DETERMINING THE ANTIMICROBIAL AND ANTICANCER POTENTIAL OF SEVEN SELECTED SOUTH AFRICAN PLANTS

by

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DECLARATION

I, Yonela Vakele, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

06 November 2023

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ABSTRACT

Different traditional cultures use traditional medicine to prevent, diagnose, improve, or treat illness. It is estimated that 80% of South Africans use traditional medicines as the primary care need and only few of South African medicinal plants have been exploited to their full potential in terms of commercialization. Varieties of phytochemicals (secondary metabolites) that may play a vital role on health improvement are found in plants. Healing and awareness of medicinal plants is an old practice. Thus, in search for treatment of human-related diseases, people used plants in nature as drugs. However, several challenges such as the safety, preparation methods and commercialization were faced. This has led to the search for antimicrobial, antioxidants and anticancer drugs from plant sources. In this study, aqueous and organic (ethanol) plant extracts of leaf and root of *Bulbine frutescens*, *Bulbine natalensis*, *Chlorophytum comosum*, *Elytropappus rhinocerotis, Kniphofia uvaria*, *Tulbaghia violacea* and corm of *Hypoxis hemerocallidea* were screened for antimicrobial, antioxidant and anticancer activities.

For antimicrobial activity, plant extracts were screened against four microorganisms (*Eschericia coli, Bacillus cereus, Aspergillus niger* and *Candida albicans*). It was observed that shoots and roots of *Bulbine frutescens*, *Bulbine natalensis* and shoots of *Chlorophytum comosum* showed visible/clear zones of inhibition against *E. coli*, *B. cereus* and *C. albicans*, however other selected plant species showed no activity against tested microorganisms.

For antioxidant activity, 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical scavenging assay was used for the assessment of antioxidant activity of these selected plants. All plants were extracted with methanol and Trolox was used as the standard. *E. rhinocerotis* and *H. Hemerocallidea* showed the highest antioxidant activity of 115.22% and 114.88% respectively.

For anticancer activity, the cytotoxicity effect of plant extracts (ethanol and aqueous) at final concentration of 100 µg/ml was assessed against human hepatocellular (HepG2), human colon (Caco-2) and human cervical (HeLa) cell lines using the MTT assay. The results showed that the ethanol extract of the plant materials exhibited high cytotoxicity against the three human cell lines, HepG2, Caco-2 and HeLa tested. The 100% ethanol extract of roots and shoots for all plant extracts displayed high activity against HepG2 compared to the 50% ethanol extract which indicated low anticancer activity. Moderate cytotoxicity was observed in the Caco-2 cell line for 50% ethanol extract. Aqueous extracts showed very little activity on all three human cell lines tested. In this study, it was concluded that antimicrobial,

antioxidants and anticancer properties are present in the selected plant organs tested which, however, it warrants further study on the phytochemical properties.

Key words: Apoptosis, antibacterial, antifungal, medicinal plants, secondary metabolites.

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DEDICATION

I dedicate this thesis to:

- My late father Welcome Zandisile Kalashe, who always encouraged me to study. May your beautiful soul continue to rest in peace Mqhwane, tswele-tswele.
- My mother and my cheer leader Mandisa Victoria Vakele, this may come as a surprise to you, but I know you're proud of me. Thank you for being my greatest support.
- My rainbow baby girl (daughter) Milukhanye Khayone Vakele, may this be a reminder to you that "Yonke into inexesha layo", may you look at my life journey and remember that patience and perseverance is the key to success, may you never give-up on anything you put your mind into no matter what.

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
MIC	Minimal Inhibitory Concentration
DPPH	2,2-diphenyl-l-picrylhydrazyl
MTT assay	3, 4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
NCI	National Cancer Institute
%	Percentage
g	Grams
rpm	Revolution per minute
hrs	Hours
°C	Degrees Celsius
ml	Millilitre
mg	Milligram
mM	Macromole
DMSO	Dimethyl Sulfoxide
µg	Micro-gram
cfu	Colony forming unit
mm	Millimetre
µl	Microliter

μm	Micro mole
DMEM	Dulbecco's Modified Eagle Medium
CPUT	Cape Peninsula University of Technology
CO_2	Carbon dioxide
FBS	Foetal bovine serum
Caco	Human Colon
HepG2	Human Hepatocellular
PDA	Potato Dextrose Agar
IC_{50}	Half-maximal cytotoxic concentration

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CHAPTER 1
INTRODUCTION AND BACKGROUND OF THE RESEARCH

1.1 Introduction

Southern Africa has over 30 000 species of higher plants and it is believed that medicinal plants are an important aspect and part of the daily lives of many South African cultural heritages (Van Wyk *et al.*, 2009). For centuries, numerous cultures have been relying on indigenous medicinal plants for their primary health care needs (Gurib-Fakim *et al.*, 2010; King & Veilleux, 1996). Various studies have shown that plant species such as *Bulbine frutescens*, *Bulbine natalensis*, *Chlorophytum comosum*, *Elytropappus rhinocerotis*, *Hypoxis hemerocallidea*, *Kniphofia uvaria* and *Tulbaghia violacea* have been used as traditional medicines for decades (Drewes *et al.*, 2008; Van Wyk *et al.*, 2009; Singh & Reddy, 2012). These South African indigenous plant species come from different families such as Asphodelaceae, Alliaceae, Hypoxidaceae, Agavaceae and Asteraceae. Medicinal plants or their secondary metabolites have been known for directly or indirectly playing a vital role in the health of people to fight diseases (Wink *et al.*, 2005).

Developing countries are currently facing global morbidity and mortality which are mostly reported to be caused by infectious diseases as well as multidrug-resistant related diseases. Different parts of the world have reported antimicrobial properties found on medicinal plants (Ahmad & Beg, 2001). These medicinal plants are used to treat infectious diseases while mitigating many side effects that are often linked with synthetic antimicrobials. Previous studies have shown the chemical profile and composition of medicinal plants that revealed the complexity and variety of compounds contributing to the various uses of plants in treating numerous ailments including life-threatening diseases such as HIV/AIDS, cancer and diabetes (Harris, 2003; Harris, 2004; Hölscher, 2009; Hutchings *et al.*, 1996; Van Jaarsveld, 2012). However, the safety, preparation methods and commercialization of these medicinal plants are among several setbacks faced.

The selected plants; *Bulbine frutescens*, *Bulbine natalensis*, *Chlorophytum comosum*, *Elytropappus rhinocerotis*, *Hypoxis hemerocallidea*, *Kniphofia uvaria* and *Tulbaghia violacea* are commonly known to be medicinal plants. Bulbine plants are administered to treat rashes, itches, wounds, burns, cracked lips and skin; and their roots and stems are also believed to contain anticancer compounds such as anthraquinones (chrysophanol and knipholone) (Singh & Reddy, 2012). *Hypoxis* species have a phytochemical substance called hypoxoside, an inactive compound that is converted to rooperol and has potent

pharmacological properties relevant to cancer, inflammations and human immunodeficiency virus (HIV). Leaves of *T. violacea* are reported to treat oesophagus cancer and tuberculosis (Van Wyk *et al.*, 2009). Pharmacological research of the South African flora also gained momentum as the scientific understanding of medicinal plants remains unknown (Van Wyk, 2002; Jeyaseelan *et al.*, 2012). Nevertheless, traditional medicine is now accepted as an alternative form of health care and the development of innovative drugs is needed. Thus, the study of these selected South African plants for potential biological activity and toxicity by preliminary bioassay screening was of significance.

1.2 Statement of the research problem

Medicinal plants are regarded as a large source of therapeutic phytochemicals that may lead to the development of innovative drugs. The discovery and development of natural antimicrobial and anticancer agents to address the problem of antibiotic resistance are of utmost importance. The purpose of this study was to determine the antimicrobial and anticancer properties of *Bulbine frutescens*, *Bulbine natalensis*, *Chlorophytum comosum*, *Elytropappus rhinocerotis*, *Hypoxis hemerocallidea*, *Kniphofia uvaria* and *Tulbaghia violacea* utilizing preliminary bioassay screening and activities.

1.3 Background of the study

Southern Africa is an important focal point of botanical and cultural diversity but only a few plant species have been fully commercialised as medicinal products (Street & Prinsloo, 2012; Van Wyk, 2019). It is estimated that there are 200 000 indigenous traditional medical practitioners in South Africa and about 3500 species of higher plants are used as traditional medicines (Gericke, 2002). This rich cultural diversity produces different medicinal systems that are practiced either formal or informal in different parts of the country and about 60% of South Africans consult traditional healers (Van Wyk *et al.*, 2009) in preference or addition to western medical doctors, especially in the rural areas (Van Wyk *et al.*, 1997). According to Dold and Cocks (2002), an estimation of R270 million a year is being generated from the indigenous plant-derived medicine trade in South Africa. These medicinal plants are commonly used in traditional healthcare to treat a range of ailments (Watt and Breyer-Brandwijk, 1962).

Several medicinal plants are known to be a large source of antimicrobial molecules. Their extracts are widely used for the treatment of numerous infections as they have a possible antimicrobial activity. These molecules are likely to be screened and marketed for herbal

industries as raw materials (Renisheya *et al.*, 2011). The attention towards medicinal plants use has gained momentum after some authorities and professionals observed more side effects of synthetic drugs compared to their benefits (Bushra *et al.*, 2012). International and local initiatives, however, developed a major interest in traditionally used medicinal plants and actively exploring the botanical resources of Southern Africa. This is intending to decipher the pharmacologically active compounds in indigenous plants (Gurib-Fakim *et al.*, 2010; Rybicki *et al.*, 2012). An entrepreneur from Johannesburg, R. W. Liebenberg, first developed phytosterols from *Hypoxis hemerocallidea* into a patent and commercialized product, Harzol®, for the treatment of benign prostate hypertrophy (Drewes *et al.*, 2008). Active compounds found in *Hypoxis* include rooperol (Drewes *et al.*, 1984) which has patented anti-cancer activity (Drewes & Liebenberg, 1983) and phytosterols, which are still associated with over-the-counter immune-boosting products (Pegel, 1973; Pegel, 1997).
Van Wyk *et al.* (2009) also reported that a large part of the day-to-day medicine is still derived from plants, and large volumes of plants or their extracts are sold in the informal and commercial sectors of the economy. It is with a high possibility that many of these plants contain chemical substances of vital pharmacological effects. The opportunity for bioprospecting of plant compounds for novel pharmaceuticals, however, remains (Street & Prinsloo, 2012). The importance of the region's diverse medicinal plants lies not only in their chemotherapeutic value in traditional healthcare but also in their potential as sources of new chemical entities for drug discovery.

1.4 Hypothesis

The main hypothesis is that bioactivity is demonstrated by *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea.*
Other hypothesis:

(i) Antibacterial activity of *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea* is demonstrated using the MIC method.

(ii) Antifungal activity of *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea* is demonstrated using the MIC method.

(iii) Antioxidant activity of *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea* is demonstrated using the DPPH method.

(iv) Cytotoxicity and anticancer activity of *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea* are demonstrated using the MTT assay.

1.5 Aim

Th is study aimed to determine the bioactivity of *Bulbine frutescens, Bulbine natalensis, Chlorophytum comosum, Elytropappus rhinocerotis, Hypoxis hemerocallidea, Kniphofia uvaria and Tulbaghia violacea* by bioassay screening.

1.6 Objectives

(i) To determine the antibacterial activity of *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea* using minimum inhibitory concentration (MIC) assay.

(ii) To determine the antifungal activity of *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea* using MIC assay.

(iii) To determine the antioxidants activity of *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea* using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) method.

(iv) To determine the cytotoxicity and anticancer activity of *B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinocerotis, H. hemerocallidea*, *K. uvaria* and *T. violacea* using 3, 4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Antimicrobial agents or drugs have been known to play a major role in fighting infectious disease-related illnesses in both humans and animals (Mayer *et al*., 2009). However, the prolonged use and misuse of these drugs against bacterial infections has led to the development of drug resistance amongst these diseases (Mabona *et al*., 2013; Guschin *et al*., 2015; Martin *et al*., 2015). Besides antimicrobial drug resistance, patients are also subjected to a higher risk of synthetic drugs; due to the harmful side effects, these drugs may produce (Valle Jr. *et al*., 2015). As a way forward, several studies have emerged investigating new antimicrobial agents from various sources to combat microbial resistance (Balouiri *et al*., 2016; Jeyaseelan *et al*., 2012, Takaidza *et al*., 2015). A huge range of complex and diverse compounds can be obtained from plants as well as other natural sources, hence studies have focused on investigating plant extracts, essential oils, pure secondary metabolites as well as new potential antimicrobial agents (Mabona *et al*., 2013; Nazzaro *et al*., 2013).

To address the challenge of development of drug resistant, new advances are in need to identify new antimicrobial activity from potential plant-natural products (Bodede *et al*., 2020; Belete, 2019). Plant-originated antimicrobials have the effect of treating infectious diseases while mitigating side effects that are often associated with synthetic drugs (Mahady, 2005; Takaidza *et al*., 2015). Furthermore, these medicinal plants also produce therapeutic properties through antimicrobial activities.

2.2 Plants as medicinal agents

Medicinal plants commonly known as herbs, herbal medicines, pharmacologically active plants or phytomedicine remain the dominant form of medicine in most countries. Over a quarter of the earth’s population depend primarily on raw plant products to meet their daily health care needs (Barrett & Kieffer, 2001). Most of the plant materials collected are usually used fresh to obtain the extract from the whole plant or parts of it, which could be leaves, roots, flowers or fruit. In the case of woody forms, mostly the bark, roots and other parts are used. Carminatives such as ginger, cloves and coriander are also usually added as fresh or dried materials (Rao & Arora, 2004).

