Staden, J. van, Stafford, G.I., Jäger, A.K., 2012. Can phylogeny predict chemical diversity and potential medicinal activity of plants ? A case study of Amaryllidaceae. BMC Evol. Biol. This 12. https://doi.org/10.1186/1471-2148-12-182

Rosière, R., Berghmans, T., De Vuyst, P., Amighi, K., Wauthoz, N., 2019. The position of inhaled chemotherapy in the care of patients with lung tumors: Clinical feasibility and indications according to recent pharmaceutical progresses. Cancers (Basel). 11, 1–13. https://doi.org/10.3390/cancers11030329

Roy, M., Liang, L., Xiao, X., Feng, P., Ye, M., Liu, J., 2018. Lycorine: A prospective natural lead for anticancer drug discovery. Biomed. Pharmacother. 107, 615–624. https://doi.org/10.1016/j.biopha.2018.07.147

Rustan, A.C., Drevon, C.A., 2005. Fatty Acids : Structures and Properties. Encycl. Life Scienecs. https://doi.org/10.1038/npg.els.0003894

Saad, H.-E., El-Sharkawy, S., Shier, W.T., 1995. Biological Activities of Pyrrolidinoindoline Alkaloids from Calycodendron milnei. Planta Med. 61, 313–316. https://doi.org/10.1055/s-2006-958090

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing

phylogenetic trees. Mol. Biol. Evol. 4, 406–25. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Salim, A., Chin, Y., Kinghorn, A., 2008. Drug Discovery from Plants A.A. Bioact. Mol. Med. Plants 1–25. https://doi.org/10.1007/978-3-540-74603-4

Santos, S.N., Ferraris, F.K., 2012. Endophytic fungi from Combretum leprosum with potential anticancer and antifungal activity. Symbiosis 109–117. https://doi.org/10.1007/s13199-012-0218-7

Santoyo, G., Moreno-Hagelsieb, G., Carmen Orozco-Mosqueda, del M., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183, 92–99. https://doi.org/10.1016/j.micres.2015.11.008

Sardul Singh, S., Suneel, K., Aharwal, R.P., 2014. Isolation and identification of endophytic fungi from Ricinus communis linn . And their antibacterial activity. Int. J. Res. Pharm. Chem. 4, 611–618.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Latha, L.Y., 2011. Extraction , Isolation and Characterization of Bioactive Compounds from Plants ' Extracts. African J. Tradit. Complement. Altern. Med. · 8, 1–10. https://doi.org/10.4314/ajtcam.v8i1.60483

Sasidharan, S., Yoga Latha, L., Kwan, Y.P., Lachumy, S.J., 1993. Screening Methods in the Study of Fungicidal Property of Medicinal Plants. Fungic. Plant Anim. Dis. 107-.

Sauerschnig, C., Doppler, M., Bueschl, C., Schuhmacher, R., 2018. Methanol Generates Numerous Artifacts during Sample Extraction and Storage of Extracts in Metabolomics Research. Metabolites 8, 1–19. https://doi.org/10.3390/metabo8010001

Sawaya, A.C.H.F., Abreu, I.N., Andreazza, L., Eberlin, M.N., Mazzafera, P., 2010. Pilocarpine and related alkaloids in pilocarpus vahl (rutaceae), in: N. M. Cassiano (Ed.), Alkaloids: Properties, Applications. Nova Science Publishers, Inc, pp. 63–80.

Saxena, M., Saxena, J., Nema, R., Singh, D., Gupta, A., 2013. Phytochemistry of medicinal plants. J. Phamacognosy Phytochem. 1, 168–182. https://doi.org/10.1016/0300-9084(96)82199-7

Scholz, M., Gatzek, S., Sterling, A., Selbig, O., Fiehn, J., 2004. Metabolite fingerprinting : detecting biological features by independent component analysis. Bioinformatics 20, 2447–2454. https://doi.org/10.1093/bioinformatics/bth270

Schwikkard, S., van Heerden, F.R., 2002. Antimalarial activity of plant metabolites. Nat. Prod. Rep. 19, 675–692. https://doi.org/10.1039/b008980j

Sebola, T.E., Ndinteh, D.T., Mavumengwana, V., 2016a. A contribution to the phytochemical and

antibacterial characteristics of Crinum macowanii bulbs extracts. University of Johannessburg.

Sebola, T.E., Ndinteh, D.T., Niemann, N., Mavumengwana, V., 2016b. Metal Analysis, Phytochemical and Antibacterial Investigation of Crinum Macowanii Bulb, in: International Conference on Advances in Science, Engineering, Technology and Natural Resources. https://doi.org/10.15242/iae.iae1116435

Sebola, T.E., Okereafor, N.C.U., Tapfuma, K.I., Mekuto, L., Green, E., Mavumengwana, V., 2019. Evaluating antibacterial and anticancer activity of crude extracts of bacterial endophytes from Crinum macowanii Baker bulbs. MicrobiologyOpen. 8, 1–10. https://doi.org/10.1002/mbo3.914

Seca, A.M.L., Pinto, D.C.G.., 2018. Plant Secondary Metabolites as Anticancer Agents : Successes in Clinical Trials and Therapeutic Application. Int. J. Mol. Sci. 19, 1–22. https://doi.org/10.3390/ijms19010263

Selim, M.H., Elshikh, H.H., Saad, M.M., Mostafa, E.E., Mahmoud, M.A., 2016. Purification and characterization of a novel thermo stable L-methioninase from Streptomyces sp. DMMMH4 and its evaluation for anticancer activity. J. Appl. Pharm. Sci. 6, 53–60. https://doi.org/10.7324/JAPS.2016.60708

Selim, M.H., Karm Eldin, E.-Z., Saad, M.M., Mostafa, E.-S.E., Shetia, Y.H., Anise, A.A.H., 2015. Purification, Characterization of L-Methioninase from Candida tropicalis , and Its Application as an Anticancer . Biotechnol. Res. Int. 2015, 1–10. https://doi.org/10.1155/2015/173140

Selvamohan, T., Shibila, V.R.S., Kishore, S., 2012. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Adv. Appl. Sci. Res. 3, 3374–3381.

Sen, A., Batra, A., 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant:melia azedarach L. Int. J. Curr. Pharm. Res. 4, 67–73.

Senyuva, H.Z., Gilbert, J., 2007. Rapid Analysis of Crude Fungal Extracts for Secondary Metabolites by LC / TOF-MS – A New Approach to Fungal Characterization Application. USA.

Shahidullah, M., Al-Mujahidee, M., Nasir Uddin, S.M., Shahadat Hossan, M., Hanif, A., Bari, S., Rahmatullah, M., 2009. Medicinal plants of the Santal tribe residing in Rajshahi district, Bangladesh. Am. J. Sustain. Agric. 3, 220–226.

Shakya, A.K., 2016. Medicinal plants : Future source of new drugs. Int. J. Herb. Med. 4, 59–64.

Shanmugam, G., Dubey, A., Ponpandian, L.N., Rim, S.O., 2018. Genomic Insights into Nematicidal Activity of a Bacterial Endophyte , Raoultella ornithinolytica MG against Pine Wilt Nematode. Plant Pathol. J. 34, 250–255.

Shiva Kameshwari, M.., B, M., Saraswathi.K.J, T., 2015. Isolation and identification of endophytic fungi from urginea indica , a medicinal plant from diverse regions of South India . Int. J. Latest Res. Sci. Technol. 4, 75–80.

Shrestha, P., Adhikari, S., Lamichhane, B., Shrestha, B.G., 2015. Phytochemical Screening of the Medicinal Plants of Nepal. J. Environ. Sci. Toxicol. Food Technol. 1, 11–17.

Singh, A., 2008. Phytochemicals of Gentianaceae : A Review of Pharmacological Properties. Int. J. Pharm. Sci. Nanotechnol. 1, 33–36.

Singh, S.K., 2015. Role of Endophytes in Pharmaceutical Industry. J. Microbiol. Biotechnol. 4, 6–7.

Sinha, T., 2018. Tumors: Benign and Malignant. Cancer Ther. & Oncol. Int. J. 10, 1–3. https://doi.org/10.19080/CTOIJ.2018.10.555790

Skibiński, R., Trawiński, J., 2017. Application of an untargeted chemometric strategy in the impurity profiling of pharmaceuticals: An example of amisulpride. J. Chromatogr. Sci. 55, 309–315. https://doi.org/10.1093/chromsci/bmw186

Sobiczewski, P., 2008. Bacterial diseases of plants : Epidemiology , diagnostics and control. Zemdirbyste-Agriculture 95, 151–157.

Solis, M.J.L., Yurkov, A., Cruz, T.E. dela, Unterseher, M., 2015. Leaf-inhabiting endophytic yeasts are abundant but unevenly distributed in three Ficus species from botanical garden greenhouses in Germany. Mycol. Prog. 14, 1–10. https://doi.org/10.1007/s11557-014-1019-6

Soltis, P.S., Soltis, D.E., 2003. Applying the Bootstrap in Phylogeny Reconstruction. Stat. Sci. 18, 256–267.

South African National Department of Health, 2017. National Cancer Strategic Framework 2017-2022. Pretoria, South Africa.

Stankovic, N., Senerovic, L., Ilic-Tomic, T., Vasiljevic, B., Nikodinovic-Runic, J., 2014. Properties and applications of undecylprodigiosin and other bacterial prodigiosins. Appl. Microbiol. Biotechnol. 98, 3841–3858. https://doi.org/10.1007/s00253-014-5590-1

Statistics South Africa, 2015. Millenium Development Goals: Country report 2015. Pretoria, South Africa.

Stierle, A.C., Cardellina, J.H., Strobel, G.A., 1988. Maculosin, a host-specific phytotoxin for spotted knapweed from Alternaria alternata. Proc. Natl. Acad. Sci. 85, 8008–8011. https://doi.org/10.1073/pnas.85.21.8008

Stolker, A.A.M., Niesing, W., Fuchs, R., Vreeken, R.J., Niessen, W.M.A., Brinkman, U.A.T., 2004. Liquid chromatography with triple-quadrupole and quadrupole-time-of-flight mass spectrometry for the determination of micro-constituents - A comparison. Anal. Bioanal. Chem. 378, 1754–1761. https://doi.org/10.1007/s00216-003-2485-x

Strobel, G., 2018. The Emergence of Endophytic Microbes and Their Biological Promise. J. Fungi 4, 1–9. https://doi.org/10.3390/jof4020057

Strobel, G., Daisy, B., 2003. Bioprospecting for Microbial Endophytes and Their Natural Products. Microbiol. Mol. Biol. Rev. 67, 491–502. https://doi.org/10.1128/MMBR.67.4.491

Strobel, G.A., 2003. Endophytes as sources of bioactive products. Microbes Infect. 5, 535–544.

Sudha, V., Govindaraj, R., Baskar, K., Al-dhabi, N.A., Duraipandiyan, V., 2016. Biological properties of Endophytic Fungi. BRAZILIAN Arch. Biol. Technol. AN 59, 1–7.

Sun, B., Chen, A., Gao, W., Zhou, Y., Liu, H., 2013. Endophytic fungi associated with the medicinal plant , Achyranthes bidentata Blume (Amaranthaceae). African J. Microbiol. Res. 7, 1357–1365. https://doi.org/10.5897/AJMR12.1461

Sun, X., Guo, L., 2012. Endophytic fungal diversity : review of traditional and molecular techniques. Mycology 31, 65–76.

Szarc Vel Szic, K., Op De Beeck, K., Ratman, D., Wouters, A., Beck, I.M., Declerck, K., Heyninck, K., Fransen, E., Bracke, M., De Bosscher, K., Lardon, F., Van Camp, G., Vanden Berghe, W., 2014. Pharmacological levels of Withaferin A (Withania somnifera) trigger clinically relevant anticancer effects specific to triple negative breast cancer cells. PLoS One 9, 1–17. https://doi.org/10.1371/journal.pone.0087850

Takos, A.M., Rook, F., 2013. Towards a Molecular Understanding of the Biosynthesis of Amaryllidaceae Alkaloids in Support of Their Expanding Medical Use. Int. J. Mol. Sci. 14, 11713–11741. https://doi.org/10.3390/ijms140611713

Tallini, L.R., Torras-Claveria, L., Borges, W. de S., Kaiser, M., Viladomat, F., Zuanazzi, J.A.S., Bastida, J., 2018. N-oxide alkaloids from crinum amabile (amaryllidaceae). Molecules 23, 1–11. https://doi.org/10.3390/molecules23061277

Tamošiune, I., Stanien, G., Haimi, P., Stanys, V., Rugienius, R., Baniulis*, D., 2018. Endophytic Bacillus and Pseudomonas spp . Modulate Apple Shoot Growth , Cellular Redox Balance ,

and Protein Expression Under in Vitro Conditions. Front. Plant Sci. 9, 1–14. https://doi.org/10.3389/fpls.2018.00889

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6 : Molecular Evolutionary Genetics Analysis Version 6 . 0 30, 2725–2729. https://doi.org/10.1093/molbev/mst197

Tan, J., Zheng, M., Duan, S., Zeng, Y., Zhang, Z., Cui, Q., Zhang, J., Hong, T., Bai, J., Du, S., 2018. Chemical Profiling and Screening of the Marker Components in the Fruit of Cassia fistula by HPLC and UHPLC/LTQ-Orbitrap MSn with Chemometrics. Molecules 23, 1–18. https://doi.org/10.3390/molecules23071501

Taneja, N., Sethi, S., Tahlan, A.K., Kumar, Y., 2019. Introductory Chapter: Stepping into the Post-Antibiotic Era—Challenges and Solutions, in: Antimicrobial Resistance - A Global Threat, Yashwant Kumar, IntechOpen. p. 13. https://doi.org/http://dx.doi.org/10.5772/57353

Tapfuma, K.I., Mekuto, L., Mellisa, M., Mavumengwana, V., 2019a. The LC-QTOF-MS / MS analysis data of detected metabolites from the crude extract of Datura stramonium leaves. Data Br. 25, 1–4. https://doi.org/10.1016/j.dib.2019.104094

Tapfuma, K.I., Uche-Okereafor, N., Sebola, T.E., Hussan, R., Mekuto, L., Makatini, M.M., Green, E., Mavumengwana, V., 2019b. Cytotoxic activity of crude extracts from Datura stramonium's fungal endophytes against A549 lung carcinoma and UMG87 glioblastoma cell lines and LC-QTOF-MS/MS based metabolite profiling. BMC Complement. Altern. Med. 19, 1–12. https://doi.org/10.1186/s12906-019-2752-9

Taylor, J.L.S., Elgorashi, E.E., Maes, A., Gorp, U. Van, Kimpe, N. De, Staden, J. Van, Verschaeve, L., 2003. Investigating the Safety of Plants Used in South African Traditional Medicine : Testing for Genotoxicity in the Micronucleus And Alkaline Comet Assays. Environ. Mol. Mutagen. 154, 144–154. https://doi.org/10.1002/em.10184

Tenorio-Salgado, S., Tinoco, R., Vazquez-Duhalt, R., Caballero-Mellado, J., Perez-Rueda, E., 2013. Identification of volatile compounds produced by the bacterium Burkholderia tropica that inhibit the growth of fungal pathogens. Bioengineered 4, 236–243.

Terhonen, E., Blumenstein, K., Kovalchuk, A., Asiegbu, F.O., 2019. Forest Tree Microbiomes and Associated Fungal Endophytes : Functional Roles and Impact on Forest Health. Forests 10, 1–33. https://doi.org/10.3390/f10010042

Thermo Scientific, 2013. Methods Optimization in Accelerated Solvent Extraction. https://doi.org/10.1080/09638288.2016.1189610

Thomas, L. V., Yu, S., Ingram, R.E., Refdahl, C., Elsser, D., Delves-Broughton, J., 2002. Ascopyrone P, a novel antibacterial derived from fungi. J. Appl. Microbiol. 93, 697–705. https://doi.org/10.1046/j.1365-2672.2002.01751.x

Tidke, S.A., Kumar, R.K.., Ramakrishna, D., Kiran, S., Kosturkova, G., Gokare, R.A., 2017. Current Understanding of Endophytes : Their Relevance , Importance , and Industrial Potentials. J. Biotechnol. Biochem. 3, 43–59. https://doi.org/10.9790/264X-03034359

Tiwari, P., Kumar, B., Mandeep, K., Kaur, G., Kaur, H., 2011. Phytochemical screening and Extraction: A Review. Int. Pharm. Sci. 1, 98–106.

Tiwaskar, M., Manohar, T., 2017. Rational for Antibiotics – Guidelines, in: Medicine Update 2017. pp. 6–15.

Torras-Claveria, L., Tallini, L., Viladomat, F., Bastida, J., 2017. Research in natural products : Amaryllidaceae ornamental plants as sources of bioactive compounds. Recent Adv. Pharm. Sci. VII 69–82.

Tram, N.T.N., Titorenkova, T. V., Bankova, S. V., Handjieva, N.V., Popov, S.S., 2002. Crinum L. Amaryllidaceae. Fitoterapia 73, 183–208.

Truong, D.-H., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H., Nguyen, H.C., 2019. Evaluation of the Use of Different Solvents for Phytochemical Constituents , Antioxidants , and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. J. Food Qual. 1–9.

Tsoukalas, N., Aravantinou-Fatorou, E., Baxevanos, P., Tolia, M., Tsapakidis, K., Galanopoulos, M., Liontos, M., Kyrgias, G., 2018. Advanced small cell lung cancer (SCLC): new challenges and new expectations. Ann. Transl. Med. 6, 1–8. https://doi.org/10.21037/atm.2018.03.31

Tugizimana, F., Piater, L., Dubery, I., 2013. Plant metabolomics: A new frontier in phytochemical analysis. S. Afr. J. Sci. 109, 1–11. https://doi.org/10.1590/sajs.2013/20120005

Tymon, L., Inglis, D., 2009. What is an Endophyte? https://doi.org/10.2307/2246769

Ullah, A., Mushtaq, H., Ali, U., Ali, E., Mubeen, S., 2018. Screening , isolation , biochemical and plant growth promoting characterization of endophytic bacteria. Microbiol. Curr. Res. 2, 61–68.

Uzma, F., Mohan, C.D., Hashem, A., Konappa, N.M., Rangappa, S., Kamath, P. V., Singh, B.P., Mudili, V., Gupta, V.K., Siddaiah, C.N., Chowdappa, S., Alqarawi, A.A., Abd_Allah, E.F., 2018. Endophytic Fungi — Alternative Sources of Cytotoxic Compounds : A Review. Front. Pharmacol. 9, 1–37. https://doi.org/10.3389/fphar.2018.00309

van Goietsenoven, G., Andolfi, A., Lallemand, B., Cimmino, A., Lamoral-theys, D., Gras, T.,

Abou-donia, A., Dubois, J., Lefranc, F., Kornienko, A., Kiss, R., Evidente, A., 2010. Amaryllidaceae Alkaloids Belonging to Different Structural Subgroups Display Activity against Apoptosis-Resistant Cancer Cells †. J. Nat. Prod. 73, 1223–1227.

Van Wyk, A.S., Prinsloo, G., 2018. Medicinal plant harvesting , sustainability and cultivation in South Africa. Biol. Conserv. 227, 335–342. https://doi.org/10.1016/j.biocon.2018.09.018

Venieraki, A., Dimou, M., Katinakis, P., 2017. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. Hell. Plant Prot. J. 10, 51–66. https://doi.org/10.1515/hppj-2017-0006

Ventola, C.L., 2015. The Antibiotic Resistance Crisis Part 1 : Causes and Threats 40, 277–283.

Venugopalan, A., Srivastava, S., 2015. Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol. Adv. 33, 873–887. https://doi.org/10.1016/j.biotechadv.2015.07.004

Verdoorn, I.C., 1973. The genus Crinum in southern Africa. Bothalia 11, 27–52.

Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S., Klaassen, C.H.W., Perrone, G., Seifert, K.A., Varga, J., Yaguchi, T., Samson, R.A., 2014. Identification and nomenclature of the genus Penicillium. Stud. Mycol. 78, 343–371. https://doi.org/10.1016/j.simyco.2014.09.001

Volkmann, M., Gorbushina, A.A., 2006. A broadly applicable method for extraction and characterization of mycosporines and mycosporine-like amino acids of terrestrial, marine and freshwater origin. FEMS Microbiol. Lett. 255, 286–295. https://doi.org/10.1111/j.1574-6968.2006.00088.x

Wade, L.. jnr, 2010. Amino acids, peptides, and proteins, in: Organic Chemistry 7th Edition. pp. 1153–1199.

Wang, H., Li, Y., Huang, Y., Zhao, C., Cheung, H., 2018. Chemical Profiling of Lobelia chinensis with High-Performance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry (HPLC/Q-TOF MS) Reveals Absence of Lobeline in the Herb. Mol. Ther. Oncolytics 23, 1–13. https://doi.org/10.3390/molecules23123258

Wang, J., Yue, Y. De, Tang, F., Sun, J., 2012. Screening and analysis of the potential bioactive components in rabbit plasma after oral administration of hot-water extracts from leaves of Bambusa textilis mcclure. Molecules 17, 8872–8885. https://doi.org/10.3390/molecules17088872

Wang, P., Yuan, H.-H., Zhang, X., Li, Y.-P., Shang, L.-Q., Yin, Z., 2014. Novel lycorine derivatives as anticancer agents: synthesis and in vitro biological evaluation. Molecules 19,

2469–2480. https://doi.org/10.3390/molecules19022469

Want, E.J., 2018. LC-MS Untargeted Analysis, in: Theodoridis, G.A., Gika, H.G., Wilson, I.D. (Eds.), Metabolic Profiling Methods and Protocols. Humana Press, New York, NY, U.S.A, pp. 99–116.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. Medicinal and Poisonous Plants, second. ed, Nature. E & S.Livingstone Ltd, London. https://doi.org/10.1038/196609b0

Widyawati, S.P., Budianta, T.D.W., Kusuma, F.A., Wijaya, E.L., 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia less leaves extracts. Int. J. Pharmacogn. Phytochem. Res. 6, 850–855.

Wietz, M., Månsson, M., Bowman, J.S., Blom, N., Ng, Y., Gram, L., 2012. Wide Distribution of Closely Related , Antibiotic-Producing Arthrobacter Strains throughout the Arctic Ocean. Appl. Environ. Microbiol. 78, 2039–2042. https://doi.org/10.1128/AEM.07096-11

Williams, V.L., Raimondo, D., Crouch, N.R., Cunningham, A.B., Scott-Shaw, C.R., Lötter, M., Ngwenya, A.M., Brueton, V.., 2016. Crinum macowanii Baker. National Assessment: Red List of South African Plants version 2017. [WWW Document]. South African Natl. Biodivers. Inst. Red List South African Plants. URL http://redlist.sanbi.org.www57.cpt1.host-h.net/species.php?species=2087-19 (accessed 9.18.19).

Williams, V.L., Victor, J.E., Crouch, N.R., 2013. Red Listed medicinal plants of South Africa: Status, trends, and assessment challenges. South African J. Bot. 86, 23–35. https://doi.org/10.1016/j.sajb.2013.01.006

Wina, E., Susana, I.W.R., Tangendjaja, B., 2010. Biological Activity of Tannins from Acacia mangium Bark Extracted by Different Solvents. Media Peternak. 33, 103–107. https://doi.org/10.5398/medpet.2010.33.2.103

World Health Organization, 2019. Don’t let tobacco take your breath away.

World Health Organization, 2002. WHO Traditional Medicine Strategy 2002-2005, World Health Organisation Geneva. https://doi.org/WHO/EDM/TRM/2002.1

Wu, W., Han, X., Wu, C., Wei, G., Zheng, G., Li, Y., Yang, Y., Yang, L., He, D., Zhao, Y., Cai, Z., 2018. Vernodalol mediates antitumor effects in acute promyelocytic leukemia cells. Oncol. Lett. 15, 2227–2235. https://doi.org/10.3892/ol.2017.7544

Xiao, J.F., Zhou, B., Ressom, H.W., 2012. Metabolite identification and quantitation in LC-MS/MS-based metabolomics. TrAC - Trends Anal. Chem. 32, 1–14.

https://doi.org/10.1016/j.trac.2011.08.009

Xie, G., Ni, Y., Su, M., Zhang, Y., Zhao, A., Gao, X., Liu, Z., Long, X., Jia, W., 2008. Application of Ultra-Performance LC-TOF MS Metabolite Profiling Techniques to the Analysis of Medicinal Panax Herbs. Metabolomics 4, 248–260.

Xie, J., Wu, Y., Zhang, T., Zhang, M., Zhu, W., Gullen, E.A., Wang, Z.-J., Cheng, Y.-C., Zhang, Y.-X., 2017. New and bioactive natural products from an endophyte of Panax notoginseng †. R. Soc. Chem. Adv. 7, 38100–38109. https://doi.org/10.1039/c7ra07060h

Xu, J., 2010. Secondary Metabolites from the Endophytic Fungus Pestalotiopsis sp. JCM2A4 and its Microbe-Host Relationship with the Mangrove Plant Rhizophora mucronata. Heinrich-Heine-Universität Düssedldorf.

Xu, Z., Chang, L., 2017. Amaryllidaceae, in: Identication and Control of Common Weeds: Volume 3. Springer Nature, Gateway East, Singapore, pp. 877–886.

Xue, J.H., Xu, L.X., Jiang, Z.H., Wei, X., 2012. Quinazoline Alkaloids from Streptomyces michiganensis. Chem. Nat. Compd. 48, 839–841. https://doi.org/10.1007/s10600-012-0397-8

Yadav, R.N.S., Agarwala, M., 2011. Phytochemical analysis of some medicinal plants. J. Phytol. 3, 10–14. https://doi.org/10.1021/np800144q

Yaish, M.W., 2016. Draft Genome Sequence of Endophytic Bacterium Enterobacter asburiae PDA134 , Isolated from Date Palm (Phoenix dactylifera L .) Roots. Genome Announc. 4, 1–2. https://doi.org/10.1128/genomeA.00848-16.Copyright

Yakandawala, D.M.D., Samarakoon, T.M., 2006. An Empirical Study on the Taxonomy of Crinum Zeylanicum (L.) L. and Crinum Latifolium L. (Amaryllidaceae) Occurring in Sri Lanka. Ceylon J. Sci. (Biological Sci. 35, 53–72.

Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., Wang, Q., 2018. Response of Plant Secondary Metabolites to Environmental Factors. Molecules 23, 1–26. https://doi.org/10.3390/molecules23040762

Yoshioka, T., Wada, T., Uchida, N., Maki, H., Yoshida, H., Ide, N., Kasai, H., Hojo, K., Shono, K., Maekawa, R., Yagi, S., Hoffman, R.M., Sugita, K., 1998. Anticancer efficacy in vivo and in vitro, synergy with 5-fluorouracil, and safety of recombinant methioninase. Cancer Res. 58, 2583–2587.

Yuan, H., Ma, Q., Ye, L., Piao, G., 2016. The Traditional Medicine and Modern Medicine from Natural Products. Molecules 21, 2–18. https://doi.org/10.3390/molecules21050559

Yusnawan, E., 2013. The Effectiveness of Polar and Non Polar Fractions of Ageratum Conyzoides L . To Control Peanut Rust Disease and Phytochemical Screenings of Secondary Metabolites. J. Hama dan Penyakit Tumbuh. Trop. 13, 159–166.

Zanders, E.D., Svensson, F., Bailey, D.S., 2019. Therapy for glioblastoma: is it working? Drug Discov. Today 24, 1193–1201. https://doi.org/10.1016/j.drudis.2019.03.008

Zeng, W., Li, W., Han, H., Tao, Y., Yang, L., Wang, Z., Chen, K., 2014. Microbial Biotransformation of Gentiopicroside by the Endophytic Fungus Penicillium crustosum 2T01Y01. Appl. Environ. Microbiol. 80, 184–192. https://doi.org/10.1128/AEM.02309-13

Zhang, J., Chen, Z., Huang, X., Shi, W., Zhang, R., Chen, M., Huang, H., Wu, L., 2019. Insights on the Multifunctional Activities of Magnolol. Biomed Res. Int. 2019, 1–15. https://doi.org/10.1155/2019/1847130

Zhang, M., Das, C., Vasquez, H., Aguilera, D., Zage, P.E., Gopalakrishnan, V., Wolff, J.E., 2006. Predicting Tumor Cell Repopulation After Response : Mathematical Modeling of Cancer Cell Growth. Anticancer Res. 26, 2933–2936.

Zhao, J., Ma, J., 2016. Phytochemicals and Biological Activities of the Genus Cinnamomum. J. Pharmacogn. Phytochem. 4, 27–34.

Zheng, M., 2016. Classification and Pathology of Lung Cancer. Surg. Oncol. Clin. N. Am. 25, 447–468. https://doi.org/10.1016/j.soc.2016.02.003

Zhou, Yunfei, Zhou, Yan, Shingu, T., Feng, L., Chen, Z., Ogasawara, M., Keating, M.J., Kondo, S., Huang, P., 2011. Metabolic Alterations in Highly Tumorigenic Glioblastoma. J. Biol. Chem. 286, 32843–32853. https://doi.org/10.1074/jbc.M111.260935

Zhu, Z., Schultz, A.W., Wang, J., Johnson, C.H., Yannone, S.M., Patti, G.J., Siuzdak, G., 2013. Liquid chromatography quadrupole time-of-flight mass spectrometry characterization of metabolites guided by the METLIN database. Nat. Protoc. 8, 451–460. https://doi.org/10.1038/nprot.2013.004

Zinniel, D.K., Lambrecht, P., Harris, N.B., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C.A., Arunakumari, A., Barletta, R.G., Vidaver, A.K., 2002. Isolation and Characterization of Endophytic Colonizing Bacteria from Agronomic Crops and Prairie Plants †. Appl. Environ. Microbiol. 68, 2198–2208. https://doi.org/10.1128/AEM.68.5.2198

Zonyane, S., Makunga, N.P., Vuuren, S.F. Van, 2013. The antimicrobial interactions of Agathosma crenulata, Dodonaea viscosa and Eucalyptus globulus combination and their chemical profiling. Stellenbosch University.

Zubair, M.S., Anam, S., Lallo, S., 2016. Cytotoxic activity and phytochemical standardization of Lunasia amara Blanco wood extract. Asian Pac. J. Trop. Biomed. 6, 962–966. https://doi.org/10.1016/j.apjtb.2016.04.014

CHAPTER THREE:

ISOLATION AND IDENTIFICATION OF CULTURABLE MICROFLORA AND/OR MYCOFLORA ENDOPHYTES FROM *CRINUM MACOWANII* BAKER*

* This chapter presents the isolation, morphological and molecular identification of endophytes from *C. macowanii* bulbs and leaves.

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Abstract

Crinum macowanii is a medicinal plant native to east and southern Africa with a variety of uses. The medicinal properties of the bulbs and leaves have led to the over-harvesting and exploitation of the plant, and alternative plant part substitution needs to be studied to prevent the extinction of this resource. This study was aimed at isolating and characterizing endophytic microflora and/or mycoflora from *C. macowanii*. The bulbs and leaves were surface sterilized using validated techniques and the isolated culturable endophytes were identified using morphological data and molecular techniques (internal transcribed spacer (ITS) region for fungi and 16S rRNA for bacteria). The following bacterial endophytes were successfully isolated and identified, *Acinetobacter guillouiae*, *Pseudomonas moraviensis*, *Pseudomonas* sp., *Rahnella aquatilis*, *Bacillus cereus*, *Novosphingobium* sp., *Raoultella ornithinolytica*, *Burkholderia tropica*, *Pseudomonas palleroniana*, *Pseudomonas putida*, *Bacillus safensis*, *Enterobacter asburiae*, *Pseudomonas cichorii* and *Arthrobacter pascens*. *Filobasidium magnum*, *Alternaria alternata*, *Penicillium chrysogenum*, *Alternaria tenuissima* and *Penicillium* sp. were the fungal endophytes isolated and characterized. From the results obtained, both bacteria and fungi co-exist in the bulbs and leaves of *C. macowanii*. To the best of our knowledge, this is the first report on the endophytic mycoflora in *C. macowanii*. Further studies on the isolation of bioactive secondary metabolites should be conducted.

Keywords: *C. macowanii*, Endophytes, Medicinal plant, Phylogenetic analysis, Microflora, Mycoflora

3.1 Introduction

Endophytes are microorganisms that reside within plant tissues which do not harm the host plant or provoke any manifestation of disease (Rhoden *et al.*, 2015; Venugopalan and Srivastava, 2015; Martinez-Klimova *et al*., 2017). Endophytic microorganisms have an endosymbiotic relationship with the host plant (Abdalla and Mcgaw, 2018). The microbes gain access to plant tissues located between the cell wall and membrane through tissue wounds, stomata, lenticels, root cracks, production of cell wall degradative enzymes and penetration via root hair cells (Ali *et al.*, 2014; Abdalla and Mcgaw, 2018).

Endophytes have been isolated from the surface-sterilized tissues of different plant parts, including roots, stems, leaves, flowers, fruits, and seeds (Strobel, 2018).The population make up of endophytes is higher in roots than other aboveground tissues found in stems and leaves (Mercado-blanco and Lugtenberg, 2014; Anjum and Chandra, 2015). Martín-García *et al.* (2011) and Jia *et al.* (2016) reported that the age of the host plant, geographic location and abiotic factors such as temperature, humidity and illumination influence the distribution pattern of endophytes and impact the type of endophytic communities found. Interest in endophytes has been growing exponentially due to endophytic secondary metabolites utilization for natural products in medicine and agriculture (Strobel and Daisy, 2003; Fouda *et al.*, 2015).

Endophytes have been reported to be repositories of novel natural bioactive compounds used as antiarthritic, antimicrobial, anticancer, antidiabetic, anti-insect, and immunosuppressant agents (Baker and Satish, 2012; Singh, 2015). The use of alternative sources such as plants has proven to have drawbacks such as overharvesting and extinction (Monakisi *et al*., 2007).

Crinum macowanii Baker is a medicinal plant from the *Amaryllidaceae* family and has been used for a number of applications including but not limited to treating itchy rashes, boils, acne, backache, venereal disease, inflamed sores, swelling of the body and urinary tract problems, as well as to increase lactation in women and cows (Watt and Breyer-Brandwijk, 1962; Nair *et al*., 2000; Elgorashi *et al*., 2003; Maroyi, 2016). According to the literature surrounding this topic, *C. macowanii* has been extensively harvested for its medicinal properties, and therefore substitute approaches should be used to obtain the bioactive compounds similar to those obtained from the plant parts. The aim of this study was to isolate and identify culturable endophytes from *C. macowanii* bulbs and leaves.

3.2 Materials and methods

3.2.1 Sample collection

Fresh, healthy *C. macowanii* bulbs and leaves showing no apparent symptoms of disease or herbivore damage were collected from the Walter Sisulu National Botanical Garden (Roodepoort, Gauteng, South Africa, 26°05'10.4"S 27°50'41.5"E). Mr. Solomon Nenungwi from the South African National Biodiversity Institute formally identified the plant material used in this study and a voucher specimen (BTNST02) was deposited in the herbarium at the University of Johannesburg. After harvesting the samples were placed in sterile polyethylene bags at 4°C for storage and transferred to the laboratory at the University of Johannesburg before being thoroughly washed with sterile distilled water and used within hours of harvesting.

3.2.2 Isolation of endophytic microflora and/or mycoflora

The bulbs and leaves were surface-sterilized separately using the method described by Jasim *et al*. (2014) with slight modifications. The samples (bulbs and leaves) were cut separately into small pieces. Briefly, each sample (approximately 10 g) was treated with 5% Tween 20 (Sigma-Aldrich, South Africa) (enough to cover the plant material) and vigorously shaken for 5 minutes. Tween 20 was removed by rinsing several times with sterile distilled water, followed by disinfection with 50 ml of 70% ethanol for 1 minute. Traces of the ethanol were removed by rinsing with sterile distilled water five times. The sample was then treated with 1% Sodium Hypochlorite (NaClO) for 10 minutes and again rinsed five times with sterile distilled water. The last rinse was used as a control and 100 µL of this was plated on Potato Dextrose agar (PDA) (HiMedia, USA) and Nutrient Agar (NA) (Oxoid, USA). The sample was then macerated in sterilized phosphate buffered saline (PBS). The macerated sample was serially diluted up to 10^{-3} dilution, and each dilution inoculated (using a spread plate method) in triplicate on NA and PDA. The NA plates were incubated at 25ºC (IncoTherm, Labotec, Johannesburg, South Africa) and the PDA plates were incubated at 30ºC (IncoTherm, Labotec, Johannesburg, South Africa). Growth was monitored periodically for 5 days for the NA plates and 7 days for the PDA plates. Effectiveness of the sterilization was monitored on the wash control plate, with growth indicating poor sterilization. Under such circumstances, the plates for the plant part were discarded and the sterilization repeated. Distinct colonies were selected and subcultured in suitable media (NA for bacteria and PDA for fungi) to obtain pure isolates. Pure fungal isolates were preserved by cultivating them on sterile filter paper and storing at 4ºC. This was repeated every two months to maintain the purity and viability of the isolates

(Morales, 2008). The preservation of pure bacterial isolates was done according to a method by Joshi and Nongkhlaw (2015) with modification, briefly, the isolates were placed in 50% glycerol at a ratio of 500 µL glycerol:500 µL overnight broth culture and kept at -80ºC.

3.2.3 Morphological identification of endophytic microflora and/or mycoflora

3.2.3.1 Endophytic microflora

Pure colonies were subjected to Gram staining to establish morphological characteristics such as shape and Gram stain reaction. Gram stain slides were observed using a compound bright-field microscope (OLYMPUS CH20BIMF200) with 1000x magnification.

