



## ***Hypoxis colchicifolia* as a potential nutraceutical to address non-communicable diseases**

**Submitted in complete fulfillment for the Degree of Master of Applied Sciences  
(Food Science and Technology) in the Department of Biotechnology and Food  
Technology, Faculty of Applied Sciences, Durban University of Technology,  
Durban, South Africa**

**Suggessan Moodley  
MAppSc (Food Science and Technology)**

**2020**

**PROMOTER/ SUPERVISOR : Prof. John Jason Mellem**  
**CO-PROMOTER/ CO-SUPERVISOR : Prof. Himansu Baijnath**

## Reference Declaration

---

I, Mr. Suggessan Moodley – 21233614 and Prof. John Jason Mellem do hereby declare that in respect of the following dissertation – Title: *Hypoxis colchicifolia* as a potential nutraceutical to address non-communicable diseases

1. As far as we ascertain:

- a) no other similar dissertation exists;
- b) the only similar dissertation(s) that exist(s) is/are referenced in my dissertation as follows:

---

---

---

2. All references as detailed in the dissertation are complete in terms of all personal communication engaged in and published works consulted.

Signature of ~~student~~

Date

Signature of promoter/ supervisor

Date

Signature of ~~co~~-promoter/ co-supervisor

Date

## Authors Declaration

---

This study presents original work by the author. It has not been submitted in any form to another academic institution. Where use was made of the work of others, it has been duly acknowledged in the text. The research described in this dissertation was carried out in the Department of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, South Africa, under the supervision of **Prof. John Jason Mellem** and **Prof. Himansu Baijnath**.

Student's signature

## Acknowledgements

---

It is my pleasure to thank and show my appreciation to all the people who made this thesis a reality

- My supervisor Prof. J.J Mellem, for being a model example of a researcher, and for giving me the freedom and allowing me to grow as a researcher. Also, for always driving me to do my best and having the faith in me and my study.
- My co-supervisor Prof. H. Baijnath, for attaining plant material, always being there to aid and direct me when needed; and for always encouraging me to “press on”.
- I would like to thank my parents for driving the importance of a good education and for affording me the privilege of being a full time student all these years. It’s only with your guidance, prayers and blessings that I have come this far in my life.
- Thanks to my twin brothers for always assisting me when I needed help and advice with computation.
- I’m grateful to my dear friends Shivon, Depika and Melissa for their assistance, encouragement and friendship over the years. It made this chapter of my life a beautiful one.
- Gratitude to the staff and my fellow post graduate students at the Department of Biotechnology and Food Technology, Durban University of Technology for their support and assistance during this study.
- Thanks to family and friends for their support and inspiration.
- ADA and Bertie Levenstein and National Research Foundation (NRF) for their financial support.

## **Publications**

---

### ***Publications***

- Moodley, S., Dwarka, D., Baijnath, H. and Mellem, J. 2020. Antioxidant and phenolic constituents of *Hypoxis colchicifolia*. *The Annals of the University Dunarea de Jos of Galati, Fascicle VI – Food Technology* 44(2)

### ***Conferences***

- Moodley, S., Dwarka, D., Baijnath, H. and Mellem, J. 2019. *Hypoxis colchicifolia* extracts as potential nutraceuticals to address non-communicable diseases. *Food Safety and Quality Symposium*. Holiday Inn Garden Court, Durban, 28 November 2019

## Preface

---

The following dissertation is organized into five chapters and is presented as follows:

**Chapter 1:**

Introduction (Presents the problem statement, the aims and objectives of the study and the contribution to the knowledge gap)

**Chapter 2:**

Literature review (Review of previous studies, the finding of the studies and the gaps in the research)

**Chapter 3:** (Research objective 1 & 2)

Antioxidant activity and phenolic content of *Hypoxis colchicifolia*.

**Chapter 4:** (Research objective 3 & 4)

*In vitro* anticancer, anti-hypertensive and anti-hyperglycaemic activities of *Hypoxis colchicifolia*.

**Chapter 5:**

General Discussion

## Table of Contents

<b>Abstract .....</b>	<b>9</b>
<b>Chapter 1: Introduction .....</b>	<b>10</b>
<b>Chapter 2: Literature Review .....</b>	<b>12</b>
2.1. History of plants and medicine .....	12
2.2. Africa and the use of traditional medicine .....	12
2.3. Plant secondary metabolites and medicine .....	14
2.4. <i>Hypoxis</i> species.....	17
2.4.1. Botanical Information.....	17
2.4.2. Traditional and contemporary use .....	17
2.4.3. Biological activity.....	18
2.5. <i>Hypoxis colchicifolia</i> .....	20
2.5.1. Taxonomy .....	20
2.5.2. Known constituents.....	21
2.5.2.1. Hypoxoside and rooperol.....	21
2.6. The role of medicinal plants in non-communicable diseases.....	22
2.6.1. Free radicals, oxidative stress and antioxidants .....	23
2.6.2. Analytical methods for determining the antioxidant properties.....	24
2.6.2.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay.....	24
2.6.2.2. Phosphomolybdenum (PM) Assay .....	25
2.6.2.3. 2,2'-azinobis(3-ethylbenzothiazolline)-6-sulfonic acid (ABTS) assay ..	25
2.6.2.4. Ferric Reducing Antioxidant Power (FRAP) assay.....	25
2.6.2.5. Cupric ion reducing antioxidant capacity (Cuprac) Assay .....	25
2.7. Oxidative stress related conditions .....	25
2.7.1. <i>Diabetes mellitus</i> .....	25
2.7.1.1. Conventional management of diabetes .....	26
2.7.1.2. Plant based treatment .....	27
2.7.2. Hypertension .....	27
2.7.2.1. Angiotensin mechanism.....	28

2.7.2.2. Medicinal plant-based therapy .....	29
2.7.3. Cancer .....	29
2.7.3.1. Importance of apoptosis in cancer treatment.....	30
2.7.3.2. Conventional cancer treatment.....	31
2.7.3.3. Plant based anticancer treatment.....	32
<b>Chapter 3: Antioxidant activity and phenolic content of <i>Hypoxis colchicifolia</i> .....</b>	<b>33</b>
3.1. Introduction .....	33
3.2. Methodology.....	35
3.2.1. Collection of plant material.....	35
3.2.2. Preparation of plant material.....	35
3.2.2.1. Fresh Corm.....	35
3.2.2.2. Dried Samples .....	35
3.2.3. Phytochemical Screening .....	35
3.2.4. Brine Shrimp Lethality Assay.....	36
3.2.5. Total Phenol Content.....	36
3.2.6. Antioxidant Activity .....	37
3.2.6.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay.....	37
3.2.6.2. 2,2'-azinobis(3-ethylbenzothiazolline)-6-sulfonic acid (ABTS) assay ..	37
3.2.6.3. Phosphomolybdenum (PM) Assay .....	38
3.2.6.4. Cupric ion reducing antioxidant capacity (Cuprac) Assay .....	38
3.2.6.5. Ferric Reducing Antioxidant Power (FRAP) assay.....	38
3.2.7. Statistical analysis.....	39
3.3. Results and Discussion .....	39
3.3.1. Phytochemical Screening .....	39
3.3.2. Brine Shrimp lethality Assay .....	40
3.3.3. Total Phenol Content.....	41
3.3.4. Antioxidant Activity .....	42
3.3.4.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay.....	42
3.3.4.2. 2,2'-azinobis(3-ethylbenzothiazolline)-6-sulfonic acid (ABTS) assay ..	45
3.3.4.3. Phosphomolybdenum (PM) Assay .....	47



3.3.5. Cupric ion reducing antioxidant capacity (Cuprac) Assay.....	48
3.3.6. Ferric Reducing Antioxidant Power (FRAP) assay.....	49
3.4. Conclusion .....	50
<b>Chapter 4: <i>In vitro</i> anti-cancer, anti-hypertensive and anti-hyperglycaemic activities of <i>Hypoxis colchicifolia</i> .....</b>	<b>52</b>
4.1. Introduction .....	52
4.2. Methodology.....	54
4.2.1 Collection of plant material .....	54
4.2.2. Preparation of plant material .....	54
4.2.3. Extraction of plant material.....	54
4.2.4. Anti-diabetic Screening.....	54
4.2.4.1. Alpha amylase Inhibition Assay .....	54
4.2.4.2. Alpha glucosidase Inhibition Assay.....	55
4.2.5. Anti-hypertension (ACE inhibition assay) .....	55
4.2.6. Cytotoxicity screening (MTT Assay).....	56
4.2.7. Statistical analysis.....	56
4.3. Results and Discussion .....	57
4.3.1. Alpha amylase inhibition .....	57
4.3.2. Alpha glucosidase inhibition.....	59
4.3.3. ACE Inhibition.....	61
4.3.4. MTT Cytotoxicity .....	63
4.4. Conclusion .....	68
<b>Chapter 5: General Discussion .....</b>	<b>69</b>
<b>References .....</b>	<b>74</b>

## Abstract

---

Cancer, hypertension and hyperglycaemia affect millions of individuals worldwide, with many succumbing to these diseases. Conventional medicine and treatments currently used to manage these diseases, although highly effective, have major side effects. *Hypoxis colchicifolia* is a traditional medicinal plant used in Southern African against an array of ailments. Providing a rationale and assessing the toxicity of the plant is essential for future use as a natural alternative to allopathic medicine. In this study *H. colchicifolia* corm and leaf extracts were qualitatively assessed for their phytochemical constituents. The total phenolic content was determined using the Folin Ciocalteu method, and toxicity screened using the brine shrimp lethality assay. The antioxidant potential was evaluated using the DPPH (1,1-diphenyl-2-picryl-hydrazyl), ABTS (2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid), PM (Phosphomolybdenum), CUPRAC (Cupric ion reducing antioxidant capacity) and FRAP (Ferric Reducing Antioxidant Power) radical scavenging methods. This study also evaluated the *in vitro* anti-diabetic ( $\alpha$ -amylase and  $\alpha$ -glucosidase), antihypertensive [ACE (angiotensin-converting enzyme)] and anticancer potential of *H. colchicifolia* corm as well as leaf solvent extracts (acetone, methanol and aqueous). All extracts showed the presence of key phytochemical constituents and produced no toxicity. There was a clear difference in the total phenolic content of the leaves compared to the corms. Extracts showed good antioxidant potential with different extracts inhibiting different free radicals, indicating selective antioxidant scavenging capacity. The extracts of leaves and corms cannot be used interchangeably as there are differences in the phytochemical composition. Results showed that *H. colchicifolia* extracts have a moderate anti-diabetic and anti-hypertensive potential. The acetone extract of fresh corms had the best  $\alpha$ -amylase potential with an  $IC_{50}$  of 337  $\mu\text{g/mL}$  and acetone extract of fresh corms leading the  $\alpha$ -glucosidase inhibition with an  $IC_{50}$  of 22.06  $\mu\text{g/mL}$ . The methanol extract of dried corms exhibited the greatest ACE inhibition with an  $IC_{50}$  of 368.20  $\mu\text{g/mL}$ . Methanol extract of dried leaves showed the least cytotoxicity against non-cancerous cell line HEK-293. Methanol extract of dried leaves inhibited MCF-7 cell line with an  $IC_{50}$  of 3.24  $\mu\text{g/mL}$ , which was lower than that of the positive control, camptothecin ( $IC_{50}$ , 8.44  $\mu\text{g/mL}$ ). All extracts exhibited a greater inhibitory potential in A549 cells than camptothecin ( $IC_{50}$ , 304.20  $\mu\text{g/mL}$ ), with aqueous extract of dried corms having the greatest potential of  $IC_{50}$  32.22  $\mu\text{g/mL}$ . This study reveals that *H. colchicifolia* has potential to act as a therapeutic.

## Chapter 1: Introduction

---

Chronic and cancerous diseases affect millions of individuals worldwide, with many succumbing to these or having to live with the major side effects associated with the therapeutics used for treatment. Oxidative stress, inflammation and cardiac diseases are interconnected, with individuals generally exhibiting more than one chronic disease. Conventional medicine and treatments used in inhibiting as well as managing these diseases, although highly effective, have significant side effects linked to their mode of action. This results in individuals suffering with short-term and long-term side effects, while paradoxically getting relief from their chronic disease. Hence the importance of developing an alternative treatment that will impede these chronic diseases without exhibiting side effects and toxicity.

According to the World Health Organization (WHO), poverty and the lack of modern medicine force 65-80% of the world's population to depend exclusively on plants for principle healthcare (WHO, 2018). Synthetic and pharmaceutical drugs for chronic and infectious diseases are unaffordable to populations in developing countries; hence the reliance on traditional plant-based medicines as their main source of healthcare. In developed countries affordability may not be an issue, however the array of side effects that pharmaceutical drugs exhibit is an issue of concern. Plant based medicines are therefore being accepted as a form of alternative or complementary medicine. Herbal medicines are generally non-toxic exhibiting limited side effects compared to conventional pharmaceutical drugs (Jamshidi-Kia et al., 2018).

Due to the inaccessibility of healthcare in South Africa, traditional medicine plays an essential role ensuring access to some form of medical treatment. Traditional healers service approximately 80% of the sub-Saharan population, with 10 000 traditional healers practicing in South Africa alone (Elujoba et al., 2005). The herbal trade industry in South Africa is worth approximately R500 million per annum, with over 80 species of African traditional medicinal plants being traded internationally. The importance of phytomedicines has been recently recognized and sparked scientific investigation, as the therapeutic functionality of medicinal plants is limited.

Many traditional plants are being used without any scientific validation. An increasing interest in African traditional plants and medicine has grasped the attention of government, private sector, institutions and pharmaceuticals, to justify the scientific value of African traditional medicinal plants and verify the therapeutic effect of their medicines.

In South Africa *Hypoxis* species have been used in traditional herbal medicines, widely distributed in Southern Africa, categorized by strap like leaves and bright yellow star shaped flowers (Singh, 2007). The tuberous rootstock is commonly referred to as the 'African Potato' owing to its potato like appearance with extracts used for the treatment, management and control of an array of human ailments such as the treatment of barrenness, bad dreams, heart ailments, nausea and to destroy pests as well as vermin in food. The dried corms (tuber of plant) are boiled in water and the extract ingested as a tonic. In other species of *Hypoxis*, only the corms are used exclusively with leaves discarded once harvested. *Hypoxis colchicifolia* leaves and corms have not been scientifically evaluated previously. Therefore, the aim of this study was to investigate the potential biological activity of *H. colchicifolia* and to establish the therapeutic use of *H. colchicifolia* corm and leaf extracts as an alternative treatment for non-communicable diseases. To achieve this, the objectives were:

1. To establish the presence of phytochemical constituents in the corms and leaves by a qualitative phytochemical screen; as well as to establish the safe use of the *H. colchicifolia* extracts.
2. To determine the antioxidant potential of the *H. colchicifolia* extracts by 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), Ferric Reducing Antioxidant Power (FRAP), Cupric ion reducing antioxidant capacity (CUPRAC) and Phosphomolybdenum (PM) antioxidant assay.
3. To determine the anticancer activity of *H. colchicifolia* crude extracts against cancer cells A549, HEK-293 and MCF-7.
4. To establish the anti-diabetic and anti-hypertensive effects of *H. colchicifolia* extracts.

## Chapter 2: Literature Review

---

### 2.1. History of plants and medicine

The history of using plants as medicine dates back to ancient times where Indian, Chinese and African civilizations provided written evidence of the use of plants for the treatment of various diseases (Fabricant and Farnsworth, 2001; Jamshidi-Kia et al., 2018; Khan, 2014). Quinine from *Cinchona* bark were amongst the earliest discoveries, thus leading to an interest in plants and the invention of new medicines (Phillipson, 2001). A series of natural products isolated from higher plants have been used as clinical agents and many are still used today such as quinine, morphine, codeine, digoxin, atropine and hyoscyne (Halberstein, 2005). Currently, medicinal plants are becoming widely acknowledged and are increasing in demand with at least one tenth of plant species (over 50 000 species) used in pharmaceutical and cosmetic products (Mamedov, 2012). Medicinal plants are mainly harvested from wild populations, with the demand ever increasing on an annual basis.

### 2.2. Africa and the use of traditional medicine

In Africa, traditional medicine is used by 80% of the population as a primary source of healthcare due to their socio-economic heritage (Kelmanson et al., 2000; Elujoba et al., 2005; Kale, 1995). Traditional medicine can be described as the direct application of plant, animal or mineral material for healing purposes and may be investigated, rationalized as well as explained scientifically. Majority of Africans use traditional medicine as the first choice of treatment for common ailments (Abdullahi, 2011). However, African traditional medicine has advantages and disadvantages viz. it is cheaper than orthodox medicine, highly accessible and widely accepted, is a potential source of new plant derived drugs and is usually made from natural plant material making it readily absorbed by the body (Samie et al., 2005; Sofowora, 1996). There is however a lack of scientific evidence for their efficacy and dosages.

**Table 2.1 : Common South African plants used in traditional medicine**

Scientific name (common name)	Part of plant used	Uses	Reference
<i>Aloe ferox</i> (Cape aloe)	Leaves	Treatment of arthritis, eczema, stomach pain, conjunctivitis, hypertension, stress and skin irritation.	(Van Wyk, 2008; Chavan et al., 2013; Wintola and Afolayan, 2015; Van Wyk, 2015)
<i>Siphonochilus aethiopicus</i> (Natal ginger)	Fresh roots/ Rhizomes	Treat influenza, colds, asthma, malaria, helps women during menstruation and as an appetite suppressant.	(Van Wyk, 2008; Van Wyk, 2015)
<i>Ericephalus africanus</i> (Wild rosemary)	Leaves	Treat asthma and infections of the throat and lung	(Van Wyk, 2008)
<i>Lessertia frutescens</i> (Cancer Bush)	Leaves	Treat poor appetite, fever, indigestion, peptic ulcers, gastritis, cancer, dysentery, colds, influenza, diabetes, asthma, coughs, chronic bronchitis, kidney and liver conditions, urinary tract infections, rheumatism, heart failure and anxiety.	(Van Wyk, 2015)
<i>Harpagophytum procumbens</i> (Devil's claw)	Tubers	Treat diseases of the kidneys, bladder and liver, also used to stimulate appetite and aid in indigestion.	(Van Wyk, 2015)
<i>Hypoxis hemerocallidea</i> (African potato)	Tubers	Treat benign prostate hypertrophy, urinary tract infections and testicular tumours. To treat dizziness, nervous and bladder disorders, heart weakness and depression.	(Van Wyk, 2008; Wintola and Afolayan, 2015)
<i>Hoodia gordonii</i> (Hoodia)	Stem	An appetite suppressant, thirst quencher and mood enhancer. As a cure for hemorrhoids, tuberculosis, severe abdominal cramps, hypertension, diabetes and indigestion	(Van Wyk, 2008; Van Wyk, 2015)
<i>Helichrysum odoratissimum</i> (Everlasting)	Twigs and leaves	Treat coughs, colds, headaches, infections, fever and menstruation pain.	(Wintola and Afolayan, 2015)
<i>Sceletium tortuosum</i> (Canna)	Leaves	Treat depression and anxiety.	(Van Wyk, 2015)
<i>Tulbaghia violacea</i> (Wild garlic)	Rhizomes and leaves	Treat fever, rheumatism, constipation, asthma, cough, colds, pulmonary tuberculosis, intestinal worms and to treat cancer of the oesophagus.	(Elgorashi et al., 2003; Wintola and Afolayan, 2015)
<i>Artemisia afra</i> (Wild wormwood)	Roots, stems and leaves	Treat colds, coughs, fever, colic, loss of appetite, earache, headaches, intestinal worms, malaria, respiratory tract infections, influenza, sore throat, asthma, pneumonia, indigestion, gastritis, flatulence, constipation, gout and measles.	(Van Wyk, 2008; Amusan et al., 2005; Van Wyk, 2015)
<i>Warburgia salutaris</i> (Pepperbark tree)	Entire plant	Treat colds, respiratory tract complaints, and fever, malaria, influenza, coughs, and abdominal pain, constipation, cancer, rheumatism and stomach ulcers.	(Elgorashi et al., 2003; Van Wyk, 2008; Van Wyk, 2015)
<i>Eucomis autumnalis</i> (Pineapple flower)	Bulbs	Heal fractures, fever, hangover, urinary complaints, colic, stomachache, syphilis and flatulence.	(Van Wyk, 2015)
<i>Pelargonium sidoides</i> (Pelargonium)	Entire plant	Treat cough, chest trouble, bronchitis. Fever, sore throat, fatigue and weakness.	(Van Wyk, 2008)
<i>Moringa oleifera</i> (Drumstick tree)	Entire plant	Treat headaches, wounds, insect bites, fungal skin infections, gastric ulcers, diarrhea, liver problems, joint pain and malnutrition.	(Van Wyk, 2015)

Indigenous plants are the primary material used with at least 771 plant species recorded in South Africa (Dold and Cocks, 2002). An estimated 20 000 tons of indigenous plant material are being harvested from forests, grasslands and woodlands annually, with only a small fraction of the plants being cultivated (Street et al., 2008). Sustainability is a concern as 86% of the plant parts harvested lead to the death of the entire plant. This scarcity of indigenous plant material is causing a rise in the price of plant materials used in traditional medicine (Taylor et al., 2001). Table 2.1 represents the most common South African plants used in traditional medicine.

### **2.3. Plant secondary metabolites and medicine**

Plants are rich sources of compounds that can be used to develop drugs. These compounds are usually found in the leaves, roots, seeds, flowers, fruits, skins or the entire plant. Materials that are produced and stored in these plants are referred to as secondary metabolites, and may have physiological effects on living organisms (Bourgaud et al., 2001; Wink, 2015). Secondary metabolites in the plant serve as defense in protection from ecological harm. These metabolites are synthesized for a particular need and are created by modified synthetic pathways of primary metabolites (Balunas and Kinghorn, 2005). Biological studies of secondary metabolites have revealed a broad spectrum of both physiological and pharmacological properties.

Active compounds in most plants possess direct or indirect therapeutic effects. Phenolics, terpenoids and alkaloids are the three large molecule families considered, with glycosides, saponins as well as tannins being part of them according to their specific structure (Bourgaud et al., 2001). A large number of plant secondary metabolites (Table 2.2) have been identified and distinguished from primary metabolites such as nucleic acids, amino acids, carbohydrates and fats. A characteristic mix of chemicals produced varies in each plant family, genus as well as species and are at times used to taxonomically classify plants (Kabera et al., 2014). The isolation of plant secondary metabolites as medicine began in the 19<sup>th</sup> century with active compounds such as morphine being isolated from opium (Balunas and Kinghorn, 2005).

Sterols are amphiphilic molecules consisting of hydroxyl groups forming the hydrophilic heads, sterane skeletons with side chains forming hydrophobic tails. Sterols found in plants are known as phytosterols. Sterols found in mammals are known as cholesterol and plays a vital role in the function and structure of cell membranes, bile production, hormone precursor and in the immune system. Over 250 phytosterols and their related compounds have been identified (Boukes et al., 2008). Phytosterols differ from cholesterol in being alkylated at C-24 with C1 or C2 substitutes. Naturally, plants contain sterols and their associated sterolins or glycosides which are destroyed by glycosidic enzymes. Phytosterols cannot be synthesized in the human body and are consumed via diet. The commonly found phytosterols are sitosterol, campesterol and stigmasterol. Inhibition of several cell lines by phytosterols have been shown in studies (Boukes et al., 2008)

Phenolic compounds (tannins, flavonoids and phenolic acids) are secondary plant metabolites highly significant for human health found in fruits, vegetables, tea, coffee, fruit juices, wines and other plant based products (Martins et al., 2011). Phenolic acids occur mainly in bound form as components of complex structures such as lignans and hydrolysable tannins or derivatives of sugars. Two main classes of phenolic acids exist: hydroxybenzoic and hydroxycinnamic acids. The antioxidant activity of phenolic acids is related to the quality, number and position of the hydroxyl groups in the molecule (Brodniewicz and Gryniewicz, 2012). Phenolic acids are involved in repair and adaptive systems and can act preventively and therapeutically in various diseases. The relationship between total phenolic and flavonoid content and antioxidant, anti-inflammatory and antibacterial activities is strong in many plants (Balasundram et al., 2006). Plant phenols show *in vitro* antioxidant activity, inhibiting lipid peroxidation by serving as chain breaking preoxyl-radical scavengers (Miguel, 2010). Phenols directly scavenge reactive oxygen species, and those phenols that possess two adjacent hydroxyl groups bind metal ions, such as iron and copper (Michalak, 2006).