The use of medicinal plants in ethno medicine is gaining much interest for treating common diseases and it is now supported with scientific evidence where the study on medicinal

plants shows some extraction procedures and consequent assays performed (Azanwida, 2015). These plants are regarded as a large source of therapeutic phytochemicals that may lead to the development of innovative drugs. Phytochemicals from plants (i.e. phenolic and flavonoids) have been reported to have preventative properties against cancer (Venugopal & Liu, 2012). Furthermore, the high content of phytochemicals has antioxidant activities which are known for the prevention of age-related diseases mostly caused by oxidative stress. Research interest seems to increase on the industrial application as natural antimicrobial compounds in, for example, skin products as a substitute to synthetic products (Mukherjee *et al.*, 2011), food processing and preservation (Tajkarimi *et al.*, 2010).

Natural antimicrobials can be obtained from different sources including plants, animals, bacteria, algae and fungi. Several studies related to plant antimicrobials have demonstrated the efficacy of plant-derived compounds (Hayek *et al.*, 2013; Gyawali and Ibrahim, 2012; Tajkarimi *et al.*, 2010).

2.3 Active components of plants

The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in plants. These compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesised and deposited in specific parts or all parts of the plant (Joseph & Raj, 2010). They are stored in various plant parts that are not utilized by the plant but are essential for humans to get therapeutic effects like preventing chronic diseases such as cancer, diabetes and coronary heart disease (Saxena *et al.*, 2013). Generally, leaves are the favourable storage site for desired compounds. Fruits also contain a substantial amount of active ingredients and thus are often consumed as juice via oral administration to obtain the desired compounds. Other parts of plants that can be extracted for therapeutic compounds are roots, aerial parts, flowers, seeds, stem barks, etc. (Chan *et al.*, 2012). Plant secondary metabolites are used as the basis to produce valuable synthetic compounds in pharmaceuticals, cosmetics, and more recently nutraceuticals (Van Wyke *et al.*, 2015; Wink *et al.*, 2012). Various extraction methods and phytochemical screening methods are developed to identify the complete history of the plant constituents' especially preliminary identification of the phylogroups (Das & Gezici, 2018). The biological significance and potential health effects of these metabolites serve as antioxidant, anticancer, anti-ageing, anti-atherosclerotic, antimicrobial and anti-inflammatory activities which makes them a potential source of new drugs, antibiotics, insecticides and herbicides (Crozier *et al.*, 2006).

2.4 Extraction methods of plant material

The processing of bioactive constituents from plants starts with the pre-extraction and extraction procedures. Extraction is the separation of medicinally active plant portions using selective solvents through standard procedures (Handa *et al.*, 2008). The purpose of this is to separate the soluble plant metabolites from insoluble cellular marc (residue). The initial crude extracts using these methods contain a complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing.

1.1 Selected South African medicinal plants

2.5.1 *Bulbine frutescens* (Asphodelaceae)

Several species of the genus, *Bulbine*, are used as traditional medicines. *B. frutescens* is a small shrub with branched, woody stems, with finger-shaped succulent leaves that vary from green to yellowish or greyish green (Van Wyk *et al.*, 2009) as shown in Figure 2.1. It occurs widely in Northern Cape, Western and Eastern Cape. It is propagated in spring by seeds, cuttings or by division of clumps (Harris, 2003). *Bulbine frutescens'* leaves are used for the treatment of wounds, burns, rashes, itches, ringworms, cracked lips and herpes. B. frutescens has been commonly known to be used in the treatment of diarrhoea (Coopoosamy, 2011) as well as aesthetic purposes (Abegaz *et al.*, 2002). This species also produces a variety of polyketide derivatives, including anthraquinone (Van Wyk *et al.*, 1995) which comprises antimicrobial, antioxidant and anticancer activity.

Figure 2.1: *Bulbine frutescens.*

2.5.2 *Bulbine natalensis* (Asphodelaceae)

This species is mostly known as *B. latifolia* and has rosette fleshy, thornless, yellowish-green leaves as shown in Figure 2.2. It is widely distributed in the eastern and northern parts of South Africa (Van Wyk *et al*., 2009). *Bulbine natalensis* is easily propagated from seeds during warmer seasons. It is used medicinally to quell vomiting, diarrhoea, convulsions, venereal diseases, diabetes, rheumatism, urinary complaints and blood disorders (Jaarsveld, 2005; Van Wyk *et al*., 1997, Yakubu & Afolayan, 2010). *Bulbine natalensis* has been found to raise testosterone levels in men (Roberts, 2012). Some anthraquinones (Bae *et al*., 2016) and tetracyclic triterpenes (Mbambo *et al*., 2012) have also been found in this plant.

Figure 2.2: *Bulbine natalensis.*

2.5.3 *Chlorophytum comosum* (Agavaceae)

Cholorophytum comosum, one of 38 species of *Chlorophytum* species in South Africa (Archer, 2003), is an evergreen perennial that grows up to 1 m with star-shaped white flowers as shown in Figure 2.3. It is widely distributed in Swellendam, Western Cape to the Soutpansberg in the Limpopo province. The plant is used for medicinal purposes (Hutchings *et al*., 1996) and can be propagated vegetatively by division or from the plantlets on the inflorescence (Van Jaarsveld, 2012). Traditionally, roots of *Chlorophytum* species are alleged to possess various pharmacological utilities like immunomodulation, adaptogenic, aphrodisiac and anti-stress properties due to saponins as one of the important phytochemical constituents. They also contain both monodesmosidic saponins (oligosaccharide chain attached at C3 position) and bidesmosidic saponins (an additional sugar moiety at the C26 or C28 positions). Saponins are a group of naturally occurring plant

glycosides and their presence has been reported in many plant families to possess significant anti-cancer properties (Marais & Reilly, 1978).

Figure 2.3: *Chlorophytum comosum.*

2.5.4 *Elytropappus rhinocerotis* (Asteraceae)

Elytropappus rhinocerotis, now called *Dicerothamus rhinocerotis*, is an erect bush that grows up to 1 m with greyish-green leaves and bears small flowers as shown in Figure 2.4. This shrub is widely distributed in Western, Northern and Eastern Cape provinces (Van Wyk *et al.*, 2009). Infusions of its young branches are used as a traditional cape medicine for indigestion, dyspepsia, ulcers and stomach cancer (Watt & Breyer-Brandwijk, 1962; Bergh & Emms, 2006) and can be propagated from smoke-treated seeds in autumn. The twigs of *Elytropappus* r*hinocerotis* are widely used in the Cape region of South Africa to treat foot odour, perspiration and also itchy, chilblained and burning feet. However, no antimicrobial studies have hitherto been published on this popular Cape herbal medicine, which is also used for a wide range of ailments (Hulley *et al.*, 2018).

Figure 2.4: *Elytropappus rhinocerotis.*

2.5.5 *Hypoxis hemerocallidea* (Hypoxidaceae)

Hypoxis hemerocallidea, commonly known as "African Potato" is *a* tuberous perennial with strap-like leaves and produces yellow star-shaped flowers as shown in Figure 2.5. This species is widely distributed in the grassland areas of South Africa and is used as a traditional medicine for a variety of ailments such as prostate hypertrophy, urinary tract infections and testicular tumors, and is also used to build up the immune system of patients suffering from cancer and HIV (Hölscher, 2009). Corm division is a more rapid and successful method of propagating this plant. This plant is one of the 50 most important medicinal plants in the African Herbal Pharmacopoeia (Brendler *et al.*, 2010). It is believed to have antibacterial activity (Katerere and Eloff, 2008) reported treating prostate cancer (Gillmer and Symmonds, 1999).

Figure 2.5: *Hypoxis hemerocallidea.*

2.5.6 *Kniphofia uvaria* (Asphodelaceae)

Kniphofia uvaria is of the Asphodelaceae family and is best known for its tubular red or orange florescence as shown in Figure 2.6. Most *Kniphofia* species are evergreen while few of them are deciduous and grow again in the early summer. Previous phytochemistry studies have shown that *Kniphofia sp*. contains anthraquinones, flavonoids and alkaloids, which have bioactivities including anticancer or antimalarial activities (Wube *et al*., 2005, Habtemariam, 2010). *Kniphofia uvaria* can be propagated by seeds or division (Stern, 2002) and has previously been proved to have antioxidant activity on their roots.

Figure 2.6: *Kniphofia uvaria.*

2.5.7 *Tulbaghia violacea (*Alliaceae)

Tulbaghia violacea is commonly known as the “Wild Garlic” and is a bulbous plant that grows up to 0.5 m with hairless, long, strap-shaped, fleshy leaves. It bears mauve or pale-purple flowers as shown in Figure 2.7. *Tulbaghia violacea* is used as a traditional medicine for fever and colds, asthma, tuberculosis and the leaves are used to treat cancer of the oesophagus. This plant may be propagated either by seeds in spring or by dividing larger clumps (Harris, 2004, Van Wyk, 1997). Most people use this plant locally to treat multiple infections (Thamburan *et al*., 2006). It also treats type-1 diabetes, fever and colds, paralysis, hypertension, asthma, tuberculosis, oesophagus cancer, inflammation and other ailments (Lyantagaye, 2011). Many studies have also indicated the plant to have antifungal and antibacterial properties (Nteso & Pretorius, 2006).

Figure 2.7: *Tulbaghia violacea.*

1.2 Biological activities of plants

Traditional pharmacopoeias have made researchers be more interested not only in determining the scientific rationale for the plant's usage but also in the discovery of novel compounds of medicinal value and their cytotoxicity activities. The use of traditional plant medicines for so many years, most people consider it safe, but it must not be taken for granted that these treatments are void of potentially toxic, mutagenic and/or carcinogenic properties and it is recommended that pharmacological studies should always be accompanied by toxicology screening (Taylor *et al*., 2001 and Cos *et al*., 2006).

2.6.1 Antimicrobial (antibacterial and antifungal) activity of plants

Poor sanitation and low level of hygiene expose people to pathogens, which make them vulnerable to microbial infections and diseases. Local and indigenous plants are often used to treat such infections (Taylor *et al*., 2001), hence being screened by laboratories to rationalize their use in traditional medicines (Aremu *et al*., 2012; Shinwari *et al*., 2013; Tetyana *et al*., 2002). Nearly all the diseases affecting millions of people are still caused by microorganisms, regardless of the understanding of the life cycle and control of many pathogens. It is well known that South Africa has a great number of endemic aromatic plants such as those of the Rutaceae, Lamiaceae, Asteraceae families and many more (Lawrence, 2006). However, most studies have focused on screening a specific genus or species where the antimicrobial activity is reported together with the chemical composition of the distilled oil. For instance, Aremu & Van Staden (2013) reported that various solvent extracts of

Tulbaghia species have been tested against a range of microorganisms affecting both humans and plants.

Developing countries are currently facing global morbidity and mortality, accounting for about 50% of all deaths in tropical countries, which are mostly caused by infectious diseases as well as antibiotic-resistant related diseases (WHO, 2003). The discovery and development of natural antimicrobial agents has become an utmost importance to address the problem of antibiotic resistance (Edward-Jones, 2013). Different parts of the world have reported antimicrobial properties found on medicinal plants (Ahmad & Beg, 2001; Abu-Shanab *et al.*, 2008).

Medicinal plants produce therapeutic properties that have vital properties such as antimicrobial activity. There have also been some medical literature documentations concerning the importance of traditional medicinal plants as alternatives to synthetic antibacterial and antifungal medications. Most of these publications come from those countries that are treating various diseases for practical and economic reasons using herbal medicine (Panda, 2014; Raut & Karuppayil, 2014; Elfahmi *et al.*, 2014; Tan & Chan, 2014). Identification of new natural products with antimicrobial activities as well as expansion of antibiotic chemical diversity may lead to chemicals for new drug development (Nascimento *et al.*, 2000). Essential oils and plant extracts based on ethnomedicinal uses are potential sources of new antimicrobial compounds against microbial strains. The combined use of plant extracts or essential oils and antibiotics are useful in decreasing drug-resistant problems (Mahboubi & Ghazian Bidgoli, 2010).

2.6.2 Anticancer activity of plants

Cancer is a life-threatening disease that severely affects the human population (Greenwell & Rahman, 2015) and millions of people are diagnosed with it (Ma & Yu, 2007). It is characterized by the uncontrollable or unstopped tumours of malignant cells in the human body that has the potential to be metastatic (Ochwang' *et al.*, 2014). Currently, this disease is being treated with chemotherapy, radiotherapy and chemically derived drugs which are believed to put a lot of strain and health damage on a patient. Hence, the focus is on alternative treatments against cancer (Cancer Research UK, 2018). The use of natural products as anticancer agents was recognized by the U.S. National Cancer Institute (NCI) in the 1950s (Cragg & Newman, 2005). Some medicinal plants have been revealed to have both chemo preventive and/or therapeutic effects on breast cancer (Mantle *et al.*, 2000) and skin cancer, and most clinically useful anti-cancer agents are sourced from plant-derived compounds (Cragg *et al.*, 2005) as plants have a history in the treatment of cancer (Reddy,

et al., 2003). Researchers are investigating nanomaterial-based drugs from terrestrial plants extracts for the treatment of diseases, cancer included (Sivaraj *et al.*, 2014) and certain plants have a history in the treatment of cancer (Reddy, *et al.*, 2003).

