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An assessment of bioactive compound profiles and biological activities in selected *Bulbine* species under short-term exposure to concurrent elevated carbon dioxide and temperatures

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Declaration

I, Thabiso Katlego Teffo, hereby declare that this thesis is my original work submitted for the degree of Doctor of Philosophy to the University of the Witwatersrand, Johannesburg. It has never been submitted to any other University for any other degree or examination.

The described experimental work in this doctoral project was carried out at the University of the Witwatersrand, Johannesburg, under the supervision of Dr Ida Risenga (School of Animal, Plant and Environmental Sciences), Mr Phillemon Ramalepe (WITS School of Education), and Associate Professor Shalini Dukhan (School of Animal, Plant and Environmental Sciences).

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Dedication

This thesis is dedicated to my late sister Kgomotso Cynthia Teffo. Love you always and forever.

Abstract

South Africa has been identified as a climate change hotspot by the Intergovernmental Panel on Climate Change (IPCC), primarily due to rising temperatures over the past four decades. Anthropogenic activities, particularly industrialization, have contributed significantly to the increase in greenhouse gases, notably atmospheric carbon dioxide (CO_2). These changes pose a potential threat to plant life by altering physiological and metabolic processes. Medicinal plants, in particular, may be highly sensitive to such environmental shifts, which can affect their phytochemical composition and therapeutic potential. While most studies have examined the effects of individual abiotic factors on medicinal plants, natural ecosystems subject plants to simultaneous environmental stresses. This study aimed to evaluate the effects of concurrent elevated CO_2 and temperature on the phytochemical content and biological activities of *Bulbine abyssinica*, *Bulbine frutescens*, and *Bulbine natalensis*. Mature potted plants were subjected to elevated CO_2 (600 and 800 ppm) and high day/night temperatures (40/30 °C and 45/35 °C) for eight days in a controlled climate simulation chamber. Plant organs, including leaves, roots, and underground stems, were harvested at intervals (48, 96, 144, and 192 hours) for analysis. Control plants were maintained at ambient CO_2 (400 ppm) and temperatures (27/25 °C).

Methanol extracts of plant parts were screened for ten phytochemical groups and quantitatively assessed for total phenolic, flavonoid, tannin, and proanthocyanidin content using UV-Vis spectrophotometry. Antioxidant activity was evaluated using DPPH, hydrogen peroxide, and metal chelation assays. Antimicrobial potential was investigated via agar diffusion and minimum inhibitory concentration (MIC) assays against several Gram-positive and Gram-negative bacteria and yeasts.

Results showed that leaf extracts generally exhibited a greater diversity of phytochemical groups than underground organs. Notably, the underground stem of *B. abyssinica* and *B.*

natalensis accumulated higher tannin content under stress conditions, while *B. frutescens* leaves showed enhanced proanthocyanidin levels. Elevated CO_2 and temperature increased the accumulation of bioactive compounds across species relative to controls. Enhanced antioxidant activity was observed, especially for H_2O_2 scavenging and iron chelation, with underground organs often outperforming leaves. Antibacterial activity under elevated CO_2 and temperature was moderate (10–19 mm inhibition zones) overall, with enhanced MIC responses observed at higher stress levels (800 ppm CO_2 and 45/35 °C). Among the tested microbes, *Enterococcus faecalis* showed consistent inhibition across all species.

In conclusion, short-term exposure to concurrent elevated CO_2 and temperature significantly influences the synthesis of phytochemicals and enhances certain biological activities in *Bulbine* species. These findings highlight species-specific and organ-specific responses that may inform future conservation and pharmaceutical utilization strategies under climate change scenarios.

Abbreviations and symbols

DEA	Department of Environmental Affairs
KZN	KwaZulu-Natal
NOAA	National Oceanic and Atmospheric Administration
IPCC	Intergovernmental Panel on Climate Change
CO_2	Carbon dioxide
GHG	Greenhouse gases
GtC	Gigatonnes of carbon
PgC	Pentagram of carbon
DPPH	2, 2 diphenylpicrylhydrazyl
H_2O_2	Hydrogen peroxide
$ONOO^-$	Peroxynitrite radical
ROS	Reactive oxygen species
$AlCl_3$	Aluminium chloride
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
Na_2CO_3	Sodium carbonate
yr	Year
g	Gram
mM	Millimolar

mm	Millimetre
ml	Millilitre
µL	Microlitre
%	Percentage
˚C	Degrees Celsius
ppm	Parts per million
mg	Milligram
µg	Microgram
UV	Ultraviolet
TPC	Total phenolic content
TFC	Total flavonoid content
TTC	Total tannin content
TPAC	Total proanthocyanidin content
AA	Antioxidant activity
MIC	Minimum inhibitory concentration
pH	Potential of hydrogen
TSB	Tryptone soya broth
TGB	thioglycolate broth
INT	Iodonitrotetrazolium
ATCC	American Type Culture Collection

ANOVA	Analysis of Variance
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent
CE	Catechin Equivalent
DMSO	Dimethyl sulfoxide

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were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

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Contributions from thesis

Publication record

Published papers:

1) Teffo TK, Dukhan S, Ramalepe P and Risenga I (2021). Possible implications of climate change on the medicinal properties of *Bulbine* species with a particular focus on *Bulbine abyssinica*, *Bulbine frutescens*, and *Bulbine natalensis* in South Africa. Journal of Pharmacognosy and Phytochemistry, 10(5):49-56. DOI: https://doi.org/10.22271/phyto.2021.v10.i5a.14208

2) Teffo TK, Dukhan S, Ramalepe P and Risenga I (2022). Comparative Phytochemical Analysis, Antioxidant and Antibacterial Activities in the Leaves, Underground Stems and Roots of *Bulbine abyssinica*. Biomedical and Pharmacology Journal, 15(3), 1323-1335. DOI: https://dx.doi.org/10.13005/bpj/2469

3) Teffo TK, Dukhan S, Ramalepe P and Risenga I (2024). Phytochemical analysis and biological activities of various parts of *Bulbine natalensis* (Baker): A comparative study. Journal of Herbmed Pharmacology, 13(1): 52-60. DOI: 10.34172/jhp.2024.44650

4) Teffo TK, Dukhan S, Ramalepe P and Risenga I (2024). Phytochemical Profile and Bioactivity of the Methanolic Leaf and Root Extracts of South African *Bulbine frutescens*. Indonesian Journal of Pharmacy, 35(1):105-115. DOI: https://doi.org/10.22146/ijp.6025

5) Teffo TK, Dukhan S, Ramalepe P and Risenga I (2024). Evaluation of the secondary metabolites and bioactivity of South African *Bulbine natalensis* under simultaneous elevated carbon dioxide and temperatures. Biomedical and Pharmacology Journal, 17(3): 1670-1700. DOI: https://dx.doi.org/10.13005/bpj/2975

6) Teffo TK, Dukhan S, Ramalepe P, Van Vuuren S and Risenga I (2024).Short-term exposure to combined elevated carbon dioxide and temperatures influences the antimicrobial activity of selected *Bulbine* species. South African Journal of Botany, 180 (2025), 199-206. DOI: https://doi.org/10.1016/j.sajb.2025.03.010 0254-6299

Manuscripts submitted/to be submitted:

1) The potential effects of concurrent elevated carbon dioxide and high temperatures on the secondary metabolites and bioactive properties of *Bulbine frutescens*. The manuscript will be submitted to The South African Journal of Science for review.

2) The synchronous effect of elevated carbon dioxide and high temperatures on the phytochemical content, antioxidant, and antibacterial properties of *Bulbine abyssinica*. The manuscript will be submitted to the Caspian Journal of Environmental Sciences for review.

Conferences

Article presentation: Teffo TK, Dukhan S, Ramalepe P and Risenga I (2022). Comparative Phytochemical Analysis, Antioxidant and Antibacterial Activities in the Leaves, Underground Stems and Roots of *Bulbine abyssinica*. Biomedical and Pharmacology Journal, 15(3), 1323-1335. South African Association of Botanists, 18 January 2022.

Thesis structure

The format of this thesis is comprised of five chapters. **Chapter one** includes the general background of the study, discussing the general conceptualisation and context of the research. The rationale and novelty, as well as the study's aim, objectives, research questions, and hypotheses are included in this section.

Chapter two includes a detailed literature review encompassing the botanical description of *Bulbine frutescens*, *Bulbine abyssinica*, and *Bulbine natalensis*, their ethnomedicinal uses and phytopharmacological properties. A thorough discussion on the environmental conditions that could potentially affect the properties of medicinal plant species relative to the study is also included in this chapter. This chapter has been published in the form of a review article, but has been modified for the context of this thesis.

The third and fourth chapters have been arranged in a manner that discusses complementary parameters. **Chapter three** investigates the effects of concurrent elevated CO_2 and temperatures on the antimicrobial activity of various plant organs of *B. frutescens*, *B. abyssinica*, and *B. natalensis*. **Chapter four** assesses the influence of concurrent elevated CO_2 and temperatures on the phytochemical profile and in-vitro antioxidant activity of each study species. The phytochemical profile, antioxidant, and qualitative antibacterial activities of the control data for each species have been published. One manuscript investigating the effects of concurrent elevated CO_2 and temperatures on the phytochemical content and bioactivity of *B. natalensis* has been published. A second manuscript discussing the combined effects of elevated CO_2 and temperatures on the antimicrobial activities of all species has been accepted for publication. Two additional manuscripts have been submitted for review in several journals.

The **fifth chapter** covers the general discussion, which looks at the overarching findings of the study, and relating them back to the aim, as well as the concluding statement and future recommendations.

CHAPTER ONE

Introduction

This chapter contains the background of the study, as well as the rationale and novelty, research questions, aim, objectives, and hypotheses.

1.1 General background

Ethnomedicine is the oldest form of therapeutic agents that have been used worldwide. The indigenous knowledge regarding their health-promoting benefits have been carried over many generations throughout communities (Khan, 2014; Marrelli, 2021). More than 50 000 medicinal plant species, about 1/10th of the global numbers, are utilised in pharmacotherapy in today's world. The countries which have the highest recorded population of medicinal plants are China, India, Colombia, South Africa and the United States of America (Chen *et al*., 2016). An estimated three-quarters of developing countries are reliant on medicinal plants for primary healthcare (Djordjevic, 2018; Rasethe *et al*., 2019). This high dependency is a result of the limited accessibility and affordability of adequate healthcare, as well as the convenient accessibility of herbal medicine (Shewamene *et al*., 2017; Rasethe *et al*., 2019). It has been reported that about 6000 medicinal plants are used in Africa (Rasethe *et al*., 2019). This has led to a large trading market of these plants in the continent. South Africa's herbal medicine trade generates an estimated value of R2.9 billion per year, which makes up 5.6% of the country's budget allocated to the Department of Health (Mander *et al*., 1998; Rasethe *et al*., 2019). Medicinal plants are well-known to produce secondary metabolites, or phytochemicals, which are active components responsible for their therapeutic properties (Bhat, 2021). Compounds such as tannins, steroids, alkaloids, flavonoids and polyphenols have been documented to exert antioxidant, antimicrobial, anti-malarial, anti-diabetic, anti-arthritic, and anti-carcinogenic properties, which further proves their significance in ethnomedicine and drug development (Vivekraj *et al*., 2017; Bhat, 2021).

Various abiotic factors such as drought, temperature and light intensity (Suzuki *et al*., 2014), and biotic factors such as herbivory and microbial invasion, hinder the growth and development (Ncube *et al*., 2012). These factors can negatively impact the physiological activities of plants such as pigmentation, transpiration and enzyme activity, which could induce cellular and tissue

apoptosis (Onyekachi *et al*., 2019). Plants being immobile, they have to develop strategies in order to counteract the effects imposed by the environment, including the synthesis of secondary metabolites (Ncube *et al*., 2012). Abiotic factors greatly influence the production, quality and quantity of secondary metabolites (Coley, 1987; Ncube *et al*., 2012). Two of the greatest contributors to climate change are temperatures and atmospheric carbon dioxide. Temperatures can accelerate protein denaturation and cell membrane distress, which can affect the concentration of secondary metabolites, which results in the slow response to injury of plant tissues and morphology (Zobayed *et al*., 2005; Ncube *et al*., 2012). The drastic changes in atmospheric carbon dioxide are becoming a concern, with projections reporting that the global atmospheric CO_2 concentrations will have increased by nearly 50%, with ranges falling between 730-1020 ppm (parts per million) over the next five decades (Ainsworth *et al.,* 2006; De Souza *et al.,* 2008). Evidence shows that elevated CO_2 increases the usage of carbohydrates by respiration, which leads to the increase in carbon fixation, further increasing energy and biochemical precursors to promote leaf growth (Ainsworth *et al.,* 2006). Based from the aforementioned statements, one can already deduce the different impacts between extreme temperatures and elevated CO_2 on plants. Both abiotic stresses have contrasting effects, with CO_2 clearly promoting plant growth whereas temperature at extreme levels compromises plants (Rangaswamy *et al.,* 2021).

There has been some emergence in the research on environmental implications on medicinal plant phytochemistry and bioactivity (Olennikov et al., 2017; Al Jaouni *et al*., 2018; Al-Huqail *et al*., 2020; Singini *et al*., 2023; Mashigo *et al*., 2023; Adeleye and Risenga, 2024; Basson *et al*., 2024). Most of the investigations however, have focused on the impacts of a single environmental stressor, rather than in combination, considering that these species endure multiple abiotic conditions at the same time (Ncube *et al*., 2012). Additionally, research of this scope in the South African context are very limited, hence the importance in expanding the

knowledge and scientifically documenting the possible impacts of climate change on the phytopharmacological profile of South African medicinal plants. For this research study, the phytochemical profile, in-vitro antioxidant, and antimicrobial activities of *Bulbine abyssinica*, *Bulbine frutescens* and *Bulbine natalensis* were investigated under the concurrent exposure of elevated carbon dioxide and temperatures. Each of the species are used for treating skin ailments such as rashes, wounds and ringworms, and diabetes-related illnesses. There have been some reports on *B. abyssinica*, *B. frutescens* and *B. natalensis*, on the phytochemical content (Yakubu *et al.,* 2009; Shikalepo *et al.,* 2018; Bodede & Prinsloo*,* 2020) and the scientific investigation of their medicinal properties, which range from antioxidant, antimicrobial, anti-inflammatory and cytotoxic activities (Mocktar, 2000; Kibiti *et al.,* 2015; Rachuonyo *et al.,* 2016; Kandari, 2016; Odeyemi *et al.,* 2018; Ghuman *et al.,* 2019; Lucas *et al.,* 2020; Teffo *et al*., 2022; Teffo *et al*., 2024 a; b). However, considering the drastic changes on climate and potential dramatic impacts on phytopharmacological activities on medicinal plants such as *B. abyssinica*, *B. frutescens* and *B. natalensis*. To date, no studies have been performed to determine the potential responses to concurrent abiotic factors. By including elements of environmental sciences, phytopharmacological research will expand and contribute new knowledge within the phytopharmacology discipline. The contributions of this study could also provide valuable information that could lead to the readiness of climate smart conservation strategies, and possible future revision of the species' medicinal profiles under long term exposure to natural holistic environments. Various industries can also learn from the results of this study by finding other means of attaining plants for product development, such as plant tissue culture and artificial environment cultivation in order to obtain the desired bioactivity required to improve products.

1.2 Rationale and novelty of the study

Climate change greatly affects the biological activity of medicinal plants, by altering the production of secondary metabolic compounds (Soni *et al*., 2015). The influence of abiotic factors on the phytopharmacological properties of some medicinal plants have been documented over the past years, which highlights the necessity of performing such analyses, to report on how they could be affected in the near future. Few studies have reported on the comparative analysis of various plant organs of *B. abyssinica*, *B. frutescens* and *B. natalensis* (Teffo *et al.,* 2022; Teffo *et al* 2024a; 2024b), and to date, there are limited reports on how the combination of elevated temperatures and CO_2 could impact the phytochemical profile, and bioactivity of each species. This is where the current research aims to fill in the knowledge gap through novel findings. Since the whole plant is exposed to the changes in its surrounding environment, it is essential to evaluate all plant parts in order to determine a correlated response and impact on the potency of the species. Usually, only one plant organ which is most popular in ethnomedicine, is selected as the focus in medicinal plant studies. This provides limited context on the entire therapeutic profile of species. Additionally, with the inclusion of environmental stressors, it further emphasizes the importance of documenting the responses of whole plant organisms and how it affects their medicinal properties. The subterranean organs play an important role in plant adaptive response, which aid in their resilience and sustainability. In ethnomedicine, multiple plant parts are utilised for treating a range of ailments, which further proves the need to assess plant parts individually. This provides an idea on the range and ability of each plant organ's therapeutic properties, and how this correlates to the efficacy of the whole organism. Underground plant organs seldom receive attention in the context of phytomedicine, but with the application of environmental factors, their inclusion in the investigation of medicinal plant species elucidates more on the response adaptations, in addition to the configuration of their beneficial profile. Secondly, under abiotic stress, plants

usually respond differently depending on the severity and exposure period of the stressor, thus making it imperative to report on the secondary metabolite activity of medicinal plants and its influence on their bioactivity and adaptive strategies they develop to prevent excessive oxidative stress and poor medicinal activity.

The study had proposed to answer the following research questions:

- How great of an effect will concurrent elevated CO_2 and temperatures have on the synthesis and concentration of several phytochemical compound groups in *B. abyssinica*, *B. frutescens* and *B. natalensis*?
- What will be the result of the antioxidant activity of *B. abyssinica*, *B. frutescens* and *B. natalensis* under concurrent elevated CO_2 and temperatures?
- Will *B. abyssinica*, *B. frutescens* and *B. natalensis* retain, improve or show a poor antimicrobial activity against various human-pathogenic microbes under concurrent elevated CO_2 and temperatures?
- Which of the plant parts from each species will show the greatest increase or decrease in the phytochemical profile and biological activities under concurrent elevated CO_2 and temperatures?

1.3 Aim of the study

The aim of the study was to investigate the effects of concurrent elevated CO_2 (600ppm and 800ppm) and temperatures (40/30 ºC and 45/35 ºC day/night) on the phytochemical profile, antioxidant and antimicrobial activity of *B. abyssinica*, *B. frutescens* and *B. natalensis*.

1.4 Objectives of the study

- To determine the qualitative and quantitative phytochemical profile from the leaves and roots of *B. frutescens*, the leaves, underground stems and roots of *B. abyssinica*, and the leaves, corms and roots of *B. natalensis* under episodic exposure to concurrent elevated CO_2 and high temperatures.
- To determine the in-vitro antioxidant activity of various plant parts of *B. abyssinica*, *B. frutescens* and *B. natalensis* harvested under episodic exposure to concurrent elevated CO_2 and temperatures.
- To investigate the qualitative antibacterial activity of the crude extracts of various plant parts of *B. abyssinica*, *B. frutescens* and *B. natalensis* harvested under episodic exposure to concurrent elevated CO_2 and temperatures, against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, using agar-well diffusion technique.
- To assess the antimicrobial activity of various plant parts of *B. abyssinica*, *B. frutescens* and *B. natalensis* harvested from episodic exposure to concurrent elevated CO_2 and temperatures, against Gram-positive *S. aureus*, *Bacillus cereus* and *Enterococcus faecalis*, Gram-negative *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, as well as *Candida albicans* and *Cryptococcus neoformans* yeast strains, using minimum inhibitory concentration (MIC).

1.5 Hypotheses of the study

- The qualitative phytochemical screening of *B. abyssinica*, *B. frutescens* and *B. natalensis* will show a greater number of compounds present as a response to the concurrent elevated CO_2 and temperatures, compared to the control samples.

- The concentrations of the total phenolics, flavonoids, tannins and proanthocyanidins will be greater under concurrent elevated CO_2 and temperatures.
- Each of the species will exhibit a stronger antioxidant and antimicrobial activities under elevated CO_2 and temperatures.
- The leaves from each species will possess an overall higher phytochemical content and biological activity in comparison to the underground plant organs in both control and experimental conditions.

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CHAPTER TWO

Literature Review

This chapter provides a literature review covering the botanical information and phytopharmacological properties of *B. frutescens*, *B. abyssinica*, and *B. natalensis*. The rationale of the study (potential effects of climate change) is also discussed in detailed context.

This chapter has been published as a review article in the Journal of Pharmacognosy and Phytochemistry (DOI: https://doi.org/10.22271/phyto.2021.v10.i5a.14208). For the purpose of this thesis, some of the contents in this chapter have been modified. The full article can be found in Appendix A.

2.1 Botanical description of the genus *Bulbine*

The genus *Bulbine* belongs to the Asphodelaceae family which occurs in southern and tropical Africa, Australia and New Zealand (Klopper *et al.,* 2010). The species are characterized by thick storage roots with mesomorphic or occasionally succulent leaves, and inflorescences which can either be centrally branched or unbranched (Mocktar, 2000). The *Bulbine* species also have distinct yellow flowers mounted on long stems with the plants ranging from bushy caulescent to dwarf groups (Bodede and Prinsloo, 2020). These plants have been described as water-wise plants, a general characteristic of succulent plants (Bodede and Prinsloo, 2020). The species within the *Bulbine* genus have been used since the 18th century for the treatment of various ailments, back when the British and Dutch settlers arrived in southern Africa (Coopoosamy, 2011). In traditional medicine, *Bulbine* species are used to treat rheumatism, venereal infections caused by bacteria and fungi, but most commonly for wounds and other skin ailments such as burns, scars, cuts and rashes (Coopoosamy *et al.,* 2012; Bodede and Prinsloo, 2020). Species that are found in southern Africa include *Bulbine abyssinica*, *Bulbine capitata*, *Bulbine frutescens*, *Bulbine latifolia*, *Bulbine narcissifolia* and *Bulbine natalensis* (Bodede and Prinsloo, 2020). The description of the species that will be studied in this research follows, i.e. *Bulbine abyssinica*, *Bulbine frutescens* and *Bulbine natalensis*.

2.1.1 *Bulbine abyssinica* (Rich.)

Bulbine abyssinica is a succulent, perennial herb, which grows in small clusters and has soft, grass-like leaves which can grow as long as 35 centimetres, and are dark green in colour (Figure 2.1) (Kibiti and Afolayan 2015; Teffo *et al.* 2021). The flowers are bright yellow and star-shaped with bearded stamens which mature from the bottom of the inflorescence. *B. abyssinica* is commonly distributed across the Eastern Cape and Kwa Zulu-Natal provinces, and can also

be found in other African countries such as ESwatini, Lesotho, Angola, and Ethiopia (Teffo *et al.,* 2021). The whole parts of *B. abyssinica* (leaves, stems, roots and flowers) have medicinal uses in the traditional sector that range from the treatment of skin-related conditions such as wounds, scars and burns, to vaginal and bladder infections, dysentery, rheumatism, bilharzia, infertility as well as the management of diabetes mellitus related illnesses (Teffo *et al.,* 2021). In addition to the traditional uses of *B. abyssinica*, the species is also used in ethnoveterinary medicine for the treatment of gastrointestinal parasites, blood cleansing and internal sores in animals, specifically cattle (Maphosa and Masika, 2010; McGaw *et al.,* 2020; Rizwan *et al.,* 2021).

Figure 2.1 Photographic images of the leaves (A), underground stems (B), and roots (C) of *B. abyssinica.*

2.1.2 *Bulbine frutescens* (Willd.)

Bulbine frutescens is described as a subshrub with subterranean roots, and onion-like resemblance of green leaves which either grow erect or slightly erect inflorescences (Figure 2.2) (Teffo *et al.,* 2021). *B. frutescens* is mostly found in the Eastern Cape, Northern Cape, and the Western Cape provinces (Vakele *et al.,* 2022). The species is often used to treat acne, burns, cold sores, insect bites and ringworms and is known to be a good anti-coagulant (Coopoosamy

and Naidoo, 2012; Teffo *et al.,* 2021). The species is also used to treat diarrhoea (Vakele *et al.,* 2022). *B. frutescens* is one of the few species within the *Bulbine* genus that is used traditionally by the indigenous people for the management of diabetes related illnesses in South Africa (Erasto *et al.,* 2005). Similar to *B. abyssinica*, this species is also used as an anti-helminthic for cattle (Maphosa and Masika, 2010).

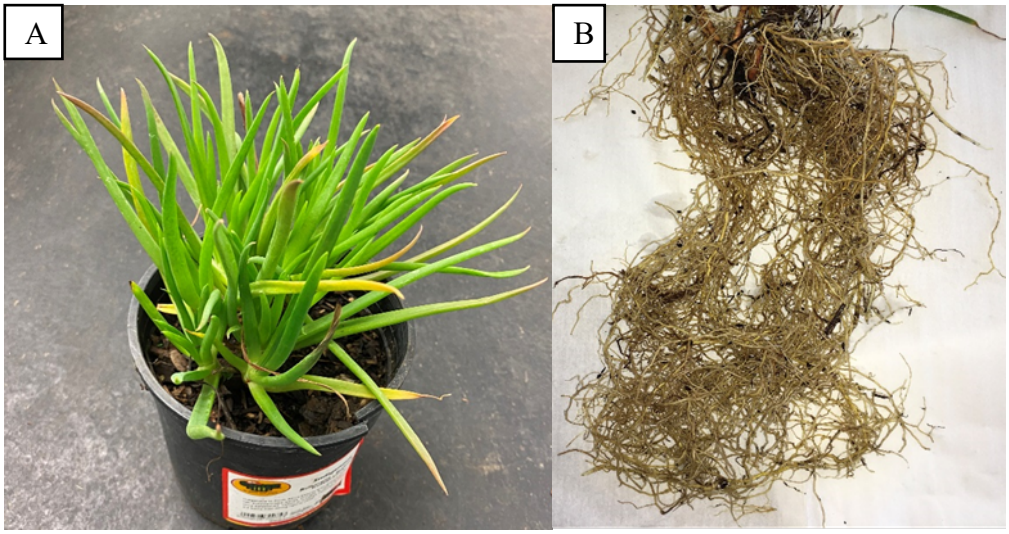

Figure 2.2 Photographic images showing the leaves (A) and roots (B) of *B. frutescens*.

2.1.3 *Bulbine natalensis* (Baker)

Bulbine natalensis is a perennial succulent plant indigenous to South Africa, characterised by its evergreen, fleshy leaves forming a rosette pattern, similar to the *Aloe* species (Figure 2.3) (Musara and Aladejana, 2020). The species is mostly distributed in the eastern and northern regions of the country, but can also be found in Kwa Zulu-Natal and the Western Cape provinces (Musara and Aladejana, 2020; Vakale *et al.,* 2022). *B. natalensis* is regarded as a medicinal plant because of its wide range of uses in traditional medicine. The leaves are mostly used for the treatment of wounds, rashes, ringworms and other skin ailments due to the presence of gel fluid (Teffo *et al.,* 2021). The underground stem is commonly used as a testosterone enhancer, which increases men's fertility (Yakubu *et al.,* 2009; Teffo *et al.,* 2021). The roots

of *B. natalensis* are used to treat gastrointestinal, venereal and blood disorders, as well as diabetes (Yakubu and Afolayan, 2009; Lazarus, 2012; Teffo *et al.,* 2021).

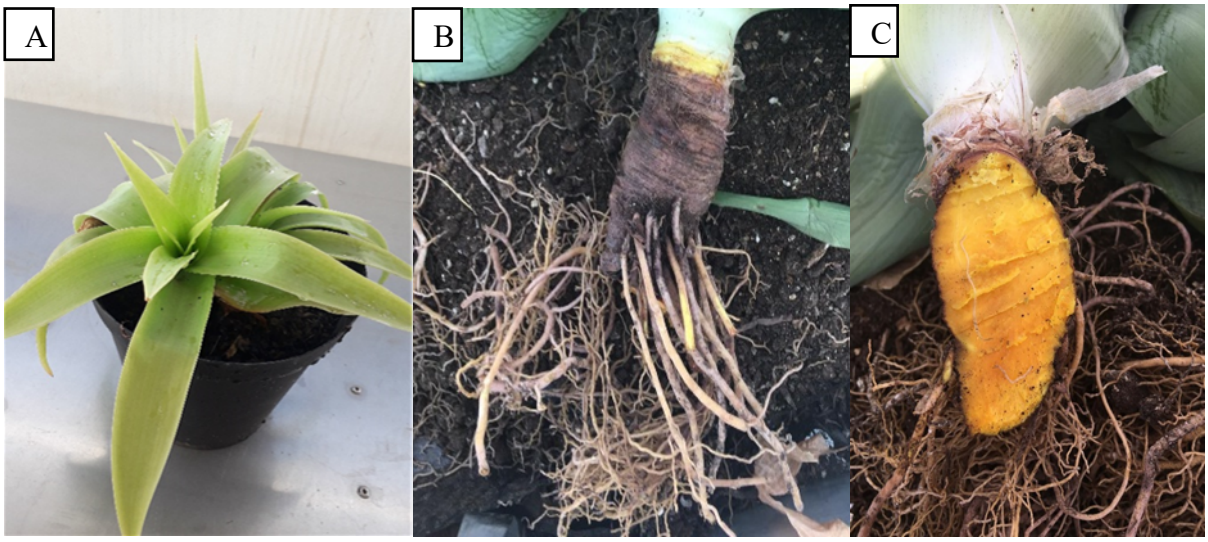

Figure 2.3 Photographic images showing the leaves (A), corm (B and C), and roots (C) of *B. natalensis*.

2.2 Pharmacological activity of *B. abyssinica*, *B. frutescens* and *B. natalensis*

Numerous studies have reported that *B. abyssinica*, *B. frutescens* and *B. natalensis* have more possible applications, other than their use for the treatment of skin-related ailments. These species have been studied for their potential management and treatment of HIV/AIDS (Shikalepo *et al.,* 2018), diabetes mellitus (Erasto *et al.,* 2005; Oyedemi *et al.,* 2009) and breast cancer (Kushwaha *et al.,* 2019). All the plant parts of *B. abyssinica* and *B. natalensis* (leaves, stems and roots) have been reported for traditional uses, however, in the case of *B. frutescens*, only the leaves and roots are used. The medicinal properties of these three species have been outlined below in table 2.1.

Table 2.1 Reported pharmacological activity of *B. abyssinica*, *B. frutescens*, *B. natalensis*.

Plant species	**Plant parts**	**Properties/Treatment**	**Reference**
Bulbine abyssinica (Rich.)	Whole plant (leaves, flowers, stems, roots)	Antifungal	Mocktar (2000); Mwiti and Afolayan (2016)
		Antimicrobial, antioxidant, anti-inflammatory	Kibiti and Afolayan (2015)
	Leaves, roots	Antibacterial	Mocktar (2000)
	Leaves	Antioxidant, antidiabetic	Odeyemi *et al* (2018)
	Leaves, underground stems, roots	Antioxidant, antibacterial	Teffo *et al* (2022)
Bulbine frutescens (Willd.)	Bulb	Breast cancer	Kushwaha *et al* (2019)
	Leaves	Wound healing	Pather (2009); Pather *et al* (2011)
		Antimicrobial	Lucas *et al* (2020)
		HIV/AIDS, antioxidant	Shikalepo *et al* (2018)

		Antimicrobial	Rachuonyo *et al* (2016)
	Leaves, roots	Antimicrobial, antifungal	Mocktar (2000) Teffo *et al* (2024b)
		Antioxidant	Ghuman *et al* (2019) Teffo *et al* (2024b)
	Roots	Antidiabetic	Erasto *et al* (2005)
Bulbine natalensis (Baker.)	Whole plant (leaves, corms, roots)	Antibacterial and antifungal	Mocktar (2000)
	Seeds	Antibacterial	Kandari (2018)
	Leaves	Wound healing	Pather (2009)
	Corms	Male sexual dysfunction (fertility)	Yakubu *et al* (2009)
	Roots	Diabetes	Erasto *et al* (2005)
		Antioxidant and anti-inflammatory	Ghuman *et al* (2019)
	Leaves, corms, roots	Antioxidant, antibacterial	Teffo *et al* (2024a)

2.3 Secondary metabolism of medicinal plants

Plants produce a wide range of secondary metabolites which aid in the maintenance of plant perennial growth, as well as inducing flowering and fruiting (Teoh, 2016). The primary role of phytochemicals however, is plant communication with the environment and plant defence (Hartmann, 2007; Jan *et al*., 2021). Secondary metabolites mitigate environmental stressors such as ultra-violet (UV) radiation, drought, and temperature (Akula and Ravishankar, 2011; Jan *et al*., 2021). The biosynthesis and subsequent accumulation of secondary metabolites are species and geographically specific, meaning that even similar species growing in different locations with different environments will not have the same responses in the production of secondary metabolites (Radusiene *et al*., 2012; Khare *et al*., 2020). Since plants are sessile and lack any form of an immune system, secondary metabolites are produced for their protection. In unfavourable conditions, more than 100 000 secondary metabolites are produced through various metabolic pathways (Khare *et al*., 2020). The primary metabolic pathways in plants responsible for the production of carbohydrates and proteins, initiate the synthesis of secondary metabolites (Jan *et al*., 2021). Under abiotic and biotic stress, the shikimate pathway (Figure 2.4) is activated to synthesize amino acids such as tyrosine and phenylalanine, which maximises the production of secondary metabolites (Parker *et al*., 2009; Jan *et al*., 2021). Some of the phytocompounds produced by medicinal plants, which promote their therapeutic properties include polyphenols, flavonoids, tannins, alkaloids and glycosides (Saxena *et al*., 2013).

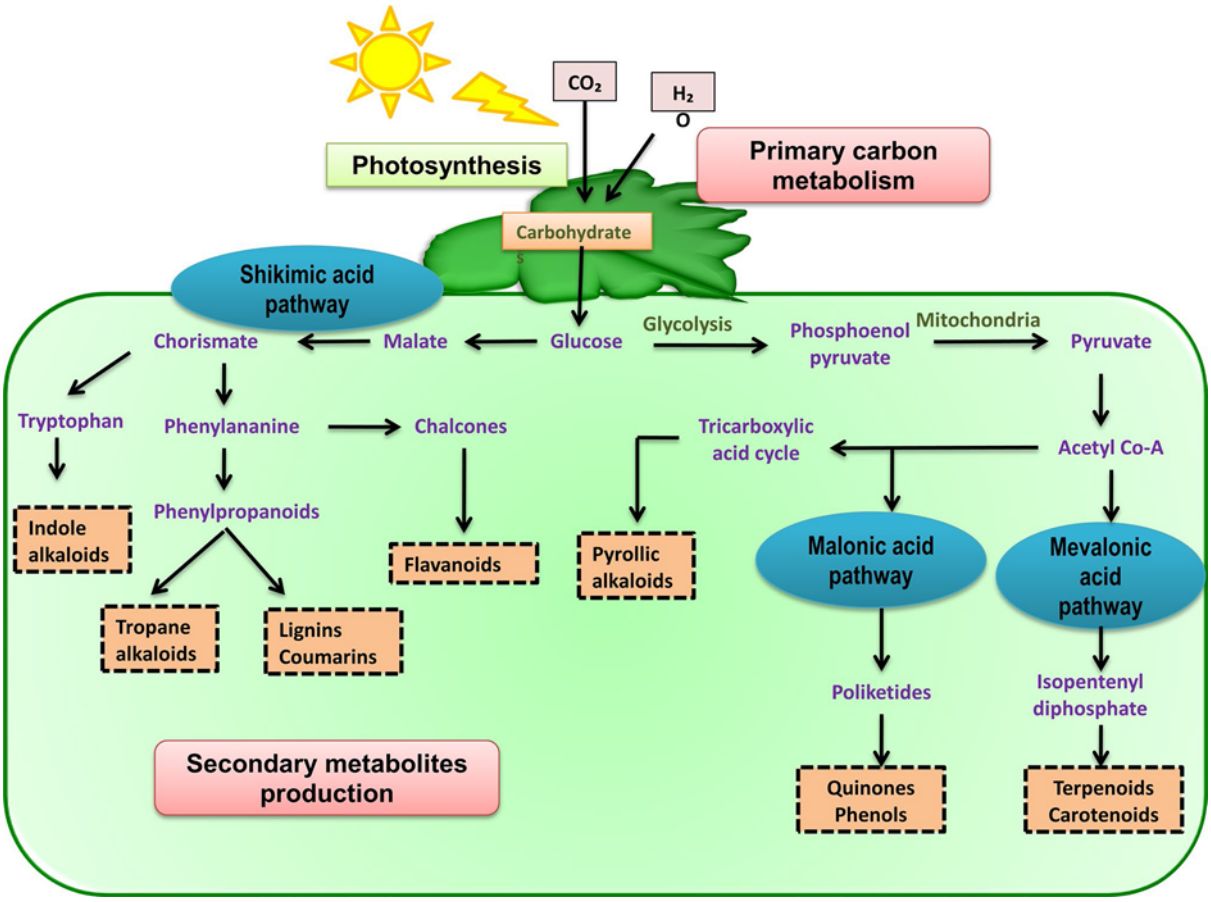

Figure 2.4: Secondary metabolite synthesis in conjunction with primary metabolism in plants.