3.2.3.2 Endophytic mycoflora

The identification of the fungal isolates was determined by macroscopic and microscopic studies as previously described (Navi *et al*.,1999; Pitt and Hocking,2009). Briefly, each isolate was grown on PDA agar for five days at 25ºC. After incubation, phenotypic characteristics such as the colony colour on the reverse and front, colony diameter, mycelia and the conidia were analyzed. For the microscopic characteristics, a wet mount for each isolate was prepared using lactophenol cotton blue stain. The microscopic-morphological character analyzed were the conidia, vesicles, stipes and philiades and metulae. A drop of the stain was placed onto a microscope to form a suspension with needle picked growth of the isolate. The suspension was covered with a cover slip and viewed under a light microscope (BX51, Ultra 20 soft imaging system (Olympus, Japan).

3.2.4 Molecular identification

3.2.4.1 Extraction of genomic DNA

Pure colonies of each isolate obtained from the NA and PDA plates were inoculated into nutrient broth and grown overnight at 30°C. Cultures were centrifuged at 13000 x g for 5 minutes and the supernatant discarded. DNA extraction was done using a ZR Fungal/Bacterial Kit™ (Zymo Research, catalog NO R2014) according to the manufacturer's instructions.

3.2.4.2 Polymerase chain reaction and sequencing

Polymerase chain reaction (PCR) was done to amplify the 16S rRNA gene and the Internal Transcribed Spacers (ITS) regions. For the bacterial endophyte, the following primers were used, 16S-27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 16S-1492R:5'-CGGTTACCTTGTTACGACTT-3', and the for the fungal endophytes, the primers used were ITS1 5'TCCGTAGGTGAACCTGCGG 3' and ITS4 5' TCCTCCGCTTATTGATATGC 3', using DreamTaq™ DNA polymerase (Thermo Scientific™). PCR products were gel extracted (Zymo Research, Zymoclean™ Gel DNA Recovery Kit) and sequenced in the forward and reverse directions on the ABI PRISM™ 3500xl Genetic Analyser. The PCR products were cleaned with ExoSAP-it™ following the manufacturer's recommendations. Purified sequencing products (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™) were analyzed using CLC Main Workbench 7, followed by a BLAST search (NCBI) (Kuklinsky-Sobral *et al*., 2005). The sequencing was performed at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

3.2.5 Phylogenetic analysis

The obtained sequences were screened for chimeras using DECIPHER23 and subjected to BLAST analysis using the National Center for Biotechnology Information (NCBI) database against the 16S rDNA sequence database (bacteria and archaea) to identify the closest related species. Species with 98–100% similarities were selected for phylogenetic analysis. Alignments of nucleotide sequences were performed using MUSCLE with default options. The positions containing gaps or missing nucleotide data were eliminated. Phylogenetic trees were constructed using a Neighbour-Joining (NJ) method (Saitou and Nei, 1987) based on the Tamura-Nei model (Tamura *et al*., 2013). A total of 1000 replications were used for bootstrap testing. All branches with greater than 50% bootstraps were considered to be significant (Soltis and Soltis, 2003). All evolutionary analyses were conducted in MEGA 7.0 (Kumar *et al*., 2016). The 16S rRNA (bacteria) and the ITS regions (fungi) of the isolates identified in the study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with the accession numbers as stated in Tables 3.1 and 3.2. The assigned names of the isolates were based on the BLAST homology percentages as well as the phylogenetic results.

3.3 Results and Discussion

3.3.1 Isolation of endophytic microflora and/or mycoflora

3.3.1.1 Isolation of endophytic microflora from the bulbs and leaves

Very few plants have been explored for their endophytic variety as most are found in distinct biological niches (Shiva Kameshwari *et al*., 2015). As the control plates did not reveal any fungal/bacterial growth, it was concluded that the isolates reported were true endophytes of the plant under study. In this study, a total of 8 bacterial endophytes Table 3.1 were isolated from the bulb of *C. macowanii*, with 9 bacterial endophytes isolated from the leaves of *C. macowanii* and 5 fungal endophytes isolated from both the leaves and bulbs of *C. macowanii* Table 3.2.

Table 3.1: The identities and morphological characteristics of the isolated bacterial endophytes from *C. macowanii* bulbs and leaves

Plant part	Sample code	Assigned bacterial Name	GenBank accession number	Similarity (%)	Gram reaction	Colony morphology (pigmentation, texture, form)
Bulbs	TES 01C	*Acinetobacter guillouiae*	MF943224	99	- rod	Faint yellow, viscid, circular
	TES 01E	*Pseudomonas moraviensis*	MF943225	100	- rod	Faint yellow, mucoid, circular
	TES 01F	*Pseudomonas* sp.	MF943226	88	- rod	Cream white, mucoid, circular
	TES 03A	*Rahnella aquatilis*	MF943229	99	- rod	Cream white, pasty, rhizoid
	TES 03B	*Bacillus cereus*	MF943230	99	+ rod	Cream white, moist, rhizoid
	TES 03C	*Bacillus cereus*	MF943231	99	+ rod	Cream white, moist, rhizoid
	TES 04A	*Novosphingobium* sp.	MF943232	99	- rod	Cream white, viscid, circular
	TES 09B	*Rahnella aquatilis*	MF943238	99	- rod	Pale yellow, viscid, circular
	TES 11A	*Raoultella ornithinolytica*	MF943240	99	- rod	Cream white, mucoid, filamentous
	TES 12A	*Burkholderia tropica*	MF943241	98	- rods	Pale yellow, viscid, circular
	TES 13A	*Rahnella aquatilis*	MF943242	100	- rods	Pale white, rugose, filamentous
Leaves	TES 02B	*Raoultella ornithinolytica*	MF943227	99	- rod	Cream white, moist, circular
	TES 02C	*Acinetobacter guillouiae*	MF943228	100	- rod	Cream white, dry, circular
	TES 05A	*Pseudomonas* sp.	MF943233	99	- rods	White, dry, filamentous
	TES 05B	*Pseudomonas palleroniana*	MF943234	100	- rods	Pale yellow, viscid, filamentous
	TES 06A	*Pseudomonas putida*	MF943235	99	- rods	Milky white, moist, circular

TES 07A	*Bacillus safensis*	MF943236	100	+ rod	Milky white, viscoid, circular
TES 07B	*Bacillus safensis*	MF943237	100	+ rod	White, dry, circular
TES 10A	*Enterobacter asburiae*	MF943239	99	- rod	Pale yellow, moist, filamentous
TES 14A	*Pseudomonas cichorii*	MF943243	99	- rods	Yellow, moist, circular
TES 15A	*Arthrobacter pascens*	MF943244	99	+ rods	Cream white, viscid, circular

3.3.1. Isolation of endophytic mycoflora from the bulbs and leaves

Table 3.2: The identities and morphological characteristics of the isolated fungal endophytes from *C. macowanii* bulbs and leaves

Sample code	Assigned fungal name	GenBank accession number	Plant tissue	ITS Similarity (%)	Taxonomic classification (Division, Family)
TES 01A	*Filobasidium magnum*	MF925700	Bulb	100	Basidiomycota, Filobasidiaceae
TES 01B	*Alternaria alternata*	MF925701	Bulb	100	Ascomycota, Pleosporaceae
TES 02A	*Filobasidium magnum*	MF925702	Leaves	100	Basidiomycota, Filobasidiaceae
TES 04B	*Penicillium chrysogenum*	MF925703	Bulb	100	Ascomycota, Trichocomaceae
TES 05C	*Alternaria tenuissima*	MF925704	Leaves	100	Ascomycota, Pleosporaceae
TES 08A	*Penicillium chrysogenum*	MF925705	Bulb	100	Ascomycota, Trichocomaceae
TES 09A	*Penicillium* sp.	MF925706	Bulb	95	Ascomycota, Trichocomaceae

3.3.2 Morphological identification of endophytes from *C. macowanii*

Endophytic bacteria are an unexplored yet diverse group of micro-organisms with a symbiotic association with plants, and are promising sources of biologically active agents (Raghu, 2012). Ullah *et al*. (2018) reported that roots and bulbs harbour a greater number of endophytes with a diverse population than other parts of the plant. This was not the case in this study, as both the bulbs and leaves harboured diverse endophytes and in greater numbers.

Micro-morphological analysis of the culturable bacteria endophytes showed that nine Gram-negative rods and two Gram-positive rods were extracted from the bulbs and seven Gram-negative rods three Gram-positive rods were derived from the leaves. Pigmentation of the

different colonies varied from faint yellow, cream white, pale yellow, pale white, cream white, milky white and white. The texture of the endophyte colonies were either viscid, mucoid, pasty, moist, rugose or dry. Circular, rhizoid and filamentous were the observed form of the endophytic colonies. All isolated bacterial endophytes were rod shaped, with more Gram-negative than Gram-positive isolates. Ngoma *et al*. (2014) reported that more Gram-negative bacterial endophytes are regularly isolated than Gram-positive bacterial endophytes. This would support the results observed in this study. Biotic and abiotic factors have been reported to influence the type of endophytic communities found. In contrast, host genotype has been deemed to be the foremost influencer of the endosphere as a whole (Terhonen *et al*., 2019). This would explain the morphology and Gram reactions of the isolated bacterial endophytes.

From the results obtained, seven fungal endophytes were isolated, two from the leaves (TES02A and TES05C) and five from the bulbs (TES01A, TES01B, TES04B, TES08A and TES09A). The macroscopic illustrations of the isolates (Figure 3.1) displayed different phenotypic observations. To the best of our knowledge, this is the first report on the isolation of endophytic fungi from *C. macowanii*.

The following macroscopic observations were seen on the PDA plates: isolate TES01B exhibited a green colony colour with pale black and wrinkled mycelia, while isolate TES04B showed a green to grey colour in the centre with a white colour on the edges, and isolate TES08A displayed a green colony colour with a woolly white centre which was covered mycelium. Isolate TES05C grew slowly, and colonies appeared as white and woolly with a yellow centre and the margins appeared in filiform with a raised elevation and a filamentous form, whereas isolate TES09A grew rapidly with a green colony colour with brown and glaucous edges and wrinkled mycelia with a velvety texture. Isolate TES01A grew rapidly, showing a grey and dark green colony colour with a velvety texture and an irregular form and a flat elevation and undulate margins. TES02A colonies were cream white in colour with smooth and slimy texture, with an irregular form and a flat elevation and entire margins.

Fungi are known to produce pigments such as carotenoids, melanins, flavins, phenazines, quinones via the polyketide synthase and the mevalonate pathway (da Costa Souza *et al.*, 2016; Narsing Rao *et al*., 2017; Pombeiro-Sponchiado *et al.*, 2017), and this would explain the different colony colours observed.

The lactophenol blue stain revealed that isolate TES01B was non-filamentous, while isolates TES04B and TES08A were both heavily sporulating. Isolate TES05C and TES09A were filamentous and isolates TES01A and TES02A both had ovoidal conidia in short chains. Isolate TES02A colonies appeared more like bacterial colonies, suggesting that TES02A might be a yeast and not a mould, as the cells were unicellular and no true hyphae was observed, and it appeared white and thread-like (Powers-Fletcher *et al*., 2016).

The observed micromorphology characteristics alone cannot be used to successfully identify and characterize fungal species and therefore molecular techniques such as DNA sequencing have to be used to correctly identify the isolated fungal endophytes (Sun and Guo, 2012).

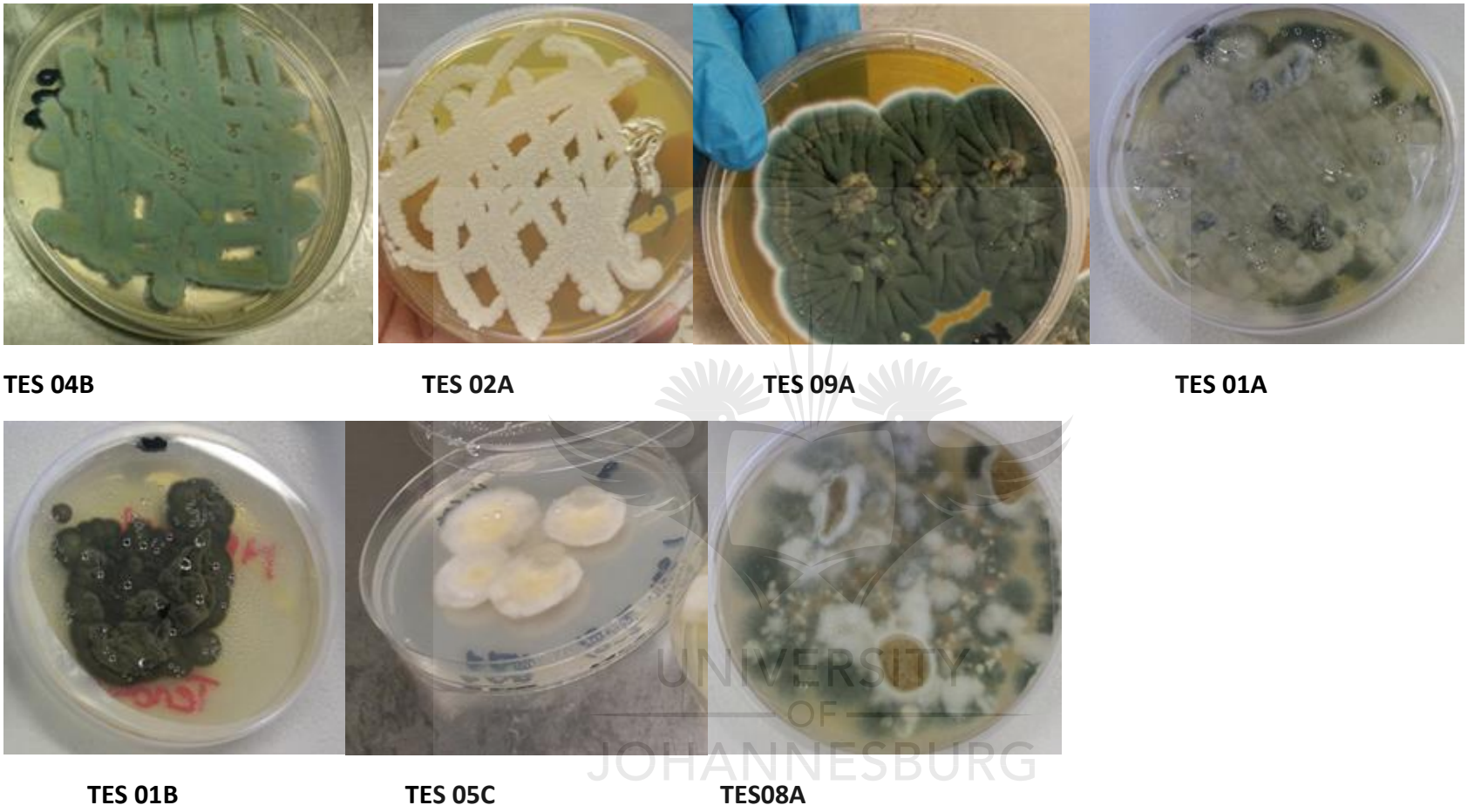

Figure 3.1: Macroscopic illustrations portraying the morphology of the isolated fungal endophytes

3.3.3 Phylogenetic relationship of endophytes from *C. macowanii*

3.3.3.1 Phylogenetic relationship of bacteria endophytes from *C. macowanii*

The BLAST search results of the 16S rDNA gene sequence revealed that the isolated endophytes belong to diverse bacterial genera such as *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Rahnella*, *Novosphingobium*, *Raoultella*, *Arthrobacter*, *Burkholderia* and *Enterobacter* as seen in Figure 3.2. Menpara & Chanda (2013) and Rhoden *et al.* (2015) reported that *Pseudomonas*, *Acinetobacter*, *Staphylococcus*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pantoea* and *Agrobacterium* are the most predominant endophytes in medicinal plants, which supports our observed results.

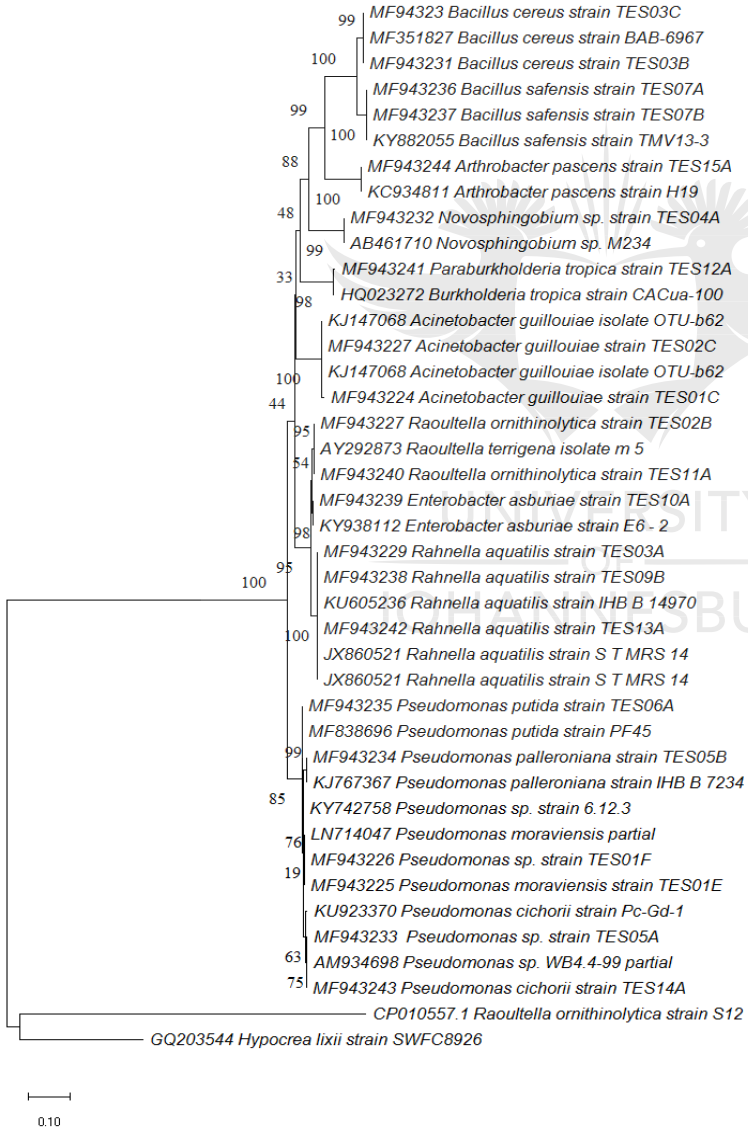

Figure 3.2: Neighbour-joining tree based on 16S rRNA gene sequence of ten endophytic bacteria isolated from *C. macowanii* bulb and leaves and other similar species selected from GenBank

Sequence identity of two of our isolates TES03B and TES03C showed a 99% similarity to *Bacillus cereus* BAB-6967. Rhoden *et al.* (2015) reported that *Bacillus* endophytes have been isolated from sunflowers, potatoes and cotton, and they assist host plants in phosphate solubilization and auxin production. Our endophytic isolates TES07A TES07B displayed a 100% similarity to *Bacillus safensis* TMV13-3. *Bacillus* spp. endophytes have been previously isolated from *C. macowanii* bulbs (Morare *et al*., 2018). TES01C and TES02C displayed a sequence identity of 100% to *Acinetobacter guillouiae* OTU-b62. Different *Acinetobacter* species have been isolated from potato cultivars (Ramírez-Bahena *et al*., 2014). Morare *et al*. (2018) reported the isolation of *Acinetobacter* spp. from *C. macowanii* bulbs. This supports our observed results.

TES15A isolates revealed a 100% similarity to *Arthrobacter pascens* H19. Nalini and Prakash (2017) reported the isolation of *Arthrobacter* spp from ethnomedicinal plants in Southern India. A 99% similarity was observed between our isolate TES04A and *Novosphingobium* sp. M234. Endophytic *Novosphingobium* sp. has been reported to grow in rice plants and promotes the growth of rice (Rangjaroen *et al*., 2017). Isolates TES02B and TES11A had a similarity of 95% to *Raoultella terrigena* m 5. Endophytic *Raoultella ornithinolytica* has been isolated from mountain-cultivated ginseng plants (Shanmugam *et al*., 2018). A 98% similarity was observed between our isolate TES10A and *Enterobacter asburiae* E6 – 2. Yaish, (2016) reported that endophytic *Enterobacter asburiae* has been isolated from date palm and that it promotes plant growth. Endophytic isolates TES03B, TES09B and TES13A displayed a 100% similarity with *Rahnella aquatilis* IHB B 14970 and *Rahnella aquatilis* S T MRS 14. *Rahnella aquatilis* endophytes have been isolated from wheat and oak and promote plant growth (Mercado-Blanco and Lugtenberg, 2014). Our isolate TES12A had a 98% similarity to *Burkholderia tropica* CACua-100. *Burkholderia tropica* species are known to escalate plant nutrient availability through nitrogen fixation or phosphate solubilization (Tenorio-Salgado *et al.*, 2013).

To the best of our knowledge, this is the first report on the isolation of *Arthrobacter pascens Burkholderia tropica, Raoultella ornithinolytica, Enterobacter asburiae, Rahnella aquatilis* and *Novosphingobium* sp. from *C. macowanii*.

Pseudomonas are common bacteria associated with plants that have been isolated from a number of plants species and tissues, displaying positive effects on host plant growth such as reducing drought stress and producing plant hormones such as 1-aminocyclopropane-1-carboxylic acid (ACC) and Indole-3-acetic acid (IAA) and acting as biocontrol agents (Jasim *et al.*, 2014; Rhoden *et al.*, 2015; Eljounaidi *et al*, 2016; Tamošiune *et al.*, 2018). Isolate TES01F displayed a 76% similarity with *Pseudomonas* sp. strain 6.12.3, whereas isolate TES05A showed a 63% similarity with *Pseudomonas* sp. WB4.4-99.

Isolate TES06A displayed a 57% similarity with *Pseudomonas putida* PF45. Asif *et al.* (2016) reported on the isolation of endophytic *Pseudomonas putida* from mango orchards. Isolate TES05B showed a 99% similarity with *Pseudomonas palleroniana* IHB B 7234, and isolate TES01E displayed a 19% similarity with *Pseudomonas moraviensis* partial. Endophytes *Pseudomonas palleroniana* and *Pseudomonas moraviensis* have been isolated from bananas and have been reported to fix free nitrogen, solubilize phosphates and produce siderophores *in vitro* (Ngamau *et al*., 2012). Endophytic isolate TES14A showed a 75% similarity with *Pseudomonas cichorii* Pc-Gd-1. Ramírez-Bahena *et al*. (2014) reported the isolation of endophytic *Pseudomonas cichorii* from potato cultivar. To the best of our knowledge *Pseudomonas* have not been recorded in *C. macowanii* prior to this study.

Even though most of the isolated endophytes are known to be plant pathogens, such as *Pseudomonas* and *Arthrobacter* (Sobiczewski, 2008), Kandel *et al*. (2017) reported that *Acinetobacter, Bacillus, Burkholderia, Enterobacter, Pseudomonas* and *Rahnella* colonize and co-exist with host plants, and this was observed in our results.

3.3.3.2 Phylogenetic relationship of fungal endophytes from *C. macowanii*

The BLAST search of the 16S rRNA gene sequences resulted in varying fungal genera; the isolates were classified as three genera, namely *Penicillium, Alternaria* and *Filobasidium,* as seen in Figure 3.3. Fungal endophytes have positive effects on host plants, such as improving drought tolerance, plant growth and producing protective compounds, and their isolation and identification is significant (Egan *et al.*, 2016; Khan *et al.*, 2017). Strobel (2018) reported that most fungal genera

are known to be plant pathogens, which can also be isolated as endophytes that do not cause any disease to the host plant.

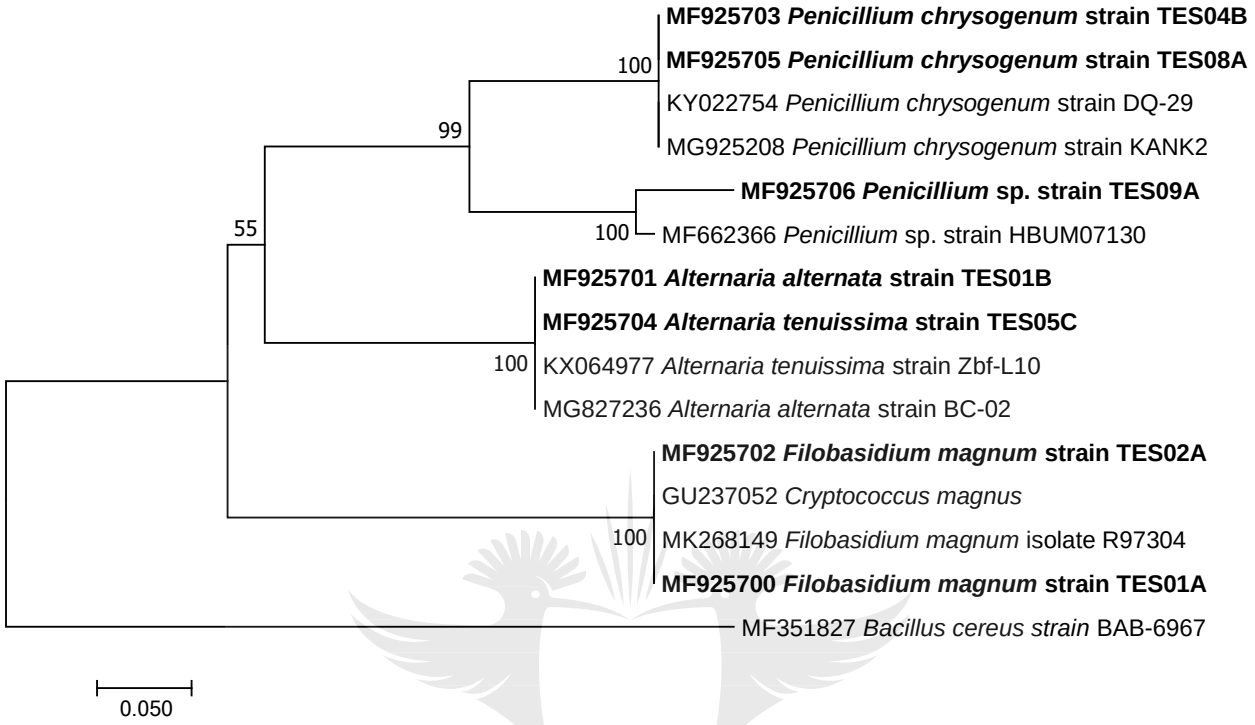

Figure 3.3: Neighbour-joining tree based on 16S ITS gene sequence of endophytic fungi isolated from *C. macowanii* bulbs and leaves and other similar species selected from GenBank

Fungal isolates TES04B and TES08A displayed a 100% similarity with *Penicillium chrysogenum* strain DQ-29 and *Penicillium chrysogenum* strain KANK2, respectively. Endophytic *Penicillium chrysogenum* has been isolated from the medicinal plant *Asclepias sinaica* (Fouda *et al*., 2015). The combination of the secondary and primary identification of TES04B and TES08A strongly suggests that this isolate is *Penicillium chrysogenum*. This is supported by the report of Pitt and Hocking (2009). Isolate TES09A showed a 100% similarity with *Penicillium* sp. strain HBUM07130. Xie *et al.*, (2017) describe the isolation of endophytic fungus *Penicillium* sp from *Panax notoginseng*. The macromorphology , micromorphology and sequence marker (ITS) concur with previous studies (Visagie *et al*., 2014) and strongly suggest that the isolate TES09A is *Penicillium* sp.

Isolates TES01B and TES05C displayed a 100% similarity with *Alternaria alternata* strain BC-02 and *Alternaria tenuissima* strain Zbf-L10, respectively. *Alternaria tenuissima* has been isolated from the leaves of the medicinal plant *Ricinus communis* (Sardul Singh *et al*., 2014). This correlates with our results in that *Alternaria tenuissima* TES05C was isolated from the leaves of *C. macowanii*. Endophytic *Alternaria alternate* have been isolated from a number of plants such as the medicinal trees *Aegle marmelo*, *Schinus terebinthifolius* and the medicinal plant *Indigofera* 'enneaphylla' (Martinez-Klimova *et al*., 2017). The molecular and morphological identification of TES01B suggest that this isolate is *Alternaria alternate* and the TES05C isolate is *Alternaria tenuissima*. This supported by the report of Pitt and Hocking, (2009) and Navi *et al.* (1999).

Isolates TES02A and TES01A showed a 100 % similarity with *Cryptococcus magnus* and *Filobasidium magnum* isolate R97304, respectively. Endophytic *Cryptococcus magnus* has been isolated from different Ficus species (Solis *et al*., 2015). Sun *et al.* (2013) described the isolation of *Cryptococcus magnus* species from the leaves, roots and stems of the medicinal plant *Achyranthes bidentata* Blume. Endophytic *Filobasidium magnum* has been isolated from the fruits of *Malus domestica* and *Pyrus communis* (Glushakova and Kachalkin, 2017). The combination of the secondary and primary identification of TES01A and TES02A strongly suggest that this isolate is *Cryptococcus magnus*, also known as *Filobasidium magnum*.

Based on the sequencing of the ITS region and phylogenetic evolution, the fungal isolates in this study were found to belong to the *Penicillium*, *Alternaria* and *Cryptococcus* genera.

3.4 Conclusion

The study revealed the presence and cohabitating of endophytic bacteria and fungi in the medicinal plant *C. macowanii*. The isolated endophytes have been reported to occur in other plants as beneficial endophytes. There is a diversity of endophytic microflora and mycoflora in the plant and this has broadened our knowledge of the microbial community of *C. macowanii*. Further studies need to be conducted to isolate bioactive secondary metabolites from the endophytes.

References

Abdalla, M.A., Mcgaw, L.J., 2018. Bioprospecting of South African Plants as a Unique Resource

for Bioactive Endophytic Microbes. Front. Pharmacol. 9, 1–18. https://doi.org/10.3389/fphar.2018.00456

Ali, S., Duan, J., Charles, T.C., Glick, B.R., 2014. A bioinformatics approach to the determination of genes involved in endophytic behavior in Burkholderia spp . J. Theor. Biol. 343, 193–198. https://doi.org/10.1016/j.jtbi.2013.10.007

Anjum, N., Chandra, R., 2015a. Endophytic bacteria : Optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J. Pharamaceutical Clin. Res. 8, 233–238.

Asif, H., Studholme, D.J., Khan, A., Aurongzeb, M., Khan, I.A., Kamran Azim, M., 2016. Comparative genomics of an endophytic Pseudomonas putida isolated from mango orchard. Genet. Mol. Biol. 39, 465–473.

Baker, S., Satish, S., 2012. Endophytes : Natural Warehouse of Bioactive Compounds. Drug Invent. Today 4, 548–553.

da Costa Souza, P.N., Grigoletto, T.L.B., de Moraes, L.A.B., Abreu, L.M., Guimarães, L.H.S., Santos, C., Galvão, L.R., Cardoso, P.G., 2016. Production and chemical characterization of pigments in filamentous fungi. Microbiol. (United Kingdom) 162, 12–22. https://doi.org/10.1099/mic.0.000168

Egan, J.M., Kaur, A., Raja, H.A., Kellogg, J.J., Oberlies, N.H., Cech, N.B., 2016. Antimicrobial fungal endophytes from the botanical medicine goldenseal (Hydrastis canadensis). Phytochem. Lett. 17, 219–225. https://doi.org/10.1016/j.phytol.2016.07.031

Elgorashi, Esameldin E., Drewes, S.E., Morris, C., Van Staden, J., 2003. Variation among three Crinum species in alkaloid content. Biochem. Syst. Ecol. 31, 601–615. https://doi.org/10.1016/S0305-1978(02)00222-3

Eljounaidi, K., Kyu, S., Bae, H., 2016. Bacterial endophytes as potential biocontrol agents of vascular wilt diseases – Review and future prospects. Biol. Control 103, 62–68. https://doi.org/10.1016/j.biocontrol.2016.07.013

Fouda, A.H., Hassan, S.E., Eid, A.M., Ewais, E.E., 2015. Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss .). Ann. Agric. Sci. 60, 95–104. https://doi.org/10.1016/j.aoas.2015.04.001

Glushakova, A.M., Kachalkin, A. V, 2017. Endophytic Yeasts in Malus domestica and Pyrus communis Fruits under Anthropogenic Impact. Microbiology 86, 128–135. https://doi.org/10.1134/S0026261716060102

Jasim, B., Joseph, A.A., John, C.J., Mathew, J., Radhakrishnan, E.K., 2014. Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of Zingiber officinale. 3 Biotech 4, 197–204. https://doi.org/10.1007/s13205-013-0143-3

Joshi, S.R., Nongkhlaw, F.M.W., 2015. L-Asparaginase and antioxidant activity of endophytic bacteria associated with ethnomedicinal plants. Indian J. Biotechnol. 14, 59–64.

Kandel, S.L., Joubert, P.M., Doty, S.L., 2017. Bacterial Endophyte Colonization and Distribution within Plants. Microorganisms 5, 1–26. https://doi.org/10.3390/microorganisms5040077

Khan, A.L., Gilani, S.A., Waqas, M., Al-hosni, K., Al-khiziri, S., Kim, Y., Ali, L., Kang, S., Asaf, S., Shahzad, R., Hussain, J., Lee, I., Al-harrasi, A., 2017. Endophytes from medicinal plants and their potential for producing indole acetic acid , improving seed germination and mitigating oxidative stress *#. J. Zhejiang Univ. B (Biomedicine Biotechnol. 18, 125–137.

Kuklinsky-Sobral, J., Arajo, W.L., Mendes, R., Pizzirani-Kleiner, A.A., Azevedo, J.L., 2005. Isolation and characterization of endophytic bacteria from soybean (Glycine max) grown in soil treated with glyphosate herbicide. Plant Soil 273, 91–99. https://doi.org/10.1007/s11104-004-6894-1

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33, 1870–4. https://doi.org/10.1093/molbev/msw054

Maroyi, A., 2016. A review of ethnoboatany, therapeutic value, phytochemistry and pharmacology of Crinum macowanii Baker_ A highly traded bulbous plant in Southern Africa. J. Ethnopharmacol. 194, 595–608. https://doi.org/10.1016/j.jep.2016.10.046

Martín-García, J., Espiga, E., Pando, V., Diez, J.J., 2011. Factors Influencing Endophytic Communities in Poplar Plantations. Silva Fenn. 45, 169–180.

Martinez-Klimova, E., Rodríguez-Peña, K., Sánchez, S., 2017. Endophytes as sources of antibiotics. Biochem. Pharmacol. 134, 1–17. https://doi.org/10.1016/j.bcp.2016.10.010

Menpara, D., Chanda, S., 2013. Endophytic Bacteria- Unexplored Reservoir of Antimicrobials for Combating Microbial Pathogens. Microb. Pathog. Strateg. Combat. them Sci. Technol. Educ. 1095–1103.

Mercado-Blanco, J., Lugtenberg, B.J.J., 2014. Biotechnological Applications of Bacterial Endophytes. Curr. Biotechnol. 3, 60–75. https://doi.org/10.2174/2211550111302666003 8

Monakisi, C.M., 2007. Knowledge and Use of Tradition Medicinal Plants By the Setswan a -Speaking Community of Kimberley , Northern Cape of South Africa. October. University of

Stellenbosch.

Morales, A., 2008. A simple way to preserve fungal cultures. Ithaca, New York.

Morare, R.F., Ubomba-Jaswa, E., Serepa-Dlamini, M.H., 2018. Isolation and identification of bacterial endophytes from Crinum macowanii Baker. African J. Biotechnol. 17, 1040–1047. https://doi.org/10.5897/AJB2017.16350

Nair, J.J., Machocho, A.K., Campbell, W.E., Brun, R., Viladomat, F., Codina, C., Bastida, J., 2000. Alkaloids from Crinum macowanii. Phytochemistry 54, 945–950. https://doi.org/10.1016/S0031-9422(00)00128-X

Nalini, M.S., Prakash, H.S., 2017. Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Lett. Appl. Microbiol. 64, 261–270. https://doi.org/10.1111/lam.12718

Narsing Rao, M.P., Xiao, M., Li, W.J., 2017. Fungal and bacterial pigments: Secondary metabolites with wide applications. Front. Microbiol. 8, 1–13. https://doi.org/10.3389/fmicb.2017.01113

Navi, S.S., Bandyopadhyay, R., Hall, A.J., Bramel-Cox, P.J., 1999. A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain. Andhra Pradesh, India.

Ngamau, C.N., Matiru, V.N., Tani, A., Muthuri, C.W., 2012. Isolation and identification of endophytic bacteria of bananas (Musa spp .) in Kenya and their potential as biofertilizers for sustainable banana production. African J. Microbiol. Res. 6, 1–10.

Ngoma, L., Mogatlanyane, K., Babalola, O.O., 2014. Screening of Endophytic Bacteria towards the Development of Cottage Industry : An in Vitro Study. J. Hum. Ecol. 47, 45–63.

Pitt, J.I., Hocking, A.., 2009. Fungi and Food Spoilage, third. ed. Springer Dordrecht Heidelberg London, London New York.

Pombeiro-Sponchiado, S.R., Sousa, G.S., Andrade, J.C.R., Lisboa, H.F., Gonçalves, R.C.R., 2017. Production of Melanin Pigment by Fungi and Its Biotechnological Applications, in: Miroslav Blumenberg, IntechOpen. pp. 47–60. https://doi.org/http://dx.doi.org/10.5772/57353

Powers-Fletcher, M., Kendall, B., Griffin, A., Hanson, K., 2016. Filamentous fungi. Microbiol. Spectr. 4, 1–29. https://doi.org/10.1128/microbiolspec.DMIH2-0002-2015.Correspondence

Raghu, J., 2012. Endophytic bacterial association and plant secondary metabolite production. University of Agricultural Sciences, Bangalore.

Ramírez-Bahena, Martha-Helena Carmen, T., Peix, A., 2014. Bacterial Endophytes Inhabiting Potato Plants (Solanum tuberosum L.), in: Mandhatri, P.G. and S. (Ed.), Agricultural Research Updates. Nova Science Publishers, New York, pp. 3–31.