Secondary metabolites that are derived from plants are being investigated for the development of new clinical drugs to treat this disease (Greenwell & Rahman, 2015) and their ability to inhibit growth or initiate apoptosis cancerous cells. Furthermore, these compounds have properties that inhibit the proliferation of cancer cells and induce apoptotic cell death. Some plants have been reported to be sources of natural antioxidants that can protect against oxidative stress and play a vital role in the chemoprevention of cancer. The medicinal properties of traditional plants are mainly attributed to the presence of flavonoids, but may also be influenced by other organic and inorganic compounds such as coumarins, phenolic acids and antioxidant micronutrients, e.g. Cu, Mn, Zn (Repetto & Llesuy, 2002).

1.3 Test microorganisms

2.7.1 Escherichia coli

Escherichia coli is a common commensal organism causing conditions such as gastroenteritis, cystitis, meningitis, peritonitis, and septicemia in people and animals. Some species of Escherichia are part of the normal microbiota in humans and animals and occur in the environment. They are excellent indicators of antimicrobial resistance (Aarestrup *et al.*, 2008); a reliable indicator of resistance in salmonella (Erb *et al.*, 2007). The cumulative misuse of antimicrobial agents contributes to *E. coli* developing resistance and has led to the search for new antimicrobials such as medicinal plants extracts (Akram *et al.*, 2007) as they have previously proved to have antimicrobial activity against enteropathogenic bacteria (Fullerton *et al.*, 2011).

2.7.2 Bacillus cereus

Bacillus cereus is a Gram-positive, aerobic-to-facultative, spore-forming bacterium that is commonly found in foods and natural environments. It causes foodborne diseases through the production of diarrheal and emetic toxins (Ehling-Schulz *et al.*, 2004; Granum and Lund, 1997). *Bacillus cereus* produces spores that survive for long periods in foods and on food-contact surfaces because they are more resistant than vegetative cells to heat treatment and chemical sanitiser (Kreske *et al.*, 2006b; Ryu and Beuchat, 2005).

2.7.3 Aspergillus niger

Aspergillus spp are opportunistic molds, known to cause allergic and invasive disorders. The genus consists of approximately 180 species, 33 of which have been linked with human diseases (Segal *et al.*, 1998; Perfect *et al.*, 2001). Bellini *et al.* (2003) and Anupama *et al.* (2007) explained *Aspergillus niger* as the third most common species linked with invasive pulmonary aspergillosis. *Aspergillus niger* plays a major role in the production of otomycosis and mycotoxin (Baker, 2006; Schuster, *et al.*, 2002) and often causes deterioration of stored food material, as well as growing upon a wide range of organic substrates (Barrios *et al.*, 1997; Mishra & Dubey, 1994; Paster *et al.*, 1990).

2.9.4 Candida albicans

Candida albicans is a yeast-like fungus that is commensals in healthy humans but can cause systemic infections in immunocompromised individuals (Pfaller *et al.*, 2007). Shao *et al.* (2007) described *C. albicans* as a normal microbiota mostly found in the mucosal cavity, vagina and gastrointestinal tract of individuals. High rates of fungal infections have led to the increase of antimicrobial resistance (Arendrup *et al.*, 2005; Espinel - Ingruff *et al.*, 2009), including *C. albicans,* and that has led to the search for new antifungal agents (White *et al.*, 1998; Sardi *et al.*, 2011). Several factors and activities that contribute to the pathogenic potential of this fungus include the secretion of hydrolases, the yeast-to-hypha transition, contact sensing and thigmotropism, biofilm formation, phenotypic switching (Mayer *et al.*, 2013). Herbal extracts from medicinal plants have been used to treat infectious diseases such as candidiasis in developing nations (Geyid *et al.*, 2005). Other studies carried out have shown that herbal extracts are generally effective against *C. albicans* (Pramila *et al.*, 2012; Hussein *et al.*, 2014).

CHAPTER 3
MATERIALS AND METHODS

3.1 Experimental design

This study was conducted within the Department of Biotechnology Laboratory, Cape Peninsula University of Technology (CPUT), District Six Campus, South Africa. The experimental procedure includes preparation of plant extracts/fractions, media preparation and screening of extracts for antibacterial and antifungal activities against selected microbial cultures.

3.2 Collection of plants material and storage

Plant materials were obtained from Shadowlands Wholesale Nursery at Kuilsriver, Cape Town, South Africa. The plant material used in this study as shown in Table 3.1 were *Bulbine frutescens*, *Bulbine natalensis*, *Chlorophytum comosum*, *Elytropappus rhinocerotis*, *Hypoxis hemerocallidea*, *Kniphofia uvaria* and *Tulbaghia violaceae.* Plants specimens were transplanted into potting soil immediately after collection and kept in an environmentally controlled greenhouse for steady and healthy growth for twelve weeks.

Table 3.1: Plants used on the assay

Plant species	Family	Growth form	Plant Parts used
B. frutescens	Asphodelaceae	Succulent	Leaves, roots
B. natalensis	Asphodelaceae	Succulent	Leaves, roots
C. comosum	Agavaceae	Groundcover	Leaves, roots
E. rhinocerotis	Asteraceae	Shrub	Leaves
H. hemerocallidea	Hypoxidaceae	Bulb	Corm
K. uvaria	Asphodelaceae	Perennial	Leaves, roots
T. violacea	Alliaceae	Bulb	Leaves, roots

3.3 Preparation of plant material

After 12 weeks of storage, different organs (shoots, roots, and corms/or tubers) were removed individually from all selected plants. All those individually separated plant organs were washed and rinsed thoroughly under the tap to remove all dirt. They were left in a rack over the sink to drip-dry. The dried plant material as shown in Figures 3.8 and 3.9 were placed on a table and covered with a newspaper exposed to sunlight before air-drying. Once

dried, plant organs were blended into powder using a 10.2 grinding mill electric blending machine (Lab equip Ltd, USA). Powdered plant material was then properly sealed in separate transparent plastic bags (Figure 3.10) and stored at room temperature until further use. The drying and grinding of plant material were performed within the facilities of the Department of Horticultural Sciences, CPUT, Bellville campus, South Africa.

Figure 3.1: Washed shoots and roots of *T. violacea.*

Figure 3.2: Dried corm of *H. hemerocallidea***.**

Figure 3.3: Powdered shoots of *E. rhinocerotis*.

3.4 Plant extraction using various solvent system

Three types of solvent namely: distilled water, 50% and 100% ethanol were prepared for the extraction of plant material as shown in Table 3.2. The aqueous extracts were prepared by suspending 25 g of powdered plant material in 200 ml of sterile distilled water in a flask and shaken at 160 rpm for 24 - 48 hrs at 37°C in a shaking incubator (Merck, South Africa). The extracts were then centrifuged at the speed of 4000 rpm for 20 minutes. Filtrates were freeze-dried for 24 - 48 hrs using a freeze-dryer (Snijders Scientific, Netherlands). The organic extracts were prepared by suspending 25 g of powdered plant material in 100 ml of ethanol in a flask. For the 50% ethanol extracts, fractions were shaken at 160 rpm for 24 - 48 hrs, centrifuged and then rotatory evaporated (Buchi Rotavapor R-200, United States) to remove traces of ethanol. The extracts were freeze-dried for 24 - 48 hrs. The 100% ethanol extracts were prepared as previously discussed with fractions open to allow the ethanol to evaporate completely. Extracts were centrifuged at the speed of 4000 rpm for 20 minutes and then rotatory evaporated at 50°C hot bath (Figure 3.4). The rotatory evaporator was set at a moderate speed (200 rpm) and the heating bath (B-490) was set to 50°C. The extracts were rotatory evaporated when the refrigerated bath calculator (BL-30) reached 4°C which enables the machine to be able to pull ethanol from the extract. For all extracts, 1 mg of the freeze-dried aqueous extracts and rotatory evaporated ethanol (50 and 100%) extracts was dissolved in 1 ml of 10 mM plant tissue culture grade Dimethyl Sulfoxide (DMSO; Sigma-

Aldrich, South Africa), using a 2 ml Eppendorf tube, to yield a concentration of 1000 µg/ml. All extracts were mixed properly using a vortex mixer (LabFriend, South Africa) to have a homogenous mixture prior to assay. The extracts were stored at -20°C until when required for use.

Table 3.2: Types of solvent systems used for plant extracts.

Extract	Degree of polarity	Solvent
Aqueous	1	Sterile-distilled water
Organic	0.327	50% sterile-distilled water + 50% ethanol
Organic	0.654	100% ethanol

B

C
100%
100%

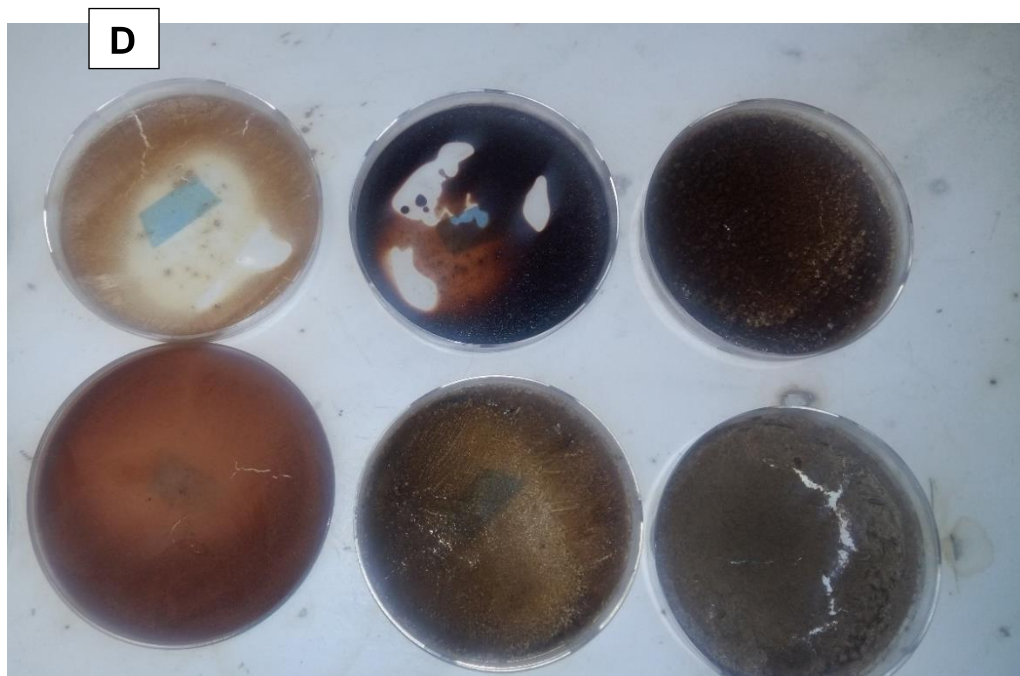

Figure 3.4: Rotatory evaporaring process of 50% and 100% Ethanol extracts. A= Centrifuged plant extracts, B= Rotatory evaporator used for 50 and 100% ethanol extracts, C= Plant ectract after evaporated, D= Freeze-dried aqueous extracts.

3.5 Antimicrobial screening and minimum inhibitory concentration (MIC)

3.5.1 Preparation of media

The media used in the study are Potato Dextrose Agar, Blood Agar Base, Tryptone Soy Agar, and Nutrient Agar (Table 3.3). All media were prepared and used according to the manufacturer's instructions (Merck & Co, South Africa).

Table 3.3: Media used for sub-culturing of microorganisms

Microorganism	Group/class	Media used	pH
E. coli	Gram-negative Bacteria	Blood agar base	7.2
B. cereus	Gram-positive Bacteria	Blood agar base	7.2
C. albicans	Yeast (Fungi)	Tryptone soy agar	7.3
A. niger	Mould (Fungi)	Potato dextrose agar	5.6

3.5.2.1 Potato Dextrose Agar (PDA)

Potato Dextrose Agar was used to culture *A. niger*. It was prepared by dissolving 30 g of PDA agar powder in 1000 ml of distilled water. A turbid solution obtained was then heated to clear transparent solution with continuous shaking to dissolve the agar completely. pH was adjusted to 5.6 as per the manufacturer's instructions. Then the media was sterilized in an autoclave at 121°C for 15-20 minutes. The sterilized media were then allowed to cool to 50 ^{o}C and later pour into sterile agar plates under sterile laminar flow where it was left for 10 - 20 minutes to solidify.

3.5.2.2 Blood Agar Base

A blood agar base was used for the gram-positive bacteria, *E. coli* and *B. cereus*. The blood agar base was prepared by dissolving 33 g in 1000 ml of water. A turbid solution obtained was then heated to clear transparent solution with continuous shaking to dissolve the agar completely. pH was adjusted to 7.2 as per the manufacturer's instructions. Then the media was sterilized in an autoclave at 121°C for 15-20 minutes. The sterilized media were then allowed to cool to 50°C and later pour into sterile agar plates under sterile laminar flow where it was left for 10-20 minutes to solidify.

3.5.2.3 Tryptone soy agar

Tryptone soy agar was used to culture *C. albicans.* Tryptone soy agar was prepared by dissolving 26.6 g in 700 ml and pH adjusted to 7.3. All agar media were then autoclaved and poured onto the plate to solidify as previously described above.

3.5.2 Growth and culturing of microorganisms for antimicrobial screening

The selected microorganisms, *Escherichia coli* (ATCC51813), *Bacillus cereus* (ATCC11778), *Candida albicans* and *Aspergillus niger* were obtained from the Department of Biotechnology, CPUT, Cape Town campus. All microorganisms were identified by the Gram stain method to determine the morphology (shape, cell type, and negative/positive) of the microorganism. All microbial cultures were pre-cultured in nutrient broth, shaken and incubated overnight (24 hrs) in a rotatory shaker at 37°C (Figure 3.12). Microbial cultures were sub-cultured for optimal growth and viability every two weeks. Inoculum spores were adjusted using a spectrophotometer. The cultures were used when 5 x 10^5 CFU/ml was obtained. This was calculated by this formula: (number of colonies x dilution factor) / volume of the culture plate. These were done in triplicates. Cell counting was done by the means of streaking the microorganism from the old plate into a freshly prepared media under sterile laminar flow.