Polyphenols

This major compound group is the most abundant of secondary metabolites, and range from simple compounds comprised of one aromatic ring, to complex polymers (Teoh, 2016). Some examples of polyphenols include phenolic acids, tannins, coumarins, and flavonoids (Saxena *et al*., 2013). Polyphenols are highly aromatic, as a result of the presence of a cyclic carbon ring (Teoh, 2016). Phenolics are categorized into soluble and bound forms. Soluble phenolic compounds are produced in the endoplasmic reticulum and stored in vacuoles of plant cells, and bound phenolic compounds are transformed from soluble phenolics in the cell wall, through the binding with molecules via glycosidic and ester bonds (Gan *et al*., 2019; Jan *et al*., 2021).

Flavonoids

Flavonoids are the major subgroup of polyphenolic compounds and are comprised of three carbon bridges which connect two aromatic rings. They are commonly found in high concentrations in the epidermis of leaves and the skin of fruits (Kumar *et al*., 2020). General functions of flavonoids include flower petal pigmentation for the purpose of attracting pollinators, as well as UV-filtration and nitrogen fixation in higher plants (Kabera *et al*., 2014). Flavonoids are subdivided into six classes, namely flavones, flavonols, isoflavones, anthocyanidins, flavanones (Kumar *et al*., 2020). Flavonoids are well-known for their uses in pharmaceutical and cosmetic industries because of their anti-inflammatory, anti-carcinogenic, antioxidant, and enzyme inhibiting properties (Walker et al., 2000; Jan *et al*., 2021).

Tannins

Tannins are made up of oligomer and polymer groups which can form complexes with proteins, carbohydrates and minerals (Kabera *et al*., 2014). This group has a molecular weight between 500 and 3000 Da (Kumar *et al*., 2020). Tannins are concentrated in the roots, tree bark, stems, and leaves (Constabel *et al*., 2014), and are toxic to herbivores, so they act as repellents, giving plants a bitter taste (Jan *et al*., 2021). Tannins are subdivided into two classes, namely hydrolysable (e.g. gallotannins and ellagitannins) and condensed tannins or proanthocyanidins (Constabel *et al*., 2014). Tannins are considered one of the most potent medicinal compounds, with properties such as anti-diarrhoic, anti-tumour (of the stomach and duodenum) and anti-inflammatory (Fujiki *et al*., 2012; Kabera *et al*., 2014). The antioxidant activity of tannins is reported to be greater than that of structurally similar phenolics due to their high molecular weight and degree of polymerization and their affinity to chelate metal ions such iron, lead and copper (Constabel *et al*., 2014).

Proanthocyanidins

Proanthocyanidins are polymers synthesized by the condensation of two or more flavan-3-ol units. The structure is made of two aromatic benzyl rings attached to three carbon atoms forming an oxygenated heterocyclic ring (C6-C3-C6) (Mannino *et al*., 2021). Proanthocyanidins are commonly found within the vacuoles of plant cells (Harding, 2019; Mannino *et al*., 2021). They are, however, mostly distributed in the endothelial layer of seed coats and in the epidermis and vascular bundles of leaves, making proanthocyanidins protective agents against environmental conditions (Lu *et al*., 2021; Mannino *et al*., 2021). Proanthocyanidins have been associated with the treatment of age-associated diseases such as obesity, neurodegeneration, diabetes and arthritis (Figure 2.5) (Nie and Sturzenbaum, 2019). Cardiovascular diseases, cancers, inflammatory-related conditions, and allergies have also been described as some of the illnesses that can be managed and treated with proanthocyanidins (Nie and Sturzenbaum, 2019).

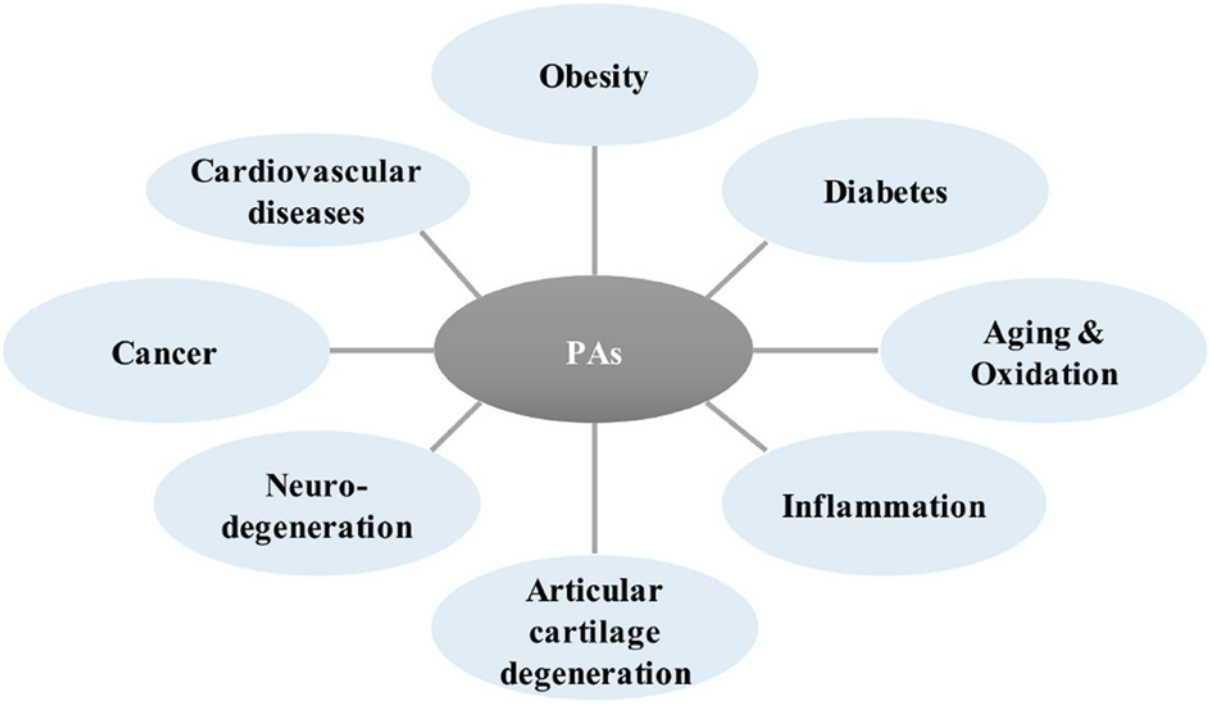

Figure 2.5: Various pharmacological activities of proanthocyanidins.

Coumarin

This is a group of polyphenols comprised from the fusion of a benzene ring and α-pyrone rings, commonly found in all vascular plants, although highly concentrated in plant seeds (Jan *et al*., 2021). Naturally-occurring coumarins are classified into four major groups, such as, simple coumarins, furanocoumarins, pyranocoumarins, and benzocoumarins (Annunziata *et al*., 2020; Heghes *et al*., 2022). The bioactivity of coumarins have been documented over the years, and findings have shown them to possess antitumour (Vianna *et al*., 2012), photochemotherapy (Harvey *et al*., 1988); anti-HIV (Kostova et al., 2006), anti-inflammatory, anticoagulant, and central nervous system stimulatory activities (Figure 2.6) (Akkol et al., 2020). Coumarins are currently studied for their high anticancer properties, and have shown great success in the treatment of breast and prostate cancers, leukaemia, malignant melanoma, and renal cell carcinomas (Akkol *et al*., 2020).

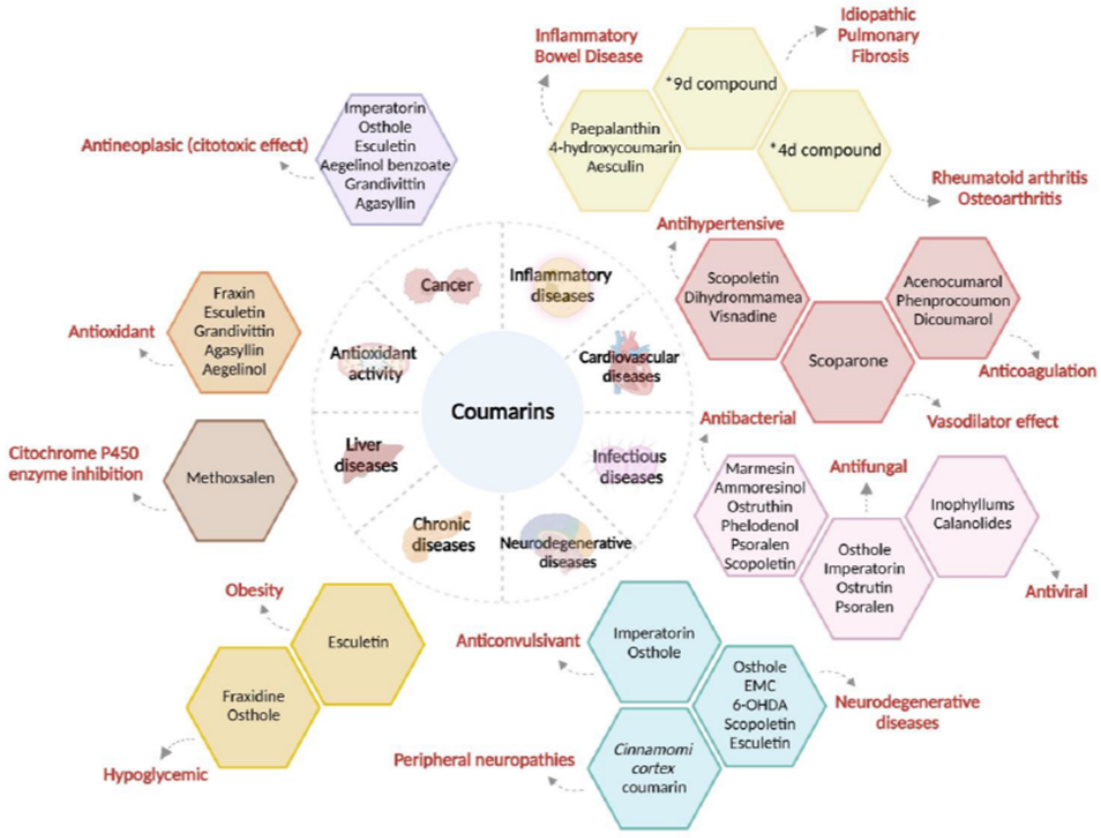

Figure 2.6: Some of the pharmacological activities of coumarins and coumarin derivatives.

Terpenoids

The largest group of secondary metabolites are known as the terpenoids (Yazaki *et al*., 2017). They contain oxygen molecules formulated through the removal or addition of methyl groups (Pandey *et al*., 2017; Masyita *et al*., 2022). Terpenoids are sub-grouped into nine classes, each based on the number carbon atoms in their chemical structure: hemiterpenoids (C5), monoterpenoids (C10), homoterpenoids (C11, 16), sequiterpenoids (C15), diterpenoids (C20), sesterpenoids (C25), triterpenoids (C30), tetraterpenoids (C40) and polyterpenoids (C >40) (Boncan et al., 2020). Terpenoids have a range of therapeutic properties, such as anticancer, antimicrobial, anti-inflammatory, antioxidant and anti-allergic activities (Masyita *et al*., 2022).

Saponins

Saponins are organic compounds that are abundantly found in the plant kingdom. Their chemical structure consists of one glycosidic linkage between aglycone and a sugar moiety at C-3 (El Aziz *et al*., 2019). Saponins are classified into three groups, namely, triterpenoid glycosides, steroid glycosides, and alkaloid glycosides (El Aziz *et al*., 2019). Pharmacological studies have reported that saponins possess anti-inflammatory, vasoprotective, hypocholesterolemic, hypoglycaemic, anti-parasitic, antifungal, anticancer, and antiviral properties (Mugford and Osbourn, 2013). As a result of their foaming characteristics, saponins are applied in beverage and cosmetic industries. They are also used in the production of fire extinguishers (Mugford and Osbourn, 2013).

Glycosides

Cardiac glycosides are a group of structurally diverse compounds, with a steroidal core framework (Prassas and Diamandis, 2008). In plants, this group of compounds widely distributed, though in uneven concentrations in the seeds, leaves, bark, roots, and stems (Morsy, 2017). Given their name, cardiac glycosides have been associated with the treatment of

cardiovascular illnesses, as they are known to inhibit the activity of Na-K-ATPase, which results in the increased myocardial contraction forces (Morsy, 2017). There has been sufficient research looking into the anticancer potential of glycosides. It has been reported that they have the ability of impairing cell proliferation and initiating apoptosis (Khan *et al*., 2019).

Volatile oils

Volatile oils are aromatic hydrocarbons and oxygenated derivative compounds, and are often contained in the secretory cells or cavities of flowers, leaves, stems, branches, seeds, fruits, and roots of plants (Korinek *et al*., 2021). Volatile oils have multiple medicinal properties such as antibacterial, anti-tumour, antioxidant, and anti-inflammatory activities (Saviuc *et al*., 2015; Sharifi-Rad *et al*., 2017; Korinek *et al*., 2021). These compounds are popularly associated with the cosmetics industry, as they are used in the production of perfumes and perfume-related products such as soaps and shampoos (Korinek *et al*., 2021).

Steroids

Plant steroids have a similar chemical structure and bioactivity as cholesterol (Berger *et al*., 2004). They contain a steroid nucleus consisting of four carbon rings (tetracyclic 5α or 5β-gonane skeleton) with methyl substituents attached to it at C-10 and C-13, and an alkyl side chain at C-17 (Yerlikaya *et al*., 2023). Plant steroids classified into nine groups: phytosterols, brassinosteroids, withasteroids, ecdysteroids, steroidal alkaloids, steroidal saponins, cardenolides, cucurbitacins, and phytoecdysteroids (Kreis and Muller-Uri, 2010; Beg *et al*., 2011; Yerlikaya *et al*., 2023). Plant steroids have shown to possess a multitude of health-promoting properties, such as anti-inflammatory, anticancer, and antioxidant activities (Berger *et al*., 2004).

The presence of the abovementioned phytochemicals have been identified, isolated and quantified from *B. abyssinica*, *B. frutescens*, and *B. natalensis* by various authors (Table 2.2)

Table 2.2: Phytochemical groups identified from various parts of *B. abyssinica*, *B. frutescens*, and *B. natalensis*.

Compound group	**Species and plant part**	**References**
***Bulbine* flavonoids**		
Galangin	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Quercetin	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Quercetin 3-O-(6″-malonyl-glucoside) 7-O-glucoside	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Kaempferol-3-O-rutinoside	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Rutin	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Variabiloside A	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Eriodictyol	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Astilbin	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Pratensein	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Ψ-Tectorigenin	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Petunidin	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Petunidin-3-O-rutinoside	*B. abyssinica* leaves	Odeyemi *et al* (2018)
Cyanidin-3-O-rutinoside	*B. abyssinica* leaves	Odeyemi *et al* (2018)
***Bulbine* phenolics**		
Psolaren	*B. abyssinica* leaves	Odeyemi *et al* (2018)
8-hydroxy-6-methylxanthone-1-carboxylic acid	*B. frutescens* roots	Abdissa *et al* (2014)
8-C-glycosyl aloesol	*B. natalensis* rhizomes	Bae *et al* (2019)
ρ-coumaric acid	*B. frutescens* roots	Cutillo *et al* (2006); Bringmann *et al* (2008)
dihydro- ρ-coumaric acid	*B. frutescens* roots	Cutillo *et al* (2006); Bringmann *et al* (2008)
vanillic acid	*B. frutescens* roots	Cutillo *et al* (2006); Bringmann *et al* (2008)
***Bulbine* triterpenes**		
Ergosterol	*B. natalensis* underground stem	Mbambo *et al* (2012)
Campesterol	*B. natalensis* leaves	Bodede *et al* (2020)
β-Sitosterol	*B. natalensis* underground stem and rhizomes	Mbambo *et al* (2012); Bae *et al* (2019); Bodede *et al* (2020)

Sitosterol 3-O-β-D-glucoside	*B. natalensis* rhizomes	Bae *et al* (2019)
Stigmasterol	*B. natalensis* underground stem	Mbambo *et al* (2020)
Glutinol	*B. natalensis* leaves	Bodede *et al* (2020)
Taraxerol	*B. natalensis* leaves	Bodede *et al* (2020)
Tannins	*B. natalensis* leaves	Lazarus (2012)
	B. natalensis rhizomes	Yakubu *et al* (2009)
	B. natalensis underground stem, roots	Teffo et al (2024a)
	B. abyssinica whole plant	Kibiti and Afolayan (2018)
	B. abyssinica leaves, underground stem, roots	Teffo *et al* (2022)
Saponins	*B. abyssinica* whole plant	Kibiti and Afolayan (2018)
	B. abyssinica leaves, underground stem, roots	Teffo *et al* (2022)
	B. natalensis leaves	Lazarus (2012)
	B. natalensis roots	Yakubu *et al* (2009)
	B. natalensis underground stems	Teffo *et al* (2024a)
Phlobatannins	*B. natalensis* leaves, underground stem, roots	Teffo *et al* (2024a)
	B. abyssinica leaves, underground stem, roots	Teffo *et al* (2022)
	B. frutescens leaves, roots	Teffo *et al* (2024b)
Steroids	*B. natalensis* leaves, underground stem, roots	Teffo *et al* (2024a)
	B. abyssinica leaves, underground stem, roots	Teffo *et al* (2022)
	B. frutescens leaves, roots	Teffo *et al* (2024b)
Glycosides	*B. natalensis* leaves, underground stem, roots	Teffo *et al* (2024a)
	B. abyssinica leaves, underground stem, roots	Teffo *et al* (2022)

	B. frutescens leaves, roots	Teffo *et al* (2024b)
Volatile oils	*B. natalensis* leaves, underground stem, roots	Teffo *et al* (2024a)
	B. abyssinica leaves, underground stem, roots	Teffo *et al* (2022)
	B. frutescens leaves, roots	Teffo *et al* (2024b)
Proanthocyanidins	*B. abyssinica* whole plant	Kibiti and Afolayan (2018)
	B. natalensis leaves, underground stem, roots	Teffo *et al* (2024a)
	B. abyssinica leaves, underground stem, roots	Teffo *et al* (2022)
	B. frutescens leaves, roots	Teffo *et al* (2024b)

2.4 Antioxidant activity

2.4.1 Oxidative stress and radicals

In all biological systems, oxidative stress is described as an imbalance between the production and subsequent accumulation of free radicals or reactive oxygen species (ROS) and the quenching of said radicals via antioxidants (Santos-Sanchez *et al*., 2019). In humans, ROS is produced either through normal biochemical reactions, exposure to the environment and/or dietary xenobiotics (Nimse and Pal, 2015). Also, exposure to radiation, ozone and air or industrial pollutants, as well as lifestyle factors such as cigarette smoking, contribute to the

accumulation of ROS (Kumar *et al*., 2021). Endogenous sources of ROS such as inflammation, xanthine oxidase and peroxidase enzymes, can also cause a variety of inflammatory diseases such as arthritis, lupus and vasculitis, as well as different classes of ischemia, respiratory disorders, hypertension and neurological disorders (Lobo *et al*., 2010). Plants are also affected by UV-radiation and herbivory, which induce the oxidative stress in their cells and tissues (Dumanovic *et al*., 2021). In the absence of, or limited availability of antioxidants in plants and animals, there is a high probability of lipid peroxidation, protein denaturation, nucleic acid oxidation and programmed cell death (Gill and Tuteja, 2010; Dumanovic *et al*., 2021).

In plant cells, there are a number of ROS produced during normal physiological conditions, however, four of these are reported to be the most active in generating oxidative stress:

2.4.1.1 Hydrogen peroxide

At high concentrations, H_2O_2 induces toxicity in plant cells. Under normal circumstances, it plays an integral part in signal transduction, polymerization, and cell wall lignin formation (Moural *et al*., 2017; Pandey *et al*., 2017; Dumanovic *et al*., 2021). Hydrogen peroxide is also capable of crossing the cell membrane and reacting with transition metal ions such as iron and copper, producing hydroxyl ions, which are more toxic (Dumanovic *et al*., 2021). Primary metabolic functions like photosynthesis can be inhibited by H_2O_2, further reducing CO_2 fixation by 50%, affecting the Calvin cycle (Dumanovic *et al*., 2021).

2.4.1.2 Singlet oxygen

Since plants contain high quantities of chlorophyll, which is an essential pigment in photosynthesis, they are vulnerable to oxidative stress as a result of the singlet oxygen. This radical is abundantly found in leaves, and in the excited state of chlorophyll, singlet oxygen accumulation occurs due to the sufficient energy supply to molecular oxygen as a result of a limited presence of a radical scavenger (Dumanovic *et al*., 2021). Singlet oxygen is said to be

the main cause for chlorophyll bleaching, leading to light-induced loss of photosystem II activity (Dumanovic *et al*., 2021).

2.4.1.3 Superoxide anion

This free radical is formed through single-electron reduction of molecular oxygen in the cell. The anion is then converted to H_2O_2 via the enzyme superoxide dismutase (Dumanovic *et al*., 2021). Iron-sulphur clusters of proteins are often damaged and become inactivated because of the over-accumulation of H_2O_2, as a mechanism of inhibiting superoxide anion production (Schieber and Chandel, 2014). Superoxide anion radicals aid in producing hydroxyl and peroxynitrite ($ONOO^-$) radicals, and is a by-product of mitochondrial respiration (Aziz *et al*., 2019).

2.4.1.4 Hydroxyl radical (OH^-)

Hydroxyl radicals have an affinity for genetic instability by oxidizing DNA, proteins amino acids, lipids, sugars, and metals (Aziz *et al*., 2019). The radical is produced when H_2O_2 reacts with a transition metal such as iron or copper via the Fenton reaction (Fenton, 1984; Dumanovic *et al*., 2021).

In plant cells, two of the major sites of ROS production are the chloroplasts and the mitochondria, followed by peroxisomes, endoplasmic reticulum, and apoplasts (Figure 2.7) (Kasote *et al*., 2015; Dumanovic *et al*., 2021). Photosynthesis is the primary cause of ROS formation in plants, mainly at the photosystem I and II of chloroplasts, the membrane and matrix of peroxisomes, as well as the electron transport chain of the mitochondria (Gill and Tuteja, 2010; Kasote *et al*., 2015). Hydrogen peroxide, superoxide anion and nitric oxide radicals are mostly produced in peroxisomes, whereas the photosystems I and II in the electron transport chain produce superoxide anion and singlet oxygen radicals respectively, via the

Mehler reaction (Kasote *et al*., 2015; Dumanovic *et al*., 2021). Under abiotic and biotic stress, these sites over-produce ROS in plant tissues, which induce physiological changes in plants.

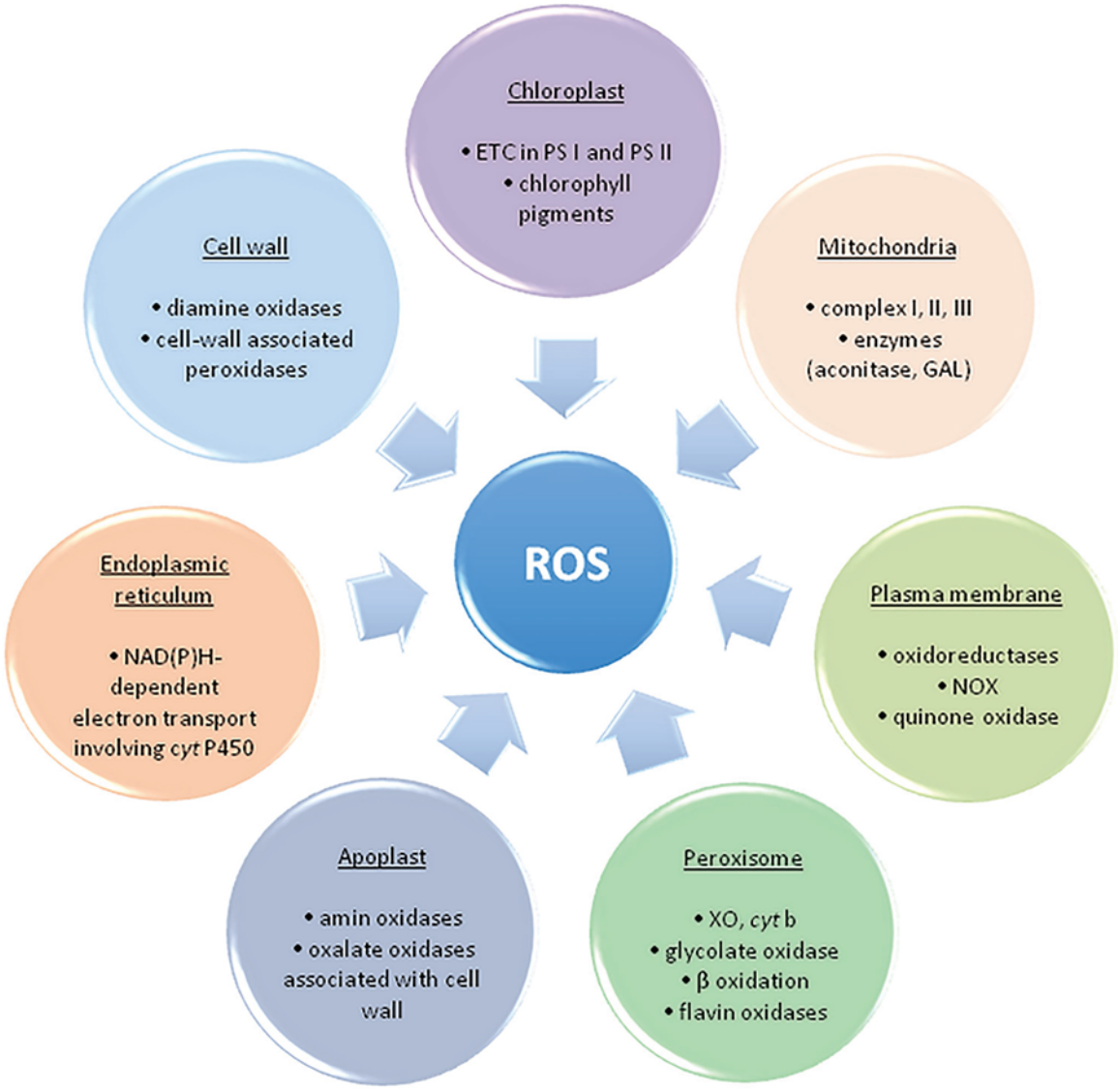

Figure 2.7: Sites of ROS production in plant cells.

2.4.2 Plant antioxidant system

Over the years, medicinal plants have been used in ethnomedicine in most developing countries, as they are generally harmless and have little to no side-effects when consumed (Pammi *et al*., 2023). The bioactivity of medicinal plants is given rise to the presence of

antioxidants. These are described as compounds that donate electrons to free radicals, as they are usually unstable, resulting in their action to inhibit cellular damage (Lobo *et al*., 2010). Medicinal plants produce low and high molecular weight secondary metabolites which play a defence role against various environmental and biotic stressors (Kasote *et al*., 2015). Their main action in these plants involve radical scavenging, thus preventing cellular damage by oxidation (Pammi *et al*., 2023). Secondary metabolites are either actively or passively synthesized, in such a way that they are continuously available or they accumulate under various stressors (Kasote *et al*., 2015). Plant antioxidants are comprised of two classes, enzymatic antioxidants such as superoxide dismutase, catalase and glutathionine, and non-enzymatic antioxidants such as ascorbic acid, melatonin, tocopherols and tocotrinols (vitamin E) and uric acid (Lobo *et al*., 2010). Phenolic compounds such as flavonoids, lignans, stilbenes, and tannins are some of the major groups of non-enzymatic antioxidants found in plants (Kumar *et al*., 2021). Most of these phytocompounds have shown great medicinal activity, as reported by many authors, as they possess antimicrobial, antidiabetic, anti-inflammatory, antiviral, and anti-carcinogenic properties (Figure 2.8) (Yakubu *et al*., 2012; Kibiti and Afolayan, 2015; Shikalepo *et al*., 2018; Teffo *et al*., 2022).

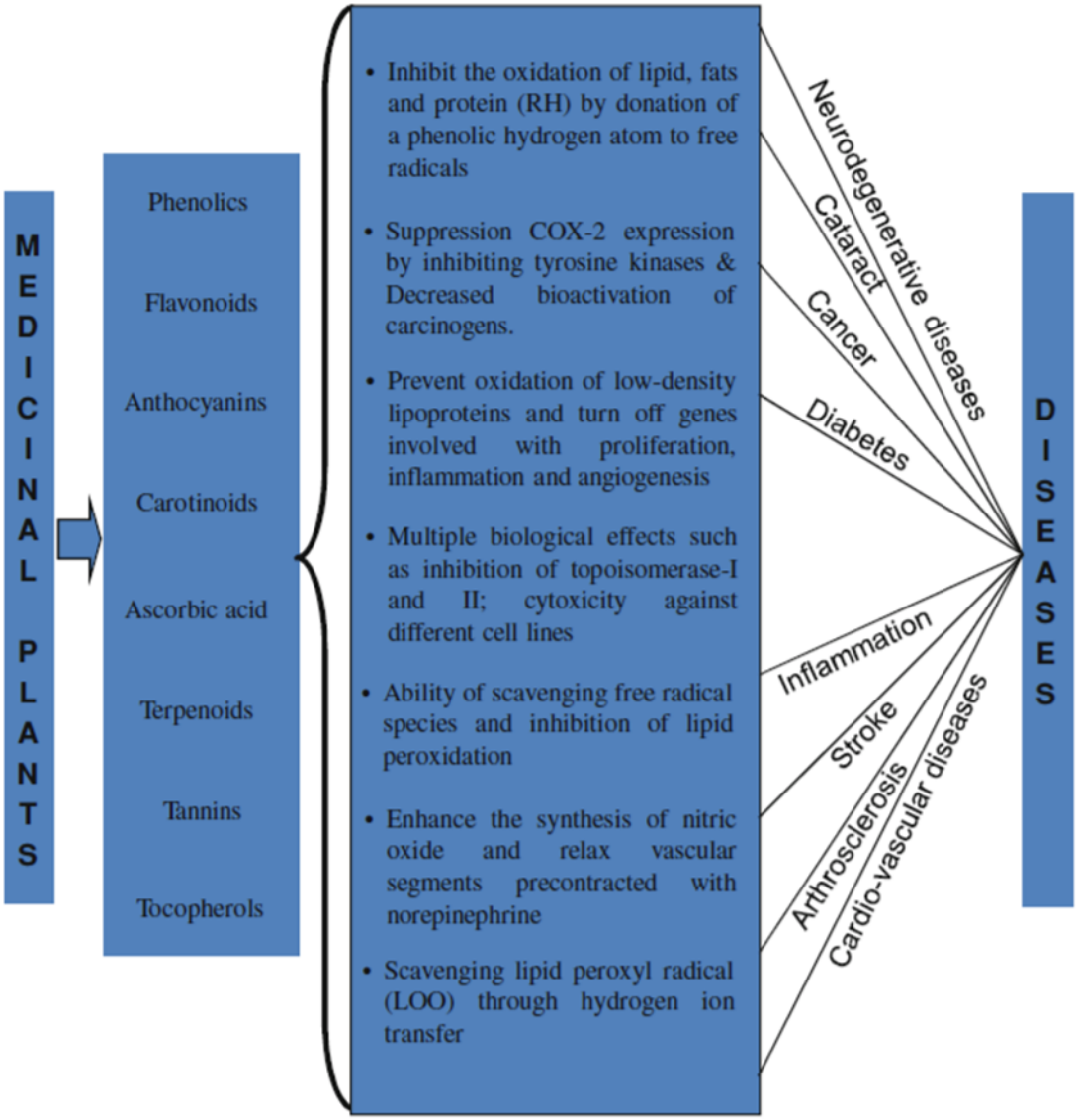

Figure 2.8: Medicinal plant phytochemical antioxidant mechanism of action.

The application and uses of synthetic antioxidants have since been normalised in modern medicine, as well as in other industries such as food and cosmetics (Lobo *et al*., 2010). Some of the common synthetic antioxidants include butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), and N-acetylcysteine (NAC) (Kumar *et al*., 2021). The downside to synthetic antioxidants is their instability and volatile nature, especially at high temperatures, as well as their high risk of inducing carcinogenesis, which is why there is a great need to shift to sourcing natural antioxidants from medicinal plants (Figure 2.9) (Lobo *et al*.,

2010). Medicinal plants, when consumed in any form, are absorbed much easier with barely any side-effects, whereas synthetic drugs are not completely broken down nor absorbed into the bloodstream, hence they cause mild to severe side-effects (Pammi *et al*., 2023). Research has shown significant documentation of the antioxidant activity of *B. abyssinica* (Kibiti and Afolayan, 2015; Teffo *et al.,* 2022), *B. frutescens* (Shikalepo *et al.,* 2018; Ghuman *et al.,* 2019; Teffo *et al.,* 2024b), and *B. natalensis* (Ghuman *et al.,* 2019; Teffo *et al.,* 2024a).

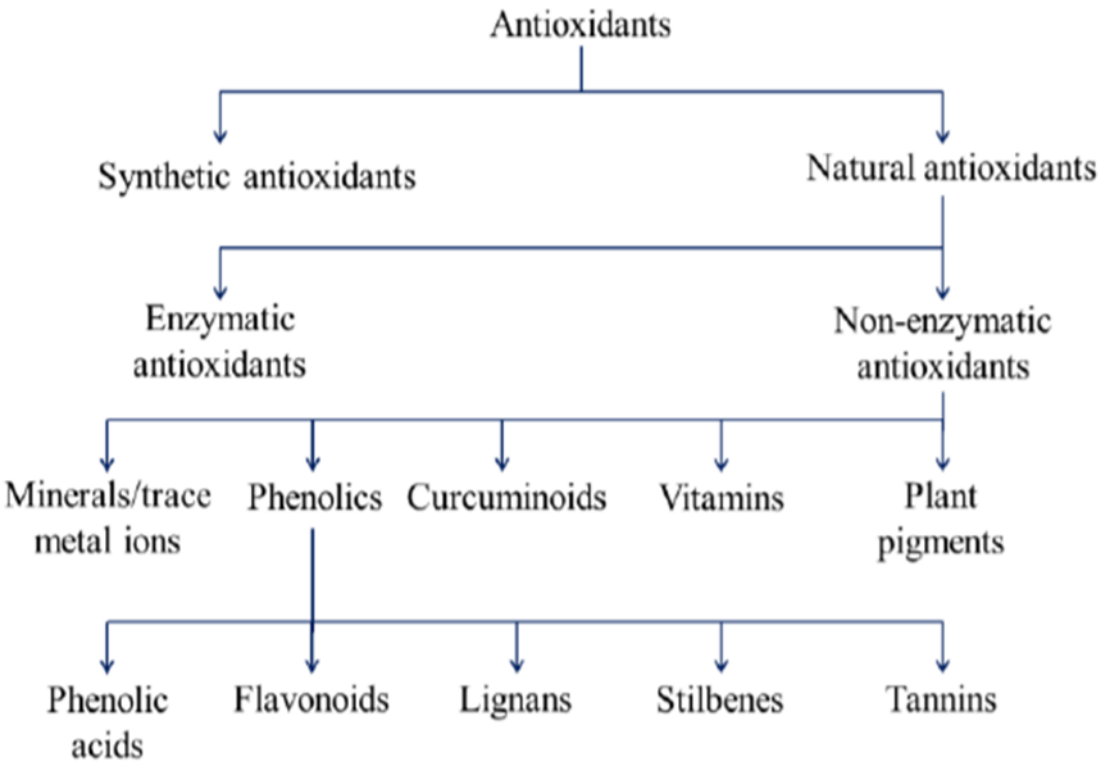

Figure 2.9: The different classes of antioxidants

2.5 Microorganisms and antimicrobial resistance

In our current time, antibiotics are continuously dispensed and used, sometimes irresponsibly. This unfortunately has led to the sudden increase in resistant microbial strains (Breijyeh *et al*., 2020). It has been estimated that antimicrobial resistance will account for approximately 10 million deaths worldwide in the next two and a half decades (O'Neill, 2016; Lucien *et al*.,

2021). The evolution of microorganisms involves the diversion of antibiotics outside of microbial cells, or through the modification of their external structures, which prevent the drugs from attaching to the microbes (Breijyeh *et al*., 2020). For the purpose of this study, bacterial and fungal microbes were investigated to determine the antimicrobial activity of the study species.