Rangjaroen, C., Sungthong, R., RerkaSem, B., Teaumroong, N., NoiSangiam, R., SaiSamorn, L., 2017. Untapped Endophytic Colonization and Plant Growth-Promoting Potential of the Genus Novosphingobium to Optimize Rice Cultivation. Microbes and Enviroments 32, 84–87. https://doi.org/10.1264/jsme2.ME16112

Rhoden, S.A., Garcia, A., Santos e Silva, M.C., Azevedo, J.L., Pamphile, J.A., 2015. Phylogenetic analysis of endophytic bacterial isolates from leaves of the medicinal plant Trichilia elegans A . Juss . (Meliaceae). Genet. Mol. Res. 14, 1515–1525.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–25. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sardul Singh, S., Suneel, K., Aharwal, R.P., 2014. Isolation and identification of endophytic fungi from Ricinus communis linn . And their antibacterial activity. Int. J. Res. Pharm. Chem. 4, 611–618.

Shanmugam, G., Dubey, A., Ponpandian, L.N., Rim, S.O., 2018. Genomic Insights into Nematicidal Activity of a Bacterial Endophyte , Raoultella ornithinolytica MG against Pine Wilt Nematode. Plant Pathol. J. 34, 250–255.

Shiva Kameshwari, M.., B, M., Saraswathi.K.J, T., 2015. Isolation and identification of endophytic fungi from Urginea Indica , a medicinal plant from diverse regions of South India . Int. J. Latest Res. Sci. Technol. 4, 75–80.

Singh, S.K., 2015. Role of Endophytes in Pharmaceutical Industry. J. Microbiol. Biotechnol. 4, 6–7.

Sobiczewski, P., 2008. Bacterial diseases of plants : Epidemiology , diagnostics and control. Zemdirbyste-Agriculture 95, 151–157.

Solis, M.J.L., Yurkov, A., Cruz, T.E. dela, Unterseher, M., 2015. Leaf-inhabiting endophytic yeasts are abundant but unevenly distributed in three Ficus species from botanical garden greenhouses in Germany. Mycol. Prog. 14, 1–10. https://doi.org/10.1007/s11557-014-1019-6

Soltis, P.S., Soltis, D.E., 2003. Applying the Bootstrap in Phylogeny Reconstruction. Stat. Sci. 18, 256–267.

Strobel, G., 2018. The Emergence of Endophytic Microbes and Their Biological Promise. J. Fungi 4, 1–9. https://doi.org/10.3390/jof4020057

Strobel, G., Daisy, B., 2003. Bioprospecting for Microbial Endophytes and Their Natural Products.

Microbiol. Mol. Biol. Rev. 67, 491–502. https://doi.org/10.1128/MMBR.67.4.491

Sun, B., Chen, A., Gao, W., Zhou, Y., Liu, H., 2013. Endophytic fungi associated with the medicinal plant , Achyranthes bidentata Blume (Amaranthaceae). African J. Microbiol. Res. 7, 1357–1365. https://doi.org/10.5897/AJMR12.1461

Sun, X., Guo, L., 2012. Endophytic fungal diversity : review of traditional and molecular techniques. Mycology 31, 65–76.

Tamošiune, I., Stanien, G., Haimi, P., Stanys, V., Rugienius, R., Baniulis*, D., 2018. Endophytic Bacillus and Pseudomonas spp . Modulate Apple Shoot Growth , Cellular Redox Balance , and Protein Expression Under in Vitro Conditions. Front. Plant Sci. 9, 1–14. https://doi.org/10.3389/fpls.2018.00889

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6 : Molecular Evolutionary Genetics Analysis Version 6 . 0 30, 2725–2729. https://doi.org/10.1093/molbev/mst197

Tenorio-Salgado, S., Tinoco, R., Vazquez-Duhalt, R., Caballero-Mellado, J., Perez-Rueda, E., 2013. Identification of volatile compounds produced by the bacterium Burkholderia tropica that inhibit the growth of fungal pathogens. Bioengineered 4, 236–243.

Terhonen, E., Blumenstein, K., Kovalchuk, A., Asiegbu, F.O., 2019. Forest Tree Microbiomes and Associated Fungal Endophytes : Functional Roles and Impact on Forest Health. Forests 10, 1–33. https://doi.org/10.3390/f10010042

Ullah, A., Mushtaq, H., Ali, U., Ali, E., Mubeen, S., 2018. Screening , isolation , biochemical and plant growth promoting characterization of endophytic bacteria. Microbiol. Curr. Res. 2, 61 68.

Venugopalan, A., Srivastava, S., 2015. Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol. Adv. 33, 873–887. https://doi.org/10.1016/j.biotechadv.2015.07.004

Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S., Klaassen, C.H.W., Perrone, G., Seifert, K.A., Varga, J., Yaguchi, T., Samson, R.A., 2014. Identification and nomenclature of the genus Penicillium. Stud. Mycol. 78, 343–371. https://doi.org/10.1016/j.simyco.2014.09.001

Watt, J.M., Breyer-Brandwijk, M.G., 1962. Medicinal and Poisonous Plants, second. ed, Nature. E & S.Livingstone Ltd, London. https://doi.org/10.1038/196609b0

Xie, J., Wu, Y., Zhang, T., Zhang, M., Zhu, W., Gullen, E.A., Wang, Z.-J., Cheng, Y.-C., Zhang, Y.-X., 2017. New and bioactive natural products from an endophyte of Panax notoginseng †.

R. Soc. Chem. Adv. 7, 38100–38109. https://doi.org/10.1039/c7ra07060h
Yaish, M.W., 2016. Draft Genome Sequence of Endophytic Bacterium Enterobacter asburiae PDA134 , Isolated from Date Palm (Phoenix dactylifera L .) Roots. Genome Announc. 4, 12. https://doi.org/10.1128/genomeA.00848-16.

CHAPTER FOUR:

EVALUATING THE ANTIBACTERIAL AND ANTICANCER ACTIVITY OF CRUDE PLANT EXTRACTS AND CRUDE ENDOPHYTE EXTRACTS*

* This chapter documents how the biological activity of the crude plant extracts and crude endophyte extracts were evaluated. Antibacterial tests were carried out on pathogenic bacteria strains and an anticancer assay on two cancer cell lines to investigate the biological activity of the different crude extracts.

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Abstract

The over-use of plants for medicinal purposes has led to undue stress on wild species due to over harvesting, and therefore some of these have become extinct. Plants produce bioactive compounds with broad medicinal applications. Endophytes are known to biosynthesize secondary metabolites, which are similar in characteristics to those of the host plant. This study aimed to evaluate the antibacterial and anticancer activities of the crude plant extracts and crude endophyte extracts from C. *macowanii* bulbs and leaves. Crude endophyte extracts were extracted using ethyl acetate, while methanol: dichloromethane (1:1) was used to obtain crude extracts from the bulbs and leaves. The antibacterial activity of the crude extract from each endophyte was investigated against selected pathogenic strains using the broth microdilution method, and the anticancer activity against U87MG Glioblastoma cells and A549 Lung carcinoma cells was determined by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. The crude endophytes extract from *Pseudomonas* sp. from the bulbs showed antibacterial activity of 0.125 mg/ml against *B. subtilis*, *S. epidermidis* and *M. marinum*. The crude endophyte extracts from *Arthrobacter pascens* displayed antibacterial activity of 0.0625 mg/ml against *B. subtilis*. Crude endophyte extracts from *Bacillus cereus* showed cell reduction of 56% at 100 µg/ml against A549 lung carcinoma cells whereas *Bacillus safensis* and *Penicillium chrysogenum* crude endophyte extracts showed cell reduction of 50% at concentrations of 100 µg/ml and 38% cell reduction at 12.5 µg/ml concentration, respectively, against U87MG glioblastoma cells. In conclusion, crude extracts isolated from endophytic bacteria and fungi obtained from *Crinum macowanii* bulbs and leaves showed activity against both Gram-positive and Gram-negative pathogenic bacteria, while *Bacillus cereus* and *Bacillus safensis* crude extracts displayed anti-cancer activity. Further studies on the purification and isolation of bioactive compounds of the crude endophyte extracts should be conducted to obtain pure bioactive compounds for further biological testing.

Keywords: *C. macowanii*, Endophytes**,** Antibacterial, Anticancer, MIC, MTS assay.

4.1 Introduction

Plants are known to produce secondary metabolites, which are small organic molecules not necessarily required for the growth, development and reproduction of the plant, but for protection of the plant against harm (Kabera *et al.*, 2014; Seca and Pinto, 2018). Secondary metabolites are low molecular weight compounds and are grouped according to pathways used for synthesis (Pagare *et al.*, 2016; Seca and Pinto, 2018). Secondary metabolites such as morphine, quinine and vincristine have medicinal uses such as analgesic, antimalarial and anti-tumour (Grech *et al*., 2009). van Wyk and Prinsloo (2018) reported that medicinal plants are prone to extinction due to overuse and over harvesting as many are slow-growing, grow in specific habitats and are slow-reproducing. Due to the extensive cultivation of plants to obtain secondary metabolites, strategies such as using other plant parts substitution or organisms should be studied (Monakisi, 2007; van Wyk and Prinsloo, 2018).

Endophytes are microorganisms that reside within plant tissues, and some are known to produce bioactive compounds required in host-endophyte relationships (Strobel, 2003). Endophytes are able to biosynthesize the same secondary metabolites as their host plants by inserting their own DNA into the host plant's genome (Venieraki *et al*., 2017; Abdalla and Mcgaw, 2018). Bioactive compounds such as taxol, emodin and hypercin have been isolated from endophytes (Gouda *et al*., 2016). Abdalla and Mcgaw (2018) reported that secondary metabolites from endophytes have been used in the discovery of antibiotics, antimalaria and anticancer drugs. The promising and potential bioactivities of the *Crinum macowanii* plant as reported by Maroyi (2016), has led to exploring the plant for endophytes to produce potential drugs against cancer and pathogenic bacteria.

The emerging of infectious diseases worldwide due to bacteria and viruses still poses a serious public health concern, claiming the lives of half a million of people a year and amounting to 25% of total deaths worldwide (Nii-Trebi, 2017). Even with the discovery and production of new and improved antibiotics, the tolerance and resistance of pathogenic microorganisms to drugs has increased exponentially (Davies and Davies, 2010). Ventola (2015) stated that the causes of antibiotic resistance include overuse, inappropriate prescribing and extensive agricultural use. There is, therefore, an imminent need to discover and develop new drugs to combat antimicrobial resistance (Rajbhandary, 2014).

Gliomas are common primary central nervous system (CNS) tumours mostly affecting the brain (Paolillo *et al*., 2018). Glioblastomas are aggressive cancers with poor prognosis and an average

patient survival of 18 months (Denicolaï *et al*., 2014). Lung cancer has been deemed to be one of the most prevalent cancers in the developed world and has a very poor survival rate, since most patients are diagnosed at a stage when curative treatment is impossible, and it is also one of the most difficult cancers to diagnose (Neal *et al*., 2015). Kumar *et al.* (2014) stated that the discovery and development of chemotherapeutic agents is vital in the treatment of cancer, since currently available therapies are ineffective and have side effects.

There is an urgent need for the discovery of new antibiotics and chemotherapeutic agents with minimum effect on the environment, and moderate toxicity effects on patients (Strobel and Daisy, 2003). Bioactive secondary metabolites obtained by bioprospecting could be alternative sources of therapeutic compounds against antibiotic resistance and cancer (Jalgaonwala & Mahajan 2011; Menpara & Chanda 2013; Tidke *et al*., 2017). The aim of this study was to evaluate the antibacterial and anticancer activities of the crude plant and crude endophyte extracts isolated from *C. macowanii* bulbs and leaves.

4.2 Materials and methods

4.2.1 Sample collection

Fresh, healthy *C. macowanii* bulbs and leaves showing no apparent symptoms of disease or herbivore damage were collected from the Walter Sisulu National Botanical Garden (Roodepoort, Gauteng, South Africa, 26°05'10.4"S 27°50'41.5"E). After harvesting the samples were placed in sterile polyethylene bags and transferred to the laboratory at 4°C before being thoroughly washed with sterile distilled water and used within hours of harvesting.

4.2.2 Extraction of crude plant extracts and crude endophytes extracts

4.2.2.1 Extraction of crude extracts from *C. macowanii* bulbs and leaves

C. macowanii bulbs and leaves were washed, chopped into small pieces and air-dried at room temperature. The dried plant material was blended into a fine powder using a commercial blender. Crude extracts were obtained according to Yadav and Agarwala (2011). Briefly, 150 g of the prepared plant material was mixed with 2 L of a 50:50 methanol: dichloromethane solution. This was allowed to shake for 3 days on a platform shaker (Amerex Gyromax, Temecula, CA, USA) at

200 rcf (relative centrifugal force). The solution was filtered through Whatman No. 1 filter paper, then the filtrate was evaporated on a rotatory evaporator and allowed to air dry in a desiccator. The crude extracts were used for antibacterial, anticancer assays and metabolite profiling.

4.2.2.2 Extraction of crude extracts from bacterial endophytes

For each endophytic bacterium, 2 L of broth was measured into a 4 L Erlenmeyer flask leaving room for aeration, and autoclaved at 121°C for 15 min. Each 4 L flask was inoculated with one of the endophytic bacterium listed in Table 3.1, shaken at 200 rcf and incubated at 30°C, an ideal temperature for the growth of the bacterial endophytes (Sardul Singh *et al*., 2014). After 7 days of cultivation, sterile XAD-7- HP resin (20 g/L) (Sigma, Johannesburg, South Africa, BCBR6696V) was added to the culture for 2 h, shaking at 200 rcf. The resin was filtered through cheesecloth and washed three times with 300 mL of acetone for each wash. The acetone soluble fraction was concentrated using a rotary evaporator and a dark yellowish viscous extract was obtained, which was transferred into a measuring cylinder. Depending on the volume, ethyl acetate was added in a ratio of 1:1 (v/v). The mixture was vigorously shaken for about 10 min, decanted into a separating funnel, allowed to separate and each phase collected in a conical flask. This process was repeated until the dark yellowish viscous liquid was obtained. After the acetone was removed it became a very light-yellow liquid. The ethyl acetate fraction was evaporated using a rotary evaporator and the brown extract obtained was stored in an amber bottle in a cool dry place until analysis was done. The brown crude extracts were used for antibacterial, anticancer assays and metabolite profiling.

4.2.2.3 Fermentation and extraction of crude extracts from fungal endophytes

A modified method of Prabavathy and Nachiyar, (2014), was used to grow the isolates and extract crude extracts. For each fungal isolate as listed in Table 3.2, 2 L of potato dextrose broth (PDB) (HiMedia Laboratories, Mumbai, India) was measured into a 4 L Erlenmeyer flask leaving room for aeration and autoclaved at 121°C for 15 min. Each 4 L flask was inoculated with one of the endophytic fungi, shaken at 100 rcf and incubated at 25°C (Amerex Gyromax, ISS, Carlifonia, USA) for 14 days. After fermentation, the broth was separated from the mycelia by first filtering with a three-layered muslin cloth followed by using a Whatman No.1 filter paper (Sigma-Aldrich,

Johannesburg, South Africa). The filtrate was mixed with ethyl acetate. Depending on the volume of the filtrate, ethyl acetate was added in a ratio of 1:1 (v/v). The mixture was vigorously shaken for about 10 min, decanted into a separating funnel, allowed to separate and each phase collected in a conical flask. The ethyl acetate fraction was evaporated using a rotary evaporator and the extract obtained was stored in an amber bottle in a cool dry place until analysis was done. The crude extracts were used for antibacterial anticancer assays and metabolite profiling.

4.2.3 Antibacterial evaluation of *Crinum macowanii* crude extracts (bulbs and leaves) and crude endophyte (bacteria and fungi) extracts

4.2.3.1 Sample preparation

The crude plant extract and crude endophyte extracts were weighed separately into empty autoclaved McCartney bottles to ensure sterility. A minimal amount of dimethyl sulfoxide (DMSO) (0.1%) was used to dissolve the crude extracts, and Mueller-Hinton (MH) broth was added to bring the volume of the dissolved crude extract to a concentration of 32 mg/ml as the stock solution.

4.2.3.2 Microtiter plate assay

Micro serial dilution was used to check for the lowest inhibition concentration of the samples to specific pathogenic bacterial species, namely, *Bacillus cereus* (ATCC10876), *Bacillus subtilis* (ATCC19659), *Streptococcus epidermidis* (ATCC14990), *Staphylococcus aureus* (ATCC25923), *M. smegmatis* (ATCC21293), *Mycobacterium marinum* (ATCC927), *Enterobacter aerogenes* (ATTC13048), *Escherichia coli* (ATCC10536), *Klebsiella pneumoniae* (ATCC10031), *Proteus vulgaris* (ATCC 33420) and *Proteus aeruginosa* (ATCC10145). This was done following a method described by Andrews (2001) and Sebola *et al*. (2016). The antibiotic Streptomycin was used as the positive control and was prepared by weighing 0.032 mg in 1 ml of sterile distilled water, while 0.1% DMSO was used as a negative control.

Serial dilutions were carried out using the MH broth from 16 mg/ml down to 0.031 mg/ml, which was the lowest inhibition observed. The experiment was carried out in five repeats using a 96-well microtiter plate. The outer wells of the plate were filled with sterile dH_2O (sdH_2O). The inoculum (100 µL) was added into each well that did not contain the sdH_2O. The diluted crude extract

samples (100 µL) were added in five wells horizontally and the concentrations decreased in vertical order from 16 mg/ml down to 0.031 mg/ml. The plates were covered and incubated overnight at 37°C. After incubation, 10 µL of 0.02% (w/v) resazurin sodium salt dye solution was added to the wells and the resulting solution incubated for another two hours. On reduction, resazurin changes colour from blue to pink to clear as oxygen becomes limited within the medium, indicating metabolism and the viability of bacterial cells, as well as no effect of the crude extracts on the bacteria. Any well with a known concentration showing a slight colour change was used as MIC. The wells were visually inspected for colour changes.

4.2.4 Anticancer evaluation of *Crinum macowanii* crude extracts (bulbs and leaves) and crude endophytes (bacteria and fungi) extracts

The crude plant extracts and crude endophyte extracts were weighed in Eppendorf tubes made up with 0.1% DMSO, sonicated to aid dissolution, and a stock solution of 200 µg/ml was made. Serial dilutions were done according to McCauley *et al*. (2013) and Artun *et al*. (2016). Briefly, dilutions were carried out using growth media from 100 µg/ml to 3.13 µg/ml. An MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) *in vitro* cancer cytotoxicity assay was carried out to determine a change in cell viability by colour change. The MTS compound (yellow) is metabolized by viable cells to form a dark, purple-coloured compound, while dead cells turn the colour of the MTS compound pink. The samples were run in duplicate across three plates (n=6) and the average values obtained were reported. The U87MG (glioblastoma) cells and A549 (lung carcinoma) cells were grown by means of normal tissue culture techniques using Dulbecco's Modified Eagle Medium (Merk, Johannesburg, SA) supplemented with 15% foetal bovine serum (FBS) (Merck, Johannesburg, SA). The cells (1×10^5 cells/ml) were incubated in 96 well plates at 37°C overnight, with the subsequent addition of the crude extracts and secondary metabolites, in concentrations of 100 µg/ml, 50.0 µg/ml, 25.0 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml and 0 µg/ml. The cells were left to incubate for 4 days, whereupon MTS (5 µL) (Promega, Madison, WI, USA) was added to the cells. The absorbance values were measured at 490 nm after 1 h, 2 h and 4 h incubation periods. Cell viability was then calculated using the formula:

$$\% \text{ Cell viability} = (E_a - B_a)/ (C_a - B_a) \times 100$$

where E_a is absorbance of the extract, B_a is absorbance of the blank and C_a is the absorbance of the control (Handayani *et al*., 2018). The positive control used for all conducted tests was auranofin, as it is able to inhibit thioredoxin reductase as well as the ubiquitin–proteasome system (UPS), by targeting proteasome-associated deubiquitinase, thereby inducing lung cancer cell apoptosis by selenocystine (Fan *et al*., 2014; Roder & Thomson, 2015; Coussens *et al*., 2017). Untreated cells mixed with MTS and the solubilizing buffer were used as the negative control.

4.3 Results and Discussion

4.3.1 Antibacterial evaluation of crude extracts

Bacterial endophytes have been less explored for their metabolic potential due to the small quantity of bacterial endophytes currently being cultured from various plants and plant parts, however, this is not the case for fungal endophytes (Brader *et al*., 2014).

4.3.1.1 Antibacterial evaluation of crude bacteria endophyte extracts from the bulbs

The lower MIC (0.125 mg/ml) was observed mostly from the *Pseudomonas sp.* crude extract against *B. subtilis*, *S. epidermidis* and *M. marinum*. The crude extracts of most of the endophytes showed MIC values higher than 0.125 mg/ml, as seen in Table 4.1. *S. epidermidis* was susceptible against the crude bulb extract and an MIC value of 0.125 mg/ml was observed.

Table 4.1: Antibacterial evaluation of *C. macowanii* crude bulb extract and crude endophyte extracts from the bulb

	Test organism with MIC (mg/mL)										
Crude extract	*B. cereus*	*B. subtilis*	*S. epidermidis*	*S. aureus*	*M. smegmatis*	*M. marinum*	*E. aerogenes*	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*
T1	**0.500**	16.00	**0.125**	8.00	**0.500**	**0.250**	16.00	8.00	>16.00	>16.00	>16.00
T2	8.00	**1.00**	4.00	16.00	**1.00**	**0.500**	**1.00**	**0.500**	**0.500**	>16.00	**0.250**
T3	4.00	4.00	16.00	8.00	4.00	16.00	16.00	8.00	16.00	8.00	16.00
T4	**0.250**	2.00	**0.500**	**1.00**	8.00	**0.125**	**0.500**	8.00	6.00	4.00	>16.00
T5	**0.500**	**0.125**	**0.125**	**0.500**	8.00	**0.125**	16.00	>16.00	4.00	16.00	8.00
T6	**1.00**	2.00	4.00	**0.125**	4.00	>16.00	2.00	8.00	2.00	>16.00	4.00
T7	>16.00	4.00	8.00	16.00	4.00	>16.00	16.00	>16.00	>16.00	8.00	**1.00**
T8	1.00	>16.00	16.00	8.00	8.00	>16.00	4.00	2.00	**1.00**	4.00	16.00
T9	**0.500**	**0.250**	4.00	2.00	16.00	>16.00	8.00	>16.00	>16.00	4.00	2.00
	Positive control MIC (µg/mL)										
T10	0.031	0.031	0.062	0.031	0.062	0.062	0.125	0.125	0.125	0.062	0.031

T1 = *C. macowanii* bulbs, T2 = *Raoultella ornithinolytica*, T3 = *Acinetobacter guillouiae*, T4 = *Pseudomonas moraviensis*, T5 = *Pseudomonas* sp., T6 = *Rahnella aquatilis*, T7 = *Novosphingobium* sp., T8 = *Bacillus. cereus*, T9 = *Burkholderia tropica*, T10 = Positive Control Streptomycin.

C. macowanii bulbs have been used to treat diseases or ailments caused by bacteria (Maroyi, 2016). Rabe & Van Staden (1997) tested methanol extracts of *C. macowanii* bulbs on a number of bacteria such as *E. coli, K. pneumoniae, S. aureus* and *S. epidermis* and observed no antibacterial activities. The results obtained in this study indicate the inhibition of *B. cereus, S. epidermidis* and *M. smegmatis* at 0.500 mg/ml, 0.125 mg/ml and 0.500 mg/ml, respectively, which concur with the study of Sebola *et al*. (2016). Inhibition greater than 16 mg/ml, observed in *K. pneumoniae, P. vulgaris* and *P. aeruginosa*, were not considered to be inhibitory. The observed antibacterial results support documented ethnomedicinal uses of *C. macowanii* bulb such as treatment of diarrhoea, cleansing of blood and the treatment of boils and sores on skin (Maroyi, 2016). The results obtained prompted the antibacterial evaluation of the crude endophyte extracts from *C. macowanii* bulbs for potential drug prospects (Abdalla and Mcgaw, 2018). The use of alternative sources for bioactive compound similar to those produced by *C. macowanii* will prevent the over harvesting and over use of this plant (Baker and Satish, 2012).

Endophytic bacteria have been reported to produce a number of secondary metabolites, such as alkaloids, steroids, terpenoids, peptides and flavonoids, with antibacterial, antifungal and cytotoxic properties (Raghu, 2012). The endophytic crude extracts tested in this study showed anti-bacterial activity against selected pathogenic strains. To the best of our knowledge, this study is the first to report on the isolation of crude extracts from endophytes of *Raoultella ornithinolytica, Acinetobacter guillouiae, Rahnella aquatilis and Novosphingobium* sp. and their antibacterial activity.

Raoultella ornithinolytica crude extract had MIC values ranging from 0.250–16 mg/ml, with the most significant inhibition observed for *K. pneumoniae, E. coli* and *P. aeruginosa* at concentrations of 0.500 mg/ml, 0.500 mg/ml and 0.250 mg/ml, respectively. The results show promising antibacterial activity, as crude extracts of activity <1 mg/ml are deemed to have noteworthy antibacterial properties (Zonyane *et al*., 2013). Crude extracts from *Bacillus cereus* showed MIC values of between 1–16 mg/ml, inhibiting *K. pneumoniae* at 1 mg/ml. Crude extracts from *B. cereus* have been reported to possess antibacterial activity against a wide range of pathogenic microbes such as *E. coli* and *K. pneumoniae* (Kumar *et al*., 2013). This supports the findings in this study.

Pseudomonas moraviensis crude extract showed MIC values of between 0.125–>16 mg/ml. The crude extract showed activity against *B. cereus, S. epidermidis*, *S. aureus*, *M. marinum* and *E. aerogenes* at concentrations of 0.250 mg/mL, 0.500 mg/ml, 1.00 mg/ml, 0.125g/ml and 0.500 mg/ml, respectively. *P. moraviensis* crude extract had no significant activity on *P. aeruginosa*, showing values of >16 mg/ml (highest tested concentration). *Pseudomonas moraviensis* has been reported to possess antibacterial proteins and peptides such as bacteriocins (De Mot and Ghequire, 2014). Matthijs *et al*. (2014) and Mohamed *et al*. (2015) reported that *Pseudomonas* sp. produced antimicrobials such as mupirocin, pyrrolnitrin and pyoluteorin. This could explain the antibacterial results of *Pseudomonas moraviensis* and *Pseudomonas* sp. crude extracts observed in this study. This may suggest that extracts from *Pseudomonas moraviensis* could be used as an antibacterial agent.

Rahnella aquatilis crude extract had MIC values ranging between 0.125–>16 mg/ml. *S. aureus* was inhibited at 0.125 mg/ml. El-Hendawy *et al*. (2003) reported that *Rahnella aquatilis* strains obtained from soil were able to produce bacteriocin, which inhibited the culture of different Gram-positive and Gram-negative bacteria. *Novosphingobium* sp. crude extract had MIC values ranging from 1–>16 mg/ml, showing a lack of activity against the tested organisms. *Burkholderia tropica* crude extract had MIC values ranging from 0.250–>16 mg/ml. *B. subtilis* was inhibited at 0.250 mg/ml. *M. marinum*, *E. coli* and *K. pneumoniae* had MIC values of >16 mg/ml, which was deemed non-inhibitory. *Burkholderia* has been reported to show biocontrol and plant growth-promoting characteristics (Ho and Huanga, 2015).

It was observed in this study that the Gram-positive bacteria species were more susceptible to the antibacterial compounds in the crude extracts than the Gram-negative bacteria. This could be attributed to the difference in the cell walls of both groups of bacteria, as Gram-negative bacteria are known to be resistant to most antibiotics due to their outer membrane, which tends to expel antibiotics from the cells by acting as a selective barrier (in contrast to that of their Gram-positive counterparts) (Delcour 2009; Iannello *et al*., 2014).

4.3.1.2 Antibacterial evaluation of crude bacteria endophyte extracts from the leaves

The lowest MIC (0.0625 mg/ml) was observed from the *Arthrobacter pascens* crude extract against *B. subtilis.* The crude extracts of most of the endophytes showed MIC values below 1.00 mg/ml. The leaves' crude extracts displayed noteworthy activity against both Gram-positive and Gram-negative bacteria as seen in Table 4.2.

Table 4.2: Antibacterial evaluation of *C. macowanii* crude leaves extract and crude endophyte extracts from the leaves

	Test organism with MIC (mg/mL)										
Crude extracts	*B. cereus*	*B. subtilis*	*S. epidermidis*	*S. aureus*	*M. smegmatis*	*M. marinum*	*E. aerogenes*	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*
T1	2.00	**0.500**	**1.00**	**0.250**	**0.500**	2.00	8.00	**0.250**	**0.250**	**0.500**	**0.125**
T2	8.00	1.00	4.00	16.00	**1.00**	**0.500**	**1.00**	**0.500**	**0.500**	>16.00	**0.250**
T3	4.00	4.00	16.00	8.00	**0.500**	16.00	16.00	8.00	16.00	8.00	16.00
T4	**0.500**	**0.125**	**0.125**	**0.500**	8.00	**0.0125**	16.00	>16.00	4.00	16.00	8.00
T5	>16.00	2.00	4.00	4.00	8.00	**0.500**	**1.00**	2.00	**0.500**	**0.250**	**1.00**
T6	>16.00	4.00	8.00	16.00	4.00	>16.00	16.00	>16.00	>16.00	8.00	1.00
T7	>16.00	**0.125**	2.00	>16.00	4.00	**0.500**	16.00	**0.250**	2.00	4.00	>16.00
T8	4.00	16.00	8.00	2.00	**0.500**	**0.125**	8.00	**0.500**	**0.125**	16.00	2.00
T9	8.00	**0.250**	16.00	**1.00**	**0.125**	8.00	4.00	**1.00**	**0.500**	**1.00**	**0.125**
T10	2.00	**0.0625**	4.00	**1.00**	16.00	>16.00	**1.00**	**0.500**	>16.00	2.00	4.00
	Positive control MIC (µg/mL)										
T11	0.031	0.031	0.062	0.031	0.062	0.062	0.125	0.125	0.125	0.062	0.031

T1= *C. macowanii* leaves, T2= *Raoultella ornithinolytica,* T3= *Acinetobacter guillouiae,* T4= *Pseudomonas* sp., T5= *Pseudomonas palleroniana,* T6= *Pseudomonas putida,* T7= *Bacillus safensis,* T8= *Enterobacter asburiae,* T9= *Pseudomonas cichorii,* T10= *Arthrobacter pascens,* T11=Positive Control Streptomycin

Maroyi (2016) reported that the leaves of *C. macowanii* have been used traditionally to cleanse blood and increase its supply, to treat coughs, kidney and bladder diseases in humans and in animals, as well as diarrhoea. The results obtained in this study showed the inhibition of *B. subtilis*, *M. smegmatis* and *P. vulgaris* at 0.500 mg/ml, *S. aureus, E. coli* and *K. pneumoniae* were inhibited at 0.250 mg/ml and *S. epidermidis* and *P. aeruginosa* at 1.00 mg/ml and 0.125 mg/ml, respectively. This would explain the antibacterial results observed, which correlates with the leaves' documented ethnomedicinal uses. Elgorashi *et al*. (2003) reported that *S. aureus, E. coli* and *K. pneumoniae* were inhibited by a final concentration of 250 μg ml^{-1} by alkaloids such as crinine, cherylline, crinamidine, 3-O-Acetylhamayne and bulbispermine, which have been isolated from *C. macowanii* leaves. To the best of our knowledge, this study is the first to report on the extraction of crude extract from leaves of *C. macowanii* and its antibacterial activity.

The cultivation of plants to obtain bioactive compounds has led to drawbacks such as over-harvesting of plants to obtain bioactive compounds; different environmental conditions can produce low yields, and total synthesis and semi-synthesis are challenging due to their complex structures (Venugopalan and Srivastava, 2015). A number of endophytic microorganisms have produced anticancer, antimicrobial, antidiabetic, insecticidal, and immunosuppressive compounds (Abdalla and Mcgaw, 2018). Plants growing in a variety of places could possibly harbour endophytes with novel natural products (Baker and Satish, 2012; Abdalla and Mcgaw, 2018).

Raoultella ornithinolytica crude extract had MIC values ranging from 0.250–16 mg/ml, with the most significant inhibition observed for *K. pneumoniae*, *E. coli* and *M. marinum* at concentrations of 0.500 mg/ml, and *P. aeruginosa* was inhibited at concentrations of 0.250 mg/ml. Shanmugam *et al.* (2018) reported the presence of microcin genes from *Raoultella ornithinolytica*. Microcin is an antibacterial peptide produced by Enterobacteria (Garcia-Bustos *et al.*, 1985). This could explain the observed results.

Acinetobacter guillouiae crude extract showed MIC values of between 0.500–16 mg/ml. The crude extract showed activity against *M. marinum* 0.500 mg/ml. To the best of our knowledge, this is the first report on the antibacterial activity of crude endophyte extracts from *Acinetobacter guillouiae.*

Bacillus safensis crude extract showed MIC values of between 0.125–>16 mg/ml. The crude extract showed activity against *B. subtilis*, *M. marinum* and *E. coli* at concentrations of 0.125 mg/ml, 0.500 mg/ml and 0.250 mg/ml, respectively. Crude endophytic extracts of *Bacillus safensis* isolated from *Ophioglossum reticulatum* L. have displayed antibacterial activity against *Staphylococcus aureus*

and *Escherichia coli* (Mukherjee *et al*., 2017). This is in agreement with the results obtained in this study, where *E. coli* was inhibited at concentrations of 0.125 mg/ml.

Enterobacter asburiae crude extract showed MIC values of between 0.125–16 mg/ml. The crude extract showed activity against *M. smegmatis* and *E. coli* at concentrations of 0.500 mg/ml and *M. marinum*, and *K. pneumoniae* at concentrations of 0.125 mg/ml. Akinsanya *et al*. (2017) reported that endophytic crude extracts of *Enterobacter asburiae* displayed antibacterial activity against *K. pneumoniae*, *E. coli*, *S. aureus* and *B. cereus*. *Enterobacter* strains have been reported to produce antibacterial lipopeptides with a broad activity (Mandal *et al*., 2013). This supports the results obtained in this study.

Arthrobacter pascens crude extract showed MIC values of between 0.0625–>16 mg/ml. The most active inhibition was against *B. subtilis* at 0.0625 mg/ml. The crude extract showed activity against *S. aureus* and *E. aerogenes* at concentrations of 1.00 mg/ml. Arthrobacilin, an antibacterial compound produced by *Arthrobacter* spp, showed inhibition against *S. aureus* (Wietz *et al.*, 2012; Neu *et al.*, 2014). This could explain the antibacterial activity observed.

Pseudomonas sp. crude extract showed MIC values of between 0.0625–>16 mg/ml. The most active inhibition was against *M. marinum* at 0.0625 mg/ml. Mupirocin, produced by *Pseudomonas* strains, has been reported to possess antibacterial activity (Matthijs *et al*., 2014).

Pseudomonas palleroniana crude extract showed MIC values of between 0.250–>16 mg/ml. The most active inhibition was against *P. vulgaris* at 0.250 mg/ml. The crude extract showed activity against *E. aerogenes* and *P. aeruginosa* at concentrations of 1.00 mg/ml, and against *M. marinum* and *K. pneumoniae at* concentrations of 0.500 mg/ml. Endophytic crude extracts from *Pseudomonas palleroniana* have been reported to inhibit *Escherichia coli* and *Staphylococcus aureus* (Nongkhlaw and Joshi, 2015). Pyoluteorin, produced by *Pseudomonas palleroniana* strains, has been reported to possess antibacterial activity (Fathalla *et al*., 2015).

Pseudomonas putida crude extract showed MIC values of between 1.00–>16 mg/ml. The most active inhibition was against *P. aeruginosa* at 1.00 mg/ml. Antibiotics pyoluteorin, phenazine-1-carboxamide and phenazine-1-carboxylic acid have been produced by *Pseudomonas putida* strains (Fathalla *et al*., 2015). This could explain the antibacterial activity observed.

Pseudomonas cichorii crude extract showed MIC values of between 0.125–16 mg/ml. The crude extract showed activity against *E. coli* and *P. vulgaris* at concentrations of 1.00 mg/ml, *M. smegmatis* and *P. aeruginosa* at concentrations of 0.125 mg/ml, *B. subtilis* at 0.250 mg/ml and *K.*

pneumoniae at 0.500 mg/ml. To the best of our knowledge, this is the first report on the antibacterial activity of crude endophyte extracts from *Pseudomonas cichorii*.

Cos *et al.* (2006) states that a concentration of <0.1 mg/ml for a crude sample is the ideal concentration for anti-infective bioassays, whereas Zonyane *et al*. (2013) and Dzotam *et al.* (2018) recommend that crude samples with a concentration of 1.00 mg/ml, ≤100 µg/ml (0.100 mg/ml) and ≤625 µg/ml are considered threshold limits for acceptable antimicrobial activity and therefore noteworthy for minimal inhibitory concentration. Stringent end points for anti-infective bioassays need to be set to prevent false results and confusion, taking into consideration the sensitivity of extracts and test micro-organisms, extraction methods and solvents used (Baker and Satish, 2012; Abdalla and Mcgaw, 2018).

The crude endophyte extracts from *Pseudomonas* sp. and *Arthrobacter pascens* obtained from *C. macowanii* leaves had noteworthy antibacterial activity against the pathogenic bacteria used in this study and could, therefore, be used as antibacterial agents against and *M. marinum B. subtilis* infections respectively.