**Table 2.2: Classes of secondary metabolites and their uses**

Secondary Metabolite	Description	Functional Groups	Reference
<b>Terpenoids</b>	<ul style="list-style-type: none"> <li>• Are polymeric isoprene derivatives</li> <li>• Used for flavours, fragrances and spices</li> <li>• Possess antimicrobial and antiviral properties</li> </ul>	Steroids Carotenoids Gibberelic acid	(Tiwari et al., 2011)
<b>Alkaloids</b>	<ul style="list-style-type: none"> <li>• Contains basic carbon atoms</li> <li>• Are biosynthesized from amino acids such as tyrosine</li> <li>• Possess diverse pharmacological effects</li> </ul>	Quinine Caffeine Nicotine	(Tiwari et al., 2011)
<b>Phenolics</b>	<ul style="list-style-type: none"> <li>• Possess a common hydroxylated aromatic ring</li> <li>• Have antioxidant, anti-inflammatory, anti-carcinogenic and other biological activities</li> </ul>	Flavonoids Tannins	(Balasundram et al., 2006)
<b>Flavonoids</b>	<ul style="list-style-type: none"> <li>• First class polyphenol</li> <li>• Are water soluble pigments</li> <li>• Have anticancer, anti-inflammatory, antiviral and antioxidant properties</li> </ul>	Anthocyanins Flavones Flavonols	(Balasundram et al., 2006)
<b>Tannins</b>	<ul style="list-style-type: none"> <li>• Phenolic compounds that precipitate proteins</li> <li>• Antidiuretic and anti-inflammatory properties</li> </ul>	Hydrolysable tannins Condensed tannins	(Dai and Mumper, 2010)
<b>Glycosides</b>	<ul style="list-style-type: none"> <li>• Presence of aglycone</li> <li>• Possess anticancer and digestive properties</li> </ul>		(Dai and Mumper, 2010)
<b>Saponins</b>	<ul style="list-style-type: none"> <li>• Compound whose active portion forms colloidal solutions in water</li> <li>• Have antimicrobial and antioxidant properties</li> </ul>		(Tiwari et al., 2011)

## 2.4. *Hypoxis* species

### 2.4.1. Botanical Information

*Hypoxis* belongs to the family Hypoxidaceae, consisting of eight genera. The genus contains 130 species, with *Hypoxis hemerocallidea* (Figure 2.1) being the most widely known and used species. *Hypoxis hemerocallidea* Fisch. & C.A. Mey., was previously known as *Hypoxis rooperi* (Singh, 2007). *Hypoxis* grows as a perennial, stem less geophyte with a large black fibrous corm, which is bright yellow when cut, but oxidizes to a dark brown colour soon after cutting. The leaves of *Hypoxis* are organized into three ranks and are slightly hairy depending on the species. The star shaped bright yellow flowers appear between late October and January and the number per inflorescence varies from 2-12. *Hypoxis* is indigenous to Southern Africa, but has been reported growing in the meanders, grasslands and mountains of South America, Australia and Asia, showing a worldwide cosmopolitan distribution (Singh, 2006)



Figure 2.1: *Hypoxis hemerocallidea* (HLEM, 2018).

### 2.4.2. Traditional and contemporary use

*Hypoxis* corms are the sought after part of the plant for dietary and medical use with *Hypoxis* species commonly used interchangeably in traditional medicine, due to close taxonomical similarities. Table 2.3 shows commonly used *Hypoxis* species and their traditional uses. Aqueous infusions are often used as a tonic to treat dizziness in children and mental disorders in adults.

*Hypoxis* is widely used in Zulu medicine for a range of illnesses including heart disease, bad dreams, insanity, anxiety, intestinal parasites, urinary tract infections, barrenness and other ailments (Elgorashi et al., 2003; Boukes et al., 2008; Pereus et al., 2018). *Hypoxis hemerocallidea* is the most commonly used *Hypoxis* species in traditional medicine, with related species being *Hypoxis colchicifolia*, *Hypoxis obtusa*, *Hypoxis nyasica* and *Hypoxis angustifolia* which are used to treat relative ailments. *Hypoxis obtusa* is traditionally used for abdominal pains, heart pains, infertility in women, bile emesis, gonorrhoea and as an aphrodisiac (Zimudzi, 2014). *Hypoxis* have also been used to treat ailments in cattle by Tswana people. Xhosa people use fresh cut corms as a facial treatment and to treat burns. Crude unprocessed corms are sold and used by traditional healers and retail pharmacies for phytomedicines, formulations and preparations. These preparations are sold with many unsubstantiated therapeutics against HIV/AIDS, arthritis, hypertension, diabetes mellitus, asthma, cancer, psoriasis, epilepsy and tuberculosis (Zimudzi, 2014). Antiviral and antifungal formulations have been made into dermatological products with *Hypoxis* extracts used in Germany since the late 1970's for prostate adenoma and benign prostate hypertrophy treatment (Khan and Drewes, 2004)

#### **2.4.3. Biological activity**

*Hypoxis* has been assessed for its biological effects with methanolic extracts of *Hypoxis hemerocallidea* corms showing no activity against *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* (Katerere and Eloff, 2008). Acetone and ethanol extracts of freshly harvested corms and leaves have shown relatively good activity against *E. coli* and *Pseudomonas aeruginosa*. Ethanol and aqueous extracts of *Hypoxis hemerocallidea* show inhibition of *E. coli*, however it does not show a high enough therapeutic dose to be used in humans, indicating the reason for *Hypoxis* being used to treat prostatitis only, due to poor antimicrobial activity (Katerere and Eloff, 2008; Ncube et al., 2013). A study by Ojewole (2006) showed significant antinociceptive, anti-inflammatory and anti-diabetic potential of aqueous extracts of *Hypoxis hemerocallidea* corms. With extracts 50-800 mg/kg showing significant inhibition of fresh egg albumin-induced acute inflammation and caused dose related hypoglycaemia in normoglycaemic and diabetic rats (Goboza et al., 2016) as well as dose dependant antinociceptive effects against induced nociceptive pain stimuli in rats (Onanuga et al., 2018).

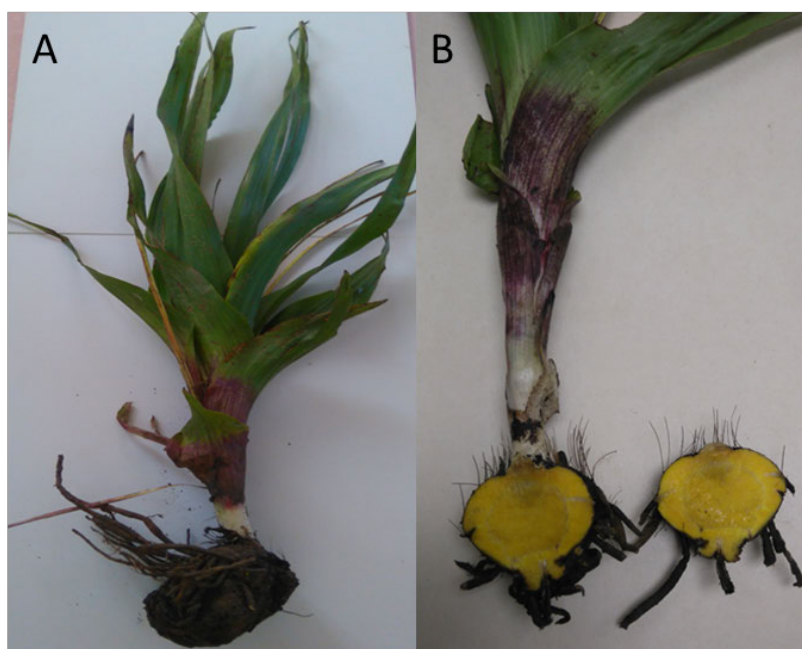
In a phytochemical screening of *Hypoxis hemerocallidea*, *Hypoxis rigidula*, *Hypoxis galpinii* and *Hypoxis obtusa* by Zimudzi (2014), methanolic extracts of the corms of all four species tested showed qualitative presence of terpenoids, saponins, cardiac glycosides, tannins as well as reducing sugars and were negative for flavonoids, alkaloids and anthraquinones. In the same study, all four species that were screened for cytotoxicity using the brine shrimp lethality bioassay were shown to be non-toxic. A study by Ramulondi et al. (2018) showed, key phytochemical compounds found in *Hypoxis*, hypoxoside and rooperol to possess good antimutagenic and cytotoxic properties in relation to other plant compounds tested in the study. The methanol extracts of *Hypoxis* possess limited toxicity when tested against Vero cells and displayed no anticancer activity in a HeLa cell line (Madikizela and McGaw, 2019).

**Table 2.3: *Hypoxis* species commonly used in South African traditional medicines**

Species	Traditional Uses	Known Chemical Constituents	Reference
<i>Hypoxis hemerocallidea</i>	Used as a cure for headaches, dizziness, dysentery, stomach ailments, bladder disorders, burns, HIV, cancer, high blood pressure, skin rashes, wounds, pimples, dermatitis, symptoms of benign prostate hypertrophy, diabetes, mental disorders and as a traditional tonic for good health.	Phytosterol, glucosides, diglucoside hypoxoside, aglycone rooperol, sterols and sterolins	(Zimudzi, 2014)
<i>Hypoxis colchicifolia</i>	Used against bad dreams, weak heart, barrenness, as a snake trap, emetic, treatment for nausea, insect bites and to destroy pests in food.	Haemanthine, hypoxoside	(Amusan et al., 2005)
<i>Hypoxis obtusa</i>	Used to treat infertility, urinary infections, abdominal pains and as an aphrodisiac.	Hypoxoside, obtusaside	(Zimudzi, 2014)
<i>Hypoxis rigidula</i>	Used to treat various ailments in humans such as asthma and arthritis, and gall sickness in cattle.	Phenolic compounds and organic acids.	(Zimudzi, 2014)
<i>Hypoxis gerrardii</i>	Used to treat dysentery and stomach ailments		(Ncube et al., 2013)
<i>Hypoxis obliquaoblique</i>	Used to treat wounds		(Ncube et al., 2013)
<i>Hypoxis argentea</i>	Used as a good luck charm, used on teats of cows to treat cracks and eaten as a food source.		(Amusan et al., 2005)

## 2.5. *Hypoxis colchicifolia*

*Hypoxis colchicifolia* (Figure 2.2) is commonly referred to as broad leaved *Hypoxis*, 'igudu', 'ingcobo' and 'ilabatheka', in Zulu. It is one of the four most sought after plant species in traditional medicine (Singh, 2007).



**Figure 2.2: *Hypoxis colchicifolia* post-harvest [A] and a cross section of *H. colchicifolia* corm [B]**

### 2.5.1. Taxonomy

*Hypoxis colchicifolia* is a South African endemic plant found distributed in both coastal and inland regions, such as the Eastern Cape, KwaZulu-Natal as well as the Free State. *Hypoxis colchicifolia* is a robust plant, relatively taller than other *Hypoxis* species, about 250-500 mm in height and growing singly, nearly glabrous, unlike other species (Singh, 2007).

The rhizome referred to as the corm is oblong or globose in shape about 40-70 mm in diameter, and often longer than wider, with contractile roots, light yellow to orange inside with an aromatic fragrance. This species possesses a false cylindrical stem with an arrangement of 4-8 clasping leaves that resembles a funnel. Leaves are flat and erect with ribbed veins.

The 200-600 mm tall leaves are bright green with it appearing purple and white near the base, which connects with corm. It has inflorescences of bright yellow flowers that crown the plant (Singh, 2007). *Hypoxis colchicifolia* grows in the most robust of habitats compared to other Southern African species (Appleton et al., 2012), preferring full sun and well-drained soil. Traditionally a decoction made by adding 100 g of sliced corms to 2 L of boiled water is taken for debility by Swazi people, with the dose being 10 mL once or twice a day. Corms are used against barrenness, heart weakness and bad dreams. Infusions of the corm are taken in small quantities as a tea to stop nausea, vomiting, anxiety, to calm the heart, improve appetite, induce good sleep and even as a treatment for diabetes (Bisi-Johnson et al., 2017). The infusion is taken in larger quantities to promote vomiting and as an enema.

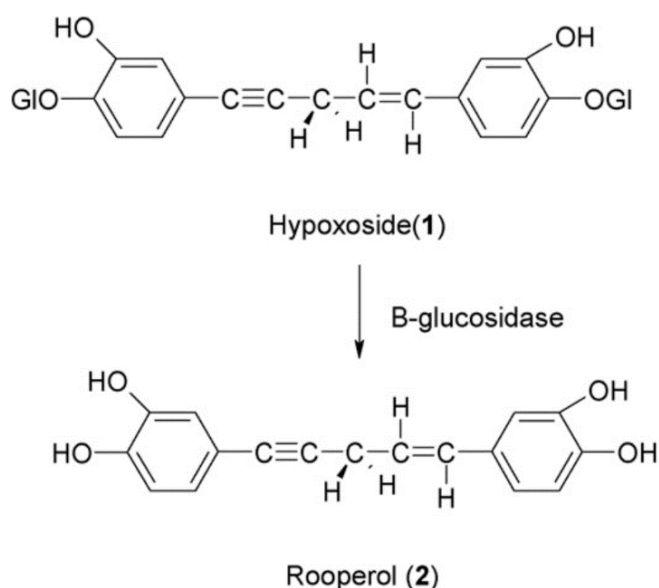
### **2.5.2. Known constituents**

*Hypoxis colchicifolia* corms have a few known constituents such as haemanthine, hypoxoside and its aglycone rooperol (Chavan et al., 2013). No phytochemical identification has been done on the leaves and flowers due to these parts not traditionally being used (Katerere and Eloff, 2008).

#### **2.5.2.1. Hypoxoside and rooperol**

A major constituent of *Hypoxis* species corms is the pent-1-en-4-yne derivative, hypoxoside (Figure 2.3) (Boukes et al., 2008). Hypoxoside is regarded as a natural product with a built-in protecting group of glucose substituents on both ends of the chain. Hypoxoside is supposed to be pharmacologically inactive, as it is presumed to be transformed into its biologically active aglycone form rooperol in the large intestine by bacterial  $\beta$ -glucosidase activity. Hypoxoside does not exhibit toxicity as an oral prodrug for cancer therapy in early clinical trials (Laporta et al., 2007). Hypoxoside shows promising anticancer activity with *in vitro* conversion catalysed by  $\beta$ -glucosidase of nontoxic hypoxoside to cytotoxic rooperol, showing growth inhibition against 60 human cell lines including melanoma, lung, breast, colon and uterus cancer cell line (Drewes et al., 2008). The active compound rooperol (Figure 2.3), is obtained via hydrolysis of hypoxoside (Khan and Drewes, 2004). The potential of rooperol as an active biological agent is yet to be fully explored.

Rooperol is an enhanced antioxidant due to the presence of two ortho phenolic groups on the benzene rings (Drewes et al., 2008). The anti-inflammatory action of rooperol and its analogues is more likely to take place through 5-lipoxygenase pathway inhibition rather than that of cyclo-oxygenase. Rooperol is capable of stimulating the synthesis of collagen type 1, exerting antimetastatic activity, and impeding tumour cell invasion.



**Figure 2.3: Chemical structure of Hypoxoside and rooperol (Drewes et al., 2008)**

## 2.6. The role of medicinal plants in non-communicable diseases

Non-communicable diseases (NCDs) or chronic diseases are not diseases that are transferred from person to person but a result of genetic, environmental and physiological factors. An unhealthy lifestyle characterized by unhealthy diet, lack of physical exercise and substance abuse increase the frequency of oxidative stress and leads to NCDs. This results in metabolic risk factors such as hyperglycaemia, hypertension and obesity, with all leading to premature death (Wafula et al., 2017). The increased risk of developing NCDs can be associated to dietary risk factors, with poor nutrition being a major factor. The consumption of high energy dense and low nutritive value foods are on the rise (Koch, 2019). Foods such as fruit and vegetables, rich in phytochemicals and antioxidants are not being consumed as required (Grosso, 2018). Antioxidants are the first line of defence against oxidative stress and the role of antioxidants needs sufficient evidence in the control of NDCs.

### 2.6.1. Free radicals, oxidative stress and antioxidants

Free radicals have beneficial effects on organisms and play a vital role in biological evolution. Oxygen radicals are crucial in actions such as gene transcription, signal transduction and regulation of soluble guanylate cyclase activity in cells (McCord, 2000). Nitrogen free radicals (NO) are part of every cellular and organ function in the body (Halliwell, 2001). These nitrogen free radicals serve as neurotransmitters when produced by neurons, and serve as important mediators of the immune response when generated by activated macrophages. However free radicals that contain iron-sulphur centres and other relative species cause oxidation of biomolecules such as amino acids, lipids, DNA and proteins which leads to cell injury (Figure 2.4) and cell death (McCord, 2000). These reactive oxygen species (ROS) are defined as molecules having an unpaired electron in the outer orbit. An antioxidant is a molecule capable of inhibiting the oxidation of another molecule. Antioxidants break the free radical chain of reactions by sacrificing their own electrons to feed free radicals, without becoming free radicals themselves (Fang et al., 2002; Duthie et al., 2003). Antioxidants are nature's way of defending cells against attack by reactive oxygen species (ROS).

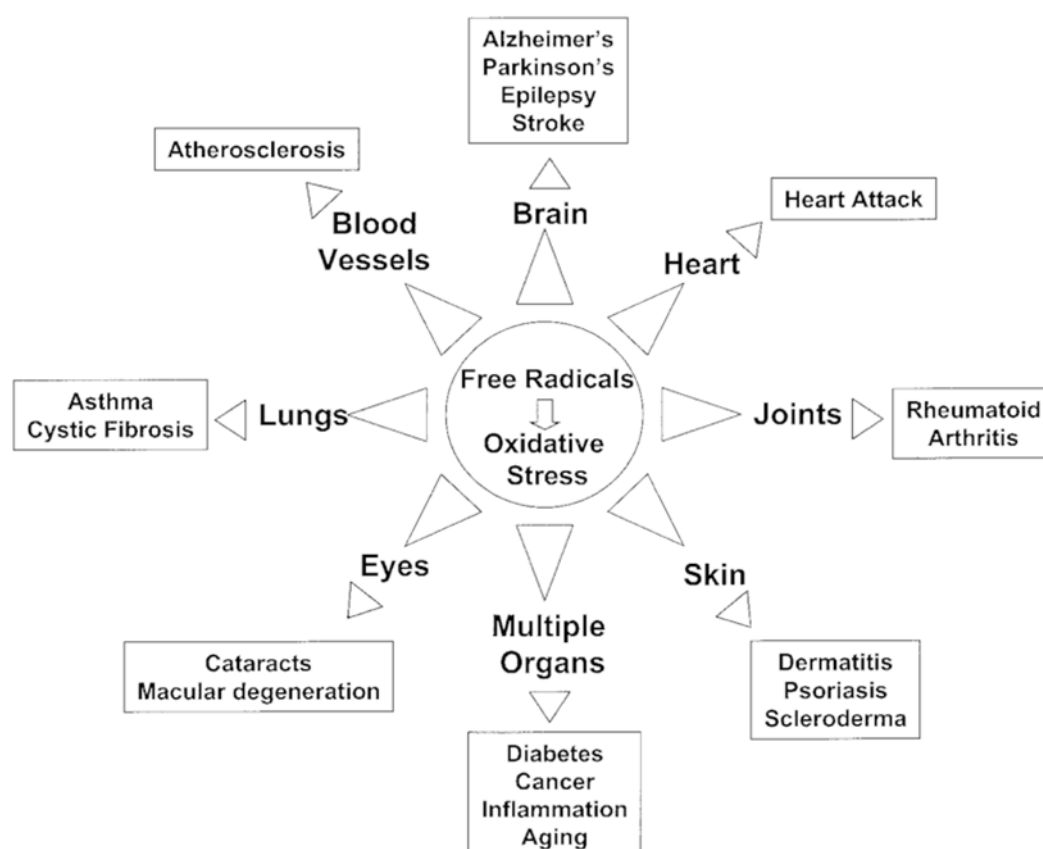


Figure 2.4 : Oxidative stress and the diseases it can cause (Evans and Goldfine, 2000).



When the free radicals in the body outnumber the antioxidant defences, this state is referred to as oxidative stress (Shahidi and Ambigaipalan, 2015). Oxidative stress causes serious cell damage leading to a variety of human diseases (Figure 2.4) such as Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer, arthritis, immunological incompetence and neurodegenerative disorder (Surveswaran et al., 2007; Pandey and Rizvi, 2009). The activity of an antioxidant is governed by its reactivity as a hydrogen electron-donor, the outcome of the antioxidant derived radical that's produced, its ability to stabilize the unpaired electron, its reactivity with other electrons and its metal chelating potential (Badarinath et al., 2010). Antioxidants work by various mechanisms; by free radical scavenging activity, donating hydrogen to radicals, metal chelating activity, reducing power, quenching singlet oxygen and inhibition of  $\beta$ -carotene (Figure 2.4).

The body naturally circulates a variety of nutrients for their antioxidant properties and manufactures antioxidant enzymes in order to control these destructive chain reactions (Hässig et al., 1999). Vitamin C, vitamin E, carotenes and lipoic acid are well known and extensively researched antioxidants. Plant polyphenols, such as tannins and flavonoids serve as plant-based antioxidants, with man relying on plants for their source of antioxidants as it is not synthesized in the body.

#### **2.6.2. Analytical methods for determining the antioxidant properties**

Most of the antioxidant assay methods only measure one of the above. *In vitro* antioxidant assays can only rank the antioxidant activity of the test compound according to their reaction system.

##### **2.6.2.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay**

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an added electron gives a maximum absorption at 517 nm (purple colour). When antioxidants react with DPPH, which is a stable free radical, and it becomes paired off in the presence of a hydrogen donor it is reduced to the DPPH and as a consequence the absorbance decreases from the DPPH. Radical to the DPPH-H form, this results in decolourization (yellow colour) with respect to the number of electrons captured (Shekhar and Anju, 2014).

#### **2.6.2.2. Phosphomolybdenum (PM) Assay**

Principle of this method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite and this causes a reduction in intensity of the blue colour. The basic principle to assess the antioxidant capacity through phosphomolybdenum assay includes the reduction of Mo (VI) to Mo (V) by the plant extract possessing antioxidant compounds (Zia-UI-Haq et al., 2013).

#### **2.6.2.3. 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assay**

Trolox equivalence antioxidant capacity (TEAC) assays use intensely coloured cation radicals of ABTS to test ability of antioxidants to quench radicals. This generates ABTS cations by first using oxidising agents such as potassium persulfate and manganese dioxide, then adding antioxidants and measuring the direct reaction with the radical (Re et al., 1999).

#### **2.6.2.4. Ferric Reducing Antioxidant Power (FRAP) assay**

When a ferric- tripyridyltriazine ( $\text{Fe}^{\text{III}}$ -TPTZ) complex is reduced to the ferrous ( $\text{Fe}^{\text{II}}$ ) form, an intense blue colour develops. The reaction is nonspecific, and any half-reaction which has a less-positive redox potential will drive  $\text{Fe}^{\text{III}}$ -TPTZ reduction. Test conditions favour reduction of the complex and, thereby, colour development, provided that an antioxidant is present (Benzie and Szeto, 1999).

#### **2.6.2.5. Cupric ion reducing antioxidant capacity (CUPRAC) Assay**

The CUPRAC method is a simple and versatile antioxidant capacity assay utilizing the copper(II)-neocuproine [ $\text{Cu}(\text{II})$ -Nc] reagent as the chromogenic oxidant. It involves mixing the antioxidant solution with solutions of  $\text{CuCl}_2$ , neocuproine, and ammonium acetate. Slowly reacting antioxidants required an incubation for colour development (Apak et al., 2008).

### **2.7. Oxidative stress related conditions**

#### **2.7.1. Diabetes mellitus**

Diabetes mellitus is a metabolic disease classified by chronic hyperglycaemia resulting from defect in insulin secretion, insulin action or both (Kharroubi and Darwish, 2015). Diabetes results from the deficiency or resistance to insulin in the human body (Thaifa et al., 2017). Diabetes mellitus poses a major global health treat and affects more than 422 million worldwide, which is an estimated 8.5% of the global population.