Figure 3.5: Freshly prepared inoculums *E. coli*, *C. albicans*, *B. cereus* and *A. niger*

3.5.3 Minimum inhibitory concentration assay

For antimicrobial screening of plant extracts, agar well diffusion assay was used with modifications as described by Irshad *et al*. (2012). Sterile blood agar base for bacteria, tryptone soy agar and potato dextrose agar for fungi were used. The medium was prepared by pouring molten agar on Petri dishes and allowing it to solidify. Afterward, 100 µl of *E. coli*, *B. cereus*, *C. albicans* as well as *A. niger* inoculums, approximately 15 x 10^5 CFU/ml, was seeded into warm molten agar and poured on the surface of the solidified agar. This was allowed to solidify, and holes of 5 mm width were made into the agar using sterile Pasteur pipettes. Crude plant extracts were prepared for each plant by dissolving 1 mg of the extract (aqueous, 50% and 100% ethanol) in 1 ml of 10 µm DMSO yielding a concentration of 1000 µg/ml. 100 µl and 200 µl of the DMSO was micro-pipetted into each well and labelled (microorganism name, plant extract, date and inoculum volume). Penicillin-streptomycin (50 µl) was also pipetted into the middle hole. This served as a standard antibiotic for the positive control, while sterilized distilled water was used as a negative control. Plates were then placed in an incubator at 37°C for 24-48 hrs and examined for the zones of inhibition using a ruler to measure the diameters. The selected microorganisms were designated arbitrarily as sensitive or resistant. Sterilized distilled water was used as a negative control. Penicillin-Streptomycin (50 µl) was used as a standard antibiotic (i.e., positive control).

3.6 Antioxidant assay DPPH

3.6.1 Principle

The assay was conducted at the Oxidative Stress Research Centre (CPUT), Bellville campus. The duration of the experiment was 2 days which included plant extraction as well as the screening of samples, using the 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) method. The antioxidant activity of selected plants was measured in terms of radical scavenging ability using the stable radical DPPH. The method was performed as described by Blois (1958) and Kedare & Singh (2011) with slight modification. It is considered as one of the standards, easy and routinely cpracticed colourimetric methods for the evaluation of antioxidant properties of pure compounds. DPPH is a stable radical in solution and appears purple colour absorbing at 515 nm in methanol but changes colour to yellow with a concomitant decrease in absorbance. The colour change is monitored by spectrophotometry. The advantage of this method is that DPPH can react with the whole sample and sufficient time given in the method allows DPPH to react slowly even with weak antioxidants. Additionally, this method may be used in aqueous and nonpolar organic solvents as well as the examination on both hydrophilic and lipophilic antioxidants.

3.6.2 Procedure

For the extraction of phytochemical extracts to perform DPPH assay, all dried and powdered plant material were weighed (90 -110 mg). Samples were all individually dispensed in test tubes filled with 10 ml of methanol and kept overnight at a temperature of 4˚C in a dark room to prevent oxidation. The next day, the mixture was centrifuged at the speed of 4000 rpm till all plant residues were removed before use.

In this study, Trolox was used as the standard sample, as it is a water-soluble man-made (synthetic) vitamin E used to reduce oxidative stress or damage. Trolox (0.0125 g) was diluted with 50 ml of 75% ethanol which was mixed until well dissolved and was made up to six dilutions. Methanolic extract (125 ul) was dispensed into each of the 96-well plates then 150 ul of DPPH solution was added. All samples were done in triplicates. Well plates were then closed and kept in the dark for about 1 hour. Plates were then taken to the plate reader, Multiskan Spectrum and the absorbance measurements were read at 515 nm. To prevent oxidation, all steps were carried out in dark and covered with aluminium foil. The percentage inhibition of the DPPH radical was calculated using the following formula:

$$X = \frac{y - c}{m} \times 100$$

Where x = DPPH inhibition (%), y and m = absorbance of the control sample and c = absorbance of a tested sample at the end of the reaction.

Data obtained from the multiscan spectrum (Thermo Electron Corporation, US) which serves as the microplate reader and takes quick absorbance measurements were analyzed using GraphPad Prism software version 5 (GraphPad Software, California, USA). Data values are expressed as mole Trolox equivalents per litre or per gram of the sample.

3.7 Cytotoxicity test for anticancer activity

3.7.1 Cell line and media

Human colon (Caco-2) (ATCC HTB-37), Human cervical (HeLa) (ATCC CCL-2) and Human Hepatocellular (HepG2) (ATCC HB-8065) cell lines were used in this study. The cells were cultured aseptically in tissue culture flasks containing Dulbecco's Modified Eagle Medium (DMEM) (Merck, South Africa) that had been already supplemented with glucose (4.5 g/L), L-glutamine (1 mM) and sodium pyruvate (0.110 g/L) by the supplier. The media was further supplemented with 10% foetal bovine serum (FBS) (Merck, South Africa) and 1% pen-strep cocktail (100 U/mL penicillin and 100 μg/mL streptomycin) (Sigma- Aldrich, South Africa). 100 µl of each cell was added to 50 and 100 µg/mL of plant extracts in a well plate and incubated for 24 hrs.

3.7.2 MTT Assay (3, 4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)

Principle

MTT assay is a method used to evaluate the cytotoxicity of compounds or to measure cell viability. The mitochondrial activity of cells is measured using spectrophotometric through the reduction of a yellow-coloured MTT salt to purple formazan crystals by the enzyme succinate tetrazolium reductase or through mitochondrial succinate dehydrogenase, both of which are present only in living metabolically active cells. The greater the purple colour of the reaction, the less the extent to which a compound (crude fraction) induces cell death in the specific reaction. Previous studies have shown that dead cells are unable to reduce MTT within 30 min of lysis. Thus, the colour reaction is a measure of cell viability (Bahuguna *et al.*, 2017; Strober, 2001). Most crude fractions are dissolved in DMSO as it is a broadly used super-solvent. No literature reports on the cytotoxicity of the DMSO on cells treated with crude fractions.

3.7.3 Cytotoxicity assay of the plant extract by MTT

The cell culture was performed under an aseptic environment, under the laminar flow hood (Labotech, South Africa). The Ultraviolet light of the laminar flow was switched on 30 minutes before cell culture work and 30 minutes after. This was followed by the swabbing of hands and the laminar flow before, after and during cell culture with 70% ethanol to ensure the environment was sterile and free of contaminating organisms. Cells (Caco-2, HeLa and HepG2) were cultured with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 1% pen-strep cocktail of 100 µg/ml penicillin and 100 µg/ml streptomycin (Sigma- Aldrich, South Africa) and incubated at 37ºC in a humidified incubator (Labotech, South Africa) containing a 5% CO_2 atmosphere. The cells were then seeded at 1 X 10^5 cells/mL density in a 96-well plate. After 24 hrs, they were treated with two different concentrations of each fraction prepared (50 and 100 µg/mL). After treatment, 10 µL of 5 mg/mL MTT (Sigma, USA) was added to each well and the plates were incubated at 37˚C for 3 hrs. The insoluble formazan crystals were solubilized by adding 100 µL of DMSO. The effect of the extracts against these tested cell lines was monitored using the EVOS XL Core imaging system (Invitrogen, South Africa).

3.7.4 Calculation of cytotoxicity

The reduction of MTT was read at 570 nm using POLARStar Omega plate reader. Data were represented as cell viability (100% any indication that all cells are alive, 80%= 20% dead cells, etc.). The formula used is as follows:

$$\text{Cytotoxicity (\%)} = 100 - \left(\frac{\text{Average absorbances of treated cells}}{\text{Average absorbances of control}}\right) \times 100\ \%$$

3.7.5 The half-maximal cytotoxic concentration (IC_{50}) values

IC_{50} is a quantitative measure that indicates how much of a particular inhibitory substance is needed to inhibit a given biological process or biological component by 50%, *in vitro*. The biological component could be an enzyme, cell, cell receptor or microorganism. The IC_{50} of the active compounds was determined on the cell line which showed the highest cytotoxicity, where 50% cell death was observed. The cells were cultured as above and treated with 50 and 100 µg/mL concentrations of the plant extracts, for 24 hrs. Following the MTT assay, the IC_{50} was estimated using GraphPad Prism software version 5 (GraphPad Software, California, USA).

CHAPTER 4

RESULTS

4.1 Antimicrobial Activity of Plants Extracts

The antibacterial and antifungal activities of the extracts of *B. frutescens* (roots and shoots), *B. natalensis* (roots and shoots), *C. comosum* (roots and shoots), *E. rhinocerotis* (shoots), *H. hemerocallidea* (corm/tuber), *K. uvaria* (roots and shoots) *and T. violacea* (roots and shoots) were determined. According to the test results, there were few inhibition zones detected against the four tested microorganisms (*E. coli, B. cereus*, *A. niger* and *C. albicans*), although the positive control showed sensitivity against the selected microbial strains as shown in Figures 4.1- 4.5 and Tables 4.1- 4.2.

Table 4.1: Antimicrobial activity of plant shoots extracts shown as zones of inhibition.

Tested Microbes	**Extract**	*Bulbine frutescens*		*Bulbine natalensis*		*Chlorophytum comosum*		*Elytropappus rhinocerotis*		*Kniphofia uvaria*		*Tulbaghia violacea*		Control (Standard)
		100 µl	**200** µl	**100** µl	**200** µl	**100** µl	**200** µl	**100** µ	**200** µl	**100** µl	**200** µl	**100** µl	**200** µl	**100** µl
	Zone of Inhibition (mm)													
E. coli	WE	5.2 ± 0.25	5.3 ± 0.57	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	50% EE	5.6 ± 057	7.3 ± 0.57	NI	NI	7.6 ± 0.57	8.3 ± 0.57	NI	NI	NI	NI	NI	NI	20 ±0
	100% EE	12.3 ± 0.57	9.6 ± 0.5	NI	NI	10 ± 0	13.6 ± 5.6	NI	NI	NI	NI	NI	NI	20 ± 0
B. cereus	WE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	50% EE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	100% EE	7.6 ± 0.57	7.6 ± 0.57	5.6 ± 0.57	7.9 ± 0.57	7.6 ± 0.57	10.3 ± 0.57	NI	NI	NI	NI	NI	NI	20 ± 0
C. albicans	WE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	50% EE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	100% EE	NI	NI	5.6 ± 057	8.0 ± 0	10.0 ± 0	10.0 ± 0.28	NI	NI	NI	NI	NI	NI	20 ± 0
A. niger	WE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	50% EE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	100% EE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

Values are presented as mean ± SD of triplicates, WE- Water Extract, EE- Ethanol Extract, NI- No Inhibition, mm- diameter of zones of inhibition.

Table 4.2: Antimicrobial activity of plant roots extracts shown as zones of inhibition.

Tested Microbes	Extract	*Bulbine frutescens*		*Bulbine natalensis*		*Chlorophytum comosum*		*Hypoxis hemerocallidea*		*Kniphofia uvaria*		*Tulbaghia violacea*		Control (Standard)
		100 µl	**200** µl	**100** µl	**200** µl	**100** µl	**200** µl	**100** µl	**200** µl	**100** µl	**200** µl	**100** µl	**200** µl	**100** µl
	Zone of Inhibition (mm)													
E. coli	WE	5.03 ± 0.057	5.9 ± 0.11	5.1 ± 0.15	5.2 ± 0.2	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	50% EE	6.6 ± 0.55	7.6 ± 0.57	6.1 ± 0.1	5.9 ± 0.58	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	100% EE	8.6 ± 0.57	9.9 ± 0.05	9.2 ± 1.07	10.5 ± 0.5	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
B. cereus	WE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	50% EE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	100% EE	7.6 ± 0.57	7.9 ± 0.1	6.6 ± 0.57	8 ± 0.1	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
C. albicans	WE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	50% EE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
	100% EE	10.1 ± 0.1	13.9 ± 0.057	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	20 ± 0
A. niger	WE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	50% EE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	100% EE	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

Values are presented as mean ± SD of triplicates, WE- Water Extract, EE- Ethanol Extract, NI- No Inhibition, mm- diameter of zones of inhibition.

Observation of antimicrobial on extracts of all tested plant species indicated that interaction between 50% ethanol, 100% ethanol and aqueous (Water) extracts of selected plants and test microorganisms, only a few plant extracts were significant, as shown in Figures 4.1- 4.5, Tables 4.1 - 4.2, others showed no visible zones of inhibition even after 24 - 48 hours incubation period at a temperature of 37°C. However, the Penicillin-streptomycin used as a positive control was more active against *E. coli*, *B. cereus* and *C. albicans* but not against *A. niger*.