4.3.1.3 Antibacterial evaluation of crude fungi endophyte extracts from the bulbs and leaves

The lowest MIC (0.125 mg/ml) was observed from *Penicillium* sp. crude extract against *B. cereus*, *S. epidermidis*, *S. aureus*, *M. marinum* and *E. coli*. *Filobasidium magnum* crude extracts showed the highest MIC against *K. pneumoniae* and *P. aeruginosa* compared to the other tested crude extracts, as seen in Table 4.3.

Table 4.3: Antibacterial evaluation of crude endophytes fungal extracts

	Test organism with MIC (mg/mL)										
Crude extract	*B. cereus*	*B. subtilis*	*S. epidermidis*	*S. aureus*	*M. smegmatis*	*M. marinum*	*E. aerogenes*	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*
T1	8.00	16.00	**0.500**	8.00	**0.500**	**0.500**	16.00	8.00	>16.00	8.00	>16.00
T2	**0.125**	**1.00**	4.00	**0.125**	8.00	**0.500**	**1.00**	**0.500**	**0.500**	>16.00	8.00
T3	**1.00**	4.00	2.00	**0.125**	**1.00**	16.00	16.00	**0.500**	16.00	8.00	16.00
T4	**0.125**	2.00	2.00	**1.00**	**1.00**	**0.125**	**0.500**	**0.500**	6.00	4.00	>16.00
T5	**0.125**	2.00	**0.125**	**0.125**	2.00	**0.125**	16.00	0.125	4.00	8.00	8.00
	Positive control MIC (µg/mL)										
T6	0.031	0.031	0.062	0.031	0.062	0.062	0.125	0.125	0.125	0.062	0.031

T1= *Filobasidium magnum*, T2= *Alternaria tenuissima*, T3= *Penicillium chrysogenum*, T4= *Alternaria alternate*, T5= *Penicillium* sp., T6 = Positive Control Streptomycin.

Fungal endophytes are known to produce antimicrobial metabolites, including antibiotics, which have effects on a number of disease-causing microbes (Sardul Singh *et al*., 2014; Abdalla and Mcgaw, 2018). The endophytic crude extracts tested in this study showed noteworthy anti-bacterial activity against selected pathogenic strains.

Alternaria tenuissima crude extract had MIC values ranging from 0.125–>16 mg/ml, with the most significant inhibition observed for *B. cereus* and *S. aureus* at concentrations of 0.125 mg/ml. The *Alternaria tenuissima* crude extract also showed antibacterial activity of 1.00 mg/ml against *B. subtilis* and *E. aerogenes*, with 0.500 mg/ml against *M. marinum*, *E. coli* and *K. pneumoniae*. Extracts from *Alternaria tenuissima* have been reported to possess antibacterial activity against a wide range of pathogenic microbes, including *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *Enterococcous* sp. and *S. typhi* (Sardul Singh *et al*., 2014). This supports the findings in this study.

Penicillium chrysogenum crude extract had MIC values ranging from 0.125–16 mg/ml, with the most significant inhibition observed for *S. aureus* at concentrations of 0.125 mg/ml. *B. cereus*, *M. smegmatis*, and *E. coli* displayed inhibition at concentrations of 1.00 mg/ml, 1.00 mg/ml and 0.500 mg/ml, respectively. Onyegeme-Okerenta *et al.* (2009) reported that crude extracts of *Penicillium chrysogenum* had inhibited isolated *E. coli* and *B. subtilis* at varying concentrations of less than 1.50 mg/ml. *Penicillium chrysogenum*, also known as *Penicillium notatum*, has been known to produce penicillin G and penicillin V antibiotics, which are active against Gram-positive bacteria, furthermore, Devi *et al*. (2012) indicated that diketopiperazine has been isolated from *Penicillium chrysogenum* and showed activity against Gram-negative bacteria. This concurs with the results obtained in this study.

Alternaria alternate crude extracts had MIC values ranging from 0.125–>16 mg/ml, with the most significant inhibition observed for *B. cereus* and *M. marinum* at concentrations of 0.125 mg/ml. *S. aureus* and *M. smegmatis* showed inhibition at concentrations of 1.00 mg/ml, and *E. aerogenes* and *E. coli* showed inhibition at concentrations of 0.500 mg/ml. Bioactive compounds from *Alternaria alternate* have inhibited *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *E. coli* at varying concentrations (Kamal *et al*., 2015). This supports the findings in this study.

Penicillium sp. crude extracts had MIC values ranging from 0.125–16 mg/ml. An inhibition at concentration of 0.125 mg/ml was observed for *B. cereus*, *S. epidermidis*, *S. aureus*, *M. marinum* and *E. coli*. Polyketides isolated from endophytic fungus *Penicillium* sp. have shown antibacterial activity against *S. aureus, E. coli* and *B. subtilis* (Jouda *et al*., 2014). A number of antibacterial compounds have been isolated from endophytic fungus *Penicillium* sp., and this concurs with the results obtained in this study (Koolen *et al*., 2012).

Pusztahelyi *et al*. (2015) reported that ascomycetes have more secondary metabolism genes than any other fungal phylum. This would explain the results observed in this study, as

endophytic fungi from the ascomycete phylum (*Alternaria alternate*, *Penicillium chrysogenum*, *Alternaria tenuissima*, *Penicillium chrysogenum* and *Penicillium* sp.) displayed greater antibacterial activity than isolates from the Basidiomycota division (*Filobasidium magnum*).

Filobasidium magnum crude extracts had MIC values ranging from 0.500–>16 mg/ml. The most significant inhibition was observed for *S. epidermidis*, *M. smegmatis* and *M. marinum* at concentrations of 0.500 mg/ml. To the best of our knowledge, this study is the first to report on the antibacterial activity of endophytic fungus *Filobasidium magnum*, also known as *Cryptococcus magnus*.

4.3.2 Anticancer evaluation of crude extracts

4.3.2.1 Anticancer evaluation of crude bacteria endophyte extracts from the bulbs against A549 lung carcinoma cells

Crude bulb endophyte extracts showed varying activities against A549 lung carcinoma cells. *Bacillus cereus* crude extracts showed a 56% cell reduction at a concentration of 100 µg/ml, whereas *Rahnella aquatilis* crude extracts displayed a cell reduction of 46% at a concentration of 50 µg/ml, as depicted in Figure 4.1.

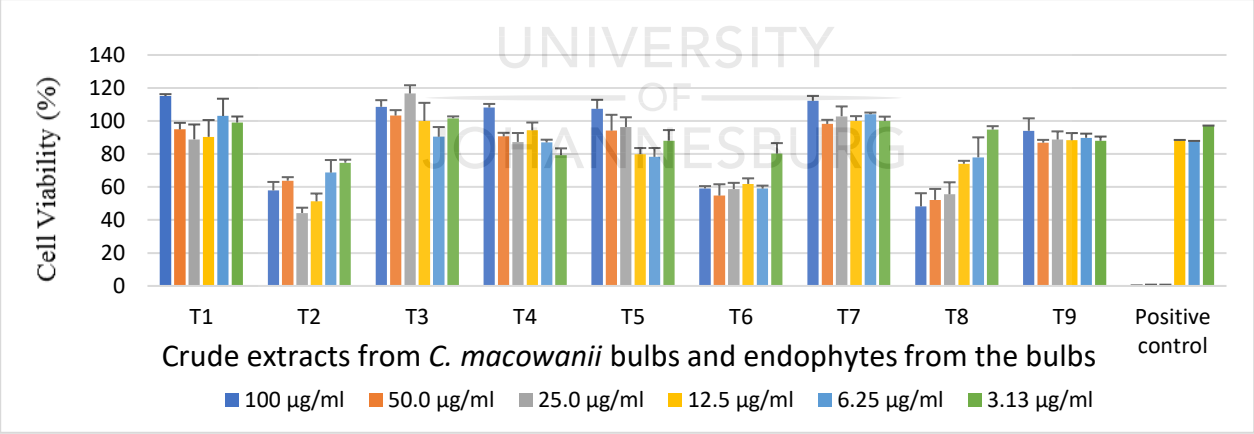

Figure 4.1. Cytotoxic activity of endophytic-derived crude extracts and bulb crude extracts on A549 lung carcinoma cells tested at different concentrations ranging from 100–3.13 µg/ml. The positive control used was auranofin. T1 = *C. macowanii* bulbs, T2 = *Raoultella ornithinolytica*, T3 = *Acinetobacter guillouiae*, T4 = *Pseudomonas moraviensis*, T5 = *Pseudomonas* sp., T6 = *Rahnella aquatilis*, T7 = *Novosphingobium* sp., T8 = *Bacillus cereus*, T9 = *Burkholderia tropica*

Bacillus cereus crude extract exhibited a low cell reduction of 56% at a concentration of 100 µg/ml on the A549 lung carcinoma cell lines. Other species of *Bacillus cereus* have been reported to display cytotoxicity on a human cervical cancer cell line (HeLa) and a breast cancer cell line (MCF-7) (Ferdous *et al.*, 2018). This could explain the results obtained in this study. *Raoultella ornithinolytica* crude extract exhibited a low cell reduction of 43% at 100 µg/ml on the A549 lung carcinoma cell lines,

despite a recovery of cell viability when the cells were exposed to a low concentration of 3.13 µg/ml. This observation is supported by studies carried out by Zhang *et al*., (2006) where the researchers reported that after a low dose of chemotherapy, tumour tissue has a propensity to regrow, causing tumour repopulation. Hutf & Grady (1996) stated that anticancer drugs used for lung carcinoma cells have concentration-effect relationships, and this could explain our results.

4.3.2.2 Anticancer evaluation of crude bacterial endophyte extracts from the bulbs against UMG87 glioblastoma cells

Acinetobacter guillouiae crude endophyte extract showed promising activity when tested against UMG87 glioblastoma cells, showing cell reduction of 52% at a concentration of 25.0 µg/ml, as shown in Figure 4.2.

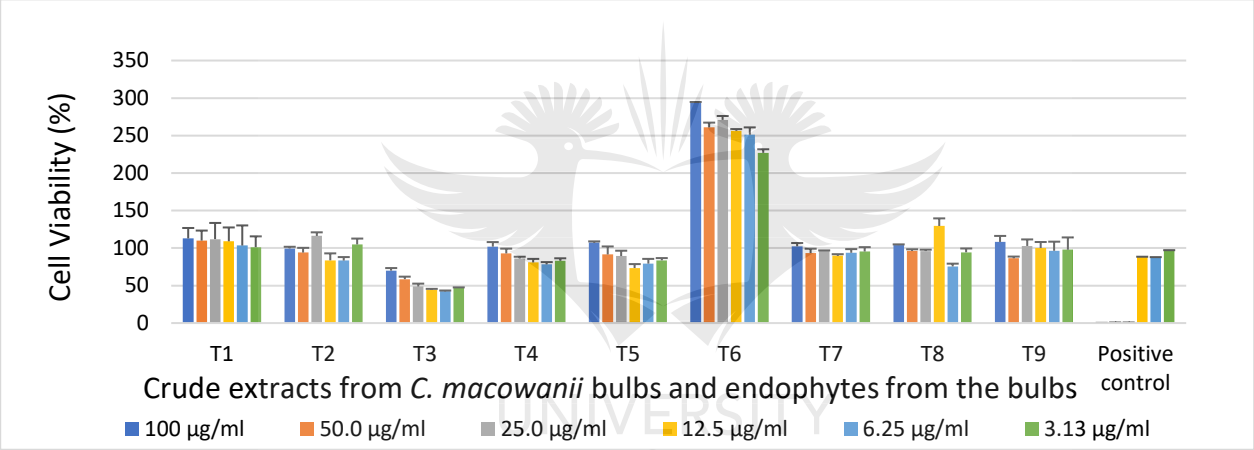

Figure 4.2. Cytotoxic activity of endophytic-derived crude extracts and bulb crude extracts on UMG87 glioblastoma cells tested at different concentrations ranging from 100–3.13 µg/ml. The positive control used was auranofin. T1 = *C. macowanii* bulbs, T2 = *Raoultella ornithinolytica*, T3 = *Acinetobacter guillouiae*, T4 = *Pseudomonas moraviensis*, T5 = *Pseudomonas* sp., T6 = *Rahnella aquatilis*, T7 = *Novosphingobium* sp., T8 = *Bacillus cereus*, T9 = *Burkholderia tropica*

Greenwell & Rahman (2015) and Seca & Pinto (2018) reported that secondary metabolites (alkaloids, brassinosteroids and taxols) from plants possess anticancer properties, and most are currently in clinical trials or being used in therapeutic applications. The *C. macowanii* crude bulb extract showed a cell viability of above 80% against A549 lung carcinoma and an above 100% cell viability was observed on UMG87 glioblastoma cell lines for all the concentrations tested, indicating no activity on those cell lines. In contrast, Maroyi (2016) reported that alkaloids such as crinamine, bulbispermine and lycorine isolated from *C. macowanii* have cytotoxic activity against human oral epidermoid carcinoma KB cells, apoptosis resistant cell lines and BLS mouse melanoma cells.

Acinetobacter guillouiae crude extract showed notable activity on UMG87 glioblastoma cell lines, with 58% cell death at 6.25 µg/ml. However, increased concentrations of *Acinetobacter guillouiae* crude extract showed an increase in cell viability. The extracts had a lower cell viability compared to that of the positive control at this concentration. To the best of our knowledge, this is the first report on the anticancer activity of crude endophytes extracts from *Acinetobacter guillouiae*.

A puzzling finding was the increased cell viability in the UMG87 glioblastoma cell in response to the *Rahnella aquatilis* crude extract. However, UMG87 glioblastoma cell lines are known to undergo hypoxia, resulting in metabolic abnormalities such as increased uptake of glucose and acid resistance; this increased glucose uptake and high aerobic glycolysis induces proliferation of cancer cells (Zhou *et al*., 2011; Jiang, 2017). This would explain the high cell viability of 100% and above on the glioblastoma cell lines. Further studies are needed to elucidate this phenomenon. The activities observed from the methanol/dichloromethane crude plant extracts also could have been caused by artifacts, as Sauerschnig *et al.*, (2018) observed that artifacts are generated by methanol during sample extraction and storage.

4.3.2.3 Anticancer evaluation of crude bacterial endophyte extracts from the leaves against A549 lung carcinoma cells

Pseudomonas putida and *Bacillus safensis* crude extracts showed a 47% and 50% cell reduction, respectively, against lung carcinoma cells at a concentration of 100 µg/ml (Figure 4.3).

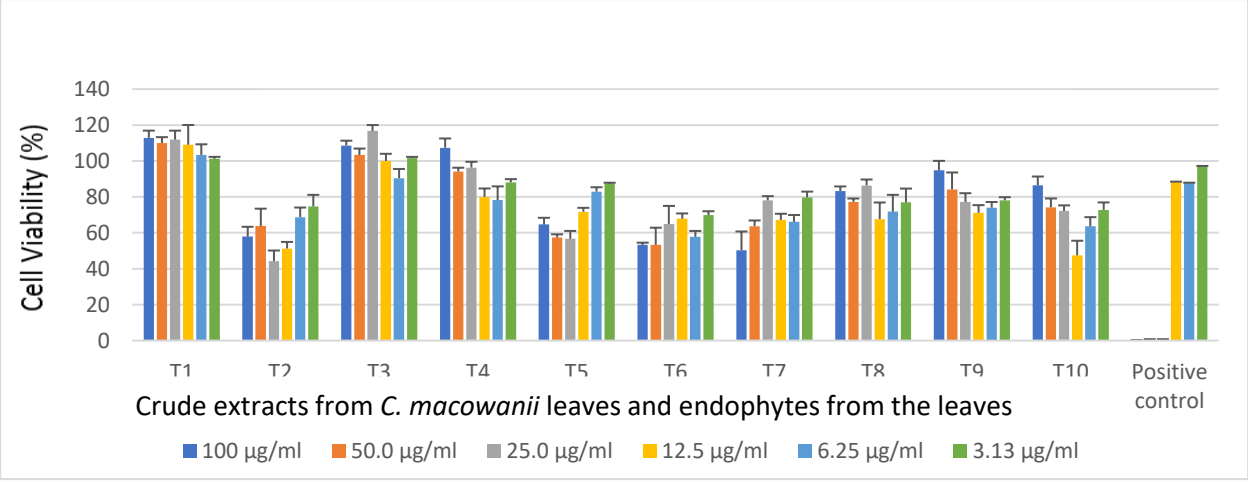

Figure 4.3. Cytotoxic activity of endophytic-derived secondary metabolites and crude extracts on A549 lung carcinoma cells tested at different concentrations ranging from 100–3.13 µg/ml. The positive control used was auranofin. T1= *C. macowanii* leaves, T2= *Raoultella ornithinolytica*, T3= *Acinetobacter guillouiae*, T4= *Pseudomonas* sp., T5= *Pseudomonas palleroniana*, T6= *Pseudomonas putida*, T7= *Bacillus safensis*, T8= *Enterobacter asburiae*, T9= *Pseudomonas cichorii*, T10= *Arthrobacter pascens*

Bacillus safensis crude extracts displayed noteworthy activity against A549 lung carcinoma cells with 50% cell reduction at 100 µg/ml. Ferdous *et al.* (2018) reported that crude extracts of *Bacillus safensis* isolated from sea sponges had anticancer activity against HepG2 (hepatocellular carcinoma), HCT (colon carcinoma) and MCF 7 (breast carcinoma). This could explain the results observed, and crude endophyte extracts from *Bacillus safensis* can be used as anticancer agent against lung cancer.

Pseudomonas sp. are known to produce anticancer compounds and have been reported to possess activity against a number of human cancer cell lines (Gross and Loper, 2009; Michelsen *et al.*, 2015). *Pseudomonas* sp., and *Pseudomonas cichorii* displayed no noteworthy activity against UMG87 glioblastoma cells or A549 lung carcinoma cells. *Pseudomonas palleroniana* crude extracts displayed 36% cell reduction at 100 µg/ml against A549 lung carcinoma cells.

Crude endophyte extracts from *Pseudomonas putida* displayed 47% cell reduction at 100 µg/ml against A549 lung carcinoma cells. Asif *et al.* (2016) reported that *P. putida* TJ151 is able to produce fluorouracil, which is a bioactive aromatic compound and is an anticancer drug. L-methioninase, an enzyme produced by *Pseudomonas putida*, has shown anticancer activity against leukaemia, liver HepG2, breast MCF-7, lung A549, prostate PC3 and colon HCT116 cell lines (Selim *et al.*, 2015; Selim *et al.*, 2016). Yoshioka *et al.* (1998) stated that methioninase from *P. putida* and 5-fluorouracil work synergistically to inhibit tumour growth, explaining the activity observed.

4.3.2.4 Anticancer evaluation of crude bacterial endophyte extracts from the leaves against UMG87 glioblastoma cells

Acinetobacter guillouiae crude extracts showed a 42% reduction of UMG87 glioblastoma cells at a concentration of 6.25 µg/ml, and *Arthrobacter pascens* crude extracts displayed cell reduction of 37% at a concentration of 12.5 µg/ml, as shown in Figure 4.4.

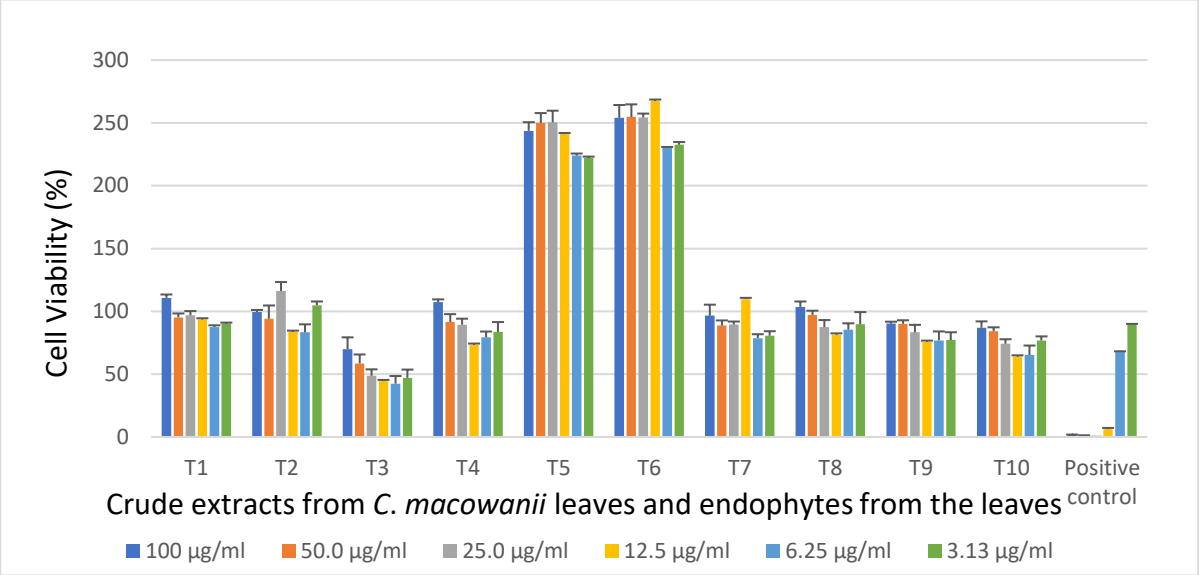

Figure 4.4. Cytotoxic activity of endophytic-derived secondary metabolites and crude extracts on UMG87 glioblastoma cells tested at different concentrations ranging from 100–3.13 µg/ml. The positive control used was auranofin. T1= *C. macowanii* leaves, T2= *Raoultella ornithinolytica*, T3= *Acinetobacter guillouiae*, T4= *Pseudomonas* sp., T5= *Pseudomonas palleroniana*, T6= *Pseudomonas putida*, T7= *Bacillus safensis*, T8= *Enterobacter asburiae*, T9= *Pseudomonas cichorii*, T10= *Arthrobacter pascens*

To the best of our knowledge, this is the first report on the anticancer activity of crude extracts from *C. macowanii* leaves. No noteworthy activities were observed from the leaves' crude samples against both cell lines used in this study. Bioactive compounds such as lycorine, pretazettine, crinamine, augustine and galanthamine are noted to appear in *C. macowanii* leaves and have been reported to possess anticancer activity (Fennell and Staden, 2001; Nair and van Staden, 2013; Maroyi, 2016).

To the best of our knowledge, this is the first report on the anticancer activity of crude endophytes extracts from *Pseudomonas palleroniana*, *Bacillus safensis*, *Enterobacter asburiae*, *Arthrobacter pascens*, *Acinetobacter guillouiae* and *Pseudomonas cichorii*.

Acinetobacter guillouiae crude endophyte extract was the only tested sample that exhibited anticancer activity against UMG87 glioblastoma cells, with a 31% cell reduction at 100 µg/ml and 53% cell reduction at 3.13 µg/ml, posing as a possible anticancer agent against brain cancer.

The crude endophytic extracts of *Enterobacter asburiae*, *Pseudomonas* sp., *Arthrobacter pascens* and *Arthrobacter pascens* and *Pseudomonas palleroniana* displayed no noteworthy activity against UMG87 glioblastoma cells or A549 lung carcinoma cells. The extracts should be tested on other cancer cell lines to determine their activity.

Crude endophyte extracts from *Raoultella ornithinolytica* displayed 43% cell reduction at 100 µg/ml against A549 lung carcinoma cells. Protein complexes from *R. ornithinolytica* have shown

anticancer activity against the HeLa cell line, the human endometrioid ovarian cancer line (TOV 112D ATCC CRL-11731) and the human breast adenocarcinoma line (T47D ECACC 85102201), resulting in cytopathic effect and reduction in cell numbers (Fiolka *et al.*, 2013; Fiołka *et al.*, 2015).

Crude endophyte extracts of *Raoultella ornithinolytica*, *Pseudomonas palleroniana*, *Pseudomonas putida* and *Bacillus safensis* can be further purified and tested for their anticancer activity against other types of cancer cell lines.

4.3.2.5 Anticancer evaluation of crude fungi endophyte extracts from the bulbs and leaves against A549 lung carcinoma cells

Crude endophytic fungal extracts showed cell viabilities above 80% against A549 lung carcinoma cells. In contrast, the *Filobasidium magnum* crude extract showed a 25% cell reduction of lung carcinoma cells at a concentration of 12.5 µg/ml (Figure 4.5).

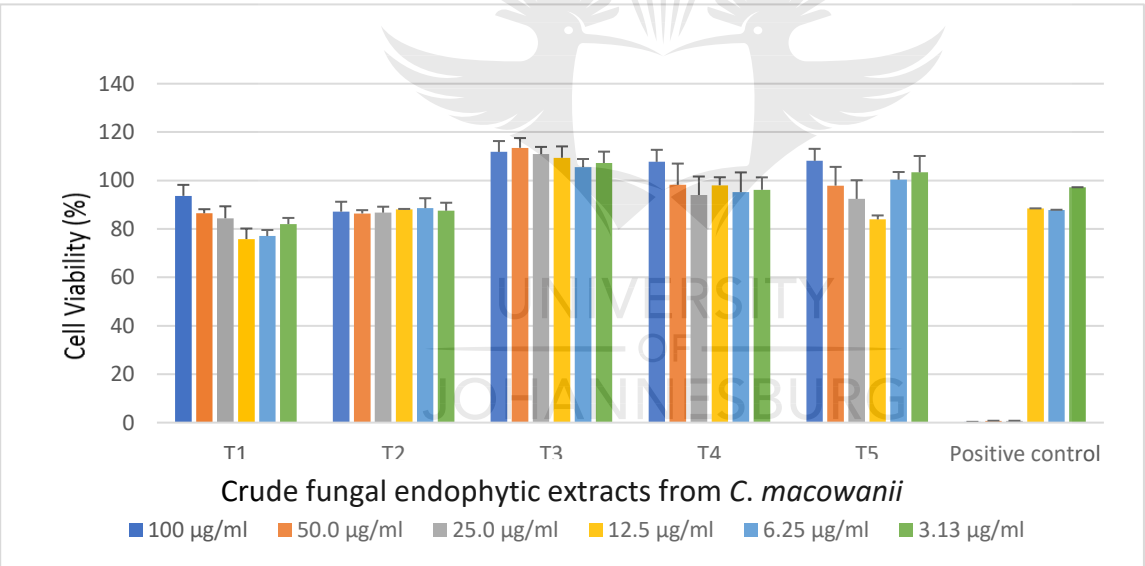

Figure 4.5. Cytotoxic activity of fungal endophytic-derived secondary metabolites on A549 lung carcinoma cells tested at different concentrations ranging from 100–3.13 µg/ml. The positive control used was auranofin. T1= *Filobasidium magnum*, T2= *Alternaria tenuissima*, T3= *Penicillium chrysogenum*, T4= *Alternaria alternate*, T5= *Penicillium* sp.

Anticancer agents such as brefeldin A, xylariaquinone A and tauranin have been isolated from fungal endophytes and are active against a number of cancer cell lines including HeLa, Vero cells and MCF-7 (Kharwar *et al.*, 2011).

4.3.2.6 Anticancer evaluation of crude fungi endophyte extracts from the bulbs and leaves against UMG87 glioblastoma cells

Penicillium chrysogenum crude extract showed cell reduction of 38% at 12.5 µg/ml. All tested extracts had cell viabilities above 94% at 100 µg/ml. *Filobasidium magnum* crude extract showed a 23% reduction of lung carcinoma cells at a concentration of 12.5 µg/mL (Figure 4.6).

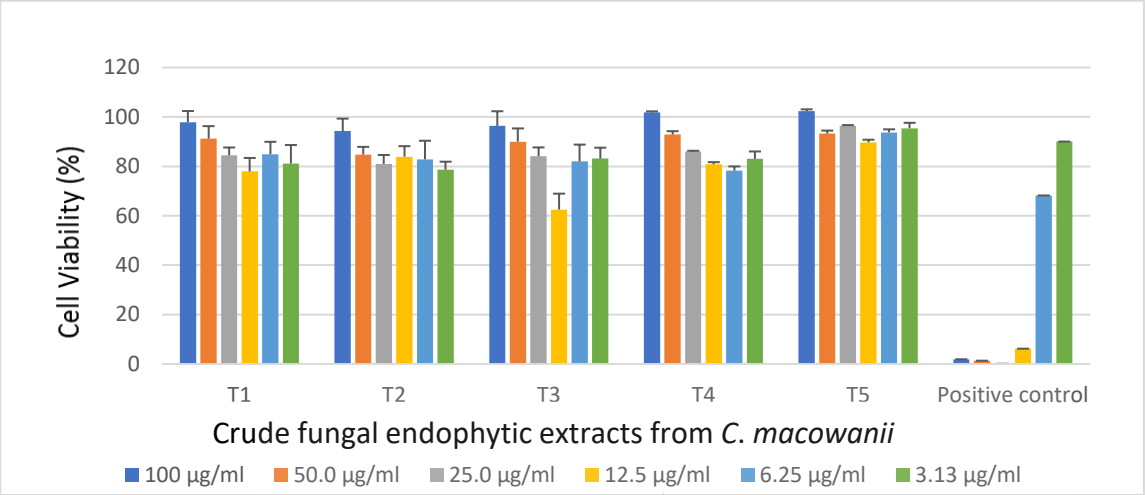

Figure 4.6. Cytotoxic activity of fungal endophytic-derived secondary metabolites on UMG87 glioblastoma cells tested at different concentrations ranging from 100–3.13 µg/ml. The positive control used was auranofin. T1= *Filobasidium magnum*, T2= *Alternaria tenuissima*, T3= *Penicillium chrysogenum*, T4= *Alternaria alternate*, T5= *Penicillium* sp.

Alternaria tenuissima crude extracts showed a cell viability of above 86% against A549 lung carcinoma and an above 81% cell viability was observed on UMG87 glioblastoma cell lines for all the concentrations tested, indicating no activity on those cell lines. Ismaiel *et al.* (2017) reported that paclitaxel, also known as taxol, produced by endophytic fungus, *Alternaria tenuissima*, was active against A549 cells at a concentration of 0.78 µg/ml. *Alternaria alternate* crude extracts showed a cell viability of above 94% against A549 lung carcinoma and a cell viability of above 78% was observed against UMG87 glioblastoma cell lines for all the concentrations tested, indicating no activity on those cell lines. Alternariol-10-methyl ether a mycotoxin produced by fungal *Alternaria alternate* has been reported to be active against A549 cells at >100 µM (Uzma *et al*., 2018).

Penicillium chrysogenum crude extracts showed a cell viability of above 105% against A549 lung carcinoma and an above 38% cell reduction was observed on UMG87 glioblastoma cell lines for all the concentrations tested. Chloctanspirone A isolated from endophytic *Penicillium chrysogenum* was active against A549 cells at a concentration of 39.7 µM (Bladt *et al*., 2013).

Penicillium sp. crude extracts showed a cell viability of above 83% against A549 lung carcinoma and an above 89% cell viability was observed on the UMG87 glioblastoma cell lines for all the concentrations tested, indicating no activity on those cell lines. Bladt *et al.* (2013) reported that

compounds from *Penicillium* sp. are active against A549 cells, which was not observed in this study. *Filobasidium magnum* crude extracts showed a cell viability of above 75% against A549 lung carcinoma and an above 77% cell viability was observed on UMG87 glioblastoma cell lines for all the concentrations tested, indicating no activity on those cell lines. To the best of our knowledge, this is the first report on the anticancer activity of crude fungal extracts from *Filobasidium magnum.*

Factors such as drought or heat stress and environmental factors affect secondary metabolisms, and interaction between the host and fungus and environmental factors regulate the production of secondary metabolites in a cooperative manner (Pusztahelyi *et al*., 2015). This can alter the production of certain secondary metabolites and would explain why isolates previously reported to have activity did not display any activity in this study. Calvo *et al.* (2002) reported that fungal development is linked to the production of fungal secondary metabolites, and different secondary metabolites are produced at different stages of fungal development, mostly at the later stages of fungal development such as sporulation, a fungal developmental process. In sporulation, pigments and secondary metabolites required for spore formation and activate sporulation, respectively, are produced. The metabolites reported to have anticancer properties could be produced at different stages of the fungal development and our isolates may not have reached those developmental stages at the time of harvesting. This would explain the results observed.

4.4 Conclusion

Crude endophyte (bacteria and fungi) extracts displayed some notable inhibitory activities against Gram-positive and Gram-negative bacterial species. Crude extracts from bacteria endophytes exhibited significant anticancer activities against lung and brain cancer compared to the crude fungal extracts. From the results obtained, it can be concluded that crude extracts obtained from endophytes (bacteria and fungi) isolated from the medicinal plant *C. macowanii* bulbs and leaves can biosynthesize bioactive compounds and can be bio prospected for medical application into antibacterial and anticancer agents.

References

Abdalla, M.A., Mcgaw, L.J., 2018. Bioprospecting of South African Plants as a Unique Resource for Bioactive Endophytic Microbes. Front. Pharmacol. 9, 1–18. https://doi.org/10.3389/fphar.2018.00456

Akinsanya, A.M., Ting, S.A., Sanusi, Om., 2017. Extraction methods and TLC-bioautography for evaluation of antimicrobial activities of endophytic bacteria from medicinal plants. LASU J. Med. Sci. 2, 1–7.

Andrews, J.M., 2001. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 1, 5–16.

Artun, F.T., Karagoz, A., Ozcan, G., Melikoglu, G., Anil, S., Kultur, S., Sutlupinar, N., 2016. In vitro anticancer and cytotoxic activities of some plant extracts on HeLa and Vero cell lines. J. Balk. Union Oncol. 21, 720–725.

Asif, H., Studholme, D.J., Khan, A., Aurongzeb, M., Khan, I.A., Kamran Azim, M., 2016. Comparative genomics of an endophytic Pseudomonas putida isolated from mango orchard. Genet. Mol. Biol. 39, 465–473.

Baker, S., Satish, S., 2012. Endophytes : Natural Warehouse of Bioactive Compounds. Drug Invent. Today 4, 548–553.

Bladt, T.T., Frisvad, J.C., Knudsen, P.B., Larsen, T.O., 2013. Anticancer and Antifungal Compounds from Aspergillus, Penicillium and Other Filamentous Fungi. Molecules 18, 11338–11376. https://doi.org/10.3390/molecules180911338

Brader, G., Stephane, C., Birgit, M., Mitter, B., Trognitz, F., Sessitsch, A., 2014. Metabolic potential of endophytic bacteria. Curr. Opin. Biotechnol. 30–37. https://doi.org/10.1016/j.copbio.2013.09.012

Calvo, A.M., Wilson, R.A., Bok, J.W., Keller, N.P., 2002. Relationship between Secondary Metabolism and Fungal Development. Microbiol. Mol. Biol. Rev. 66, 447–459. https://doi.org/10.1128/MMBR.66.3.447

Cos, P., Vlietinck, A.J., Berghe, D. Vanden, Maes, L., 2006. Anti-infective potential of natural products : How to develop a stronger in vitro ‘ proof-of-concept .’ J. Ethnopharmacol. 106, 290–302. https://doi.org/10.1016/j.jep.2006.04.003

Coussens, N.P., Braisted, J.C., Peryea, T., Sittampalam, G.S., Simeonov, A., Hall, M.D., 2017. Small-Molecule Screens : A Gateway to Cancer Therapeutic Agents with Case Studies of Food and Drug Administration – Approved Drugs. Pharmacol. Rev. 479–496.

Davies, J., Davies, D., 2010. Origins and Evolution of Antibiotic Resistance. Microbiol. Mol. Biol. Rev. 74, 417–433. https://doi.org/10.1128/MMBR.00016-10

De Mot, R., Ghequire, M.G.K., 2014. Ribosomally encoded antibacterial proteins and peptides from Pseudomonas. Fed. Eur. Microbiol. Soc. 38, 523–568. https://doi.org/10.1111/1574-6976.12079

Delcour, A.H., 2009. NIH Public Access. Biochim. Biophys. Acta 1794, 808–816. https://doi.org/10.1016/j.bbapap.2008.11.005.Outer

Denicolaï, E., Baeza-kallee, N., Tchoghandjian, A., Carré, M., Colin, C., Jiglaire, C.J., Mercurio, S., Beclin, C., Figarella-branger, D., 2014. Proscillaridin A is cytotoxic for glioblastoma cell lines and controls tumor xenograft growth in vivo. Oncotarget 5, 10934–10948.

Devi, P., Rodrigues, C., Naik, C.G., 2012. Isolation and Characterization of Antibacterial Compound from a Mangrove-Endophytic Fungus , Penicillium chrysogenum MTCC 5108. Indian J. Microbiol. 52, 617–623. https://doi.org/10.1007/s12088-012-0277-8

Dzotam, J.K., Simo, I.K., Bitchagno, G., Celik, I., Sandjo, L.P., Tane, P., Kuete, V., 2018. In vitro antibacterial and antibiotic modifying activity of crude extract, fractions and 3',4',7-trihydroxyflavone from Myristica fragrans Houtt against MDR Gram-negative enteric bacteria. BMC Complement. Altern. Med. 18, 1–9. https://doi.org/10.1186/s12906-018-2084-1

El-Hendawy, H.., Osman, M.., Sorour, N.., 2003. Characterization of Two Antagonistic Strains of Rahnella aquatilis Isolated from Soil in Egypt. Folia Microbiol. 48, 799–804. https://doi.org/10.1007/BF02931517

Elgorashi, E E, Zschocke, S., Staden, J. Van, 2003. The anti-inflammatory and antibacterial activities of Amaryllidaceae alkaloids. South African J. Bot. 69, 448–449. https://doi.org/10.1016/S0254-6299(15)30329-X

Fan, C., Zheng, W., Fu, X., Li, X., Wong, Y., Chen, T., 2014. Enhancement of auranofin-induced lung cancer cell apoptosis by selenocystine , a natural inhibitor of TrxR1 in vitro and in vivo. Nat. Publ. Gr. 5, 2–11. https://doi.org/10.1038/cddis.2014.132

Fathalla, A.M., Samy, A.M.A.E.-A., Metwaly, A.B., Ramadan, E.M., 2015. Characterization of Antifungal Metabolites from Antagonistic Fluorescent Pseudomonads. J. Appl. Plant Prot. 4, 13–21. https://doi.org/10.21608/japp.2015.7593

Fennell, C.W., van Staden, J., 2001. Crinum species in traditional and modern medicine. J. Ethnopharmacol. 78, 15–26.