Diabetes is rising in prevalence in middle to low income countries and has a mortality rate of more than 80% in developing countries (Zheng et al., 2018). According to the International Diabetes Federation (IDF), 7% of South African adults (21-79 years of age), which is an estimate of 3.85 million of the population, are diabetic (Atlas, 2015). This estimate merely touches the surface of a deeper problem, due to a group of the population being undiagnosed. An alarming 60-80% of the diabetic population in South Africa die before the age of 60. Diabetes is a major cause of blindness, kidney failure, stroke, limb amputation and heart attacks. In a hyperglycaemic condition, the excessive glucose will not only metabolize from glycolysis but the polyol pathway in which glucose is converted to sorbitol. Hyperglycaemia is a condition characterized by an abnormal excess of sugar in the blood. It is linked to the onset of type 2 diabetes and associated with hypertension. Hyperglycaemia has been linked to the onset of cardiovascular diabetic complications and triggers the generation of free radicals as well as oxidation-related damage to various organs by stimulating oxidative stress. Oxidative stress has been repetitively shown to be a hallmark of many diseases linked to metabolic or vascular disorders including diabetes and hypertension (De Boer et al., 2017). Hence it is important to control both blood glucose levels and cellular redox status for managing diabetic complications.

#### **2.7.1.1. Conventional management of diabetes**

Conventional anti-diabetic drugs regulate and manage the blood glucose levels below the normal range by either supplementing insulin, increasing insulin secretion from the pancreas, improving insulin sensitivity or decreasing glucose absorption from the intestinal tract and glucose uptake by cells (Rasouli et al., 2017). Insulin secretagogues such as sulfonylureas and meglitinides; insulin sensitizers such as biguanides, metformin as well as thiazolidinediones;  $\alpha$ -glucosidase inhibitors such as miglitol and acarbose, are the type of glucose lowering drugs that are currently used (Adinortey et al., 2019). Majority of these glucose lowering drugs have severe side effects including severe hypoglycaemia, idiosyncratic liver cell injury, lactic acidosis, digestive discomfort, headaches, permanent neurological deficit, dizziness and even death. Conventional treatments for diabetes without side effects are still a huge challenge. However, there are about 800 medicinal plants used in the control of diabetes in alternative treatment methods, with 450 of them being scientifically validated and about 100 of which the mechanism of action has been established (Shapiro and Gong, 2002).

#### **2.7.1.2. Plant based treatment**

There are hundreds of different natural plant-based medicines being used to combat diabetes with only a few that exhibit reliable evidence of effectiveness. The phytochemicals of the plant-based medicines work through various metabolic pathways which involve glucose or glucose derivatives as the substrate or product. They affect glucose metabolism, pentose phosphate pathways, absorption of glucose via alimentary canal and increase the insulin production and release. The plant-based medicines modulate blood sugar levels via various mechanisms and exhibit efficiency similar to that of conventional medicine; and serve as hypoglycaemic agents, insulin sensitizers, carbohydrate absorption inhibitors. Common plants that are effective against hyperglycaemia are *Panax ginseng* (Ginseng), *Momordica charantia* (Bitter melon), *Coptis chinensis*, *Trigonella foenum-graecum* (Fenugreek), *Lagerstroemia speciosa*, *Gymnema sylvestre*, *Cinnamomum cassia* (Cinnamon) and *Agaricus* mushroom (Shapiro and Gong, 2002). Plant sources that possess phytochemicals including flavonoids, alkaloids, glycosides, glycolipids, polysaccharides, peptidoglycans, amino acids, carbohydrates and saponins have been reported to have hypoglycaemic activity (Guasch-Ferré et al., 2017).

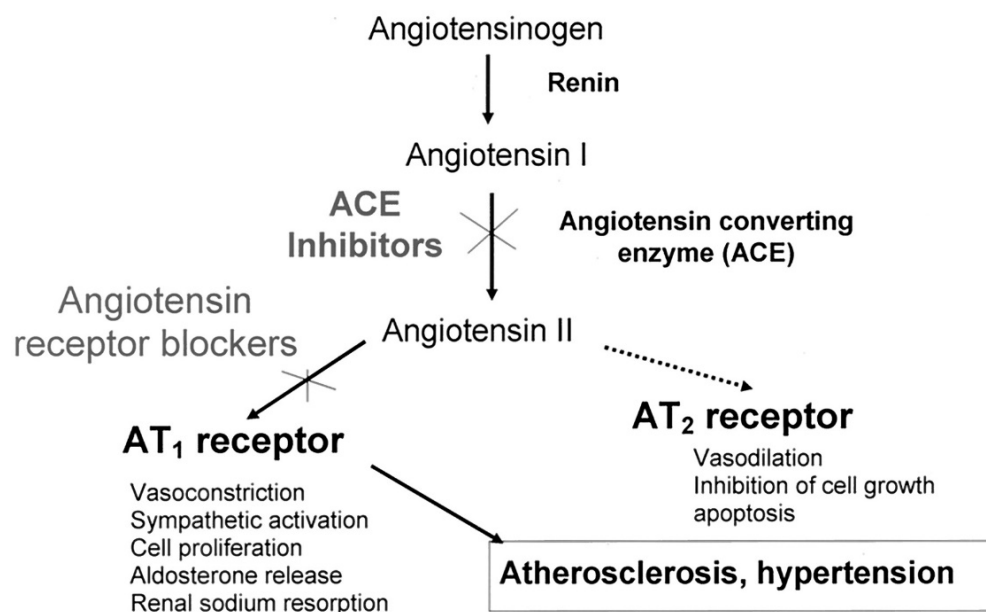
#### **2.7.2. Hypertension**

Hypertension refers to blood pressure higher than normal blood pressure, which is 120 over 80 mm of Mercury (mmHg), with hypertension being anything higher (De Boer et al., 2017). Hypertension is a global health concern, with the WHO suggesting that the increase in salt intake due to the processed food industry is the key role player in the high prevalence of hypertension (WHO, 2018). Hypertension leads to severe complications and increase the risk of stroke, heart disease and death (Gupta et al., 2017b). An unhealthy diet, lack of physical exercise, tobacco usage and hefty alcohol consumption leads to raised blood pressure, blood glucose levels and obesity; and aids the risk of becoming hypertensive (Gupta et al., 2017a; Gupta and Xavier, 2018; Sajeew and Soman, 2018). In 2015 over 1.1 billion people worldwide were recorded as being hypertensive, with 7.5 million people dying due to hypertension related heart disease and stroke (Tripathy et al., 2017). Currently it is estimated that 40% of South Africans are hypertensive with approximately half of them unaware of their condition.

Diuretics, beta blockers, alpha blockers, calcium channel blockers, central agonists, peripheral adrenergic inhibitors are drug types used to help lower blood pressure as hypertensive treatment (Seedat and Rayner, 2012). However, angiotensin 1- converting enzyme (ACE) inhibitors are the most widely used group of drugs used to lower blood pressure. Such ACE inhibitors are captopril, fosinopril, ramipril and enalapril (Khan and Kumar, 2019). Antihypertensive drugs may be highly effective but cause many adverse side effects such as reduced renal function, dry cough, skin problems, nausea, fatigue, angioedema, dizziness, hypotension and renal impairment (Patel and Gamit, 2018).

### 2.7.2.1. Angiotensin mechanism

The renin angiotensin system (RAS) plays a crucial role in the blood pressure regulation with angiotensin 1- converting enzyme (ACE) and renin as the main regulators, controlling the RAS pathway (Figure 2.5). Blood pressure regulation is due to renin converting angiotensinogen to angiotensin I (AT-1), which is converted by ACE to angiotensin II (Ang II). Angiotensin II is a potent vasoconstrictor, which induces the release of aldosterone, which increases the sodium concentration and blood pressure. Bradykinin is a potent vasodilator, when it is hydrolyzed by ACE it leads to the inability of blood vessels to relax following contraction. Therefore, limiting the formation of angiotensin II and the destruction of bradykinin by inhibiting ACE will contribute to lowering blood pressure (He et al., 2013).



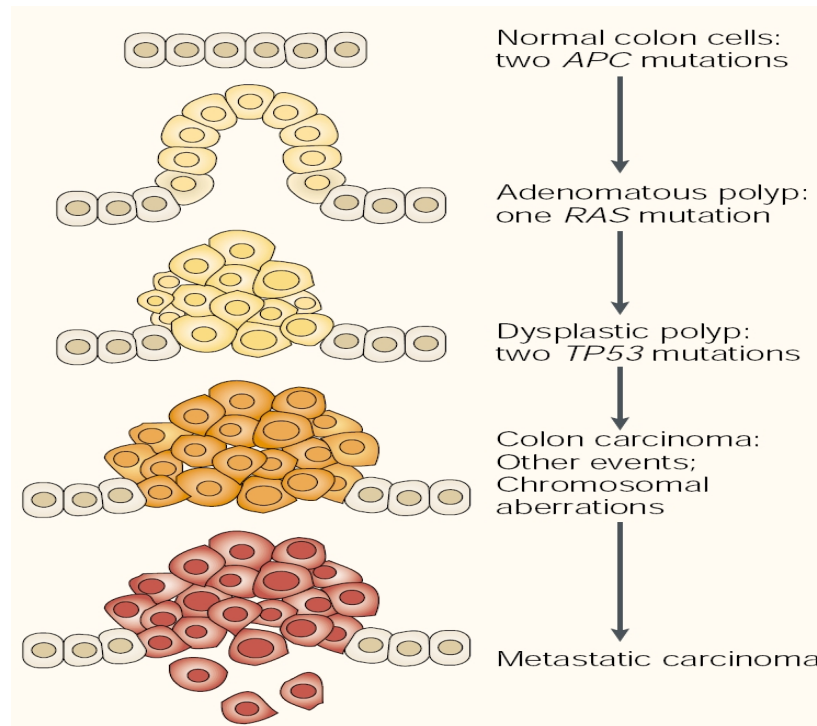
**Figure 2.5: Angiotensin pathway (Association, 2018)**

### **2.7.2.2. Medicinal plant-based therapy**

Many studies have shown that plant extracts that are rich in phytochemicals are effective in inhibiting ACE, but identification of single compounds that are responsible is lacking (Balasuriya and Rupasinghe, 2011). Secondary plant metabolites exhibit antihypertensive effects, with many common plants being researched as natural alternatives. Examples include *Allium sativum*, *Cuminum cyminum*, *Cinnamomum zeylanicum* and many others that are traditionally used around the world (Ranilla et al., 2010; Al Disi et al., 2016; Anwar et al., 2016). Plant derived compounds such as terpenoids and polyphenolic compounds like hydrolysable tannins, flavonoids, procyanidins, xanthones and caffeoylquinic acid derivatives are known to possess *in vitro* ACE inhibitory activities (Braga et al., 2007).

### **2.7.3. Cancer**

Cancer is the second leading cause of mortality worldwide with an estimated 9.6 million cancer related deaths in 2018. Deaths from cancer in low to middle income countries account for 70% of the total estimate. This is mainly due to the lack of appropriate cancer treatment infrastructure and the prevalence of infectious diseases caused during cancer diagnosis (Khelifi et al., 2013). The most common cancers affecting the global population, in order of most commonly reported cases are lung, breast, colorectal, prostate, skin cancer (non-melanoma) and stomach cancer. Lung, colorectal, stomach, liver and breast cancer are the most common forms of cancer that caused mortality in 2018. Cancer arises from the transformation of normal healthy cells to tumour cells leading to a malignant tumour (Figure 2.6). This is a result due to personal genetics and the types of external agents, which are physical carcinogens, chemical carcinogens and biological carcinogens. Physical carcinogens refer to ultraviolet and ionizing radiation; chemical carcinogens are substances from asbestos, aflatoxin, arsenic and compounds found in tobacco smoke; and biological carcinogens are infections from certain bacteria, parasites and viruses.



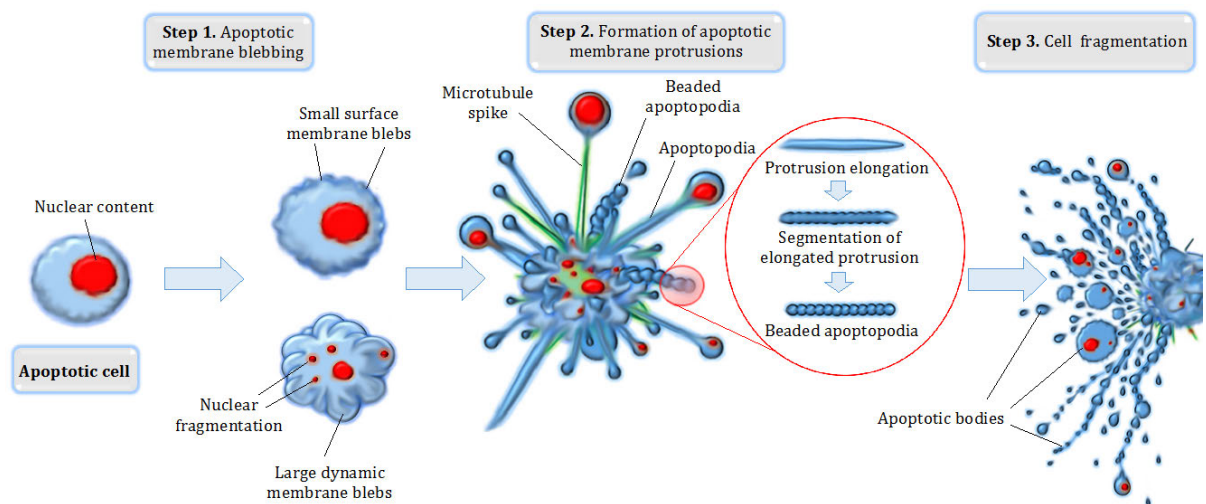
**Figure 2.6: Mutation and tumour formation due to mutations caused by carcinogens (Nature, 2014).**

Metastasis is when a tumour spreads to different parts of the body, growing, invading and destroying the healthy cell tissue. Once this takes place, it becomes highly serious and extremely difficult to treat. Ultimately, cancer is the result of uncontrollable cell growth without death, as normal cells grow, divide and die. Cancer cells do not experience apoptosis, but instead continue growing and dividing leading to masses of abnormal cells growing out of control.

#### **2.7.3.1. Importance of apoptosis in cancer treatment**

A good anticancer drug should be able to induce apoptosis. Apoptosis is the physiological processing of cell death, which is vital for normal cell development and functioning of multicellular organisms. Apoptosis, or programmed cell death (Figure 2.7), evolved as a rapid and irreversible process to efficiently eliminate dysfunctional cells. Apoptosis is signaled by two main independent pathways which are caspase cascade and stress activated protein kinase pathways. Caspase cascade is either activated from the surface of the cell or the mitochondria (Green and Reed, 1998).

Both apoptosis pathways converge on a common mechanism of cell destruction, which is activated by cysteine proteases that cleave proteins at aspartate residues (Makin and Dive, 2001). The dismantling and removal of dead cells is done by proteolysis of major cellular constituents, DNA degradation and phagocytosis of the neighbouring cells (Cotter, 2009).



**Figure 2.7: Apoptosis mechanism of programmed cell death (Smith et al., 2017).**

### 2.7.3.2. Conventional cancer treatment

Conventional treatments are allopathic treatments that are commonly used as the main treatment of an illness due to its proven effectiveness and reliability of its efficiency. Conventional treatments for cancer include:

- i. Surgery - The physical removal of cancerous tumours
- ii. Radiation - high doses of radiation are used to kill cancer cells and shrink tumours.
- iii. Chemotherapy - uses drugs to kill cancer cells
- iv. Hormone therapy - Uses hormones to slow or stop the growth of cancers like breast and prostate cancer.
- v. Stem cell transplant - This is done to restore lost blood forming stem cells in patients who have destroyed them during chemotherapy or radiotherapy.

Conventional cancer treatments such as chemotherapy and radiation cause an array of side effects; this often affects healthy tissue and organs.



Side effects caused are anaemia, bleeding and bruising, loss of appetite, delirium, constipation, swelling, fertility issues, fatigue, hair loss, nausea as well as vomiting, pain, nervous problems, skin changes, nail changes, urinary difficulties, bladder problems and sleep disruptions.

#### **2.7.3.3. Plant based anticancer treatment**

Phytochemical evaluation of many medicinal plants and herbs are investigated to understand their anti-tumour action against various types of cancer. About 60% of anticancer agents that are currently used come from natural sources. These include vinca alkaloids, taxanes, podophyllotoxin, camptothecin, anthracyclines and others (Patel, 2016; Assaf et al., 2013). The use of plant material as anticancer agents began with the isolation of vinca alkaloids, vinblastine and vincristine from *Catharanthus roseus* (Chavan et al., 2013). This initial discovery advanced the clinical use of plant based anticancer agents. Vinblastine and vincristine are currently used in combination with other cancer chemotherapeutic drugs in the treatment of cancers like leukaemia's, testicular cancers, lymphomas, and lung and breast cancers. Paclitaxel was discovered from the bark of the *Taxus brevifolia* tree and has been used to treat non-cancerous cases by Native American tribes until it was discovered to be significantly active against ovarian, lung and breast cancers (Chavan et al., 2013). Camptothecin isolated from *Camptotheca* initially showed severe bladder toxicity, however topotecan and irinotecan, semisynthetic derivatives of camptothecin are used in the treatment of ovarian, colorectal and small cell lung cancers (Chavan et al., 2013). Epipodophyllotoxin is an isomer of podophyllotoxin which was isolated from the roots of *Podophyllum* species and is an active antitumour agent. Semisynthetic derivatives of epipodophyllotoxin, etoposide and teniposide are used in treating lymphomas, bronchial and testicular cancers (Chavan et al., 2013).

## Chapter 3: Antioxidant activity and phenolic content of *Hypoxis colchicifolia*

---

### Abstract

*Hypoxis* species are used extensively in traditional medicine in southern Africa. *Hypoxis colchicifolia* is one of four main species that are used to treat an array of ailments. *Hypoxis colchicifolia* corms are traditionally eaten and brewed as a tea to treat diabetes, stop nausea as well as vomiting and as a tonic for good health. Traditionally the corms are used, with the leaves being discarded upon harvesting. Providing a rationale and indicating the toxicity of the plant is essential for future use of the plant as a natural alternative to allopathic medicine. In this study *H. colchicifolia* corm and leaf extracts were qualitatively assessed for their phytochemical constituents. The total phenolic content was determined using the Folin Ciocalteu method, with the toxicity screened using the brine shrimp lethality assay. The antioxidant potential was evaluated using the DPPH, ABTS, PM, CUPRAC and FRAP radical scavenging methods. All extracts showed the presence of key phytochemical constituents and showed no toxicity. There was a major difference in the total phenolic content of the leaves compared to the corms, with corm extracts possessing a much higher total phenolic content. Extracts showed good antioxidant potential with different extracts inhibiting different free radicals' indicating selective antioxidant scavenging. The extracts of leaves and corms cannot be used interchangeably as there are differences in the phytochemical composition. According to this study protocol, all extracts exhibit promising antioxidant potential, which validates the use of this species as a tonic for good health.

### 3.1. Introduction

Antioxidants are nature's way of defending cells against attack by reactive oxygen species (ROS). When the free radicals in the body outnumber the antioxidant defenses, this state is referred to as oxidative stress (Shahidi and Ambigaipalan, 2015). Oxidative stress causes serious cell damage; leading to a variety of human diseases like Alzheimer's, Parkinson's, atherosclerosis, cancer, arthritis, immunological incompetence and neurodegenerative disorder (Surveswaran et al., 2007; Pandey and Rizvi, 2009). The body naturally circulates a variety of nutrients for their antioxidant properties and manufactures antioxidant enzymes in order to control these destructive chain reactions (Hässig et al., 1999).

Vitamin C, vitamin E, carotenes and lipoic acid are well known and well researched antioxidants. Most of these antioxidants are found in the foods and beverages that we consume and supplement our health and wellbeing.

Plant secondary metabolites are usually classified by their biosynthetic pathways. Phenolics, terpenoids and alkaloids are the three large molecule families considered, with glycosides, saponins as well as tannins being part of these groups according to their specific structure (Bourgaud et al., 2001). Biological studies of secondary metabolites have revealed a broad spectrum of physiological and pharmacological properties. The antioxidant potential of a plant extract is due to the various free radical scavenging molecules present (Choi et al., 2002), with each phytochemical constituent in the extract having certain biological activities due to their chemical structure (Tiwari et al., 2011).

*Hypoxis colchicifolia* is commonly referred to as broad leaved *Hypoxis*, 'inkomfe', 'igudu', 'ingcobo' and 'ilabatheka', in Zulu, is found in both coastal and inland regions, such as the Eastern Cape, KwaZulu-Natal as well as the Free State. *Hypoxis colchicifolia* is a robust plant, relatively taller than other *Hypoxis* species, about 250-500 mm in height and growing singly, nearly glabrous, unlike other *Hypoxis* species (Singh, 2006; Singh, 2007). It is one of the four most sought after plant species in traditional medicine (Ncube et al., 2013). *Hypoxis colchicifolia* corms have a few known constituents such as haemanthine, hypoxoside and its aglycone rooperol (Amusan et al., 2005; Chavan et al., 2013). The phytochemical identification has not been carried out on the leaves and flowers, with most studies focusing on the corms due to its extensive use in traditional medicine. The leaves are not used traditionally, though they may contain important phytochemical constituents that are beneficial to human health. Therefore, this study is aimed to compare the phytochemical composition, toxicity and antioxidant potential of *H. colchicifolia* corm and leaf extracts. *In vitro* antioxidant assays can only classify antioxidants for their particular reaction mechanism; therefore, the use of multiple antioxidant assays is necessary in antioxidant screening.

## **3.2. Methodology**

### **3.2.1. Collection of plant material**

*Hypoxis colchicifolia* was collected and identified using taxonomic keys by Professor H. Baijnath from the School of Life Science, University of KwaZulu-Natal (UKZN). Sampling site was allocated in Mooiriver, Kwa-Zulu Natal, South Africa. A voucher specimen of the authenticated plant material was deposited to the Ward Herbarium (WARD) at UKZN (Westville campus) (Voucher Moodley & Baijnath No 1.).

### **3.2.2. Preparation of plant material**

#### **3.2.2.1. Fresh Corm**

Corms of *Hypoxis colchicifolia* were washed, dried, peeled and grated. Grated fresh corms were ground to a pulp using a mortar and pestle. The fresh corm (150 g) pulp was mixed with different solvents (acetone, methanol, distilled water) in the ratio 1:4 w/v. This was stirred for 48 h on a rotary shaker then samples were filtered using Whatman No. 1 filter paper. The solvents were then evaporated using a Bauchi Rotary evaporator with the remaining extract air dried further.

#### **3.2.2.2. Dried Samples**

Dried corms were peeled, grated and allowed to dry completely. The dried corms were coarsely ground in an industrial grinder (Retsch GmbH, West Germany), then stored in labelled Schott bottles in cool dark place until further use. Leaves were thoroughly washed and dried completely. The dried leaves were then ground. The milled sample was stored in an airtight bottle until further use. Milled corms (20 g) and leaves (20 g) were extracted in a ratio 1:20 using acetone, methanol and distilled water. Samples were allowed to shake at 200 rpm for 48 h on a rotary shaker and filtered. Solvents from the respective filtrates were evaporated using rotary evaporator to concentrate the extract.

### **3.2.3. Phytochemical Screening**

Phytochemical screening was conducted using standard qualitative methods by Tiwari et al. (2011) with minor modifications. Test for alkaloids: Extracts were treated with Dragendroff's reagent, formation of a red precipitate indicated presence of alkaloids.

Test for saponins: Five millilitres of the extract were shaken vigorously and observed for a stable persistent froth, three drops of olive oil were added and shaken vigorously after which the formation of an emulsion observed. Test for tannins: 10 mL of aqueous solution of extracts were boiled and filtered. A few drops of 0.1% ferric chloride were added and observed for a blue-black or brownish green colouration. Ferric chloride test: extracts were treated with 3-4 drops of ferric chloride solution. Formation of a blue black colour indicated presence of phenols. Test for Terpenoids: Extracts were suspended in chloroform and concentrated sulphuric acid was carefully added to form a layer. A reddish brown colour at the interface indicated the presence of terpenoid. Test for cardiac glycosides: An aqueous solution of extracts was added to 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 mL of concentrated sulphuric acid. Formation of a brown ring indicated presence. Test for reducing sugars: Extracts were boiled with Benedict's reagent, an orange to red precipitate indicated presence of reducing sugars.

#### **3.2.4. Brine Shrimp Lethality Assay**

The safety of the extracts was tested using the Brine Shrimp lethality Assay method by Meyer et al. (1982) with minor modifications. Artificial seawater was made by adding 23 g NaCl, 11 g  $MgCl_2 \cdot 6H_2O$ , 4 g  $Na_2SO_4$ , 1.3 g  $CaCl_2 \cdot 2H_2O$  and 0.7 g KCl to distilled water, this was then made up to 1 L with the pH adjusted to 9 using 0.1 M  $Na_2CO_3$  solution. Extracts were re-suspended in 2% DMSO, in artificial seawater. Samples were made in 100  $\mu g/mL$  to 100000  $\mu g/mL$  concentrations. Brine shrimp were hatched in artificial seawater over a period of 48 h by adding 100 mg brine shrimp eggs to 100 mL artificial seawater. This was incubated at room temperature. The assay was conducted by adding 10 brine shrimp nauplii into 4.9 mL artificial seawater and 100  $\mu L$  sample in a 6-well plate. Potassium chromate (same concentration as sample) was used as a positive control, and the artificial seawater in place of the sample was used as the negative control.