Results of antimicrobial test on shoots of the selected plants showed that *Bulbine frutescens* shoot on 100% ethanol solvent had a greater zone of inhibition of 12.3 mm (100 µl) and 9.6 mm (200 µl). The 50% ethanol extracts showed 5.6 mm (100 µl) and 7.3 mm (200 µl), whereas aqueous extracts had the least antimicrobial activity of 5.2 mm (100 µl) and 5.3 mm (200 µl) against *E. coli*. A 7.6 mm (100 µl) and 7.6 mm (200 µl) inhibition was observed for 100% ethanol shoot extract of *B. frutescens* against *B. cereus.*

For results obtained from the roots of selected plants, *Bulbine frutescens* showed 8.6 (100 µl) and 9.9 (200 µl) zone of inhibition average from the 100% ethanol solvent, 6.6 (100 µl) and 7.6 (200 µl) from 50% ethanol extracts, and 5.0 (100 µl) and 5.9 (200 µl) from aqueous extracts against *E. coli*. An average of 7.6 (100 µl) and 7.9 (200 µl) from the 100% ethanol solvent against *B. cereus*, and 10.1 (100 µl) and 13.9 (200 µl) against *C. albicans* was recorded.
On the other side, *Bulbine natalensis* roots showed an average of 9.2 (100 µl) and 10.5 (200 µl) from 100 % ethanol solvent, 6.1 (100 µl) and 5.9 (200 µl) from 50 % ethanol solvent and 5.1 (100 µl) and 5.2 (200 µl) from aqueous extracts against *E. coli. B. natalensis* also showed an average of 6.6 (100 µl) and 8 (200 µl) from 100 % ethanol solvent against *B. cereus.*

4.2 Antioxidant Activity by DPPH Assay

The DPPH assay was carried out to determine which of the seven plants whose extract had the highest free radical scavenging capacity. The shoots methanolic extracts of *E. rhinocerotis presented* the highest antioxidant activity of 115.22% when compared to the other plant extracts and Trolox (standard control). In addition, the shoot methanolic extracts of *K. uvaria* (17.11%) and *C. comosus* (15.12%) showed high antioxidants in comparison to the *T.violacea* (8.44%), *B. natalensis* (8.14%) and *B. frutescens* (7.87%).

In the root extracts, the roots methanolic extracts of *H. hemerocallidea* showed the highest activity of 114.8%, and *B. natalensis* (33.19 %) when compared to other plants and standard

control. This was followed by the roots methanolic extracts of *B. frutescens* (26.33%), *C. comosus* (10.3 %), *K. uvaria* (9.62%) and *T. violacea* (5.06%).

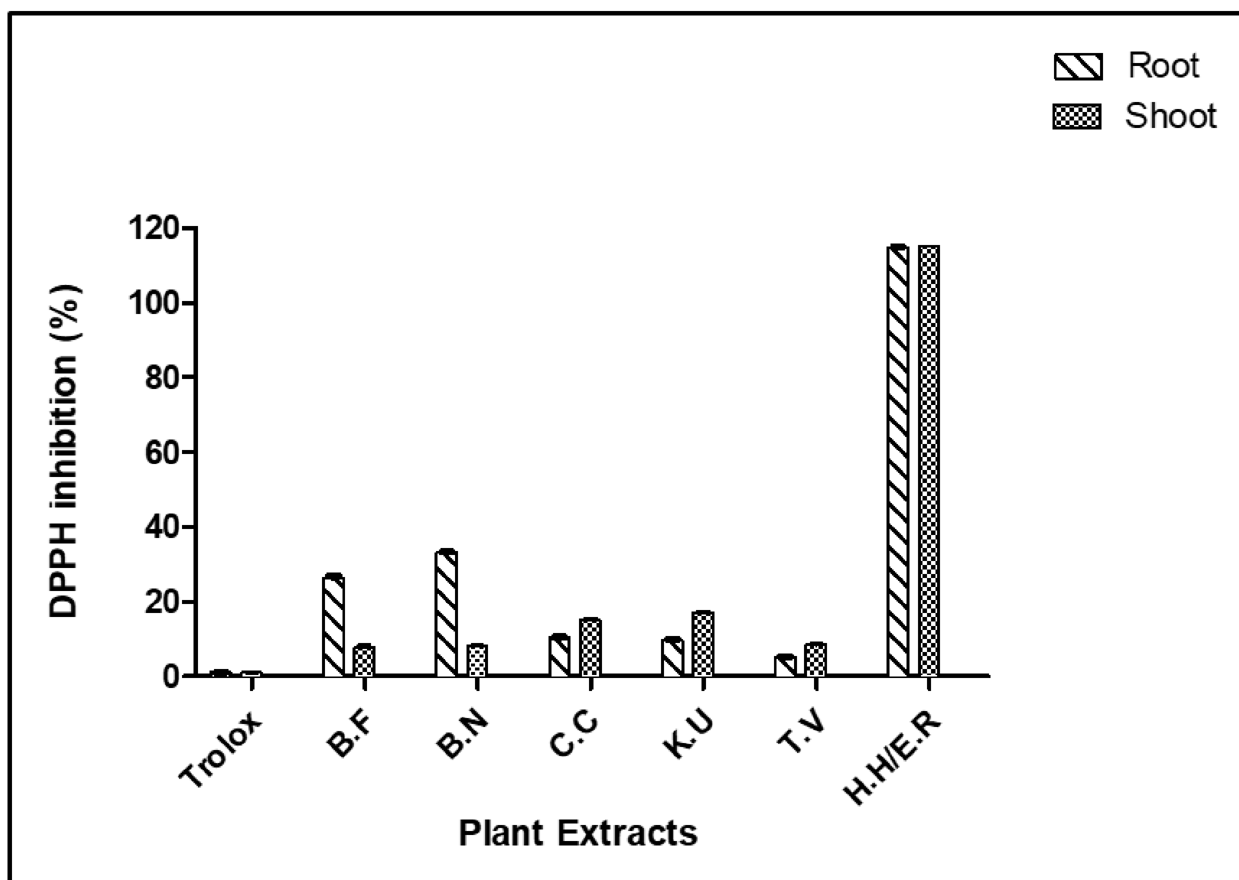

Figure 4.1: Graphical analysis showing the DPPH inhibition effect (%) of roots and shoots in: B.f= *Bulbine frutescens*, B.n= *Bulbine natalensis*, C.c= *Chlorophytum comosum*, K.u= *Kniphofia uvaria*, T.v= *Tulbaghia violacea*, H.h= *Hypoxis hemerocallidea* (corm), *E.r= Elytropappus rhinocerotis* and Control: Trolox. (n = 3).

4.3 Cytotoxicity of roots plant extracts

The cytotoxicity effect of ethanol (50% and 100%) and aqueous (water) extracts of roots of *B. frutescens, B. natalensis, C. comosum, K. uvaria and T. violacea* were determined on human hepatocellular (HepG2), human colon (Caco-2) and human cervical (HeLa) cell lines using MTT assay as shown in Table 4.3. This study showed that some plant extracts exhibited cytotoxicity against the two human cell lines tested, HepG2 and Caco-2.

4.3.1 *B. frutescens*

For water extracts, 8% cytotoxicity against Caco-2 was obtained while HepG2 and HeLa showed no cytotoxicity. For 50% ethanol extracts, 4% and 35% cell death were obtained in HepG2 and HeLa, respectively. Caco-2 showed no cytotoxicity. For 100% ethanol extracts,

66% and 17% cytotoxicity were shown in HepG2 and Caco-2, while HeLa showed no cell death.

4.3.2 *B. natalensis*

For water extracts, only both HepG2 and HeLa showed a 25% cytotoxic effect of *B. natalensis*. For 50% ethanol extracts, only HepG2 showed cytotoxicity (24%) while Caco-2 and HeLa reported no cell death. For the 100% ethanol extracts, 8% cytotoxicity was obtained against Caco-2, 32% against HepG2 and 0% against HeLa.

4.3.3 *C. comosum*

For water extracts, 35% cytotoxicity against Caco-2 was obtained, 13% against HepG2 and 8% against HeLa. For 50% ethanol extracts, 23% cytotoxicity against Caco-2 was obtained, 24% against HepG2 and 5% against HeLa. For 100% ethanol extracts, 86% cytotoxicity was obtained against Caco-2, 88% against HepG2 and 45% against HeLa.

4.3.4 *K. uvaria*

For water extracts, Caco-2 showed 11% cytotoxic effect of K. *uvaria*, 38% against HepG2 and 2% against HeLa. For 50% ethanol extracts, only HepG2 showed 44% cytotoxicity while Caco-2 and HeLa reported no cell death. For 100% ethanol extracts, 34% cytotoxicity was obtained against Caco-2, 75% against HepG2 and 0% against HeLa.

4.3.5 *T. violacea*

For water extracts, Caco-2, HepG2 and HeLa showed no cell death. For 50% ethanol extracts, Caco-2 showed 9% cytotoxic effect, 12% against HepG2 and 0% against HeLa. For 100% ethanol extracts, 75% cytotoxicity was obtained against Caco-2, 88% against HepG2 and 29% against HeLa.

4.4 Cytotoxicity of shoot plant extracts

4.4.1 B. frutescens

For water extracts, only Caco-2 showed cytotoxicity (10%) of *B. frutescens*, while both HepG2 and HeLa reported no cell death. For 50% ethanol extracts, 0% cytotoxicity against Caco-2 was obtained, 27% against HepG2 and 0% against HeLa. For 100% ethanol extracts, 22% cytotoxicity was obtained against Caco-2, 69% against HepG2 and 32% against HeLa.

4.4.2 *B. natalensis*

For water extracts, 10% cytotoxicity against Caco-2 was obtained, 39% against HepG2 and no cell death was reported on HeLa. For 50% ethanol extracts, HepG2 showed 16% cytotoxicity and no cell death was reported on both Caco-2 and HeLa. For 100% ethanol extracts, 45% cytotoxicity was obtained against Caco-2, 71% against HepG2 and 48% against HeLa.

4.4.3 *C. comosum*

For water extracts, 19% cytotoxicity against Caco-2 was obtained, 1% against HepG2 and 0% against HeLa. For 50% ethanol extracts, 23% cytotoxicity against Caco-2 was obtained, 10% against HepG2 and 25% against HeLa. For 100% ethanol extracts, 42% cytotoxicity was obtained against Caco-2, 86% against HepG2 and 0% against HeLa.

4.4.4 *E. rhinocerotis*

For water extracts, HepG2 showed a 17% cytotoxic effect on *E. rhinicerotis*, and no cell death reported on Caco-2 and HeLa. For 50% ethanol extracts, 27% cytotoxicity against Caco-2 was obtained, 0% cytotoxic effect was reported on both HepG2 and HeLa. For 100% ethanol extracts, 67% cytotoxicity was obtained against Caco-2, 94% against HepG2 and 63% against HeLa.

4.4.5 *K. uvaria*

For water extracts, 12% cytotoxicity against Caco-2 was obtained, 1% against HepG2 and 10% against HeLa. For 50% ethanol extracts, HepG2 reported 26% cytotoxicity and 0% against Caco-2 and HeLa. For 100% ethanol extracts, 84% cytotoxicity was obtained against Caco-2, 86% against HepG2 and 75% against HeLa.

4.4.6 *T. violacea*

For water extracts, 34% cytotoxicity against HepG2 was reported and Caco-2 and HeLa showed no cell death. For 50% ethanol extracts, 9% against HepG2 was obtained, 0% against both Caco-2 and HeLa. For 100% ethanol extracts, 74% cytotoxicity was obtained against Caco-2, 84% against HepG2 and 22% against HeLa.

4.5 Cytotoxicity of corm plant extract

4.5.1 *H. hemerocallidea*

For water extracts, 0% cytotoxicity obtained against Caco-2 was obtained, HepG2 and HeLa. For 50% ethanol extracts, 0% cytotoxicity against Caco-2 was obtained, 8% against HepG2 and 0% against HeLa. For 100% ethanol extracts, 0% cytotoxicity was obtained against Caco-2, 15% against HepG2 and 0% against HeLa.

Table 4.3: Cytotoxicity of the phytochemical extracts (roots and shoots) of the indigenous plants on Caco-2, HeLa and HepG2 cells.

Plant name (Botanical)	Extract	Cytotoxicity (%)					
		Caco-2		HeLa		HepG2	
		Root	Shoots	Root	Shoots	Roots	Shoots
Bulbine frutescens	WE	10.00	13.25	0.00	0.00	0.00	0.00
	50% EE	0.00	0.00	35.32	0.00	5.72	29.37
	100% EE	20.10	22.58	0.00	32.39	67.83	71.56
Bulbine natalensis	WE	0.00	13.25	23.16	0.00	23.16	40.31
	50% EE	0.00	0.00	0.00	0.00	26.34	17.97
	100% EE	8.52	44.50	0.00	50.24	33.73	72.87
Chlorophytum comosum	WE	37.31	20.54	10.10	0.00	13.5	0.00
	50% EE	23.20	24.58	5.85	26.90	22.41	13.78
	100% EE	**88.20**	43.43	45.23	0.00	**90.47**	22.31
Kniphofia uvaria	WE	15.60	17.66	5.50	15.89	40.50	4.65
	50% EE	0.00	0.00	0.00	0.00	46.61	24.85
	100% EE	33.61	**85.43**	0.00	75.30	77.23	**84.43**
Tulbaghia violacea	WE	0.00	0.00	0.00	0.00	0.00	32.34
	50% EE	10.10	0.00	0.00	0.00	13.54	11.12
	100% EE	77.23	72.89	34.20	21.50	**90.20**	**85.43**

WE: water extracts; EE: Ethanol extract; Extracts that caused more than 80% cytotoxicity on the cell lines assayed are in bold. Cytotoxicity (%) was presented as the mean of three replicates.