Ferdous, U.., Shishir, M.., Khan, S.., Hoq, M.., 2018. Bacillus spp .: Attractive Sources of Anti-cancer and Anti-proliferative Biomolecules. Microb. Bioact. 1, 033–045. https://doi.org/10.25163/microbbioacts.11005B0408130818

Fiołka, M.J., Grzywnowicz, K., Rzymowska, J., Lewtak, K., Szewczyk, R., Mendyk, E., Keller, R., 2015. Antitumour and apoptotic effects of a novel Tris-peptide complex obtained after isolation of Raoultella ornithinolytica extracellular metabolites. J. Appl. Microbiol. 118, 1357–1369. https://doi.org/10.1111/jam.12806

Fiolka, M.J., Lewtak, K., Rzymowska, J., Grzywnowicz, K., Hulas-Stasiak, M., Sofińska-Chmiel, W., Skrzypiec, K., 2013. Antifungal and anticancer effects of a polysaccharide-protein complex from the gut bacterium Raoultella ornithinolytica isolated from the earthworm Dendrobaena veneta. Pathog. Dis. 69, 49–61. https://doi.org/10.1111/2049-632X.12056

Garcia-Bustos, J.F., Pezzi, N., Mendez, E., 1985. Structure and Mode of Action of Microcin 7 , an Antibacterial Peptide Produced by Escherichia coli. Antimicrob. Agents Chemother. 27, 791–

797.

Gouda, S., Das, G., Sen, S.K., Shin, H., Patra, J.K., 2016. Endophytes : A Treasure House of Bioactive Compounds of Medicinal Importance. Front. Microbiol. 7, 1–8. https://doi.org/10.3389/fmicb.2016.01538

Grech, A., Baldacchino, A., Tufignio, M., 2009. Secondary Metabolites and their Exploitation in Medicine. Maltese Fam. Dr. 18, 8–13.

Greenwell, M., Rahman, P.K.S.M., 2015. Europe PMC Funders Group Medicinal Plants : Their Use in Anticancer Treatment. Int. J. Pharm. Sci. Res. 6, 4103–4112. https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12.Medicinal

Gross, H., Loper, J.E., 2009. Genomics of secondary metabolite production by Pseudomonas spp Genomics of secondary metabolite production by Pseudomonas spp †. Nat. Prod. Rep. 26, 1408–1446. https://doi.org/10.1039/b817075b

Handayani, D., Rivai, H., Mulyana, R., Suharti, N., Rasyid, R., Hertiani, T., 2018. Antimicrobial and Cytotoxic Activities of Endophytic Fungi Isolated from Antimicrobial and Cytotoxic Activities of Endophytic Fungi Isolated from Mangrove Plant Sonneratia alba Sm. J Appl Pharm Sci 8, 49–53. https://doi.org/10.7324/JAPS.2018.8207

Ho, Y.-N., Huanga, C.-C., 2015. Draft Genome Sequence of Burkholderia cenocepacia Strain 869T2, a Plant-Beneficial Endophytic Bacterium. Genome Announc. 3, 1–2. https://doi.org/10.1128/genomeA.01327-15

Hutf, A.J., Grady, J.O., 1996. Review Drug chirality : a consideration of the significance of the stereochemistry of antimicrobial agents. J. Antimicrob. Chemother. 37, 7–32.

Iannello, C., Bastida, J., Bonvicini, F., Antognoni, F., Gentilomi, G.A., Poli, F., 2014. Chemical composition, and in vitro antibacterial and antifungal activity of an alkaloid extract from Crinum angustum Steud. Nat. Prod. Res. https://doi.org/10.1080/14786419.2013.877903

Ismaiel, A.A., Ahmed, A.S., Hassan, I.A., El-Sayed, E.-S.R., El-Din, A.-Z.A.K., 2017. Production of paclitaxel with anticancer activity by two local fungal endophytes , Aspergillus fumigatus and Alternaria tenuissima. Appl. Microbiol. Biotechnol. 101, 5831–5846. https://doi.org/10.1007/s00253-017-8354-x

Jalgaonwala, R.E., Mahajan, R.T., 2011. Evaluation of hydrolytic enzyme activities of endophytes from. J. Agric. Technol. 2011 7, 1733–1741.

Jouda, J., Kusari, S., Lamshöft, M., Mouafo, F., Douala, C., Wandji, J., Spiteller, M., 2014. Fitoterapia Penialidins A – C with strong antibacterial activities from Penicillium sp ., an endophytic fungus harboring leaves of Garcinia nobilis. Fitoterapia 98, 209–214. https://doi.org/10.1016/j.fitote.2014.08.011

Kabera, J.N., Semana, E., Mussa, A.R., He, X., 2014a. Plant Secondary Metabolites: Biosynthesis,

Classification, Function and Pharmacological Properties. J. Pharm. Pharmacol. 2, 377–392. https://doi.org/10.1016/0300-9084(96)82199-7

Kamal, S.A., Hamza, L.F., Hameed, I.H., 2015. Antibacterial activity of secondary metabolites isolated from Alternaria alternata. African J. Biotechnol. 14, 2972–2994. https://doi.org/10.5897/AJB2015.14906

Kharwar, R.N., Mishra, A., Gond, S.K., Stierle, A., Stierle, D., 2011. Anticancer compounds derived from fungal endophytes : their importance andfuture challenges. Nat. Prod. Rep. 28, 1208–1228. https://doi.org/10.1039/c1np00008j

Koolen, H.H.F., Soares, E.R., Silva, F.M.A. da, Souza, R.A. de A. e A.D.L. de, 2012. An antimicrobial alkaloid and other metabolites produced by penicillium sp. an endophytic fungus isolated from Mauritia flexuosa L. f. Quim. Nova 35, 771–774.

Kumar, M.L.V., Thippeswamy, B., Kuppust, I.J., Virupaksha, J.H., 2013. Evaluation of analgesic activities of Bacillus. Int. J. Chem. Sci. 12, 272–280.

Kumar, V., Rai, S., Gaur, P., 2014. Endophytic Fungi : Novel Sources of Anticancer Molecules. https://doi.org/10.1007/978-81-322-1575-2

Mandal, S.M., Sharma, S., Pinnaka, A.K., Kumari, A., Korpole, S., 2013. Isolation and characterization of diverse antimicrobial lipopeptides produced by Citrobacter and Enterobacter. BMC Microbiol. 2013, 13, 1–9.

Maroyi, A., 2016a. A review of ethnoboatany, therapeutic value, phytochemistry and pharmacology of Crinum macowanii Baker_ A highly traded bulbous plant in Southern Africa. J. Ethnopharmacol. 194, 595–608. https://doi.org/10.1016/j.jep.2016.10.046

Matthijs, S., Vander Wauven, C., Cornu, B., Ye, L., Cornelis, P., Thomas, C., Ongena, M., 2014. Antimicrobial properties of producing the antibiotic mupirocin. Res. Microbiol. 165, 695–704. https://doi.org/10.1016/j.resmic.2014.09.009

McCauley, J., Zivanovic, A., Skropeta, D., 2013. Bioassays for anticancer activities. Methods Mol. Biol. 1055, 191–205.

Menpara, D., Chanda, S., 2013. Endophytic Bacteria- Unexplored Reservoir of Antimicrobials for Combating Microbial Pathogens. Microb. Pathog. Strateg. Combat. them Sci. Technol. Educ. 1095–1103.

Michelsen, C.F., Jensen, H., Venditto, V.J., Hennessy, R.C., Stougaard, P., 2015. Bioactivities by a crude extract from the Greenlandic Pseudomonas sp . In5 involves the nonribosomal peptides , nunamycin and nunapeptin. PeerJ 1476, 1–16. https://doi.org/10.7717/peerj.1476

Mohamed, R., Avis, T., Tsopmo, A., 2015. Distribution of antimicrobial lipopeptides in Bacillus and Pseudomonas spp ., two genera with antagonistic effects against plant pathogens. Carleton University Ottawa, Ontario.

Monakisi, C.M., 2007. Knowledge and Use of Tradition Medicinal Plants By the Setswan a - Speaking Community of Kimberley , Northern Cape of South Africa. October. University of Stellenbosch.

Mukherjee, A., Das, R., Pal, A., Paul, A.K., 2017. Antibacterial Activity of Endophytic Bacillus safensis Isolated from Ophioglossum reticulatum L . Microbiol. Res. J. Int. 18, 1–12. https://doi.org/10.9734/MRJI/2017/31923

Nair, J.J., van Staden, J., 2013. Pharmacological and toxicological insights to the South African Amaryllidaceae. Food Chem. Toxicol. J. 62, 262–275. https://doi.org/10.1016/j.fct.2013.08.042

Neal, R.D., Robbé, I.J., Lewis, M., Williamson, I., Hanson, J., 2015. The complexity and dif fi culty of diagnosing lung cancer : fi ndings from a national primary-care study in Wales. Prim. Health Care Res. Dev. 16, 436–449. https://doi.org/10.1017/S1463423614000516

Neu, A.K., Månsson, M., Gram, L., Prol-García, M.J., 2014. Toxicity of Bioactive and Probiotic Marine Bacteria and Their Secondary Metabolites in Artemia sp . and Caenorhabditis elegans as Eukaryotic Model Organisms. Appl. Environ. Microbiol. 80, 146–153. https://doi.org/10.1128/AEM.02717-13

Nii-Trebi, N.I., 2017. Emerging and Neglected Infectious Diseases : Insights , Advances , and Challenges. Hindawi BioMed Res. Int. 2017, 1–15.

Nongkhlaw, W.M.F., Joshi, S.R., 2015. Original Article Investigation on the bioactivity of culturable endophytic and epiphytic bacteria associated with ethnomedicinal plants. J. Infect. Dev. Ctries. 9, 954–961. https://doi.org/10.3855/jidc.4967

Onyegeme-Okerenta, B.M., Chinedu, S.N., Okafor, U.A., Okochi, V.I., 2009. Antibacterial Activity of Culture Extracts of Penicillium chrysogenum PCL501: Effects of Carbon Sources. Online J. Heal. Allied Sci. 8, 1–7.

Pagare, Saurabh, Bhatia, M., Tripathi, N., Pagare, Sonal, Bansal, Y.K., 2016. Secondary metabolites of plants and their role : Overview. Curr. Trends Biotechnol. Pharm. 9, 293–304.

Paolillo, M., Boselli, C., Sergio, S., 2018. Glioblastoma under Siege: An Overview of Current Therapeutic Strategies. Brain Sci. 8, 1–13. https://doi.org/10.3390/brainsci8010015

Prabavathy, D., Nachiyar, C.V., 2014. Cytotoxicity of Ethyl Acetate Extract of Endophytic Aspergillus fumigatus on A549 Cell Lines. Biosci. Biotechnol. Res. ASIA, 11, 797–802.

Pusztahelyi, T., Holb, I.J., Pócsi, I., 2015. Secondary metabolites in fungus-plant interactions. Front. Plant Sci. 6, 1–23. https://doi.org/10.3389/fpls.2015.00573

Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 56, 81–87. https://doi.org/10.1016/S0378-8741(96)01515-2

Raghu, J., 2012. Endophytic bacterial association and plant secondary metabolite production.

University of Agricultural Sciences, Bangalore.

Rajbhandary, S., 2014. Healing Traditions of the Himalayas 21–36. https://doi.org/10.1007/978-81-322-1925-5

Roder, C., Thomson, M.J., 2015. Auranofin : Repurposing an Old Drug for a Golden New Age 13–20. https://doi.org/10.1007/s40268-015-0083-y

Sardul Singh, S., Suneel, K., Aharwal, R.P., 2014. Isolation and identification of endophytic fungi from Ricinus communis linn . And their antibacterial activity. Int. J. Res. Pharm. Chem. 4, 611–618.

Sauerschnig, C., Doppler, M., Bueschl, C., Schuhmacher, R., 2018. Methanol Generates Numerous Artifacts during Sample Extraction and Storage of Extracts in Metabolomics Research. Metabolites 8, 1–19. https://doi.org/10.3390/metabo8010001

Sebola, T.E., Ndinteh, D.T., Mavumengwana, V., 2016. A contribution to the phytochemical and antibacterial characteristics of Crinum macowanii bulbs extracts. University of Johannessburg.

Seca, A.M.L., Pinto, D.C.G.., 2018. Plant Secondary Metabolites as Anticancer Agents : Successes in Clinical Trials and Therapeutic Application. Int. J. Mol. Sci. 19, 1–22. https://doi.org/10.3390/ijms19010263

Selim, M.H., Elshikh, H.H., Saad, M.M., Mostafa, E.E., Mahmoud, M.A., 2016. Purification and characterization of a novel thermo stable L-methioninase from Streptomyces sp. DMMMH4 and its evaluation for anticancer activity. J. Appl. Pharm. Sci. 6, 53–60. https://doi.org/10.7324/JAPS.2016.60708

Selim, M.H., Karm Eldin, E.-Z., Saad, M.M., Mostafa, E.-S.E., Shetia, Y.H., Anise, A.A.H., 2015. Purification, Characterization of L-Methioninase from Candida tropicalis , and Its Application as an Anticancer . Biotechnol. Res. Int. 2015, 1–10. https://doi.org/10.1155/2015/173140

Shanmugam, G., Dubey, A., Ponpandian, L.N., Rim, S.O., 2018. Genomic Insights into Nematicidal Activity of a Bacterial Endophyte , Raoultella ornithinolytica MG against Pine Wilt Nematode. Plant Pathol. J. 34, 250–255.

Strobel, G., Daisy, B., 2003. Bioprospecting for Microbial Endophytes and Their Natural Products. Microbiol. Mol. Biol. Rev. 67, 491–502. https://doi.org/10.1128/MMBR.67.4.491

Strobel, G.A., 2003. Endophytes as sources of bioactive products. Microbes Infect. 5, 535–544.

Tidke, S.A., Kumar, R.K.., Ramakrishna, D., Kiran, S., Kosturkova, G., Gokare, R.A., 2017. Current Understanding of Endophytes : Their Relevance , Importance , and Industrial Potentials. J. Biotechnol. Biochem. 3, 43–59. https://doi.org/10.9790/264X-03034359

Uzma, F., Mohan, C.D., Hashem, A., Konappa, N.M., Rangappa, S., Kamath, P. V., Singh, B.P., Mudili, V., Gupta, V.K., Siddaiah, C.N., Chowdappa, S., Alqarawi, A.A., Abd_Allah, E.F.,

2018. Endophytic Fungi — Alternative Sources of Cytotoxic Compounds : A Review. Front. Pharmacol. 9, 1–37. https://doi.org/10.3389/fphar.2018.00309

Van Wyk, A.S., Prinsloo, G., 2018. Medicinal plant harvesting , sustainability and cultivation in South Africa. Biol. Conserv. 227, 335–342. https://doi.org/10.1016/j.biocon.2018.09.018

Venieraki, A., Dimou, M., Katinakis, P., 2017. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. Hell. Plant Prot. J. 10, 51–66. https://doi.org/10.1515/hppj-2017-0006

Ventola, C.L., 2015. The Antibiotic Resistance Crisis Part 1 : Causes and Threats 40, 277–283.

Venugopalan, A., Srivastava, S., 2015. Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol. Adv. 33, 873–887. https://doi.org/10.1016/j.biotechadv.2015.07.004

Wietz, M., Månsson, M., Bowman, J.S., Blom, N., Ng, Y., Gram, L., 2012. Wide Distribution of Closely Related , Antibiotic-Producing Arthrobacter Strains throughout the Arctic Ocean. Appl. Environ. Microbiol. 78, 2039–2042. https://doi.org/10.1128/AEM.07096-11

Yadav, R.N.S., Agarwala, M., 2011. Phytochemical analysis of some medicinal plants. J. Phytol. 3, 10–14. https://doi.org/10.1021/np800144q

Yoshioka, T., Wada, T., Uchida, N., Maki, H., Yoshida, H., Ide, N., Kasai, H., Hojo, K., Shono, K., Maekawa, R., Yagi, S., Hoffman, R.M., Sugita, K., 1998. Anticancer efficacy in vivo and in vitro, synergy with 5-fluorouracil, and safety of recombinant methioninase. Cancer Res. 58, 2583–2587.

Zhang, M., Das, C., Vasquez, H., Aguilera, D., Zage, P.E., Gopalakrishnan, V., Wolff, J.E., 2006. Predicting Tumor Cell Repopulation After Response : Mathematical Modeling of Cancer Cell Growth. Anticancer Res. 26, 2933–2936.

Zhou, Yunfei, Zhou, Yan, Shingu, T., Feng, L., Chen, Z., Ogasawara, M., Keating, M.J., Kondo, S., Huang, P., 2011. Metabolic Alterations in Highly Tumorigenic Glioblastoma. J. Biol. Chem. 286, 32843–32853. https://doi.org/10.1074/jbc.M111.260935

Zonyane, S., Makunga, N.P., Vuuren, S.F. Van, 2013. The antimicrobial interactions of Agathosma crenulata, Dodonaea viscosa and Eucalyptus globulus combination and their chemical profiling. Stellenbosch University.

CHAPTER FIVE:

UNTARGETED SECONDARY METABOLITE PROFILING OF CRUDE PLANT EXTRACTS AND CRUDE ENDOPHYTE (BACTERIA AND FUNGI) EXTRACTS USING LIQUID CHROMATOGRAPHY COUPLED TO A QUADRUPOLE TIME-OF-FLIGHT WITH TANDEM MASS SPECTROMETRY (LCQTOF MS) *

* This chapter depicts the phytochemical analysis of the crude plant extracts and crude endophyte extracts. Although the chemical profile of the plant has been reported, the intent here was to use LC-QTOF-MS to identify the secondary metabolites produced by the isolated endophytes.

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Abstract

Endophytes are known to produce similar secondary metabolites to their host plants. These secondary metabolites possess antimicrobial, antiviral, antiparasitic, antioxidants and anticancer properties. Untargeted secondary metabolite profiling was performed using liquid chromatography coupled to a quadrupole time-of-flight with tandem mass spectrometry (LC-QTOF-MS). A C_{18} column was used in reverse phase UHPLC to separate the secondary metabolites from the bulbs, leaves and their endophyte crude extracts. Electrospray ionization in positive mode was used to ionize the secondary metabolites and fragments using collision-induced dissociation in automatic MS/MS mode. The spectra of the detected precursor ions and their fragments were used to screen the crude extracts in compound library databases. The results revealed the presence of unique alkaloidal constituents known to occur in *C. macowanii*. This included lycorine, crinamine, crinamidine and crinine, which were identified from plant parts and endophytes at different retention times and intensities. Secondary metabolites such as belladine, maculosin, magnolol, isoquinoline and melosatin A were also identified from different endophyte extracts, and their biological activities have been previously reported. In conclusion, endophytes isolated from *C. macowanii* produce secondary metabolites similar and dissimilar to those the host plant, with reported biological activities. Further work should be done to isolate these bioactive metabolites for drug development.

Keywords: Secondary metabolite, LC-QTOF-MS, Untargeted metabolite profiling, *C. macowanii*, Endophytes

5.1 Introduction

Endophytes have been isolated from different types of plant tissues and not only do they influence the health and growth of the host plant, they also produce bioactive compounds of interest in agriculture, medicine and commerce (Doty, 2017; Strobel, 2018). Bioactive secondary metabolites from endophytes can be grouped into different chemical groups such as alkaloids, flavonoids, quinones, steroids and terpenoids depending on their metabolic pathways *(*Tidke *et al*., 2017). These bioactive secondary metabolites have been reported to possess antimicrobial, antiviral, antiparasitic, antioxidant and anticancer properties (Sudha *et al.*, 2016; Tidke *et al.*, 2017). Xie *et al.* (2008) reported that bioactive secondary metabolites consist of compounds that differ in size, type and polarity, and analytical techniques have to be used to isolate and characterize compounds of interest.

The isolation and characterization of bioactive secondary metabolites help in distinguishing between new and already identified bioactive secondary metabolites, which assists in the development and discovery of new drug leads (Chikezie *et al*., 2015). Metabolite fingerprinting detects intracellular metabolites from the spectra of total compositions, whereas metabolite profiling is done to identify detected metabolites from large group of metabolites belonging to a specific metabolic pathway, or a class of compounds (Scholz *et al.*, 2004; Ncube *et al.*, 2016). These technologies assist in the identification of the vital compounds, and different techniques are used to achieve this *(*Hounsome *et al*., 2008). Several LC-MS/MS methods for compound detection in samples from endophytes and or medicinal plants have confirmed the presence of active compounds (Xiao *et al*., 2012). However, when working only in MS/MS mode, the instrument needs to be tuned to detect a limited set of the most relevant targeted compounds; other compounds, which can be present in complex matrices, are then not detected. These metabolites should be taken into consideration since they may also show activity against pathogenic microorganisms. Therefore, there also is a need for untargeted screening.

Techniques such as nuclear magnetic resonance (NMR), liquid chromatography (LC) or gas chromatography (GC), coupled with mass spectrometry (MS), are used for metabolite profiling (Xie *et al.*, 2008; Chikezie *et al*., 2015). Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) is an analytical technique used to screen, detect and identify crude extracts of biological samples for the characterization of secondary metabolites (Zhu *et al.*, 2013; Jayakrishnan and Benjamin, 2016). LC-TOF-MS is currently one of the most common techniques for non-target analysis. Quadrupole mass analysers TOF (QTOF) are distinguished by high precision with sensitivity, compared to tandem four-scan instruments in full scan mode. For secondary fungal metabolite analysis, various methods based on TOF-MS have been applied.

Wang *et al.* (2018) reported that the high sensitivity and high mass accuracy of LC-Q-TOF-MS assists in giving a detailed chemical profile of possibly unknown compounds in a crude extract. LC-Q-TOF-MS obtains accurate molecular mass combined with tandem mass spectrometry (MS/MS) spectra to be detected and identified, with the chemical compounds arranged in peaks (Xie *et al.*, 2008; Stolker *et al.*, 2004; Tan *et al.*, 2018). This makes it an ideal technique for the detection and identification of secondary metabolites in crude extracts, with potential for drug development and discovery (Senyuva and Gilbert, 2007 ; Xie *et al.*, 2008). The aim of this section was to perform secondary metabolite profiling of crude extracts from *C. macowanii* bulbs and leaves, and crude endophyte (bacteria and fungi) extracts which showed noteworthy antibacterial and anticancer activity in chapter 4.

5.2 Materials and methods

5.2.1 Extraction of crude plant extracts (bulbs and leaves) and fermentation of endophyte (bacteria and fungi) secondary metabolites

The crude plant extracts and crude endophyte extracts were prepared as described by Sebola *et al*. (2019). The concentrated extract was then prepared for LC-MS analysis by dissolving in HPLC grade methanol at 1 mg/ml (w/v), followed by sonicating for 10 minutes, and finally filtering through 0.22 µm polyvinylidene fluoride (PVDF) membrane syringe filters into 1 ml LC auto-sampler vials (Morare *et al.*, 2017; Changwa *et al.*, 2018).

5.2.1.1 Extraction of crude extracts from *C. macowanii* bulbs and leaves

C. macowanii bulbs and leaves were washed separately, chopped into small pieces and air-dried at room temperature. The dried plant material was blended into a fine powder using a commercial blender. Crude extracts were obtained according to Yadav and Agarwala (2011). Briefly, 150 g of the prepared plant material was mixed with two litres of a 50:50 methanol:dichloromethane solution. This was allowed to shake for 3 days on a platform shaker (Amerex Gyromax, Temecula, CA, USA) at 200 rcf. The solution was filtered through Whatman No. 1 filter paper; the filtrate was evaporated on a rotatory evaporator and allowed to air dry in a desiccator. HPLC grade methanol was used as a negative control. The concentrated extract was then prepared for LC-MS analysis by dissolving in HPLC grade methanol at 1 mg/ml (w/v), followed by sonicating for 10 minutes, and finally filtering through 0.22 µm polyvinylidene fluoride (PVDF) membrane syringe filters into 1 ml LC auto-sampler vials (Changwa *et al.*, 2018; Wang *et al.*, 2018).

5.2.1.2 Fermentation and extraction of crude extracts from bacterial endophytes

Two litres of nutrient broth (NB) were measured into a 4 L Erlenmeyer flask, leaving room for aeration, and autoclaved at 121°C for 15 min. Each 4 L flask was inoculated with one of the endophytic bacteria isolated from the bulbs and leaves as listed in Table 3.1, shaken at 200 rcf and incubated at 30°C, an ideal temperature for the growth of the bacterial endophytes (Sardul Singh *et al*., 2014). A negative containing nutrient broth (NB) only, was also prepared and exposed to the same conditions. After 7 days of cultivation, sterile XAD-7- HP resin (20 g/L) (Sigma, Johannesburg, South Africa, BCBR6696V) was added to the culture for 2 h, shaking at 200 rcf. The resin was filtered through cheesecloth and washed three times with 300 ml of acetone for each wash. The acetone soluble fraction was concentrated using a rotary evaporator and a dark yellowish viscous extract was obtained, which was transferred into a measuring cylinder. Depending on the volume, ethyl acetate was added in a ratio of 1:1 (v/v). The mixture was vigorously shaken for about 10 min, decanted into a separating funnel, allowed to separate and each phase collected in a conical flask. This process was repeated until the dark yellowish viscous liquid obtained after removing the acetone became a very light-yellow liquid. The ethyl acetate fraction was evaporated using a rotary evaporator and the brown extract obtained was stored in an amber bottle in a cool dry place until analysis was done (Maloney *et al*., 2009). The concentrated extract was then prepared for LC-MS analysis by dissolving in high profile liquid chromatography (HPLC) grade methanol at 1 mg/ml (w/v), followed by sonicating for 10 minutes, and finally filtering through 0.22 µm polyvinylidene fluoride (PVDF) membrane syringe filters into 1 ml LC auto-sampler vials (Morare *et al.*, 2017; Changwa *et al.*, 2018).

5.2.1.3 Fermentation and extraction of crude extracts from fungal endophytes

A modified method of Prabavathy and Nachiyar (2014) was used to grow the isolates, ferment and extract crude extracts. Two litres of potato dextrose broth (PDB) (Himedia) were measured into a 4 L Erlenmeyer flask, leaving room for aeration, and autoclaved at 121°C for 15 min. Each 4 L flask was inoculated with one of the endophytic fungi isolated from the bulbs and leaves as listed in Table 3.2, shaken at 100 rcf and incubated at 25°C (Amerex Gyromax, ISS, Carlifonia, USA) for 14 days. A negative containing potatoes dextrose broth (PDB) only was also prepared and exposed to the same conditions. After fermentation the broth was separated from the mycelia by first filtering with a three-layered muslin cloth, followed by using a Whatman No.1 filter paper (Sigma-Aldrich, Johannesburg, South Africa). The filtrate was washed three times with 300 ml acetone for each wash. The acetone soluble fraction was concentrated using a rotary evaporator and a dark yellowish viscous extract was obtained, which was transferred into a measuring cylinder. The extract was

mixed with ethyl acetate as the organic solvent. Depending on the volume of the extract, ethyl acetate was added in a ratio of 1:1 (v/v). The mixture was vigorously shaken for about 10 min, decanted into a separating funnel, allowed to separate and each phase collected in a conical flask. The ethyl acetate fraction was evaporated using a rotary evaporator and the extract obtained was stored in an amber bottle in a cool dry place until analysis was done. The concentrated extract was then prepared for LC-MS analysis by dissolving in HPLC grade methanol at 1 mg/ml (w/v), followed by sonicating for 10 minutes, and finally filtering through 0.22 µm polyvinylidene fluoride (PVDF) membrane syringe filters into 1 ml LC auto-sampler vials (Senyuva and Gilbert, 2007**;** Changwa *et al.*, 2018).

5.2.2 LC-QTOF-MS analysis

5.2.2.1 Instrumentation

The analysis of the crude plant extracts and crude endophyte extracts was done on an LC-QTOF system by parameters as seen in Table 5.1 with a Dionex UltiMate 3000 UHPLC (Thermo Scientific, Darmstadt, Germany) coupled to a Compact™ QTOF (Bruker Daltonics, Bremen, Germany), which uses an electrospray ionization (ESI) interface, following previously reported methods (Hoffmann *et al.*, 2014; Changwa *et al.*, 2018; Want, 2018 and Tapfuma *et al.*, 2019). Instrument operation control and acquisition was done using HyStar software version 2.1 (Therom Scientific, Darmstadt, Germany). The analytical run was set at 40 minutes and the mobile phase gradient flow profiles is seen in Table 5.2.

Table 5.1 Parameters of the LC-QTOF-MS/MS system

Specification	Setting
Column	Raptor ARC-18 column with dimensions of 2.7 µm (particle size), 2.1 mm (internal diameter), 100 mm (length) and 90 Å (pore size) (Restek, Bellefonte, USA
Injection volume	5 µL
Operating	reverse phase ultra-high-performance liquid chromatography (RP-UHPLC)
Capillary voltage at	4.5 kV
End plate offset	–500 V
Dry heater nebulizer gas pressure	1.8 Bar
Scan	50 to 1300 m/z

Table 5.2 Gradient flow profiles of the mobile phase

Time (min)	Flow (ml/min)	Solvent A [0.1% formic acid in H_2O (v/v)]	Solvent B [0.1% formic acid in acetonitrile (v/v)]
0-2	300	95	5
2-30	300	5	95
30-40	300	95	5

Data processing

Spectral data processing was performed on Bruker Compass DataAnalysis software version 4.3 (Bruker Daltonics, Brem Hub, California, USA). MetFrag web tool version 2.1 software (Git Hub, California,USA) was used to compare fragment patterns of fragmented ions with those from compound databases, namely PubChem, ChemSpider and KEGG (Blaženovi *et al*., 2018). Additional databases used were METLIN (Scripps Research, California, USA) and KNapSAcK (Kanaya Laboratory, Japan) (Zhu *et al*., 2013). Blanks containing methanol for plant extracts, NB for bacteria endophyte extracts and PDB for fungi endophyte extracts were also analyzed under the same conditions. Comparison of the two base chromatograms (from the crude extracts, endophyte extracts and the controls) allowed for filtering out impurities from the growth medium (Tapfuma *et al.*, 2019).

5.3 Results and Discussion

Bioactive compounds such as alkaloids, flavonoids, steroids and terpenoids have been isolated and identified from plants, and are used for a number of applications including human therapy, veterinary application and agriculture (Shrestha *et al*., 2015).

5.3.1 Untargeted metabolite fingerprinting from crude bulbs and leaves extracts

Plants produce a vast array of diverse secondary metabolites, and the different characteristic mix of the secondary metabolites can be used to classify plants into different taxonomic characters (Kabera *et al.*, 2014; Guerriero *et al.*, 2018). Secondary metabolites have been reported to display biological activities (Dias *et al*., 2012). Chromatography techniques have been used for the

identification, isolation and characterization of many bioactive secondary metabolites from plant cells (Gurib-Fakim, 2006). Untargeted metabolite fingerprinting revealed the presence of a number of secondary metabolites from *C. macowanii* bulbs and leaves as seen in Table 5.3.

Table 5.3 MS-MS data and the identification results of the constituents from *C. macowanii* bulbs and leaves by LC-Q-TOF-MS

Plant part	Identification	RT (min)	m/z	Ms/Ms fragments	Chemical Formula
Bulbs	1. Arborinine	18.40	286.1077		$C_{16}H_{15}N_1O_4$
	2. Brusatol	19.01	521.2019	344.1618, 330.1462	$C_{26}H_{32}O_{11}$
	3. Istamycin C1	6.49	432.2805		$C_{19}H_{37}N_5O_6$
	4. Glepidotin B	8.18	341.1390	266.0937, 251.0914, 250.0836	$C_{20}H_{20}O_5$
	5. Boschnaloside	27.57	345.1526	303.1438, 302.136, 276.1567, 275.1489, 274.1411	$C_{16}H_{24}O_8$
Leaves	6. Brugine	6.31	274.0933	259.1059	$C_{12}H_{19}N_1O_2S_2$
	7. Arbutin	5.71	273.0962	245.102, 163.0601, 145.0495,	$C_{12}H_{16}O_7$
	8. Bilobalide	25.55	327.1081		$C_{15}H_{18}O_8$
	9. Melicopicine	5.64	330.1351		$C_{18}H_{19}N_1O_5$
	10. Ginkgolide B	33.71	425.1439	354.1309, 350.0996, 303.1227	$C_{20}H_{24}O_{10}$

The secondary metabolites (1) Arborinine, (2) Brusatol, (3) Istamycin C1, (4) Glepidotin B and (5) Boschnaloside were identified from the bulbs, and (6) Brugine, (7) Arbutin, (8) Bilobalide, (9) Melicopicine and (10) Ginkgolide B were identified from the leaves, as depicted in Table 5.3. Arborinine, a acridone alkaloid, has been isolated from plants of the family Rutaceae, and possesses anticancer effects (Piboonprai *et al*., 2018). Brusatol a quassinoid, has shown antimalaria properties (Schwikkard and van Heerden, 2002). Istamycin C1 is a aminoglycoside antibiotic isolated from *Streptomyces* tenjimariensis (Davies, 2007) and the flower extract of *Allium* atroviolaceum (Khazaei *et al*., 2017). A dihydroflavonol glepidotin B, which has been isolated from Glycyrrhiza Zepidota, has displayed antimicrobial effects and is inactive against anti-viral assays (Mitscher *et al.*, 1983; Manfredi *et al.*, 2001). An iridoid glycoside boschnaloside was identified, which has been isolated previously from *Boschniakia rossica*, with antidiabetic effects (Lin *et al*., 2019), as well as from *Incarvillea emodi* (Bignoniaceae) which showed antibacterial activity (Rana *et al*., 2012).

Not much work has been done on the leaves of *C. macowanii*, and to the best of our knowledge this is the first report of the identification of compounds from the leaves of *C. macowanii* using LC-Q-TOF-MS. Brugine is a tropane alkaloid and has been isolated from the stem and bark of *Bruguiera cylindrica* (Bick, 1996; Nebula *et al*., 2013) and has anticancer properties (Boopathy and Kathiresan, 2010). The hydroquinone glucoside arbutin, has been found in different plants (Pop *et al*., 2009) and has significant antibacterial, antioxidant, anti-inflammatory and antitumour properties (Migas and Krauze-Baranowska, 2015). Bilobalide is a terpenic trilactone found in the leaves of *Ginkgo biloba* and exerts neuro- protective effects (Venieraki *et al*.,2017) and has shown antifungal properties against *Pneumocystis carinii* (Atzori *et al*., 1993). An acridone alkaloid melicopicine has been isolated from *Melicope fareana* (Aniszewski, 2007b) and this has anthelmintic and antibacterial activities (Muema, 2013). Ginkgolide B is a terpenic lactone found in *Ginkgo biloba* plants (Nakanishi, 2005) and it has anti-inflammatory properties, (Omar, 2013; Nicoletti and Fiorentino, 2015).

5.3.2 Untargeted metabolite fingerprinting from crude endophytes extracts

Endophytes are known to produce chemical compounds such as alkaloids, quinones and steroids, with antimicrobial and anticancer properties (Gouda *et al*., 2016). Table 5.4 shows some of the secondary metabolites isolated from *C. macowanii* endophytes that exhibited significant antibacterial and/or anticancer activity.

Table 5.4 Untargeted secondary metabolites from *C. macowanii* endophytes

Sample	Compound name	Rt (min)	m/z	Molecular formula	Intensity	Biological activity	Sample	Compound name	Rt (min)	m/z	Molecular formula	Intensity	Biological activity
TES01E	Proscillaridin A	14.35	531.3000	$C_{30}H_{42}O_8$	10214	Anticancer (Denicolaï *et al.*, 2014)	TESO5A and TES01F	Piperidine	36.39	86.0966	$C_5H_{11}N_1$	871	Bactericidal (Chikezie *et al.*, 2015)
	Belladine	17.52	316.1930	$C_{19}H_{25}N_1O_3$	11535	Anti-bacterial (He et al., 2015b)		Maculosin	30.54	261.1237	$C_{14}H_{16}N_2O_3$	2661	Antimicrobial activities (Cain *et al.*, 2003)
TES02B and TES11A	Mangostin	24.49	411.1775	$C_{24}H_{26}O_6$	1433	Antimicrobial properties (Hutchings *et al.*, 1996)	TES05B	Withaferin A	19.77	471.2705	$C_{28}H_{38}O_6$	726	Anti-tumour activity (Szarc Vel Szic *et al.*, 2014)
	Hodgkinsine	27.55	519.3267	$C_{33}H_{38}N_6$	8906	Antibacterial (Saad *et al.*, 1995)		Magnolol	22.49	267.1373	$C_{18}H_{18}O_2$	3506	Antimicroorganism (Zhang *et al.*, 2019)
TES03B and TES03C	Ascopyrone P	6.86	145.0489	$C_6H_8O_4$	8902	Antibacterial activity (Thomas *et al.*, 2002)	TES09B and TES13A	Gentianine	3.56	176.0708	$C_{10}H_9N_1O_2$	50608	Antibacterial properties (Singh, 2008)
	(+)-Syringaresinol	28.25	419.1724	$C_{22}H_{26}O_8$	482	Anti-cancer agent (El-Desouky and Gamal-Eldeen, 2009)		Pilocarpine	4.06	209.1287	$C_{11}H_{16}N_2O_2$	30986	Treatment of glaucoma (Sawaya *et al.*, 2010)
TES15A	Pedilstatin	5.48	513.2808	$C_{30}H_{40}O_7$	1687579	Cancer cell growth inhibitor (Duran-Pena *et al.*, 2014)	TES01A and TES02A	Undecylprodigiosin	33.24	394.2815	$C_{25}H_{35}N_3O_1$	681501	Antibacterial and antifungal (Stankovic *et al.*, 2014)
	Isoquinoline	2.10	130.0651	$C_9H_7N_1$	25670	Antibacterial (Mahady, 2005)		Isodomedin	4.31	393.2269	$C_{22}H_{32}O_6$	5657	Cytotoxic (Ohsaki *et al.*, 2012)
TES04B and TES08A	Vernodalol	4.26	393.1579	$C_{20}H_{24}O_8$	5305	Anticancer activity (Wu *et al.*, 2018)							
	Melosatin A	28.49	354.1686	$C_{21}H_{23}N_1O_4$	9406	Antimicrobial (Nithya *et al.*, 2012)							

Note TES01E= *Pseudomonas moraviensis*, TESO5A and TES01F= *Pseudomonas* sp., TES02B and TES11A= *Raoultella ornithinolytica*, TES05B= *Pseudomonas palleronia*, TES03B and TES03C= *Bacillus cereus*, TES09B and TES13A= *Rahnella aquatilis*, TES15A= *Arthrobacter pascens*, TES01A and TES02A= *Filobasidium magnum*, TES04B and TES08A =*Pencillum chrogenum*

Plants growing in distinctive environments harbour endophytic microbes with the potential to produce novel bioactive compounds (Patil *et al*., 2016). Endophytes provide possibilities for the production of known and novel biologically active secondary metabolites, as the process is reproducible, continuous and cost-effective (Kusari *et al*., 2012). Gunatilaka (2006) reported that from 128 plant-associated microorganisms, over 400 natural products were characterized, and these had novel structures and/or useful biological activities (Gunatilaka, 2006).