#### **3.2.5. Total Phenol Content**

The total phenol content was determined according to the method by Ainsworth and Gillespie (2007) with minor modifications. Folin Ciocalteu reagent (10%, 2.5 mL) was added to 500  $\mu L$  of sample (1 mg/mL), subsequently 2 mL of 2%  $Na_2CO_3$  was also added. The reaction was then incubated at 45°C for 15 min.

Thereafter, the absorbance was read at 765 nm. A gallic acid standard curve using 100-1000 µg/mL concentrations of gallic acid was used with results expressed as milligram of gallic acid equivalents (mg GAE per g).

### 3.2.6. Antioxidant Activity

#### 3.2.6.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

The DPPH assay was performed according to the method by Oboh (2006) with minor modifications. DPPH (0.1 mM, 1 mL) was prepared in methanol and thereafter added to 1 mL of samples (200, 400, 600, 800 and 1000 µg/mL). The reaction mix was kept in the dark for 30 min, and finally the absorbance was read at 517 nm. Rutin (1 mg/mL) was used as a positive control, and methanol was used as a negative control.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance}(\text{control}) - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

#### 3.2.6.2. 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assay

The ABTS free radical scavenging ability was determined according to the method by Re et al. (1999) with minor modifications. Briefly, ABTS solution was prepared by mixing equal parts of 7 mM ABTS and 2.45 mM Potassium persulphate for 16 h. The absorbance of this solution was read at 734nm. The solution was then diluted to a concentration that gave an absorbance of 0.7 (±0.02) at 734 nm. Diluted ABTS (3mL) solution was added to 1 mL of sample (200, 400, 600, 800, 1000 µg/mL). Samples were read at 734 nm after 5 min of incubation. Rutin (1 mg/mL) was used as a positive control and methanol was used as a negative control.

*Percentage scavenging*

$$= \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

#### 3.2.6.3. Phosphomolybdenum (PM) Assay

The PM assay was conducted using the method by Sudha et al. (2011) with minor modifications. A molybdate solution was made by adding 1 mL each of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate to 20 mL of distilled water. The final volume was made up to 50 mL. Molybdate solution (1 mL) was added to 1 mL of sample (200, 400, 600, 800, 1000 µg/mL). This was then incubated at 95°C for 90 min. Samples were cooled and read at 695 nm. Rutin (1 mg/mL) was used as a positive control, and methanol used as a negative control.

$$\text{Inhibition}(\%) = \text{Absorbance}(\text{sample}) / \text{Absorbance}(\text{control}) \times 100$$

#### 3.2.6.4. Cupric ion reducing antioxidant capacity (CUPRAC) Assay

The CUPRAC assay was performed according to the method by Phatak and Hendre (2014). Briefly 1 mg of 10 mM cupric chloride, 7.5 mM neocuprine (made in methanol), 1mM ammonium acetate buffer (pH 7) and 2 mL of distilled water were added to 100 µL of sample (200, 400, 600, 800, 1000 µg/mL). Samples were incubated at room temperature for 30 min and the absorbance read at 450 nm. Rutin (1mg/mL) was used as a positive control, and methanol was used as a negative control.

#### 3.2.6.5. Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was conducted according to the method by Benzie and Strain (1996) with minor modifications. A 300 mM acetate buffer was made by adding 3.1 g sodium acetate trihydrate to 16 mL glacial acetic acid. The pH was adjusted to 3.6 and final volume made to 1 L with distilled water. Freshly prepared 10 mM TPTZ (2, 4, 6-tripyridyl-s- triazine) in 40 mM HCL and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O in distilled water were made. TPTZ reagent was prepared by adding 10 parts of acetate buffer to 1 part each of TPTZ and FeCl<sub>3</sub> solution to make up the FRAP reagent. FRAP reagent (3 mL) was added to 100 µL sample (200, 400, 600, 800, 1000 µg/mL). Samples were read at 593 nm, incubated at 37°C and read again after 4 min. Ascorbic acid was used as the standard and the FRAP value for Ascorbic acid is 2.

$$\text{FRAP Value of Sample} = \frac{\Delta \text{Absorbance}_{593}(\text{sample})}{\Delta \text{Absorbance}_{593}(\text{standard})} \times \text{Frap value of the standard}$$

### 3.2.7. Statistical analysis

Results were analysed by ANOVA (Graph Pad Prism software, San Diego, CA, USA). All analysis was done in triplicate; mean $\pm$ standard deviation was calculated. IC<sub>50</sub> was also calculated using Graph Pad Prism. The lower the IC<sub>50</sub> concentration, the more potent the extract is as a therapeutic agent.

## 3.3. Results and Discussion

### 3.3.1. Phytochemical Screening

All *H. colchicifolia* extracts tested positive for saponins, tannins, flavonoids, terpenoids, phenols and reducing sugars (Table 3.1). All acetone and methanol extracts were positive for cardiac glycosides and anthraquinones. Only corm extracts were positive for alkaloids, with all leaf extracts not possessing alkaloids.

**Table 3.1: Phytochemical constituents in *H. colchicifolia* extracts using different solvent systems**

Test	FCA	FCM	FCAQ	DLA	DLM	DLAQ	DCA	DCM	DCAQ
Alkaloids	+	+	+	-	-	-	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	-	+	+	-	+	+	-
Terpenoids	+	+	+	+	+	+	+	+	+
Anthraquinones	+	+	-	+	+	-	+	+	-
Phenols	+	+	+	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+	+	+	+

Fresh corm acetone extract (FCA). Fresh corm methanol extract (FCM). Fresh corm aqueous extract (FCAQ). Dried leaf acetone extract (DLA). Dried leaf methanol extract (DLM). Dried leaf aqueous extract (DLAQ). Dried corm acetone extract (DCA). Dried corm methanol extract (DCM). Dried corm aqueous extract (DCAQ).

In a study by Zimudzi (2014), crude methanolic extracts of *Hypoxis hemerocallidea*, *Hypoxis obtusa*, *Hypoxis rigidula* and *Hypoxis galpini* tested positive for reducing sugars, tannins, cardiac glycosides, saponins and terpenoids; and negative for flavonoids, alkaloids and anthraquinones. *Hypoxis colchicifolia* corm and leaf extracts in our study tested positive for all the phytochemical constituents tested, with the only exception being the absence of alkaloids in the leaves.



Phytochemical constituents identified in the study have some association with biological activities attributed to plant chemistry. Tannins possess antinociceptive, antioxidant and anti-inflammatory properties; terpenoids have anti-inflammatory and anti-microbial properties. Saponins possess anticancer, anti-diabetic, anti-inflammatory, anti-microbial and antioxidant properties; with cardiac glycosides being reported to be important in treating heart ailments (Tiwari et al., 2011). Assessment of the aerial and subterranean parts of *Hypoxis hemerocallidea* by Katerere and Eloff (2008) show definite difference with the biological activity and chemistry of these parts and cannot be used interchangeably. *Hypoxis hemerocallidea* corms and leaves tested for chemical composition on TLC showed them to be distinctly different, with leaf samples being more complex than that of corms. There were no apparent difference in the chemical composition of ethanol and acetone extracts of either corms or leaves in the study by (Katerere, 2013, Katerere and Eloff, 2008).

### 3.3.2. Brine Shrimp lethality Assay

All *H. colchicifolia* extracts tested at different concentrations showed no mortality of the brine shrimp indicating the non-toxic effect that extracts possess (Table 3.2). Potassium chromate used as the positive control was lethal from the lowest concentration tested.

**Table 3.2: Brine shrimp lethality assay, Percentage death by *H. colchicifolia* extracts by different solvent systems**

Concentration (µg/mL)	FCA	FCM	FCAQ	DLA	DLM	DLAQ	DCA	DCM	DCAQ	Positive control
0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	100
1000	0	0	0	0	0	0	0	0	0	100
10000	0	0	0	0	0	0	0	0	0	100
100000	0	0	0	0	0	0	0	0	0	100

Fresh corm acetone extract (FCA). Fresh corm methanol extract (FCM). Fresh corm aqueous extract (FCAQ). Dried leaf acetone extract (DLA). Dried leaf methanol extract (DLM). Dried leaf aqueous extract (DLAQ). Dried corm acetone extract (DCA). Dried corm methanol extract (DCM). Dried corm aqueous extract (DCAQ). Positive control used was Potassium chromate. Values represent mean of replicate reading (n=3)

Jooste (2012) showed that corms of *H. hemerocallidea* showed cytotoxicity on brine shrimp lethality at 10, 100, 1000 µg/mL concentrations however, Ramulondi et al. (2018) found that an aqueous extract of *H. hemerocallidea* corms had a low brine shrimp lethality assay mortality percentage of 4 and 5% after 24 and 48 h respectively. The organic solvent extract had a higher mortality of 29 and 54% mortality after 24 and 48 h respectively. A study by Zimudzi (2014), revealed the extracts of *Hypoxis* corms are nontoxic to brine shrimp nauplii. In this study, with the highest concentration tested being 100 000 µg/mL, showed all extracts tested to be nontoxic

### 3.3.3. Total Phenol Content

The methanol extract of dried corms of *H. colchicifolia* has the highest phenolic content from the extracts evaluated ( $204.80 \pm 1.73$  mg/g); with the aqueous extract of dried leaves having the lowest phenolic content ( $103.67 \pm 1.15$  mg/g). Statistical analysis of the results showed no significant difference found between FCA and FCM, FCAQ and DCM, DLA and DLM; and DCA and DCAQ (Table 3.3). The rest of extracts tested had a significant difference with each other  $p < 0.0001$ .

**Table 3.3: Total phenolic content of *H. colchicifolia* extracts by different solvent systems**

Sample	Solvent type	Total Phenol GAE (gallic acid equivalent) mg/g
<b>Fresh Corms</b>	Acetone	$186.53 \pm 5.08^c$
	Methanol	$186.87 \pm 0.81^c$
	Aqueous	$198.53 \pm 1.89^a$
<b>Dried Leaves</b>	Acetone	$112.80 \pm 0.33^g$
	Methanol	$115.13 \pm 3.45^g$
	Aqueous	$103.67 \pm 1.15^i$
<b>Dried Corms</b>	Acetone	$157.47 \pm 2.05^e$
	Methanol	$204.80 \pm 1.73^a$
	Aqueous	$160.60 \pm 1.23^e$

Data represent mean  $\pm$  standard deviation (n=3). Superscript letters indicates significant difference ( $p < 0.0001$ ).

The total phenolic content of the three fresh corm extracts were similar and showed no significant difference. The total phenolic content of the leaf extracts were lower ( $\pm 50\%$ ) than that of the fresh and dried corm extracts. The aqueous extract of dried leaves had the lowest phenolic content. In a study by Laher et al. (2013), the total phenolic content of fresh *H. hemerocallidea* corms and leaves had a significantly higher total phenolic content than that of dried corms and leaves.

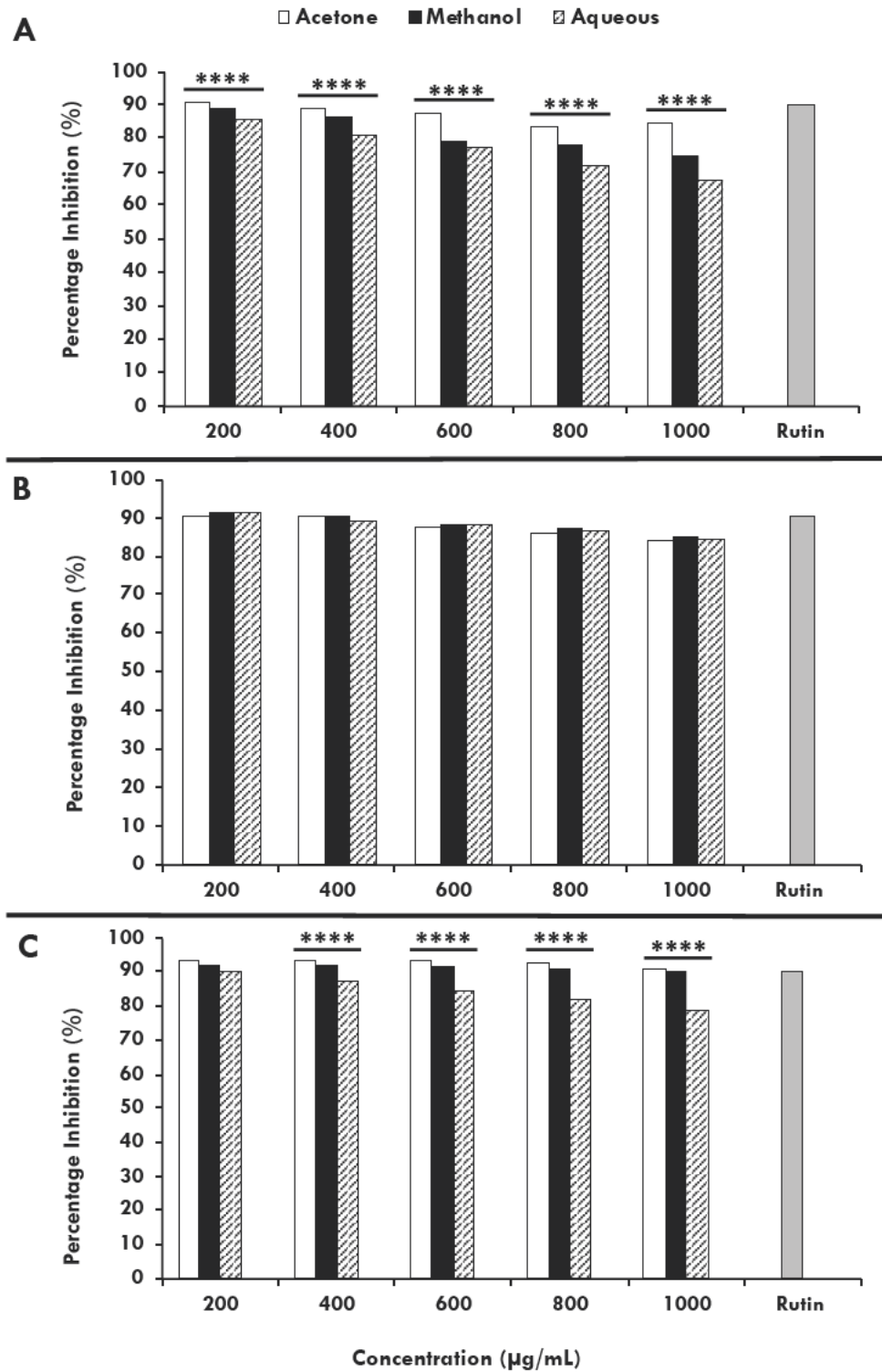
Phenolic compounds are considered responsible for antioxidant activity and effective free radical scavenging; hence the quantity of total phenols present gives an indication of the sample's antioxidant ability. Fresh corms had a total phenolic content of 173.59 mgGAE/g. Fresh *H. hemerocallidea* leaves had higher flavonoid content than that of dried leaves. An assessment of seven *Hypoxis* species for the total phenolic content by Nsibande et al. (2018) found the total phenolic content ranging from 134.79 to 396  $\mu\text{g/g}$  in corm extracts of *Hypoxis* species evaluated. In the study *H. hemerocallidea* had a total phenolic content of 204.56  $\mu\text{g/g}$  with these findings in line with those of this study

### **3.3.4. Antioxidant Activity**

#### **3.3.4.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay**

The acetone extract of dried corms of *H. colchicifolia* shows the highest DPPH free radical inhibition potential with the fresh corm aqueous extract exhibiting the poorest inhibition potential (Figure 3.1). The  $\text{IC}_{50}$  for DPPH in descending order are as follows: fresh corm aqueous (23.16  $\mu\text{g/mL}$ ), fresh corm methanol (21.25  $\mu\text{g/mL}$ ), dried corm aqueous (20.16  $\mu\text{g/mL}$ ), dried corm methanol (18.91  $\mu\text{g/mL}$ ), dried leaf aqueous (18.76  $\mu\text{g/mL}$ ), fresh corm acetone (18.72  $\mu\text{g/mL}$ ), dried leaf methanol (18.44  $\mu\text{g/mL}$ ), dried leaf acetone (17.89  $\mu\text{g/mL}$ ) and dried corm acetone (17.56  $\mu\text{g/mL}$ ). The  $\text{IC}_{50}$  of the positive control rutin was 21.79  $\mu\text{g/mL}$ . There was no significant difference in the results of DLA and DLM, DLA and DLAQ; and DLM and DLAQ. Indicating that there was no significant difference between all leaf extracts. The fresh and dried corm extracts had significant difference ( $p < 0.0001$ ) between each other as well as the leaf extracts and the positive control. Dried leaf extracts although possessed a lower total phenolic content had as good a DPPH scavenging potential compared to the other extracts tested. Acetone extracts of the fresh corms, dried leaves as well as dried corms had better potential than aqueous and methanol extracts.

The Acetone extract of dried corms had the lowest IC<sub>50</sub> for DPPH scavenging. There was no significant difference between each of the nine extracts and the extracts compared to rutin. A study by Laporta et al., (2007) showed extracts of *H. hemerocallidea* have greater antioxidant activity to extracts of green tea and olive leaf (Laporta et al., 2007). An antioxidant inhibition screening against DPPH and ABTS free radicals by Madikizela and McGaw (2019) found that aqueous extracts of *H. colchicifolia* corms were most effective against free radicals, with *H. colchicifolia* showing a weak DPPH scavenging potential. Acetone, ethanol, hot and cold-water extracts were evaluated, with aqueous extracts having the lowest IC<sub>50</sub> values for antioxidant inhibition. Hot water extract of *H. colchicifolia* had an IC<sub>50</sub> of 12.18 µg/mL against ABTS free radicals and the cold-water extract had an IC<sub>50</sub> of 19.75 µg/mL against DPPH free radicals.

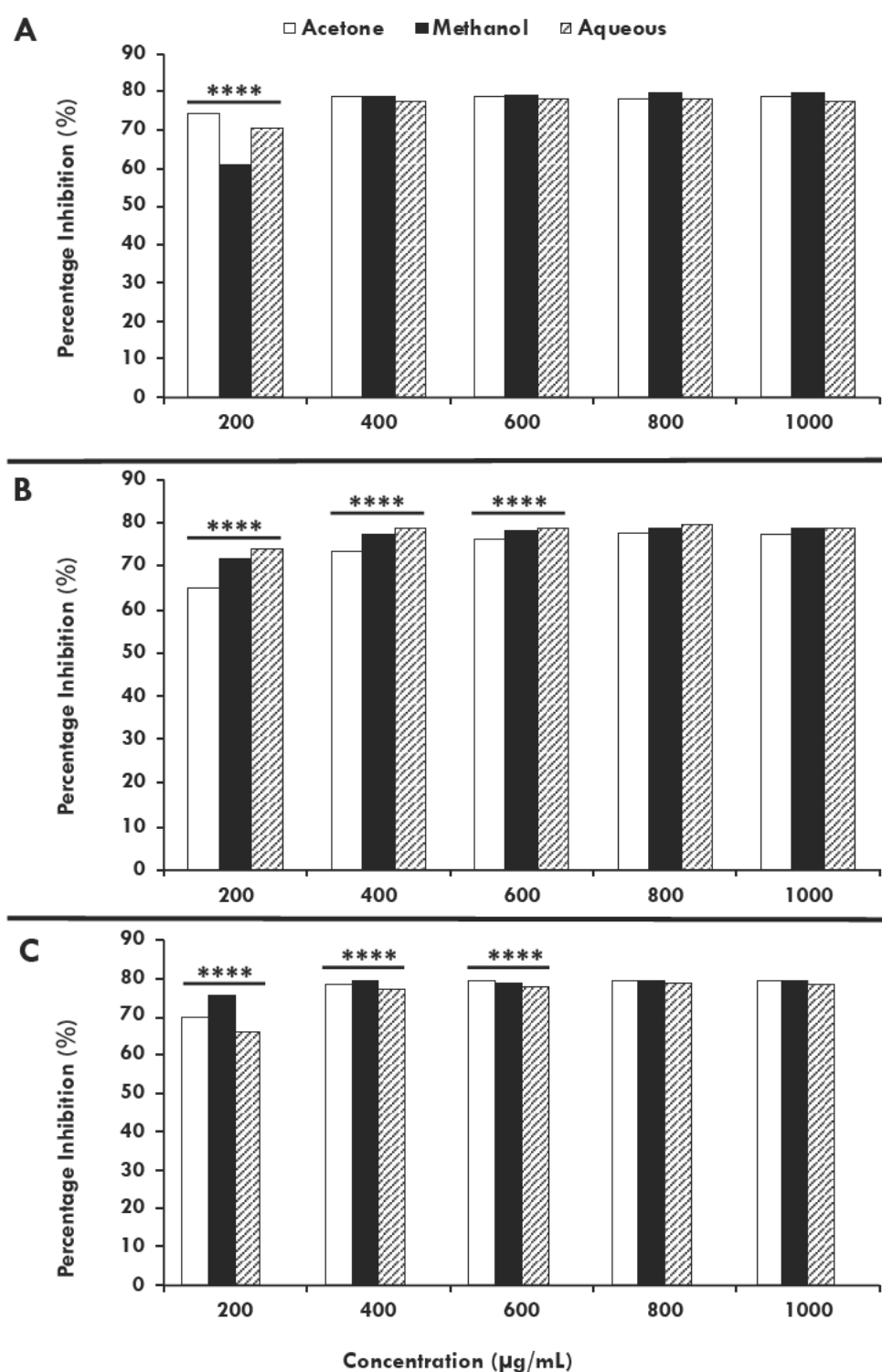


**Figure 3.1: DPPH Inhibition by *H. colchicifolia* extracts [A - solvent extracts of fresh corms; B- solvent extracts of dried leaves; C- solvent extracts of dried corms]. Values represent mean  $\pm$  standard deviation of replicate reading (n=3) ( $p < 0.0001$ ).**

#### **3.3.4.2. 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assay**

The methanol extract of dried leaves exhibited the greatest inhibitory effect (Figure 3.2). The  $IC_{50}$  for ABTS inhibition in descending order are as follows: fresh corm methanol (153  $\mu\text{g/mL}$ ), fresh corm acetone (120.1  $\mu\text{g/mL}$ ), dried corm aqueous (118.6  $\mu\text{g/mL}$ ), dried corm methanol (106.4  $\mu\text{g/mL}$ ), dried corm acetone (106.4  $\mu\text{g/mL}$ ), fresh corm aqueous (104.5  $\mu\text{g/mL}$ ), dried leaf acetone (89.98  $\mu\text{g/mL}$ ), dried leaf aqueous (85.57  $\mu\text{g/mL}$ ) and dried leaf methanol (74.46  $\mu\text{g/mL}$ ). The  $IC_{50}$  for the positive control of rutin was 30.69  $\mu\text{g/mL}$ . Leaf extracts were most effective against ABTS cations, with the methanol extract having the lowest  $IC_{50}$  followed by aqueous and acetone extract. Fresh corm extracts were least effective in scavenging ABTS radicals. There was a significant difference between all extracts tested, including the positive control.

Assessment of *Hypoxis argentea* for antioxidant potential using DPPH and ABTS methods found ethanol and aqueous extracts of corm had high antioxidant potential and had an ABTS inhibition of more than 90% at a 500  $\mu\text{g/mL}$  concentration. Extracts showed moderate DPPH scavenging potential (Akinrinde et al., 2018b). These findings were similar to that of this study, as corm extracts exhibited high antioxidant potential.



**Figure 3.2: ABTS Inhibition by *H. colchicifolia* extracts [A - solvent extracts of fresh corms; B- solvent extracts of dried leaves; C- solvent extracts of dried corms]. Values represent mean  $\pm$  standard deviation of replicate reading (n=3) ( $p < 0.0001$ ).**

### 3.3.4.3. Phosphomolybdenum (PM) Assay

The acetone extract of dried corms of *H. colchicifolia* have shown to have the highest TEAC against phosphomolybdenum free radicals with the aqueous extract of the fresh corms exhibiting the lowest potential (Table 3.4). All acetone extracts had a higher potential than that of rutin ( $150.53 \pm 1.76 \mu\text{g/mL}$ ). Acetone extract of dried corms inhibited PM free radicals better than other extracts and had the highest trolox equivalent. There was a significant difference ( $p < 0.0001$ ) between all extracts tested, including the positive control.