Gram-negative bacteria are highly resistant to antibiotics, which creates a significant issue in the health and overall well-being of individuals. This group of microorganisms is mostly associated with morbidity and mortality in hospitals (Hormozi *et al*., 2018; Oliveira and Reygaert, 2019). Their physiology enables them to have such a high level of resistance. Gram-negative bacteria consist of an outer membrane composed of lipopolysaccharide, a thin peptidoglycan layer, and an inner membrane. The lipopolysaccharide outer membrane is responsible for protecting the internal environment of Gram-negative bacteria, by preventing successful entry of antibiotic compounds (Breijyeh *et al*., 2020). Contrastingly, Gram-positive bacteria lack an outer membrane, however, they possess a much thicker peptidoglycan layer surrounding their plasma membrane (Silhavy *et al*., 2010; Jubeh *et al*., 2020). The pathogenicity of Gram-positive bacteria is due to the release of toxins, which cause tissue damage, exacerbating disease. They can also manipulate the immune response by altering microbiome behaviour, contributing to antibiotic resistance (Romero *et al*., 2011; Sharma *et al*., 2023).

Aside from bacteria, fungal infections have also shown changes in the pattern of antifungal resistance. Fungal infections can occur in more than one mode of transmission, such as inhalation, cutaneous and subcutaneous, mucosal, and ingestion (Badiee and Hashemizadeh, 2014). Opportunistic infections can further suppress the immune system, and these are especially common with immunocompromised patients living with HIV and cancer, among others (Reddy *et al*., 2022). Factors such as sexual reproduction, horizontal gene transfer, and

adaptive phenotypic plasticity, are said to aid in antifungal resistance (Hokken *et al*., 2019; Reddy *et al*., 2022).

Phytochemical compounds could become a valuable substitute for synthetic antimicrobial agents, as they have minimal to no side effects. This study aimed to assess the antimicrobial potential of *B. frutescens*, *B. abyssinica*, and *B. natalensis* under elevated CO_2 and high temperatures, against six bacterial, and two fungal pathogens described below.

2.5.1 Gram-positive bacteria

2.5.1.1 Staphylococcus aureus

Staphylococcus aureus is said to form colonies among the human population, which is why it is regarded as a potent opportunistic pathogen responsible for high morbidity rates worldwide (Howden *et al*., 2023). About 30% of *S. aureus* colonies are found in the nasal cavity, however, they also frequent in other areas of body including the skin, throat and intestine (Howden *et al*., 2023). *S. aureus* is most commonly associated with infective endocarditis, osteoarticular skin and soft tissue, and pleuropulmonary infections and is the leading cause of bacteremia (Tong *et al*., 2015).

2.5.1.2 Bacillus cereus

Bacillus cereus is a spore forming, rod-shaped bacterium found in the natural environment. Normally discovered in fresh and marine waters, vegetables, decaying organic matter and the intestinal tract of invertebrates, the bacterium leaches into soil and food products, which results in their colonization of the human intestine via contamination (Bottone, 2010). As a result, *B. cereus* is commonly associated with food poisoning, particularly diarrheal and emetic syndromes (Liu *et al*., 2020). Extra-intestinal infections associated with *B. cereus* include

systemic (bacteremia and septicemia), eye-infections (panophthalmitis and endophthalmitis), brain-related infections (meningioenchephalitis) and endocarditis (Messelhauβer and Ehling-Schulz, 2018).

2.5.1.3 Enterococcus faecalis

This facultative anaerobic bacterium exists commonly in a single, paired, or a chain of cocci (Hashem *et al*., 2021). The pathogen is mostly found in the gastrointestinal tract and the oral cavity (Rosen *et al*., 2018). Some of the infections that are caused by *E. faecalis* include urinary tract infections (UTI's), meningitis, bacteremia, wound-infections, and neonatal infections (Anderson *et al*., 2016). *E. faecalis* has been greatly associated with dental disorders such as periodontitis, peri-implantitis, and caries (Kouidhi *et al*., 2011; Dahlen *et al*., 2012; Rams *et al*., 2013; Anderson *et al*., 2016).

2.5.2 Gram-negative bacteria

2.5.2.1 Klebsiella pneumoniae

Klebsiella pneumoniae is a facultative anaerobic, encapsulated bacterial pathogen which colonizes the nasal cavity and digestive tract in humans (Chang *et al*., 2021). *K. pneumoniae* is a major Gram-negative bacterial pathogen, causing bacteremia, resulting in high mortality rates, mostly affecting immunocompromised individuals (Imai *et al*., 2019; Kot *et al*., 2023). This pathogen is also responsible for one-third of Gram-negative infections, such as cystitis, pneumoniae, pyogenic liver abscesses, urinary tract infections, and endogenous endophthalmitis (Effah *et al*., 2020).

2.5.2.2 Pseudomonas aeruginosa

This bacterial pathogen is found in the environment, from soil, water, and plants (Wu and Li, 2015; Liao *et al*., 2022). *P. aeruginosa* can withstand temperatures between 4 and 42 °C, making it suitably resilient to any environment, even in nutrient deficient conditions (Liao *et al*., 2022). Pneumonia is the most common infection caused by *P. aeruginosa*, followed by UTI's, bacteremia, and surgical site infections. About 7.1-7.3% of healthcare associated infections are caused by *P. aeruginosa* (Weiner *et al*., 2016; Reynolds and Kollef, 2021). *P. aeruginosa* has also been reported to be a complication in burn patients, since a moist environment is required for the unit, it contributes to the infections which in severe cases, could lead to sepsis and death (Reynolds and Kollef, 2021).

2.5.3 Fungal microorganisms

2.5.3.1 Candida albicans

Candida albicans exists in various forms, such as hyphae, pseudohyphae, and chlamydospores. Due to the fungal diversity of *C. albicans*, the pathogen grows in either single-cell cultures, micro-colonies, or biofilms (Cottier and Hall, 2020; Macias-Paz *et al*., 2023). *C. albicans* accommodates in the gastrointestinal, oral, and vaginal mucosa (Macias-Paz *et al*., 2023). Immunocompromised individuals can however, become infected based on the high rate of colonization of *C. albicans*, causing oral and vulvovaginal candidiasis (Mayer *et al*., 2013). When *C. albicans* grows abnormally, it can greatly impair the immune system, resulting in opportunistic infections of the viscera, or visceral candidiasis (Jabra-Rizk *et al*., 2016; Talapko *et al*., 2021).

2.5.3.2 Cryptococcus neoformans

Cryptococcus neoformans is found mostly in bird faeces, soil, and decaying wood. Humans become infected when inhaling dust containing faeces (Zhao *et al*., 2023). The pathogen is one of the few members of the *Cryptococcus* family known to cause cryptococcosis, which greatly contributes to the high mortality rate of HIV infected individuals. Treatment of cryptococcosis has shown to be difficult, especially in developing countries (Zhao *et al*., 2023). Cryptococcal infections manifest in three forms, namely cryptococcal pneumonia, cryptococcal meningitis, and cryptococcemia (Zhao *et al*., 2023). Due to its infective nature, and the health level of immunocompromised individuals, *C. neoformans* can also affect the skin, eyes, bone, and prostate (Maziarz and Perfect, 2016). Due to the use of *Bulbine* in traditional medicine for their wound-healing potential, *B. abyssinica*, *B. frutescens* and *B. natalensis* have received attention in antimicrobial research. Various plant parts have been assessed of their potential activities against several bacterial and fungal microorganisms (See table 2.1).

2.6 Effects of atmospheric carbon dioxide and temperatures on the bioactivity of medicinal plants

2.6.1 Atmospheric carbon dioxide

The burning of fossil fuels over the years has increased the concentrations of greenhouse gases (GHGs), more especially CO_2 (Hansen *et al*., 2013). Prior to the first Industrial Revolution, atmospheric CO_2 levels were approximately 280 ppm, about 597 GtC (gigatonnes of carbon). This concentration has since increased nearly two-fold, with reports of over 400 ppm (Roduner and Rohwer, 2020). The combustion of fossil fuels also changes the patterns and use capture of infrared radiation from the Earth's surface, which further warms the atmosphere (Davis,

2017). Coal is the largest contributor to the increase in GHGs like CO_2 into the atmosphere, as it serves as the primary energy source in the world (Nunes, 2023). The continuous rise in anthropogenic emissions of CO_2, could lead to the instability of natural processes such as the carbon cycle responsible for the balance in CO_2 concentrations (Alsarhan *et al*., 2021; Nunes, 2023). This could affect weather patterns, causing frequent episodes of heavy rainfall, floods and drought (Davis, 2017). Long term effects of elevated CO_2 could exacerbate global warming by making temperatures more sensitive to rising CO_2 (Davis, 2017), although this would not be the first time whereby atmospheric CO_2 increases gradually overtime.

Approximately 100 million years ago, during the Cretaceous period, atmospheric CO_2 was estimated to be at 1000 ppm, and several thousand ppm more in the Paleozoic era (Qiu *et al*., 2023; Nunes, 2023). At the time however, it was necessary for evolution to occur on Earth, leading to the current environment and climate we have today (Nunes, 2023). It is worth noting that the higher levels of CO_2 during the Earth's evolution occurred over longer time periods, compared to the faster rate in today's period due to anthropogenic activities dating back to the first Industrial Revolution (Steffen, 2003; Steffen et al., 2007; Nunes, 2023). Global concentrations are estimated on the basis of the industrial emissions of CO_2 and changes in land use patterns in the Northern and Southern hemispheres (Tomizuka, 2013). Atmospheric CO_2 is reported to change with latitude, thus it is not uniform throughout the globe. The Northern hemisphere has the highest concentration of atmospheric CO_2 compared to the Southern hemisphere (Tomizuka, 2013). It has been reported that the estimated industrial CO_2 emissions since 1850 was higher in the Northern hemisphere. In 2000, the emissions had reached 7 PgC/yr (pentagrams of carbon) (Tomizuka, 2013). Between 1850 and 1950, the CO_2 emissions based on land use changes showed ranges of 0.5 and 1 PgC/yr for the Northern hemisphere, but, in 2000 the Southern hemisphere recorded the highest estimate CO_2 emissions with 0.8 PgC/yr (Tomizuka, 2013). Cheng *et al* (2022) provided global historical atmospheric

CO_2 concentrations between 1890 and 1999, and 2004-2013 (Figure 2.10). The map shows that the Northern hemisphere had averages between 314 and 387 ppm, compared to < 313 and 387 ppm in the Southern hemisphere. In this century, concentrations had increased with 386 to > 395 ppm in the North and 315-389 ppm in the South.

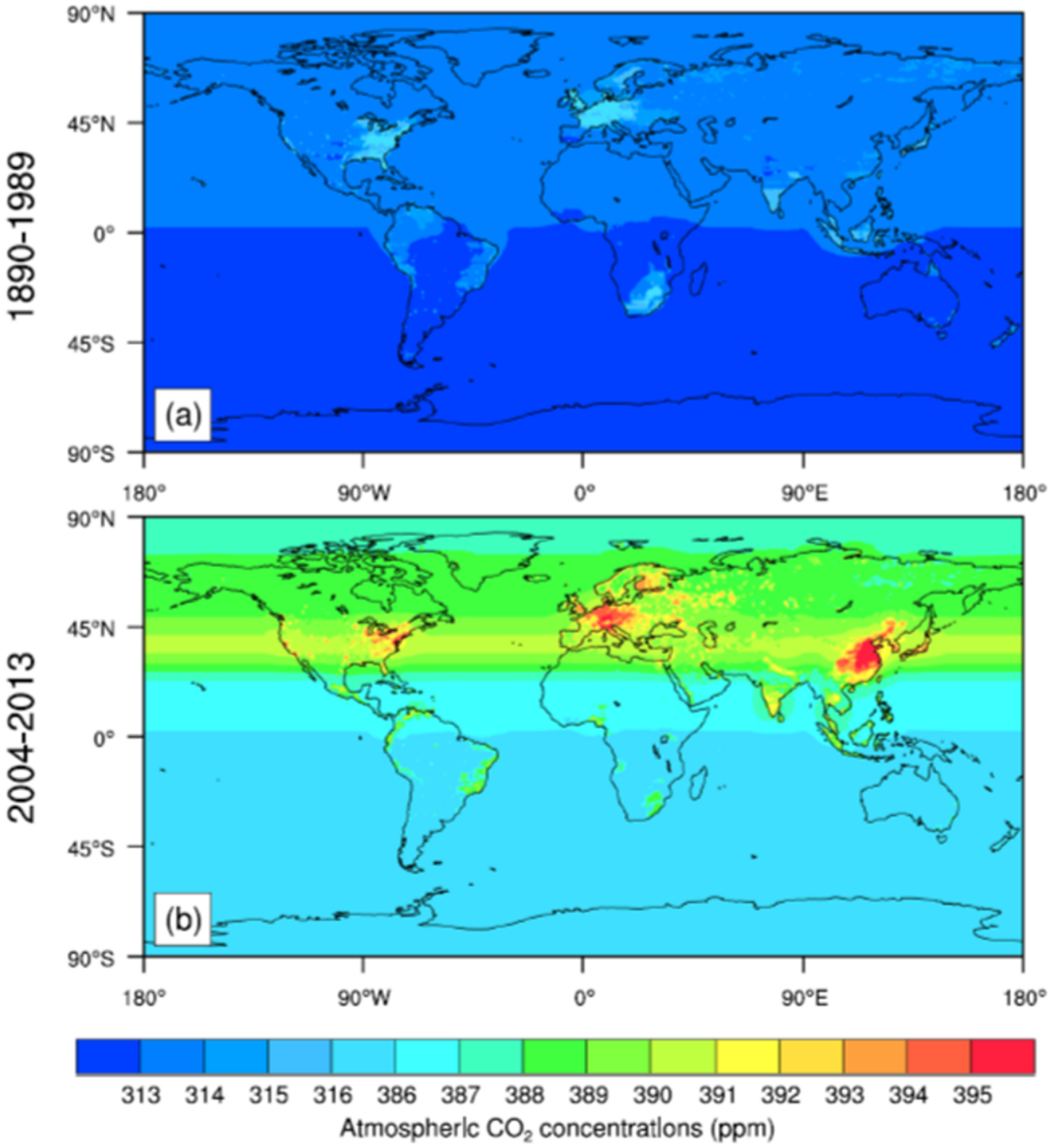

Figure 2.10: Atmospheric CO_2 concentrations from historical data (Cheng *et al*., 2022).

Future estimates of CO_2 concentrations had been derived from scenarios using socioeconomic and representative concentration pathways (SSP-RSP) spatial reconstruction (Cheng et al., 2022) (Figure 2.11 – 2.12). Based on the eight scenarios, the CO_2 concentrations are estimated to range between 437 and > 578 ppm in the Northern hemisphere, and 431-574 ppm in the Southern hemisphere between 2041 and 2060. Towards the late 21st century (2081-2100) CO_2 concentrations are predicted to fall between 399-1030 ppm in the North, and 399-1015 ppm in the South.

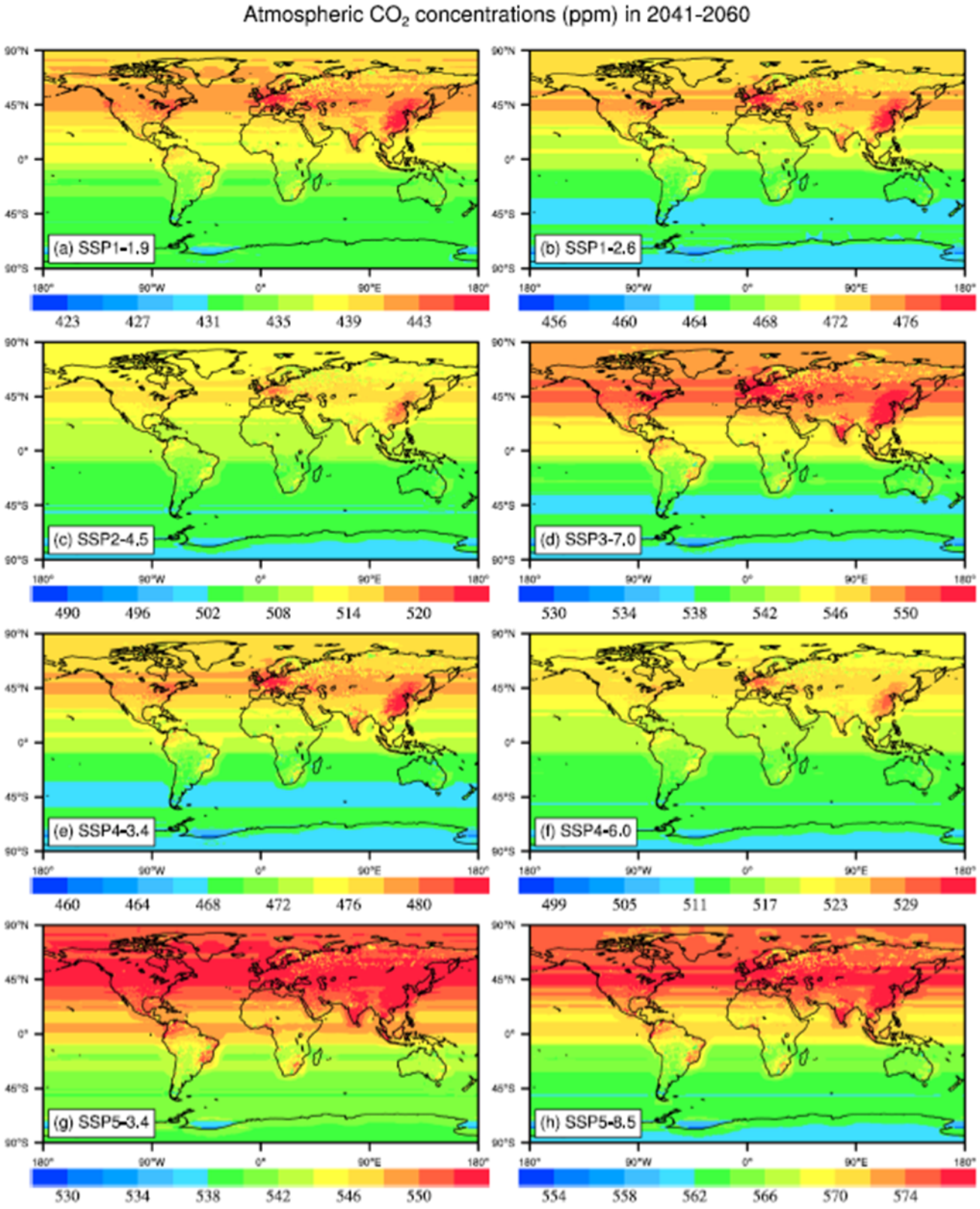

Figure 2.11: Predicted estimates of atmospheric CO_2 between 2041 and 2060 (Cheng *et al.*, 2022).

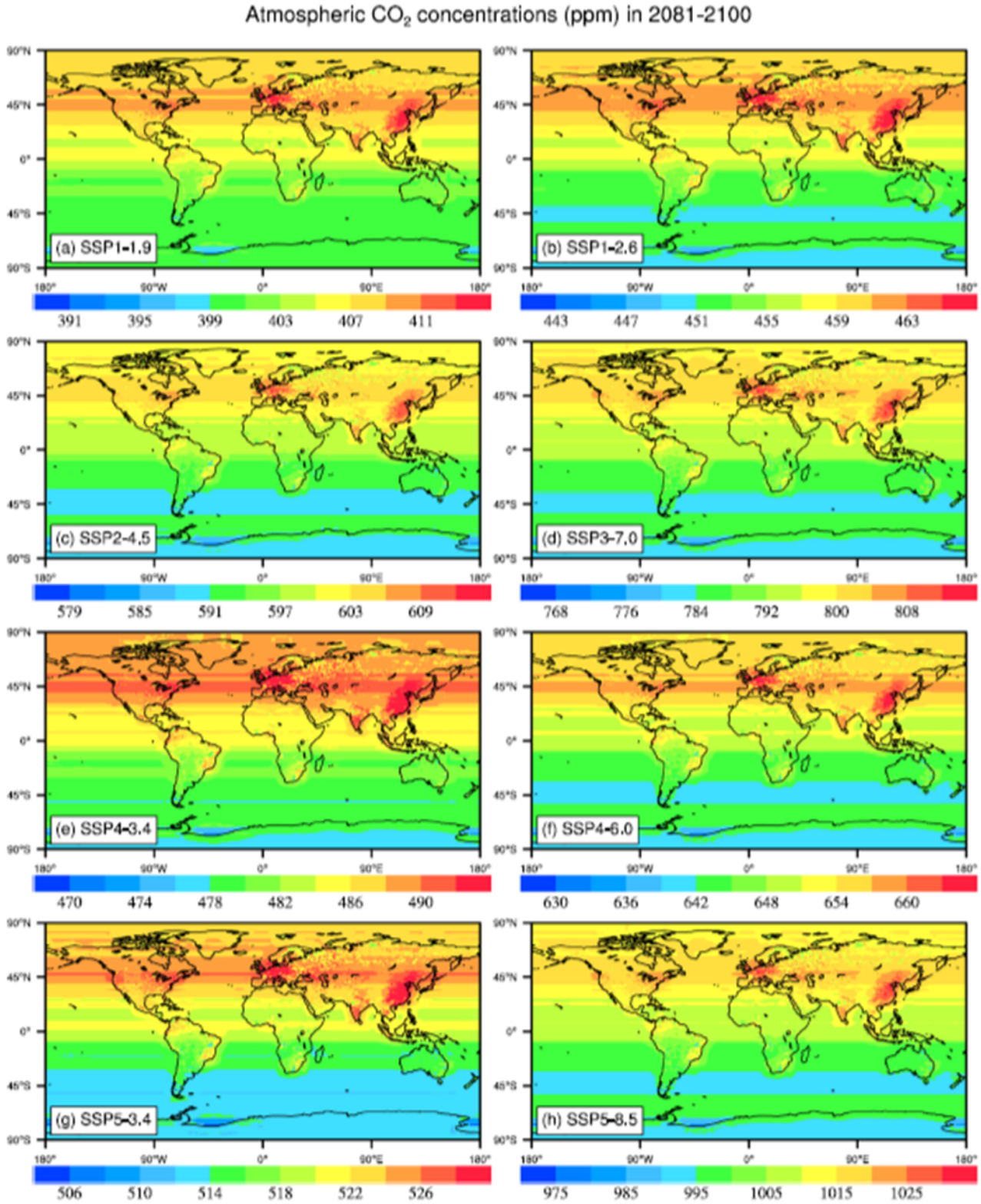

Figure 2.12: Predicted estimates of atmospheric CO_2 between 2081 and 2100 (Cheng *et al*., 2022).

Carbon dioxide is the primary GHG important for the growth and development of plants on Earth. An increase in the concentrations could result plants restricting water loss during

transpiration, leading to a greater ratio between carbon-gain to water loss (Prentice *et al.,* 2001). The carbon fixing enzyme Rubisco will increase photosynthesis, which increase plant biomass. Plants could experience physiological and morphological changes, which could impact species distribution and competition (Prentice *et al.,* 2001).

2.6.2 Elevated temperatures and heatwaves

The combustion of GHGs contributes to a rise in global temperatures, which heavily impacts human well-being, socio-economic, ecological, and environmental factors (Mengistu *et al*., 2023). Global temperatures are expected to be higher, compared to pre-industrial levels. This means that weather events around the world could drastically change in the coming years (Field *et al*., 2012). In the 20th century, global temperatures had increased by 0.7 °C and according to the IPCC, temperatures are predicted to increase between 1 and 6 °C (Bedair *et al*., 2023). The vegetation in South Africa could face extreme vulnerability due to rising temperatures (Lawal *et al.,* 2019). Southern Africa is identified as a "climate change hotspot" because of its warm and dry conditions (Engelbrecht *et al*., 2022). South Africa already has experienced temperatures 2 °C higher compared to 1981-2010 (Bedair *et al*., 2023). The country is also predicted to experience higher levels of warming compared to the global average (Mengistu *et al*., 2023). Temperatures are estimated to be 4 °C higher than the maximum average in the southern regions and 6 °C higher in the central, northern, and western regions of the country by the end of the 21st century (Kapwata *et al*., 2018; Chersich and Wright, 2019).

The increase in temperatures has also increased the frequency and duration of associated weather conditions such as heatwaves and drought (Mengistu *et al*., 2023). The heatwave threshold in South Africa is higher along the western parts of the country, with temperatures ranging between 32 and 44 °C during the summer (December-February), as well as the north-

eastern Lowveld (between 32-40 °C) (Mbokodo *et al*., 2020). In the North-West, Limpopo and north eastern Mpumalanga, the occurrences of heatwaves from 1983-2012 were between 13 and 30 during summer. Gauteng and most parts of the Free State, Kwa-Zulu Natal as well as central and western regions of the Northern Cape and central Western Cape, have experienced 1-12 heatwave events (Figure 2.13) (Mbokodo *et al*., 2020). The observed average duration of heatwaves between 1983 and 2012 showed the northern parts of the Northern Cape, Free State, the western KZN, Gauteng and Mpumalanga to have experienced 4-6 days of heatwaves. The extreme northern areas of the Northern Cape and Limpopo, as well as the North-West provinces have had 6-7 days of heatwaves (Mbokodo *et al*., 2020). The southern areas of the country have had shorter heatwave periods (3-5 days) on average.

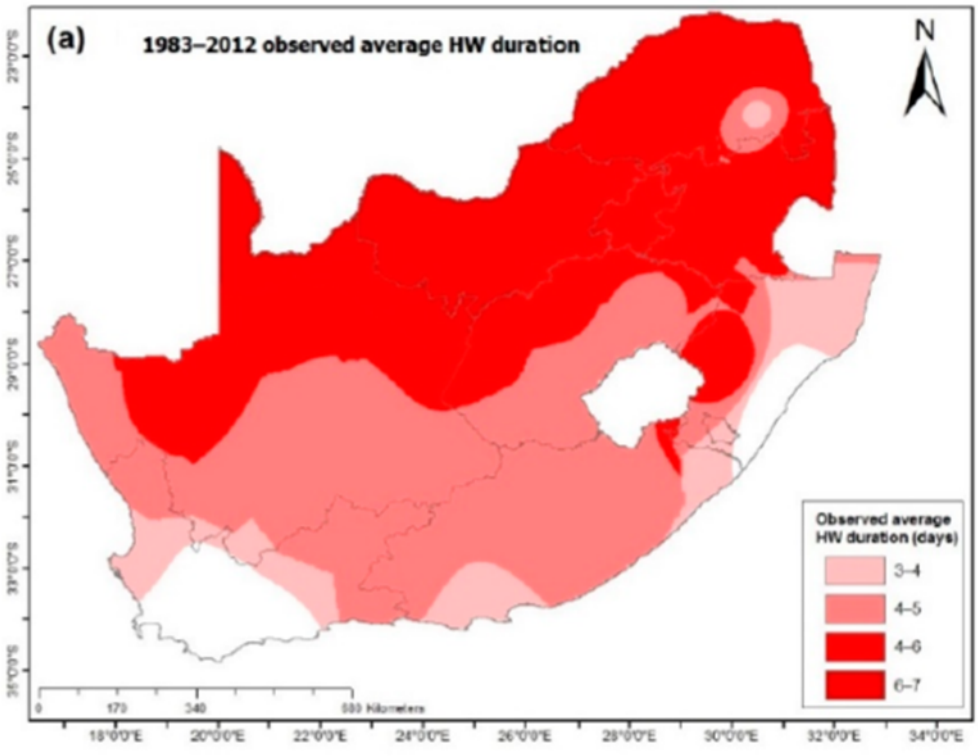

Figure 2.13: Observed average duration of heatwaves in South Africa between 1983 and 2012 (Mbokodo *et al*., 2020).

Climate simulations predicted that the frequency of heatwaves between 2010 and 2039 will fall between 30 and 80 occurrences across the country, with higher frequencies in the northern and

eastern areas (Figure 2.14) (Mbokodo *et al*., 2020). During the mid-century (2040-2069) the frequency is expected to increase, especially along the southern and eastern Cape regions (100 heatwave events), averaging to three events every summer (Mbokodo *et al*., 2020). About a 50% increase in the occurrences of heatwaves is predicted during 2070-2099, with 80-110 events estimated for the coastal regions of South Africa, and 50-80 events along the central and northern parts.

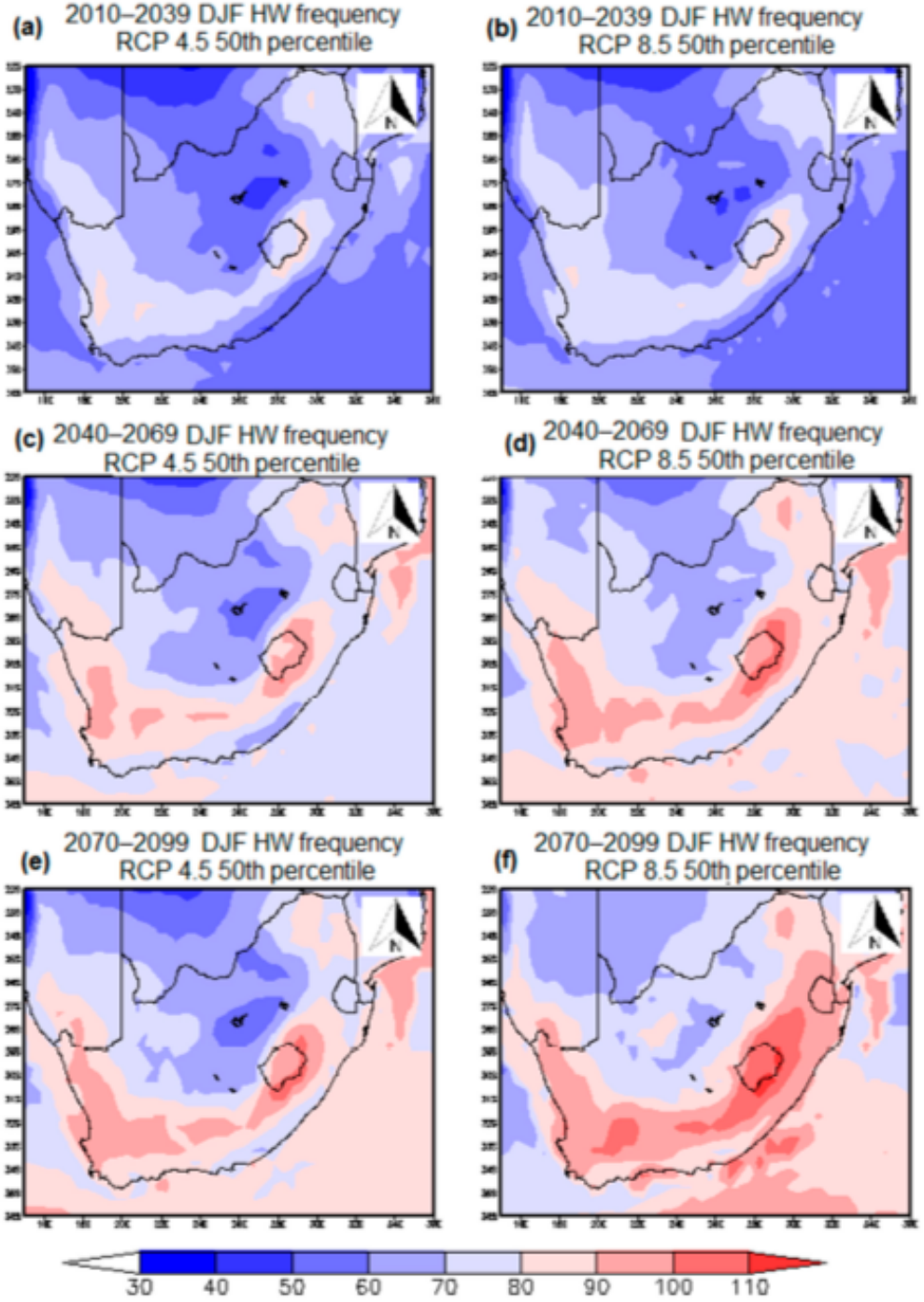

Figure 2.14: Simulated heat wave frequency projections during the summer period (December-February) in South Africa from 2010-2039, 2040-2069, and 2070-2099 (Mbokodo *et al*., 2020).

In South Africa's current climate, the average span of heatwaves is 3-4 days. This is predicted to increase to one week, more so in the central parts of the country in the summer between 2010 and 2039 (Mbokodo *et al*., 2020). The duration of heatwaves are estimated to increase

more inland than along coastal regions, with periods of 9-10 days, in the north western parts in summer. Between 2040 and 2069, about 100 heatwave events are expected in South Africa, more along the south and eastern coastal areas. Heatwaves are also predicted to span over one week along the coast (Mbokodo *et al*., 2020). Towards the late 21st century, heatwave frequencies are projected to be 50% higher compared to the present day climate. The duration is also predicted to last well over 10 days (Mbokodo *et al*., 2020).

2.6.3 Effects of elevated CO_2 and temperatures on medicinal plants

This drastic change in South Africa's climate could negatively impact the growth, development and bioactivity of medicinal plants. Temperature is the greatest factor influencing the rate of plant growth, since each species has its own threshold where they can develop at optimal rates (Wani *et al*., 2017). Should unfavourable conditions occur, plants could experience altered signalling and physiological regulation of metabolic processes, including their defence responses (Wani *et al*., 2017). This could affect the therapeutic uses of medicinal plants, rendering them useless due to the reduced bioactivity.