The rich biodiversity of bacterial endophytes allows for the development and discovery of drugs (Andryukov *et al*., 2019). In addition, their short life cycle and adaptability to genetic modification enable the unique production of secondary metabolites (Narsing Rao *et al*., 2017). Therefore, the isolation and identification of endophytic bacterial secondary metabolites is of interest, and research needs to be increased to fill the knowledge gap. Proscillaridin A is a cardiac glycoside (Pereira *et al*., 2019), and has been isolated from the plant family Hyacinthaceae/Liliaceae (Kamboj *et al*., 2013). To the best of our knowledge, this is the first time proscillaridin A has been identified from the endophytic bacterial source *Pseudomonas moraviensis*. Belladine is an alkaloid isolated from the *Crinum* species (Refaat *et al.*, 2012); this is plausible as the endophyte, *Pseudomonas moraviensis* MF943225, can metabolize secondary compounds from the host plant (*Crinum macowanii*) as stated by Ludwig-Muller (2015). Piperidine has been isolated from *Piper nigrum* (Aniszewski, 2007a) and to the best of our knowledge, this is the first report on the identification of piperidine from an endophytic bacterial source.

Maculosin is a quinoline alkaloid, and has been isolated from plants (*Helietta longifoliata*) (Aniszewski, 2007a), bacteria (*Aristabacter necator)* (Cain *et al*., 2003) and fungi (*Alternaria alternata*) (Stierle *et al*., 1988). Mangostin is a xanthonoid from the Mangosteen plant (Gutierrez-Orozco and Failla, 2013). Hodgkinsine is an alkaloid from *Hodgkinsonia frutescens* (Ghisalberti *et al*., 1998). Withaferin A is a steroidal lactone isolated from *Withania somnifera* from the Solanaceae family (Antony *et al*., 2014). To the best of our knowledge, maculosin, mangostin, hodgkinsine and withaferin A have been identified for the first time from endophytic bacterial sources in this study.

Magnolol is a lignan isolated from plants belonging to the *Magnoliaceae* family (Ghisalberti *et al*., 1998). To the best of our knowledge, this is the first report on the identification of magnolol from an endophytic bacterial source. The fungal metabolite

ascopyrone P has been isolated from fungi belonging to the Pezizales order (Thomas *et al*., 2002). Andreassen and Lundt (2006) reported that ascopyrone P (APP), is found in small amounts in nature and is formed by the breakdown of 1,5-anhydro-D-fructose (AF). Even though AF was initially detected in fungi, Kuhn *et al.* (2006) note that small amounts of AF have been found in bacteria, algae and higher plants. This would explain why ascopyrone P was identified from the crude endophytic secondary metabolites of *Bacillus cereus*.

(+)-Syringaresinol is a lignan found in different plants, namely, *Lasiosiphon eriocephalus* and *Cinnamomum burmanii* (Bhandurge *et al.*, 2013; Zhao and Ma, 2016) and from the endophytic fungus *Annulohypoxylon ilanense* (Nicoletti and Fiorentino, 2015). To the best of our knowledge, this is the first report on its identification from an endophytic bacterial source. Gentianine is a terpenoid alkaloid isolated from *Gentiana* spp (Ghisalberti *et al*., 1998). Pilocarpine is an imidazole alkaloid from the *Pilocarpus* plant species (Sawaya *et al*., 2010). Pedilstatin is a diterpenoid isolated from the *Pedilanthus* sp plant species (Pettit *et al*., 2002). To the best of our knowledge, no studies have reported gentianine, pilocarpine or pedilstatin being identified from endophytic bacterial sources. Isoquinoline alkaloids are found in the Amaryllidaceae plant family (Bastida *et al*., 2011) and Fennell and van Staden (2001) have reported on the occurrence of isoquinoline alkaloids from *Crinum* plant species. It is not surprising that isoquinoline was identified from the endophytic *Arthrobacter pascens* isolated from *C. macowanii*, as the endophyte can metabolize secondary compounds from the host plant (Chutulo and Chalannavar, 2018).

Kaul *et al*. (2012) reported that fungal endophytes produce more secondary metabolites than any other endophytic microorganism class, which makes it ideal for discovering novel bioactive compounds. Undecylprodigiosin is an alkaloid produced by Streptomyces bacteria (Stankovic *et al*., 2014). Isodomedin is a diterpenoid isolated from Rabdosia pseudoirrorata (Lí and Chen, 1990). Vernodalol is a sesquiterpene lactone from Vernonia amygdalina (Erasto *et al*., 2006). Melosatin A is an alkaloid from Melochia tomentosa (Kapadia *et al*., 1980). As far as we are aware, no studies have reported the identification of undecylprodigiosin, isodomedin, vernodalol or melosatin A from endophytic fungal sources.

The qualitative analysis, detection sensitivity, mass accuracy and resolution of LC-Q-TOF-MS makes it an ideal tool for the identification of secondary metabolites from endophytes

(Zhu *et al.*, 2013; Pang *et al.*, 2016). The untargeted analysis of crude extracts of *C. macowanii* bulbs and leaves and the crude endophytic (bacteria and fungi) extracts used in this study displayed the presence of secondary metabolites from the plants and endophytes. Ludwig-Muller (2015) and Chutulo and Chalannavar (2018) reported that host plants and endophytes interact on different metabolic levels, being: 1. endophytes or host inducing metabolism of host/ host plant; 2. host plant and endophyte sharing parts of their metabolic pathways and; 3. host plant or endophyte can metabolize secondary metabolites from the endophyte or host plant. In this way, host plants and endophytes are able to produce similar secondary metabolites which perform distinct functions.

5.3.3 Similar secondary metabolite identified from crude bulbs and leaves extracts and crude endophytes extracts

Endophytes are known to use the same precursors as host plants to produce secondary metabolites similar to the host plant (Venieraki *et al*. 2017; Khare *et al*. 2018), This is observed in Table 5.5.

Table 5.5 Similar secondary metabolites identified from *C. macowanii* bulbs and leaves and their endophytes

Compound group	Identification	Chemical Formula	m/z	MS/MS Fragments		Sample																
						Bulb	Leaves	TES01A	TES05C	TES08A	TES02C	TES02B	TES03B	TES01E	TES03A	TES05A	TES04A	TES05B	TES06A	TES07A	TES12A	TES10A	TES14A	TES15A
Lycorine type Alkaloid	Lycorine	$C_{16}H_{17}N_1O_4$	288.1236	270, 252, 222, 177, 147, 119, 95	RT (min)	16.93	1.92	17.29	17.30	17.31	13.76	7.63	2.78	10.46	7.23	36.79	36.76	23.94	23.98	36.75	2.41	7.23	7.23	7.37
					Intensity	8224	486005	8452	2863	7140	18087	1080	6009	81650	17389	1302	1145	6270	1313	1364	265063	9580	17389	5482
Crinine type Alkaloid	Crinamine	$C_{17}H_{19}N_1O_4$	302.1401	290, 289, 288, 287, 287, 286, 286, 272	RT (min)	19.88	19.14	18.46	18.50	25.58	14.05	18.06	2.30	14.06	7.43	17.99	17.99	17.96	17.97	17.98	2.75	7.43	7.43	7.43
					Intensity	20992	769052	21249	25016	12029	28199	10375	24398	27987	117502	1424	2569	20621	19562	3439	21376	141602	117502	174857
Angustine-type Alkaloid	Angustine	$C_{20}H_{15}N_3O_1$	314.1388	228, 187, 175, 173, 159, 143, 128, 115	RT (min)	10.33	10.35	8.63	26.00	26.04	19.23	25.97	2.59	19.26	11.07	25.99	26.00	25.96	25.69	25.98	3.52	3.64	11.07	8.17
					Intensity	62619	97876	16586	8273	9750	14549	3701	5953	14179	2448	885	712	3689	1578	1111	56878	14980	2474	49817
Galanthamine type Alkaloid	Powelline	$C_{17}H_{19}N_1O_4$	302.1389	290, 258, 246, 220, 217	RT (min)	19.86	19.14	18.46	18.38	25.58	14.05	18.06	2.30	14.06	7.43	17.99	17.99	17396	17.97	17.98	2.76	7.42	7.43	7.43
					Intensity	20992	769053	21249	25016	12029	28199	10755	23298	2987	117502	4124	2700	20621	19562	3439	21376	141602	117502	172533
Quinoline type Alkaloid	Lunacrine	$C_{16}H_{19}N_1O_3$	274.1442		RT (min)	15.34	6.30	15.57	15.51	15.52	11.32	15.31	36.78	15.32	6.24	15.29	15.28	15.24	15.26	15.26	6.24	6.25	6.24	6.27
					Intensity	75305	166753	107477	110520	90511	79144	81686	608	64430	520671	15230	8699	75573	67194	11098	78324	573048	520671	586649
Crinine type Alkaloid	Crinamidine	$C_{17}H_{19}N_1O_5$	318.1348	300, 286, 268, 250, 227, 209, 199, 149	RT (min)	17.84	17.57	15.75	15.73	15.73	15.50	15.49	16.22	15.52	6.31	15.48	15.45	15.43	15.44	15.44	6.31	6.31	6.32	7.27
					Intensity	186394	459458	33935	32548	32000	17115	42899	619	14515	156970	11953	6620	52333	59262	8127	33014	207077	166970	205820
Crinine type Alkaloid	Crinine	$C_{16}H_{17}N_1O_3$	272.1278	270, 254, 228, 214, 199,187, 173, 115	RT (min)	17.17	5.71	16.34	16.32	8.94	11.67	16.04	6.86	28.86	6.59	16.01	16.01	15.97	15.99	15.97	1.56	7.30	6.58	6.60
					Intensity	2801	142521	2798	2685	3203	2148	4516	3260	2690	11000	2160	1119	1594	7916	1742	69923	25795	11000	9192
Lignan	Justicidin B	$C_{21}H_{16}O_6$	365.1033		RT (min)	0.74	17.05	1.73	1.53	13.57	31.32	25.14	0.82	8.84	5.62	4.39	0.87	31.22	36.69	36.37	3.84	3.89	10.37	5.68
					Intensity	9723	7234	3701	3090	5905	2970	3437	2195	960	1468	355	291	1489	449	408	7894	5128	1977	36302
Mycosporine-like amino acids	Mycosporine	$C_{11}H_{19}N_1O_6$	262.1297	246, 166, 137, 118	RT (min)	1.14	4.40	1.16	1.13	1.15	9.07	6.08	2.93	6.01	2.92	9.81	9.79	9.76	9.79	9.78	2.45	2.93	2.92	3.52
					Intensity	10503	2505	16500	17872	11382	4884	5439	181475	2369	36938	1371	833	6486	6710	1243	196261	256544	36938	114841
Quinazoline type Alkaloid	Vasicinol	$C_{11}H_{12}N_2O_2$	205.0977	187, 185, 169, 160, 159, 134	RT (min)	4.17	4.18	21.73	21.71	21.74	21.69	14.01	2.25	21.69	8.91	21.71	21.69	21.68	21.70	21.51	2.21	2.25	8.91	9.00
					Intensity	82083	82662	9406	9031	9518	9984	28559	527158	9133	288400	6969	5606	9831	9371	10458	1327645	290247	288400	241815

Note TES01A = *Filobasidium magnum*, TES05C = *Alternaria tenuissima*, TES08A = *Penicillium chrysogenum*, TES02C = *Acinetobacter guillouiae*, TES02B = *Raoultella ornithinolytica*, TES03B = *Bacillus cereus*, TES01E = *Pseudomonas moraviensis*, TES03A = *Rahnella aquatilis*, TES05A = *Pseudomonas* sp., TES04A = *Novosphingobium* sp., TES05B = *Pseudomonas palleroniana*, TES06A = *Pseudomonas putida*, TES07A = *Bacillus safensis*, TES12A = *Burkholderia tropica*, TES10A = *Enterobacter asburiae*, TES14A = *Pseudomonas cichorii*, TES15A = *Arthrobacter pascens*

Lycorine, an alkaloid previously isolated from *C. macowanii* (Nair *et al*., 2000), was identified in all samples tested. Elgorashi *et al*. (2002) reported that the leaves of *C. macowanii* contain more lycorine than the bulbs and other plant parts such as roots and flowers. This supports the findings in this study, as the leaves had a greater intensity than that of the bulbs. Endophytes isolated from the bulbs TES01E and TES12A also exhibited greater intensities than the other endophyte samples. This is not surprising as alkaloids are primarily concentrated within the bulbs of plants (Acosta *et al*., 2014) and endophytes can metabolize secondary metabolites from the host plant (Ludwig-Muller, 2015).

Crinamine is an alkaloid previously isolated from the roots of *C. macowanii* (Elgorashi *et al*., 2002). High levels of crinamine have been detected in the bulbs of *C. macowanii* (Elgorashi *et al*., 2002). From the results obtained in this study, the leaves and endophytes from the leaves (TES10A, TES14A and TES15A) had higher intensities than the other samples tested. This could be due to the fact that endophytes can metabolize secondary metabolites from the host plant (Chutulo and Chalannavar, 2018).

Crinine is a crinine-type alkaloid which has been isolated from different *Crinum* species, including *C. macowanii* Baker, *C. moorei* Hook F., C. *powellii* Hort. and *C. bulbispermum* Milne. (Refaat *et al.*, 2012). Elgorashi *et al*. (2002) reported that bulbs of *C. macowanii* contain high levels of crinine, followed by the roots, and that the leaves contain the least amount of the alkaloid crinine. The results obtained in this study show that the leaves had a higher intensity than the other samples, followed by TES03A and TES14A, which are endophytes from the bulbs and leaves, respectively. This was expected as crinine is a popular alkaloid of the *Crinum* plant species and the *Amaryllidaceae* family, and it is widely distributed in different plant parts (Blumenthal, 2009). Crinine-type alkaloid crinamidine (Refaat *et al.*, 2012) has been isolated from different plants of the Amaryllidaceae family (He et al., 2015b) as well as from the bulbs, flowering stalks, leaves and roots of *Crinum macowanii* (Maroyi, 2016a). **Crinamidine** is found in whole plant parts of the *Crinum* species (Tram *et al*., 2002). **Powelline** is an alkaloid reported to occur in *C. macowanii* (Nair *et al*., 2013) and Ndhlala *et al.* (2013) has reported its occurrence from the bulbs.

Plants from the Amaryllidaceae family are known to synthesize unique alkaloidal constituents (Nair *et al*., 2013), which are produced by members of this family only (Ghosal *et al*., 1985). The

identified alkaloids, lycorine, crinamine, crinine, crinamidine and powelline, are true alkaloids of the Amaryllidaceae family (Elgorashi, 2000; Jin and Yao, 2019) and this supports the results obtained, as *Crinum macowanii* belongs to this plant family. The availability of these alkaloids has led to the overuse and over-harvesting of *C. macowanii* to obtain these bioactive compounds (Venugopalan and Srivastava, 2015; Yang *et al.*, 2018).

The high demand of bioactive secondary metabolites has prompted further research for *in vitro* production of secondary metabolites, including plant cell culture and microbial fermentation. Unfortunately, restrictions such as genomic instability, low yields, and scale-up difficulties of plant cells cultures have made this difficult (Cardoso *et al*., 2019). Microbial fermentation requires culture medium which is relatively simple and inexpensive, largely consisting of industrial by-products/wastes, making it advantageous over other methods (Venugopalan and Srivastava, 2015). In this study high intensity levels of the alkaloids lycorine, crinamine, powelline, crinamidine and crinine were observed in the leaves compared to the bulbs; this was expected as alkaloids (secondary metabolites) play a defensive role in plants against biotic stress such as attack by herbivores and pathogens (Jain *et al*., 2019) and leaves are the first point of contact for most herbivores and pathogens.

Angustine is an alkaloid previously isolated from plants of the Rubiaceae and Loganiaceae families (Phillipson and Hemingway, 1974; Liew *et al.*, 2012). To the best of our knowledge, this is the first time angustine has been identified from *C. macowanii* plants and its endophytes. The quinolone type alkaloid **lunacrine** has been reported to occur in Lunasia amara Blanco from the Rutaceae plant family (Zubair *et al*., 2016). Lloyd *et al*. (2018) stated that lunacrine is mostly found on the bark of plants, while the results obtained in this study indicated high intensity from the leaves, fungi endophytes (TES01A and TES05C) and bacterial endophytes (TES10A, TES14A, TES15A and TES03A). This shows a wide distribution of the quinolone alkaloid. **Vasicinol** is an alkaloid from *Adhatoda zeylanica* Medic. (Ahmad *et al*., 2009). Crude endophyte extracts of *Burkholderia tropica* (TES12A) had a high intensity compared to other samples; this is not surprising as some quinazoline alkaloids are known to be produced by microbes (Xue *et al*., 2012). Amaryllidaceae and rubiaceae plants are both angiosperms, and the occurrence of alkaloids in these plant families can be treated as a general family characteristic (Li and Willaman, 1968).

Justicidin B is a lignans which occurs in plants from the Acanthaceae, Euphorbiaceae, Rutaceae and Linaceae families (Hemmati and Seradj, 2016). Lignans have been reported to occur in Amaryllidaceae plants (Nair and Staden, 2014; Nair *et al.*, 2017). Ionkova (2007) reported that lignans occur in low concentrations in plants, which concurs with our study as low concentrations were observed on our plant samples. However, bacterial endophytic sample TES15A (*Arthrobacter pascens*) had a higher intensity than the other samples. Justicidin B was isolated for the first time from a bacterium *Nocardia* sp. ALAA 2000 by El-Gendy *et al*., 2008, and the identification of justicidin B from our endophytic sample TES15A (*Arthrobacter pascens*) is a notable occurrence, as the natural abundance of bioactive lignans is rare and their chemical synthesis is not yet possible (Ionkova, 2007).

Mycosporine is a secondary metabolite found in prokaryotic (cyanobacteria) as well as eukaryotic (microalgae, yeasts, and fungi) microorganisms (Oren and Gunde-Cimerman, 2007) which use the shikimate pathway for synthesis (Pusztahelyi *et al*., 2015). Fungi such as *Coniosporium*, *Sarcinomyces* and *Botryosphaeria* are known to produce mycosporine (Volkmann and Gorbushina, 2006), however low concentrations were observed in the endophytic fungi of this study. This could be due to the growth media used and the fermentation/growth conditions, as these have a noticeable impact on the production of secondary metabolite (Anuhya *et al*., 2017). The extraction method and solvent used for extraction also determines the type and quantity of secondary metabolites produced (Sasidharan *et al.*, 1993; Sasidharan *et al*., 2011). Demain and Fang (2000) stated that secondary metabolites are produced by organisms to function as survival mechanisms. The high intensity values of samples TES03B, TES12A and TES15A are justifiable, as mycosporine are reported to improve the ability of cells to tolerate abiotic stress factors such as salinity, dehydration, and temperature (Chrapusta *et al*., 2017). Regulatory factors including carbon sources, nitrogen sources, phosphate, NaCl, trace elements and different parameters such as temperature, pH, incubating time intervals and extraction solvents can influence secondary metabolism and its yield and abundance (Dwivedi *et al*., 2019).

Extraction methods and extraction solvents used influence the type of secondary metabolites extracted and play a key role in the recovery of secondary metabolites (Le *et al*., 2018). The grinding of plant samples helps to render samples with a smaller particle size, which increases surface contact between samples and extraction solvents (Thermo Scientific, 2013). The soaking

of the plant material softens and breaks down the plant's cell walls to release the soluble phytochemicals (Azwanida, 2015). Solvent type and strength influence the extraction yields of secondary metabolites (Ngo *et al*., 2017). The use of polar extraction solvents methanol and dichloromethane solution tend to extract more polar molecules such as alkaloids (Adhikari *et al*., 2018). The solvent polarity determines the type, composition, and biological activity of secondary metabolites (Widyawati *et al*., 2014). As polar solvents used for extraction have been shown to extract secondary metabolite biological activity (Ncube *et al*., 2008; Nazemi *et al.*, 2017), methanol: dichloromethane and ethyl acetate were used for the extraction of plant and endophytes secondary metabolites, respectively, in this study.

Sebola *et al*. (2016) reported the identification of fatty acids, sterols, volatile oils, alkaloids, and flavonoids from *C. macowanii* bulbs when methanol: dichloromethane was used as the extraction solvent. Different extraction solvents produce various yields (Truong *et al*., 2019) and so polar solvents were used for extraction. Yusnawan (2013) reported that polar solvents can increase cell permeability and penetrate cells, extracting more intracellular secondary metabolites.

Maroyi (2016) reported that extracts of dichloromethane-methanol were able to yield alkaloid and non-alkaloid compounds from *Crinum macowanii* with various biological activities. Ethyl acetate was used for the extraction of endophytes secondary metabolites as it is the best solvent to extract the culture filtrate from fungi and/or bacteria (Purwestri *et al.*, 2016; Ibrahim *et al.*, 2017). Xu (2010) indicated that secondary metabolites extracted by ethyl acetate extract are more easily separated and isolated using chromatography techniques, compared to other organic solvents. The extraction solvent not only influences the yield of the secondary metabolites (Felhi *et al*., 2017), but also the affinity of the compounds to the solvent (Aires, 2012).

From the results obtained, varying retention times were observed between the plant (bulbs and leaves) and endophyte (bacteria and fungi) samples, even though the same chromatography conditions were used. Factors such as the affinity of the compounds to the extraction solvents used (Aires, 2012), the change in polarity of the sample being analyzed and fragments that make up the secondary metabolites detected (Moco *et al*., 2006) and the formation of secondary metabolite complexes with the extraction solvents used, all influenced the different retention times observed, as they can create a sample matrix (Naz *et al*., 2017).

This study revealed that plants parts (bulbs and leaves) are able to produce secondary metabolites similar to those produced by their endophytes and that endophytes revealed the identification of bioactive secondary metabolites. The identified metabolites from endophytes are from different compound groups (Ludwig-Muller, 2015).

Correlating antibacterial and anticancer activity of the crude extracts to LCQTOF MS data

Lycorine has been reported to possess antibacterial activity and cytotoxic and antitumour activities (Khalifa *et al*., 2018). This would explain the observed antibacterial and/or anticancer activity of the crude endophyte extracts. Crinine-type alkaloid crinamidine and powelline alkaloids have been reported to possess antibacterial, antitumour and anticancer activity (Brueton, 2013; Jerald James Nair and Staden, 2014; Maroyi, 2016). Vasicinol and Brefeldin A have been reported to possess antibacterial and anticancer properties, respectively (Jain *et al*., 2011; Farias *et al*., 2019). The observed biological activities could be attributed to the synergistic effects of these compounds, as secondary metabolites display more biological activities when in crude extracts compared to a pure compound (Olufunke, 2012).

5.4 Conclusion

Endophytes isolated from the bulbs and leaves of *C. macowanii* are able to synthesize similar secondary metabolites to their host plant. Biologically active secondary metabolites were identified from the isolated endophytes, and therefore there is no need to over harvest *C. macowanii* plants for their medicinal properties. This is a promising lead for drug discovery and bioprospecting. Further extraction of secondary metabolites from endophyte is still needed.

References

Acosta, K., Pigni, N., Oleas, N., Bastida, J., 2014. Identification of the alkaloids of stenomesson aurantiacum (Kunth) herb an amaryllidaceae species from the ecuadorian andes. Pharmacologyonline 3, 178–183.

Adhikari, P., Pandey, A., Agnihotri, V., Pande, V., 2018. Selection of solvent and extraction method for determination of antimicrobial potential of Taxus wallichiana Zucc. Res. Pharm. 8, 1–9. https://doi.org/10.25081/rip.2018.v8.3487

Ahmad, S., Garg, M., Ali, M., Singh, M., Athar, T., Ansari, S.H., 2009. A phyto-pharmacological

overview on Adhatoda zeylanica Medic . syn . A . vasica (Linn .) Nees. Nat. Prod. Radiance 8, 549–554.

Aires, A., 2012. Phenolics in Foods: Extraction, Analysis and Measurements. Intech 72–75. https://doi.org/10.1016/j.colsurfa.2011.12.014

Andreassen, M., Lundt, I., 2006. A new chemical synthesis of Ascopyrone P from 1,5-anhydro-d-fructose. Carbohydr. Res. 341, 1692–1696. https://doi.org/10.1016/j.carres.2006.03.037

Andryukov, B., Mikhailov, V., Besednova, N., 2019. The Biotechnological Potential of Secondary Metabolites from Marine Bacteria. J. Mar. Sci. Eng. 7, 1–16.

Aniszewski, T., 2007a. Definition, Typology and Occurrence of Alkaloids, in: Alkaloids – secrets of life alkaloid chemistry, biological significance, applications and ecological role. Elsevier, Amsterdam, The Netherlands, pp. 40–53.

Aniszewski, T., 2007b. Alkaloids – secrets of life alkaloid chemistry, biological significance, applications and ecological role.

Antony, M.L., Lee, J., Hahm, E.R., Kim, S.H., Marcus, A.I., Kumari, V., Ji, X., Yang, Z., Vowell, C.L., Wipf, P., Uechi, G.T., Yates, N.A., Romero, G., Sarkar, S.N., Singh, S. V., 2014. Growth arrest by the antitumor steroidal lactone withaferin a in human breast cancer cells is associated with down-regulation and covalent binding at cysteine 303 of β-tubulin. J. Biol. Chem. 289, 1852–1865. https://doi.org/10.1074/jbc.M113.496844

Anuhya, G., Jyostna, V., Aswani, K.Y., Bodaiah, B., Sudhakar, P., 2017. Influence of physico-chemica parameters on secondary metabolite production by marine fungi. Int. J. Curr. Pharm. Res. 9, 112–118. https://doi.org/10.22159/ijcpr.2017v9i5.22152

Atzori, C., Bruno, A., Chichino, G., Bombardelli, E., Scaglia, M., Ghione, M., 1993. Activity of bilobalide, a sesquiterpene from Ginkgo biloba, on Pneumocystis carinii. Antimicrob. Agents Chemother. 37, 1492–1496. https://doi.org/10.1128/AAC.37.7.1492

Azwanida, N., 2015. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med. Aromat. Plants 4, 1–6. https://doi.org/10.4172/2167-0412.1000196

Bastida, J., Berkov, S., Torras Clavería, L., Pigni, N.B., Andradre, J.P. De, Martínez, V., Codina Mahrer, C., Viladomat Meya, F., 2011. Chemical and biological aspects of Amaryllidaceae alkaloids, Recent Advances in Pharmaceutical Sciences.

Bhandurge, P., Rajarajeshwari, N., Ganapaty, S., Pattanshetti, S., 2013. The Gnidia genus: A review. Asian J. Biomed. Pharm. Sci. 3, 1–31.

Bick, I.R.C., 1996. Alkaloids from Australian Flora, in: Pelletier, S.W. (Ed.), Alkaloids" Chemical and Biological Perspectives. Elsevier, pp. 94–116.

Blumenthal, M., 2009. African Natural Plant Products : A Foreword to the Science and Challenges, in: Juliani, H.R., Simon, J., Ho, C.-T. (Eds.), African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality. ACS Pulbication series, Rutgers, The State University of New Jersey, pp. 157–166.

Boopathy, N.S., Kathiresan, K., 2010. Anticancer Drugs from Marine Flora : An Overview. J. of Oncology 2010, 1–18. https://doi.org/10.1155/2010/214186

Brueton, V.J., 2013. Trade in commonly used medicinal bulbs: value and ecological implications. University of the Witwatersrand.

Cain, C.C., Lee, D., Iii, R.H.W., Henry, A.T., Jr, E.J.C., Wani, M.C., Wall, M.E., Oberlies, N.H., Iii, J.O.F., 2003. Synergistic Antimicrobial Activity of Metabolites Produced by a Nonobligate Bacterial Predator. Antimicrob. Agents Chemother. 47, 2113–2117. https://doi.org/10.1128/AAC.47.7.2113

Cardoso, J.C., de Oliveira, M.E.B.S., Cardoso, F. de C.I., 2019. Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. Hortic. Bras. 37, 124–132. https://doi.org/10.1590/s0102-053620190201

Changwa, R., Abia, W., Msagati, T., Nyoni, H., Ndleve, K., Njobeh, P., 2018. Multi-Mycotoxin Occurrence in Dairy Cattle Feeds from the Gauteng Province of South Africa : A Pilot Study Using UHPLC-QTOF-MS / MS. Toxicon 10, 1–21. https://doi.org/10.3390/toxins10070294

Chikezie, P.., Ibegbulem, C.., Mbagwu, F.., 2015. Medicinal & Aromatic Plants Medicinal Potentials and Toxicity Concerns of Bioactive Principles. Med. Aromat. Plants 4, 1–15. https://doi.org/10.4172/2167-0412.1000202

Chrapusta, E., Kaminski, A., Duchnik, K., Bober, B., Adamski, M., Bialczyk, J., 2017. Mycosporine-Like Amino Acids: Potential health and beauty ingredients. Mar. Drugs 15, 1–29. https://doi.org/10.3390/md15100326

Chutulo, E.C., Chalannavar, R.K., 2018. Endophytic Mycoflora and Their Bioactive Compounds from Azadirachta Indica :A Comprehensive Review. J. Funct. Foods 4, 1–12. https://doi.org/10.3390/jof4020042

Demain, A.L., Fang, A., 2000. The Natural Functions of Secondary Metabolites, in: Scheper, T. (Ed.), Advances in Biochemical Engineering / Biotechnology. Springer, pp. 1–33. https://doi.org/10.1016/j.jbiotec.2007.09.006

Denicolaï, E., Baeza-kallee, N., Tchoghandjian, A., Carré, M., Colin, C., Jiglaire, C.J., Mercurio, S., Beclin, C., Figarella-branger, D., 2014. Proscillaridin A is cytotoxic for glioblastoma cell lines and controls tumor xenograft growth in vivo. Oncotarget 5, 10934–10948.

Doty, S.L., 2017. Functional Importance of the Plant Endophytic Microbiome : Implications for Agriculture , Forestry , and Bioenergy, in: Doty, S.L. (Ed.), Functional Importance of the Plant Microbiome. Springer International Publishing, Cham, Switzerland, pp. 1–5. https://doi.org/10.1007/978-3-319-65897-1

Dwivedi, G.R., Sisodia, B.S., Shikha, 2019. Secondary Metabolites: Metabolomics for Secondary Metabolites, in: Gupta, V.K., Pandey, A. (Eds.), New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier, Amsterdam, Netherlands, pp. 337–339. https://doi.org/10.1016/b978-0-444-63504-4.00022-0

El-Desouky, S.K., Gamal-Eldeen, A.M., 2009. Cytotoxic and anti-inflammatory activities of some constituents from the floral buds of Syringa patula. Pharm. Biol. 47, 872–877. https://doi.org/10.1080/13880200902950785

El-Gendy, M.M.A., Hawas, U.W., Jaspars, M., 2008. Novel bioactive metabolites from a marine derived bacterium Nocardia sp. ALAA 2000. J. Antibiot. (Tokyo). 61, 379–386. https://doi.org/10.1038/ja.2008.53

Elgorashi, E.E., 2000. alkaloids from three South African Crinum species. University of Natal Pietermaritzburg.

Elgorashi, E.E., Drewes, S.E., van Staden, J., 2002. Organ-to-organ and seasonal variation in alkaloids from Crinum macowanii. Fitoterapia 73, 490–495. https://doi.org/10.1016/S0367-326X(02)00164-8

Erasto, P., Grierson, D.S., Afolayan, A.J., 2006. Bioactive sesquiterpene lactones from the leaves of Vernonia amygdalina. J. Ethnopharmacol. 106, 117–120. https://doi.org/10.1016/j.jep.2005.12.016

Farias, K.J.S., Machado, P.R.L., de Almeida Júnior, R.F., Lopes da Fonseca, B.A., 2019. Brefeldin A and Cytochalasin B reduce dengue virus replication in cell cultures but do not protect mice against viral challenge. Access Microbiol. 1, 1–8. https://doi.org/10.1099/acmi.0.000041

Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., Kadri, A., 2017. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. Food Sci. Technol. 37, 483–492. https://doi.org/10.1590/1678-457x.23516

Ghisalberti, E.L., Pennacchio, M., Alexander, E., 1998. Survey of secondary plant metabolites with cardiovascular activity. Pharm. Biol. 36, 237–279. https://doi.org/10.1076/phbi.36.4.237.4583

Ghosal, S., Saini, K.S., Razdan, S., 1985. Crinum alkaloids: their chemistry and biology. Phytochemistry 24, 2141–2156.

Gouda, S., Das, G., Sen, S.K., Shin, H., Patra, J.K., 2016. Endophytes : A Treasure House of Bioactive Compounds of Medicinal Importance. Front. Microbiol. 7, 1–8. https://doi.org/10.3389/fmicb.2016.01538

Gunatilaka, A.A.L., 2006. Natural Products from Plant-associated Microorganisms: Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence. Journak Nat. Prod. 69, 509–526. https://doi.org/10.1021/np058128n.Natural

Gutierrez-Orozco, F., Failla, M.L., 2013. Biological Activities and Bioavailability of Mangosteen Xanthones: A Critical Review of the Current Evidence. Nutr. 2013, 5, 3163–3183. https://doi.org/10.3390/nu5083163

He, M., Qu, C., Gao, O., Hu, X., Hong, X., 2015. Biological and pharmacological activities of amaryllidaceae alkaloids. RSC Adv. 5, 16562–16574. https://doi.org/10.1039/c4ra14666b

Hemmati, S., Seradj, H., 2016. Justicidin B: A promising bioactive lignan. Molecules 21, 1–20. https://doi.org/10.3390/molecules21070820

Hoffmann, T., Krug, D., Huttel, S., Muller, R., 2014. Improving Natural Products Identification through Targeted LC-MS/ MS in an Untargeted Secondary Metabolomics Work flow. Anal. Chem. 86, 10780−10788. https://doi.org/10.1021/ac502805w

Hounsome, N., Hounsome, B., Tomos, D., Edwards-Jones, G., 2008. Plant Metabolites and Nutritional Quality of Vegetables. J. Food Sci. 73, 48–65. https://doi.org/10.1111/j.1750-3841.2008.00716.x

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.., 1996. Zulu medicinal plants: an inventory. University of Natal Press, Pietermaritzburg., Pietermaritzburg.

Ibrahim, M., Kaushik, N., Sowemimo, A., Chhipa, H., Koekemoer, T., Van De Venter, M., Odukoya, O.A., 2017. Antifungal and antiproliferative activities of endophytic fungi isolated from the leaves of markhamia tomentosa. Pharm. Biol. 55, 590–595. https://doi.org/10.1080/13880209.2016.1263671

Ionkova, I., 2007. Biotechnological Approaches for the Production of Lignans. Pharamacognosy Reviwes 1, 57–68.

Jain, A., Choubev, S., Singour, P.K., Rajak, H., Pawar, R.S., 2011. Sida cordifolia (Linn) - An overview. J. Appl. Pharm. Sci. 1, 23–31.

Jain, C., Khatana, S., Vijayvergia, R., 2019. Bioactivity of secondary metabolites of various plants: A review. Int. J. Pharm. Sci. Res. 10, 494–504. https://doi.org/10.13040/IJPSR.0975-8232.10(2).494-04

Jayakrishnan, T., Benjamin, S., 2016. Purpureocillium lilacinum Strain BP13 Produces Flavoglaucin. Electron. J. Biol. 12, 472–476.

Kamboj, A., Rathour, A., Kaur, M., 2013. Bufadienolides and their medicinal utility: a review. Int. J. Pharm. Pharm. Sci. 5, 20–27.

Kapadia, G.J., Shukla, Y.N., Basak, S.P., Sokolosk, E.A., Fales, H.M., 1980. The melosatins-a novel class of alkaloids from Melochza Tomentosa. Tetrahedron 36, 2441–2447.

Kaul, S., Gupta, S., Ahmed, M., Dhar, M.K., 2012. Endophytic fungi from medicinal plants : a treasure hunt for bioactive metabolites. Phytochem. Lett. 1–19. https://doi.org/10.1007/s11101-012-9260-6

Khalifa, M.F., Attia, E.Z., Fahim, J.R., Kamel, M.S., 2018. An overview on the chemical and biological aspects of lycorine alkaloid. J. Adv. Biomed. Pharm. Sci. 1, 41–49.