**Table 3.4: Trolox equivalence antioxidant capacity (TEAC) of *H. colchicifolia* extracts against phosphomolybdenum free radicals**

Sample	Concentration ( $\mu\text{g/mL}$ )	Acetone	Methanol	Aqueous
Fresh corm extracts	0	0	0	0
	200	$54.30 \pm 1.32^{ab}$	$80.11 \pm 1.32^{bc}$	$68.82 \pm 0.88^{ac}$
	400	$109.14 \pm 3.07^{ab}$	$95.16 \pm 0.44^b$	$96.24 \pm 3.07^{ac}$
	600	$132.26 \pm 5.27^{ab}$	$113.98 \pm 0.88^b$	$110.22 \pm 3.07^{ac}$
	800	$152.69 \pm 1.76^{ab}$	$124.73 \pm 0.88^{bc}$	$103.76 \pm 3.07^{ac}$
	1000	$178.49 \pm 7.90^{ab}$	$130.11 \pm 3.51^{bc}$	$106.45 \pm 4.39^{ac}$
Dried leaf extracts	0	0	0	0
	200	$74.73 \pm 3.07^a$	$72.94 \pm 2.92^a$	$65.05 \pm 1.32^{bc}$
	400	$117.74 \pm 7.46^{ab}$	$76.88 \pm 0.44^a$	$82.80 \pm 4.39^{bc}$
	600	$151.61 \pm 2.63^{ab}$	$104.30 \pm 2.63^{ac}$	$67.20 \pm 2.19^{bc}$
	800	$162.90 \pm 4.83^{ab}$	$106.45 \pm 2.63^a$	$105.91 \pm 1.32^{bc}$
	1000	$162.37 \pm 0.88^{ab}$	$147.85 \pm 2.19^{ac}$	$116.67 \pm 4.83^{bc}$
Dried corm extracts	0	0	0	0
	200	$107.53 \pm 3.51^{ac}$	$63.44 \pm 0.88^b$	$58.60 \pm 0.44^{ac}$
	400	$127.96 \pm 0.00^{ac}$	$70.43 \pm 1.32^b$	$66.13 \pm 0.44^{ac}$
	600	$143.01 \pm 1.76^{ac}$	$81.18 \pm 1.32^b$	$84.41 \pm 1.32^{ac}$
	800	$160.22 \pm 0.88^{ac}$	$88.71 \pm 0.44^b$	$91.94 \pm 1.32^{ac}$
	1000	$180.65 \pm 2.63^{ac}$	$131.72 \pm 0.44^{bc}$	$120.43 \pm 6.15^{ac}$

Values represent mean  $\pm$  standard deviation of replicate reading ( $n=3$ ). Superscript letters indicate significant difference ( $p < 0.0001$ ).



### 3.3.5. Cupric ion reducing antioxidant capacity (Cuprac) Assay

All extracts had a poor TEAC compared to that of rutin ( $978.79 \pm 3.89 \mu\text{g/mL}$ ) however, the methanol extract of fresh corms of *H. colchicifolia* had the highest TEAC against cupric ion free radicals with the methanol extract of dried leaves having the lowest TEAC potential (Table 3.5). There was no significant difference between FCA and FCM, DLM and DCM; and DCM and DCAQ. The rest of extracts tested had a significant difference ( $p < 0.0001$ ) when compared to each other and the positive control.

**Table 3.5: Trolox equivalence antioxidant capacity (TEAC) of *H. colchicifolia* extracts against cupric ion free radicals**

Sample	Concentration ( $\mu\text{g/mL}$ )	Acetone	Methanol	Aqueous
Fresh corm extracts	0	0	0	0
	200	$107.78 \pm 9.79^b$	$105.03 \pm 8.92^{ac}$	$42.65 \pm 2.83^{bc}$
	400	$184.84 \pm 10.72^b$	$185.29 \pm 5.77^{ac}$	$99.07 \pm 2.59^{bc}$
	600	$254.55 \pm 5.54^b$	$261.89 \pm 2.97^{ac}$	$118.79 \pm 4.05^{bc}$
	800	$325.65 \pm 1.72^b$	$303.17 \pm 9.98^{ac}$	$136.68 \pm 1.95^{bc}$
	1000	$359.13 \pm 3.61^b$	$362.80 \pm 3.24^{ac}$	$225.66 \pm 9.08^{bc}$
Dried leaf extracts	0	0	0	0
	200	$27.97 \pm 5.15^{ab}$	$36.23 \pm 4.05^a$	$7.44 \pm 1.13^{ab}$
	400	$70.63 \pm 2.25^a$	$58.24 \pm 7.37^{ac}$	$57.79 \pm 7.48^{ab}$
	600	$124.29 \pm 3.89^a$	$90.81 \pm 3.61^a$	$81.18 \pm 5.54^{ab}$
	800	$187.59 \pm 2.97^a$	$110.53 \pm 7.86^{ac}$	$118.33 \pm 8.13^{ab}$
	1000	$228.87 \pm 16.85^{ab}$	$167.87 \pm 14.49^{ac}$	$188.05 \pm 3.24^{ab}$
Dried corm extracts	0	0	0	0
	200	$49.53 \pm 7.89^a$	$38.98 \pm 2.25^b$	$8.25 \pm 2.83^a$
	400	$104.11 \pm 12.01^a$	$66.96 \pm 7.97^b$	$60.08 \pm 1.72^a$
	600	$183.00 \pm 14.23^{ab}$	$95.40 \pm 6.83^b$	$98.15 \pm 20.81^{ac}$
	800	$227.95 \pm 10.07^{ab}$	$135.76 \pm 1.72^b$	$133.01 \pm 2.39^{ac}$
	1000	$288.49 \pm 3.43^{ab}$	$176.12 \pm 13.96^b$	$248.13 \pm 12.15^{ac}$

Values represent mean  $\pm$  standard deviation of replicate reading ( $n=3$ ). Superscript letters indicate significant difference ( $p < 0.0001$ )

In a study by Güçlü et al. (2006) on methanol extracts of dry and fresh apricots, showed that TEAC against cupric ion free radicals ranged between 2.67 and 52.47  $\mu\text{mol/g}$ . Methanol extracts of 21 Macedonian medicinal plants tested against cupric ion free radicals by Tusevski et al. (2014) had results ranging from 52.89 to 1068.58  $\mu\text{mol/g}$  TEAC with *Origanum vulgare* having the highest equivalent. A study by Zengin et al. (2015) on solvent extracts of three medicinal plants (*Hedysarum varium*, *Onobrychis hypargyrea* and *Vicia truncatula*) showed  $\text{EC}_{50}$  values ranging between 0.69 and 3.01 mg/mL with *Hedysarum varium* having the best cupric potential in the study.

### 3.3.6. Ferric Reducing Antioxidant Power (FRAP) assay

The methanol extract of *H. colchicifolia* dried corms had the highest ferric ion free radical scavenging ability with the acetone extract of the dried leaves having the lowest scavenging ability (Table 3.6). There was no significant difference found between the following extracts: FCA and DLA, FCA and DLM, DLA and DLM, DLM and DCA, DLM and DCAQ; and DCA and DCAQ. The rest of extracts tested had a significant difference ( $p < 0.0001$ ) with each other and the positive control.

In this study, methanol extract of dried corms gave the highest ferric ion scavenging potential, with acetone extract of fresh corms also having a greater scavenging potential than that of rutin ( $2.31 \pm 0.10 \mu\text{mol/mL}$ ). In this study, acetone extracts generally had poor potential; however, there were no significant differences with some of the other solvent extracts.

Aqueous extracts of *H. hemerocallidea* and active compound hypoxoside were evaluated for their antioxidant potential against DPPH and FRAP free radicals by Nair et al. (2007). *Hypoxis hemerocallidea* extracts showed dose dependent free radical scavenging with hypoxoside not having any antioxidant potential when tested alone (Nair et al., 2013). No single assay can capture the different modes of action of the antioxidant. Antioxidants work by various mechanisms; by free radical scavenging activity, donating hydrogen to radical's metal chelating activity, reducing power, quenching singlet oxygen and inhibition of  $\beta$ -carotene (Badarinath et al., 2010).

**Table 3.6: Trolox equivalence antioxidant capacity (TEAC) of *H. colchicifolia* extracts against ferric ion free radicals**

Sample	Concentration (µg/mL)	Acetone	Methanol	Aqueous
	0	0	0	0
<b>Fresh corm extracts</b>	200	0.46±0.02 <sup>a</sup>	0.68±0.05 <sup>a</sup>	0.31±0.10 <sup>a</sup>
	400	0.52±0.01 <sup>a</sup>	0.88±0.35 <sup>a</sup>	0.66±0.07 <sup>ab</sup>
	600	1.05±0.17 <sup>a</sup>	1.22±0.09 <sup>a</sup>	0.83±0.10 <sup>a</sup>
	800	1.30±0.16 <sup>ac</sup>	1.73±0.11 <sup>a</sup>	2.28±0.16 <sup>ab</sup>
	1000	1.36±0.17 <sup>ab</sup>	2.26±0.19 <sup>a</sup>	2.49±0.17 <sup>ab</sup>
	0	0	0	0
<b>Dried leaf extracts</b>	200	0.52±0.01 <sup>a</sup>	0.44±0.03 <sup>a</sup>	0.41±0.01 <sup>a</sup>
	400	0.64±0.02 <sup>a</sup>	0.80±0.07 <sup>a</sup>	0.63±0.01 <sup>a</sup>
	600	0.83±0.07 <sup>a</sup>	1.12±0.11 <sup>ab</sup>	1.39±0.15 <sup>a</sup>
	800	1.07±0.15 <sup>a</sup>	1.39±0.09 <sup>ab</sup>	1.75±0.10 <sup>a</sup>
	1000	1.24±0.11 <sup>a</sup>	1.44±0.13 <sup>ab</sup>	2.00±0.04 <sup>ac</sup>
	0	0	0	0
<b>Dried corm extracts</b>	200	0.68±0.10 <sup>a</sup>	0.73±0.11 <sup>a</sup>	0.60±0.01 <sup>a</sup>
	400	1.05±0.05 <sup>a</sup>	1.19±0.13 <sup>a</sup>	0.93±0.06 <sup>a</sup>
	600	1.32±0.35 <sup>a</sup>	1.65±0.06 <sup>a</sup>	1.06±0.05 <sup>ac</sup>
	800	1.43±0.31 <sup>a</sup>	2.05±0.11 <sup>ab</sup>	1.29±0.28 <sup>ac</sup>
	1000	1.54±0.13 <sup>a</sup>	2.78±0.11 <sup>ab</sup>	1.38±0.19 <sup>ac</sup>

Values represent mean±standard deviation of replicate reading (n=3). Superscript letters indicate significant difference ( $p<0.0001$ ).

### 3.4. Conclusion

The composition of *Hypoxis colchicifolia* corm and leaf extracts have shown to be phytochemically different, with the solvent used for extracting being the determining factor. Only the acetone and methanol extracts have shown to have a full spectrum of phytochemical constituents. The plant is shown to be nontoxic and safe for use, with extracts exhibiting promising antioxidant potential with acetone and methanol corm extracts showing the most favourable results. This may be one of the reasons that only the corms are used in traditional medicine.

The leaf extracts have moderate phenolic content and may have promising biological activity even though the antioxidant ability is limited. Further studies are recommended to assess the quantitative phytochemical composition and the full potential of *H. colchicifolia* as an antioxidant.

## Chapter 4: *In vitro* anti-cancer, anti-hypertensive and anti-hyperglycaemic activities of *Hypoxis colchicifolia*

---

### Abstract

Economic challenges associated with non-communicable diseases and the sociocultural outlook of many patients especially in Africa have increased dependence on traditional herbal medicines for these diseases. *Hypoxis colchicifolia* is a traditional medicinal plant used in southern Africa against an array of ailments. This study evaluated the *in vitro* anti-diabetic ( $\alpha$ -amylase and  $\alpha$ -glucosidase), anti-hypertensive (angiotensin-converting enzyme) and anticancer potential of *H. colchicifolia* corm as well as leaf (acetone, methanol and aqueous) extracts. Results showed that extracts have a moderate anti-diabetic and anti-hypertensive potential, with great anti-cancer potential. The acetone extract of both fresh and dried corms produced significant  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition with ACE inhibited predominantly by the dried corm methanolic extract ( $IC_{50}$  368.2  $\mu$ g/mL). Methanolic extract of dried leaves showed the least cytotoxicity against the non-cancerous cell line HEK-293 while exhibiting the highest inhibition of MCF-7 cells ( $IC_{50}$  3.24  $\mu$ g/mL). All extracts exhibited a greater inhibitory potential in A549 cells than the positive control camptothecin ( $IC_{50}$  304.2  $\mu$ g/mL). This study reveals that *H. colchicifolia* has therapeutic potential as an anti-diabetic and anti-cancer agent.

### 4.1. Introduction

Non-communicable diseases (NCD) are the leading cause of death globally, with the top killers that together account for more than 80% of all precipitate NCD deaths including hypertension (17.9 million deaths annually), cancer (9.0 million) and diabetes (1.6 million) (Forouzanfar et al., 2016). Similarly, in South Africa, diabetes, cancer and hypertension remain the greatest causes of morbidity. Conventional treatment for each of these NCD do exist, however, these drugs have numerous side effects. Furthermore, despite the prevalence and burden of these disorders, a large proportion of people with such problems do not receive treatment (Abegunde et al., 2007). Treatment remain largely inaccessible predominantly in the developing countries due to their exorbitant price tag as well as weaknesses in the health care systems, hence, they depend, even if nominally, on alternative therapies such as traditional herbal medicines. In certain parts of Africa, traditional medicine remains the most employed method of healthcare because of their accessibility to the community (Kiringe, 2006).

The importance of phytomedicines has recently sparked scientific investigations, as the therapeutic functionality of medicinal plants is limited. Common plants that are effective and tested against hyperglycaemia are *Panax ginseng* (Ginseng), *Momordica charantia* (Bitter melon), *Coptis chinensis*, *Trigonella foenum-graecum* (Fenugreek), *Lagerstroemia speciosa*, *Gymnema sylvestre*, *Cinnamomum cassia* (Cinnamon) and *Agaricus campestris* mushrooms (Shapiro and Gong, 2002). Kamtekar et al. (2014) found that plant extracts that are rich in phytochemical secondary metabolites such as flavonoids and phenolics have the potential to control diabetes due to the alpha amylase inhibition potential of these compounds. Odhav et al. (2010) found that traditional African vegetables such as *Centella asiatica*, *Ceratotheca triboba*, *Cleome monophylla* and others were effective in inhibiting  $\alpha$ -amylase. Plant derived compounds such as terpenoids and polyphenolics are known to possess *in vitro* ACE inhibitory activities (Braga et al., 2007). Ranilla et al. (2010) found that peppers and spices (*Cuminum cyminum*, *Zingiber officinale*, *Curcuma longa* and *Cinnamomum zeylanicum*) had significant ACE inhibition due to the phenolic compounds present and can significantly aid in lowering hypertension. About 60% of anticancer agents that are currently used come from natural sources (Cragg and Newman, 2005). These include vinca alkaloids, taxanes, podophyllotoxin, camptothecin, anthracyclines (Assaf et al., 2013; Patel, 2016).

However, the quest to find the ideal anticancer drug, which kills cancer cells while having minimal effect on normal cells, endures. *Hypoxis colchicifolia* is commonly referred to as broad leaved *Hypoxis*, 'inkomfe', 'igudu', 'ingcobo' and 'ilabatheka', in Zulu. It is one of the four most sought after plant species in traditional medicine. *H. colchicifolia* corms are used against barrenness, heart weakness and bad dreams. Infusions of the corm are drunk in small quantities as a tea to stop nausea, vomiting, anxiety, to calm the heart, improve appetite, induce good sleep and even as a treatment for diabetes (Bisi-Johnson et al., 2017). *H. colchicifolia* leaves have not been scientifically validated previously even though the corms are used extensively in traditional medicine. The leaves may contain therapeutic potential like that of the corms. Therefore, this study aimed at investigating the potential biological activity of *H. colchicifolia* leaves and corms, by determining the anticancer activity of *H. colchicifolia* extracts against cancer cells A549, HEK-293 and MCF-7 and establish the anti-diabetic and anti-hypertensive effects of *H. colchicifolia* extracts.

## **4.2. Methodology**

### **4.2.1 Collection of plant material**

*Hypoxis colchicifolia* was collected and identified using taxonomic keys by the School of Life Science, University of KwaZulu-Natal. The sampling site was located in Mooiriver, KwaZulu-Natal, South Africa with voucher specimens of the authenticated plant material deposited in the Ward Herbarium (WARD) at UKZN (Westville campus) (Voucher specimen number: Moodley & Baijnath No 1.).

### **4.2.2. Preparation of plant material**

Fresh as well as dried corms and leaves of *Hypoxis colchicifolia* were washed, cut and allowed to air dry. Plant material was then coarsely ground in an industrial grinder (Retsch GmbH, West Germany), and stored in labelled Schott bottles in cool dark condition for further use.

### **4.2.3. Extraction of plant material**

The fresh corms (150 g), dried corms (20 g) and leaves (20 g) were extracted using different solvents (acetone, methanol, distilled water) at the ratio of 1:4 w/v, for 48 h on a rotary shaker and filtered using Whatman No. 1 filter paper. Filtrates were then evaporated using a Buchi rotary evaporator with resulting extract air dried further.

### **4.2.4. Anti-diabetic Screening**

#### **4.2.4.1. Alpha amylase Inhibition Assay**

Alpha amylase inhibition was tested using the method by Ranilla et al. (2009) with minor modifications. Sodium Potassium Tartrate solution was made by adding 12 g of  $\text{KNa}_2\text{C}_4\text{H}_4.4\text{H}_2\text{O}$  to 8 mL of 2 M NaOH and heated till dissolved. Twenty millilitres of 96 mM 3,5 Dinitrosalicylic acid (DNS) solution was made in distilled water and was heated till dissolved. The DNS solution was then added to the Sodium Potassium Tartrate solution with the addition of 8 mL distilled water. This was allowed to stir in the dark overnight ( $\pm 16$  h). A 20 mM sodium phosphate buffer was made up with 6 mM NaCl. The extracts were suspended in the sodium phosphate buffer (1 mg/mL concentration). Starch solution (1%) was made in sodium phosphate buffer. One millilitre of 1% soluble starch solution was added to 1 mL of sample (200, 400, 600, 800, 1000  $\mu\text{g/mL}$ ) and was incubated for 5 min.

Thereafter 1 mL of 1 unit/mL  $\alpha$ -amylase solution made in sodium phosphate buffer was added and incubated for 3 min. DNS solution (1 mL) was added to the reaction mixture and the reaction was thereafter boiled for 15 min at 100°C. The samples were then cooled to room temperature and 9 mL of distilled water was added. The samples were then transferred to a 96-well plate and read at 540 nm. Absorbance values were converted into percentage Inhibition using the following equation:

$$Inhibition(\%) = \frac{Absorbance\ 540(control) - Absorbance\ 540(sample)}{Absorbance\ 540\ (control)} \times 100$$

#### 4.2.4.2. Alpha glucosidase Inhibition Assay

The  $\alpha$ -glucosidase assay was conducted using the method by Ranilla et al. (2009) with minor modifications. A 50  $\mu$ L sample (200, 400, 600, 800, 1000  $\mu$ g/mL) was added to 50  $\mu$ L of 0.1 M potassium phosphate buffer (pH 6.9) and 100  $\mu$ L of 1 U/mL  $\alpha$ -glucosidase enzyme solution (in 0.1 M potassium phosphate buffer, pH 6.9) was added. This was then incubated at 25°C for 10 min. Following pre-incubation, 50  $\mu$ L of 5 mM p-nitrophenyl  $\alpha$ -D-glucopyranoside solution (in 0.1 M potassium phosphate buffer) was then added. This was further incubated at 25°C for 5 min. The control used was the buffer in place of sample and the blank was the buffer in place of the enzyme. The absorbance was read at 405 nm before and after incubation using a micro plate reader, with percentage inhibition calculated using the following equation:

$$Inhibition(\%) = \frac{\Delta Absorbance_{405}\ (control) - \Delta Absorbance_{405}\ (sample)}{\Delta Absorbance_{405}\ (control)} \times 100$$

#### 4.2.5. Anti-hypertension (ACE inhibition assay)

The ACE inhibition assay was conducted according to Li et al. (2005) and Chen et al. (2013) with minor modifications. Twenty microlitres of sample (200, 400, 600, 800, 1000  $\mu$ g/mL) was suspended in sodium borate buffer, 50  $\mu$ L of 5 mM HHL (in 0.1M sodium borate buffer) and 0.3 M sodium chloride (pH 8.3). This was then pre-incubated at 37°C for 30 min. Thereafter 10  $\mu$ L (1 U/mL) ACE solution was added to initiate the reaction. This reaction was incubated at 37°C for 30 min.



One hundred microlitres of 1 M HCl was added to stop the reaction and absorbance read at 492 nm. The sample blank was buffer in place of enzyme solution and the sample control buffer in place of sample.

$$Inhibition (\%) = \frac{Absorbance_{492} (control) - Absorbance_{492} (sample)}{Absorbance_{492} (control) - Absorbance_{492} (blank)} \times 100$$

#### 4.2.6. Cytotoxicity screening (MTT Assay)

Human embryonic kidney (HEK-293), breast cancer (MCF-7) and human lung cancer (A549) cell lines were obtained from the Department of Human Physiology at the University of KwaZulu-Natal, Westville campus and grown at 37°C in a humidified incubator under 5% CO<sub>2</sub> in Dulbecco's modified Eagle's medium (DMEM). The 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of the isolates. The MTT assay was conducted according to Dwarka et al. (2017) with minor modifications. Briefly, cells (50 µL) (1x10<sup>2</sup> cells/mL) as well as 50 µL DMEM were seeded in 96-well flat bottom plates and incubated for 24 h at 37°C in a humidified incubator under 5% CO<sub>2</sub>. Cells were then treated with 50 µL of sample extract prepared in 5% DMSO (7.8-1000 µg/mL) and incubated for 24 h. Camptothecin was used as the positive control. MTT reagent (20 µL, 5 mg/mL) was added to the cells and incubated (4 h at 37°C). Finally, 100 µL of DMSO was added to each well in order to solubilize the formazan salt formed. The absorbance was read at 570 nm on a micro plate spectrophotometer (Multiscan Go, Thermo Scientific) and the percentage viability determined using the formula:

$$\% Cell viability = \frac{Absorbance of treated cells}{Absorbance of untreated cells} \times 100$$

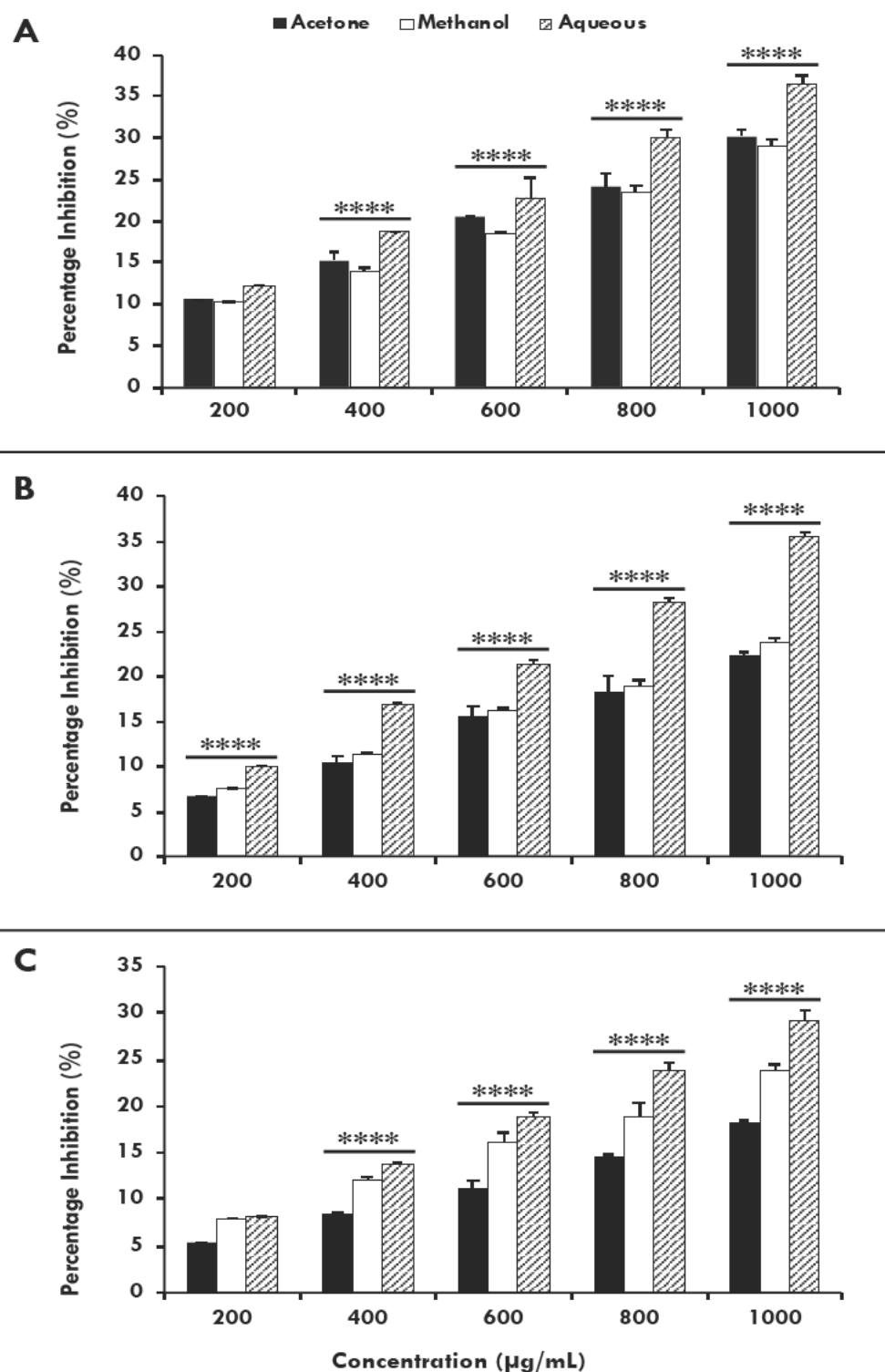
#### 4.2.7. Statistical analysis

Results were analysed by ANOVA (Graph Pad Prism software, San Diego, CA, USA). All analyses were done in triplicate, mean±standard deviation was calculated. IC<sub>50</sub> was also calculated using Graph Pad Prism.