The assessment on the phytopharmacological properties of medicinal plants subjected to various environmental factors has received significant attention. A study by Sharma and Singh (2021) investigated the biochemical response of *Asparagus racemosus* to elevated CO_2. Under three concentrations (400 ± 4, 600 ± 12, and 800 ± 16 μmol CO_2 mol-1), chlorophyll, protein, and carbohydrate contents had increased in the leaves, stems, and roots. However, the concentration of ascorbic acid decreased. Although a significant amount of research was conducted for their study, phytopharmacological analysis of *A. racemosus* was not performed. Saleh *et al* (2018) discovered that the antioxidant capacity, antibacterial, and anticancer activities of parsley and dill had improved considerably under elevated CO_2 (627 ± 24 μmol

CO_2 mol-1 air). This was in conjunction with the reported increase in total phenolics, total flavonoids, and vitamins A and E. The effect of elevated CO_2 (600 ppm) on *Portulacaria afra* leaves over a three-month exposure period displayed an increase in the total flavonoid content, antioxidant activity, and the inhibitory properties against *Cutibacterium acnes*, compared to the ambient conditions (Basson *et al.,* 2024). Analysis of the whole plant would have been more beneficial, to get a better understanding of how CO_2 influences properties in *P. afra*. Elevated CO_2 (5-15%) had also improved the enzymatic and non-enzymatic antioxidants, total phenolic and flavonoid contents in wolfberry (*Lycium barbarum* L.) (Liang *et al.,* 2021). *Carpobrotus edulis* leaves exposed to low (15/10 °C) and high (45/35 °C) temperatures showed an increase in terpenoids from the methanol extracts, and volatile oils from the hexane extracts over a 144-hour exposure period (Mashigo *et al.,* 2024). The presence of tannins however, was lower under high temperatures compared to low temperatures and the control (25/15 °C). In addition, the methanol extracts of *C. edulis* leaf samples harvested at 144 hours (high temperatures) showed noteworthy activity against cervical cancer cell lines. The aqueous leaf extracts of *P. afra* that were collected under cold temperature and water deficit simulation (10/15 °C) had the strongest antidiabetic activity, compared to the stems and roots (Adeleye and Risenga, 2024). Conversely, the ethyl acetate and aqueous stem extracts from extreme cold (0/5 °C) and high temperatures (45/35 °C) paired with drought stress, exhibited the greatest α-amylase inhibition. These studies show that the exposure to elevated CO_2 and temperature variations improves the therapeutic properties in several medicinal plant species. This could be due to the accumulation of bio-compounds, which are responsible for eliciting this effect. In most cases, however, the studies either put focus on one environmental stressor even though they occur simultaneously in nature. Another shortfall in the cited studies is that the focus was mostly on leaves, seedlings of plants. Considering how atmospheric gases are responsible for trapping heat and radiation within the Earth's atmosphere, investigating how changes in carbon

dioxide and temperatures could influence the overall physiological and bioactive attributes of medicinal plants is crucial. Assessment of the entire organism is also of great importance, since the natural environment they are found in not only affects a singular plant organ. Furthermore, this can provide information that can result in the cultivation of tolerant plant species with the goal of sustainable preservation for future generations.

2.7 Research Experimental Design

2.7.1 Climate simulation setup

During the month of February 2021, mature potted *Bulbine frutescens*, *Bulbine abyssinica*, and *Bulbine natalensis* specimens (85 samples per species) were purchased from the Witkoppen Nursery in Randburg, Gauteng Province, South Africa. Each of the species were taken to the climate simulation chambers (CONVIRON, PGW40) for the remainder of the month under ambient conditions of 400 parts per million (ppm) CO_2 concentration, combined with a temperature range of 27/25 °C (day/night). Following the acclimation period, 20 samples of each species were placed across four different chambers, each with simulations of concurrent elevated CO_2 and temperatures. Two of the chambers had CO_2 concentrations of 600ppm, while the other two were set at 800ppm. Both concentrations of CO_2 were paired with temperature settings of 40/30 °C and 45/35 °C respectively. The experiment was conducted over a period of eight days, and five samples per species were harvested at 48-hour intervals (Table 2.3). Harvest periods were selected based on future estimations of heat waves in South Africa (Mbokodo et al., 2020). All samples were regularly watered during both the acclimation and experimental segments. Parameters including relative humidity (60%) and light intensity (160 nits/level 1) were kept constant. The abovementioned conditions were strongly aligned with extensive documentation from various scientific sources of current climate records and

future predictions (South African Department of Environmental Affairs, 2019; Chersich and Wright, 2019; NOAA, 2020; Mbokodo *et al* 2020; Van der Walt and Fitchett, 2021).

Table 2.3: Illustration of the experimental design, showing treatment conditions and harvest period frequency

	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**
CO_2 concentration (ppm)	420	600	600	800	800
Temperature/s (°C day/night)	27/25 °C	40/30 °C	45/35 °C	40/30 °C	45/35 °C
Harvest frequency (hours)	Once	48 96, 144, 192	48 96, 144, 192	48 96, 144, 192	48 96, 144, 192

Sample size per treatment: 20 pot plants per species; five pot plants harvested for each period.

2.7.2 Biological assessment

Qualitative and quantitative methods were applied in this study in combination with case and experimental studies. Methanol extracts from various *B. abyssinica*, *B. frutescens* and *B. natalensis* plant parts harvested at 48-hour intervals (48, 96, 144, 192 hours) were utilised to determine the possible variations in the phytochemical profile under combined elevated CO_2 and temperatures. Analysis of the plant organs' antioxidant and antimicrobial activities were also compared against the experimental conditions.

2.7.3 Approaches to data analysis

Environmental conditions driven by climate change take long periods of time for any change to occur. It was because of this reason that case studies and a combination of qualitative and quantitative experimental methods were used to obtain primary data (Kumatongo and Muzata, 2021). The simulation of concurrent elevated CO_2 and high temperatures allowed for the observation of possible changes in the phytochemical profile, in-vitro antioxidant, and antimicrobial activities of *B. abyssinica*, *B. frutescens*, and *B. natalensis*. By exposing each species to these conditions for a period of eight days and conducting analyses on their phytopharmacological properties, the objectives of the study were achieved. The experimental parameters chosen for this study were repeated three times with the intention of producing statistically significant results (Creswell, 2013). Experimental research design needs to ensure validity and reliability (Kumatongo and Muzata, 2021). The consistency of each experiment, including the application of measurements, analytical tests and instruments, demonstrated reliability. The validity of the experiment was increased by conducting a pilot study on each objective, to minimize the chances of experimental bias. This helped to avoid any possibility of making changes during the course of the experiment, while also providing an opportunity to master the use of the instruments.

The advantage of combining case studies with experimental studies is that it presents an opportunity to focus on multiple species and simulate environmental conditions that take a relatively long time to observe any changes. Because plants vary when it comes to their response to the environment, the results obtained in this study were not used to standardize the combined effects of CO_2 and high temperatures on plants. The qualitative data from the study was subjected to statistical analysis through a series of one way analysis of variance (ANOVA) and Tukey post-hoc tests to report on any significance. The research problem of this study was addressed through achieving the objectives, highlighting the novelty.

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CHAPTER THREE

Effects of concurrent elevated CO_2 and temperatures on the antimicrobial activities of *B. frutescens*, *B. abyssinica*, and *B. natalensis*

This chapter provides details on the investigation of the antimicrobial activities of the leaves and roots of *B. frutescens*, *B. abyssinica*, and *B. natalensis*, as well as the underground stems and corms of *B. abyssinica* and *B. natalensis* respectively, under concurrent elevated CO_2 and temperatures.

The contents of the control data from the qualitative antibacterial assay each species have been published in several articles:

Biomedical and Pharmacology Journal (DOI: https://dx.doi.org/10.13005/bpj/2469)

Journal of Herbmed Pharmacology (DOI: 10.34172/jhp.2024.44650)

Indonesian Journal of Pharmacy (DOI: https://doi.org/10.22146/ijp.6025)

A manuscript outlining the findings from the experimental treatment conditions of concurrent elevated CO_2 and temperatures on the antibacterial activity in *B. natalensis* has been published in the Biomedical and Pharmacology Journal (DOI: https://dx.doi.org/10.13005/bpj/2975).

A manuscript outlining the minimum inhibitory activity of each study species has been published in the South African Journal of Botany (DOI: https://doi.org/10.1016/j.sajb.2025.03.010 0254-6299).

3.1. Introduction

The development of synthetic antibiotics has significantly improved the livelihoods of humans by reducing the recurrence of microbial infections (Aslam *et al.,* 2018; Rossolini *et al.,* 2014; Owusu *et al.,* 2021). However, antibiotics have been extensively used over the years, resulting in a surge of antimicrobial resistance (Vaou *et al.,* 2021). Furthermore, synthetic antibiotics have been known to cause unsettling side effects such as nausea, vomiting, abdominal pain, and dizziness (Mohsen *et al.,* 2020; Matotoka *et al.,* 2023). Chandra *et al* (2017) reported that due to the genetic changes of micro-organisms aiding in their resistance, the effectiveness of antibiotics could be lost within half a decade. As a result, there has been an increase in the search for alternative antimicrobial sources found in the natural environment. (Anand *et al.,* 2019; Owusu *et al.,* 2021). Ethnomedicine has been used over many centuries in primary healthcare, especially in Third World countries, where traditional medicine is favoured over Western medicine because of their cost-effectiveness and availability (Teffo *et al.,* 2022). A large diversity of secondary metabolic compounds, are produced by plants. Compounds such as polyphenols, tannins, alkaloids, flavonoids and terpenoids contribute to the therapeutic properties of medicinal plants. These include antimicrobial, anticancer, and antioxidant activities (Wendakoon *et al.,* 2012). More than 30 000 plant species have been documented in South Africa, and about 3000 of these are medicinal, and mostly of endemic origin (Van Wyk *et al.,* 1997; Mulholland, 2005; Van Vuuren, 2008). Some of the most commonly used medicinal plant species include *Bulbine frutescens*, *Bulbine abyssinica,* and *Bulbine natalensis*. Their therapeutic uses range from skin conditions such as rashes and wound-healing, to viral, gastrointestinal, urinary tract infections, as well as diabetes management (Bodede and Prinsloo, 2020). Given the uses of medicinal plants for the treatment and management of illnesses and diseases, one factor is known to impact both human and plant life, and that is climate change. This phenomenon is

often linked to an increase in atmospheric carbon dioxide (CO_2) and global temperatures. It has been reported that CO_2 concentrations could reach high levels of up to 900 ppm (parts per million) by the end of the 21st century (Kumar *et al.,* 2020). Additionally, global warming is predicted to increase between 3-5 ˚C each decade (Walia et al., 2022). Currently, the average summertime temperatures in South Africa stands at 27 ˚C, which has increased at a minimum of 1.5 times the global average (Ziervogel *et al.,* 2014; Van der Walt and Fitchett, 2021). Most parts of the country, according to the South African Weather Service (2019), are expected to have temperatures of up to 47 ˚C. This change in temperature conditions contributes to the duration of heatwaves, which currently spans at least four days. However, with climate change, heatwaves are projected to increase two-fold, to one week (Lyon, 2009). The factors could negatively impact the bioactivity of medicinal plants. Climate change also has direct consequences to human health. Micro-organisms are said to be highly opportunistic, hence their response to climate change could increase the cost of healthcare and the burden of multiple infectious diseases (Tiedje *et al.,* 2022). High temperatures are associated with antimicrobial resistance, since microbial growth rates are dependent on specific weather conditions (Philipsborn *et al.,* 2016; Magnano San Lio et al., 2023). Although sufficient research on the antimicrobial activity of *B. frutescens*, *B. abyssinica* and *B. natalensis* has been documented to date (Mocktar, 2000; Mbambo *et al.,* 2012; Yakubu *et al.,* 2012; Rachuonyo *et al.,* 2016; Mwiti and Afolayan, 2016; Kandari, 2018; Lucas *et al.,* 2020; Teffo *et al.,* 2022; 2024 (a); 2024 (b)) there are no available reports that focused on the influence of environmental factors such as concurrent elevated CO_2 and temperatures on the antimicrobial activities of these plant species. This research aimed to investigate the effects of concurrent elevated CO_2 and high temperatures on the antimicrobial activity of various parts of *B. frutescens*, *B. abyssinica* and *B. natalensis*. By conducting this analysis, I can document how the species adapt to climate change based on their ability to either retain,

improve, and/or deteriorate their antimicrobial activity. The findings could provide insight on the effects climate change poses on the therapeutic properties of medicinal plants, and aid in developing harvesting strategies for indigenous communities such as specific harvest times when the bioactivity is of quality standard and plant organ harvest protocols to preserve the diversity of the species.

3.2. Materials and methods

3.2.1 Crude plant extraction

The crude extraction method described by Hassan (2018) was adopted for the determination of the antimicrobial activity. An Erlenmeyer flask was initially weighed prior to the addition of 100g of powdered plant material, which was also weighed. An organic solvent (80% methanol) was poured into the flask with the powdered material. Autoclaved cotton wool was used to seal the flask, which was incubated at 37 ˚C for 24 hours. After the incubation period, the supernatant was extracted and re-suspended in acetone, vortexed to ensure efficient mixing of the contents in the beaker, and stored at 4 ˚C.

3.2.2 Antimicrobial activity

Microbial culture preparation

Six bacterial micro-organisms, namely, *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 27858) were selected for the antibacterial portion of the analysis. *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 90112) were selected to assess the antifungal activity.

The microbial strains are used as reference strains of the American Type Culture Collection and were obtained from the Department of Pharmacy and Pharmacology at the University of the Witwatersrand, Johannesburg. Each of the microorganisms were selected based on their prevalence in disease and infections in South Africa.

Minimal inhibitory concentration (MIC) assay

To evaluate the minimal inhibitory concentration of *B. frutescens*, *B. natalensis,* and *B. abyssinica*, a two-fold serial dilution technique was followed. Using a 96-well micro-titre plate, 100 µL of tryptone soya broth [TSB] was added into the wells. A volume of 100 µL of the plant extract was added into the first well. In the last three wells, a positive control (0.01 mg/ml of ciprofloxacin for bacteria and 0.1 mg/ml of nystatin for yeasts), negative control (acetone) and culture control were added. The positive control was used to note the antimicrobial susceptibility of the tested pathogens, whereas the negative control was used to determine whether the micro-organisms were having an inhibitory effect to the solvent. The culture control ensured that microbial growth occurred. A serial dilution was followed, down the plate at concentrations of 16, 8, 4, 2, 1, 0.5, and 0.125 mg/ml. Following this, 100 µL of a 0.5 McFarland turbidity standard of culture was added into each well. Once this process was completed, sterile adhesive film was used to seal the micro-titre plates and incubated at 37 ˚C. The plates containing the bacterial pathogens were incubated for 24 hours, whereas those containing yeast pathogens were incubated for 48 hours. After the incubation, a volume of 400 µL of *p*-iodonitrotetrazolium (INT) violet solution was added to the wells. The solution indicated microbial growth by exhibiting a pink-purple colour in the well.

3.3. Results

3.3.1 Minimum inhibitory concentration

Two of the commercial antimicrobial drugs were used as positive controls for this study were ciprofloxacin and nystatin for the bacterial and fungal pathogens respectively. Ciprofloxacin showed better inhibition against Gram-negative bacteria, with MIC values of 0.31 µg/ml reported against *K. pneumoniae* and *P. aeruginosa*. *S. aureus*, *E. coli* and *E. faecalis* were inhibited at the same MIC (0.63 µg/ml). *C. neoformans* was the most susceptible fungal pathogen against nystatin (0.31 µg/ml), compared to *C. albicans* (1.25 µg/ml).

3.3.1.1 Bulbine abyssinica

a) Leaves

Bulbine abyssinica leaf extracts displayed a greater variation in the inhibitory activity against *B. cereus*, compared to *S. aureus* and *E. faecalis*. The MIC reported from *B. cereus* inhibition varied between 0.5 and 2 mg/ml between treatments of concurrent elevated CO_2 and temperatures (Figure 3.1). However, the variation was most notable under 600 ppm & 40/30 ˚C (0.5 mg/ml at 0, 96 and 144 hrs, and 2 mg/ml at 192 hrs). *S. aureus* showed similar variation against *B. abyssinica* leaves under 800 ppm & 40/30 ˚C (1 mg/ml at 0 hrs and 4 mg/ml at 192 hrs).

For the Gram- negative pathogens, *B. abyssinica* leaves had the greatest inhibitory effect against *E. coli* under 600 ppm and 800 ppm 40/30 ˚C. The variation in the MIC was reported between 1 and 4 mg/ml from several harvest periods (Figure 3.1). The activity of *B. abyssinica* leaves had notable variation under 800 ppm & 40/30 ˚C between 48 and 144 hrs (MIC = 1

mg/ml), and 0 and 192 hrs (MIC = 4 mg/ml). The leaves exposed to the 600 ppm & 45/35 ˚C treatment showed comparatively greater difference in the MIC against *P. aeruginosa* (0.5 mg/ml at 144 and 192 hrs, and 2 mg/ml at 48 hrs) than *E. coli* and *K. pneumoniae*.

The antifungal activity of *B. abyssinica* leaves against *C. albicans* showed MIC variations of 1 and 4 mg/ml from 0-48 hours and 144-192 hours under 600ppm & 40/30 ˚C and between 0, 48 and 192 hours under 800ppm & 40/30 ˚C respectively (Figure 3.1). The same observations were reported for the MIC of the leaves against *C. neoformans* between 0 and 144 hours (1 mg/ml) and 192 hours (4 mg/ml) from the 800ppm & 40/30 ˚C treatment.

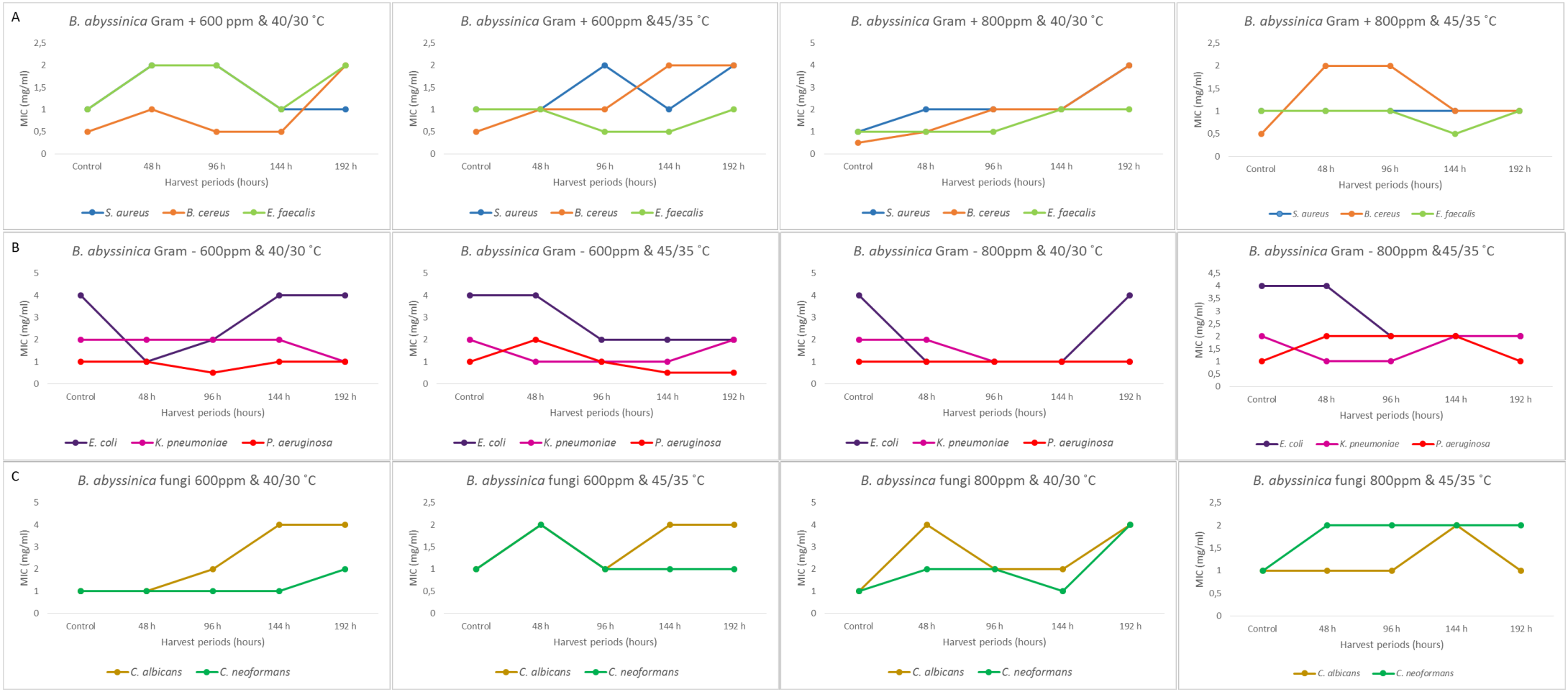

Figure 3.1 Minimum inhibitory concentration of *B. abyssinica* leaves under concurrent elevated CO_2 and high temperatures. Row A = Gram-positive bacteria; Row B = Gram-negative bacteria; Row C = Fungal microorganisms.

b) Underground stems

B. abyssinica underground stems had shown an inhibitory concentration eight-times greater against *S. aureus* between 0 hrs (4 mg/ml) and 0.5 mg/ml (48 hrs- 600 ppm & 40/30 ˚C; 144 hrs- 800 ppm & 45/35 ˚C – Figure 3.2). The largest variation however, was reported under 600 ppm & 45/35 ˚C, with the MIC decreasing from 48 and 96 hrs (16 mg/ml) to 144 hrs (1 mg/ml). *E. faecalis* had a variation between 2 mg/ml (0 hrs) to 0.5 mg/ml across all other harvest periods, with the greatest effect reported under 800 ppm & 45/35 ˚C.

The greatest difference in the MIC of *E. coli* against *B. abyssinica* underground stems was reported under 600 ppm & 45/35 ˚C, with the concentration decreasing from 16 mg/ml (48 hrs) to 0.5 (96 hrs). *P. aeruginosa* showed some variation in the MIC under 800 ppm & 45/35 ˚C, between 0 hrs (2 mg/ml) and 96 hrs (0.5 mg/ml). Overall, *E. coli* was most affected by *B. abyssinica* stems under 600 ppm & 45/35 ˚C and both 800 ppm treatments compared to other tested Gram-negative bacteria (Figure 3.2).

Candida albicans and *C. neoformans* expressed similar variation in the MIC against *B. abyssinica* stems under 600 ppm & 45/35 ˚C (2 mg/ml at 0 hrs and 0.5 mg/ml at 48 hrs- *C. albicans*) and 800 ppm & 45/35 ˚C (4 mg/ml at 0 hrs to 1 mg/ml at 96 and 192 hrs- *C. neoformans*) respectively (Figure 3.2).

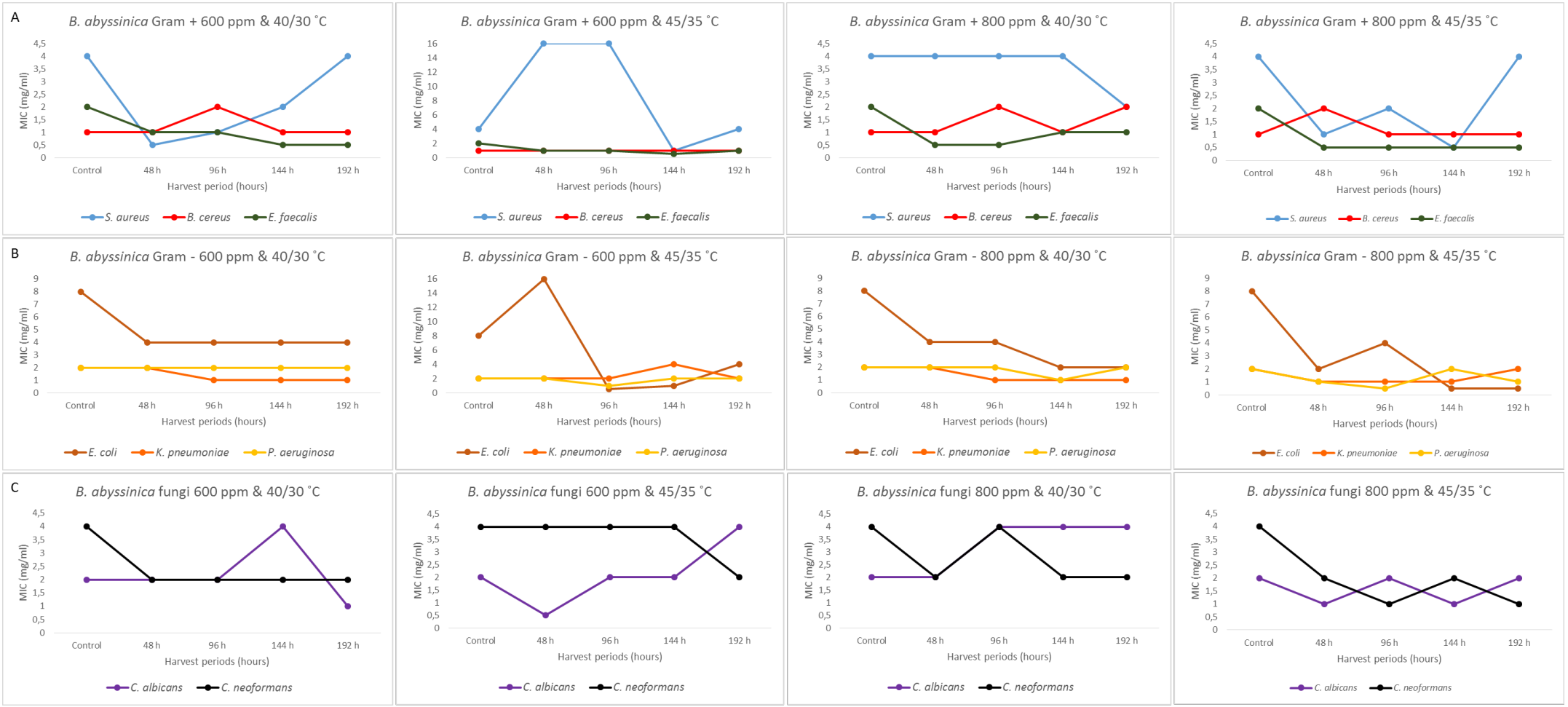

Figure 3.2 Minimum inhibitory concentration of *B. abyssinica* underground stems under concurrent elevated CO_2 and high temperatures. Row A = Gram-positive bacteria; Row B = Gram-negative bacteria; Row C = Fungal microorganisms.

c) Roots

The MIC values reported from the inhibition against *S. aureus* had shown variation in three treatments (600 ppm & 45/35 ˚C, 800 ppm & 40/30 ˚C and 45/35 ˚C). The most notable difference across these treatments were between 48 hrs (0.5 mg/ml) and 96-144 hrs (8 mg/ml). Other variations included MIC values from 48-96 hrs (8 mg/ml) to 144-192 hrs (2 mg/ml) under 800 ppm & 40/30 ˚C, and between 0 hrs (4 mg/ml) and 144 hrs (1 mg/ml) under 800 ppm & 45/35 ˚C. *B. cereus* inhibition from *B. abyssinica* roots was four ties lower at 96 hrs (0.5 mg/ml) compared to 0 hrs (2 mg/ml) under 600 ppm & 45/35 ˚C (Figure 3.3).

Escherichia coli displayed the greatest difference in the MIC against *B. abyssinica* roots under 600 ppm & 40/30 ˚C and 45/35 ˚C, in comparison to other tested Gram-negative bacteria. A four and eight-fold difference in the concentration against *E. coli* reported between 48 and 192 hrs (0.5-1 mg/ml) compared to 0 hrs (4 mg/ml) under 600 ppm 40/30 ˚C. *P. aeruginosa* had shown a difference in MIC under 600 ppm & 40/30 ˚C (2 mg/ml at 0 hrs and 0.5 mg.ml at 144 hrs), and 800 ppm & 40/30 ˚C (2 mg/ml at 0 hrs and 96 hrs, and 0.5 mg/ml at 192 hrs – Figure 3.3).

The roots harvested under both 600 ppm treatments and 800 ppm & 40/30 ˚C showed the same inhibition difference against *C. neoformans* between the control and some of the harvest periods in the abovementioned treatments (4-1 mg/ml). The strongest MIC variation against *C. albicans* was reported between the control and 192 hrs (4-0.5 mg/ml – 600 ppm & 40/30 ˚C) and 48-96 hrs (8-1 mg/ml – 800 ppm & 40/30 ˚C – Figure 3.3).

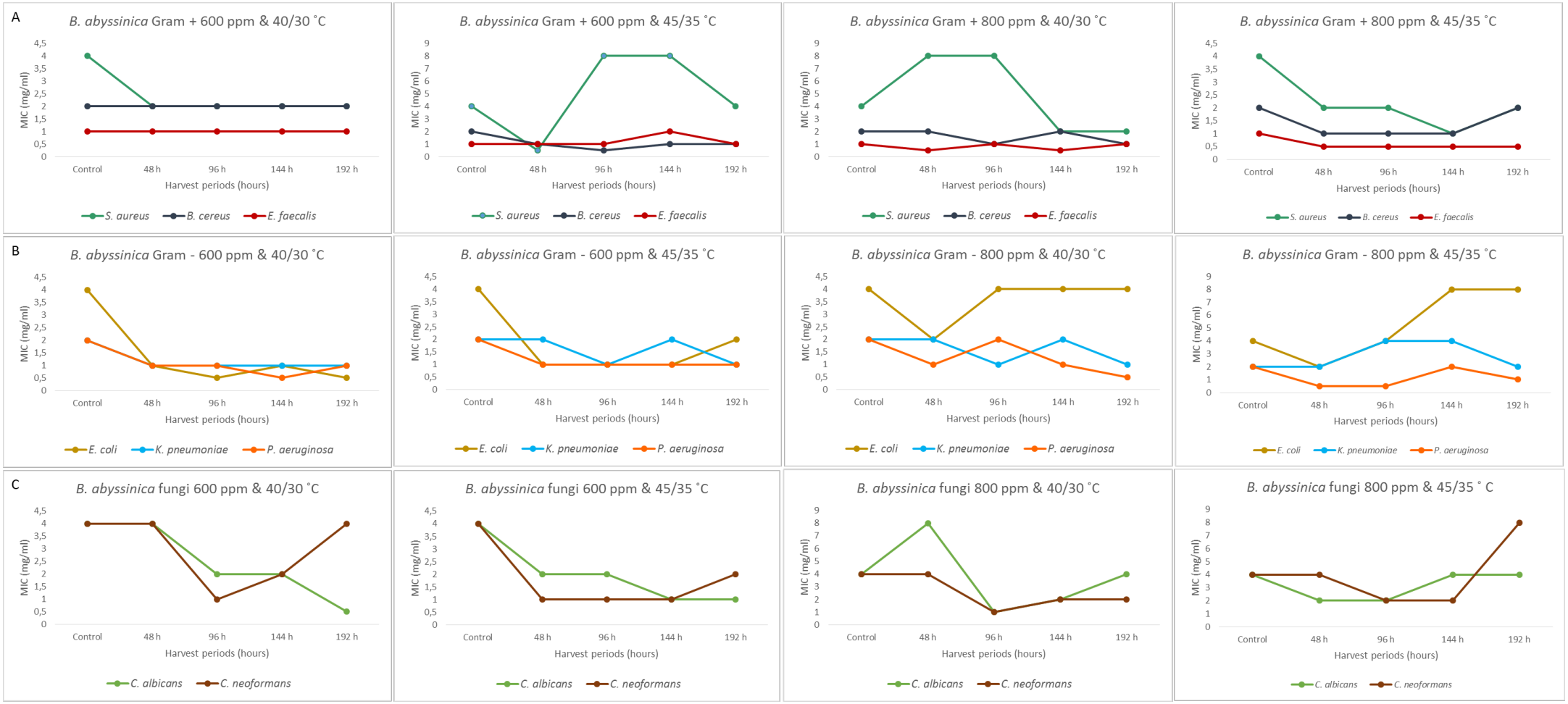

Figure 3.3 Minimum inhibitory concentration of *B. abyssinica* roots under concurrent elevated CO_2 and high temperatures. Row A = Gram-positive bacteria; Row B = Gram-negative bacteria; Row C = Fungal microorganisms.

3.3.1.2 Bulbine frutescens

a) Leaves

The inhibitory concentration of *B. frutescens* leaves against *S. aureus* displayed a greater difference in the MIC under both 800 ppm treatments (Figure 3.4). The variation had changed from 2 mg/ml (96 hrs) to 16 mg/ml (144-192 hrs) under 800 ppm & 40/30 ˚C, while a gradual decrease was reported between 48 hrs (16 mg/ml) to 144-192 hrs (2 mg/ml) under 800 ppm & 45/35 ˚C. *E. faecalis* showed comparatively greater susceptibility under 600 ppm & 45/35 ˚C, where the variation against *B. frutescens* leaves ranged between 0.5 mg/ml (48 and 192 hrs) and 4 mg/ml (144 hrs), than other treatments.

Across all tested Gram-negative bacteria, the largest difference from the inhibition against *B. frutescens* leaves was reported against *P. aeruginosa* (600 ppm & 45/35 ˚C and both 800 ppm treatments) and *K. pneumoniae* (600 ppm & 45/35 ˚C and 800 ppm & 40/30 ˚C) respectively. The inhibitory concentrations varied between 16 (control) and 1 (several harvest periods) mg/ml (Figure 3.4).

There was a notable difference in the MIC of *B. frutescens* leaves against *C. neoformans* between 48 and 144 hrs (2-8 mg/ml) under 600 ppm & 45/35 ˚C. *B. frutescens* leaves showed similar inhibition against both *C. albicans* and *C. neoformans* at 96 hrs under 800 pm & 45/35 ˚C (Figure 3.4). The reported concentrations for both fungal microorganisms were four times lower (4 mg/ml – *C. albicans*; 1 mg/ml – *C. neoformans*) compared to their respective higher MIC values (16 mg/ml – *C. albicans*; 4 mg/ml – *C. neoformans*).

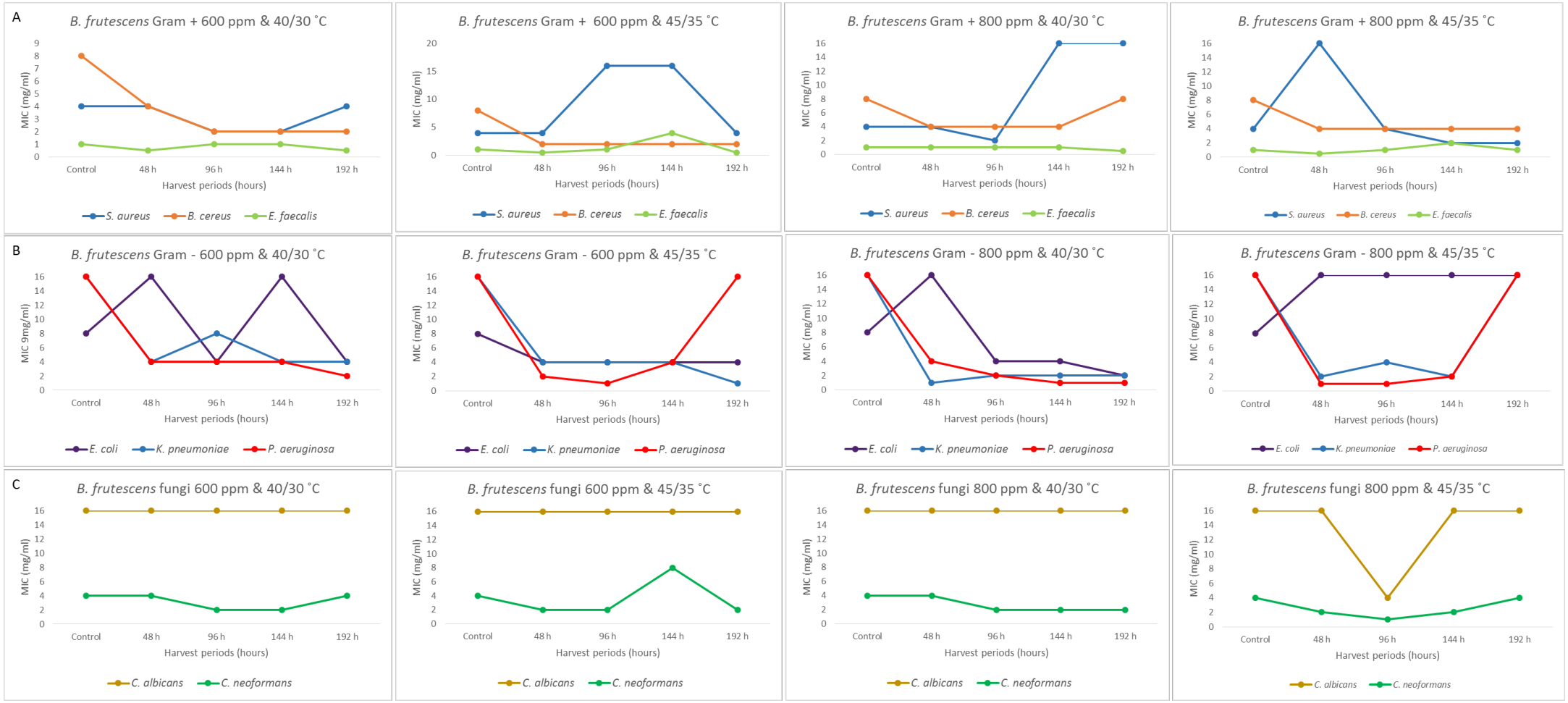

Figure 3.4 Minimum inhibitory concentration of *B. frutescens* leaves under concurrent elevated CO_2 and high temperatures. Row A = Gram-positive bacteria; Row B = Gram-negative bacteria; Row C = Fungal microorganisms.

b) Roots

Under 600 ppm & 40/30 ˚C and 800 ppm & 40/30 ˚C, *S. aureus* showed a similar inhibition pattern against *B. frutescens* roots at some harvest periods (4 to 1 mg/ml). The roots exhibited the strongest inhibitory activity under 800 ppm & 45/35 ˚C against *S. aureus* between the control (2 mg/ml) and 192 hrs (0.25 mg/ml). *E. faecalis* was inhibited at similar MICs between the control and 96 hrs from roots harvested under 600 ppm & 40/30 ˚C and 800 ppm & 45/35 ˚C (Figure 3.5).