Khare, E., Mishra, J., Arora, N.K., 2018. Multifaceted interactions between endophytes and plant: Developments and Prospects. Front. Microbiol. 9, 1–12. https://doi.org/10.3389/fmicb.2018.02732

Kuhn, A., Yu, S., Giffhorn, F., 2006. Catabolism of 1,5-Anhydro-D-Fructose in Sinorhizobium morelense S-30.7.5: Discovery, Characterization, and Overexpression of a New 1,5-Anhydro-D-Fructose Reductase and Its Application in Sugar Analysis and Rare Sugar Synthesis. Appl. Environ. Microbiol. 72, 1248–1257. https://doi.org/10.1128/AEM.72.2.1248

Kusari, S., Hertweck, C., Spiteller, M., 2012. Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chem. Biol. 19, 792–798. https://doi.org/10.1016/j.chembiol.2012.06.004

Le, A. V., Parks, S.E., Nguyen, M.H., Roach, P.D., 2018. Effect of solvents and extraction methods on recovery of bioactive compounds from defatted gac (Momordica cochinchinensis spreng.) seeds. Separations 5, 1–13. https://doi.org/10.3390/separations5030039

Li, A.H.L., Willaman, J.J., 1968. Distribution of Alkaloids in Angiosperm Phylogeny. Econ. Bot. 22, 239–252.

Lí, Y., Chen, Y., 1990. Diterpenoids from rabdosia pseudo-irrorata. J. Nat. Prod. 53, 841–844. https://doi.org/10.1021/np50070a011

Liew, S.Y., Mukhtar, M.R., Hadi, A.H.A., Awang, K., Mustafa, M.R., Zaima, K., Morita, H., Litaudon, M., 2012. Naucline, a new indole alkaloid from the bark of Nauclea officinalis. Molecules 17, 4028–4036. https://doi.org/10.3390/molecules17044028

Lloyd, M., Dapar, G., Demayo, C.G., Senarath, W.T.P.S.K., 2018. Antimicrobial and Cellular Metabolic Inhibitory Properties of the Ethanolic Extract From the Bark of "Lunas-Bagon" (Lunasia Sp.). Int. J. Pharm. Sci. Res. 9, 88–97. https://doi.org/10.13040/IJPSR.0975-8232.9(1).88-97

Ludwig-Muller, J., 2015. Plants and endophytes : equal partners in secondary metabolite production ? Biotechnol. Lett. 37, 1325–1334. https://doi.org/10.1007/s10529-015-1814-4

Mahady, G., 2005. Medicinal plants for the prevention and treatment of bacterial infections. Curr. Pharm. Des. 11, 2405–2427.

Maloney, K.N., Macmillan, J.B., Kauffman, C.A., Jensen, P.R., Dipasquale, A.G., Rheingold, A.L., Fenical, W., 2009. NIH Public Access. Org. Lett. 11, 1–7. https://doi.org/10.1021/ol901997k.Lodopyridone

Mariıa Jesus Duran-Pena, Ares, J.M.B., Collado, I.G., Hern´andez-Gal´an, R., 2014. Biologically active diterpenes containing a gem- dimethylcyclopropane subunit: an intriguing source of PKC modulators. Nat. Prod. Rep. 1–14. https://doi.org/10.1039/c4np00008k

Maroyi, A., 2016. A review of ethnoboatany, therapeutic value, phytochemistry and pharmacology of Crinum macowanii Baker_ A highly traded bulbous plant in Southern Africa. J. Ethnopharmacol. 194, 595–608. https://doi.org/10.1016/j.jep.2016.10.046

Migas, P., Krauze-Baranowska, M., 2015. The significance of arbutin and its derivatives in therapy and cosmetics. Phytochem. Lett. 13, 35–40. https://doi.org/10.1016/j.phytol.2015.05.015

Moco, S., Bino, R.J., Vorst, O., Verhoeven, H.A., Groot, J. de, Beek, T.A. van, Vervoort, J., de Vos, C.H.R., 2006. A Liquid Chromatography-Mass Spectrometry-Based Metabolome Database for Tomato. Plant Physiol. 141, 1205–1218. https://doi.org/10.1104/pp.106.078428.once

Morare, R.. ., Serepa-Dlamini, M.H., Ubomba-Jaswa, E., Mavumengwana, V., 2017. Screening of anti-microbial activity of secondary metabolites extracted from Crinum macowanii and its associated bacterial endophytes. Univrersity of Johannesburg.

Muema, S.M., 2013. Phytochemical and anthelmintic study of the root bark of teclea trichocarpa,

engl. (rutaceae). University of Nairobi.

Nair, J.J., Bastida, J., Codina, C., Viladomat, F., van Staden, J., 2013. Alkaloids of the South African Amaryllidaceae : A Review. Nat. Prod. Commun. 8, 1335–1350. https://doi.org/10.1002/chin.201430280

Nair, J.J., Machocho, A.K., Campbell, W.E., Brun, R., Viladomat, F., Codina, C., Bastida, J., 2000. Alkaloids from Crinum macowanii. Phytochemistry 54, 945–950. https://doi.org/10.1016/S0031-9422(00)00128-X

Nair, Jerald J., Staden, J. Van, 2014. Traditional usage , phytochemistry and pharmacology of the South African medicinal plant Boophone disticha (L . f .) Herb . J. Ethnopharmacol. J. 151, 12–26. https://doi.org/10.1016/j.jep.2013.10.053

Nair, J.J., Staden, J. Van, Bonnet, S.L., Wilhelm, A., 2017. Antibacterial Properties of the Family Amaryllidaceae: Evaluation of Plant Extracts in vitro. Nat. Prod. Commun. 12, 1145–1151. https://doi.org/10.1177/1934578X1701200735

Nakanishi, K., 2005. Terpene trilactones from Gingko biloba : From ancient times to the 21st century. Bioorg. Med. Chem. 13, 4987–5000. https://doi.org/10.1016/j.bmc.2005.06.014

Narsing Rao, M.P., Xiao, M., Li, W.J., 2017. Fungal and bacterial pigments: Secondary metabolites with wide applications. Front. Microbiol. 8, 1–13. https://doi.org/10.3389/fmicb.2017.01113

Naz, S., Gallart-Ayala, H., Reinke, S.N., Mathon, C., Blankley, R., Chaleckis, R., Wheelock, C.E., 2017. Development of a Liquid Chromatography-High Resolution Mass Spectrometry Metabolomics Method with High Specificity for Metabolite Identification Using All Ion Fragmentation Acquisition. Anal. Chem. 89, 7933–7942. https://doi.org/10.1021/acs.analchem.7b00925

Nazemi, M., Moradi, Y., Rezvani, G.F., Ahmaditaba, M.A., Gozari, M., Salari, Z., 2017. Antimicrobial activities of semi polar-nonpolar and polar secondary metabolites of sponge Dysidea pallescens from Hengam Island, Persian Gulf. Iran. J. Fish. Sci. 15, 200–209.

Ncube, E.N., Dubery, I.A., Steenkamp, P.A., Madala, N.E., 2016. Manipulation of secondary metabolite synthesis in Centella asiatica cells : a metabolomics study. University of Johannesburg.

Ncube, N.S., Afolayan, A.J., Okoh, A.I., 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J. Biotechnol. 7, 1797–1806. https://doi.org/10.5897/AJB07.613

Ndhlala, A.R., Ncube, B., Okem, A., Mulaudzi, R.B., Staden, J. Van, 2013. Toxicology of some important medicinal plants in southern Africa. Food Chem. Toxicol. J. 62, 609–621. https://doi.org/10.1016/j.fct.2013.09.027

Nebula, M., Harisankar, H.S., Chandramohanakumar, N., 2013. Metabolites and bioactivities of Rhizophoraceae mangroves. Nat. Products Bioprospect. 3, 207–232. https://doi.org/10.1007/s13659-013-0012-0

Ngo, T. Van, Scarlett, C.J., Bowyer, M.C., Ngo, P.D., Vuong, Q. Van, 2017. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. J. Food Qual. 1–8. https://doi.org/10.1155/2017/9305047

Nicoletti, R., Fiorentino, A., 2015. Plant Bioactive Metabolites and Drugs Produced by Endophytic Fungi of Spermatophyta. Agriculture 5, 918–970. https://doi.org/10.3390/agriculture5040918

Nithya, G., Panneerselvam, P., Nepolean, R., Selvakumar, N., Jeyavalli, A., 2012. Synthesis & biological evaluation of some novel schiff bases of isatin derivatives. Int. J. Pharm. Ther. 3, 243–246.

Ohsaki, A., Ozawa, M., Komiyama, K., Kishida, A., Isobe, T., 2012. Natural Product Communications The Cytotoxic Activity of Diterpenoids from Isodon species. Nat. Prod. Commun. 7, 977–978. https://doi.org/10.1177/1934578X1200700803

Olufunke, M.D., 2012. Developments in Phytochemistry, in: Vallisuta, P.O. (Ed.), Drug Discovery Research in Pharmacognosy. InTech Published, pp. 12–23.

Omar, S.H., 2013. Ginkgolides and Neuroprotective Effects. Nat. Prod. 3697–3741. https://doi.org/10.1007/978-3-642-22144-6

Oren, A., Gunde-Cimerman, N., 2007. Mycosporines and mycosporine-like amino acids : UV protectants or multipurpose secondary metabolites ? FEMS Microbiol. Lett. 269, 1–10. https://doi.org/10.1111/j.1574-6968.2007.00650.x

Pang, B., Zhu, Y., Lu, L., Gu, F., Chen, H., 2016. The applications and features of liquid chromatography-mass spectrometry in the analysis of traditional Chinese medicine. Evidence-based Complement. Altern. Med. 2016, 1–7. https://doi.org/10.1155/2016/3837270

Patil, R.H., Patil, M.P., Maheshwari, V.L., 2016. Bioactive Secondary Metabolites From Endophytic Fungi: A Review of Biotechnological Production and Their Potential Applications, in: Atta-ur-Rahman (Ed.) (Ed.), Studies in Natural Products Chemistry. Elsevier, pp. 189–205. https://doi.org/10.1016/B978-0-444-63601-0.00005-3

Pereira, A.S.P., Haan, H. Den, Peña-García, J., Moreno, M.M., Pérez-Sánchez, H., Apostolides,

Z., 2019. Exploring African Medicinal Plants for Potential Anti-Diabetic Compounds with the DIA-DB Inverse Virtual Screening Web Server. Molecules 24, 1–32. https://doi.org/10.3390/molecules24102002

Pettit, G.R., Ducki, S., Tan, R., Gardella, R.S., Mcmahon, J.B., Boyd, M.R., Pettit, G.R., Blumberg, P.M., Lewin, N.E., Doubek, D.L., Tackett, L.P., Williams, M.D., 2002. Isolation and Structure of Pedilstatin from a Republic of Maldives Pedilanthus. J. Nat. Prod. 65, 1262–1265. https://doi.org/10.1021/np020115b

Phillipson, J.D., Hemingway, S.R., 1974. Angustine and related alkaloids from species of mitragyna, nauclea, uncarza, and strychnos. Phytochemistry 13, 973–978.

Pop, C., Vlase, L., Tamas, M., 2009. Natural resources containing arbutin. Determination of arbutin in the leaves of Bergenia crassifolia (L.) Fritsch. acclimated in Romania. Not. Bot. Horti Agrobot. Cluj-Napoca 37, 129–132.

Prabavathy, D., Nachiyar, C.V., 2014. Cytotoxicity of Ethyl Acetate Extract of Endophytic Aspergillus fumigatus on A549 Cell Lines. Biosci. Biotechnol. Res. ASIA, 11, 797–802.

Purwestri, Y.A., Kartikasari, N., Putri, S.G., Wilson, W., Sembiring, L., 2016. Metabolic profiling of endophytic bacteria from Purwoceng (Pimpinella pruatjan Molkend) root and antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. AIP Conf. Proc. 1744, 1–7. https://doi.org/10.1063/1.4953537

Pusztahelyi, T., Holb, I.J., Pócsi, I., 2015. Secondary metabolites in fungus-plant interactions. Front. Plant Sci. 6, 1–23. https://doi.org/10.3389/fpls.2015.00573

Refaat, John, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012. Crinum ; an endless source of bioactive principles : a review , part ii . crinum alkaloids : crinine- type alkaloids. Int. J. Pharma Sci. Res. 3, 3091–3100.

Refaat, J, Kamel, M.S., Ramadan, M.A., Ali, A.A., 2012. Crinum; an endless source of bioactive principles: a review. part iii; crinum alkaloids: belladine-, galanthamine-, lycorenine-, tazettine-type alkaloids and other minor types. Int. J. Pharm. Sci. Res. 3, 3630–3638.

Saad, H.-E., El-Sharkawy, S., Shier, W.T., 1995. Biological Activities of Pyrrolidinoindoline Alkaloids from Calycodendron milnei. Planta Med. 61, 313–316. https://doi.org/10.1055/s-2006-958090

Sardul Singh, S., Suneel, K., Aharwal, R.P., 2014. Isolation and identification of endophytic fungi from Ricinus communis linn . And their antibacterial activity. Int. J. Res. Pharm. Chem. 4, 611–618.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Latha, L.Y., 2011. Extraction , Isolation and Characterization of Bioactive Compounds from Plants ' Extracts. African J. Tradit. Complement. Altern. Med. · 8, 1–10. https://doi.org/10.4314/ajtcam.v8i1.60483

Sasidharan, S., Yoga Latha, L., Kwan, Y.P., Lachumy, S.J., 1993. Screening Methods in the Study of Fungicidal Property of Medicinal Plants. Fungic. Plant Anim. Dis. 107-.

Sawaya, A.C.H.F., Abreu, I.N., Andreazza, L., Eberlin, M.N., Mazzafera, P., 2010. Pilocarpine and related alkaloids in pilocarpus vahl (Rutaceae), in: N. M. Cassiano (Ed.), Alkaloids: Properties, Applications. Nova Science Publishers, Inc, pp. 63–80.

Scholz, M., Gatzek, S., Sterling, A., Selbig, O., Fiehn, J., 2004. Metabolite fingerprinting : detecting biological features by independent component analysis. Bioinformatics 20, 2447–2454. https://doi.org/10.1093/bioinformatics/bth270

Sebola, T.E., Ndinteh, D.T., Mavumengwana, V., 2016. A contribution to the phytochemical and antibacterial characteristics of Crinum macowanii bulbs extracts. University of Johannessburg.3-4.

Sebola, T.E., Okereafor, N.C.U., Tapfuma, K.I., Mekuto, L., Green, E., Mavumengwana, V., 2019. Evaluating antibacterial and anticancer activity of crude extracts of bacterial endophytes from *Crinum macowanii* Baker bulbs. MicrobiologyOpen.8(12).1–10. https://doi.org/10.1002/mbo3.914

Senyuva, H.Z., Gilbert, J., 2007. Rapid Analysis of Crude Fungal Extracts for Secondary Metabolites by LC / TOF-MS – A New Approach to Fungal Characterization Application. USA.

Singh, A., 2008. Phytochemicals of Gentianaceae : A Review of Pharmacological Properties. Int. J. Pharm. Sci. Nanotechnol. 1, 33–36.

Stankovic, N., Senerovic, L., Ilic-Tomic, T., Vasiljevic, B., Nikodinovic-Runic, J., 2014. Properties and applications of undecylprodigiosin and other bacterial prodigiosins. Appl. Microbiol. Biotechnol. 98, 3841–3858. https://doi.org/10.1007/s00253-014-5590-1

Stierle, A.C., Cardellina, J.H., Strobel, G.A., 1988. Maculosin, a host-specific phytotoxin for spotted knapweed from Alternaria alternata. Proc. Natl. Acad. Sci. 85, 8008–8011. https://doi.org/10.1073/pnas.85.21.8008

Stolker, A.A.M., Niesing, W., Fuchs, R., Vreeken, R.J., Niessen, W.M.A., Brinkman, U.A.T., 2004. Liquid chromatography with triple-quadrupole and quadrupole-time-of-flight mass spectrometry for the determination of micro-constituents - A comparison. Anal. Bioanal.

Chem. 378, 1754–1761. https://doi.org/10.1007/s00216-003-2485-x

Strobel, G., 2018. The Emergence of Endophytic Microbes and Their Biological Promise. J. Fungi 4, 1–9. https://doi.org/10.3390/jof4020057

Sudha, V., Govindaraj, R., Baskar, K., Al-dhabi, N.A., Duraipandiyan, V., 2016. Biological properties of Endophytic Fungi. BRAZILIAN Arch. Biol. Technol. AN 59, 1–7.

Szarc Vel Szic, K., Op De Beeck, K., Ratman, D., Wouters, A., Beck, I.M., Declerck, K., Heyninck, K., Fransen, E., Bracke, M., De Bosscher, K., Lardon, F., Van Camp, G., Vanden Berghe, W., 2014. Pharmacological levels of Withaferin A (Withania somnifera) trigger clinically relevant anticancer effects specific to triple negative breast cancer cells. PLoS One 9, 1–17. https://doi.org/10.1371/journal.pone.0087850

Tan, J., Zheng, M., Duan, S., Zeng, Y., Zhang, Z., Cui, Q., Zhang, J., Hong, T., Bai, J., Du, S., 2018. Chemical Profiling and Screening of the Marker Components in the Fruit of Cassia fistula by HPLC and UHPLC/LTQ-Orbitrap MSn with Chemometrics. Molecules 23, 1–18. https://doi.org/10.3390/molecules23071501

Tapfuma, K.I., Mekuto, L., Mellisa, M., Mavumengwana, V., 2019. The LC-QTOF-MS / MS analysis data of detected metabolites from the crude extract of Datura stramonium leaves. Data Br. 25, 1–4. https://doi.org/10.1016/j.dib.2019.104094

Thermo Scientific, 2013. Methods Optimization in Accelerated Solvent Extraction. https://doi.org/10.1080/09638288.2016.1189610

Thomas, L. V., Yu, S., Ingram, R.E., Refdahl, C., Elsser, D., Delves-Broughton, J., 2002. Ascopyrone P, a novel antibacterial derived from fungi. J. Appl. Microbiol. 93, 697–705. https://doi.org/10.1046/j.1365-2672.2002.01751.x

Tidke, S.A., Kumar, R.K.., Ramakrishna, D., Kiran, S., Kosturkova, G., Gokare, R.A., 2017. Current Understanding of Endophytes : Their Relevance , Importance , and Industrial Potentials. J. Biotechnol. Biochem. 3, 43–59. https://doi.org/10.9790/264X-03034359

Tram, N.T.N., Titorenkova, T. V., Bankova, S. V., Handjieva, N.V., Popov, S.S., 2002. Crinum L. Amaryllidaceae. Fitoterapia 73, 183–208.

Truong, D.-H., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H., Nguyen, H.C., 2019. Evaluation of the Use of Different Solvents for Phytochemical Constituents , Antioxidants , and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. J. Food Qual. 1–9.

Venieraki, A., Dimou, M., Katinakis, P., 2017. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as

their hosts. Hell. Plant Prot. J. 10, 51–66. https://doi.org/10.1515/hppj-2017-0006

Venugopalan, A., Srivastava, S., 2015. Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol. Adv. 33, 873–887. https://doi.org/10.1016/j.biotechadv.2015.07.004

Volkmann, M., Gorbushina, A.A., 2006. A broadly applicable method for extraction and characterization of mycosporines and mycosporine-like amino acids of terrestrial, marine and freshwater origin. FEMS Microbiol. Lett. 255, 286–295. https://doi.org/10.1111/j.1574-6968.2006.00088.x

Wang, H., Li, Y., Huang, Y., Zhao, C., Cheung, H., 2018. Chemical Profiling of Lobelia chinensis with High-Performance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry (HPLC/Q-TOF MS) Reveals Absence of Lobeline in the Herb. Mol. Ther. Oncolytics 23, 1–13. https://doi.org/10.3390/molecules23123258

Want, E.J., 2018. LC-MS Untargeted Analysis, in: Theodoridis, G.A., Gika, H.G., Wilson, I.D. (Eds.), Metabolic Profiling Methods and Protocols. Humana Press, New York, NY, U.S.A, pp. 99–116.

Widyawati, S.P., Budianta, T.D.W., Kusuma, F.A., Wijaya, E.L., 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia less leaves extracts. Int. J. Pharmacogn. Phytochem. Res. 6, 850–855.

Wu, W., Han, X., Wu, C., Wei, G., Zheng, G., Li, Y., Yang, Y., Yang, L., He, D., Zhao, Y., Cai, Z., 2018. Vernodalol mediates antitumor effects in acute promyelocytic leukemia cells. Oncol. Lett. 15, 2227–2235. https://doi.org/10.3892/ol.2017.7544

Xiao, J.F., Zhou, B., Ressom, H.W., 2012. Metabolite identification and quantitation in LC-MS/MS-based metabolomics. TrAC - Trends Anal. Chem. 32, 1–14. https://doi.org/10.1016/j.trac.2011.08.009

Xie, G., Ni, Y., Su, M., Zhang, Y., Zhao, A., Gao, X., Liu, Z., Long, X., Jia, W., 2008. Application of Ultra-Performance LC-TOF MS Metabolite Profiling Techniques to the Analysis of Medicinal Panax Herbs. Metabolomics 4, 248–260.

Xu, J., 2010. Secondary Metabolites from the Endophytic Fungus Pestalotiopsis sp. JCM2A4 and its Microbe-Host Relationship with the Mangrove Plant Rhizophora mucronata. Heinrich-Heine-Universität Düssedldorf.

Xue, J.H., Xu, L.X., Jiang, Z.H., Wei, X., 2012. Quinazoline Alkaloids from Streptomyces michiganensis. Chem. Nat. Compd. 48, 839–841. https://doi.org/10.1007/s10600-012-0397-

8

Yadav, R.N.S., Agarwala, M., 2011. Phytochemical analysis of some medicinal plants. J. Phytol. 3, 10–14. https://doi.org/10.1021/np800144q

Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., Wang, Q., 2018. Response of Plant Secondary Metabolites to Environmental Factors. Molecules 23, 1–26. https://doi.org/10.3390/molecules23040762

Yusnawan, E., 2013. The Effectiveness of Polar and Non Polar Fractions of Ageratum Conyzoides L . To Control Peanut Rust Disease and Phytochemical Screenings of Secondary Metabolites. J. Hama dan Penyakit Tumbuh. Trop. 13, 159–166.

Zhang, J., Chen, Z., Huang, X., Shi, W., Zhang, R., Chen, M., Huang, H., Wu, L., 2019. Insights on the Multifunctional Activities of Magnolol. Biomed Res. Int. 2019, 1–15. https://doi.org/10.1155/2019/1847130

Zhao, J., Ma, J., 2016. Phytochemicals and Biological Activities of the Genus Cinnamomum. J. Pharmacogn. Phytochem. 4, 27–34.

Zhu, Z., Schultz, A.W., Wang, J., Johnson, C.H., Yannone, S.M., Patti, G.J., Siuzdak, G., 2013. Liquid chromatography quadrupole time-of-flight mass spectrometry characterization of metabolites guided by the METLIN database. Nat. Protoc. 8, 451–460. https://doi.org/10.1038/nprot.2013.004

Zubair, M.S., Anam, S., Lallo, S., 2016. Cytotoxic activity and phytochemical standardization of Lunasia amara Blanco wood extract. Asian Pac. J. Trop. Biomed. 6, 962–966. https://doi.org/10.1016/j.apjtb.2016.04.014

CHAPTER SIX:

GENERAL DISCUSSION AND CONCLUSION *

* This is the final chapter in the thesis, which draws a connection between the experimental chapters of the study and gives a brief review of the analytical and experimental work done and a general conclusion from the study. Recommendations for future work are drawn from the outcomes of the study.

6. GENERAL DISCUSSION AND CONCLUSION

6.1 GENERAL DISCUSSION

This study aimed to isolate, identify and characterize culturable microflora and/or mycoflora endophytes from *Crinum macowanii* bulbs and leaves. The crude extracts of the plant and crude endophytes were isolated, and their secondary metabolites were characterized. The antibacterial and anticancer activity of the crude extracts (plants and endophytes) were evaluated. Significant findings are described in Chapters 3 to 5.

In this study a total of 8 bacterial endophytes were isolated from the bulbs, 9 bacterial endophytes were isolated from the leaves and 5 fungal endophytes were isolated from both the leaves and bulbs of *C. macowanii* and characterized. The bacterial endophytes included genera *Acinetobacter*, *Pseudomonas, Rahnella*, *Bacillus*, *Novosphingobium*, *Raoultella*, *Burkholderia*, *Enterobacter* and *Arthrobacter* and the fungal endophytes yielded *Filobasidium*, *Alternaria* and *Penicillium* as the isolated genera. Bacterial genera such as *Pseudomonas*, *Bacillus* and *Burkholderia* are common plant endophytes (Santoyo *et al*., 2016). *Alternaria* and *Filobasidium* are common fungal endophytes isolated from medicinal plants. The bulbs and leaves had similar bacterial endophytes such as *Acinetobacter guillouiae*, *Pseudomonas* sp., *Raoultella ornithinolytica*. A similar pattern was observed with the fungal endophytes; *Filobasidium magnum* was also isolated from both the bulbs and leaves. This indicates that both bacteria and fungi co-exist in the bulbs and leaves of *C. macowanii* and this has added to our knowledge regarding the microbial community of *C. macowanii*.

The isolated endophytes (Chapter 3) did not only display the co-existing variety of the endophytes but also possible diverse bioactive compounds which could be produced by the isolated endophytes, which led to our testing of the biological activity of the different crude extracts (plants and endophytes) (Chapter 4). Crude bacteria endophyte extracts, namely, *Raoultella ornithinolytica, Pseudomonas moraviensis, Pseudomonas sp., Enterobacter asburiae, Pseudomonas cichorii* and *Arthrobacter pascens* displayed noteworthy antibacterial activity against pathogenic bacteria and they can be further explored as antibacterial agents. It was observed in this study that the Gram-positive bacterial species were more susceptible to the antibacterial compounds in the crude extracts of endophytes isolated from the bulbs than the Gram-negative bacteria, whereas the Gram-negative bacteria species were more susceptible to the antibacterial compounds in the crude extracts of endophytes isolated from the leaves, than the

Gram-positive ones. Crude endophyte extracts from *Penicillium* sp. displayed notable antibacterial activity against pathogenic bacteria, with the least inhibition compared to the other fungal extracts. *Pseudomonas putida* and *Bacillus safensis* crude extracts showed a 47% and 50% cell reduction respectively against lung carcinoma cells at a concentration of 100 µg/ml and could be further explored as anticancer agents. The observed antibacterial and anticancer activity could be attributed to a synergistic relationship between the different secondary metabolites present in the crude extracts, as the combination of bioactive metabolites is known to improve the therapeutic effects and increased efficacy of the crude extracts (Kabera *et al*., 2014a). In addition, the combined effects of the bioactive secondary metabolites is more than the sum of independent activities of pure compounds (Tiwaskar and Manohar, 2017). This synergistic effect has been used in combating drug resistance in infectious diseases and cancer (Doughari, 2012) which is why we chose to use crude extracts (Chapter 4) rather than single isolated compounds. It is therefore important that crude extracts are profiled, to determine which secondary metabolites are present (Chapter 5).

Alkaloids, including lycorine, crinamine, crinine, crinamidine and powelline, have been identified from crude plant extracts and crude endophytes extracts. This is not a new discovery, as endophytes are known to be able to synthesize phytochemicals similar to their host plants, as they imitate the chemistry of the host plant, producing the same bioactive metabolites (Santos and Ferraris, 2012; Bedi *et al*., 2017; Venieraki, Dimou and Katinakis, 2017; Uzma *et al.*, 2018). An array of different compounds with biological activities were identified from the endophyte crude extracts.

6.2 CONCLUSION

With the ongoing over-harvesting of medicinal plants to obtain bioactive extracts in South Africa, and the constant increase in infectious diseases, the conservation of medicinal plants is increasingly under strain. Therefore, new approaches to obtaining medicinal plants and herbs must be explored to ensure the eradication of infectious and resistant diseases, while preserving and conserving medicinal plants. The exploring of endophytes is a feasible approach to this challenge, as it is more advantageous than other techniques. This study has provided information on the endophyte community of *C. macowanii* and the biological activities of their crude extracts, and to some extent, the secondary metabolites within. The data generated confirm that crude endophyte

extracts have a broad spectrum on the tested pathogenic bacteria and can be further tested on other cancer cell lines. The bioprospecting of endophytes to isolate metabolites could help save the environment, as endophytes have been reported to contain similar bioactive compounds to the host plant, and in so doing medicinal plants are conserved and revenue is generated by the bioprospecting of metabolites from endophytes.

RECOMMENDATIONS

From the outcomes of the study the following recommendations for future work can be made.

- ✓ The lack of knowledge about endophytes from medicinal plants in South Africa, hinders the further exploration of these and therefore the South African National Biodiversity Institute can assist in formal documentation of endophytes as to support their strategy to develop a database to capture all economically important microbial species and increasing taxonomic capacity in South Africa by establishing a network of endophytes systematists.
- ✓ Stringent end points for anti-infective bioassays ought to be set to prevent false results and confusion, taking into consideration the sensitivity of extracts and test micro-organisms, extraction methods and solvents used. The South African Medical Research Council can spearhead such as to establish end points for anti-infective bioassays as to translate research in the drug development and discovery.
- ✓ Lack of scientific knowledge in verifying the biological activities of bioactive secondary metabolites from endophytes hinder their regulation and quality standards, and therefore adequate research still needs to be conducted regarding their mode of action and safety. Therefore, pharmacological testing is a key strategy for discovering and developing new drugs. The South African Health Regulatory Products Authority can help in ensuring that these compounds are safe, effective and is of good quality as per the acts and regulations.
- ✓ More research has to be conducted to isolate endophytes from indigenous plants in South Africa as this will be in alignment with the bioeconomy strategy the Department of Science and Innovation in South Africa which encourages the bioprospecting of microorganisms for pharmaceutical applications.

References

Bedi, A., Adholeya, A., Deshmukh, S.K., 2017. Novel Anticancer Compounds from Endophytic Fungi. Curr. Biotechnol. 6, 1–18.

https://doi.org/10.2174/2211550105666160622080 3

Doughari, J.H., 2012. Phytochemicals : Extraction Methods , Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, in: Phytochemicals - A Global Perspective of Their Role in Nutrition and Health. pp. 1–33.

Kabera, J.N., Semana, E., Mussa, A.R., He, X., 2014a. Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. J. Pharm. Pharmacol. 2, 377–392. https://doi.org/10.1016/0300-9084(96)82199-7

Santos, S.N., Ferraris, F.K., 2012. Endophytic fungi from Combretum leprosum with potential anticancer and antifungal activity. Symbiosis 109–117. https://doi.org/10.1007/s13199-012-0218-7

Santoyo, G., Moreno-Hagelsieb, G., Carmen Orozco-Mosqueda, del M., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183, 92–99. https://doi.org/10.1016/j.micres.2015.11.008

Tiwaskar, M., Manohar, T., 2017. Rational for Antibiotics – Guidelines, in: Medicine Update 2017. pp. 6–15.

Uzma, F., Mohan, C.D., Hashem, A., Konappa, N.M., Rangappa, S., Kamath, P. V., Singh, B.P., Mudili, V., Gupta, V.K., Siddaiah, C.N., Chowdappa, S., Alqarawi, A.A., Abd_Allah, E.F., 2018. Endophytic Fungi — Alternative Sources of Cytotoxic Compounds : A Review. Front. Pharmacol. 9, 1–37. https://doi.org/10.3389/fphar.2018.00309

Venieraki, A., Dimou, M., Katinakis, P., 2017. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. Hell. Plant Prot. J. 10, 51–66. https://doi.org/10.1515/hppj-2017-0006

APPENDIX I

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ORIGINAL ARTICLE

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Evaluating antibacterial and anticancer activity of crude extracts of bacterial endophytes from *Crinum macowanii* Baker bulbs

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Abstract

The results from this study revealed that crude extracts isolated from bacterial endophytes obtained from *Crinum macowanii* bulbs showed activity against both Gram-positive and Gram-negative pathogenic bacteria, while *Acinetobacter guillouiae* crude extracts displayed anticancer activity. This study aimed to isolate and characterize bacterial endophytes and their crude extracts from *C. macowanii* bulbs. Endophytes were isolated using validated surface sterilization techniques, followed by phenotypic and genotypic profiles of the isolates. Crude extracts were extracted from the endophytes using ethyl acetate, while methanol:dichloromethane (1:1) was used to obtain crude extracts from the bulbs. Antibacterial activity of crude extract from each endophyte was investigated against selected pathogenic strains using the broth microdilution method, and anticancer activity against U87MG glioblastoma and A549 lung carcinoma cells was determined by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. *Acinetobacter guillouiae*, *Pseudomonas moraviensis*, *Pseudomonas* sp., *Rahnella aquatilis*, *Bacillus cereus*, *Novosphingobium* sp., *Raoultella ornithinolytica*, and *Burkholderia tropica* were successfully isolated. The crude extracts from the majority of endophytes showed antibacterial activity, ranging from 0.125 to >16.00 mg/ml against Gram-negative and Gram-positive pathogenic bacteria. *Acinetobacter guillouiae* extracts showed a high bioactive potential against U87MG glioblastoma cell lines by reducing their growth by 50% at concentrations of 12.5, 6.25, and 3.13 μg/mL. Crude extracts isolated from *C. macowanii* bulbs showed potential for possible drug lead against common pathogenic bacteria.

KEYWORDS

antibacterial activity, anticancer activity, *Crinum macowanii* bulbs, crude extracts, endophytes

APPENDIX II

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Research Article

Antibacterial and Anticancer Activity and Untargeted Secondary Metabolite Profiling of Crude Bacterial Endophyte Extracts from *Crinum macowanii* Baker Leaves

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This study isolated and identified endophytic bacteria from the leaves of *Crinum macowanii* and investigated the potential of the bacterial endophyte extracts as antibacterial and anticancer agents and their subsequent secondary metabolites. Ethyl acetate extracts from the endophytes and the leaves (methanol: dichloromethane (1 : 1)) were used for antibacterial activity against selected pathogenic bacterial strains by using the broth microdilution method. The anticancer activity against the U87MG glioblastoma and A549 lung carcinoma cells was determined by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. Bacterial endophytes that were successfully isolated from *C. macowanii* leaves include *Raoultella ornithinolytica*, *Acinetobacter guillouiae*, *Pseudomonas* sp., *Pseudomonas palleroniana*, *Pseudomonas putida*, *Bacillus safensis*, *Enterobacter asburiae*, *Pseudomonas cichorii*, and *Arthrobacter pascens*. *Pseudomonas cichorii* exhibited broad antibacterial activity against both Gram-negative and Gram-positive pathogenic bacteria while *Arthrobacter pascens* displayed the least MIC of 0.0625 mg/mL. *Bacillus safensis* crude extracts were the only sample that showed notable cell reduction of 50% against A549 lung carcinoma cells at a concentration of 100 µg/mL. Metabolite profiling of *Bacillus safensis*, *Pseudomonas cichorii*, and *Arthrobacter pascens* crude extracts revealed the presence of known antibacterial and/or anticancer agents such as lycorine (1), angustine (2), crinamidine (3), vasicinol (4), and powelline. It can be concluded that the crude bacterial endophyte extracts obtained from *C. macowanii* leaves can biosynthesize bioactive compounds and can be bioprospected for medical application into antibacterial and anticancer agents.