### 4.3. Results and Discussion

#### 4.3.1. Alpha amylase inhibition

The  $IC_{50}$  values for  $\alpha$ -amylase inhibition (Figure 4.1) in descending order are as follows: dried leaf aqueous (392.40  $\mu$ g/mL), dried corm acetone (391.80  $\mu$ g/mL), dried corm aqueous (386  $\mu$ g/mL), dried leaf acetone (366.10  $\mu$ g/mL), dried leaf methanol (359.30  $\mu$ g/mL), fresh corm methanol (351.4  $\mu$ g/mL), fresh corm aqueous (350.90  $\mu$ g/mL), dried corm methanol (346.5  $\mu$ g/mL) and fresh corm acetone (337  $\mu$ g/mL). The  $IC_{50}$  of the positive control acarbose was 515.60  $\mu$ g/mL. The acetone extract of fresh corms had prominent-amylase inhibition, with the aqueous extract of the leaves having least effect. All extracts had a greater inhibitory potential than that of the positive control acarbose. There was no significant difference between the results of FCA and FCM, FCA and DCAQ, FCM and DCAQ, DLA and DLM, DLA and DCA; and DLM and DCM. The rest of the extracts tested had a significant difference ( $p < 0.0001$ ) with each other and the positive control. Akinrinde et al. (2018a) found that the aqueous extracts of *Hypoxis argentea* showed no *in vitro*  $\alpha$ -amylase inhibition, with no percentage inhibition of the three concentrations tested. However, a study by Alimi and Ashafa (2017), on leaf extracts of *Sutherlandia montana*,  $IC_{50}$  values ranged between 0.13 and 5.52 mg/mL for  $\alpha$ -amylase inhibition. All extracts tested had an improved inhibition than that of acarbose ( $IC_{50}$  0.24 mg/mL), with aqueous extracts of the plant displaying the best inhibition. A similar study by Nair et al. (2013) on methanol extracts of medicinal plants (*Artocarpus altilis*, *Artocarpus heterophyllus*, *Cinnamomum zeylanicum* and *Piper betel*) found that  $\alpha$ -amylase inhibition by these plants had  $IC_{50}$  values ranging between 7.058 and 130.55  $\mu$ g/mL, with *A. heterophyllus* having the greatest inhibition potential

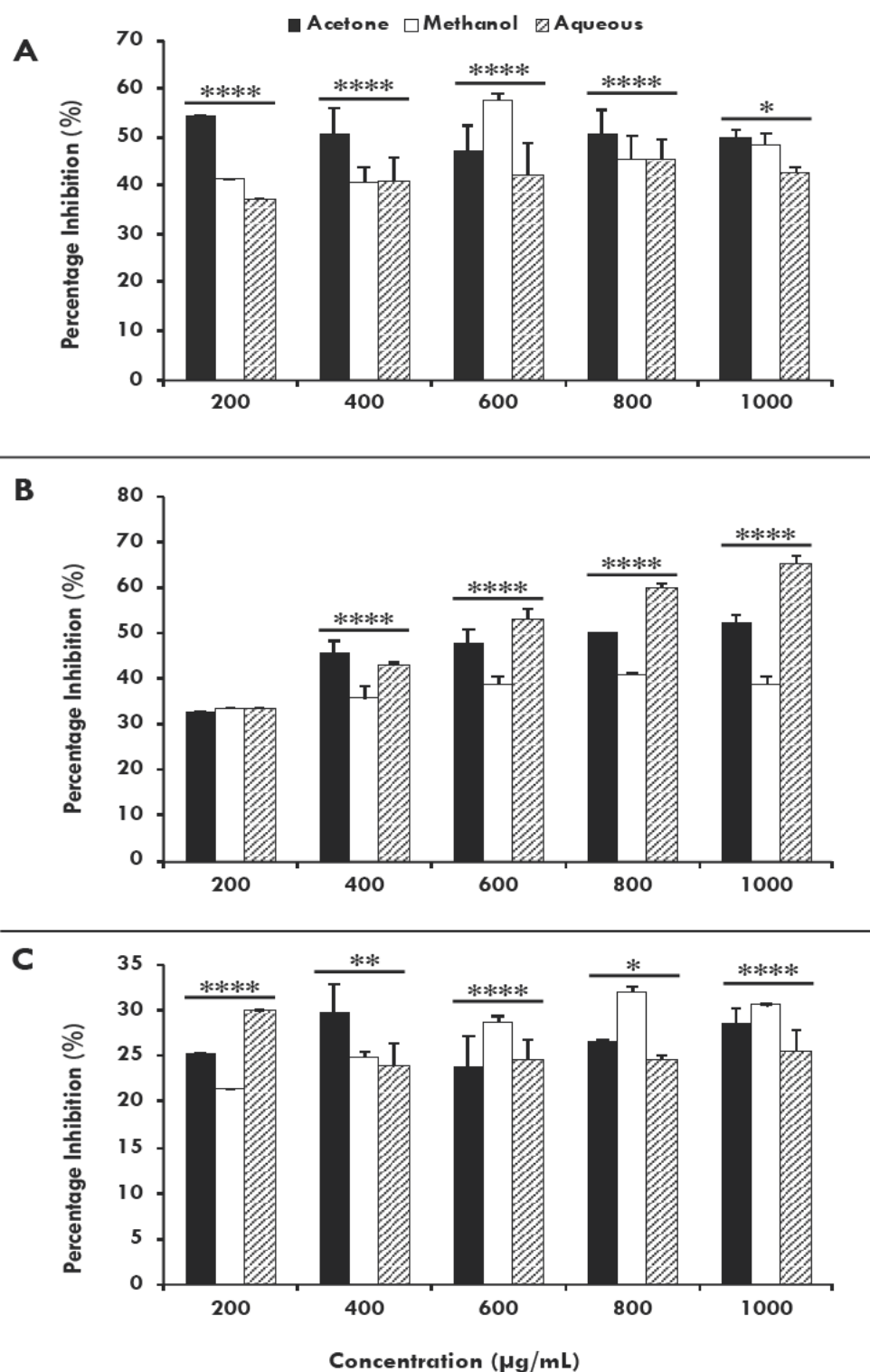


**Figure 4.1:** Alpha amylase inhibitory potential of *H. colchicifolia* extracts [A - fresh corms; B - dried leaves; C - dried corms]. Bars denote mean  $\pm$  standard deviation (n=3) ( $p < 0.0001$ ).

#### 4.3.2. Alpha glucosidase inhibition

The IC<sub>50</sub> for  $\alpha$ -glucosidase inhibition (Figure 4.2) in descending order are as follows: dried leaf aqueous (210.50  $\mu\text{g/mL}$ ), dried leaf acetone (152.90  $\mu\text{g/mL}$ ), dried corm methanol (130.80  $\mu\text{g/mL}$ ), fresh corm methanol (73.83  $\mu\text{g/mL}$ ), dried leaf methanol (63.53  $\mu\text{g/mL}$ ), fresh corm aqueous (53.89  $\mu\text{g/mL}$ ), dried corm acetone (36.67  $\mu\text{g/mL}$ ), dried corm aqueous (29.92  $\mu\text{g/mL}$ ) and fresh corm acetone (22.06  $\mu\text{g/mL}$ ). The IC<sub>50</sub> of the positive control acarbose was 118.40  $\mu\text{g/mL}$ . The aqueous extract of dried leaves had the highest  $\alpha$ -glucosidase inhibition potential. Fresh corm acetone extracts had the lowest IC<sub>50</sub>, indicating effective activity. There was no significant difference between FCAQ and DLM, DCA and DCM; and DCA and DCAQ. The rest of extracts tested had a significant difference ( $p < 0.0001$ ) with each other and the positive control. Aqueous extracts of *H. argentea* showed a very low dose dependent  $\alpha$ -glucosidase inhibition (Akinrinde et al., 2018a).

In an *in vivo* study by Oguntibeju et al. (2016), the methanol extracts of *H. hemerocallidea* corms are effective antioxidant and anti-hyperglycaemic agents, however increased concentrations of the extracts showed negative effects on the kidneys. Alpha glucosidase inhibition by *S. montana* leaf extracts had IC<sub>50</sub> values ranging between 0.05 and 0.43 mg/mL, with that of acarbose being 0.31 mg/mL. The decoction extract was most effective. Nair et al. (2013) found that medicinal plants tested for  $\alpha$ -glucosidase inhibition had good inhibition, with IC<sub>50</sub> values for the four plants tested ranging between 76.90 and 140.01  $\mu\text{g/mL}$ , similarly  $\alpha$ -amylase inhibition in the study, *A. heterophyllus* had the best inhibition potential. Sama et al. (2012) found crude ethanol extracts of *Cissus arnottiana* fruit had significant anti-diabetic potential due to the extract inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase at concentrations above 5 mg/mL.



**Figure 4.2:** Alpha glucosidase inhibitory potential of *Hypoxis colchicifolia* extracts [A - fresh corms; B - dried leaves; C - dried corms]. Bars denote mean $\pm$ standard deviation (n=3) ( $p<0.0001$ ).

#### 4.3.3. ACE Inhibition

The IC<sub>50</sub> values for ACE inhibition (Figure 4.3) in descending order are as follows: dried leaf acetone (705.60 µg/mL), fresh corm aqueous (691.20 µg/mL), dried corm acetone (628.20 µg/mL), dried corm aqueous (569.4 µg/mL), dried leaf methanol (542.60 µg/mL), dried leaf aqueous (537 µg/mL), fresh corm acetone (503 µg/mL), fresh corm methanol (439.7 µg/mL) and dried corm methanol (368.20 µg/mL). The IC<sub>50</sub> of the positive control captopril was 442.50 µg/mL. The methanol extract of *H. colchicifolia* dried corms has the lowest IC<sub>50</sub>, denoting optimal dosage for ACE inhibitory potential and the acetone extract of dried leaves had the highest IC<sub>50</sub> value. Only the methanol extract of fresh and dried corms were more effective than that of the positive control, captopril. There were no significant differences between the results of the following extracts: FCA and FCAQ, FCA and DLM, FCA and DCM, FCM and FCAQ, FCM and DCM, FCAQ and DLM, DLA and DCAQ, DLM and DCM, DCM and DCAQ. The rest of the extracts tested had a significant difference ( $p < 0.0001$ ) with each other and the positive control. This mainly denotes no significant difference between fresh corms extracts; no significant difference in between the methanolic extracts of fresh and dried corms.

Duncan et al. (1999) found that aqueous and ethanolic extracts of leaves and roots of *H. colchicifolia* tested for ACE inhibition produced poor inhibition of between 4-37% inhibitions, with both leaf extracts having a greater inhibition than that of root extracts. Arhin et al. (2019) showed a greater ACE inhibitory potential of the methanolic extracts of the leaves of *Tulbaghia acutiloba* with inhibition activity of  $76.66 \pm 1.65$  (IC<sub>50</sub> 154.23 µg/mL).

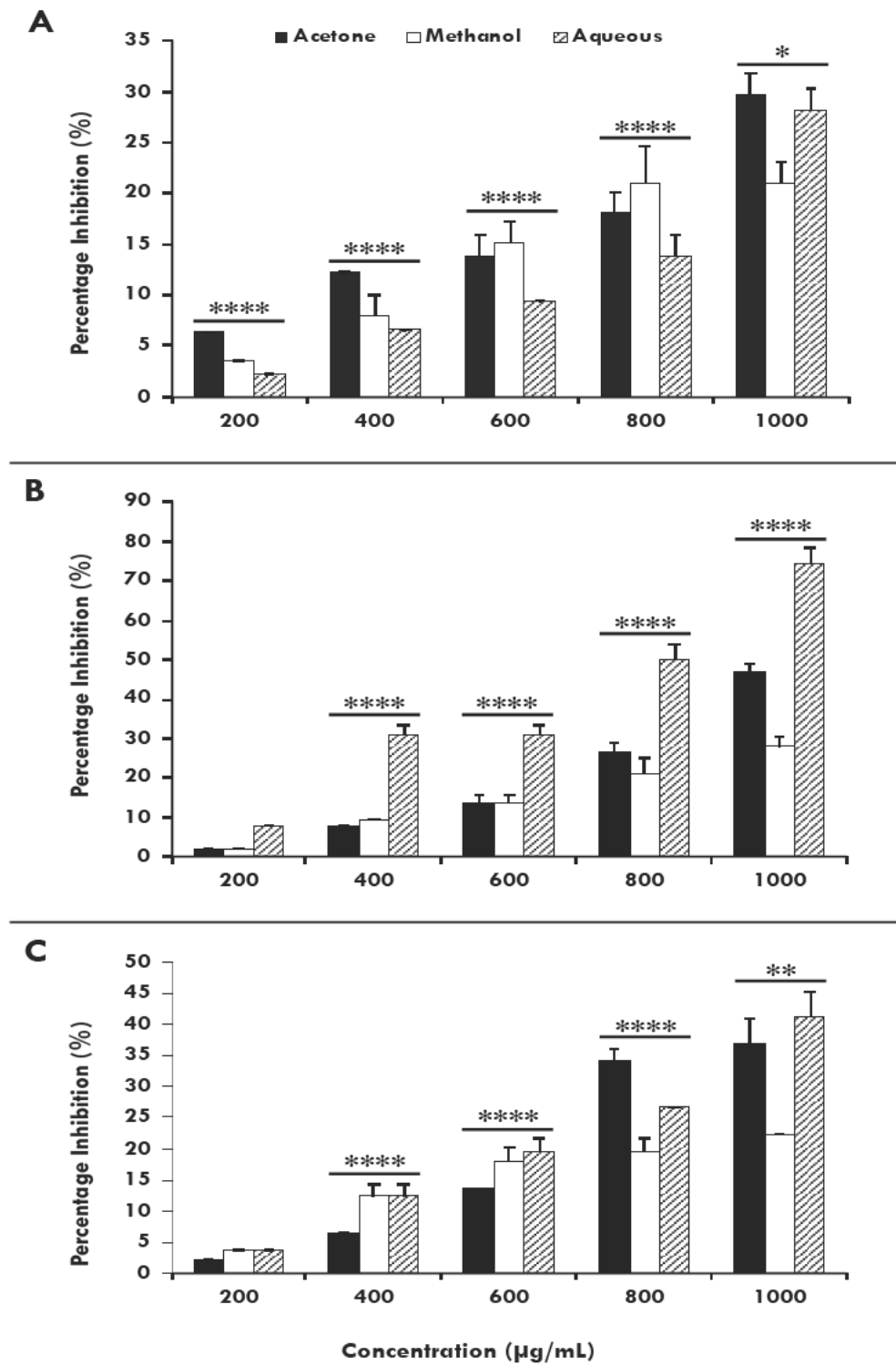


Figure 4.3: ACE Inhibitory potential of *Hypoxis colchicifolia* extracts [A - fresh corms; B - dried leaves; C - dried corms]. Bars denote mean  $\pm$  standard deviation (n=3) ( $p < 0.0001$ ).

#### 4.3.4. MTT Cytotoxicity

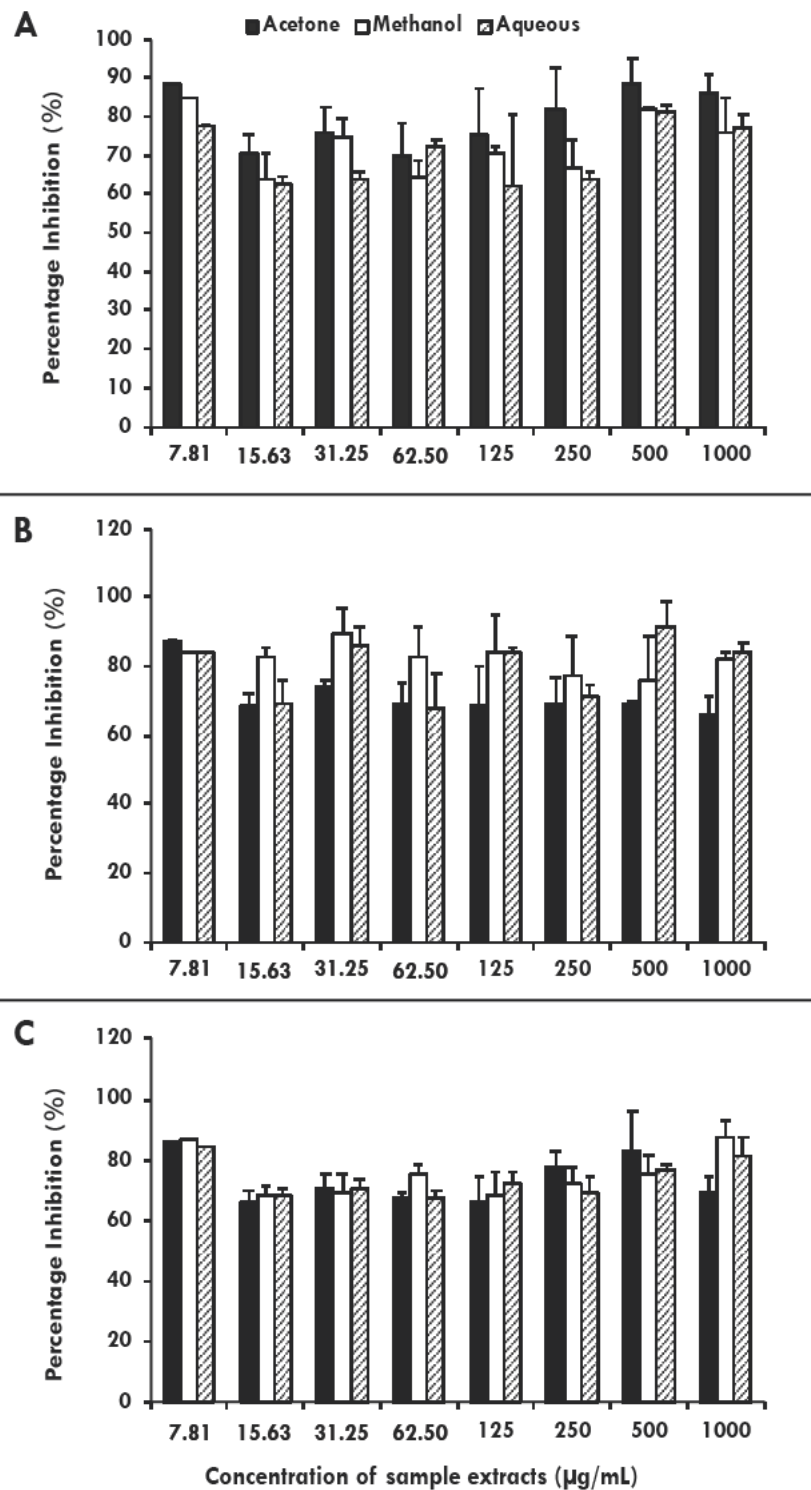
The IC<sub>50</sub> values for HEK-293 inhibition (Figure 4.4) in descending order are as follows: dried leaf methanol (14.16 µg/mL), dried leaf aqueous (11.35 µg/mL), dried leaf acetone (9.02 µg/mL), dried corm aqueous (7.99 µg/mL), fresh corm methanol (7.98 µg/mL), dried corm acetone (7.96 µg/mL), dried corm methanol (7.93 µg/mL), fresh corm aqueous (7.34 µg/mL) and fresh corm acetone (5.39 µg/mL). The IC<sub>50</sub> of the positive control camptothecin was 9.06 µg/mL. The acetone extract of *H. colchicifolia* fresh corms produced the greatest cell inhibition and the methanol extract of dried leaves had the lowest cell inhibition in HEK-293 cell line. All extracts tested showed no significant difference when compared to each other and the control. A study by Madikizela and McGaw (2018) showed that the corm extracts of *H. colchicifolia* had an LC<sub>50</sub> values of 2.48, 0.89 and 0.98 mg/mL against Vero monkey kidney cells for aqueous, ethanol and acetone extracts respectively. A study by Madikizela and McGaw (2018) showed that the corm extracts of *H. colchicifolia* had a LC<sub>50</sub> values of 2.48, 0.89 and 0.98 mg/mL against Vero monkey kidney cells for aqueous, ethanol and acetone extracts respectively.

The IC<sub>50</sub> for MCF-7 inhibition (Figure 4.5) in descending order are as follows: fresh corm acetone (9.51 µg/mL), fresh corm aqueous (7.49 µg/mL), dried corm aqueous (7.41 µg/mL), fresh corm methanol (7.28 µg/mL), dried leaf acetone (7.19 µg/mL), dried corm acetone (4.52 µg/mL), dried corm methanol (4.34 µg/mL), dried leaf aqueous (3.83 µg/mL), and dried leaf methanol (3.24 µg/mL). The IC<sub>50</sub> of the positive control camptothecin was 8.44 µg/mL. The methanol extract of *H. colchicifolia* dried leaves produced the greatest cell inhibition and the acetone extract of fresh corms had the lowest cell inhibition in MCF-7 cell line. All extracts examined showed no significant difference when compared to each other and the control. In a cytotoxicity screening of African medicinal plants, Steenkamp and Gouws (2006) found that aqueous extracts of *H. hemerocallidea* corms stimulated cell growth of DU-145 (prostate carcinoma cells), MCF-12A (non-malignant breast cancer cells) and inhibited the growth of MCF-7 cells. Boukes and van de Venter (2011) evaluated the cytotoxicity of *H. hemerocallidea*, *H. stellipilis* and *H. sobolifera* chloroform corm extracts in HeLa (cervical), HT-29 (colorectal) and MCF-7 (breast) cancer cell lines using the MTT assay. Findings suggest that *H. sobolifera* has the best overall cytotoxic effect against the cancerous cell lines screened, with *H. hemerocallidea* effectively inhibiting HT-29 and *H. stellipilis* having stimulated the growth of HeLa as well as HT-29 cells.



The IC<sub>50</sub> for A549 inhibition (Figure 4.6) in descending order are as follows: dried leaf aqueous (280 µg/mL), fresh corm aqueous (270.30 µg/mL), fresh corm acetone (228.70 µg/mL), fresh corm methanol (215.90 µg/mL), dried corm methanol (118.90 µg/mL), dried leaf acetone (95.65 µg/mL), dried corm acetone (87.07 µg/mL), dried leaf methanol (68.68 µg/mL) and dried corm aqueous (32.22 µg/mL). The IC<sub>50</sub> of the positive control camptothecin was 304.20 µg/mL.