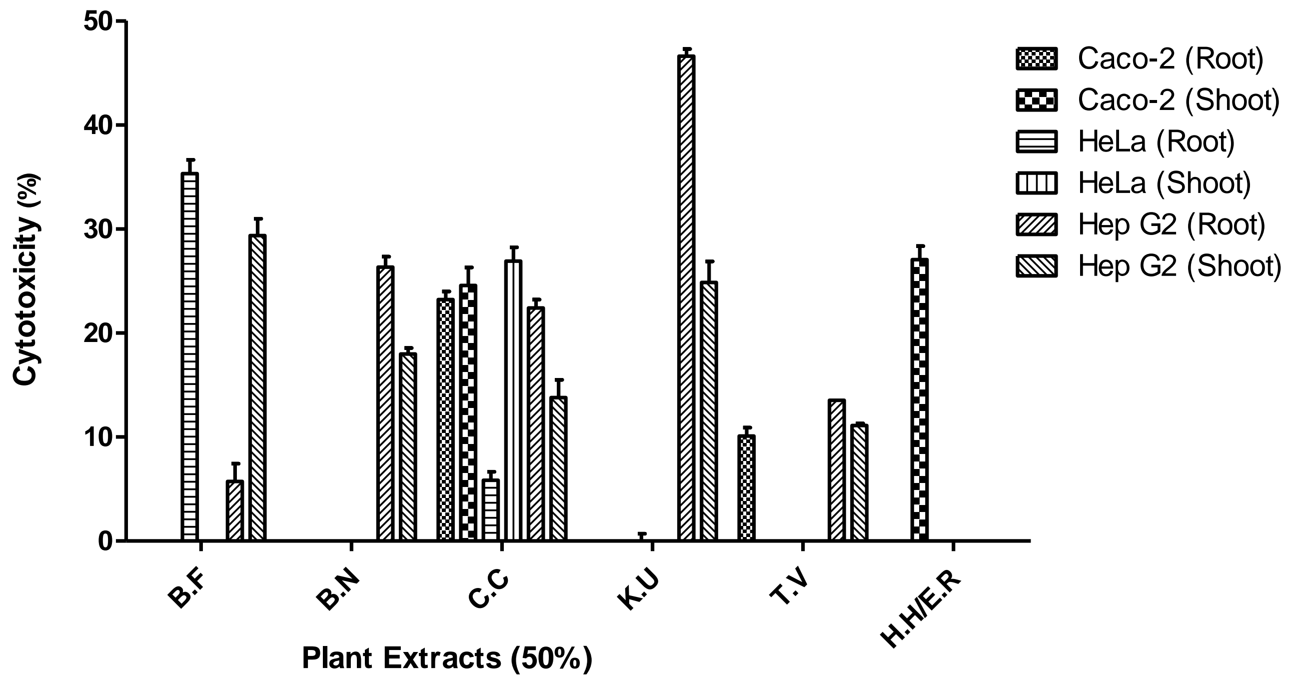

Figure 4.2: Graphical analysis showing the anti-cancer activity against human cancer cell lines. Cells were treated for 24 hours with 100 µg/mL of 50 % ethanol extracts from roots and shoots obtained from: B.f= *Bulbine frutescens*, B.n= *Bulbine natalensis*, C.c= *Chlorophytum comosum*, E.r= *Elytropappus rhinocerotis*, K.u= *Kniphofia uvaria*, T.v= *Tulbaghia violacea, H.h= Hypoxis hemerocallidea.*

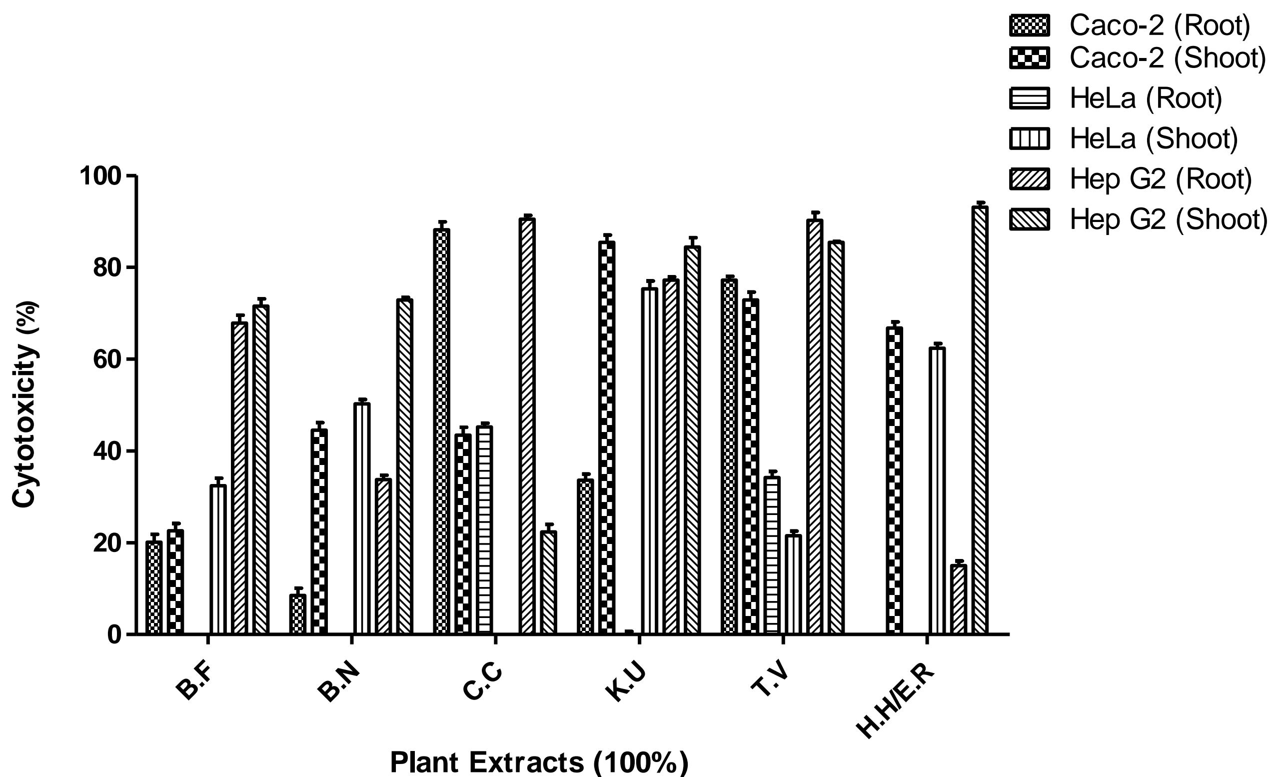

Figure 4.3: Anti-cancer activity against human cancer cell lines. Cells were treated for 24 hours with 100 µg/mL of 100 % ethanol extracts from roots and shoots obtained from: B.f= *Bulbine frutescens*, B.n= *Bulbine natalensis*, C.c= *Chlorophytum comosum*, E.r= *Elytropappus rhinocerotis*, K.u= *Kniphofia uvaria*, T.v= *Tulbaghia violacea, H.h= Hypoxis hemerocallidea.* (n = 3).

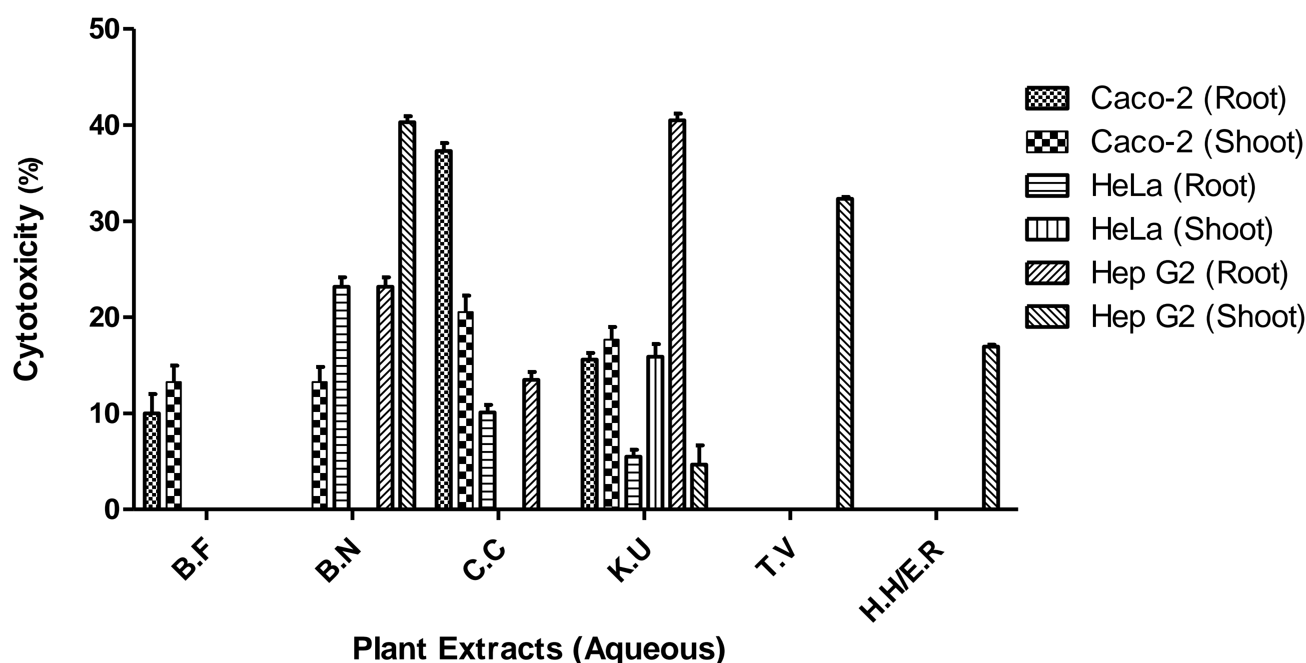

Figure 4.4: Anti-cancer activity against human cancer cell lines. Cells were treated for 24 hours with 100 µg/mL of aqueous (H_2O) extracts from roots and shoots obtained from: B.f= *Bulbine frutescens*, B.n= *Bulbine natalensis*, C.c= *Chlorophytum comosum*, E.r= *Elytropappus rhinocerotis*, K.u= *Kniphofia uvaria*, T.v= *Tulbaghia violacea, H.h= Hypoxis hemerocallidea,* DC= DMSO control, D10%= 10% DMSO as a positive control (n = 3).

4.5.2 **Determination of the Half Maximal Inhibitory Concentration (IC_{50}) for active compounds**

Fractions that showed more than 50% inhibition were selected for IC_{50} determination. Two concentrations (50 and 100 µg/mL) were prepared from each active fraction and tested against the three cell lines. IC_{50} values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line (Caco-2, HepG2 and HeLa). Results obtained from the study reported that plant extracts that showed the greatest cytotoxicity levels as well as the cell line that showed the least cell viability after the assay, which hwere further selected for IC_{50} determination, as shown in table 4.4. *Bulbine frutescens* 100% shoot

extracts showed the lowest IC_{50} value of 10.43 µg/ml concentration to produce potency against 50% of HepG2 cells. Followed by 100% *Chlorophytum comosum* shoots which need only 23.77 µg/ml concentration against HepG2 cells.

To further confirm the cytotoxicity of plant extracts showing more than 80% cytotoxicity in the cell lines, we assayed all the extracts against HepG2 which were most sensitive to the 100% ethanol plant extract to determine the dose-dependent half-maximal cytotoxic concentration and the most active plant extracts that caused 50% cell growth inhibition (IC_{50}). The 100% ethanol extracts for most of the plants showed a stronger cytotoxic effect (IC_{50} < 50 µg/ml) against the HepG2 cell. The 100% ethanol extracts for *B. frutescens* (shoot), *K. uvaria* (shoot) and *C. comosum* (root) were the most active with IC_{50} = 10.43 µg/ml, IC_{50} = 23.0 µg/ml and IC_{50} = 23.77 µg/ml respectively exhibiting the highest cytotoxicity as shown in Table 3. The *B. frutescens* (root) IC_{50} value was 32.72 µg/ml and it was the only 50% ethanol extract amongst all the plant extracts to exhibit cytotoxic effect with IC_{50} < 50 µg/ml. The shoot of *T. violacea* was shown to have significant cytotoxicity with IC_{50} < 50 µg/ml.

Table 4.4: IC_{50} values of the extracts against HepG2 cells.

Plant name (Botanical)		**Extract**	**IC_{50} (*µg/ml*) HepG2**	
			Root	**Shoots**
Bulbine frutescens		WE	>100	>100
		50% EE	>100	**34**
		100% EE	**32.72**	**10.43**
Bulbine natalensis		WE	>100	74.23
		50% EE	66.65	100
		100% EE	>100	61.19
Chlorophytum comosum		WE	>100	>100
		50% EE	>100	>100
		100% EE	**23.77**	>100
Kniphofia uvaria		WE	100	>100
		50% EE	>100	>100
		100% EE	100	**23**
Tulbaghia violacea		WE	>100	>100
		50% EE	>100	>100
		100% EE	78.6	**45.61**

WE: water extracts; EE: Ethanol extract; Extracts that caused more than 80% cytotoxicity on the cell lines assayed are in bold. Cytotoxicity (%) was presented as the mean of three replicates.

CHAPTER 5

DISCUSSION AND CONCLUSION

The potential of higher plants as a source for new drugs is gaining attention and they are well-known for the development of new chemotherapeutic agents. Medicinal plants represent a rich source of antimicrobial, antioxidants, and anticancer agents, which are a potential source of many drugs. Many studies have reported the antiviral, antibacterial, antifungal, anthelmintic, anticancer and anti-inflammatory properties found in medicinal plants (Wyk & Wink, 2004). Although hundreds of plants have been tested for their phytochemicals, the vast majority have not been adequately evaluated (Okoro *et al.*, 2019; Balandrin *et al.*, 1985), hence the need to study for more natural or organic materials to overcome challenges associated with the toxicity of synthetic drugs currently used and the emerging multidrug resistance. The seven plants selected for this study, *Bulbine frutescens*, *Bulbine natalensis*, *Chlorophytum comosum*, *Elytropappus rhinocerotis*, *Hypoxis hemerocallidea*, *Kniphofia uvaria* and *Tulbaghia violacea,* have been used locally based on their medicinal properties. Previous studies showed that they have potential antimicrobial, and antioxidant as well as anticancer activities (Bae *et al.*, 2016; Bergh & Emms, 2006; Hulley *et al.*, 2018).