Both 800 ppm treatments had a comparatively greater effect at inhibiting *E. coli* than the 600 ppm treatments. *B. frutescens* roots from the 800 ppm & 40/30 ˚C treatment showed a 16-2 mg/ml MIC variation against *E. coli* between the control and 192 hours. A similar trend was reported at 800 ppm & 45/35 ˚C, where the largest variation against the bacterial strain was noted between the control and 192 hours (16-0.5 mg/ml). *K. pneumoniae* and *P. aeruginosa* both showed similar inhibition patterns against *B. frutescens* roots, between 48 and 144 hours (4-1 mg/ml - 600 ppm & 40/30 ˚C) and control to 192 hours (2-0.5 mg/ml – 800 ppm & 45/35 ˚C) respectively (Figure 3.5).

Cryptococcus neoformans and *C. albicans* had the greatest difference in MIC reported between the same harvest periods (control-96 hours) at 600 ppm & 40/30 ˚C (2-0.5 mg/ml) and 800 ppm & 45/35 ˚C (4-1 mg/ml) respectively (Figure 3.5).

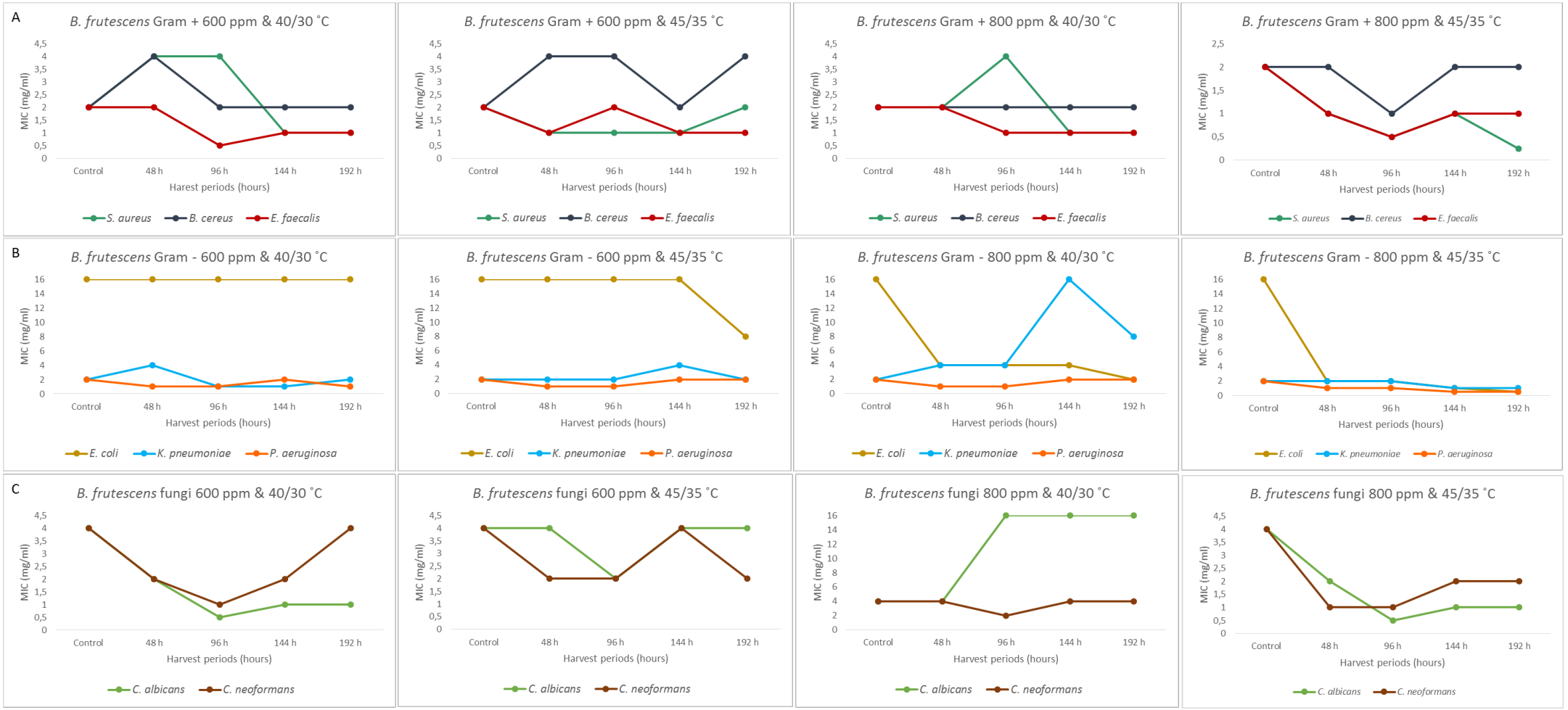

Figure 3.5 Minimum inhibitory concentration of *B. frutescens* roots under concurrent elevated CO_2 and high temperatures. Row A = Gram-positive bacteria; Row B = Gram-negative bacteria; Row C = Fungal microorganisms.

3.3.1.3 Bulbine natalensis

a) Leaves

Bulbine natalensis leaves exposed under 600 ppm & 40/30 ˚C had the greatest effect in the inhibition of *S. aureus* compared to *B. cereus* and *E. faecalis*. The MIC increased between 0 hrs (2 mg/ml) and 192 hrs (16 mg/ml). The inhibition of *E. faecalis* had greater variation against *B. natalensis* leaves under both 800 ppm treatments. The concentration values ranged between 0.5 mg/ml and 2 mg/ml (Figure 3.6).

Escherichia coli and *K. pneumoniae* had exhibited similar variations in their MICs against *B. natalensis* leaves under 600 ppm & 40/30 ˚C and both 800 ppm treatments with *E. coli* and all treatments with *K. pneumoniae* respectively (Figure 3.6). The greatest inhibitory concentrations against both Gram-negative bacteria ranged between 2 and 16 in several harvest periods. *P. aeruginosa* also displayed the same difference in the concentration from leaves exposed under 600 ppm & 45/35 ˚C, showing a gradual decrease from 0 hrs (4 mg/ml) to 192 hours (0.5 mg/ml).

The antifungal activity of *B. natalensis* leaves yielded an MIC difference that was four times greater to the concentration reported at 0 hrs against *C. albicans* (16 mg/ml to 4 mg/ml) and *C. neoformans* (4 mg/ml to 1 mg/ml) under 600 ppm & 45/35 ˚C (Figure 3.6).

Figure 3.6 Minimum inhibitory concentration of *B. natalensis* leaves under concurrent elevated CO_2 and high temperatures. Row A = Gram-positive bacteria; Row B = Gram-negative bacteria; Row C = Fungal microorganisms.

b) Underground stems

The same variation in the inhibition of *E. faecalis* against *B. natalensis* corms was noted across all treatments, with MICs indicating a decrease between 2 and 0.5 mg/ml. *B. cereus* had also shown a similar result in all treatments. However, under 800 ppm & 45/35 ˚C, the concentration decreased from 0.5 mg/ml (48-96 hrs) to 0.25 mg/ml (144-192 hrs) compared to 2 mg/ml (0 hrs – Figure 3.7).

An eight-fold decrease in the MIC of *P. aeruginosa* against *B. natalensis* corms, was reported between 48-192 hrs (16-2 mg/ml – 600 ppm & 40/30 ˚C) and 0-192 hrs (8-1 mg/ml – 800 ppm & 40/30 ˚C) respectively. The greatest difference, however, was discovered from the corms harvested between 0 and 96 hrs, under 800 ppm & 45/35 ˚C (8-0.5 mg/ml) (Figure 3.7).

Overall, *C. neoformans* showed consistent variation compared to *C. albicans*, against *B. natalensis* corms. In three of the treatments, the MIC of C. neoformans decreased from 4 mg/ml to 1 mg/ml across several harvest periods under both 600 ppm and 800 ppm & 45/35 ˚C respectively. *C. albicans* however, displayed the highest inhibitory concentration against *B. natalensis* corms under 600 ppm & 40/30 ˚C (1 mg/ml – 0 and 192 hrs to 16 mg/ml – 96 hrs) (Figure 3.7).

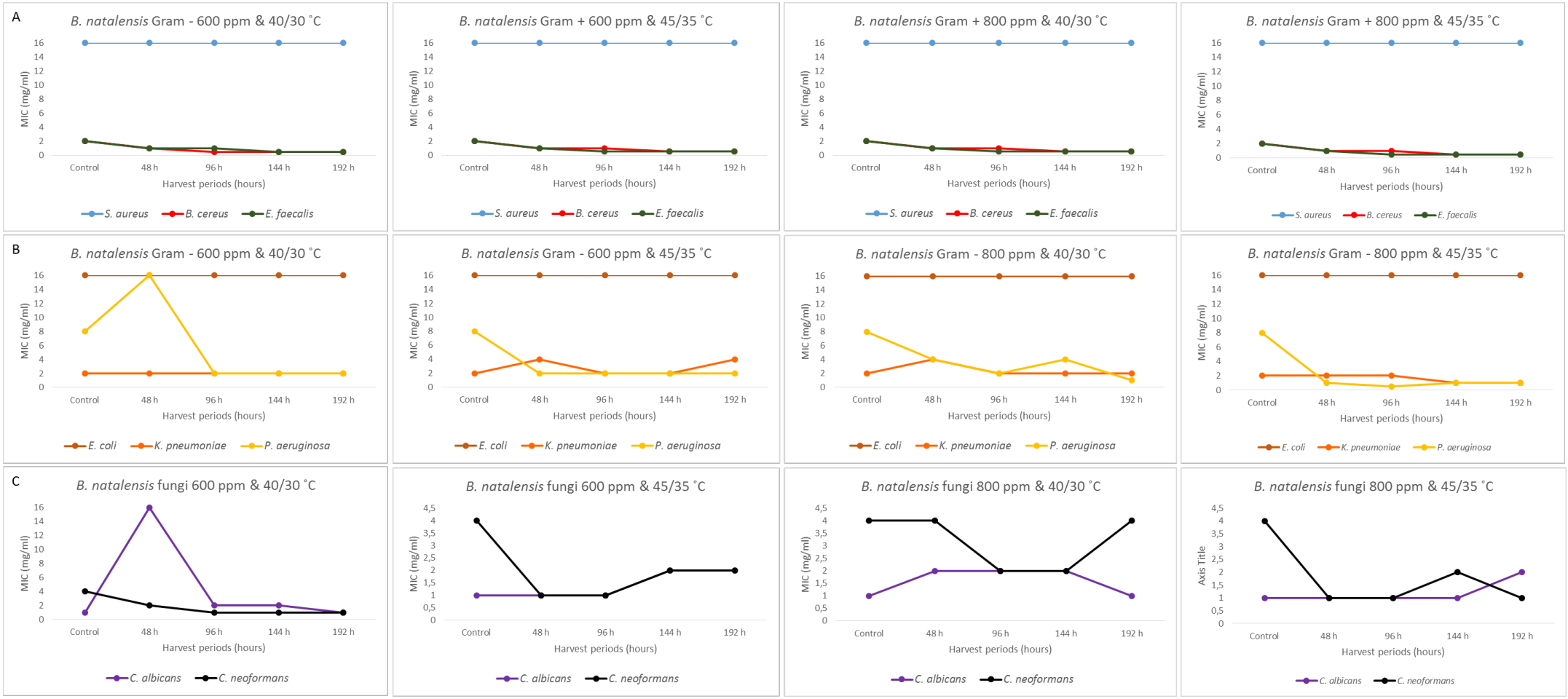

Figure 3.7 Minimum inhibitory concentration of *B. natalensis* underground stems under concurrent elevated CO_2 and high temperatures. Row A = Gram-positive bacteria; Row B = Gram-negative bacteria; Row C = Fungal microorganisms.

c) Roots

Bacillus cereus had the same inhibitory concentration against *B. natalensis* roots under both 600 ppm treatments, between 0 hrs (2 mg/ml) and some of the harvest periods (0.5 mg/ml). The variation in the MIC of *S. aureus* decreased from 16 to 2 mg/ml (600 ppm & 45/35 ˚C), to 16-2 mg/ml (800 ppm & 40/30 ˚C). The most notable difference however, was reported under 800 ppm & 45/35 ˚C (16-0.5 mg/ml- *S. aureus*) against the root extracts (Figure 3.8).

The highest variation in the MIC of *E. coli* against *B. natalensis* roots was reported from three treatments, 600 ppm & 40/30 ˚C, 800 ppm & 40/30 ˚C and 45/35 ˚C. This concentration difference ranged from 16 mg/ml (0 hrs) to 1 mg/ml in few harvest periods. There was a four times difference in the inhibition against *P. aeruginosa* across all treatments. The concentration had decreased from 4 mg/ml between 48 and 96 hrs, to 1 mg/ml (192 hrs) from roots exposed to 600 ppm & 40/30 ˚C, and 2 mg/ml (0 hrs) to 0.5 mg/ml under 600 ppm & 45/35 ˚C and both 800 ppm treatments. *K. pneumoniae* expressed varied MIC values, from 0.5 to 2 mg/ml (600 ppm & 40/30 ˚C) and 1 to 4 mg/ml (800 ppm & 40/30 ˚C) respectively (Figure 3.8).

Candida albicans had the most notable variation in its inhibitory concentration against *B. natalensis* roots between both 600 ppm treatments and 800 ppm & 40/30 ˚C, compared to *C. neoformans*. The greatest difference in the reported MICs was between 8 mg/ml (0 hrs) and 1 mg/ml (select periods – Figure 3.8).

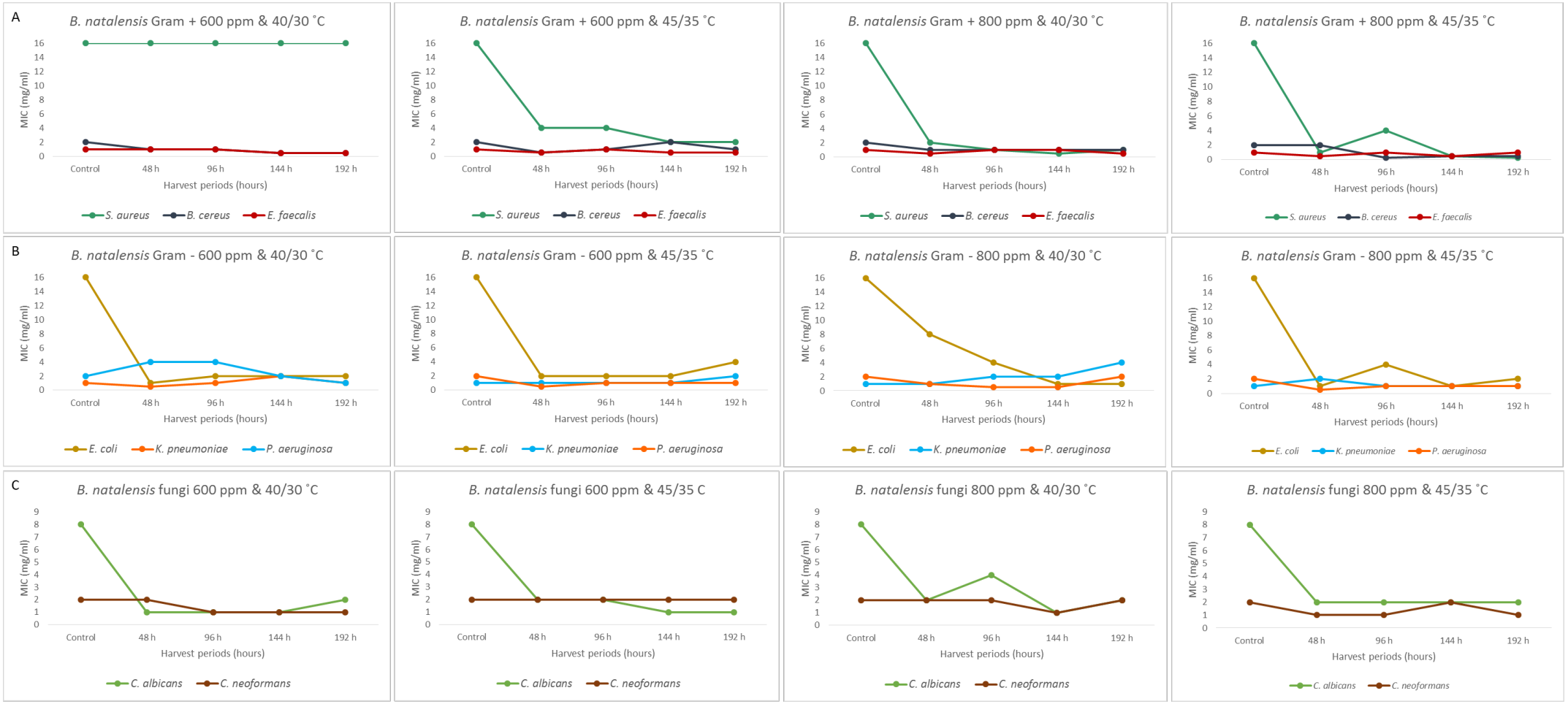

Figure 3.8 Minimum inhibitory concentration of *B. natalensis* roots under concurrent elevated CO_2 and high temperatures. Row A = Gram-positive bacteria; Row B = Gram-negative bacteria; Row C = Fungal microorganisms.

3.3.2 Qualitative antibacterial activity

3.3.2.1 S. aureus

B. frutescens leaf extracts from samples that were harvested under elevated CO_2 and high temperatures showed intermediate growth inhibitory activity against *S. aureus* (Table 3.1). The control extract, however, was susceptible to the pathogen, as shown by the zone of inhibition (20.67 ± 1.15 mm). All of the root extracts, both control (14.33 ± 0.58 mm) and harvest periods under treatment only exhibited a good activity, with values ranging between 14 and 18 mm.

The leaves and roots both showed an intermediate antibacterial activity against *S. aureus*. The leaves harvested under elevated conditions had comparatively weaker activity than the control, but the roots maintained a good activity throughout.

The leaf extracts retained their growth inhibitory activity against *S. aureus* under 800ppm & 40/30 ˚C, as the zone of inhibition values fell within the intermediate range of 10-19 mm. Susceptibility was reported for the extracts from root samples collected at 96 hours (19.67 ± 1.20 mm) and 144 hours (22.67 ± 0.88 mm).

Much like the previous three elevated CO_2 and high temperature treatments, the leaves under 800ppm & 45/35 ˚C still showed a good antibacterial activity against *S. aureus* between 48 and 192 hours. The roots followed suit, with zone of inhibition values slightly fluctuating between 12 and 16 mm from 48 to 192 hours. The ANOVA results showed significant differences overall for the leaves and roots against *S. aureus* ($p < 0.05$), however, only the leaves under elevated CO_2 and temperatures were significantly different to the control (Tukey's test), whereas some of the roots harvested under treatment conditions displayed significant difference.

Table 3.1: Growth inhibitory activity of *B. frutescens* against *S. aureus* under concurrent elevated CO_2 and temperatures.

Treatment	Harvest period	Leaves Zone of inhibition (mm)	Roots Zone of inhibition (mm)
Control		$20.67^{a} \pm 1.15$	$14.33^{b} \pm 0.58$
600ppm & 40/30 ˚C	48 h	$12.67^{c} \pm 0.33$	$14.33^{b} \pm 0.33$
	96 h	$12.33^{cd} \pm 0.33$	$16.67^{a} \pm 0.33$
	144 h	$15.00^{b} \pm 0.58$	$13.33^{b} \pm 0.67$
	192 h	$10.33^{d} \pm 0.33$	$17.67^{a} \pm 0.67$
Control		$20.67^{a} \pm 1.15$	$14.33^{a} \pm 0.58$
600ppm & 45/35 ˚C	48 h	$11.33^{b} \pm 0.33$	$13.67^{ab} \pm 0.33$
	96 h	$12.00^{b} \pm 0.58$	$15.00^{a} \pm 0.58$
	144 h	$12.00^{b} \pm 1.00$	$11.67^{b} \pm 0.33$
	192 h	$12.33^{b} \pm 0.33$	$11.33^{b} \pm 0.88$
Control		$20.67^{a} \pm 1.15$	$14.33^{c} \pm 0.58$
800ppm & 40/30 ˚C	48 h	$14.67^{b} \pm 0.67$	$16.33^{bc} \pm 0.33$
	96 h	$13.67^{b} \pm 0.33$	$19.67^{ab} \pm 1.20$
	144 h	$13.67^{b} \pm 0.33$	$22.67^{a} \pm 0.88$
	192 h	$13.67^{b} \pm 0.88$	$17.67^{bc} \pm 0.67$
Control		$20.67^{a} \pm 1.15$	$14.33^{ab} \pm 0.58$
800ppm & 45/35 ˚C	48 h	$12.67^{bc} \pm 0.33$	$13.67^{b} \pm 0.33$
	96 h	$11.67^{c} \pm 0.33$	$12.67^{b} \pm 0.67$
	144 h	$15.00^{b} \pm 0.58$	$15.67^{a} \pm 0.33$
	192 h	$12.67^{bc} \pm 0.67$	$13.67^{b} \pm 0.33$

Values are expressed as mean (mm) ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

The leaf extracts form the samples harvested under 600ppm & 40/30 ˚C all exhibited an intermediate inhibitory activity against *S. aureus*, with zone of inhibition values ranging between 12 and 15 mm. Although the control had a larger zone of inhibition (19.33 ± 0.88 mm), *B. abyssinica* leaves maintained the same activity overall (Table 3.2). A similar trend was observed with the underground stems, with all samples, including the control (15.00 ± 1.73 mm) showing an intermediate activity. A stronger activity was reported at 144 hours (21.67 ± 0.33 mm) and 48 hours (19.67 ± 0.88 mm) from the root extracts. The samples harvested at 48 and 192 hours, as well as the control (12.67 ± 0.88 mm) had an intermediary effect against *S. aureus*.

The leaves showed susceptibility against *S. aureus* after 48 hours (20.33 ± 0.33 mm), while the remaining samples displayed an intermediate activity. The zones of inhibition from the underground stem extracts showed a consistent antibacterial activity, ranging between 12 and 17 mm. The roots also had an intermediate growth inhibition against *S. aureus*, as the zones of inhibition fell between 11 and 14 mm.

Second to the zone of inhibition of the control leaf extract, a slightly higher diameter was reported for the 96-hour harvested samples (18.33 ± 0.33 mm). Overall, the leaves retained a moderate activity under 800ppm &40/30 ˚C. Both underground stems (11-16 mm) and roots (12-16 mm) had similar antibacterial activity against *S. aureus*.

The control leaf extract yielded a greater zone of inhibition compared to all samples from this treatment, however, the growth inhibition was intermediate throughout. A susceptible activity was only reported at 192 hours for the underground stems (21.00 ± 1.73 mm) and roots (20.67 ± 1.20 mm) respectively. Only the leaves showed overall significant difference in each of the

treatments ($p < 0.05$), whereas the underground stems and roots were insignificant under 600ppm & 45/35 ˚C for both plant parts and 800ppm & 40/30 ˚C for the roots ($p > 0.05$).

Table 3.2 Growth inhibitory activity of *B. abyssinica* against *S. aureus* under concurrent elevated CO_2 and temperatures.

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		$19.33^{a} \pm 0.88$	$15.00^{a} \pm 1.73$	$12.67^{c} \pm 0.88$
600ppm & 40/30 ˚C	48 h	$14.33^{b} \pm 0.33$	$15.00^{a} \pm 1.15$	$19.67^{ab} \pm 0.88$
	96 h	$12.33^{b} \pm 0.88$	$13.33^{a} \pm 0.67$	$16.67^{b} \pm 1.20$
	144 h	$13.67^{b} \pm 0.33$	$12.67^{a} \pm 0.88$	$21.67^{a} \pm 0.33$
	192 h	$15.00^{b} \pm 1.00$	$13.00^{a} \pm 2.08$	$15.33^{bc} \pm 1.45$
Control		$19.33^{ab} \pm 0.88$	$15.00^{a} \pm 1.73$	$12.67^{a} \pm 0.88$
600ppm & 45/35 ˚C	48 h	$20.33^{a} \pm 0.33$	$17.33^{a} \pm 0.88$	$12.67^{a} \pm 0.33$
	96 h	$13.33^{c} \pm 1.20$	$16.67^{a} \pm 1.20$	$11.00^{a} \pm 1.00$
	144 h	$13,33^{c} \pm 1.33$	$13.00^{a} \pm 1.00$	$13.33^{a} \pm 0.88$
	192 h	$15.00^{bc} \pm 1.15$	$12.00^{b} \pm 1.15$	$14.00^{a} \pm 1.73$
Control		$19.33^{a} \pm 0.88$	$15.00^{a} \pm 1.73$	$12.67^{a} \pm 0.88$
800ppm & 40/30 ˚C	48 h	$15.67^{a} \pm 0.88$	$14.00^{a} \pm 1.00$	$16.33^{a} \pm 1.76$
	96 h	$18.33^{a} \pm 0.33$	$16.33^{a} \pm 0.33$	$13.33^{a} \pm 0.33$
	144 h	$12.67^{b} \pm 1.45$	$14.33^{a} \pm 0.67$	$12.00^{a} \pm 0.58$
	192 h	$15.33^{ab} \pm 1.33$	$11.33^{b} \pm 0.33$	$15.00^{a} \pm 1.53$
Control		$19.33^{a} \pm 0.88$	$15.00^{a} \pm 1.73$	$12.67^{b} \pm 0.88$
800ppm & 45/35 ˚C	48 h	$12.67^{b} \pm 0.67$	$12.00^{b} \pm 1.53$	$12.67^{b} \pm 0.88$
	96 h	$16.00^{a} \pm 1.73$	$14.33^{ab} \pm 1.45$	$17.33^{ab} \pm 0.33$
	144 h	$12.67^{b} \pm 1.67$	$13.33^{b} \pm 0.88$	$13.00^{b} \pm 1.53$

	192 h	$14.67^{ab} \pm 1.45$	$21.00^{a} \pm 1.73$	$20.67^{a} \pm 1.20$

Values are expressed as mean (mg/ml) ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

The harvested leaves from this treatment all displayed an intermediate antibacterial activity against *S. aureus*, with a range between 13 and 17 mm (Table 3.3). Surprisingly, the control sample did not show any inhibitory activity, indicating bacterial resistance. The underground stems also had the same activity as the leaves, but with slightly lower zones of inhibition (10-13 mm) under 600ppm & 40/30 ˚C, even with the control having a larger zone of inhibition (15.33 ± 0.67 mm; Table 3.3). The roots also had the same intermediate activity as the leaves and underground stems. Only the roots had insignificant under 600ppm & 40/30 ˚C against the control ($p > 0.05$).

All of the harvested plant samples under 600ppm & 45/35 ˚C showed a good antibacterial activity against *S. aureus* (Table 3.3). The leaves had stronger activity under treatment than the control, while the underground stem and root extracts had slightly larger zones of inhibition under treatment compared to the control ($p < 0.05$; ANOVA). A similar pattern as the previous two treatments was observed in 800ppm & 40/30 ˚C, where all plant parts showed a moderate growth inhibitory effect against *S. aureus* (Table 3.3). The leaves and underground stems both displayed significance ($p < 0.05$), while the harvested roots were not significant to the control ($p > 0.05$).

The leaves and underground stems had small variations in the zones of inhibition. Though the leaves harvested under 800ppm & 45/35 ˚C had shown an increase in activity compared to the control, the underground stems displayed significantly smaller diameters than the control ($p < 0.05$). The root extracts from samples harvested at 96 (18.00 ± 0.58 mm) and 192 hours (17.67

± 0.33 mm; $p < 0.05$) had greater zones of inhibition than the control. Overall, a good antibacterial activity was reported from the plant extracts (Table 3.3).

Table 3.3: Growth inhibitory activity of *B. natalensis* against *S. aureus* under concurrent elevated CO_2 and temperatures.

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		N/A	$15.33^{a} \pm 0.67$	$15.67^{a} \pm 0.33$
600ppm & 40/30 ˚C	48 h	$13.00^{b} \pm 0.58$	$12.33^{b} \pm 0.33$	$14.33^{a} \pm 0.33$
	96 h	$13.33^{b} \pm 0.67$	$11.33^{b} \pm 0.88$	$13.00^{a} \pm 0.58$
	144 h	$17.33^{a} \pm 0.88$	$10.33^{b} \pm 0.33$	$11.00^{a} \pm 0.58$
	192 h	$15.33^{ab} \pm 0.33$	$12.67^{b} \pm 0.33$	$12.67^{a} \pm 1.20$
Control		N/A	$15.33^{a} \pm 0.67$	$15.67^{a} \pm 0.33$
600ppm & 45/35 ˚C	48 h	$15.67^{a} \pm 0.67$	$12.33^{bc} \pm 0.33$	$15.00^{a} \pm 0.58$
	96 h	$17.33^{a} \pm 0.88$	$10.67^{c} \pm 0.67$	$14.33^{a} \pm 0.33$
	144 h	$12.33^{b} \pm 0.33$	$11.67^{bc} \pm 0.33$	$12.67^{a} \pm 0.33$
	192 h	$16.33^{a} \pm 0.88$	$13.33^{ab} \pm 0.33$	$14.67^{a} \pm 0.67$
Control		N/A	$15.33^{a} \pm 0.67$	$15.67^{a} \pm 0.33$
800ppm & 40/30 ˚C	48 h	$12.00^{a} \pm 0.58$	$12.00^{b} \pm 0.58$	$13.00^{a} \pm 1.00$
	96 h	$11.33^{a} \pm 0.33$	$13.67^{a} \pm 0.67$	$11.67^{a} \pm 1.67$
	144 h	$10.67^{a} \pm 0.33$	$15.00^{a} \pm 0.58$	$13.33^{a} \pm 1.20$
	192 h	$10.67^{a} \pm 0.67$	$13.67^{a} \pm 0.67$	$12.00^{a} \pm 0.58$
Control		N/A	$15.33^{a} \pm 0.67$	$15.67^{b} \pm 0.33$
800ppm & 45/35 ˚C	48 h	$11.33^{a} \pm 0.67$	$10.67^{c} \pm 0.33$	$13.33^{b} \pm 0.33$
	96 h	$11.33^{a} \pm 0.88$	$13.33^{ab} \pm 0.88$	$18.00^{a} \pm 0.58$
	144 h	$11.33^{a} \pm 0.33$	$11.67^{bc} \pm 0.33$	$12.33^{b} \pm 0.33$
	192 h	$12.67^{a} \pm 0.88$	$11.33^{bc} \pm 0.33$	$17.67^{a} \pm 0.33$

Values are expressed as mean (mg/ml) ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

3.3.2.2 E. coli

The leaves were initially resistant to *E. coli* after the first harvest period, with a zone of inhibition of 6.33 ± 3.18 mm, before showing a good activity between 96 and 192 hours (Table 3.4). An intermediate activity was reported for the roots, with zone of inhibition values ranging between 10 and 14 mm. The control leaf (15.67 ± 0.67 mm) and root (13.33 ± 0.33 mm) extracts also had similar activity as the samples under 600ppm & 40/30 ˚C.

The highest zone of inhibition from the leaf samples exposed to 600ppm & 45/35 ˚C was recorded at 144 hours (16.67 ± 0.33 mm). However, the growth inhibitory action of all samples was found to be intermediate. In the roots, a good antibacterial activity was reported at 48, 144, and 192 hours. At 96 hours, the roots showed sensitivity against *E. coli* (22.33 ± 2.03 mm).

The antibacterial activity of the leaves increased at 96 hours (20.33 ± 0.33 mm) displaying sensitivity against *E. coli*. The zones of inhibition of the root extracts from samples harvested under 800ppm & 40/30 ˚C gradually decreased, with susceptibility reported at 48 (20.00 ± 1.53 mm) and 96 hours (19.67 ± 0.88 mm) respectively, followed by an intermediate activity at 144 and 192 hours.

Most of the leaf extracts from samples exposed to 800ppm & 45/35 ˚C had a good inhibitory activity against *E.coli*, apart from the 96 hour harvested sample which showed sensitivity (23.00 ± 1.53 mm). All of the root extracts had an intermediate activity against the bacterial strain. In each of the treatments of combined elevated CO_2 and temperatures, both leaves and roots of *B. frutescens* showed an overall significant difference ($p < 0.05$).

Table 3.4: Growth inhibitory activity of *B. frutescens* against *E. coli* under concurrent elevated CO_2 and temperatures

Treatment	Harvest period	Leaves Zone of inhibition (mm)	Roots Zone of inhibition (mm)
Control		$15.67^{a} \pm 0.67$	$13.33^{a} \pm 0.33$
600ppm & 40/30 ˚C	48 h	$6.33^{b} \pm 3.18$	$10.67^{b} \pm 0.33$
	96 h	$14.33^{a} \pm 0.33$	$12.67^{a} \pm 0.33$
	144 h	$15.67^{a} \pm 0.33$	$12.33^{ab} \pm 0.67$
	192 h	$16.00^{a} \pm 0.58$	$13.67^{a} \pm 0.33$
Control		$15.67^{a} \pm 0.67$	$13.33^{b} \pm 0.33$
600ppm & 45/35 ˚C	48 h	$10.33^{c} \pm 0.33$	$13.67^{b} \pm 0.67$
	96 h	$12.33^{b} \pm 0.33$	$22.33^{a} \pm 2.03$
	144 h	$16.67^{a} \pm 0.33$	$16.33^{b} \pm 0.33$
	192 h	$13.33^{b} \pm 0.33$	$16.67^{b} \pm 1.20$
Control		$15.67^{b} \pm 0.67$	$13.33^{b} \pm 0.33$
800ppm & 40/30 ˚C	48 h	$16.67^{b} \pm 0.33$	$20.00^{a} \pm 1.53$
	96 h	$20.33^{a} \pm 0.33$	$19.67^{a} \pm 0.88$
	144 h	$12.33^{c} \pm 0.33$	$17.67^{ac} \pm 0.33$
	192 h	$17.33^{b} \pm 0.88$	$14.33^{bc} \pm 0.33$
Control		$15.67^{a} \pm 0.67$	$13.33^{ac} \pm 0.33$
800ppm & 45/35 ˚C	48 h	$13.67^{a} \pm 0.33$	$14.67^{a} \pm 1.20$
	96 h	$23.00^{a} \pm 1.53$	$14.33^{ac} \pm 0.33$
	144 h	$13.67^{a} \pm 0.67$	$15.33^{a} \pm 0.88$
	192 h	$10.67^{b} \pm 0.33$	$11.33^{bc} \pm 0.33$

Values are expressed as mean (mm) ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

B. abyssinica leaves had a consistent antibacterial activity against *E. coli* in both the control (13.67 ± 0.88 mm) and under 600ppm & 40/30 ˚C, with all extracts exhibiting a moderate effect (Table 3.5). The underground stems showed stronger inhibition of *E. coli* growth after 48 hours (20.33 ± 0.88 mm), and a good activity between 96 and 192 hours, including the control (14.67 ± 0.88 mm). All of the root extracts from samples harvested under elevated conditions as well as the control (13.67 ± 1.45 mm) showed a moderate antibacterial activity against *E. coli*.