The aqueous extract of *H. colchicifolia* dried corms has the highest cell inhibition and the aqueous extract of dried leaves had minimal inhibition in A549 cell line. These results indicate that the extracts are toxic to cancerous cells while not producing a drastic decrease in normal cells. All extracts tested showed no significant difference when compared to each other and the control. This is in opposition to studies by Madikizela and McGaw (2019) who found that aqueous extracts of corms to be least toxic and had the highest IC<sub>50</sub> (2480 µg/mL) in non-cancerous Vero African monkey kidney cells. However, when tested in A549, CaCo-2, HELA and MCF-7 in different solvents (acetone, ethanol, hot and cold water), had an IC<sub>50</sub> ranging from 50-251.95 µg/mL. The anticancer potential of *H. colchicifolia* could be due to glycoside hypoxoside and rooperol activity (Chavan et al., 2013). In a study by Steenkamp and Gouws (2006), corms of *Hypoxis* were found to be non-cytotoxic against prostate cancer cells, breast cancer cells and non-malignant breast cancer cell lines at a concentration of 50 µg/mL.



**Figure 4.4:** HEK-293 cell line Inhibition by *H. colchicifolia* extracts [A - solvent extracts of fresh corms; B - solvent extracts of dried leaves; C - solvent extracts of dried corms]. Bars denote mean $\pm$ standard deviation (n=3) ( $p<0.0001$ ).

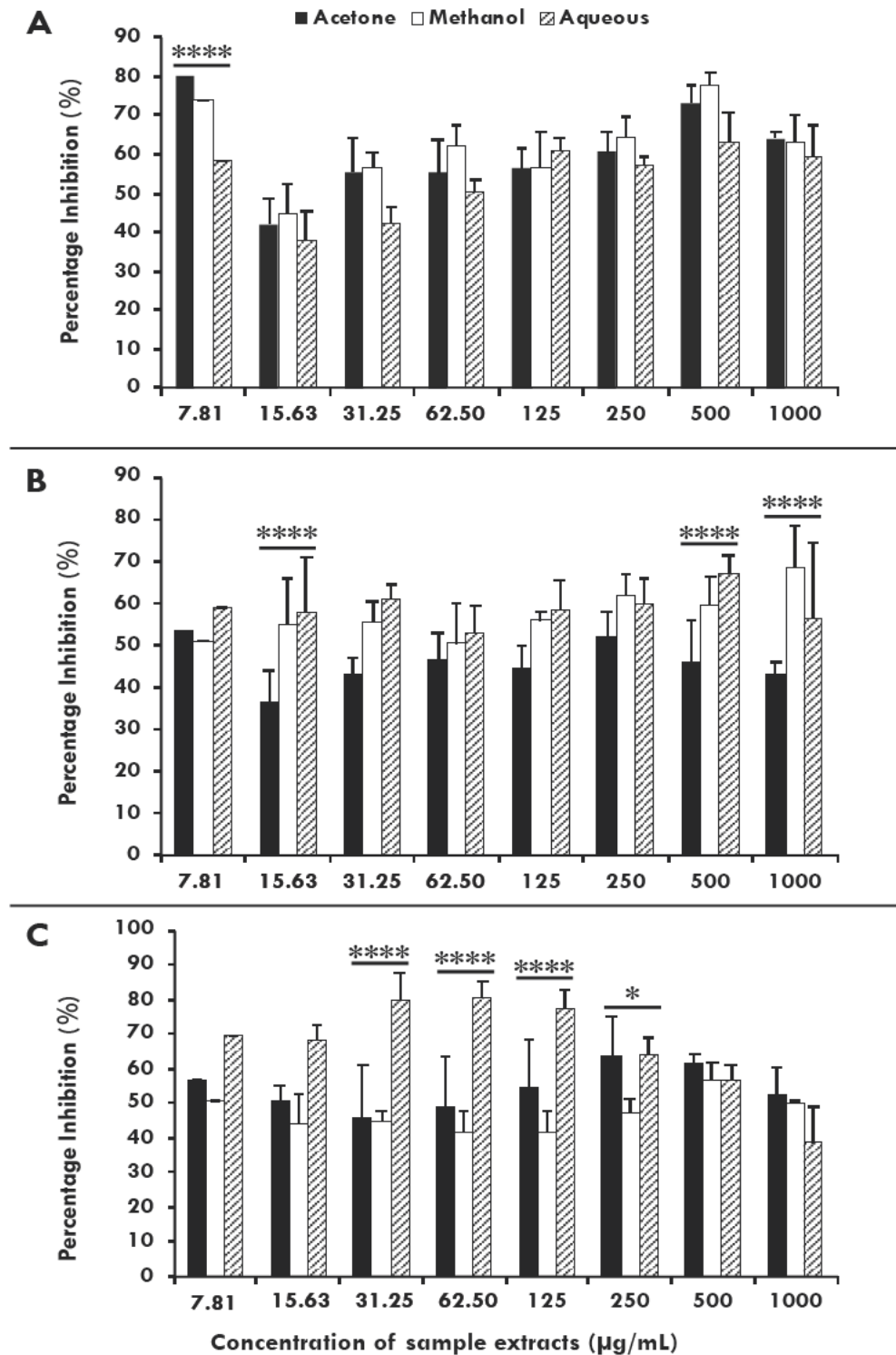


Figure 4.5: MCF-7 cell line Inhibition by *H. colchicifolia* extracts [A - solvent extracts of fresh corms; B - solvent extracts of dried leaves; C - solvent extracts of dried corms]. Bars denote mean $\pm$ standard deviation (n=3) (p<0.0001).

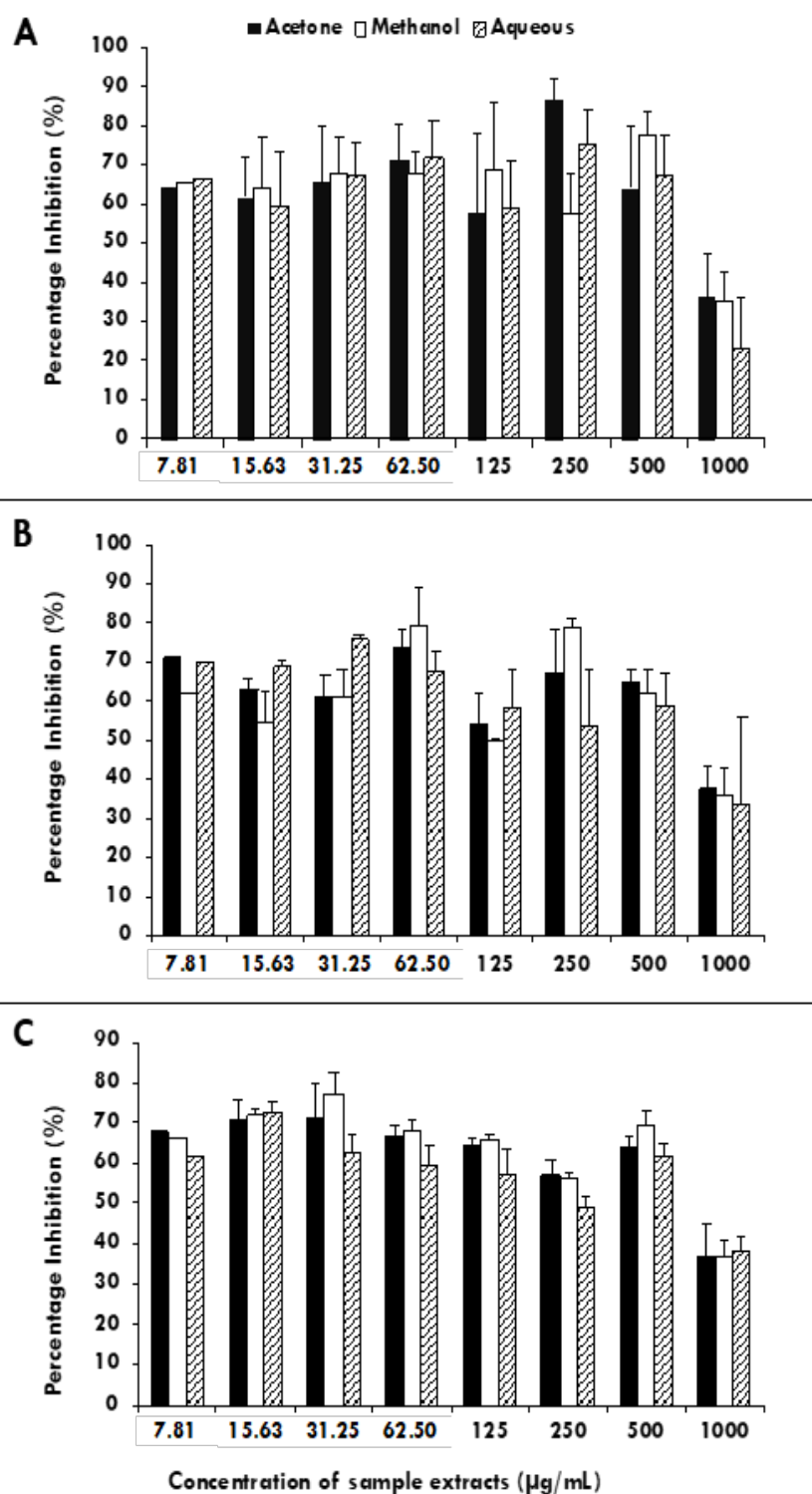


Figure 4.6: A549 cell line Inhibition by *H. colchicifolia* extracts [A - solvent extracts of fresh corms; B - solvent extracts of dried leaves; C - solvent extracts of dried corms]. Bars denote mean $\pm$ standard deviation (n=3) ( $p<0.0001$ ).

#### **4.4. Conclusion**

The fresh corms acetone extract was effective in producing anti-diabetic effects with the dried corm methanolic extract being active in hypertension suppression. Methanol extracts of dried leaves were successful in inhibiting cancerous cell lines while remaining non-toxic to noncancerous cell lines. This study shows that different parts of the plant have different capabilities as a therapeutic and cannot be used interchangeably; Although *H. colchicifolia* has potential to act as a therapeutic.

## Chapter 5: General Discussion

---

Non communicable diseases are the leading cause of mortality in South Africa and the world (WHO, 2018). A fraction of South Africans living with NCDs rely on traditional medicine due to the lack of access to health care. This study was aimed to establish the use of *Hypoxis colchicifolia* extracts as a potential alternative treatment to non-communicable diseases such as diabetes, hypertension and cancer. Traditionally the corms of the *Hypoxis* species are used exclusively. This study evaluated the plant holistically and in doing so, tapped into the unknown potential of the plant.

In one of the few studies that examined the leaves of the *Hypoxis* species, Otunola and Afolayan (2019) looked at the proximate and elemental composition of *H. hemerocallidea*. They found that the plant had a high mineral and nutrient composition, with the presence of essential trace elements. These findings may support the therapeutic potential the plant possesses. The study also suggests that the whole plant can be therapeutically used, with the leaves having as much potential as the corms.

In this study, three types of extracts were tested (fresh corms, dried leaves and dried corms), each exhibited positive and negative traits in the evaluation. No single type of extract exhibited superior potential in showing the best antioxidant potential or inhibition in *in vitro* biological screening. From the initial stage of the study, the main difference that presented itself was the difference in the phytochemical composition of the extracts, with the leaf extracts not possessing alkaloids. All aqueous extracts had no cardiac glycosides and anthraquinones present. This indicated that acetone and methanol extracts have the potential to possess the full spectrum of phytochemicals present in the plant. Although the acetone and methanol extracts have the same qualitative phytochemical composition, the quantitative composition may vary and warrants further study.

A highlight in the study was that all the extracts tested showed no toxicity against brine shrimp, which indicated the safe use of the plant. This is a positive point when compared to other *Hypoxis* species which show some level of toxicity. Jooste (2012) and Ramulondi et al. (2018), found extracts of *H. hemerocallidea* corms to have slight toxicity. The safe use of the plant reassures the traditional use of the plant as a tonic for good health, without being detrimental to the health of the user.

To test the full antioxidant potential of the extracts, five different antioxidant assays were used, each working on a different mode of action in testing the type of antioxidants the extracts possessed. In each assay using a different extract indicated superior activity, indicating the varied antioxidant potential the plant possessed. Acetone and methanol extracts of the dried corms had the most promising potential across the board, with dried leaf extracts only being more effective against ABTS free radicals and the methanolic extract of fresh corms being highly effective against cupric ion free radicals. Traditionally dried corms are brewed into a tea and drunk as a tonic for good health. This aspect of the study affirms the use of dried corm as opposed to fresh corms as a promising antioxidant. In a study by Mwinga et al. (2019), on *H. hemerocallidea*, it was found that corm extracts showed good antioxidant and antimicrobial activity, with minimum inhibitory concentration less than one mg/mL for *Shigella flexneri* and *Trichophyton tonsurans*. Gas chromatography – Mass spectrometry (GC-MS) showed the presence of phenolics, flavonoids and other bioactive compounds, which may be responsible for the activities reported. Mannathoko et al. (2017) analysed methanolic extracts of *H. hemerocallidea* corms and found that extracts exhibited good antioxidant and antimicrobial activity and concluded that the presence of phytochemicals and phenolic compounds contribute to the activities shown. Non-communicable diseases such as hyperglycaemia and hypertension are currently treated by allopathic chronic medications which carry an array of side effects. Finding a natural, safer alternative plant based treatment from a traditionally used plant is the key element of this study.

All extracts evaluated in this study showed low but good  $\alpha$ -amylase inhibition, with fresh corm acetone extract showing the most favourable inhibition. The same goes for  $\alpha$ -glucosidase inhibition, with most of the extracts working more efficiently than acarbose. The fresh corm acetone extract exhibited the best  $\alpha$ -glucosidase inhibition thus indicating that it is a suitable as an anti-diabetic agent. In this study no single type of extract had the most favourable ACE inhibition; however corm extracts, both fresh and dried, were more effective than that of leaf extracts. The methanolic extract of dried corms showed the best ACE inhibition with a lower IC<sub>50</sub> value than that of captopril thus showing a promising potential as a natural replacement to allopathic ACE inhibitors. Zulfiqar et al. (2020) found that compounds extracted from *H. hemerocallidea* showed anti-inflammatory activity by nitric oxide production inhibition in stimulated mouse macrophages.

Elbagory et al. (2019) found that aqueous extracts of *H. hemerocallidea* gold nanoparticles and biosynthesized gold nanoparticles using hypoxoside showed a reduction in pro-inflammatory cytokines in a macrophage cell line THP1, showing anti-inflammatory activity. This finding reaffirms the use of traditional plants like *Hypoxis* as treatments for inflammation.

An effective anticancer agent is one that is cytotoxic to cancerous cells while remaining nontoxic to healthy cells. In this study when extracts were tested against HEK-293 (human embryonic kidney) cell line, some extracts were more potent than camptothecin. This shows them being more toxic to the non-cancerous cells than camptothecin. The only sample type that remained less toxic were the leaf extracts, with the methanolic leaf extract being the least toxic against HEK-293 cell line. The breast cancer cell line (MCF-7) was inhibited successfully by all extracts, with the leaf extracts showing the most promising results. They possessed the lowest IC<sub>50</sub> concentrations, even lower than that of camptothecin. The methanolic leaf extract was the most potent against the breast cancer cell line.

This result provides rationale for the use of *H. colchicifolia* leaf extracts as a therapeutic alternative to breast cancer treatment. All extracts tested against A-549 (human lung cancer) cell line showed promising potential and were more efficient than camptothecin. Leaf extracts, as with MCF-7 inhibition, had the lower IC<sub>50</sub> values, however the aqueous extract of dried corms were the most potent. The aqueous extract of dried leaves although highly effective had a similar cytotoxicity to the noncancerous HEK-293, which is not favourable. This leads to the fact that the leaf extracts are the most effective anticancer agent in the study. They offer the characteristics of the ideal natural anticancer therapeutic. Although the phytochemical composition of the leaf extract is different to that of the corms, with the lack of alkaloids. It indicates that the extract as a whole leads to inhibition and not a single compound on its own. This study has successfully determined the biological activity of *H. colchicifolia* extracts and has compared the difference between extracts of dried and fresh corms; and corms and leaves. Each extract has its individual phytochemical composition and indicates this in the varying activity of each extract. A single dominant extract on its own cannot be recognized as the most suitable extract for therapeutic use (Table 5.1). For each therapeutic requirement, there is a *H. colchicifolia* extract that can be used. This study validates the use of *H. colchicifolia* and gives merit for further research.



**Table 5.1: General summary of findings**

Extract	Antioxidant						Anti-diabetic		Anti-Hypertensive	Anti-cancer		
	Total Phenolic	DPPH	ABTS	PM	CUPRAC	FRAP	$\alpha$ - Amylase	$\alpha$ -Glucosidase	ACE	HEK-293	MCF-7	A549
<b>Fresh corm acetone</b>	Good	Good	Poor	Excellent	Poor	Good	Good	Excellent	Good	Excellent	Poor	Good
<b>Fresh corm methanol</b>	Good	Good	Poor	Good	Poor	Excellent	Good	Excellent	Excellent	Good	Good	Good
<b>Fresh corm aqueous</b>	Good	Good	Poor	Poor	Poor	Excellent	Good	Excellent	Good	Good	Good	Good
<b>Dried leaf acetone</b>	Good	Good	Poor	Excellent	Poor	Good	Good	Good	Poor	Good	Good	Excellent
<b>Dried leaf methanol</b>	Good	Good	Poor	Good	Poor	Good	Good	Excellent	Good	Poor	Excellent	Excellent
<b>Dried leaf aqueous</b>	Good	Good	Poor	Good	Poor	Good	Good	Good	Good	Poor	Excellent	Good
<b>Dried corm acetone</b>	Good	Good	Poor	Excellent	Poor	Good	Good	Excellent	Good	Good	Excellent	Excellent
<b>Dried corm methanol</b>	Good	Good	Poor	Good	Poor	Excellent	Good	Good	Excellent	Good	Excellent	Excellent
<b>Dried corm aqueous</b>	Good	Good	Poor	Good	Poor	Good	Good	Excellent	Good	Good	Good	Excellent

### **Conclusion and Recommendations**

There are clear differences in the phytochemical composition of *H. colchicifolia* leaves and corms which affect subsequent biological activity of the extracts. Extracts of the corms and leaves showed selective antioxidant, anti-diabetic, antihypertensive and anticancer potential. An important outcome of the study is that extracts of *H. colchicifolia* show no toxicity. This indicated the safety of use of *H. colchicifolia*, which is currently used extensively by traditional healers in treating various ailments without any scientific rationale. The high antioxidant potential of *H. colchicifolia* extracts validates the traditional use of it being used as a tonic for good health. The free radical scavenging ability of *H. colchicifolia* gives rise to a potential antioxidant nutraceutical in combating non-communicable diseases that are caused by oxidative stress. *H. colchicifolia* extracts have a low to moderate antidiabetic potential. The effectiveness of corm extracts against angiotensin converting enzyme, which is a key enzyme in regulating blood pressure is more potent than that of captopril which is used as an allopathic ACE inhibitor and an antihypertensive agent. *H. colchicifolia* leaf extracts showed the highest cytotoxic effectiveness against cancer cell lines, while being moderately toxic against noncancerous cell lines. This study revealed that *H. colchicifolia* has potential to act as a therapeutic.

## References

---

- ABDULLAHI, A. A. 2011. Trends and challenges of traditional medicine in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 8.
- ABEGUNDE, D. O., MATHERS, C. D., ADAM, T., ORTEGON, M. & STRONG, K. 2007. The burden and costs of chronic diseases in low-income and middle-income countries. *The Lancet*, 370, 1929-1938.
- ADINORTEY, M. B., AGBEKO, R., BOISON, D., EKLOH, W., KUATSIENU, L. E., BINEY, E. E., AFFUM, O. O., KWARTENG, J. & NYARKO, A. K. 2019. Phytomedicines Used for Diabetes Mellitus in Ghana: A Systematic Search and Review of Preclinical and Clinical Evidence. *Evidence-Based Complementary and Alternative Medicine*, 2019.
- AINSWORTH, E. A. & GILLESPIE, K. M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature protocols*, 2, 875-877.
- AKINRINDE, A., KOEKEMOER, T., VAN DE VENTER, M. & BRADLEY, G. 2018a. *In vitro* investigation of potential anti-diabetic activity of the corm extract of *Hypoxis argentea* Harv. ex Baker. *Acta Pharmaceutica*, 68, 389-407.
- AKINRINDE, A. S., AFOLAYAN, A. J. & BRADLEY, G. 2018b. Phytochemical composition and antioxidant activities of *Dianthus thunbergii* Hooper and *Hypoxis argentea* Harv ex Baker: Plants used for the management of diabetes mellitus in Eastern Cape, South Africa. *Pharmacognosy magazine*, 14, 195.
- AL DISI, S. S., ANWAR, M. A. & EID, A. H. 2016. Anti-hypertensive herbs and their mechanisms of action: Part I. *Frontiers in Pharmacology*, 6, 323.
- ALIMI, A. A. & ASHAFA, A. O. T. 2017. An *in vitro* evaluation of the antioxidant and antidiabetic potential of *Sutherlandia montana* E. Phillips & R.A. Dyer leaf extracts. *Asian Pacific Journal of Tropical Biomedicine*, 7, 765-772.
- AMUSAN, O. O., SUKATI, N. A. & SHONGWE, M. S. 2005. Some phytomedicines from Shiselweni region of Swaziland. *Journal of Natural Remedies*, 5, 19-25.
- ANWAR, M. A., AL DISI, S. S. & EID, A. H. 2016. Anti-hypertensive herbs and their mechanisms of action: part II. *Frontiers in pharmacology*, 7, 50.

- APAK, R., GÜCLÜ, K., ÖZYÜREK, M. & CELİK, S. E. 2008. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*, 160, 413-419.
- APPLETON, M., ASCOUGH, G. & VAN STADEN, J. 2012. *In vitro* regeneration of *Hypoxis colchicifolia* plantlets. *South African Journal of Botany*, 80, 25-35.
- ARHIN, I., DWARKA, D., BISSESSUR, A., NAICKER, D. & MACKRAJ, I. 2019. Biochemical, Phytochemical Profile and Angiotensin-1 Converting Enzyme Inhibitory Activity of the Hydro-methanolic Extracts of *Tulbaghia acutiloba* Harv. *Journal of Natural Remedies*, 19, 221-235.
- ASSAF, A. M., HADDADIN, R. N., ALDOURI, N. A., ALABBASSI, R., MASHALLAH, S., MOHAMMAD, M. & BUSTANJI, Y. 2013. Anti-cancer, anti-inflammatory and anti-microbial activities of plant extracts used against hematological tumors in traditional medicine of Jordan. *Journal of ethnopharmacology*, 145, 728-736.
- ASSOCIATION, A. H. 2018. Available: <https://www.ahajournals.org/cms/attachment/612626c1-020d-441d-89eb-66ac974bd98b/21ff1.jpeg> [Accessed 14 April 2020].
- ATLAS, D. 2015. International diabetes federation. *IDF Diabetes Atlas, 7th edition. Brussels, Belgium: International Diabetes Federation.*
- BADARINATH, A., RAO, K. M., CHETTY, C. M. S., RAMKANTH, S., RAJAN, T. & GNANAPRAKASH, K. 2010. A review on *in-vitro* antioxidant methods: comparisons, correlations and considerations. *International Journal of PharmTech Research*, 2, 1276-1285.
- BALASUNDRAM, N., SUNDRAM, K. & SAMMAN, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food chemistry*, 99, 191-203.
- BALASURIYA, B. N. & RUPASINGHE, H. V. 2011. Plant flavonoids as angiotensin converting enzyme inhibitors in regulation of hypertension. *Functional Foods in Health and Disease*, 1, 172-188.
- BALUNAS, M. J. & KINGHORN, A. D. 2005. Drug discovery from medicinal plants. *Life sciences*, 78, 431-441.