In this present study, the antimicrobial (antibacterial and antifungal) activity of the selected plant extracts (aqueous, 50% and 100% ethanol) against four microorganisms, *E. coli (*gram-negative), *B. cereus (*gram-positive), *A. niger* and *C. albicans (*fungi) were determined and evaluated using agar well diffusion test. It was observed that shoots and roots of *Bulbine frutescens*, *Bulbine natalensis* and shoots of *Chlorophytum comosum* showed visible/clear zones of inhibition against *E. coli*, *B. cereus* and *C. albicans* after 24 - 48 hours incubation period at a temperature of 37°C. However, the standard antibiotics (Penicillin and Streptomycin), showed a clear zone of inhibition (20 mm diameter) for the *E. coli*, *B. cereus* and *C. albicans*, except for the *A. niger*.

The 50% and 100% ethanol shoot extracts of *B. natalensis* managed to inhibit 45% of the growth of gram-positive, *B. cereus* and *C. albicans* showing zone diameter ranging from 5.6 mm to 8 mm. According to a study by Yakubu *et al.* (2012), the ethanol extracts of *B. natalensis* inhibited 75% of the gram-negative bacteria tested, and the n-butanol fraction inhibited nearly 87.5% of the bacteria at MICs ranging from 3 to 10 mg/ml. At MICs of 1 and 5 mg/ml, the ethyl acetate fraction inhibited all of the tested gram-negative and gram-positive organisms completely, whereas the water extract had no effect. Similar to our study, there was no growth inhibition from the aqueous extracts of *B. natalensis* tubers against *E. coli, B. cereus* and *C. albicans* except for *Aspergillus sp.* where the aqueous extracts produced 100% inhibition at all the doses investigated.

Furthermore, Coopoosamy (2011) discovered that acetone and ethyl acetate extracts of *B. natalensis* leaf, root, and rhizome had greater antibacterial activity against both gram-positive and gram-negative bacteria tested when compared to water extracts.

Ghorpade & Thakare (2014) reported *Chlorophytum sp.* serves as an effective antimicrobial agents as the aqueous extracts of these species showed antimicrobial activity against *S. aureus*, *E. coli*, *P. vulgaris*, *B. substilis*, *A. niger* and *C. albicans*. Our study showed that for *C. comosum,* the shoot 100% ethanol solvent recorded a zone of inhibition of 10 mm (100 µl) and 13.6 mm (200 µl), while the 50% extract inhibits E. *coli* by 7.6 mm (100 µl) and 8.3 mm (200 µl). An average of 7.6 mm (100 µl) and 10.3 mm (200 µl) was also observed from 100% ethanol solvent against *B. cereus*, and 10 mm (100 µl) and 10 mm (200 µl) against *C. albicans*. This shows that 50% of the *B. cereus* and *C. albicans* were inhibited by the 100% ethanol extract of *C. comosum.*

E. rhinocerotis, *H. hemerocallidea*, *K. uvaria* and *T. violacea* showed no visible zones of inhibition against *E. coli*, *B. cereus*, *A. niger* and *C. albicans*. Results obtained on this study may be affected by many factors, which may include the agar type, salt concentration, incubation temperatures and molecular size of the antimicrobial component as well as the preparation of inoculums (Schultz & Kishony, 2013; Bidlas, *et al*., 2008). Furthermore, variations on the antimicrobial potential within studied plant species and tested microorganisms may also be due to the intrinsic properties of the species studied (Ghorpade & Thakare, 2014).

To study the antioxidant activity, the methanolic extracts of the seven selected South African plants; *B. frutescens* (roots and shoots)*, B. natalensis* (roots and shoots)*, C. comosum* (roots and shoots)*, E. rhinocerotis* (shoots)*, H. hemerocallidea* (corm)*, K. uvaria* (roots and shoots) *and T. violacea* (roots and shoots) were examined for their antioxidant capacity using DPPH assay.

Many studies have evaluated the free radical-scavenging activity of natural antioxidants (, Yokozawa *et al*., 1998, Zhu *et al*., 2001). the free-radical scavenging property is shown by a purple colour, which changes into a stable yellow compound after reacting with an antioxidant, and the extent of the reaction depends on the hydrogen donating ability of the antioxidant (Bondent *et al*., 1997). In this present study, all plants selected and tested showed varying antioxidant values. The *H. hemerocallidea* corm and E. *rhinocerotis* shoot of methanolic extracts were found to be the highest in antioxidants (115.0 ± 0.71% and 115.0 ± 5.04%) respectively. The *B. natalensis* root extract (also showed a high antioxidant of 33.0 ± 0.37%. As the values of antioxidant capacities are inversely proportional, the roots of *T.*

violacea were established to have the lowest antioxidant capacity (5 ± 0.19%). Previous study performed for the analysis and characterization of bioactive compounds has indicated that the leaves of the *T. violacea* have more active compounds than the roots (Madike *et al.*, 2017).

Furthermore, the present study screened *in vitro* cytotoxic activities of both leaves and roots from these seven selected plants (*B. frutescens*, *B. natalensis*, *C. comosum*, *E. rhinicerotis*, *H. hemerocallidea*, *K. uvaria* and *T. violacea*) against human hepatocellular (HepG2), human cervical (HeLa) and human colon (Caco-2) cell lines using the MTT cytotoxicity assay. As previously discussed, the MTT cytotoxicity assay measures the activity of the mitochondria through the reduction of a yellow-coloured MTT salt to purple formazan crystals by the enzyme succinate tetrazolium reductase or through mitochondrial succinate dehydrogenase, both of which are found in viable cells (Mossman, 1983; Reddy, *et al.*, 2006).

The results suggest that 100% ethanol extracts exhibited cytotoxicity in a cell type manner for all three extracts (aqueous, 100 % ethanol and 5 % ethanol). On shoots, *E. rhinocerotis* exhibited the most cytotoxicity on HepG2 cells, with 94% cytotoxicity, compared with 67% in Caco-2and 63% in HeLa cells. This was followed by *K. uvaria* which recorded 86% against HepG2, 84 % against Caco-2and 75% from HeLacells. *T. violacea* also exhibited 84% against HepG2, 74% against Caco-2and 22% from HeLa cells. Okoro *et al.* (2019) and Kushwaha *et al.* (2019) explained that the activities may vary according to the different polarity of extracts tested at different concentrations which might be attributed to the uneven distribution of phytochemicals within these extracts. These phytochemicals maybe supported by the ethnobotanical use of studied plants, as well as chemical constituents reported, such as carbohydrates, glycosides, alkaloids, flavonoids, terpenoids, tannins, saponins, sterols, e.t.c (Gbadamosi and Erinoso, 2015) which favours their use as biologically active ingredients against various human cancer cell lines (Srisawat *et al.*, 2013; Zhang & Li, 2007).

On roots, 100% ethanol extracts also showed high cytotoxicity compared to other extracts, where *T. violacea* recorded the highest percentage of cytotoxicity at 88% against HepG2, 75% against Caco-2 and 29% against HeLacells. *C. comosum* was with also 88% cytotoxicity against Hep-G2, 86% against CaCo-2 and 45% against HeLa cell lines. All other plant extracts recorded below 80% cytotoxicity. Butanolic root fractions of *C. comosum* demonstrated significant anti-tumor activity against tumor promoter-induced phospholipid metabolism in HeLa cell 342 lines. In another study, Matushita et al. observed apoptosis induction by Butanolic 343 extracts in four human cancer cell lines including HeLa. A possible limitation of this study could be the use of endpoint assays like MTT for cytotoxicity assays as it is labor intensive, requiring labelling steps to ensure accuracy on plates as

interference of the extract with mitochondrial function may lead to false positives. For these reasons, it is suggested that MTT should only be used as a preliminary method for screening cytotoxicity.

With about 36 extracts tested from 7 different plant species, the majority of aqueous extracts showed very little activity in the screening, with others not showing activity at all. The reduction of MTT was read at 570 nm and the cell viability (100%) indicates that all cells are alive and 80% indicates 20% dead cells etc. Plants extracted with 50% ethanol also indicated very little anticancer activity on both human cell lines tested. However, it is interesting to note that the organic extracts of the plants containing 100% ethanol, both roots and shoots, displayed potent activity against human HepG2, whilst moderate activity was observed for the Caco-2 and very low cytotoxicity levels against HeLa cell line.

CONCLUSION

The experiments described in this study establish the usefulness of preliminary bioassay screening of plants for potential biological activity and toxicity. According to this study, it can be concluded that various phytochemicals, including antioxidants and anticancer properties are present on several plant organs tested from the selected plants. However, most of plant extracts tested showed no antimicrobial potency against *E. coli*, *B. cereus*, *A. niger* and *C. albicans*.

For future studies, the combination of plant extracts is highly recommended as previous studies showed the use of combined plant extracts; *B. frutescens* and *Vernonia lasiopus*, synergistically enhanced antimicrobial activity levels (Rachuonyo, *et al.*, 2016). When these plant extracts were used separately against *E. coli*, average on zones of inhibition experienced was less than those produced when two plant extracts were combined. This suggests that combination of plant extracts can improve antimicrobial activity against some microorganism.

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APPENDICES

Appendix 1: Publication

In vitro antioxidant and cytotoxicity activities of selected indigenous South African medicinal plants

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DOI: 10.4314/ahs.v22i1.48

Keywords: In vitro antioxidant; cytotoxicity activities; South African medicinal plants; C. comosum, K. uvaria, T. violacea.

Abstract

Background: Medicinal plants are regarded as a large source of phytochemicals that may have anticancer properties. This could lead to the development of innovative drugs or alternative therapy against cancer.

Objective: This study was designed to determine the antioxidant and cytotoxicity effect of 5 selected indigenous South African medicinal plants namely; *Bulbine frutescens*, *Bulbine natalensis*, *Chlorophytum comosum*, *Kniphofia uvaria*, and *Tulbaghia violacea*.

Method: Phytochemical extracts namely; methanol, 50%, 100% ethanol, and water extracts were prepared from the root and shoot of the plants. The antioxidant effect of methanol extracts of the plant materials was performed using a DPPH assay. A preliminary cytotoxicity screening of the phytochemical extracts in the human colon (Caco-2), cervical (HeLa), and hepatocellular (HepG2) cell lines were determined followed by the half-maximal inhibitory concentration (IC50) using MTT assay.

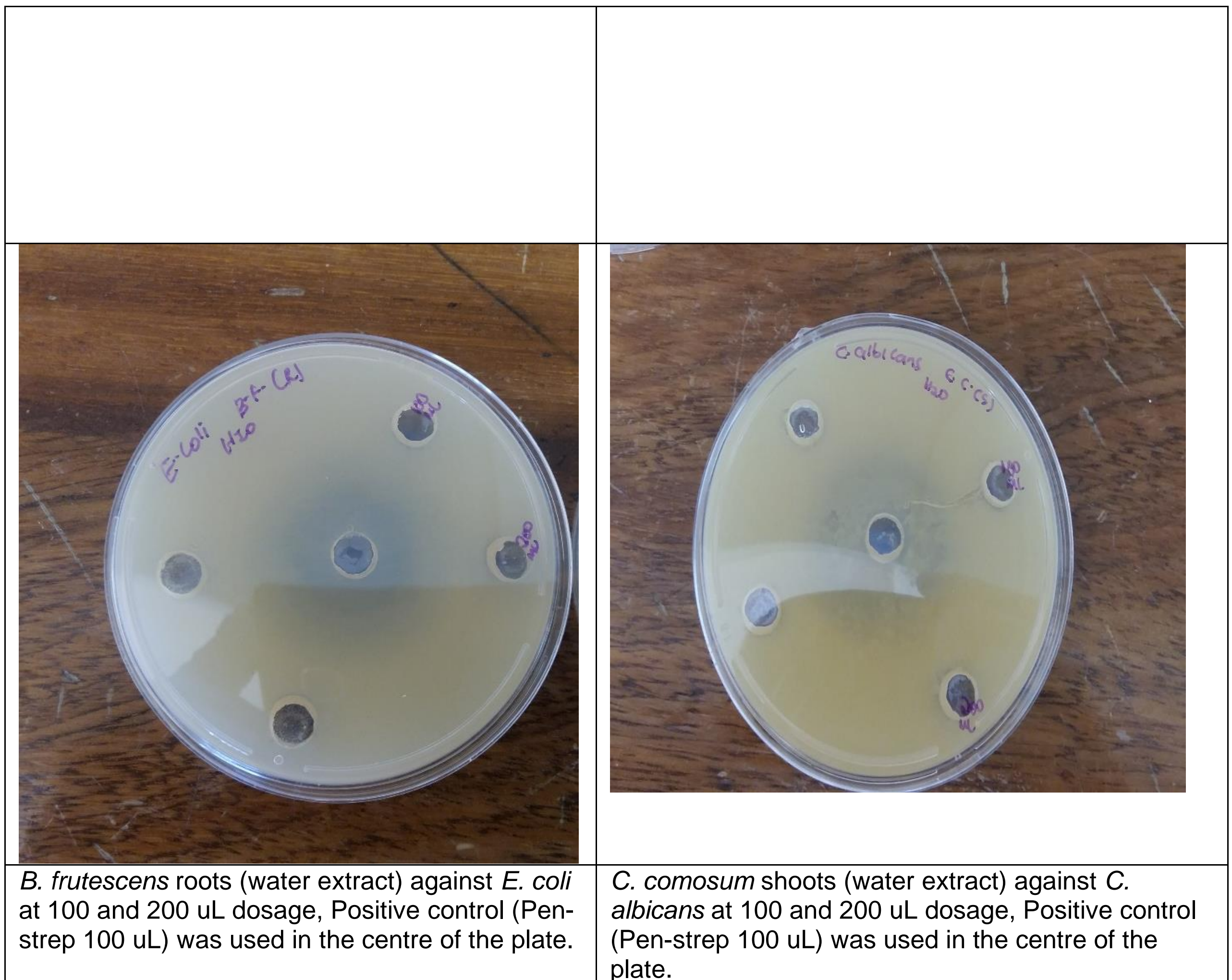
Result: The methanol root extract of *B. natalensis* and *B. frutescens* (33.20% and 26.33% respectively) and shoot extract of *K. uvaria* (17.10%) showed the highest antioxidant. Out of the 5 plants, only 100% ethanol extract of *C. comosum*, *K. uvaria*, and *T. violacea* caused more than 80% cytotoxicity in HepG2 and Caco-2 cell lines. The shoot of *B. frutescens* (10.43 µg/ml), *K. uvaria* (23.0 µg/ml), and root of *C. comosum* (23.77 µg/ml) were the most active with the highest cytotoxicity.