The zone of inhibition from the leaf extracts showed a continual increase in diameter from 48 to 96 hours, shifting from a moderate inhibitory activity to susceptibility at 144 hours (22.00 ± 1.73 mm). A consistent antibacterial activity was noted from the underground stems harvested under 600ppm & 45/35 ˚C, sharing an intermediate effect with the control. Similarly, the roots did not deviate too far with regards to the zone of inhibition values, which ranged between 13 and 15 mm.

Interestingly, the leaves were resistant to *E. coli* at 48 hours (6.33 ± 3.18 mm). The activity strengthened from 96 hours until susceptibility was reported at 144 hours (21.00 ± 1.53 mm). Both underground plant parts had shown similar antibacterial activity against *E. coli*, as only a moderate activity was noted.

Each of the harvested plant parts of *B. abyssinica* exposed to 800ppm & 45/35 ˚C exhibited intermediate growth inhibition against *E. coli*. The leaves had zones of inhibition between 10 and 13 mm, the underground stems with a slightly different range between 11-14 mm and the greatest range was reported from the roots (14-18 mm). Both the leaves and roots were only

significantly different ($p < 0.05$) under 600ppm & 40/30 ˚C and 800ppm & 45/35 ˚C, whereas the underground stems showed no significance in each treatment ($p > 0.05$).

Table 3.5: Growth inhibitory activity of *B. abyssinica* against *E. coli* under concurrent elevated CO_2 and temperatures.

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		$13.67^{a} \pm 0.88$	$14.67^{a} \pm 0.88$	$13.67^{a} \pm 0.33$
600ppm & 40/30 ˚C	48 h	$11.00^{a} \pm 1.15$	$20.33^{a} \pm 0.88$	$13.00^{a} \pm 1.53$
	96 h	$13.33^{a} \pm 0.67$	$17.33^{a} \pm 0.33$	$10.00^{a} \pm 1.73$
	144 h	$13.33^{a} \pm 0.88$	$16.00^{a} \pm 1.73$	$15.67^{a} \pm 1.45$
	192 h	$10.67^{a} \pm 0.67$	$16.67^{a} \pm 1.67$	$14.33^{a} \pm 1.33$
Control		$13.67^{b} \pm 0.88$	$14.67^{a} \pm 0.88$	$13.67^{a} \pm 0.33$
600ppm & 45/35 ˚C	48 h	$12.67^{b} \pm 1.20$	$16.00^{a} \pm 1.53$	$15.00^{b} \pm 1.00$
	96 h	$14.33^{b} \pm 0.33$	$15.33^{a} \pm 0.67$	$14.00^{a} \pm 1.73$
	144 h	$22.00^{a} \pm 1.73$	$13.67^{a} \pm 1.76$	$14.67^{a} \pm 2.03$
	192 h	$16.33^{b} \pm 0.33$	$13.67^{a} \pm 1.67$	$13.33^{a} \pm 1.86$
Control		$13.67^{ab} \pm 0.88$	$14.67^{a} \pm 0.88$	$13.67^{b} \pm 0.33$
800ppm & 40/30 ˚C	48 h	$6.33^{b} \pm 3.18$	$14.33^{a} \pm 2.19$	$17.33^{a} \pm 0.88$
	96 h	$16.33^{a} \pm 1.20$	$14.33^{a} \pm 1.33$	$14.00^{a} \pm 1.15$
	144 h	$21.00^{a} \pm 1.53$	$13.00^{a} \pm 1.15$	$16.33^{a} \pm 0.33$
	192 h	$11.67^{b} \pm 0.33$	$13.67^{a} \pm 0.33$	$14.00^{a} \pm 0.58$
Control		$13.67^{a} \pm 0.88$	$14.67^{a} \pm 0.88$	$13.67^{a} \pm 0.33$
800ppm & 45/35 ˚C	48 h	$10.67^{a} \pm 1.20$	$13.67^{a} \pm 0.88$	$15.33^{a} \pm 1.86$
	96 h	$13.33^{a} \pm 1.20$	$11.67^{a} \pm 0.88$	$18.33^{a} \pm 0.67$
	144 h	$10.67^{a} \pm 0.33$	$12.33^{a} \pm 0.33$	$15.67^{a} \pm 1.20$

	192 h	$11.00^{a} \pm 1.00$	$14.33^{a} \pm 1.76$	$14.33^{a} \pm 1.33$

Values are expressed as mean (mg/ml) ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

The control leaf extract had a greater zone of inhibition value against *E. coli* (18.33 ± 0.88 mm), and all harvested samples under 600ppm & 40/30 ˚C had zones of inhibition between 11 and 12 mm, also showing significance against the control ($p < 0.05$; Table 3.6). The underground stems and roots also had an intermediate activity in both control (15.33 ± 0.88 mm- underground stems; 13.33 ± 0.88 mm- roots) and 600ppm & 40/30 ˚C samples ($p < 0.05$; Table 3.6).

An interesting pattern was noted in the antibacterial activity of the leaves under 600ppm & 45/35 ˚C against *E. coli* (Table 3.6). The activity greatly decreased between the control and 48 hours (6.00 ± 3.00 mm; $p < 0.05$) indicating bacterial resistance. The leaves then increased in their activity, from intermediate at 96 hours to susceptible at 144 hours (20.33 ± 1.20 mm; $p < 0.05$). Slight variations in the zones of inhibition of the underground stem and root extracts were reported, but both plant parts maintained a good antibacterial activity from all harvest periods under elevated CO_2 and high temperatures ($p < 0.05$).

Each plant part showed a consistent zone of inhibition between 48 and 192 hours under 800ppm & 40/30 ˚C, showing overall significance against *E. coli* ($p < 0.05$; Table 3.6). The leaves had a range between 12 and 16 mm, the underground stems, being the most consistent, around 16-17 mm, and the roots, between 15-16 mm.

The leaves, underground stems, and roots all displayed an intermediate antibacterial activity against *E. coli* under 800ppm & 45/35 ˚C ($p < 0.05$). The control leaf extract was found to be stronger in activity, as the zone of inhibition was higher than all the extracts from the elevated

conditions. The underground stems and roots had greater zones of inhibition at 96 hours (16.67 ± 0.67 mm) and 48 hours (16.33 ± 0.33 mm) compared to their respective control (Table 3.6).

Table 3.6: Growth inhibitory activity of *B. natalensis* against *E. coli* under concurrent elevated CO_2 and temperatures.

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		$18.33^{a} \pm 0.88$	$15.33^{a} \pm 0.88$	$13.33^{ac} \pm 0.88$
600ppm & 40/30 ˚C	48 h	$11.67^{b} \pm 0.67$	$14.00^{a} \pm 0.58$	$12.33^{a} \pm 0.33$
	96 h	$11.00^{b} \pm 0.58$	$13.33^{a} \pm 0.33$	$10.33^{b} \pm 0.33$
	144 h	$11.67^{b} \pm 0.67$	$12.33^{a} \pm 0.88$	$11.33^{bc} \pm 0.33$
	192 h	$11.00^{b} \pm 0.58$	$15.33^{a} \pm 0.33$	$15.67^{a} \pm 0.33$
Control		$18.33^{ab} \pm 0.88$	$15.33^{ab} \pm 0.88$	$13.33^{a} \pm 0.88$
600ppm & 45/35 ˚C	48 h	$6.00^{c} \pm 3.00$	$13.33^{b} \pm 0.33$	$12.33^{a} \pm 0.67$
	96 h	$16.33^{ab} \pm 0.33$	$14.33^{ab} \pm 1.20$	$15.33^{a} \pm 0.88$
	144 h	$20.33^{a} \pm 1.20$	$15.33^{ab} \pm 0.33$	$13.00^{a} \pm 0.58$
	192 h	$11.33^{bc} \pm 0.33$	$17.00^{a} \pm 0.58$	$13.33^{a} \pm 0.33$
Control		$18.33^{a} \pm 0.88$	$15.33^{ac} \pm 0.88$	$13.33^{a} \pm 0.88$
800ppm & 40/30 ˚C	48 h	$14.67^{bc} \pm 0.67$	$16.33^{bc} \pm 0.33$	$16.33^{a} \pm 0.33$
	96 h	$16.33^{ab} \pm 0.33$	$16.67^{a} \pm 0.33$	$15.33^{a} \pm 0.67$
	144 h	$15.33^{ac} \pm 0.88$	$16.33^{b} \pm 0.33$	$15.67^{a} \pm 1.20$
	192 h	$12.67^{c} \pm 0.88$	$16.67^{bc} \pm 0.88$	$15.67^{a} \pm 0.67$
Control		$18.33^{a} \pm 0.88$	$15.33^{ac} \pm 0.88$	$13.33^{ac} \pm 0.88$
800ppm & 45/35 ˚C	48 h	$14.00^{ab} \pm 0.58$	$13.33^{bc} \pm 0.33$	$16.33^{a} \pm 0.33$
	96 h	$15.00^{ab} \pm 1.00$	$16.67^{a} \pm 0.67$	$15.33^{a} \pm 0.67$
	144 h	$12.67^{b} \pm 0.67$	$12.33^{b} \pm 0.33$	$15.00^{ac} \pm 1.15$

	192 h	$14.00^{ab} \pm 1.53$	$13.67^{bc} \pm 0.33$	$12.33^{bc} \pm 0.88$

Values are expressed as mean (mg/ml) ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

3.4. Discussion

Herbal medicine usually consists of dried, and in some instances, freshly harvested plant material which are often prescribed by traditional healers in the form of a tincture, to treat illness and disease (Wendakoon *et al.,* 2012). The one down-fall, however, is that medicinal plants are subject to environmental factors, which influence their metabolic processes, and could further affect their bioactivity and beneficial properties (Heydari *et al.,* 2018). The current study reported on the effect of concurrent elevated CO_2 and temperatures on the antimicrobial activity of *B. frutescens*, *B. natalensis,* and *B. abyssinica*. This is the first study on the species where climate aspects of temperatures and CO_2 were considered. *E. faecalis* was the most susceptible pathogen against all tested *Bulbine* species under elevated CO_2 and temperatures. *Enterococci* are classified as Gram-positive bacteria which are commonly found in the gastrointestinal tract (Fisher and Phillips, 2009; Hashem *et al.,* 2021). Due to its opportunistic nature, *E. faecalis* is the most common pathogen of the *Enterococci* to induce various infections, which further increases the risk for antibiotic resistance (Jett et al., 1994; Ruoff *et al.,* 1990; Hashem *et al.,* 2021). Some of the infections associated with *E. faecalis* include endocarditis, bacteremia, urinary tract infections, wound-infections and meningitis (Anderson *et al.,* 2016; Agudelo and Huycke, 2014; Noskin *et al.,* 1995; Rosen *et al.,* 2018).

Compared to *B. frutescens* and *B. natalensis*, *B. abyssinica* displayed a better antibacterial activity overall, with MIC values less than or equal to 1 mg/ml against *B. cereus*, *K. pneumoniae* and *P. aeruginosa*. The leaves and roots, as well as the leaves and underground

stems possessed stronger antimicrobial activity under 600ppm & 45/35 ˚C and 800ppm & 45/35 ˚C respectively. Kibiti and Afolayan (2015) reported that acetone extracts of whole plant *B. abyssinica* showed MIC values of 0.31 mg/ml against *S. aureus*, *P. aeruginosa* and *E. faecalis*. Although this result is comparatively better than most of the findings from the current research, one has to consider the influence of using a singular plant part, as opposed to the whole plant for decoctions. This further emphasises the importance of analysing individual plant organs to accurately document plant medicinal activities. The underground plant organs of *B. frutescens* and *B. natalensis* showed a strong antimicrobial activity under elevated CO_2 and temperatures, compared to the leaves as well as their respective controls. Additionally, the antimicrobial activities of these plant parts were stronger under 800ppm & 45/35 ˚C. Elevated temperatures can affect plant physiology and biochemistry via re-modification of their processes (Heydari *et al.,* 2018). The production of phytocompounds is both plant species and organ specific (Li *et al.,* 2020). Underground plant organs are known to accumulate secondary metabolites, which is to be expected since they are storage organs as well (Li *et al.,* 2020). However, when exposed to changing environmental conditions, this biosynthesis or accumulation could fluctuate depending on the intensity of the abiotic factor and its duration. Numerous reports about the impact of CO_2 and temperatures on the phytochemical content of several medicinal plant species have been published. Anthocyanin concentrations, as well as those of individual phenolic acids and flavonoids showed increases under elevated CO_2 (620 ppm) from basil and peppermint (Al Jaouni *et al*., 2018). Three cultivars of alfalfa (*Medicago sativa*) had displayed an increase in the total phenolic and flavonoid contents under 620 ± 42 µmol CO_2 mol-1 air ppm, compared to 400 ± 27 µmol CO_2 mol-1 air ppm (Almuhayawi *et al*., 2021).The concentrations of total phenolic, flavonoid and saponin decreased when peppermint (*Mentha piperita*) and Madagascar periwinkle (*Catharanthus roseus*) were exposed to combined drought and heat (35 ˚C) for up to two weeks (Alhaithloul *et al*., 2021).

Contrastingly, terpenoid, tannin and alkaloid content increased during the same exposure period. Mashigo *et al* (2023) had reported that the presence of tannins in methanol extracts of *Carpobrotus edulis* leaves was lower under high temperatures (45/35 ˚C) when compared to low temperature exposure (15/10 ˚C) and the control (25/15 ˚C). Antimicrobial activity is one of the few pharmacological properties that has had very limited reports from the climate change perspective. Heydari *et al* (2018) investigated the effects of heat stress on the antimicrobial activity of essential oil extracts of *Mentha* x *piperita* var. Mitcham and *Mentha arvensis*. The MIC of the essential oils from *M. piperita* samples increased at each treatment of elevated temperatures (Treatment 1 = 14-32 ˚C; Treatment 2 = 26-40 ˚C; Treatment 3 = 27-45 ˚C) against *Streptococcus epidermidis*, *S. aureus* and *B. cereus* respectively. The lowest MIC against *Bacillus subtilis* and *B. cereus* were reported from *M. arvensis* essential oil extract under 27-45 ˚C (0.1 and 2 mg/ml) respectively. In another experiment by Netshiluvhi and Eloff (2019), the antimicrobial activity of *Leonotis dysophylla*, *Tulbaghia violaceae* and *B. frutescens* were determined from 15 and 30 ˚C treatments. The MIC values from *L. dysophylla* had slightly increased at 30 ˚C against *S. aureus*, *C. albicans*, *E. coli*, *C. neoformans* and *Aspergillus fumigatus*. *T. violaceae* showed no change in the MIC against *E. coli* and *P. aeruginosa*, however, the concentration decreased against *C. neoformans* and *A. fumigatus*. *B. frutescens* showed a small increase in the MIC when tested against *S. aureus (0.52* ± 0.20 mg/ml to 0.62 ± 0.20 mg.ml), *E. faecalis* (0.42 ± 0.10 mg/ml to 0.52 mg/ml) and *A. fumigatus* (0.63 ± 0.10 mg/ml to 1.04 ± 0.10 mg/ml) between 15 and 30 ˚C respectively. Their MIC against *E. coli*, *P. aeruginosa* and *C. neoformans*, however, remained the same in both treatments. A study on the effects of elevated CO_2 on the antimicrobial activity of *Portulacaria afra* showed minimal change in the MIC values between 420 and 600 ppm over a three-month period (Basson *et al.,* 2024). Under 420 ppm, the MIC values for *S. aureus* and *P. aeruginosa* remained unchanged after three months of exposure (4000 µg/ml). *Staphylococcus*

epidermidis, *Klebsiella aerogenes* and *C. albicans* however, retained the same inhibitory concentration across both treatments (4000, 2000, and 8000 µg/ml respectively). Since both atmospheric CO_2 and temperatures exist in unison, the current study had combined these environmental factors. Studies where both parameters were applied separately builds up information on how each factor influences the antimicrobial activity of medicinal plants. Furthermore, it offers some explanation as to which parameter could be the strongest influence on the antimicrobial potential of the plant species in the current investigation. This is in consideration, that they are exposed to a variation of conditions simultaneously at any given period.

3.5. Conclusion

The effects of elevated CO_2 and temperatures on the antimicrobial activity of *B. frutescens*, *B. natalensis,* and *B. abyssinica* showed varying results. The findings showed that all species had a greater improvement in their antibacterial activity as compared to their antifungal activity under elevated conditions. Furthermore, the underground plant organs often had better antimicrobial potential under treatments of higher temperatures (600 ppm and 800 ppm 45/35 ˚C) in comparison to the leaves and their respective control samples. This provides insight into the physiological and biochemical responses of different plant species from abiotic factors, while showing how the species could be affected by environmental conditions based on individual plant organ assessment. Since this research puts focus on predicted temperature changes in the South African context, accompanied by the frequency of heatwaves, it will bring into perspective the potential impacts of the bioactive properties, not just on the study species, but of medicinal plants in the country. This further sheds light on optimal harvest periods where

antimicrobial activity is more improved, and also highlights the importance of sustainable cultivation and mitigation strategies for the preservation of the species.

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CHAPTER FOUR

The influence of concurrent elevated CO_2 and temperatures on the phytochemical profile and in-vitro antioxidant activity of *Bulbine frutescens*, *Bulbine abyssinica*, and *Bulbine natalensis*

This chapter provides details on the investigation of the phytochemical profile and antioxidant activities of the leaves and roots of *B. frutescens*, *B. abyssinica*, and *B. natalensis*, as well as the underground stems and corms of *B. abyssinica* and *B. natalensis* respectively, under concurrent elevated CO_2 and temperatures.

The contents of the control data from each species have been published in several articles:

Biomedical and Pharmacology Journal (DOI: https://dx.doi.org/10.13005/bpj/2469)

Journal of Herbmed Pharmacology (DOI: 10.34172/jhp.2024.44650)

Indonesian Journal of Pharmacy (DOI: https://doi.org/10.22146/ijp.6025)

A manuscript outlining the findings from the experimental treatment conditions of concurrent elevated CO_2 and temperatures on the phytochemical profile and antioxidant activity in *B. natalensis* has been published by the Biomedical and Pharmacology Journal (DOI: https://dx.doi.org/10.13005/bpj/2975).

4.1 Introduction

Plants are heavily subjected to all kinds of environmental stress, which can impact their metabolism (Šamec *et al*., 2021). Fortunately, plants have mechanisms built into their physiology that allows them to function adequately under constantly changing climate. Secondary metabolite compounds are produced in response to abiotic stress, through primary metabolism, as a line of defence in plants (Mahajan *et al*., 2020). Changes in temperature, humidity, UV-radiation, drought, and atmospheric gas fluctuations are some of the stressors that impact phytochemical synthesis (Kolbet *et al*., 2001; Agati *et al*., 2009; Shomali *et al*., 2022). Phytocompounds are said to be structurally and chemically diverse than primary metabolic compounds (Naikoo *et al*., 2019). Some of the major compounds produced by plants are polyphenols, which include phenolics, tannins, stilbenes, lignans, and flavonoids. These are synthesised in higher concentrations in unfavourable climatic conditions (Selmar *et al*., 2008; Naikoo *et al*., 2019). Additionally these compounds form part of the non-enzymatic antioxidant system, which help to scavenge reactive oxygen species (ROS) in order to regulate plant homeostasis (Chowdhary *et al*., 2021). In the case of abiotic stress, there is a high accumulation of ROS which leads to oxidative stress in plants. This further results in the alteration of processes such as photosynthesis, causing the initiation of programmed cell death among other issues (Demmig-Adams and Adams, 2002; Al-Gubory *et al.*, 2017). Medicinal plants are a good source of secondary metabolites, which contribute to their health benefits (Pandey *et al*., 2009; Sati *et al*., 2013). They are especially known to curb the oxidation from metabolic processes in the human body which can cause an array of illnesses that are carcinogenic, cardiovascular, and neurodegenerative in nature (Selby-Pham *et al*., 2017). Factors such as rising atmospheric CO_2 and temperatures seem to have an effect on the biochemical activity, and might even present changes in the therapeutic properties of medicinal plants. There have been several reports showing an increase in phytochemical content and antioxidant activity of

some medicinal plants (Li *et al*., 2017; Al Jaouni *et al*., 2018; Alia *et al*., 2019; Basson *et al*., 2024). The increase in temperatures, however, does show contrasting results (Alhaithloul *et al*., 2019; Rysiak *et al*., 2021; Mashigo *et al*., 2023). Both of these abiotic factors can occur simultaneously in the natural environment, so it is important to not just assess the impact of each stressor individually, but to report on how the combination of these factors manipulate the physiological responses and the implications on the bioactivity of medicinal plants. The current study looked at the effects of concurrent elevated CO_2 and temperatures on the leaves and underground organs of *B. frutescens*, *B. abyssinica*, and *B. natalensis* over an eight-day period, on their phytochemical content and antioxidant activity.

4.2 Materials and methods

4.2.1 Preparation of methanol extracts

Powdered samples of *B. frutescens, B. abyssinica, and B. natalensis* plant parts (3 g) were mixed with 25 ml of 80% methanol. The extracts were placed in a sonicating water bath mixed with 20 ml of 80% acetone, at 50 ˚C for 25 minutes. Thereafter, the extracts were centrifuged for 10 minutes at 3500 rotations per minute (rpm) in order to separate the solid material from the liquid extract (Siddhuraju & Becker, 2003; Pakade et al., 2013).

4.2.2 Ultraviolet (UV) spectrophotometric phytochemical analysis

Total phenolic content (TPC)

A Folin-Ciocalteu solution (750 µL- 1:10) mixed with a volume of 2.5 ml saturated sodium carbonate (Na_2CO_3) and 0.3 ml of the plant extracts were prepared in volumetric flasks, followed by 7 ml of distilled water to dilute the mixtures (Pakade *et al*., 2013). All vials were

incubated in a dark cabinet for a period of two hours at ambient temperature, and the absorbance of the mixtures were recorded at a wavelength of 765 nm, following incubation. A gallic acid calibration curve equation ($y = 0.0495 – 0.0259$, $r^2 = 0.9994$; Appendix A) was used to determine the total phenolic content of all extracts.

Total flavonoid content (TFC)

Methanol extracts (300 µL) of *B. natalensis* were added into 10 ml volumetric flasks, followed by 4 ml of distilled water and 300 µL of sodium nitrate ($NaNO_3$) solution. The mixtures in the flasks were left to settle for five minutes before aluminium chloride solution (3 ml) was added, followed by another six minute waiting period before adding 1 M of sodium chloride (NaOH-2 ml). All mixtures were then made up to 10 ml with distilled water (Siddhuraju & Becker, 2003; Pakade *et al*., 2013). Each flask was vortexed for 15 minutes before recording the absorbance at a wavelength of 510 nm (Iqbal & Bhanger, 2006). The total flavonoid content of the extracts were then quantified using the quercetin calibration curve equation, $y = 0.2388 – 0.0019$, $r^2 = 0.9997$ (Appendix B).

Total tannin content (TTC)

Lahare *et al* (2021) developed a method to determine the total tannin content of the MeOH extracts, which was adopted for this study. *B. natalensis* extracts (100 µL) were mixed with 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent in glass vials. A solution of 35% Na_2CO_3 was then added into each vial before filling them up to 10 ml with distilled water. The mixtures were vortexed and incubated for 30 minutes at ambient temperature, and then the absorbance of the extracts were recorded at a wavelength of 725 nm. The gallic acid calibration

curve equation (y = 0.046x – 0.0264, r^2 = 0.9833; Appendix C) was used to quantify the total tannin content of the extracts.

Total proanthocyanidin content (TPAC)

Oyedemi (2010) reported the method for quantifying the total proanthocyanidin content, which was followed in this study. Methanol extracts (500 µL) mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml of HCl were added into vials, and then vortexed before incubating for 15 minutes at ambient temperature. A wavelength of 500 nm was used to record the absorbance of the extracts, and the standard catechin calibration curve equation (y = 0.9554x + 0.0003, r^2 = 0.9927; Appendix D) was used to calculate the total proanthocyanidin content.

4.2.3 Qualitative phytochemical analysis

Preliminary screening of phytochemical compound groups were investigated using several test methods (Prabhavathi et al., 2016; Roghini & Vijayalakshmi, 2018; Ayoade et al., 2019; Tepal, 2016; Yadav & Agarwala, 2011; Gul et al., 2017). The phytocompounds of interest for this study were phenolics, saponins, volatile oils, terpenoids, steroids, tannins, flavonoids, glycosides, coumarins and phlobatannins.

4.2.4 *In-vitro* Antioxidant activity (AA)

2, 2 diphenylpicrylhydrazyl (DPPH) scavenging activity assay

The method developed by Brand-Williams (1995) was performed for this study with slight alterations. A stock solution of DPPH was prepared from 50 mg of DPPH powder and 80% methanol (100 ml), from which a work solution (1:5) was made using the stock solution and

80% methanol. Reaction mixtures consisting of 10-50 µL of the methanol extracts were mixed with 0.7 ml of the work solution and filled up to 1 ml using 80% methanol. The solutions were incubated in a cabinet for 45 minutes to avoid any interference with light before measuring the absorbance at a wavelength of 517 nm. A blank solution (80% methanol) as well as a control solution (work solution and 80% methanol) were used, and a % inhibition was obtained using the equation: scavenging (%) = [(Absample – Abblank)/Abcontrol] × 100.

Hydrogen peroxide scavenging assay

The method described by Ruch (1989) was followed for the assessment of the hydrogen peroxide scavenging potential of selected *Bulbine* under elevated CO_2 and temperatures. A hydrogen peroxide solution (40 mM) containing phosphate buffer (pH 7.4) and 30% hydrogen peroxide was prepared. The extracts (10-50 µL) were mixed with 0.6 ml of hydrogen peroxide solution (40 mM), and then incubated for 10 minutes in a dark cabinet. The absorbance of the solutions were measured at a wavelength of 230 nm. Blank (phosphate buffer) and positive control (phosphate buffer and 40 mM hydrogen peroxide) solutions were used for the analysis of the scavenging activity using the equation % scavenged $H_2O_2 = [(A_c – A_s)/A_c] \times 100$, where A_c represents the positive control solution, and A_s represents the plant extract solution mixed with hydrogen peroxide.

Metal chelating assay

Few alterations were made to the method developed by Dinis (1994) for the investigation of the iron chelating activity of the selected *Bulbine* species. Solution mixtures were prepared using plant extracts (10-50 µL) and a solution of 2 mM ferric (II) chloride (50 µL). A volume of 0.2 ml of ferrozine (5 mM) was then added to initiate a reaction. The solution mixtures were vortexed and left to stand for 10 minutes at room temperature. A blank solution consisting of

80% methanol and a positive control containing ferric (II) chloride and ferrozine were used as part of the analysis. The absorbance of the solutions were measured at 562 nm, and the percentage (%) chelation was obtained using the equation: % chelation = $[(A_0 – A_1)/A_0] \times 100$, with A_0 being the absorbance value of the positive control solution and A_1 representing the extract samples.

4.2.5 Data analysis

Three replicates were performed for the quantitative phytochemical and antioxidant activity analyses, and all values were expressed as mean ± standard error (SE). A one-way analysis of variance (ANOVA) and Tukey HSD post-hoc tests were performed to determine the overall significance across all plant parts ($p < 0.05$) and where the difference lies amongst the plant parts using R Studio® statistical software. Microsoft Excel® was used for the graphical illustration of the qualitative and quantitative phytochemical profile.

4.3 Results

4.3.1 Quantitative phytochemical content

4.3.1.1 Bulbine frutescens

a) 600ppm & 40/30 ˚C

The total phenolic content of *B. frutescens* leaves and roots were reported from various treatments of combined elevated CO_2 and temperatures (Figure 4.1 A). Under 600ppm & 40/30 ˚C, both leaves and roots had the highest concentration of total phenolics at 144 hours (2293.4 ± 24.45 mg/100g GAE- leaves and 1401.11 ± 36.71 mg/100g GAE- roots). All of the harvest periods for both plant parts were significantly different to their respective controls ($p < 0.05$).

Figure 4 illustrates the TFC of *B. frutescens* under elevated CO_2 and high temperatures (Figure 4.1 B). The leaves were reported to have their highest TFC at 144 hours, whereas the roots peaked at 48 hours, both under 600ppm & 40/30 ˚C. The leaves had a higher concentration of total flavonoids (881.4 ± 22.16 mg/100g QE) compared to the roots (850.6 ± 60.38 mg/100g QE). All the values for both plant parts were reported to be higher than their respective controls (215.0 ± 12.9 mg/100g QE for leaves and 285.3 ± 60.62 mg/100g QE for roots). Both leaves and roots were significantly different when all values from samples harvested under elevated conditions were compared to their control ($p < 0.05$).

The concentration of total tannins in the leaves decreased at 48 hours in comparison to the control (1027.50 ± 15.61 mg/100g GAE), before reaching a peak of 6345.65 ± 187.16 mg/100g GAE at 96 hours (Figure 4.1 C). All TTC values between 96 and 192 hours were significantly higher than the control ($p < 0.05$). A continual increase in the TTC was reported in the roots from 48 to 96 hours, where the highest concentration was recorded in the latter harvest period (5171.01 ± 41.49 mg/100g GAE). The concentration then gradually decreased from 144 hours to 192 hours ($p < 0.05$). Although the leaves had higher TTC compared to the roots in three of the four harvest periods under 600ppm & 40/30 ˚C, both plant parts had the highest concentration of total tannins at 96 hours.

The leaves showed an increase in the concentration of total proanthocyanidins from the control (645.8 ± 2.68 mg/100g CE) to 48 hours under 600ppm & 40/30 ˚C (983.20 ± 3.49 mg/100g CE; Figure 4.1 D). The concentration plummeted five-fold from 96 hours to 144 hours before increasing at 192 hours. An overall significant difference was reported for the leaves ($p < 0.05$). Only one of the harvest periods recorded higher TPAC in the roots than the control (296.9 ± 4.73 mg/100g CE), and that was at 192 hours (389.9 ± 1.75 mg/100g CE). The lowest

concentration was reported after 48 hours, which was four times lower than the control. Each of the roots harvested under treatment were significantly different to the control ($p < 0.05$).

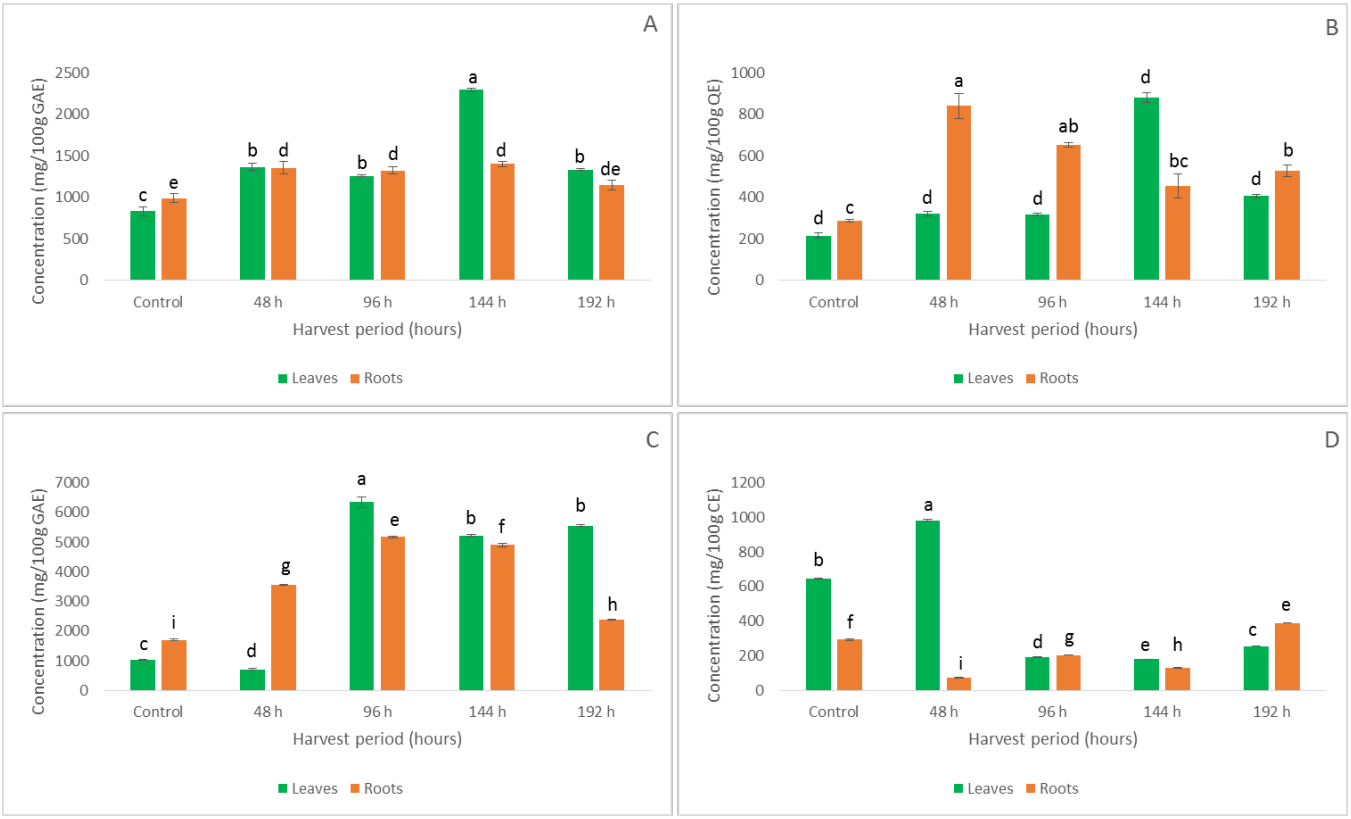

Figure 4.1: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. frutescens* leaves and roots under 600ppm & 40/30 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

b) 600ppm & 45/35 ˚C

The roots outperformed the leaves under 600ppm & 45/35 ˚C, with a TPC of 2606.1 ± 74.76 mg/100g GAE at 144 hours, compared to the leaves, which had a concentration of 1283.3 ± 215.68 mg/100g GAE at 192 hours. A significant difference was reported for the roots ($p < 0.05$), but not for the leaves, when compared to its control ($p > 0.05$; Figure 4.2 A).

The highest concentration of TF's were recorded at 144 hours for the leaves and 192 hours for the roots, under 600ppm & 45/35 ˚C (Figure 4.2 B). The TFC of the roots was slightly higher (364.7 ± 11.41 mg/100 QE) than the leaves (328.6 ± 1.11 mg/100g QE). Although a significant difference was reported for both plant parts against their control ($p < 0.05$; ANOVA), the Tukey HSD revealed that no significance was observed between the control and the leaves harvested at 144 hours.

Similar to the previous treatment of 600ppm & 40/30 ˚C, the TTC in the leaves was higher at 96 hours (3805.07 ± 3.62 mg/100g GAE; Figure 5). The concentration then decreased from 144 hours to 192 hours. All of the leaf extracts harvested under this treatment had at least 50% or higher TTC than the control ($p < 0.05$; Figure 4.2 C). The concentration of total tannins in the roots increased at 48 and 144 hours, where the content was at its highest (3469. 56 ± 8.30 mg/100g GAE). A slight decline was noted at 96 and 192 hours ($p < 0.05$). Both plant parts had a 50% split in respect to the TTC. The leaves had slightly higher concentration 96 and 144 hours, and the roots at 48 and 192 hours.

The two plant parts each had higher TPAC at two different harvest periods at 600ppm & 45/35 ˚C: the leaves at 48 and 144 hours, and the roots at 96 and 192 hours, respectively. The total proanthocyanidin concentration in the roots was significantly higher at 48 hours (319. 1 ± 0.46 mg/100g CE), followed by the control, 96 hours, 192 hours, and 144 hours ($p < 0.05$). The leaves had contrastingly greater TPAC than the roots between 96 hours and 192 hours ($p < 0.05$; Figure 4.2 D).