- BENZIE, I. F. & STRAIN, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239, 70-76.
- BENZIE, I. F. & SZETO, Y. 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of agricultural and food chemistry*, 47, 633-636.
- BISI-JOHNSON, M. A., OBI, C. L., SAMUEL, B. B., ELOFF, J. N. & OKOH, A. I. 2017. Antibacterial activity of crude extracts of some South African medicinal plants against multidrug resistant etiological agents of diarrhoea. *BMC complementary and alternative medicine*, 17, 321.
- BOUKES, G. J. & VAN DE VENTER, M. 2011. Cytotoxicity and mechanism (s) of action of *Hypoxis* spp.(African potato) against HeLa, HT-29 and MCF-7 cancer cell lines. *Journal of Medical Plants Research*, 5, 2766-74.
- BOUKES, G. J., VAN DE VENTER, M. & OOSTHUIZEN, V. 2008. Quantitative and qualitative analysis of sterols/sterolins and hypoxoside contents of three *Hypoxis* (African potato) spp. *African Journal of Biotechnology*, 7.
- BOURGAUD, F., GRAVOT, A., MILESI, S. & GONTIER, E. 2001. Production of plant secondary metabolites: a historical perspective. *Plant science*, 161, 839-851.
- BRAGA, F. C., SERRA, C. P., JÚNIOR, N. S. V., OLIVEIRA, A. B., CÔRTEZ, S. F. & LOMBARDI, J. A. 2007. Angiotensin-converting enzyme inhibition by Brazilian plants. *Fitoterapia*, 78, 353-358.
- BRODNIEWICZ, T. & GRYNKIEWICZ, G. 2012. Plant phenolics as drug leads--what is missing. *Acta Poloniae Pharmaceutica* 69, 1203-17.
- CHAVAN, S. S., DAMALE, M. G., SHAMKUWAR, P. B. & PAWAR, D. P. 2013. Traditional medicinal plants for anticancer activity. *International Journal of Current Pharmaceutical Research*, 5, 50-54.
- CHEN, J., WANG, Y., YE, R., WU, Y. & XIA, W. 2013. Comparison of analytical methods to assay inhibitors of angiotensin I-converting enzyme. *Food chemistry*, 141, 3329-3334.
- CHOI, C. W., KIM, S. C., HWANG, S. S., CHOI, B. K., AHN, H. J., LEE, M. Y., PARK, S. H. & KIM, S. K. 2002. Antioxidant activity and free radical scavenging capacity

- between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant science*, 163, 1161-1168.
- COTTER, T. G. 2009. Apoptosis and cancer: the genesis of a research field. *Nature Reviews Cancer*, 9, 501-507.
- CRAGG, G. M. & NEWMAN, D. J. 2005. Plants as a source of anti-cancer agents. *Journal of ethnopharmacology*, 100, 72-79.
- DAI, J. & MUMPER, R. J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15, 7313-7352.
- DE BOER, I. H., BANGALORE, S., BENETOS, A., DAVIS, A. M., MICHOS, E. D., MUNTNER, P., ROSSING, P., ZOUNGAS, S. & BAKRIS, G. 2017. Diabetes and hypertension: a position statement by the American Diabetes Association. *Diabetes Care*, 40, 1273-1284.
- DOLD, A. P. & COCKS, M. L. 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Science*, 98, 589-597.
- DREWES, S., ELLIOT, E., KHAN, F., DHLAMINI, J. & GCUMISA, M. 2008. *Hypoxis hemerocallidea*—Not merely a cure for benign prostate hyperplasia. *Journal of ethnopharmacology*, 119, 593-598.
- DUNCAN, A. C., JÄGER, A. K. & VAN STADEN, J. 1999. Screening of Zulu medicinal plants for angiotensin converting enzyme (ACE) inhibitors. *Journal of Ethnopharmacology*, 68, 63-70.
- DUTHIE, G. G., GARDNER, P. T. & KYLE, J. A. 2003. Plant polyphenols: are they the new magic bullet? *Proceedings of the Nutrition Society*, 62, 599-603.
- DWARKA, D., THAVER, V., NAIDU, M., KOORBANALLY, N. A. & BAIJNATH, H. 2017. *In vitro* chemo-preventative activity of *Strelitzia nicolai* aril extract containing bilirubin. *African Journal of Traditional, Complementary and Alternative Medicines*, 14, 147-156.
- ELBAGORY, A. M., HUSSEIN, A. A. & MEYER, M. 2019. The *In Vitro* Immunomodulatory Effects Of Gold Nanoparticles Synthesized From *Hypoxis hemerocallidea* Aqueous Extract And Hypoxoside On Macrophage And Natural Killer Cells. *International Journal of Nanomedicine*, 14, 9007.

- ELGORASHI, E. E., TAYLOR, J. L., MAES, A., VAN STADEN, J., DE KIMPE, N. & VERSCHAEVE, L. 2003. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. *Toxicology letters*, 143, 195-207.
- ELUJOBA, A. A., ODELEYE, O. & OGUNYEMI, C. 2005. Traditional Medicine Development for Medical and Dental Primary Health Care Delivery System in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 2, 46-61.
- EVANS, J. L. & GOLDFINE, I. D. 2000.  $\alpha$ -Lipoic acid: a multifunctional antioxidant that improves insulin sensitivity in patients with type 2 diabetes. *Diabetes technology & therapeutics*, 2, 401-413.
- FABRICANT, D. S. & FARNSWORTH, N. R. 2001. The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives*, 109, 69-75.
- FANG, Y.-Z., YANG, S. & WU, G. 2002. Free radicals, antioxidants, and nutrition. *Nutrition*, 18, 872-879.
- FOROUZANFAR, M. H., AFSHIN, A., ALEXANDER, L. T., ANDERSON, H. R., BHUTTA, Z. A., BIRYUKOV, S., BRAUER, M., BURNETT, R., CERCY, K. & CHARLSON, F. J. 2016. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The lancet*, 388, 1659-1724.
- GOBOZA, M., ABOUA, Y. G., MEYER, S. & OGUNTIBEJU, O. O. 2016. Diabetes mellitus: economic and health burden, treatment and the therapeutical effects of *Hypoxis hemerocallidea* plant. *Medical Technology SA*, 30, 39-46.
- GREEN, D. R. & REED, J. C. 1998. Mitochondria and apoptosis. *Science*, 281, 1309-1312.
- GROSSO, G. 2018. Dietary Antioxidants and Prevention of Non-Communicable Diseases. Multidisciplinary Digital Publishing Institute.
- GUASCH-FERRÉ, M., MERINO, J., SUN, Q., FITÓ, M. & SALAS-SALVADÓ, J. 2017. Dietary polyphenols, Mediterranean diet, prediabetes, and Type 2 diabetes: A narrative review of the evidence. *Oxidative medicine and cellular longevity*, 2017.

- GÜÇLÜ, K., ALTUN, M., ÖZYÜREK, M., KARADEMİR, S. E. & APAK, R. 2006. Antioxidant capacity of fresh, sun-and sulphited-dried Malatya apricot (*Prunus armeniaca*) assayed by CUPRAC, ABTS/TEAC and folin methods. *International journal of food science & technology*, 41, 76-85.
- GUPTA, R., GUPTA, V. P., PRAKASH, H., AGRAWAL, A., SHARMA, K. K. & DEEDWANIA, P. C. 2017a. 25-Year trends in hypertension prevalence, awareness, treatment, and control in an Indian urban population: Jaipur Heart Watch. *Indian heart journal*, 70, 802-807.
- GUPTA, R., RAO, R. S., MISRA, A. & SHARMA, S. K. 2017b. Recent trends in epidemiology of dyslipidemias in India. *Indian heart journal*, 69, 382-392.
- GUPTA, R. & XAVIER, D. 2018. Hypertension: The most important non communicable disease risk factor in India. *Indian heart journal*, 70, 565-572.
- HALBERSTEIN, R. A. 2005. Medicinal plants: historical and cross-cultural usage patterns. *Annals of epidemiology*, 15, 686-699.
- HALLIWELL, B. 2001. Free radicals and other reactive species in disease. *eLS*, 1-9.
- HÄSSIG, A., LINAG, W., SCHWABL, H. & STAMPFLI, K. 1999. Flavonoids and tannins: plant-based antioxidants with vitamin character. *Medical hypotheses*, 52, 479-481.
- HE, R., ALASHI, A., MALOMO, S. A., GIRGIH, A. T., CHAO, D., JU, X. & ALUKO, R. E. 2013. Antihypertensive and free radical scavenging properties of enzymatic rapeseed protein hydrolysates. *Food Chemistry*, 141, 153-159.
- HLEM, E. M. 2018. Available: [http://www.hlem.co.za/indigenous\\_plants/hypoxis\\_hemerocallidea.html](http://www.hlem.co.za/indigenous_plants/hypoxis_hemerocallidea.html) [Accessed 01 May 2020].
- JAMSHIDI-KIA, F., LORIGOOINI, Z. & AMINI-KHOEI, H. 2018. Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology*, 7.
- JOOSTE, C. S. 2012. *Brine shrimp lethality test and acetylcholine esterase inhibition studies on selected South African medicinal plants*. University of the Western Cape.



- KABERA, J. N., SEMANA, E., MUSSA, A. R. & HE, X. 2014. Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *J Pharm Pharmacol*, 2, 377-392.
- KALE, R. 1995. Traditional healers in South Africa: A parallel health care system. South Africa's health. *British Medical Journal*, 310, 1182-1186.
- KAMTEKAR, S., KEER, V. & PATIL, V. 2014. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *Journal of applied pharmaceutical Science*, 4, 61.
- KATERERE, D. 2013. *Hypoxis hemerocallidea* (African potato): A botanical whose time has come. *African natural plant products*, 11.
- KATERERE, D. & ELOFF, J. 2008. Anti-bacterial and anti-oxidant activity of *Hypoxis hemerocallidea* (Hypoxidaceae): can leaves be substituted for corms as a conservation strategy? *South African Journal of Botany*, 74, 613-616.
- KELMANSON, J. E., JÄGER, A. K. & VAN STADEN, J. 2000. Zulu medicinal plants with antibacterial activity. *Journal of ethnopharmacology*, 69, 241-246.
- KHAN, F. & DREWES, S. 2004. The African potato (*Hypoxis hemerocallidea*): a chemical-historical perspective. *South African journal of science*, 100, 425-430.
- KHAN, H. 2014. Medicinal plants in light of history: recognized therapeutic modality. *Journal of evidence-based complementary & alternative medicine*, 19, 216-219.
- KHAN, M. Y. & KUMAR, V. 2019. Mechanism & inhibition kinetics of bioassay-guided fractions of Indian medicinal plants and foods as ACE inhibitors. *Journal of traditional and complementary medicine*, 9, 73-84.
- KHARROUBI, A. T. & DARWISH, H. M. 2015. Diabetes mellitus: The epidemic of the century. *World journal of diabetes*, 6, 850.
- KHLIFI, D., SGHAIER, R. M., AMOURI, S., LAOUINI, D., HAMDI, M. & BOUAIJILA, J. 2013. Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of *Artemisia herba-alba*, *Ruta chalapensis* L. and *Peganum harmala* L. *Food and chemical toxicology*, 55, 202-208.
- KIRINGE, J. W. 2006. A survey of traditional health remedies used by the Maasai of Southern Kaijiado District, Kenya. *Ethnobotany Research and Applications*, 4, 061-074.

- KOCH, W. 2019. Dietary Polyphenols—Important Non-Nutrients in the Prevention of Chronic Noncommunicable Diseases. A Systematic Review. *Nutrients*, 11, 1039.
- LAHER, F., AREMU, A., VAN STADEN, J. & FINNIE, J. 2013. Evaluating the effect of storage on the biological activity and chemical composition of three South African medicinal plants. *South African journal of botany*, 88, 414-418.
- LAPORTA, O., PÉREZ-FONS, L., MALLAVIA, R., CATURLA, N. & MICOL, V. 2007. Isolation, characterization and antioxidant capacity assessment of the bioactive compounds derived from *Hypoxis rooperi* corm extract (African potato). *Food Chemistry*, 101, 1425-1437.
- LI, G.-H., LIU, H., SHI, Y.-H. & LE, G.-W. 2005. Direct spectrophotometric measurement of angiotensin I-converting enzyme inhibitory activity for screening bioactive peptides. *Journal of pharmaceutical and biomedical analysis*, 37, 219-224.
- MADIKIZELA, B. & MCGAW, L. 2019. *In vitro* cytotoxicity, antioxidant and anti-inflammatory activities of *Pittosporum viridiflorum* Sims and *Hypoxis colchicifolia* Baker used traditionally against cancer in Eastern Cape, South Africa. *South African Journal of Botany*, 126, 250-255.
- MADIKIZELA, B. & MCGAW, L. J. 2018. Scientific rationale for traditional use of plants to treat tuberculosis in the eastern region of the OR Tambo district, South Africa. *Journal of ethnopharmacology*, 224, 250-260.
- MAKIN, G. & DIVE, C. 2001. Apoptosis and cancer chemotherapy. *Trends in cell biology*, 11, S22-S26.
- MAMEDOV, N. 2012. Medicinal plants studies: history, challenges and prospective. *Med Aromat Plants*, 1, e133.
- MANNATHOKO, N., GEORGE, S., SOUDA, S., CHABAESELE, K. & GOERCKE, I. 2017. An *in-vitro* Analysis of the Antioxidant and Antimicrobial Properties of the Methanol Extract of *Hypoxis hemerocallidea* corm (MEHHC) from Botswana. *Microbiology Research Journal International*, 1-12.
- MARTINS, S., MUSSATTO, S. I., MARTÍNEZ-AVILA, G., MONTAÑEZ-SAENZ, J., AGUILAR, C. N. & TEIXEIRA, J. A. 2011. Bioactive phenolic compounds: production and extraction by solid-state fermentation. A review. *Biotechnology advances*, 29, 365-373.

- MCCORD, J. M. 2000. The evolution of free radicals and oxidative stress. *The American journal of medicine*, 108, 652-659.
- MEYER, B., FERRIGNI, N., PUTNAM, J., JACOBSEN, L., NICHOLS, D. & MCLAUGHLIN, J. L. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica*, 45, 31-34.
- MICHALAK, A. 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, 15.
- MIGUEL, M. G. 2010. Antioxidant activity of medicinal and aromatic plants. A review. *Flavour and Fragrance Journal*, 25, 291-312.
- MWINGA, J. L., ASONG, J. A., AMOO, S. O., NKADIMENG, S. M., MCGAW, L. J., AREMU, A. O. & OTANG-MBENG, W. 2019. *In vitro* antimicrobial effects of *Hypoxis hemerocallidea* against six pathogens with dermatological relevance and its phytochemical characterization and cytotoxicity evaluation. *Journal of ethnopharmacology*, 242, 112048.
- NAIR, S. S., KAVREKAR, V. & MISHRA, A. 2013. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *European Journal of Experimental Biology*, 3, 128-132.
- NAIR, V. D., DAIRAM, A., AGBONON, A., ARNASON, J., FOSTER, B. & KANFER, I. 2007. Investigation of the antioxidant activity of African potato (*Hypoxis hemerocallidea*). *Journal of agricultural and food chemistry*, 55, 1707-1711.
- NATURE. 2014. *Cell division and cancer* [Online]. Available: <https://www.nature.com/scitable/topicpage/cell-division-and-cancer-14046590/> [Accessed 14 April 2020].
- NCUBE, B., NDHLALA, A. R., OKEM, A. & VAN STADEN, J. 2013. *Hypoxis* (Hypoxidaceae) in African traditional medicine. *Journal of ethnopharmacology*, 150, 818-827.
- NSIBANDE, B. E., GUSTAVSSON, K.-E. & ZHU, L.-H. 2018. Analysis of Health-Associated Phytochemical Compounds in Seven *Hypoxis* Species. *American Journal of Plant Sciences*, 9, 571.
- OBOH, G. 2006. Antioxidant properties of some commonly consumed and underutilized tropical legumes. *European Food Research and Technology*, 224, 61-65.

- ODHAV, B., KANDASAMY, T., KHUMALO, N. & BAIJNATH, H. 2010. Screening of African traditional vegetables for their alpha-amylase inhibitory effect. *Journal of Medical Plants Research* 4, 1502-1507.
- OGUNTIBEJU, O. O., MEYER, S., ABOUA, Y. G. & GOBOZA, M. 2016. *Hypoxis hemerocallidea* significantly reduced hyperglycaemia and hyperglycaemic-induced oxidative stress in the liver and kidney tissues of streptozotocin-induced diabetic male wistar rats. *Evidence-Based Complementary and Alternative Medicine*, 2016.
- OJEWOLE, J. A. 2006. Antinociceptive, anti-inflammatory and antidiabetic properties of *Hypoxis hemerocallidea* Fisch. & C.A. Mey.(Hypoxidaceae) corm ['African Potato'] aqueous extract in mice and rats. *Journal of ethnopharmacology*, 103, 126-134.
- ONANUGA, I. O., JEGEDE, A. I., OFFOR, U., OGEDENGBE, O. O., NAIDU, E. C., PETER, A. I. & AZU, O. O. 2018. *Hypoxis hemerocallidea* alters metabolic parameters and hepatic histomorphology in streptozotocin-nicotinamide-induced diabetic male rats under antiretroviral therapy. *Archives of Medical Science*, 14.
- OTUNOLA, G. A. & AFOLAYAN, A. J. 2019. Proximate and elemental composition of leaf, corm, root and peel of *Hypoxis hemerocallidea*: A Southern African multipurpose medicinal plant. *Pakistan journal of pharmaceutical sciences*, 32.
- PANDEY, K. B. & RIZVI, S. I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, 2, 270-278.
- PATEL, H. H. & GAMIT, D. 2018. Angiotensin Converting Enzyme (ACE): Inhibition of Rabbit Lungs ACE *in Vitro* by *Nardostacys Jatamansi*. *International Journal of Scientific Research in Science and Technology*, 4, 1519-1523.
- PATEL, S. G. 2016. A review on medicinal plants for cancer therapy. *International Journal of MediPharm*, 2, 105-112.
- PEREUS, D., OTIENO, J., GHORBANI, A., KOCYAN, A., HILONGA, S. & DE BOER, H. 2018. Diversity of *Hypoxis* species used in ethnomedicine in Tanzania. *South African Journal of Botany*.
- PHATAK, R. S. & HENDRE, A. S. 2014. Total antioxidant capacity (TAC) of fresh leaves of *Kalanchoe pinnata*. *Journal of Pharmacognosy and Phytochemistry*, 2.

- PHILLIPSON, J. D. 2001. Phytochemistry and medicinal plants. *Phytochemistry*, 56, 237-243.
- RAMULONDI, M., DE WET, H. & VAN VUUREN, S. 2018. Toxicology of medicinal plants and combinations used in rural northern KwaZulu-Natal (South Africa) for the treatment of hypertension. *Journal of Herbal Medicine*, 100251.
- RANILLA, L. G., APOSTOLIDIS, E., GENOVESE, M. I., LAJOLO, F. M. & SHETTY, K. 2009. Evaluation of indigenous grains from the Peruvian Andean region for antidiabetes and antihypertension potential using *in vitro* methods. *Journal of medicinal food*, 12, 704-713.
- RANILLA, L. G., KWON, Y.-I., APOSTOLIDIS, E. & SHETTY, K. 2010. Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource technology*, 101, 4676-4689.
- RASOULI, H., HOSSEINI-GHAZVINI, S. M.-B., ADIBI, H. & KHODARAHMI, R. 2017. Differential  $\alpha$ -amylase/ $\alpha$ -glucosidase inhibitory activities of plant-derived phenolic compounds: a virtual screening perspective for the treatment of obesity and diabetes. *Food & function*, 8, 1942-1954.
- RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. & RICE-EVANS, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26, 1231-1237.
- SAJEEV, P. & SOMAN, B. 2018. Prevalence of noncommunicable disease risk factors among the Kani tribe in Thiruvananthapuram district, Kerala. *Indian heart journal*, 70, 598-603.
- SAMA, K., MURUGESAN, K. & SIVARAJ, R. 2012. *In vitro* alpha amylase and alpha glucosidase inhibition activity of crude ethanol extract of *Cissus arnottiana*. *Asian Journal of Plant Science and Research*, 2, 550-553.
- SAMIE, A., OBI, C., BESSONG, P. & NAMRITA, L. 2005. Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. *African journal of Biotechnology*, 4.

- SEEDAT, Y. & RAYNER, B. 2012. South African hypertension guideline 2011. *South African Medical Journal*, 102, 60-83.
- SHAHIDI, F. & AMBIGAIPALAN, P. 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects—A review. *Journal of functional foods*, 18, 820-897.
- SHAPIRO, K. & GONG, W. C. 2002. Natural products used for diabetes. *Journal of the American Pharmaceutical Association (1996)*, 42, 217-226.
- SHEKHAR, T. C. & ANJU, G. 2014. Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn. leaves. *American journal of ethnomedicine*, 1, 244-249.
- SINGH, Y. 2006. *Hypoxis* (Hypoxidaceae) in Africa: list of species and infraspecific names. *Bothalia*, 36, 13-23.
- SINGH, Y. 2007. *Hypoxis* (Hypoxidaceae) in southern Africa: taxonomic notes. *South African Journal of Botany*, 73, 360-365.
- SMITH, A., PARKES, M., ATKIN-SMITH, G., TIXEIRA, R. & POON, I. 2017. *Cell disassembly during apoptosis* [Online]. Available: [https://en.wikipedia.org/wiki/Apoptosis#cite\\_note-57](https://en.wikipedia.org/wiki/Apoptosis#cite_note-57) [Accessed 14 April 2020].
- SOFOWORA, A. 1996. Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary Medicine*, 2, 365-372.
- STEENKAMP, V. & GOUWS, M. 2006. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. *South African Journal of Botany*, 72, 630-633.
- STREET, R., STIRK, W. & VAN STADEN, J. 2008. South African traditional medicinal plant trade—challenges in regulating quality, safety and efficacy. *Journal of Ethnopharmacology*, 119, 705-710.
- SUDHA, G., SANGEETHA, P., INDHU, S. & VADIVUKKARASI, S. 2011. Antioxidant activity of ripe pepino fruit (*Solanum muricatum* Aiton). *International Journal of Pharmacy and Pharmaceutical Sciences* 3, 257-61.

- SURVESWARAN, S., CAI, Y.-Z., CORKE, H. & SUN, M. 2007. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food chemistry*, 102, 938-953.
- TAYLOR, J., RABE, T., MCGAW, L., JÄGER, A. & VAN STADEN, J. 2001. Towards the scientific validation of traditional medicinal plants. *Plant growth regulation*, 34, 23-37.
- THAIFA, M., ROSHNA, S., ARYA, U. & APARNA, G. B. 2017. A review on diabetes mellitus and diabetic neuropathy: A plant based approach. *Journal of Pharmacognosy and Phytochemistry*, 6, 506-510.
- TIWARI, P., KUMAR, B., KAUR, M., KAUR, G. & KAUR, H. 2011. Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*, 1, 98-106.
- TRIPATHY, J. P., THAKUR, J., JEET, G. & JAIN, S. 2017. Prevalence and determinants of comorbid diabetes and hypertension: Evidence from non communicable disease risk factor STEPS survey, India. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 11, S459-S465.
- TUSEVSKI, O., KOSTOVSKA, A., ILOSKA, A., TRAJKOVSKA, L. & SIMIC, S. G. 2014. Phenolic production and antioxidant properties of some Macedonian medicinal plants. *Central European Journal of Biology*, 9, 888-900.
- VAN WYK, B.-E. 2008. A broad review of commercially important southern African medicinal plants. *Journal of ethnopharmacology*, 119, 342-355.
- VAN WYK, B.-E. 2015. A review of commercially important African medicinal plants. *Journal of ethnopharmacology*, 176, 118-134.
- WAFULA, W. G., ARNOLD, O., CALVIN, O. & MOSES, M. 2017. Reactive oxygen species (ROS) generation, impacts on tissue oxidation and dietary management of non-communicable diseases: A review.
- WHO. 2018. *Noncommunicable diseases country profiles 2018* [Online]. Available: [https://www.who.int/nmh/countries/2018/zaf\\_en.pdf?ua=1](https://www.who.int/nmh/countries/2018/zaf_en.pdf?ua=1) [Accessed 01 May 2020].
- WINK, M. 2015. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, 2, 251-286.

- WINTOLA, O. A. & AFOLAYAN, A. J. 2015. An inventory of indigenous plants used as anthelmintics in Amathole district municipality of the Eastern Cape province, South Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 12, 112-121.
- ZENGİN, G., GÜLER, G. O., AKTUMSEK, A., CEYLAN, R., PICOT, C. M. N. & MAHOMOODALLY, M. F. 2015. Enzyme inhibitory properties, antioxidant activities, and phytochemical profile of three medicinal plants from Turkey. *Advances in pharmacological sciences*, 2015.
- ZHENG, Y., LEY, S. H. & HU, F. B. 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology*, 14, 88.
- ZIA-UL-HAQ, M., AHMAD, S., AMAROWICZ, R. & DE FEO, V. 2013. Antioxidant activity of the extracts of some cowpea (*Vigna unguiculata* (L) Walp.) cultivars commonly consumed in Pakistan. *Molecules*, 18, 2005-2017.
- ZIMUDZI, C. 2014. African potato (*Hypoxis* Spp): Diversity and comparison of the phytochemical profiles and cytotoxicity evaluation of four Zimbabwean species.
- ZULFIQAR, F., KHAN, S. I., ALI, Z., WANG, Y.-H., ROSS, S. A., VILJOEN, A. M. & KHAN, I. A. 2020. Norlignan glucosides from *Hypoxis hemerocallidea* and their potential *in vitro* anti-inflammatory activity via inhibition of iNOS and NF- $\kappa$ B. *Phytochemistry*, 172, 112273.