Conclusion: *C. comosum*, *K. uvaria*, and *T. violacea* possess significant cytotoxicity that is promising in developing alternative drugs against colon and liver cancers. Our results provided new pieces of evidence for antioxidant and cytotoxic activities of these plants which could be useful for developing new anticancer therapies.

Keywords: In vitro antioxidant; cytotoxicity activities; South African medicinal plants; *C. comosum*, *K. uvaria*, *T. violacea*.

Appendix 2: Antimicrobial Assay results

ANTIMICROBIAL ASSAY	
B. natalensis shoots (50% ethanol) against *E. coli* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.	*B. natalensis* roots (50% ethanol) against *E. coli* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.
B. natalensis roots (100% ethanol) against *E. coli* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.	*B. frutescens* roots (100% ethanol) against *E. coli* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.



B. frutescens roots (water extract) against *E. coli* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.	*C. comosum* shoots (water extract) against *C. albicans* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.

Figure 4.5: Zone of inhibition of *B. frutescens*, *B. natalensis* and *C. comosum* against tested microorganisms

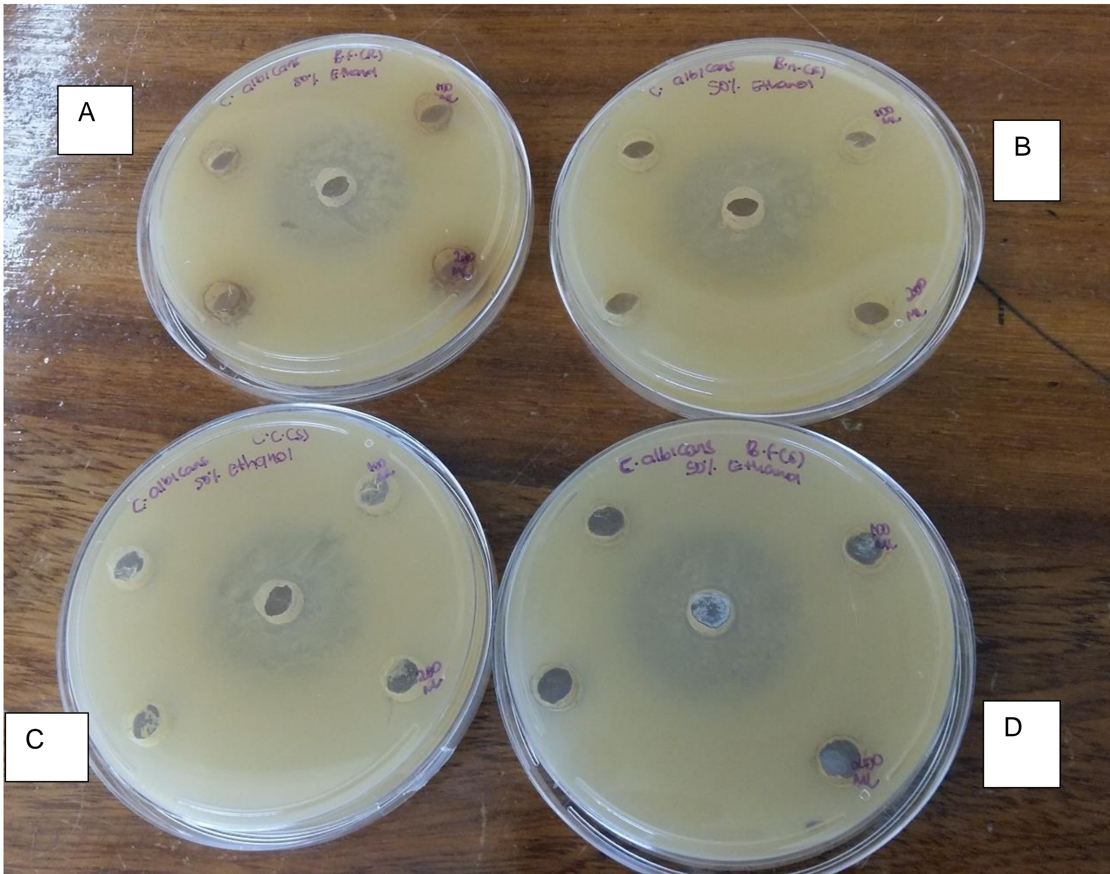


Figure 4.6: A= *B. frutescens* roots (50% ethanol), B= *B. natalensis* roots, C= C. comosum shoots, D= *frutescens* shoots. All the above plants were 50% ethanol extracts against *C. albicans* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.

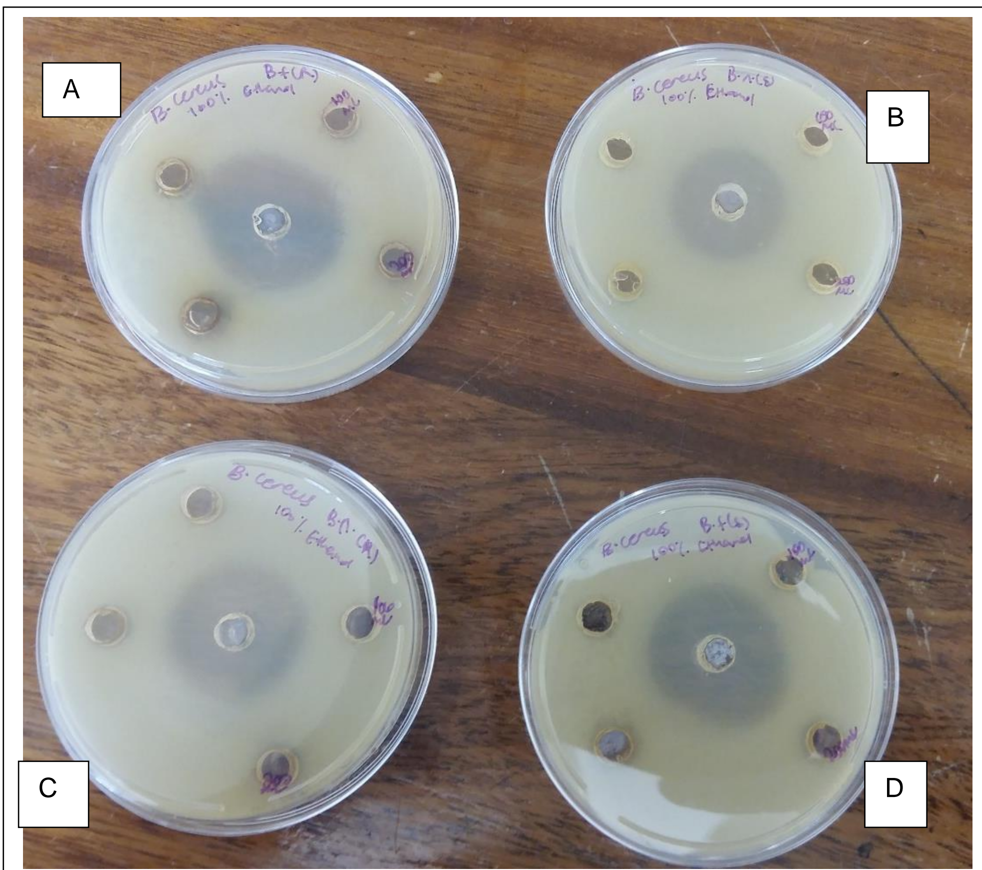


Figure 4.7: A=*B. frutescens* roots, B= *B. natalensis* shoots, C= *B. natalensis* roots, D= *frutescens* shoots. All the above plants were 100% ethanol extracts against *B. cereus* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.

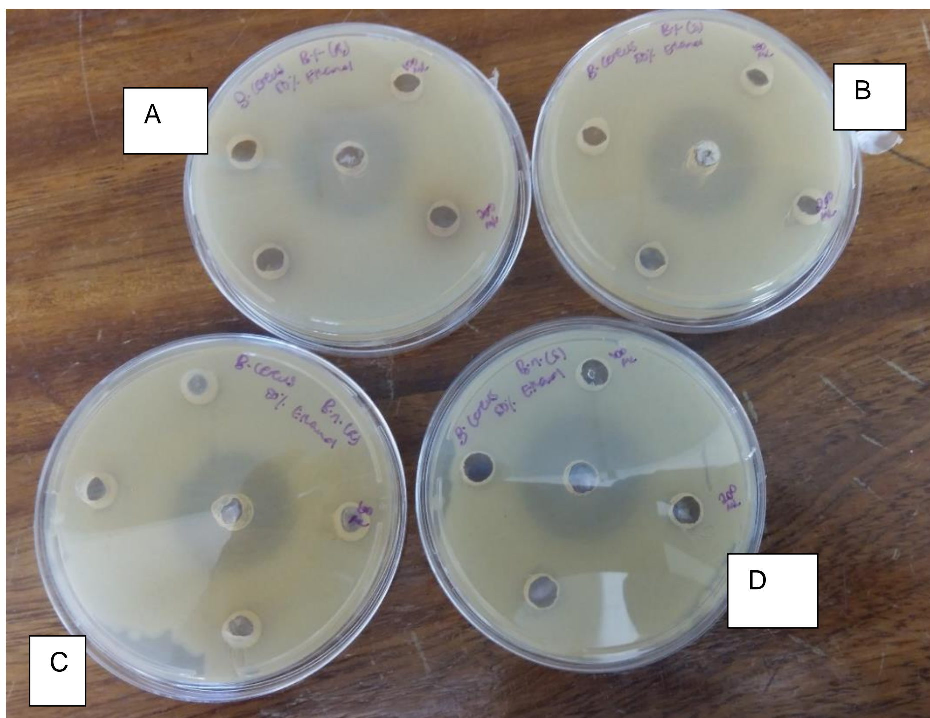


Figure 4.8: A=*B. frutescens* roots, B= *B. frutescens* shoots, C= *B. natalensis* roots, D= *natalensis* shoots. All the above plants were 50% ethanol extracts against *B. cereus* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.

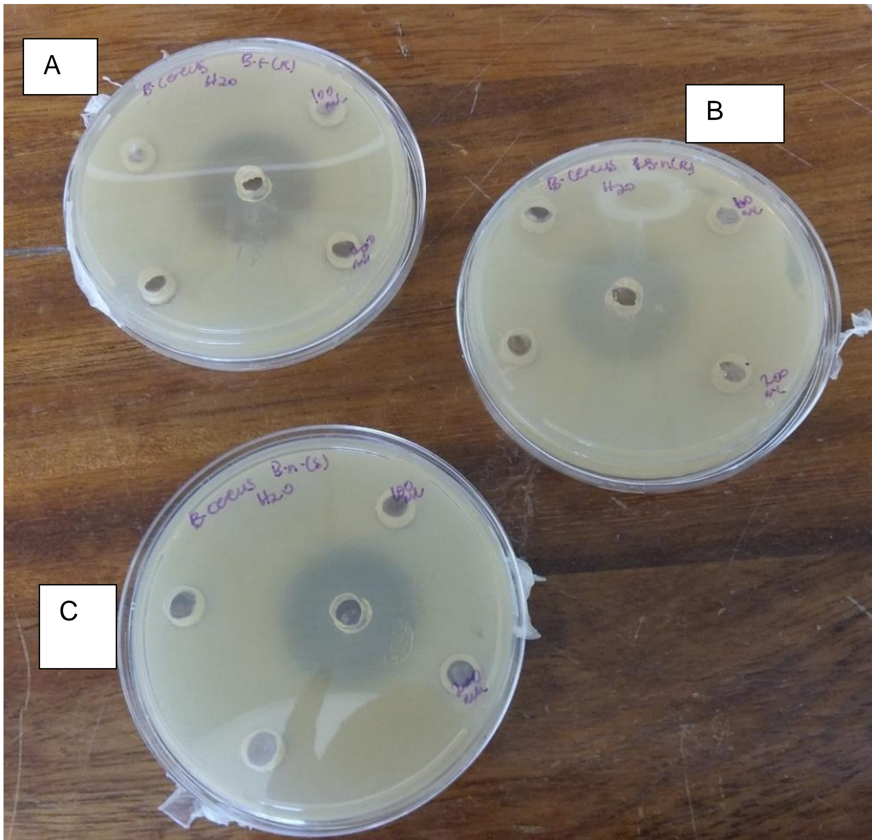


Figure 4.9: A=*B. frutescens* roots, B= *B. natalensis* roots*,* C= *B. natalensis* shoots. All the above plants were water extracts against *B. cereus* at 100 and 200 uL dosage, Positive control (Pen-strep 100 uL) was used in the centre of the plate.