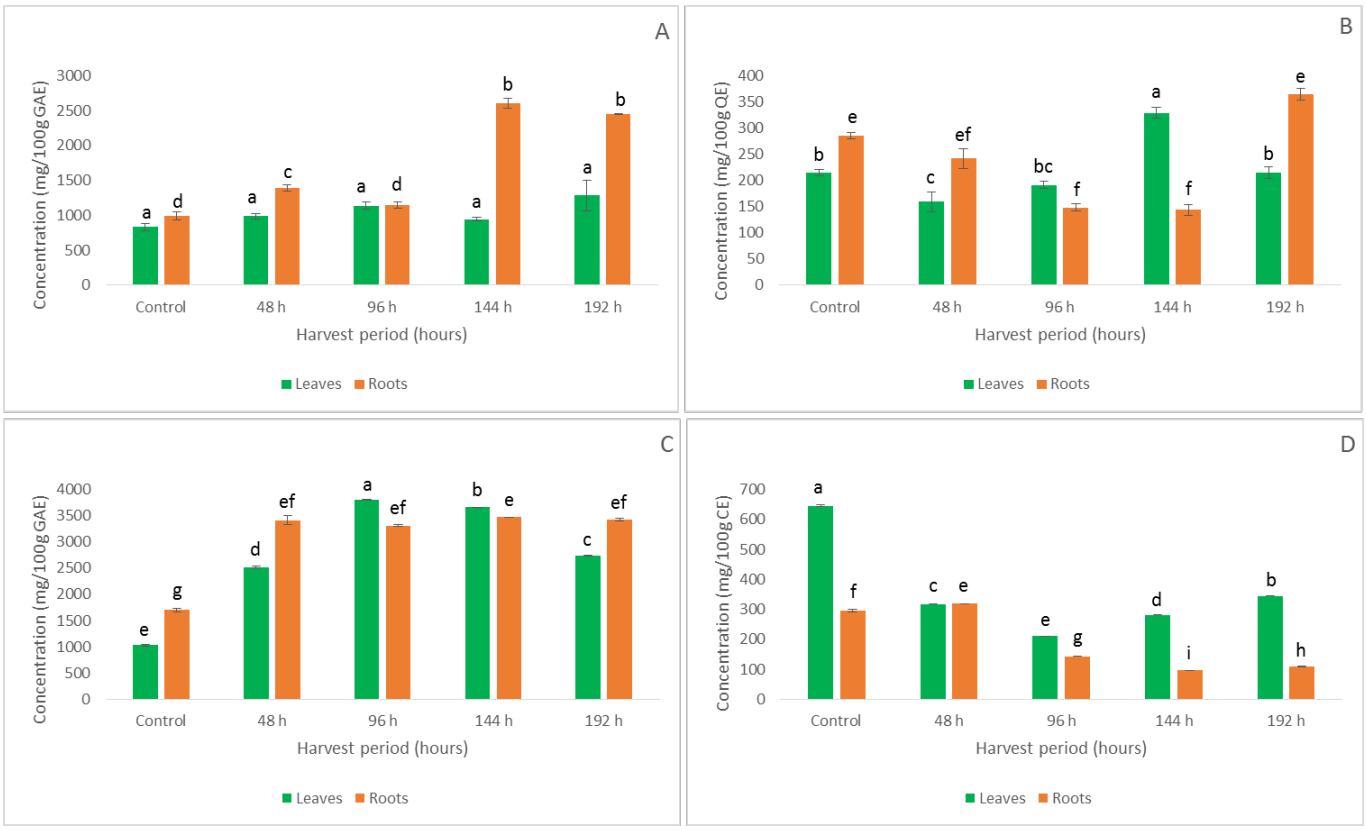

Figure 4.2: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. frutescens* leaves and roots under 600ppm & 45/35 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

c) 800ppm & 40/30 ˚C

Both plant parts recorded higher TPC's at 48 hours under 800ppm & 40/30 ˚C, with the roots once again yielding a higher concentration of 1536.1 ± 49.91 mg/100g GAE, compared to the leaves (1320.0 ± 34.16 mg/100g GAE; Figure 4.3 A). An overall significant difference was reported for both leaves and roots against their respective controls ($p < 0.05$; Tukey).

In the 800ppm & 40/30 ˚C treatment, the leaves had the overall highest TFC (569.4 ± 11.11 mg/100g QE; 192 hours) compared to the roots (298.3 ± 15.15.16 mg/100g QE; 144 hours;

Figure 4.3 B). Both plant parts were significantly different to their controls ($p < 0.05$; ANOVA).

Under 800ppm & 40/30 ˚C, the TTC in the leaves exceeded 5000 mg/100g GAE following 96 hour (5565.94 ± 3.63 mg/100g GAE) and 144 hour (5076.81 ± 46.26 mg/100g GAE) periods respectively. Overall, each harvest period displayed a higher TTC than the control ($p < 0.05$; Figure 4.3 C). The highest concentration of total tannins from the root extracts was recorded at 192 hours (5029.71 ± 25.36 mg/100g GAE), followed by 96 hours, 48 hours and 144 hours ($p < 0.05$).

There was a minute increase in TPAC at 48 hours (664.1 ± 1.76 mg/100g CE) in the leaves under 800ppm & 40/30 ˚C, from the control, which then decreased until 144 hours. All of the harvest periods showed significant difference against the control ($p < 0.05$; Figure 4.3 D). A concentration of 1031.7 ± 3.81 mg/100g CE was recorded after the 48-hour harvest of *B. frutescens* roots. This was followed by a great drop at 96 hours, over four times the difference from 48 hours. The TPAC continued to decrease until 192 hours. The roots were significantly different at 48, 144, and 192 hours ($p < 0.05$). Although the leaves had higher TPAC than the

roots between 96 and 192 hours, both plant parts exhibited higher concentrations of total proanthocyanidins at 48 hours (Figure 6).

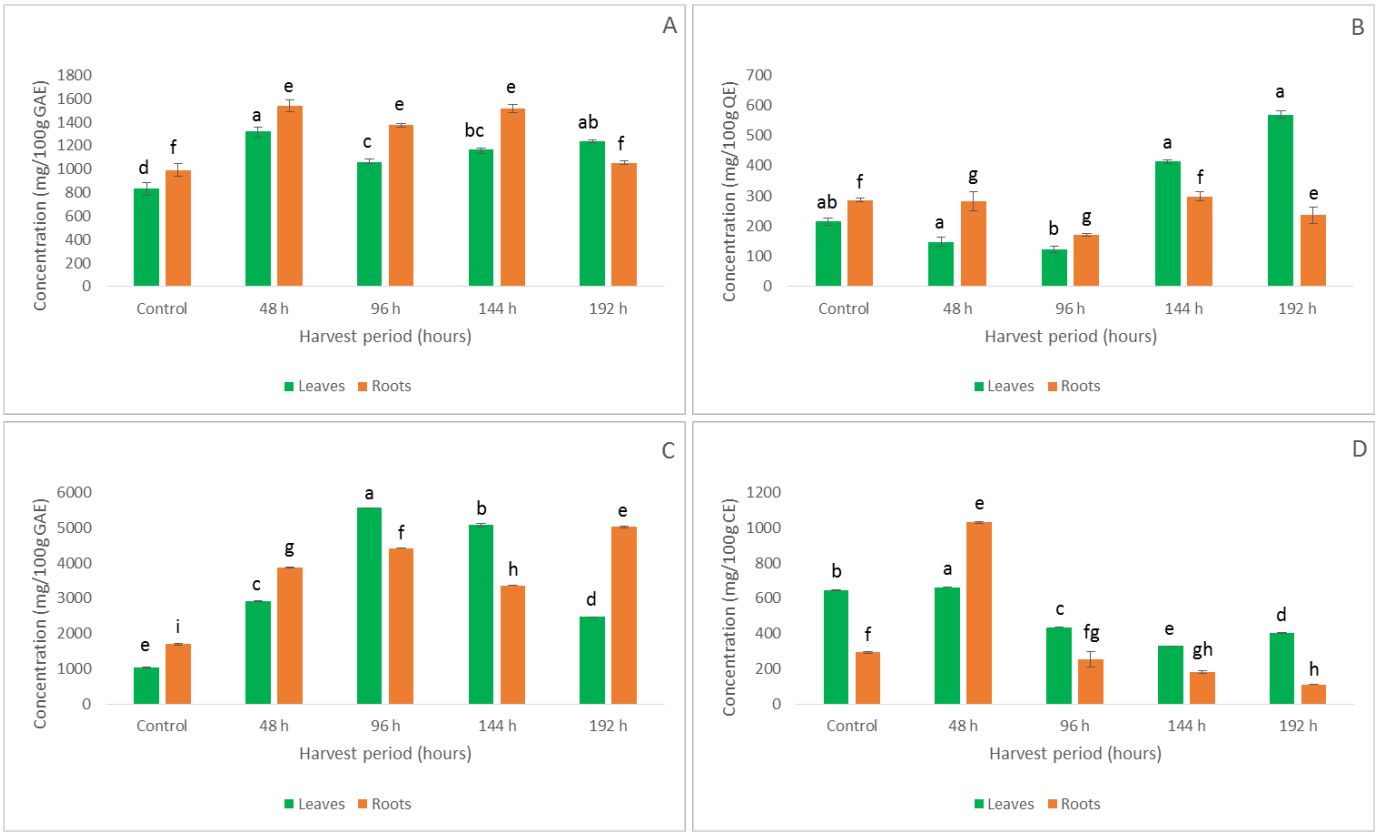

Figure 4.3: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. frutescens* leaves and roots under 800ppm & 40/30 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

d) 800ppm & 45/35 ˚C

Under 800ppm & 45/35 ˚C, the leaves and roots had reported their highest TPC at 144 hours (5040.0 ± 53.4 mg/100g GAE) and 96 hours (3483.1 ± 34.96 mg/100g GAE) respectively (Figure 4.4 A). When compared to their respective controls, each plant part, at their highest recorded concentrations, were significantly different ($p < 0.05$). Overall, all TPC values for leaves and roots from the elevated CO_2 and high temperature treatments had higher

concentrations compared to the control values, with the exception of the roots at 800ppm & 45/35 ˚C after 144 hours.

B. frutescens roots had a comparatively higher TFC of 661. 1 ± 53.65 mg/100g QE at 48 hours, than the leaves (388.1 ± 1.39 mg/100g QE; 96 hours) when exposed to 800ppm & 45/35 ˚C (Figure 4.4 B). Each plant part did show significance against their control ($p < 0.05$; ANOVA).

The concentration of total tannins in the leaves had tripled in contrast to the control, at 48 hours (3214.49 ± 13.06 mg/100g GAE). This concentration slightly changed between 96 and 144 hours before increasing at 192 hours (5312.32 ± 9.58 mg/100g GAE; $p < 0.05$). A consistent pattern in the TTC was reported in the roots between 48 and 144 hours. The TTC nearly tripled at 192 hours, with a recorded concentration of 6297.83 ± 28.76 mg/100g GAE (Figure 4.4 C). The leaves had a higher TTC in 75% of the harvest periods (48-144 hours). Both plant parts showed the highest concentration of total tannins at 192 hours, however, the roots were comparatively higher in content. *B. frutescens* control leaves and roots were significantly different to each of the samples harvested under 800ppm & 45/35 ˚C ($p < 0.05$).

The leaves that were harvested under 800ppm & 45/35 ˚C had significantly lower TPAC than the control. In this treatment, the greatest significance was reported at 192 hours (603.7 ± 0.97 mg/100g CE; $p < 0.05$) and the lowest was at 144 hours. An almost five-fold decrease in TPAC in the roots was noted between the control and 48 hours (60.54 ± 0.09 mg/100g CE; Figure 4.4 D). An inverse of the same effect was recorded at 96 hours, where the highest concentration in the treatment was reported (299.19 ± 46.69 mg/100g CE; $p < 0.05$), before a gradual decrease from 144 hours to 192 hours. The leaves contained a higher TPAC than the roots in all harvest periods under 800ppm & 45/35 ˚C.

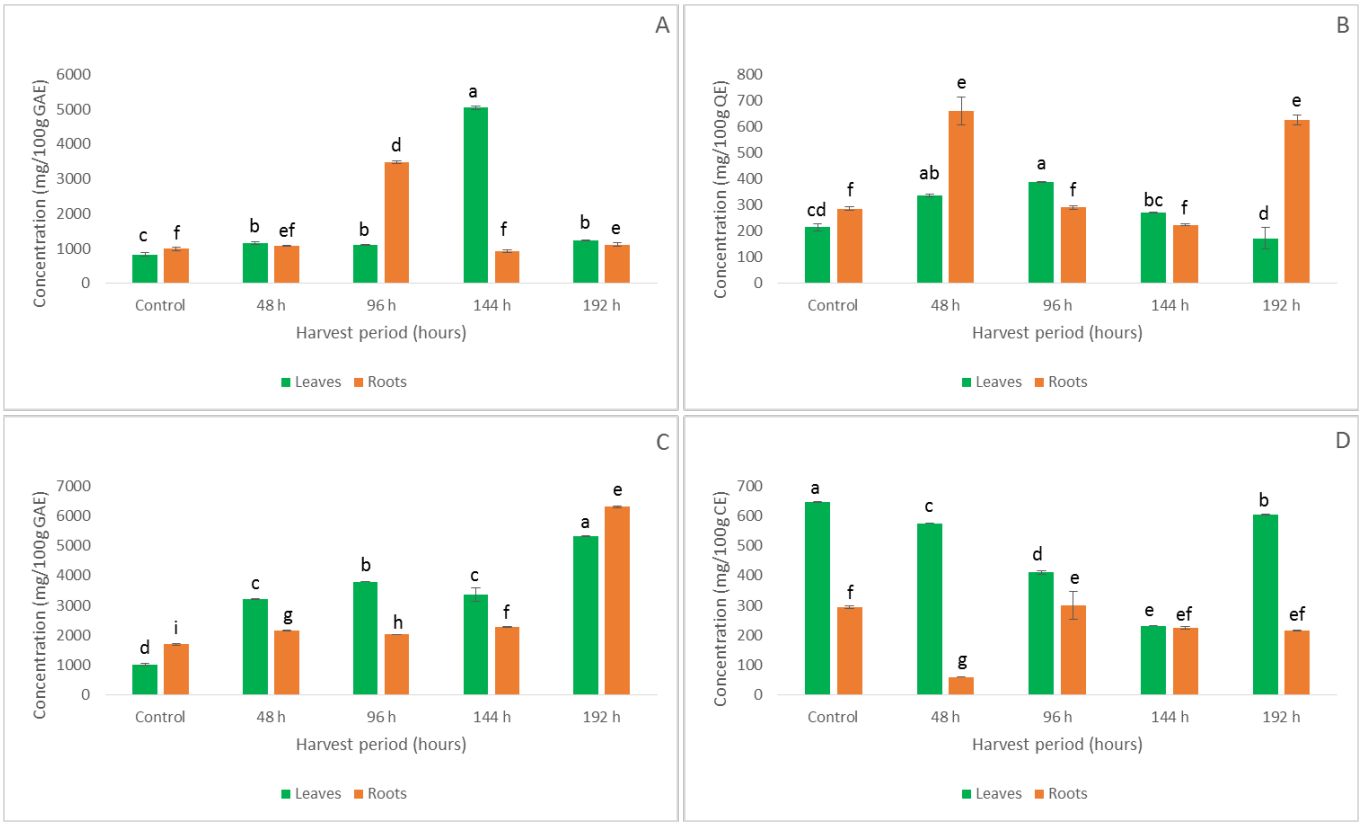

Figure 4.4: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. frutescens* leaves and roots under 800ppm & 45/35 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

4.3.1.2 Bulbine abyssinica

a) 600ppm & 40/30 ˚C

The TPC of the leaves under 600ppm & 40/30 ˚C decreased at 48 hours from the control (1841.7 ± 4.80 mg/100g GAE), following an increase at 96 hours, and then another decrease at 144 hours, before reaching its highest recorded concentration at 192 hours (2146.4 ± 102.72 mg/100g GAE; Figure 4.5 A). A one-way ANOVA showed a significant difference in this treatment against the control ($p < 0.05$; Tukey). The underground stems increased after 48 hours with a concentration of 2298.9 ± 58.73 mg/100g GAE, before decreasing at 96 hours. The TPC continued to increase from 144 hours to 192 hours. All the values from the treatment samples were higher than the control (1328.7 ± 7.20 mg/100g GAE). Significant difference was observed for the underground stems against the control ($p < 0.05$; Tukey; Figure 4.5 A). The concentration of total phenolics was higher at 96 hours (2102.8 ± 59.24 mg/100g GAE), in comparison with 144 hours, 192 hours, the control (1652.8 ± 19.20 mg/100g GAE) and 48 hours. A one-way ANOVA showed a significant difference reported for the roots ($p < 0.05$; Tukey). The underground stems had higher TPC at 48, 144 and 192 hours, compared to the leaves and roots.

A higher concentration of total flavonoids in *B. abyssinica* leaves was noted from the control sample (809.2 ± 75.6 mg/100g QE), followed by 144 hours, 96 hours, 192 hours and 48 hours. Each sample from the 600ppm & 40/30 ˚C treatment was significantly different to the control, as shown by the ANOVA ($p < 0.05$) and Tukey post-hoc tests (Figure 4.5 B). The TFC in the underground stems increased between the control (226.7 ± 0.58 mg/100g QE) and 48 hours (411.1 ± 40.91 mg/100g QE). A gradual decrease was observed at 96 and 144 hours, from which the highest TFC was recorded at 192 hours (494.9 ± 11.11 mg/100g QE). A significant difference was reported between the control and the treatment samples ($p < 0.05$; Tukey). At

144 hours, the roots had the highest concentration of total flavonoids (820.0 ± 54.37 mg/100g QE), more than double the concentration of the control (231.7 ± 33.2 mg/100g QE). This was followed by 192 hours, 96 hours, the control, and 48 hours. Only the control and the root extract from the sample harvested at 144 hours was significantly different based on Tukey's post-hoc test, however, the ANOVA revealed an overall significance between the control and the harvest periods under 600ppm & 40/30 ˚C ($p < 0.05$). The underground stems yielded greater TFC values at 48 hours and 192 hours, followed by the leaves (48 hours) and roots (144 hours; Figure 4.5 B).

An increase in the TTC in the leaves was reported at 48 hours under 600ppm & 40/30 ˚C, from the control, which then gradually declined until 144 hours. At 192 hours, the TTC increased to 6429.71 ± 32.66 mg/100g GAE, almost three times the concentration of the control (2850.00 ± 70.01 mg/100g GAE; $p < 0.05$; Figure 4.5 C). *B. abyssinica* underground stems displayed higher TTC at 48 hours (6786.59 ± 7.89 mg/100g GAE; $p < 0.05$). The concentration decreased from 96 hours to 192 hours, however, the content was reported to be nearly three times the value of the control (2678.33 ± 55.01 mg/100g GAE; $p < 0.05$; Figure 4.5 C). The highest concentration of total tannins in the roots was noted at 96 hours (6652.53 ± 1.81 mg/100g GAE), which was followed by 192 hours, 144 hours, 48 hours, and the control (2393.03 ± 55.97 mg/100g GAE; ANOVA; $p < 0.05$). Overall, the underground plant organs of *B. abyssinica* contained higher TTC than the leaves. The roots had more content between 96 and 192 hours, and the underground stems at 48 hours.

At 48 hours, a slight decrease in the TPAC was noted in comparison to the control (636.7 ± 1.67 mg/100g CE; $p < 0.05$). The concentration increased at 96 hours, where the highest content was recorded (709.6 ± 2.49 mg/100g CE). This was before a gradual decrease from 144 hours to 192 hours, of which both had lower TPAC than the control (Figure 4.5 D). The highest TPAC in the underground stems was reported at 144 hours (570.7 ± 0.76 mg/100g CE; $p <$

0.05). In descending order, followed the control (429.5 ± 0.76 mg/100g CE), 96 hours, 48 hours, and 192 hours (Figure 4.5 D). All of the harvested roots in this treatment had lower concentrations of total proanthocyanidins in contrast to the control (403.8 ± 2.03 mg/100g CE; $p < 0.05$). At 600ppm & 40/30 ˚C, the highest TPAC was reported at 96 hours (316.9 ± 0.32 mg/100g CE) and the lowest at 48 hours (172.1 ± 0.40 mg/100g CE). The leaves had greater concentrations of total proanthocyanidins in three harvest periods (48, 96 and 192 hours), and the underground stems at 144 hours (Figure 4.5 D).

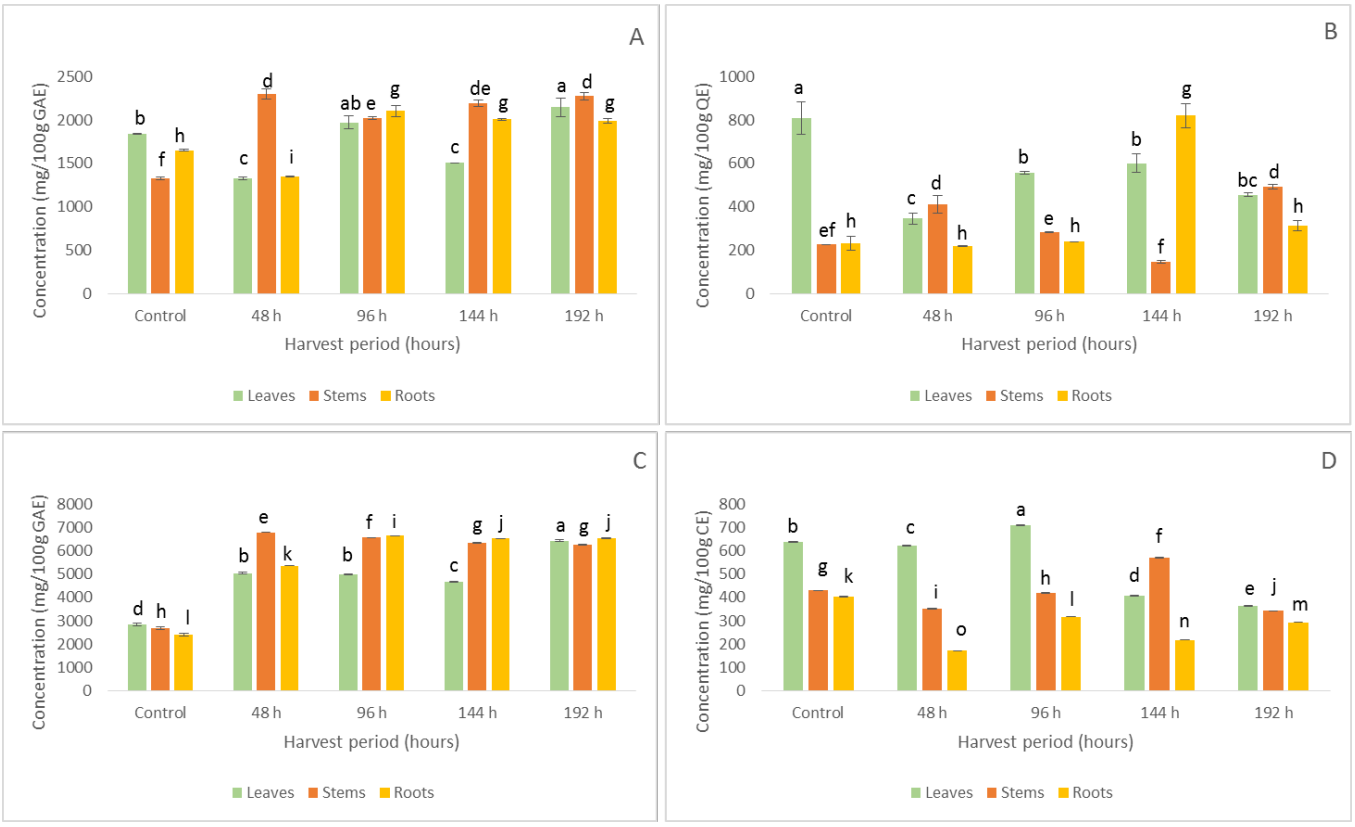

Figure 4.5: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. abyssinica* leaves, underground stems, and roots under 600ppm & 40/30 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

b) 600ppm & 45/35 ˚C

At 48 hours, the TPC in the leaves decreased to 1606.4 ± 24.29 mg/100g GAE from the control. The concentration increased to 2860.3 ± 84.60 mg/100g GAE, the highest recorded at 144 hours, followed by a decrease at 192 hours. The ANOVA showed a significant difference under 600ppm & 45/35 ˚C ($p < 0.05$; Figure 4.6 A). The concentration of total phenolics from the underground stems greatly increased from the control, to 48 hours (2405.8 ± 38.97 mg/100g GAE) and then gradually increased at 96 hours and 144 hours (3162.7 ± 18.67 mg/100g GAE), which then decreased at 192 hours. All harvest periods showed a significant difference against the control ($p < 0.05$; Tukey; Figure 4.6 A). Similar to the previous treatment, the underground stems harvested under 600ppm & 45/35 ˚C were higher in TPC than the control. The greatest significance in TPC in *B. abyssinica* roots was recorded at 192 hours (2461.9 ± 48.02 mg/100g GAE), followed by 96 hours, the control, 48 hours and 144 hours ($p < 0.05$; Tukey). The underground stems yielded higher concentrations of total phenolics between 48 hours and 144 hours, when compared against the leaves and roots. At 192 hours however, it was the roots that had the greatest TPC.

Under 600ppm & 45/35 ˚C, the leaves had a TFC of 624.2 ± 56.29 mg/100g QE at 144 hours, which was higher than the samples harvested at 96 hours, 48 hours, and 192 hours. The control still contained the highest TFC in the leaves, which was also significantly different to the samples under 600ppm & 45/35 ˚C ($p < 0.05$; Figure 4.6 B). A noticeable increase in the TFC was reported at 48 hours from the underground stems, however, the concentration decreased at 96 hours. The TFC reached was significantly higher at 144 hours (480.0 ± 13.24 mg/100g QE), which had a 70% decrease at 192 hours. All harvest periods were significantly different to the control ($p < 0.05$; Tukey; Figure 4.6 B). The overall highest concentration of total flavonoids was at 144 hours (347.2 ± 9.16 mg/100g QE), followed by 96 hours, the control, 192 hours, and 48 hours. The ANOVA showed an overall significance amongst all the values ($p < 0.05$).

The leaves had higher TFC in the last two harvest periods (144 and 192 hours), and the underground stems and roots traded off at 48 hours and 96 hours respectively.

There was an increase in TTC between 48 and 144 hours, with the latter having the greatest concentration under 600ppm & 45/35 ˚C (6770.29 ± 4.79 mg/100g GAE). The leaves exposed to these conditions had significantly higher TTC than the control ($p < 0.05$; Figure 4.6 C). The TTC in the underground stems slightly decreased from 48 hours to 96 hours, however, a gradual increase in the concentration was reported between 96 and 192 hours. A concentration of 7069.20 ± 45.07 mg/100g GAE was recorded from the underground stem samples harvested at 192 hours. All of the underground stems harvested at 600ppm & 45/35 ˚C contained at least double the total tannin content, and were significantly different to the control ($p < 0.05$; Figure 4.6 C). The roots showed a continual increase in the TTC from 48 hours to 192 hours (7228.4 ± 9.59 mg/100g GAE; $p < 0.05$). Both the underground stems and roots had higher TTC at 48 hours (underground stems), 96 hours (roots) and 192 hours (roots), compared to the leaves, which had a higher concentration at 144 hours.

There was a decrease in the TPAC between the control and 48 hours under 600ppm & 45/35 ˚C, followed by a steady increase up to 144 hours (1001.7 ± 4.23 mg/100g CE; $p < 0.05$; Figure 4.6 D). At 192 hours, the content in the leaves was reduced by nearly 50%. The TPAC was higher in comparison to the control at 96 and 144 hours ($p < 0.05$). In the underground stems, the concentration decreased from 48 hours (507.2 ± 1.11 mg/100g CE) to 192 hours (318.4 ± 0.76 mg/100g CE). The TPAC was comparatively higher between 48 and 144 hours than the control ($p < 0.05$; Figure 4.6 D). The concentration of total proanthocyanidins in the roots was reported to be three times higher at 192 hours (1343.83 ± 22.36 mg/100g CE; $p < 0.05$) than the control. The next highest content was recorded at 96 hours, then at 48 hours and 144 hours. The leaves and roots had high concentrations of total proanthocyanidins at 48 and 144 hours, and 96 and 192 hours, respectively (Figure 4.6 D).

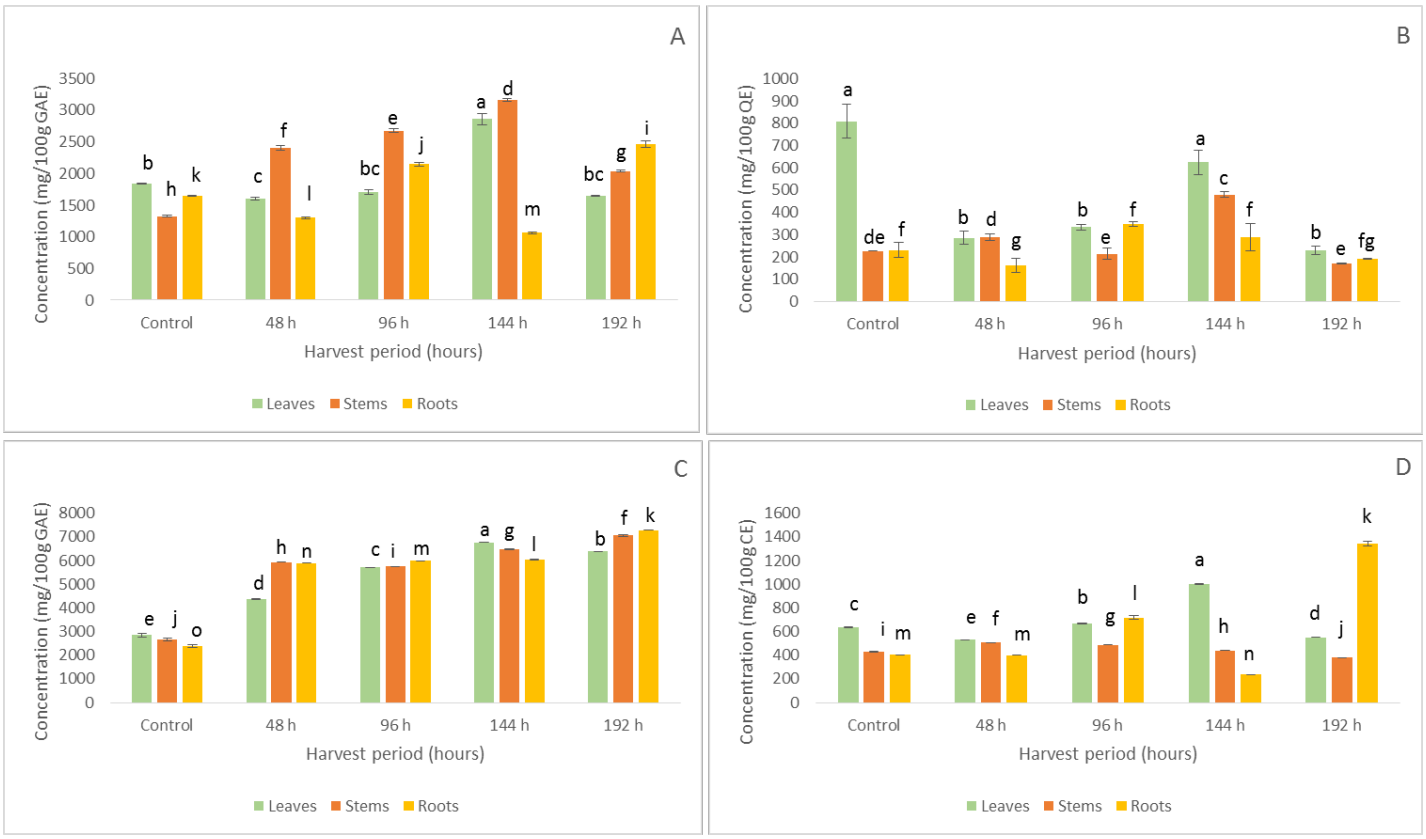

Figure 4.6: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. abyssinica* leaves, underground stems, and roots under 600ppm & 45/35 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

c) 800ppm 40/30 ˚C

A higher TPC in the leaves at 800ppm & 40/30 ˚C was reported at 144 hours (2388.9 ± 9.86 mg/100g GAE), and the lowest at 192 hours. There was a significant difference observed for all recorded values ($p < 0.05$; Figure 4.7 A). A nearly doubled concentration of total phenolics in the underground stems was recorded at 48 hours (2322.5 ± 9.66 mg/100g GAE) from the control, however, this concentration declined at 96 hours. The TPC reached its highest concentration at 144 hours (3241.7 ± 31.21 mg/100g GAE). All harvest periods were significantly different when compared to the control ($p < 0.05$; Tukey; Figure 4.7 A). Once again, the TPC of the underground stems under 800ppm & 40/30 ˚C were higher in contrast to

the control. Three harvest periods showed TPC values higher than 2000 mg/100g GAE, namely 48 hours (2506.4 ± 87.32 mg/100g GAE), 144 hours, and 192 hours. Similar to the underground stems in the same treatment, the concentrations from every harvest period of the roots was higher than and significant to the control ($p < 0.05$; Tukey). The underground stems showed a higher concentration of total phenolics from 96-192 hours, than the roots and leaves.

The TFC after 144 hours was greater in concentration (888.9 ± 110.79 mg/100g QE) than the control. TFC was comparatively lower at 96 hours, 48 hours, and 192 hours ($p < 0.05$; Figure 4.7 B). In the underground stems, the TFC decreased from the control to 48 hours, which then increased up until 144 hours (316.94 ± 13.95 mg/100g QE). The control sample did have a significant difference against each of the samples from 800ppm & 40/30 ˚C ($p < 0.05$; Tukey HSD), as well as an overall significance ($p < 0.05$; Figure 4.7 B). The harvested roots at 96 hours had most significant increase in the concentration of total flavonoids (696.4 ± 39.46 mg/100g QE), after having decreased slightly at 48 hours from the control. The TFC decreased between 96 and 144 hours and then increased again at 192 hours. Only three of the root samples from this treatment (96 hours, 144 hours, and 192 hours) were significantly different to the control ($p < 0.05$; Tukey HSD). However, there was a significant difference altogether within the treatment against the control ($p < 0.05$; ANOVA). *B. abyssinica* leaves contained greater TFC at 48 and 144 hours, while the roots had a higher content at 96 and 192 hours.

The highest concentration in the leaves was recorded following the 144-hour harvest period (5449.64 ± 32.05 mg/100g GAE). The rest of the samples under elevated conditions had values nearly double the concentration of the control ($p < 0.05$; Figure 4.7 C). A concentration of 6556.52 mg/100g GAE from the underground stems was recorded at 48 hours which slightly decreased at 96 hours. The TTC greatly decreased at 144 hours (2020.29 ± 1.81 mg/100g GAE) before tripling at 192 hours. The control was reported to be significantly different to each of the harvested samples ($p < 0.05$; Figure 4.7 C). The roots had the highest TTC at 48 hours

(8942.39 ± 390.89 mg/100g GAE), followed by 144 hours, 192 hours, 192 hours, and 96 hours, under 800ppm & 40/30 ˚C. The harvested roots possessed higher TTC under elevated CO_2 and high temperatures than the control ($p < 0.05$). *B. abyssinica* subterranean plant organs had higher TTC than the leaves. The roots had a higher content in all harvest periods, followed by the underground stems at 48, 96, and 192 hours.

The TPAC of the leaves was higher at 144 hours (523.7 ± 0.49 mg/100g CE) under 800ppm & 40/30 ˚C. All of the leaves harvested, however, were significantly lower in content than the control ($p < 0.05$; Figure 4.7 D). The TPAC in the underground stems harvested under these conditions were all higher than the control sample ($p < 0.05$), with the greatest concentration reported at 48 hours (794.9 ± 1.60 mg/100g CE) and the lowest at 96 hours (477.67 ± 13.09 mg/100g CE; Figure 4.7 D). The roots showed lower concentrations of total proanthocyanidins under elevated CO_2 and high compared to the control. The content showed no change between the control and 48 hours (403.3 ± 0.33 mg/100g CE), and lower concentrations were reported for the rest of the harvest periods ($p < 0.05$). The underground stems had higher TPAC than the leaves and roots at 48, 96, and 192 hours (Figure 4.7 D).