1. Introduction

The emergence of infectious diseases worldwide due to bacteria and viruses still poses a serious public health concern, claiming the lives of half a million people a year and amounting to 25% of the total deaths worldwide [1]. Even with the discovery and production of new and improved antibiotics, the resistance of pathogenic microorganisms to drugs has increased enormously [2]. Ventola [3] indicated that the causes of antibiotic resistance include overuse, inappropriate prescribing, and extensive agricultural use. There is, therefore, an imminent need to discover and

APPENDIX III

16S rRNA sequences of bacteria endophytes

Sample code	Accession number	Bacteria name	16S rRNA sequences
TES 01C	MF943224	*Acinetobacter guillouiae*	TGTTAGCTGCGCCACTAAAGCCTCAAAGGCCCCAACGGCTAGTASACATGGTTTACGGCATGGACTAGGGGGGTA TCTAATCCTGTTTGCTCCCCATGGTTTCGGACCTCAGCGTCAGTATTAGGCCAGATGGCTGCCTTCGCCATCGGTA TTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCATCCTCTCCCATACTCTAGCTTCCCAGTAT CGAATGCAATTCCCAAGTTAAGCTCGGGGATTTCACATCCGACTTAAAAAGCCGCCTACGCACGCTTTACGCCCA GTAAATCCGATTAACGCTCGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCTGCG AGTAACGTCCACTCACTGTAGGTATTAACTACAGGAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCC TTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGA GTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCT TTACCCCACCAACTAGCTAATCCGACTTAGGCTCATCTATTAACGCAAGGTCACAAGTGATCCCCTGCTTTCCCCC GTAGGGCGTATGCGGTATTAGCATCCCTTTCGAGATGTTGTCCCCCATTAATAGGCAGATTCCTAAGTATTACTCA CCGTCCGCCGCTAGGTCAATTACCGAAGCAA
TES 01E	MF943225	*Pseudomonas moraviensis*	TTAATGCGTTAGCTGCGCCACTAAGAGCTCAAGGCTCCCAACGGCTAGTTGACATCGTTTACGGCGTGGACTACC AGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCA CTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTACCATACTCTAGCTCG CCAGTTTTGGATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCAACTTAACGAACCACCTACGCGCGCTTT ACGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTAT TCTGTCGGTAACGTCAAAACAGCAAAGTATTAATTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAA GACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGT AGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTG AGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTC TCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGCATTA CTCACCCGTCCGCCGCTGAATTCAGGAGCAAGCTCCCTTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAG CGTTCAATCTGAGC
TES 01F	MF943226	*Pseudomonas* sp.	TTAATGCGTTAGCTGCGCCACTAAGAGCTCAAGGCTCCCAACGGCTAGTTGACATCGTTTACGGCGTGGACTACC AGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCA CTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTACCATACTCTAGCTCG CCAGTTTTGGATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCAACTTAACGAACCACCTACGCGCGCTTT ACGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTAT TCTGTCGGTAACGTCAAAACAGCAAAGTATTAATTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAA GACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGT AGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTG AGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTC TCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGCATTA CTCACCCGTCCGCCGCTGAATTCAGGAGCAAGCTCCCTTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAG CGTTCAATCTGAGC
TES 03A	MF943229	*Rahnella aquatilis*	AACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACAGCGTGGACTACCA GGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCAC CGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCTTGC CAGTTTCAAATGCAGTTCCCACGTTAAGCGCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCTTTA CGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTT CTGCGAGTAACGTCAATCACCACACGTATTAAGTRTGATGCCTTCCTCCTCGCTGAAAGTGCTTTACAACCCTAAG

			GCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTA GGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGA GCCATTACCTCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCGTGAGGTCCGAAGATCCCCCACTTTGCT CTTTCGAGGTCATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACT CACCCGTCCGCCGCTCGCCGGCAAAGTAGCAAGCTACTTTCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCC GCCAGCGTTCAATCTGAGC
TES 03B	MF943230	*Bacillus cereus*	GCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACT ACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCG CCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTC TCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCT TTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTT TCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCC GAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCC CGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTG GTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAAT TTCGAACCATGCGGTTCAAAATRTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGT TACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGT ATTAGGCACGCCGCCAGCGTTCATCCTGAG
TES 03C	MF943231	*Bacillus cereus*	GCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACT ACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCG CCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTC TCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCT TTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTT TCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCC GAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCC CGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTG GTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAAT TTCGAACCATGCGGTTCAAAATRTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGT TACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGT ATTAGGCACGCCGCCAGCGTTCATCCTGAG
TES 04A	MF943232	*Novosphingobium* sp.	ATACTTAATGCGTTAGCTGCGCCACCCAAGTACCAAGTACCCGGACAGCTASTTWTCATCGTTTACGGCGTGGAC TACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAATACTTGTCCAGTCAGTCGCCTTC GCCACTGGTGTTCTTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTGACCTCTCCAAGATTCTAG TCACCTAGTTTCAAAGGCAGTTCCGGGGTTGAGCCCCGGGCTTTCACCTCTGACTTGAGTAACCGCCTACGCGCG CTTTACGCCCAGTAATTCCGAACAACGCTAGCTCCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGAG CTTATTCTCCCGGTACTGTCATTATCATCCCGGGTAAAAGAGCTTTACAACCCTAAGGCCTTCATCACTCACGCGG CATTGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTC AGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAAGGATCGTCGGCTTGGTAGGCCATTACCCCACCAACTA CCTAATCCTACGCGGGCTCATCCCTGGGCGATAAATCTTTGGTCCGAAGACATCATCCGGTATTAGCAGTAATTTC TCACTGTTATTCCGAACCCAAGGGCAGATTCCCACGCGTTACGCACCCGTGCGCCACTAGACCCGAAGGTCTCGT TCGACTTGCATGTGTTAGGCATGCCGCCAGCGTTCGTTCTGAGCCTA
TES 09B	MF943238	*Rahnella aquatilis*	AACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACAGCGTGGACTACCA GGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCAC CGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCCTCTACAAGACTCTAGCTTGC CAGTTTCAAATGCAGTTCCCACGTTAAGCGCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCTTTA CGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTT CTGCGAGTAACGTCAATCACCACACGTATTAAGTGTGATGCCTTCCTCCTCGCTGAAAGTGCTTTACAACCCTAA GGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGT AGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTG AGCCATTACCTCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCGTGAGGTCCGAAGATCCCCCACTTTG

			CTCTTTCGAGGTCATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTA CTCACCCGTCCGCCGCTCGCCGGCAAAGTAGCAAGCTACTTTCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGC CGCCAGCGTTCAATCTGAG
TES 11A	MF943240	*Raoultella ornithinolytica*	TCGACTTAACGCGTTAGCTCCGGAAGCCACTCCTCAAGGGAACAACCTCCAAGTCGACATCGTTTACAGCGTGGA CTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTT CGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTA GCCTGCCAGTTTCGGATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGC GCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGT GCTTCTTCTGCGAGTAACGTCAATCRMYAAGGTTATTAACCTTAWYGCCTTCCTCCTCGCTGAAAGTACTTTACA ACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGC CTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGC CTAGGTGAGCCATTACCTCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCC CACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCA GACATTACTCACCCGTCCGCCGCTCGTCACCCGAAGAGCAAGCTCTTCTGTGCTACCGCTCGACTTGCATGTGTTA GGCCTGCCGCCAGCGTTCAATCTGAGC
TES 12A	MF943241	*Burkholderia tropica*	TTCACGCGTTAGCTTCGTTACTAAGGAAATGAATCCCCAACAACCAGTTGACATCGTTTAGGGCGTGGACTACCA GGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTATTGGCCCAGGGGGCTGCCTTCGCCAT CGGTATTCCTCCACATCTCTACGCATTTCACTGCTACACGTGGAATTCTACCCCCCTCTGCCATACTCCAGCGATG CAGTCACCAATGCAGTTCCCAGGTTAAGCCCGGGGATTTCACATCGGTCTTACATCACCGCCTGCGCACGCTTTA CGCCCAGTAATTCCGATTAACGCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTATT CTTCCGGTACCGTCATCCCCCGACCATATTAGGATCAGGGATTTCTTTCCGGACAAAAGTGCTTTACAACCCGAA GGCCTTCTTCACACACGCGGCATTGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGT AGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAGACCAGCTACGGATCGTCGCCTTGGTG AGCCTTTACCCCACCAACTAGCTAATCCGCCATCGGCCGCCCCTATAGCGCGAGGTCCGAAGATCCCCCGCTTTC ATCCCTAGATCGTATGCGGTATTAATCCGGCTTTCGCCGGGCTATCCCCCACTACAGGACACGTTCCGATGTATTA CTCACCCGTTCGCCACTCGCCACCAGGTGCAAGCACCCGTGCTGCCGTTCGACTTGCATGTGTAAGGCATGCCGC CAGCGTTCAATCTGAG
TES 13A	MF943242	*Rahnella aquatilis*	CGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACAGCGTGGAC TACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTC GCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAG CTTGCCAGTTTCAAATGCAGTTCCCACGTTAAGCGCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCG CTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGC TTCTTCTGCGAGTAACGTCAATCACCACACGTATTAAGTATGATGCCTTCCTCCTCGCTGAAAGTGCTTTACAACC CTAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTC CCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTA GGTGAGCCATTACCTCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCGTGAGGTCCGAAGATCCCCCAC TTTGCTCTTTCGAGGTCATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGAC ATTACTCACCCGTCCGCCGCTCGCCGGCAAAGTAGCAAGCTACTTTCCGCTGCCGCTCGACTTGCATGTGTTAGGC CTGCCGCCAGCGTTCAATCTGAGC
TES 02B	MF943227	*Raoultella ornithinolytica*	TCGACTTAACGCGTTAGCTCCGGAAGCCACTCCTCAAGGGAACAACCTCCAAGTCGACATCGTTTACAGCGTGGA CTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTT CGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTA GCCTGCCAGTTTCGGATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGC GCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGT GCTTCTTCTGCGAGTAACGTCAATCRMYAAGGTTATTAACCTTAWYGCCTTCCTCCTCGCTGAAAGTACTTTACA ACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGC CTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGC CTAGGTGAGCCATTACCTCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCC CACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCA

			GACATTACTCACCCGTCCGCCGCTCGTCACCCGAAGAGCAAGCTCTTCTGTGCTACCGCTCGACTTGCATGTGTTA GGCCTGCCGCCAGCGTTCAATCTGAGC
TES 02C	MF943228	*Acinetobacter guillouiae*	TCTACTTATCGCGTTAGCTGCGCCACTAAAGCCTCAAAGGCCCCAACGGCTAGTAGACATCGTTTACGGCATGGA CTACCAGGGTATCTAATCCTGTTTGCTCCCCATGCTTTCGTACCTCAGCGTCAGTATTAGGCCAGATGGCTGCCTT CGCCATCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCATCCTCTCCCATACTCTA GCTTCCCAGTATCGAATGCAATTCCCAAGTTAAGCTCGGGGATTTCACATCCGACTTAAAAAGCCGCCTACGCAC GCTTTACGCCCAGTAAATCCGATTAACGCTCGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGT GCTTATTCTGCGAGTAACGTCCACTCACTGTAGGTATTAACTACAGGAGCCTCCTCCTCGCTTAAAGTGCTTTACA ACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGTCCAATATTCCCCACTGCTGC CTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGC CTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTTAGGCTCATCTATTAACGCAAGGTCACAAGTGATCC CCTGCTTTCCCCCCGTAGGGCGTATGCGGTATTAGCATCCCTTTCGAGATGTTGTCCCCCATTAATAGGCAGATTCC TAAGTATTACTCACCCGTCCGCCGCTAGGTCAATTACCGAAGCAATCTCCCCCGCTCGACTTGCATGTGTTAAGCC TGCCGCCAGCGTTCAATCTGAGC
TES 05A	MF943233	*Pseudomonas* sp.	GCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTTTACGGCGTGGACTACCAGGGT ATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATGAGCCCAGGKGGTCGCCTTCGCCACTGGT GTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTGCCCTACTCTAGCTTGCCAGT TTTGGATGCAGTTCCCAGGGTGAGCCCGGGGATTTCACATTCAACTTAACAAACCACCTACGCGCGCTTTACGCC CAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCTGT CGGTAACGTCAAAATCGTCACGTATTAGGTAACGACCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACC TTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGA GTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCT TTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGT AGGACGTATGCGGTATTAGCGTCCGTTTCCGGACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCAC CCGTCCGCCGCTCTCAAGAGGTGCAAGCACCTCTCTACCGCTCGACTTGCATGTGTTAGCCTGCCGCCAGCGTTCA ATCTGA
TES 05B	MF943234	*Pseudomonas palleroniana*	TCACTTAATGCGTTAGCTGCGCCACTAAAAGCTCAAGGCTTCCAACGGCTAGTTGACATCGTTTACGGCGTGGAC TACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTC GCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTACCATACTCTAG TCAGTCAGTTTTGAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCAACTTAACAAACCACCTACGCGCG CTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGC TTATTCTGTCGGTAACGTCAAAACAGTTACGTATTAGGCAACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATC CGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTC CCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTG GTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCT TTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCA TTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAAGCTTCTCTCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGC CAGCGTTCAATCTGA
TES 06A	MF943235	*Pseudomonas putida*	AATGCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTTTACGGCGTGGACTACCAG GGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACT GGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTACCGTACTCTAGCTTGCC AGTTTTGGATGCAGTTCCCAGGTTGAGCCCGGGGCTTTCACATYCAACTTAACAAACCACCTACGCGCGCTTTAC GCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTC TGTCGGTAACGTCAAAACACTAACGTATTAGGTTAATGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAG ACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTA GGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGA GCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCT CCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGCATTAC TCACCCGTCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAG CGTTCAATCTG

TES 07A	MF943236	*Bacillus safensis*	TGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGAC TACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTC GCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAG TTTCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCC CTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGG CTTTCTGGTTAGGTACCGTCAAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGAT CCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCT CCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTT GGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCAT CCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAG GTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGT ATTAGGCACGCCGCCAGCGTTCGTCCTGAGC
TES 07B	MF943237	*Bacillus safensis*	CAGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTG GACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCC TTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCA AGTTTCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAG CCCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGT GGCTTTCTGGTTAGGTACCGTCAAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACG ATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGC CTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGC CTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTT CATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGC AGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCAT GTATTAGGCACGCCGCCAGCGTTCGTCCTGAGC
TES 10A	MF943239	*Enterobacter asburiae*	TTAACGCGTTAGCTCCGGAAGCCACTCCTCAAGGGAACAACCTCCAAGTCGACATCGTTTACGGCGTGGACTACC AGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCA CCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTG CCAGTTTCGAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTT ACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCT TCTGCGGGTAACGTCAATTGCTGAGGTTATTAACCTCAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAA GGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGT AGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTG AGCCRTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTG GTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTAT
TES 14A	MF943243	*Pseudomonas cichorii*	GGGCGGGTCACTTAATGCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTTTACGG CGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATGAGCCCAGGTGGT CGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTGCCCT ACTCTAGCTTGCCAGTTTTGGATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATTCAACTTAACAAACCACCTA CGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGC CGGTGCTTATTCTGTCGGTAACGTCAAAATCGTCACGTATTAGGTAACGACCCTTCCTCCCAACTTAAAGTGCTTT ACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGC TGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTC GCCTTGGTGAGCCTTTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCC CCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGGACGTTATCCCCCACTACCAGGCAGATTCC TAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGGTGCAAGCACCTCTCTACCGCTCGACTTGCATGTGTTAGGCC TGCCGCCAGCGTTCAATCTGAGC
TES 15A	MF943244	*Arthrobacter pascens*	CACTTAATGCGTTAGCTACGGCGCGGAAAACGTGGAATGTCCCCCACACCTAKTGCCCAACGTTTACGGCATGGA CTACCAGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAGTTAATGCCCAGAGACCTGCCTT CGCCATCGGTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCAGTCTCCCCTACATCACTCTA GTCTGCCCGTACCCACCGCAGATCCGGAGTTGAGCCCCGGACTTTCACGGCAGACGCGACAAACCGCCTACGAG

	CTCTTTACGCCCAATAATTCCGGATAACGCTTGCGCCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGC GCTTCTTCTGCAGGTACCGTCACTTTCGCTTCTTCCCTACTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTC ACGCGGCGTCGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCC GTGTCTCAGTCCCAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTGAGCCATTACCTCAC CAACAAGCTGATAGGCCGCGAGTCCATCCAAAACCACAAAAGCTTTCCACCCCCCACCATGCGATGAGGAGTCA TATCCGGTATTAGACCCAGTTTCCCAGGCTTATCCCAGAGTTAAGGGCAGGTTACTCACGTGTTACTCACCCGTTC GCCACTAATCCCCCCAGCAAGCTGGGGATCATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCATCCT GAGCTA

ITS sequences of fungal endophytes

Sample code	Accession number	Bacteria name	ITS sequences
TES 01A	MF925700	*Filobasidium magnum*	GTGGTTCTTCTTTCATATCCATAACACCTGTGCACTGTTGGATGCTTGCATCCACTTTTAAACTAAACATT ATTGTAACAAATGTAGTCTTATTATAACATAATAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCG ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA ACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAACCCTCAAACCCAA GTTTTGGATTTCGATCCATGCTTGAGTTTGGATTTGGATGTTTGCCGGTGATGAACCGACTCATCTTAAA AGTATTAGCTTGGATCTGTCTATATGACTGGTTTGACTTGGCATAATAAGTATTTTGCTGAGGACATCTT CGGATGGCCAGGACCTAGACTACTGTCTGCTAACTAAACCATCACTTTAAGTGCATCTTTGGATGTTACT CATTGTGTAACTTTGACATCTGGCCTCAAATCAAGTAGGACTACCCGCTGAACTTAAGCATATCAATA
TES 01B	MF925701	*Alternaria alternata*	ACTGGACCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTG GTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAA TAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAG TAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAA GGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTT GCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTC TCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATAC CCGCTGAACTTAAGCATATCAAAA
TES 02A	MF925702	*Filobasidium magnum*	GATTGACCATAGGCGAAGCCAGTGGTTCTTCTTTCATATCCATAACACCTGTGCACTGTTGGATGCTTGC ATCCACTTTTAAACTAAACATTATTGTAACAAATGTAGTCTTATTATAACATAATAAAACTTTCAACAAC GGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGT CATGAAACCCTCAAACCCAAGTTTTGGATTTCGATCCATGCTTGAGTTTGGATTTGGATGTTTGCCGGTG ATGAACCGACTCATCTTAAAAGTATTAGCTTGGATCTGTCTATATGACTGGTTTGACTTGGCATAATAAG TATTTTGCTGAGGACATCTTCGGATGGCCAGGACCTAGACTACTGTCTGCTAACTAAACCATCACTTTAA GTGCATCTTTGGATGTTACTCATTGTGTAACTTTGACATCTGGCCTCAAATCAAGTAGGACTACCCGCTG AACTTAAGCATATCAATA
TES 04B	MF925703	*Penicillium chrysogenum*	CTCTGGGTCCACCTCCCACCCGTGTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCC GGGGGGCTTACGCCCCCGGGCCCGCGCCCGCCGAAGACACCCTCGAACTCTGTCTGAAGATTGTAGTCT GAGTGAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGC AGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGC CCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTTCTGCCCTCAAGCACGGCTTGTGTGTTGGG CCCCGTCCTCCGATCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGT ATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTT GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAAAA
TES 05C	MF925704	*Alternaria tenuissima*	CAGGCGGGCTGGACCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTG TTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAAC

			AAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATG CGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGT ATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTC TCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAA GTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTCAACTTTTGACCTCGGATCAGG TAGGGATACCCGCTGAACTTAAGCATATCAAAA
TES 08A	MF925705	*Penicillium chrysogenum*	TCACCTCCCACCCGTGTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCCGGGGGGCT TACGCCCCCGGGCCCGCGCCCGCCGAAGACACCCTCGAACTCTGTCTGAAGATTGTAGTCTGAGTGAAA ATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAAT GCGATACGTAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGT ATTCCGGGGGGGCATGCCTGTCCGAGCGTCATTTCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCC TCCGATCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCT TTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCTCGG ATCAGGTAGGGATACCCGCTGAACTTAAGCATATCA
TES 09A	MF925706	*Penicillium* sp.	ACCGGGGCGGCCCAACCTCCCACCCTTGGGCTCTCTACACCTGGTGCTTTGGCGGGCCCACTGGGGTAG CCTGGGCGCCGGGGGACACCCGTCCCCGGGCCCGCGCCCGCCGAAGCGCTTCGTGAACCCTGATGAAG AAGGGCTGTCTGAGGACTATGAAAATTGTCAAAAGTTTCAACAATGGATCTCTTGGTTCCGGCATCGAT GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAAC GCACATTGCGCCCCCTGGCATTCCGGGGGGGCATGGCTGTCCGAGCGTCATTTCTGCCCTCAAGCACGGG TTGTGTGTTGGGTGGGGTCCCCCCCGGGGACCTGCCCAAAAGGCASCGGCGACGTCCGTGTGGCCCTCGA GGGTATGGGCCTCTGGCCCTCGGTCGGGAAGGACCTGCGGGGGTTGGGCGGRCCACATTTTCCATTATG GTTGACCGGGAGATCAGGGGGGAGTACCCGGGAGAATTTATGCATATGAATAACGCGAAGGGATCATT ACCGAGTGCGGGCCCTCGCGGCCCAACCTCCGCTTGTCTCTCTACACTGTTGCTTTGGCGGGCGCTGGG GCTCCTGGTCGCGGGGGACACCGCTGGGGGGCGCGGAACGCTTGGGAACGGATGAAAAAAGGGGCTGT CTGAGTACTATGAAAAATTGTCAAAGTTTTCAACAATGGGATCTCTTGGTTC

APPENDIX IV

A549 lung carcinoma cell line (% cell viability)

Concentration of extract	**Crude extracts of *C. macowanii* bulbs and endophytes from the bulbs**									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Positive control
100 µg/ml	115,086	57,88734	108,5932	108,1279	107,3647	59,13153	112,1532	48,21092	94,06547	0,302635
50.0 µg/ml	94,89406	63,82598	103,2871	90,62304	94,13343	54,74812	98,22931	52,17349	86,8565	0,74899
25.0 µg/ml	88,86132	44,23527	116,6621	87,3087	96,33167	58,71593	102,8244	55,57932	88,73847	0,739429
12.5 µg/ml	90,4061	51,25604	99,99365	94,36083	79,92722	61,84731	100,0956	74,09052	88,31503	88,43403
6.25 µg/ml	103,0257	68,70863	90,41917	86,96629	78,2413	58,993	104,066	77,97207	89,81014	87,86501
3.13 µg/ml	99,05267	74,70216	101,6587	79,32342	88,05364	80,23043	99,9466	94,7712	87,99875	97,16548

T1 = *C. macowanii* bulbs, T2 = *Raoultella ornithinolytica*, T3 = *Acinetobacter guillouiae*, T4 = *Pseudomonas moraviensis*, T5 = *Pseudomonas* sp., T6 = *Rahnella aquatilis*, T7 = *Novosphingobium* sp., T8 = *Bacillus cereus*, T9 = *Burkholderia tropica*

A549 lung carcinoma cell line (standard deviation)

Concentration of extract	**Crude extracts of *C. macowanii* bulbs and endophytes from the bulbs**									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Positive control
100 µg/ml	1,180538	5,163422	4,038968	2,193438	5,47376	1,374548	3,056588	8,006591	7,533912	0,022083
50.0 µg/ml	3,984578	2,115964	3,287543	2,199	9,610833	6,886456	2,46835	6,632796	1,686515	0,027747
25.0 µg/ml	9,024073	3,203355	4,998237	5,395149	5,921595	3,785594	5,994616	7,256072	4,956161	0,026984
12.5 µg/ml	10,15919	4,77491	10,99547	4,7217	3,67174	3,376106	2,825909	1,752667	4,359686	0,069462
6.25 µg/ml	10,47709	7,633729	5,910661	1,689041	5,36704	1,818307	1,040265	12,10144	2,506469	0,055246
3.13 µg/ml	3,660277	1,845636	1,118536	4,07869	6,442178	6,353227	2,692141	2,07423	2,568049	0,057887

T1 = *C. macowanii* bulbs, T2 = *Raoultella ornithinolytica*, T3 = *Acinetobacter guillouiae*, T4 = *Pseudomonas moraviensis*, T5 = *Pseudomonas* sp., T6 = *Rahnella aquatilis*, T7 = *Novosphingobium* sp., T8 = *Bacillus cereus*, T9 = *Burkholderia tropica*

A549 lung carcinoma cell line (% cell viability)

Concentration of extract		**Crude extracts of *C. macowanii* leaves and endophytes from the leaves**									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Positive control
100 µg/ml	112,8563	57,88734	108,5932	107,3647	64,62843	53,28176	50,34642	83,22327	94,79212	86,45136	0,302635
50.0 µg/ml	109,9785	63,82598	103,2871	94,13343	57,34105	53,28438	63,72666	77,21406	84,193	74,20553	0,74899
25.0 µg/ml	111,8814	44,23527	116,6621	96,33167	56,651	64,87936	78,08185	86,31805	77,2036	72,19549	0,739429
12.5 µg/ml	109,0349	51,25604	99,99365	79,92722	71,73284	67,86175	67,1926	67,63434	71,1578	47,42938	88,43403
6.25 µg/ml	103,376	68,70863	90,41917	78,2413	82,92791	57,78802	66,09741	71,75375	74,01211	63,55676	87,86501
3.13 µg/ml	101,1882	74,70216	101,6587	88,05364	87,43678	69,85088	79,6972	77,01279	78,10538	72,80451	97,16548

T1= *C. macowanii* leaves, T2= *Raoultella ornithinolytica*, T3= *Acinetobacter guillouiae*, T4= *Pseudomonas* sp., T5= *Pseudomonas palleroniana*, T6= *Pseudomonas putida*, T7= *Bacillus safensis*, T8= *Enterobacter asburiae*, T9= *Pseudomonas cichorii*, T10= *Arthrobacter pascens*

A549 lung carcinoma cell line (standard deviation)

Concentration of extract		**Crude extracts of *C. macowanii* leaves and endophytes from the leaves**									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Positive control
100 µg/ml	4,038968	5,47376	2,705232	5,163422	3,730404	1,23138	10,39666	2,602181	5,263622	4,900042	0,022083
50.0 µg/ml	3,287543	9,610833	3,640154	2,115964	1,819798	9,56673	3,149637	1,894776	9,412515	4,9258	0,027747
25.0 µg/ml	4,998237	5,921595	3,33918	3,203355	4,410856	10,10666	2,342137	3,360986	4,844191	3,094094	0,026984
12.5 µg/ml	10,99547	3,67174	4,004552	4,77491	2,181341	2,936427	3,418552	9,248209	4,262309	8,173009	0,069462
6.25 µg/ml	5,910661	5,36704	5,14695	7,633729	2,458519	3,254738	3,823012	9,388704	3,14487	5,170388	0,055246
3.13 µg/ml	1,118536	6,442178	0,609439	1,845636	0,444865	2,185748	3,252048	7,62225	1,71452	4,125061	0,057887

T1= *C. macowanii* leaves, T2= *Raoultella ornithinolytica*, T3= *Acinetobacter guillouiae*, T4= *Pseudomonas* sp., T5= *Pseudomonas palleroniana*, T6= *Pseudomonas putida*, T7= *Bacillus safensis*, T8= *Enterobacter asburiae*, T9= *Pseudomonas cichorii*, T10= *Arthrobacter pascens*

A549 lung carcinoma cell line (% cell viability)

Concentration of extract	**Crude fungal endophytic extracts from *C. macowanii***					
	T1	T2	T3	T4	T5	Positive control
100 µg/ml	93,67339	87,13357	111,8108	107,7175	108,1331	0,302635
50.0 µg/ml	86,46704	86,37817	113,5124	98,21624	97,8869	0,74899
25.0 µg/ml	84,39427	86,73104	110,9038	94,01581	92,41352	0,739429
12.5 µg/ml	75,81565	88,0275	109,4061	98,04112	83,94208	88,43403
6.25 µg/ml	77,05723	88,55289	105,5219	95,18419	100,3361	87,86501
3.13 µg/ml	81,9817	87,51258	107,2418	96,16699	103,3446	97,16548

T1= *Filobasidium magnum*, T2= *Alternaria tenuissima*, T3= *Penicillium chrysogenum*, T4= *Alternaria alternate*, T5= *Penicillium* sp.

A549 lung carcinoma cell line (standard deviation)

Concentration of extract	**Crude fungal endophytic extracts from *C. macowanii***					
	T1	T2	T3	T4	T5	Positive control
100 µg/ml	4,533912	4,105542	4,493541	4,958092	4,948652	0,022083
50.0 µg/ml	1,686515	1,383407	4,019128	8,775907	7,741074	0,027747
25.0 µg/ml	4,956161	2,455913	2,976606	7,633181	7,701132	0,026984
12.5 µg/ml	4,359686	0,232925	4,681646	3,30168	1,646238	0,069462
6.25 µg/ml	2,506469	4,129927	3,373503	8,163087	3,174947	0,055246
3.13 µg/ml	2,568049	3,314215	4,686923	5,129994	6,790502	0,057887

T1= *Filobasidium magnum*, T2= *Alternaria tenuissima*, T3= *Penicillium chrysogenum*, T4= *Alternaria alternate*, T5= *Penicillium* sp.

U87MG Glioblastoma cell line (% cell viability)

Concentration of extract	**Crude extracts of *C. macowanii* bulbs and endophytes from the bulbs**									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Positive control
100 µg/ml	112,8563	99,46788	69,94664	101,8879	107,2744	293,4731	102,3784	103,9715	108,1901	0,302635
50.0 µg/ml	109,9785	94,00657	58,52633	92,86665	91,48381	260,9107	93,26375	96,44057	86,3355	0,74899
25.0 µg/ml	111,8814	116,1508	48,74917	86,01782	89,33011	270,913	96,45458	95,98273	102,8035	0,739429
12.5 µg/ml	109,0349	83,63521	44,42633	80,8555	73,38531	256,4679	89,70386	129,4327	100,0192	88,43403
6.25 µg/ml	103,376	83,3222	42,46404	78,24864	79,2484	251,1373	93,72159	75,47827	96,39385	87,86501
3.13 µg/ml	101,1882	104,8358	47,01898	82,99517	83,61185	226,9515	95,4081	94,36162	97,81874	97,16548

T1 = *C. macowanii* bulbs, T2 = *Raoultella ornithinolytica*, T3 = *Acinetobacter guillouiae*, T4 = *Pseudomonas moraviensis*, T5 = *Pseudomonas* sp., T6 = *Rahnella aquatilis*, T7 = *Novosphingobium* sp., T8 = *Bacillus cereus*, T9 = *Burkholderia tropica*

U87MG Glioblastoma cell line (standard deviation)

Concentration of extract	**Crude extracts of *C. macowanii* bulbs and endophytes from the bulbs**									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Positive control
100 µg/ml	13,93166	2,251039	3,403353	6,075295	1,631831	1,256727	4,342226	0,94956	8,010326	0,008282
50.0 µg/ml	13,36655	6,293273	3,380512	6,378053	10,6529	6,32636	5,900735	1,955246	2,337344	0,013462
25.0 µg/ml	21,60306	4,836819	3,846474	2,442307	7,156028	5,166097	0,423795	1,890376	8,708963	0,014632
12.5 µg/ml	18,43941	9,312538	1,201452	4,712522	5,239892	2,136426	2,129519	10,13776	8,04318	0,020964
6.25 µg/ml	26,8312	4,681092	0,959334	3,022066	6,337827	9,738269	4,885196	3,731252	12,14018	0,048554
3.13 µg/ml	14,43226	7,844996	0,584737	3,349024	3,034154	4,787832	5,812306	5,1088	16,37507	0,03473

T1 = *C. macowanii* bulbs, T2 = *Raoultella ornithinolytica*, T3 = *Acinetobacter guillouiae*, T4 = *Pseudomonas moraviensis*, T5 = *Pseudomonas* sp., T6 = *Rahnella aquatilis*, T7 = *Novosphingobium* sp., T8 = *Bacillus cereus*, T9 = *Burkholderia tropica*

U87MG Glioblastoma cell line (% cell viability)

Concentration of extract		**Crude extracts of *C. macowanii* leaves and endophytes from the leaves**									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Positive control
100 µg/ml	110,666	99,46788	69,94664	107,2744	243,6905	254,0339	96,54335	103,5837	90,18972	86,95685	1,916366
50.0 µg/ml	95,22863	94,00657	58,52633	91,48381	249,8853	254,7159	88,64336	97,15068	90,04022	84,15378	1,326319
25.0 µg/ml	96,85182	116,1508	48,74917	89,33011	250,3758	254,2347	89,45158	87,38665	83,28015	74,18886	0,091567
12.5 µg/ml	93,50611	83,63521	44,42633	73,38531	240,9482	267,6007	109,7458	81,47918	75,72121	63,99503	6,196654
6.25 µg/ml	87,57008	83,3222	42,46404	79,2484	223,9569	230,6656	78,65976	85,46421	76,90317	65,27043	68,17114
3.13 µg/ml	90,34075	104,8358	47,01898	83,61185	222,261	232,5857	80,61724	89,67115	77,18815	76,82842	89,99417

T1= *C. macowanii* leaves, T2= *Raoultella ornithinolytica*, T3= *Acinetobacter guillouiae*, T4= *Pseudomonas* sp., T5= *Pseudomonas palleroniana*, T6= *Pseudomonas putida*, T7= *Bacillus safensis*, T8= *Enterobacter asburiae*, T9= *Pseudomonas cichorii*, T10= *Arthrobacter pascens*

U87MG Glioblastoma cell line (standard deviation)

Concentration of extract		**Crude extracts of *C. macowanii* leaves and endophytes from the leaves**									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Positive control
100 µg/ml	2,743927	1,631831	9,3552	2,251039	6,850321	10,20989	8,781156	4,277194	1,578144	5,056539	0,008282
50.0 µg/ml	3,102803	10,6529	7,148905	6,293273	7,920129	10,02885	4,125119	3,366496	2,856967	3,135568	0,013462
25.0 µg/ml	3,364212	7,156028	5,080765	4,836819	9,34145	3,169183	2,431131	5,667062	5,985671	3,601491	0,014632
12.5 µg/ml	1,546658	5,239892	10,09178	9,312538	4,71831	6,789377	3,778249	5,317978	0,573489	4,279833	0,020964
6.25 µg/ml	1,332327	6,337827	6,043548	4,681092	1,658016	0,196382	3,125214	5,022452	7,114955	7,525556	0,048554
3.13 µg/ml	0,710199	3,034154	6,628723	7,844996	0,907732	2,170993	3,571124	9,717471	6,166792	3,229325	0,03473

T1= *C. macowanii* leaves, T2= *Raoultella ornithinolytica*, T3= *Acinetobacter guillouiae*, T4= *Pseudomonas* sp., T5= *Pseudomonas palleroniana*, T6= *Pseudomonas putida*, T7= *Bacillus safensis*, T8= *Enterobacter asburiae*, T9= *Pseudomonas cichorii*, T10= *Arthrobacter pascens*

U87MG Glioblastoma cell line (% cell viability)

Concentration of extract	**Crude fungal endophytic extracts from *C. macowanii***					
	T1	T2	T3	T4	T5	Positive control
100 µg/ml	97,84677	94,25884	96,36115	101,8879	102,3784	1,916366
50.0 µg/ml	91,2035	84,75644	89,97949	92,86665	93,26375	1,326319
25.0 µg/ml	84,42941	81,00032	84,06501	86,01782	96,45458	0,091567
12.5 µg/ml	77,9403	83,88748	62,50473	80,8555	89,70386	6,196654
6.25 µg/ml	84,84987	82,83633	81,96738	78,24864	93,72159	68,17114
3.13 µg/ml	81,11245	78,65509	83,12598	82,99517	95,4081	89,99417

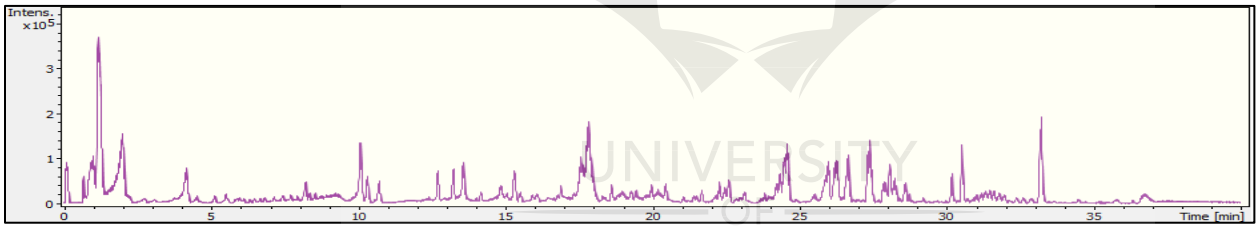
T1= *Filobasidium magnum*, T2= *Alternaria tenuissima*, T3= *Penicillium chrysogenum*, T4= *Alternaria alternate*, T5= *Penicillium* sp.

U87MG Glioblastoma cell line (standard deviation)

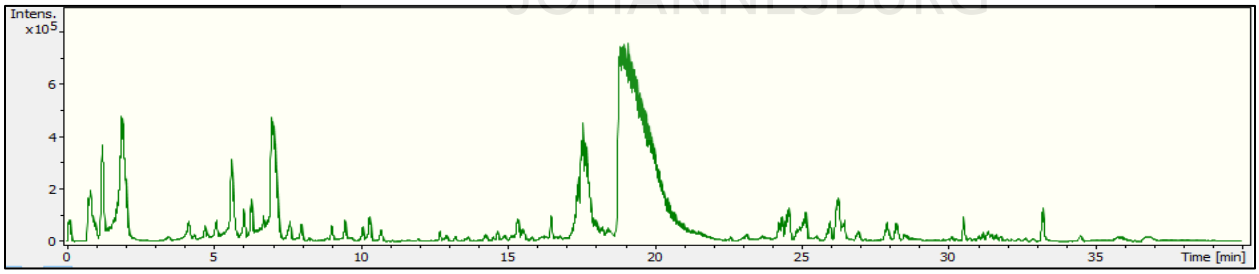
Concentration of extract	**Crude fungal endophytic extracts from *C. macowanii***					
	T1	T2	T3	T4	T5	Positive control
100 µg/ml	4,545953	5,056539	5,931657	0,361039	0,700798	0,008282
50.0 µg/ml	5,072247	3,135568	5,366548	1,369439	1,212065	0,013462
25.0 µg/ml	3,200363	3,601491	3,603064	0,28364	0,232047	0,014632
12.5 µg/ml	5,464196	4,279833	6,439409	0,837471	1,081475	0,020964
6.25 µg/ml	5,083195	7,525556	6,831197	1,72701	1,25849	0,048554
3.13 µg/ml	7,514586	3,229325	4,432256	3,030137	2,210251	0,03473

T1= *Filobasidium magnum*, T2= *Alternaria tenuissima*, T3= *Penicillium chrysogenum*, T4= *Alternaria alternate*, T5= *Penicillium* sp.

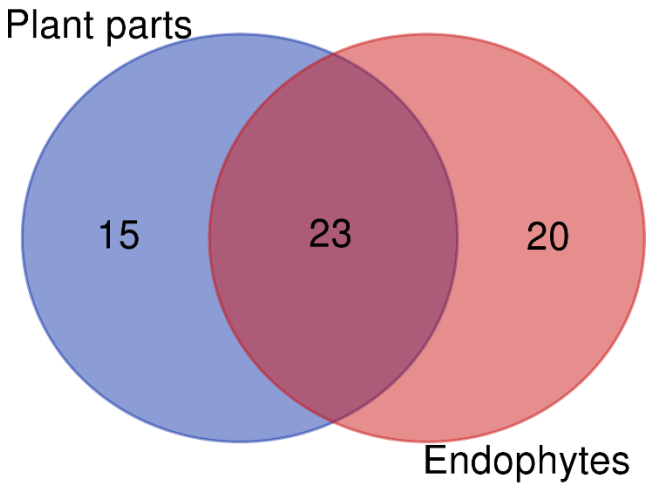
APPENDIX V



Base peak chromatogram of *C. macowanii* bulbs



Base peak chromatogram of *C. macowanii* leaves



Venn diagram comparing secondary metabolites identified from plant parts (bulbs and leaves) and endophytes (bacteria and fungi)