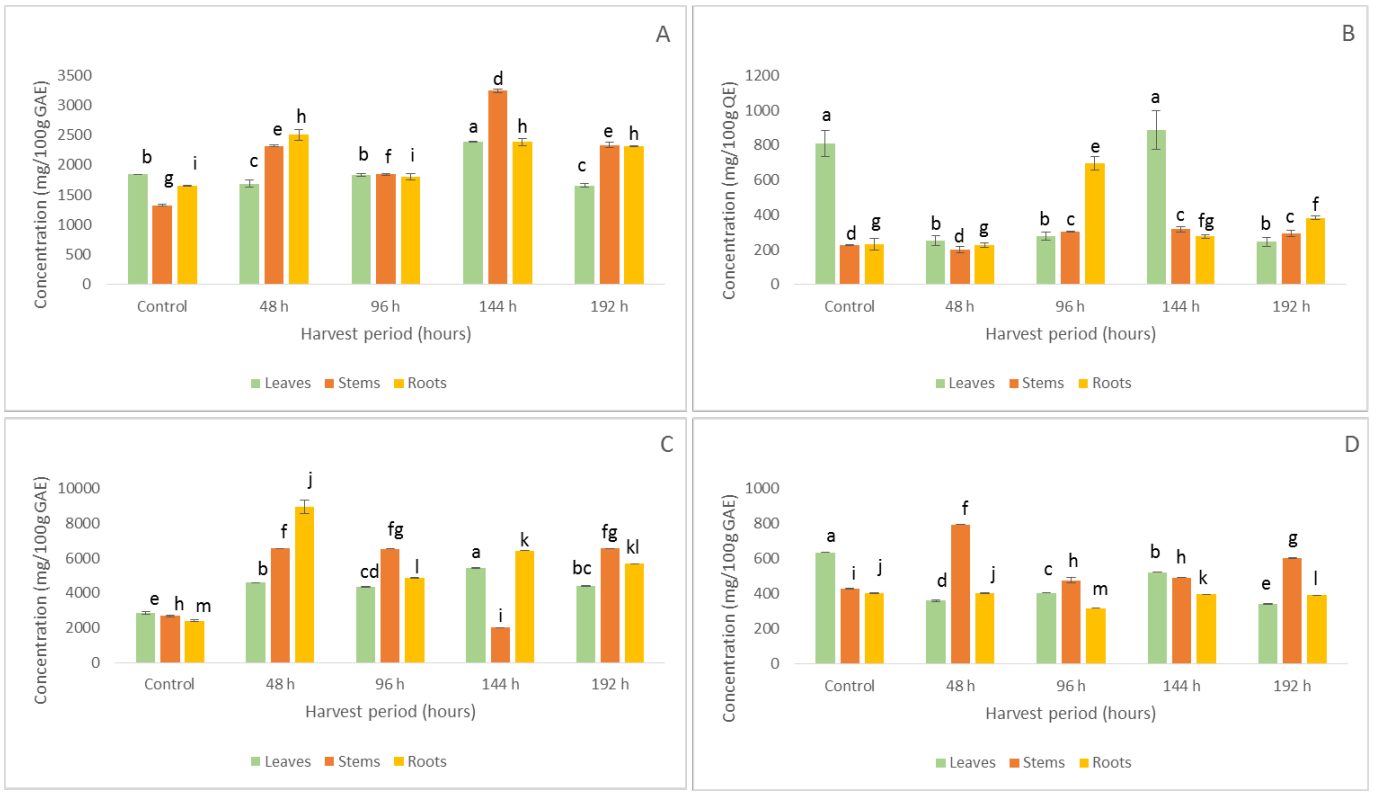

Figure 4.7: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. abyssinica* leaves, underground stems, and roots under 800ppm & 40/30 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

d) 800ppm & 45/35 ˚C

A great increase in TPC was recorded at 48 hours (2632.5 ± 14.43 mg/100g GAE) from which the concentration continued to increase, nearly doubling at 96 hours (3398.9 ± 34.96 mg/100g GAE). The TPC decreased at 144 hours, before increasing again at 192 hours. All the values showed a significant difference when compared to the control ($p < 0.05$; Tukey; Figure 4.8 A). The highest concentration of total phenolics in the underground stems was recorded after the 48-hour harvest period (2916.4 ± 70.44 mg/100g GAE). The concentration had decreased at 96 hours, and continued so at 144 hours. All TPC from the underground stems were significantly different to the control ($p < 0.05$; Tukey). A similar trend from the TPC results of the

underground stems of *B. abyssinica* was observed for the roots, where the concentration of total phenolics was higher at 48 hours (2960.8 ± 462.89 mg/100g GAE), which decreased at 96 and 144 hours. In contrast to the change in TPC from the underground stems at 192 hours, the concentration in the roots decreased from 144 hours. An overall significant difference was reported for the roots ($p < 0.05$; Tukey; Figure 4.8 A). Under 800ppm & 45/35 ˚C, all plant parts had higher concentrations of total phenolics compared to their respective controls. The underground stems recorded the greatest TPC at 48 hours, and the leaves outperformed both underground stems and roots between 96 and 192 hours.

The TFC in the leaves under 800ppm & 45/35 ˚C saw the greatest drop after 48 hours (176.1 ± 8.41 mg/100g QE), seeing an approximate quintuple difference compared to the control (Figure 4.8 B). The concentration almost tripled at 96 hours (508.06 ± 11.08 mg/100g QE) and then fluctuated between 144 hours (decrease) and 192 hours (increase). Both Tukey HSD and ANOVA tests showed a significant difference between the treatment samples and the control ($p < 0.05$). A noticeable increase in total flavonoids in the underground stems was reported at 48 hours from the control, which increased at 96 hours (452.8 ± 12.51 mg/100g QE). A steep decline followed from 144 hours to 192 hours (Figure 4.8 B). The roots contained the highest TFC at 192 hours with a concentration of 1001.1 ± 24.29 mg/100g QE, followed by 96 hours, 144 hours, 48 hours, and the control.

Under 800ppm & 45/35 ˚C, a slight decrease in the TTC was observed in the leaves between 48 hours (6712.32 ± 4.79 mg/100g GAE) and 144 hours. However, all harvest periods were significantly higher in TTC than the control ($p < 0.05$; Figure 4.8 C). The concentration of total tannins in the underground stems had nearly tripled at 48 hours from the control, however the greatest concentration was recorded at 144 hours (6753.98 ± 4.79 mg/100g GAE; $p < 0.05$).

The roots showed an interesting pattern in the TTC under 800ppm & 45/35 ˚C. The highest concentration was recorded at 48 hours (6199.64 ± 53.65 mg/100g GAE; $p < 0.05$) before greatly decreasing at 96 hours. This decline continued after the 144-hour harvest, where the lowest TTC was reported (1395.29 ± 11.02 mg/100g GAE; Figure 4.8 C). All three plant parts had somewhat consistent TTC at 48 hours, with the concentration being above 6000 mg/100g GAE. However, the leaves had comparatively greater content at 48 hours and 192 hours, followed by the underground stems, at 96 hours and 144 hours.

The leaves displayed higher concentrations of total proanthocyanidins between 48 and 144 hours (797.2 ± 0.99 mg/100g CE) at 800ppm & 45/35 ˚C, compared to the control ($p < 0.05$; Figure 4.8 D). The TPAC was higher following the 192-hour harvest of the underground stems (763.0 ± 6.59 mg/100g CE). At least two of the harvest periods (96 and 144 hours) had significantly higher content than the control ($p < 0.05$; Figure 4.8 D). A decrease in TPAC in the roots was recorded from 48 hours (486.3 ± 14.38 mg/100g CE; $p < 0.05$) to 144 hours, followed by an increase at 192 hours. The control sample was only higher in concentration than

the roots harvested at 144 hours ($p < 0.05$). The leaves (48 to 144 hours) and the underground stems (192 hours) had greater TPAC than the roots under 800ppm 45/35 ˚C (Figure 4.8 D).

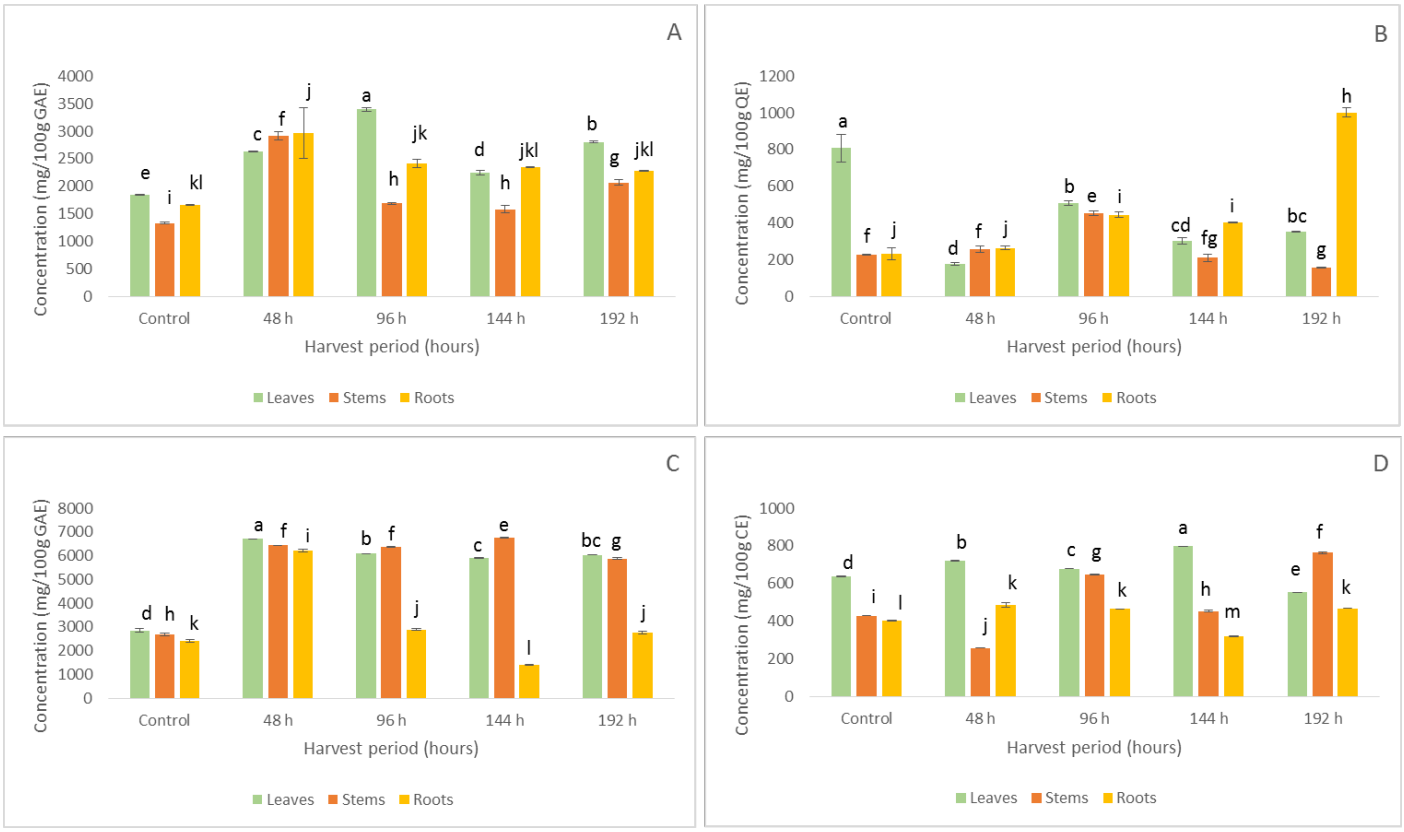

Figure 4.8: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. abyssinica* leaves, underground stems, and roots under 800ppm & 45/35 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

4.3.1.3 Bulbine natalensis

a) 600ppm & 40/30 ˚C

The TPC of *B. natalensis* leaves showed a progressive increase between the control (867.0 ± 64.50 mg/100g GAE) and 96 hours (1883.1 ± 102.43 mg/100g GAE). A decrease was noted at 144 hours, which then slightly increased at 192 hours. The control had a significantly lower ($p < 0.05$) TPC than the leaves harvested under 600ppm & 40/30 ˚C (Figure 4.9 A). The

concentration of total phenolics in the underground stems decreased from the control (1909.2 ± 4.80 mg/100g GAE) up to 144 hours ($p < 0.05$), followed by an increase, with the highest recorded concentration under 600ppm & 40/30 ˚C at 192 hours (1833.3 ± 35.16 mg/100g GAE). The highest TPC from the root samples under elevated conditions was reported at 48 hours (3718.9 ± 42.49 mg/100g GAE), which was almost quadruple the concentration of the control (1002.8 ± 24.7 mg/100g GAE). In decreasing order follows the 144-hour, 96-hour and 192-hour harvest periods, and lastly, the control ($p < 0.05$). The roots had greater TPC than the leaves and underground stems under 600ppm & 40/30 ˚C.

An increase in the TFC was noticed at 48 hours (358.9 ± 51.17 mg/100g QE) from the control (134.4 ± 21.40 mg/100g QE). The concentration slightly decreased at 96 hours, and then increased again at 144 hours, before doubling down at 192 hours (172.8 ± 15.17 mg/100g QE). An overall significant difference was noted for the leaves under 600ppm & 40/30 ˚C against the control ($p < 0.05$; Figure 4.9 B). The TFC in the underground stems at 192 hours was slightly higher (265.0 ± 3.33 mg/100g QE) in comparison to the control (259.7 ± 8.18 mg/100g QE). The three preceding harvest periods under 600ppm & 40/30 ˚C were lower in TFC by comparison. Only the stems harvested at 48 and 144 hours were significantly different to the control ($p < 0.05$). The concentration of total flavonoids in the roots increased between the control (238.3 ± 43.40 mg/100g QE) and 96 hours (897.5 ± 24.75 mg/100g QE; $p < 0.05$). At 144 hours, the TFC took a great fall (285.6 ± 5.37 mg/100g QE; $p < 0.05$) which then increased at 192 hours. The leaves and roots alternated in terms of the TFC, with the leaves having a greater content at 48 and 144 hours, and the roots at 96 and 192 hours respectively. They were also the only plant parts to contain higher TFC than their respective controls.

The highest concentration of total tannins in the leaves was recorded at 48 hours under 600ppm & 40/30 ˚C (3228.62 ± 1.81 mg/100g GAE; $p < 0.05$). This was followed by the control, with a slightly lower concentration (3008.3 ± 38.50 mg/100g GAE), 192 hours, 144 hours, and 96

hours (Figure 4.9 C). *B. natalensis* underground stems showed an increasing pattern in the TTC from the control (2890.0 ± 53.10 mg/100g GAE) to 192 hours (6958.69 ± 21.97 mg/100g GAE). Each of the samples harvested under elevated conditions were significantly different against the control ($p < 0.05$). Overall, the roots showed consistency in TTC, with slight increases and decreases under elevated CO_2 and high temperatures. The concentration was highest at 96 hours (5480.43 ± 6.27 mg/100g GAE; $p < 0.05$), followed by 48 hours, 192 hours, 144 hours, and the control (1645.0 ± 34.50 mg/100g GAE). The underground stems and roots were higher in TTC under elevated conditions compared to the leaves. Contrastingly, the leaves did have a greater content in the control.

The concentration of total proanthocyanidins in *B. natalensis* leaves had increased from 48 hours to 96 hours (294.9 ± 0.32 mg/100g CE), which then decreased in the last two harvest periods (Figure 4.9 D). The control (362. 3 ± 9.50 mg/100g CE) was significantly higher ($p < 0.05$) in TPAC than the leaves under 600ppm & 40/30 ˚C. A significantly higher concentration of total proanthocyanidins in the underground stems was reported at 192 hours (864.1 ± 34.27 mg/100g CE, $p < 0.05$) in comparison to the control (858.3 ± 1.70 mg/100g CE). The underground stems harvested between 48 and 144 hours were lower in TPAC than the control. The TPAC in the roots at 48 hours (462.2 ± 0.23 mg/100g CE) was the only harvest period that showed higher content than the control (373.3 ± 9.50 mg/100g CE) ($p < 0.05$). Overall, the results showed that the underground plant parts contained greater TPAC than the leaves.

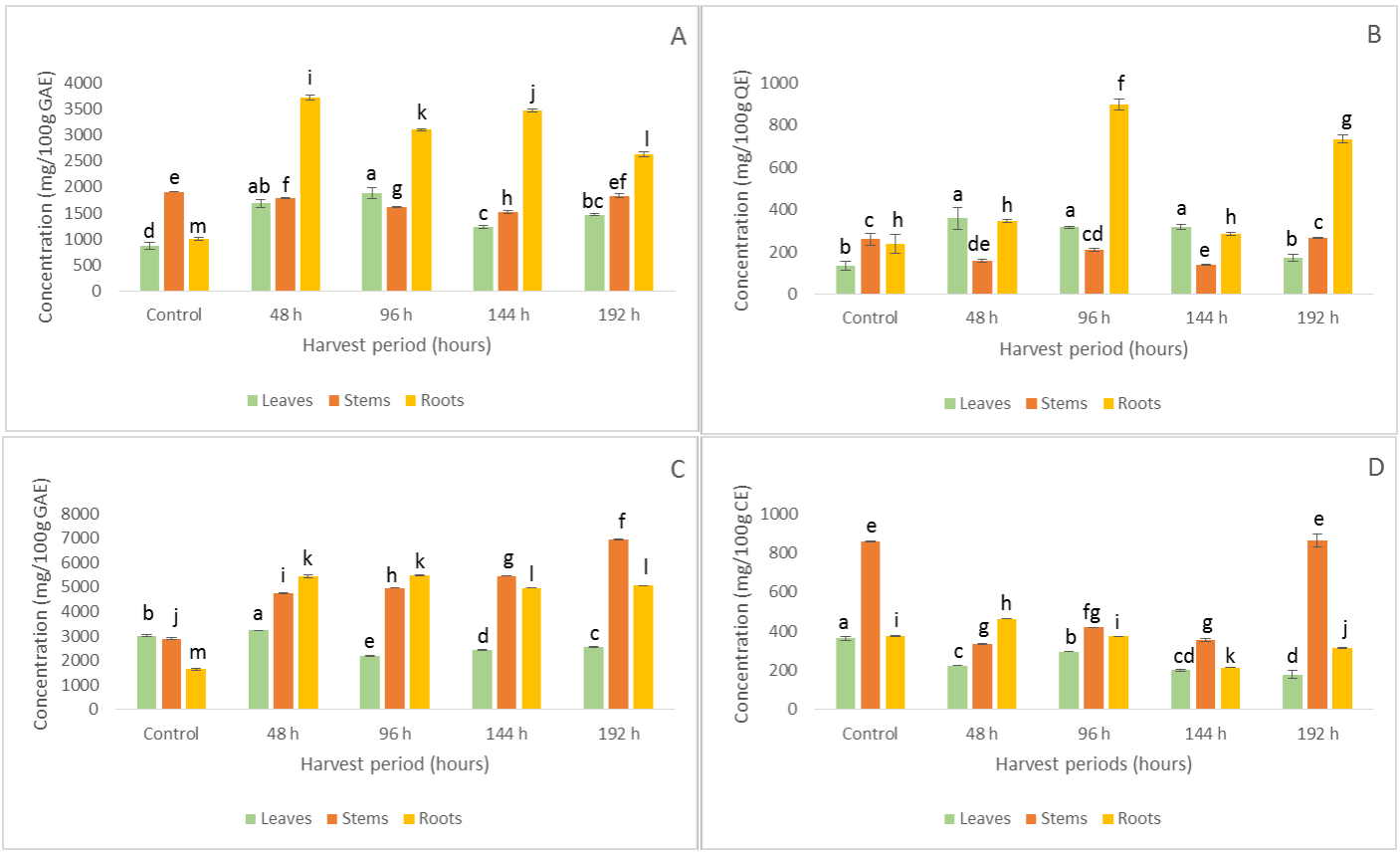

Figure 4.9: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. natalensis* leaves and roots under 600ppm & 40/30 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

b) 600ppm & 45/35 ˚C

The greatest significance was noted at 144 hours, with a TPC of 1960.8 ± 6.74 mg/100g GAE ($p < 0.05$) in the leaves harvested under 600ppm & 45/35 ˚C, which was followed by 192 hours, 48 hours, 96 hours, and the control (Figure 4.10 A). The TPC in the underground stems had doubled at 48 hours (3910.4 ± 6.73 mg/100g GAE) from the control. The highest content was recorded at 144 hours (4725.3 ± 23.33 mg/100g GAE; $p < 0.05$), following a decrease at 96 hours. The TPC then decreased from 144 hours to 192 hours. The same pattern was observed from the TPC in the leaves. The greatest decrease in TPC in the roots was reported at 96 hours

(1896.9 ± 11.66 mg/100g GAE), which was three times lower in concentration compared to 48 hours (3668.0 ± 26.94 mg/100g GAE). The TPC slightly decreased at 192 hours following a gradual increase between 96 and 144 hours. Overall, the control was significantly different to all samples harvested under elevated conditions ($p < 0.05$). All three plant parts had greater concentrations under 600ppm & 45/35 ˚C compared to their respective controls.

The leaves had a slight decrease in TFC at 48 hours (130.8 ± 5.83 mg/100g QE) from the control, before increasing at 96 hours (268.3 ± 23.12 mg/100g QE; $p < 0.05$; Figure 4.10 B). A moderate decrease was noted at 144 hours ($p < 0.05$), which was followed by another increase at 192 hours. A similar trend was observed in the underground stems, where a fluctuation in the TFC was reported. A drop in concentration from the control to 48 hours was noted ($p < 0.05$), which increased to its highest content at 96 hours (232.2 ± 1.11 mg/100g QE). The TFC decreased at 144 hours before increasing, however indifferently, at 192 hours. The greatest difference in the TFC of the roots was recorded at 144 hours, with a concentration of 423.9 ± 3.13 mg/100g QE, ($p < 0.05$) followed by the control, 192 hours, 48 hours, and 96 hours. Under 600ppm & 45/35 ˚C, the leaves showed comparatively higher TFC at 96 and 192 hours. The underground stems and roots each had higher concentrations at 48 and 144 hours respectively.

A steady increase was noted in the TTC in the leaves between 48 and 144 hours, with the latter having the greatest concentration (2808.33 ± 9.06 mg/100g GAE). The control, however, was significantly higher ($p < 0.05$; Figure 4.10 C) in TTC than every sample that was exposed to 600ppm & 45/35 ˚C. The TTC in the underground stems continued to increase from the control to 192 hours, where a concentration of 7199.64 ± 17.28 mg/100g GAE was recorded ($p < 0.05$). The concentration of TTC in the roots nearly quadrupled in concentration at 48 hours (5946.02 ± 4.79 mg/100g GAE; $p < 0.05$) from the control. Some consistency was noted between 96 and 192 hours, as the concentrations slightly differed between 100 and 250 mg/100g GAE

(ANOVA; Tukey). *B. natalensis* underground stems had greater TTC in 75% of the harvest periods (96 to 192 hours) than the roots and leaves.

A continual decrease in the concentration of total proanthocyanidins was observed in the leaves under 600ppm & 45/35 ˚C from 48 hours to 192 hours (Figure 4.10 D). The control was significantly higher in TPAC compared to the leaves exposed to elevated CO_2 and high temperatures ($p < 0.05$). The TPAC increased between 48 and 144 hours (940.4 ± 2.48 mg/100g CE) in the underground stems. The control was higher in content in contrast to 48 hours, 96 hours, and 192 hours ($p < 0.05$). The roots displayed greater concentrations of total proanthocyanidins at 192 hours (573.3 ± 0.85 mg/100g CE; $p < 0.05$), followed by the 48 hours, 96 hours, the control, and 144 hours. The leaves were lower in TPAC in this treatment compared to the species' underground plant parts.

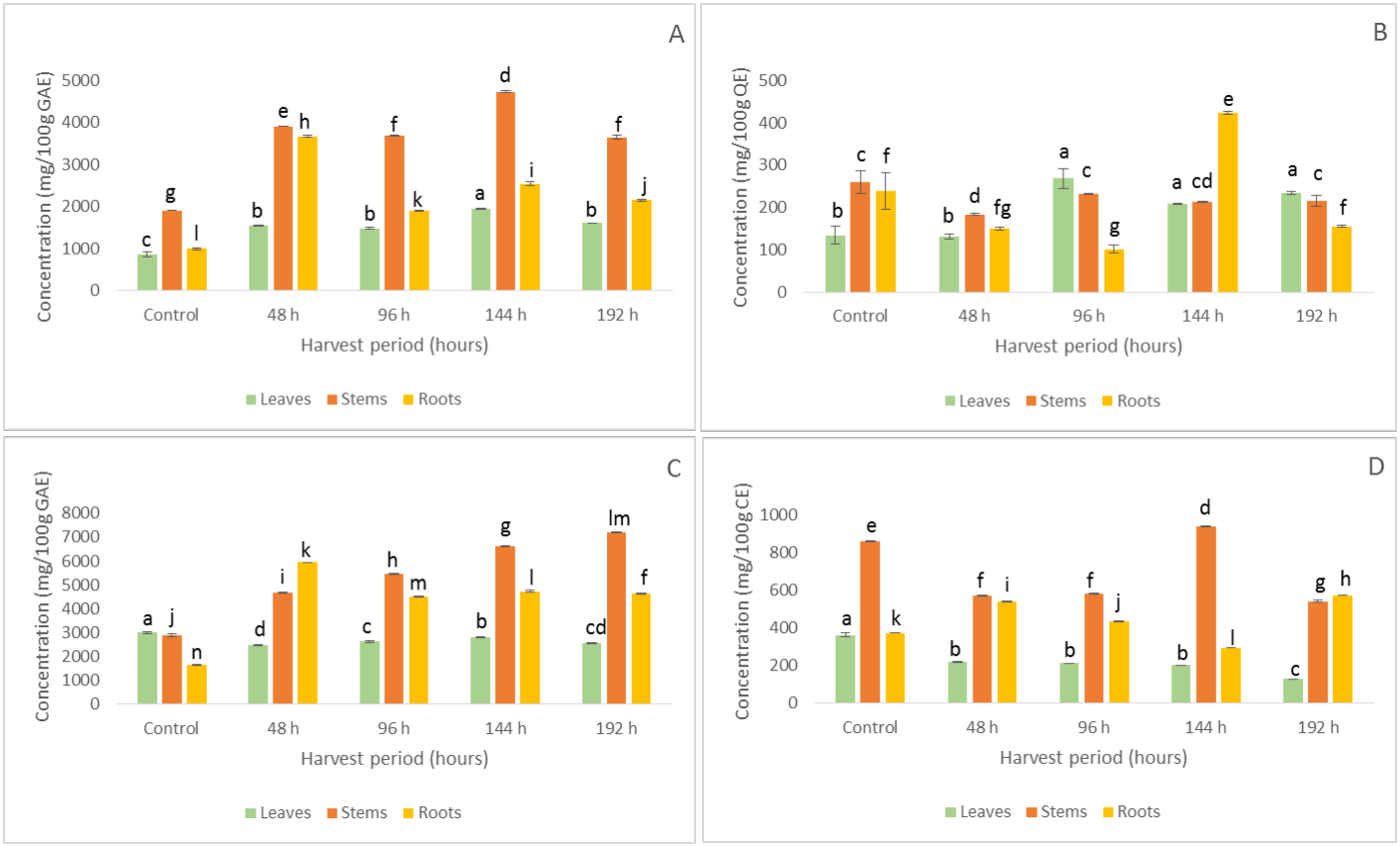

Figure 4.10: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. natalensis* leaves, corms, and roots under 600ppm & 45/35 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

c) 800ppm & 40/30 ˚C

A concentration of 2314.4 ± 17.75 mg/100g GAE was the highest recorded at 48 hours from the leaves harvested under 800pm & 40/30 ˚C ($p < 0.05$), which decreased at 96 hours (Figure 4.11 A). The TPC increased again at 144 hours before decreasing at 192 hours. The greatest significance was recorded after 96 hours, with a TPC of 6294.3 ± 26.94 mg/100g GAE ($p < 0.05$) from the underground stems. This was followed by 48 hours, 144 hours, 192 hours, and the control, which had the lowest TPC by comparison. In the roots, the TPC had doubled from the control to 48 hours, with a concentration of 2442.4 ± 151.63 mg/100g GAE, which then decreased from 96 hours to 144 hours. The highest TPC came from the roots harvested at 192 hours (3890.2 ± 84.38 mg/100g GAE; $p < 0.05$). The underground stems had higher TPC between 48 and 144 hours, compared to the leaves and roots. The roots contained a comparatively higher concentration of total phenolics, followed by the underground stems and the leaves at 192 hours.

A significant increase in total flavonoids under 800ppm & 40/30 ˚C at 48 hours was reported in the leaves, which gradually increased all the way to 192 hours, with the highest recorded concentration of 501.3 ± 40.29 mg/100g QE in the leaves (Figure 4.11 B). The control was significantly different to the harvested samples under treatment ($p < 0.05$). Compared to the leaves, *B. natalensis* underground stems displayed an inverse pattern in TFC. The greatest concentration was reported at 48 hours (607.8 ± 12.75 mg/100g QE), showing a great increase from the control. The TFC then took a downturn from 96 hours to 192 hours (356.7 ± 26.94 mg/100g QE). The control had the lowest TFC compared to each harvested sample from this

treatment. Furthermore, a significant difference was reported between 48 and 144 hours against the control ($p < 0.05$). The highest concentration of total flavonoids in the roots was reported at 96 hours (232.9 ± 24.29 mg/100g QE) followed by 48 hours, 192 hours and 144 hours, under 800ppm & 40/30 ˚C. All the above mentioned concentrations had lower TFC than the control. In the last three harvest periods (96 to 192 hours), the leaves contained greater TFC than the underground stems and roots.

A concentration of 2672.46 ± 172.13 mg/100g GAE of total tannins was recorded after the 96-hour harvest of *B. natalensis* leaves. All of the samples harvested under 800ppm & 40/30 ˚C had significantly lower TTC than the control ($p < 0.05$; Figure 4.11 C). The TTC in the underground stems gradually declined from 48 hours (6647.10 ± 7.89 mg/100g GAE) to 192 hours. The concentrations between 48 and 144 hours had at least twice the concentration of the control ($p < 0.05$). A similar pattern as in the underground stems was observed from the TTC in the roots. The concentration steadily decreased from 48 hours (5686.67 ± 6.03 mg/100g GAE) to 192 hours ($p < 0.05$). Overall, *B. natalensis* underground stems and roots both had greater TTC compared to the leaves.

Although a concentration of 307.9 ± 0.76 mg/100g CE was reported at 144 hours and was subsequently the highest TPAC in the leaves under 800ppm & 40/30 ˚C, the control was still significantly higher in content overall ($p < 0.05$; Figure 4.11 D). The TPAC in the underground stems continued to increase from 48 to 144 hours (776.1 ± 0.78 mg/100g CE) until it decreased at 192 hours, by almost half the concentration of the previous harvest period. The control, however, remained the highest in TPAC by comparison ($p < 0.05$). At least 75% of the harvested roots showed greater TPAC, at 192 hours (870.6 ± 1.13 mg/100g CE), 96 hours and 144 hours, than the control ($p < 0.05$). The subterranean plant parts of *B. natalensis* contained higher concentrations of total proanthocyanidins than the leaves.

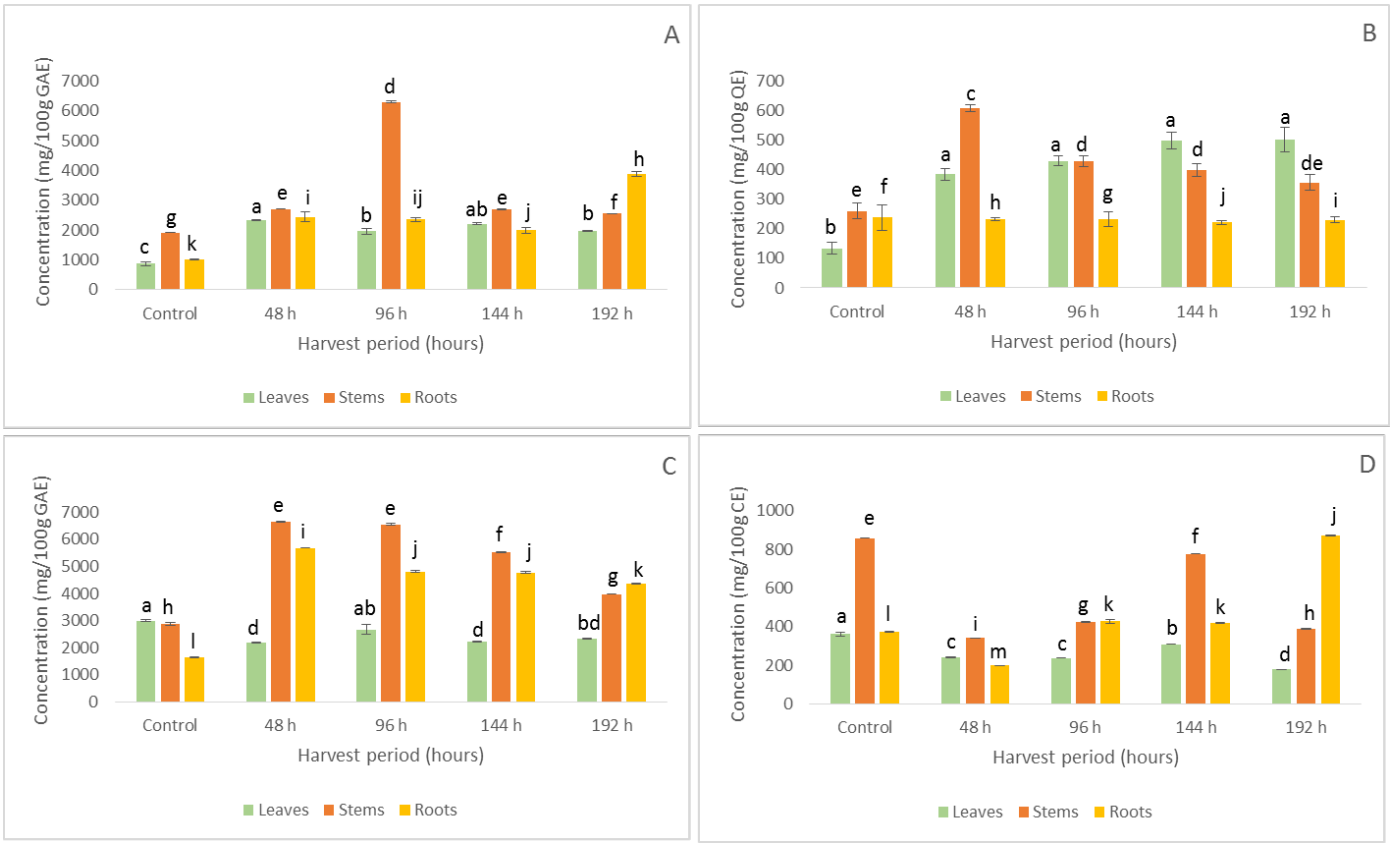

Figure 4.11: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. natalensis* leaves, corms, and roots under 600ppm & 45/35 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

d) 800ppm & 45/35 ˚C

The TPC in the leaves saw a noticeable increase from the control to 144 hours (1312.9 ± 32.04 mg/100g GAE; $p < 0.05$), and then decreased at 192 hours (Figure 4.12 A). A huge increase in total phenolics in the underground stems was reported at 48 hours (5499.7 ± 6.73 mg/100g GAE) with the next highest value at 144 hours, followed by 192 hours, 96 hours and the control ($p < 0.05$). The TPC in the roots under 800ppm & 45/35 ˚C, at first, increased from 48 to 96 hours (2422.2 ± 23.33 mg/100g GAE; $p < 0.05$), then decreased from 144 hours to 192 hours. The underground stems possessed greater TPC than the leaves and roots in all harvest periods under 800ppm & 45/35 ˚C. Although the leaves and roots were the only plant parts to have higher TPC in each concurrent elevated CO_2 and high temperature treatment in contrast to their controls, the underground stems had an overall higher TPC.

About 75% of the methanol leaf extracts of *B. natalensis* were reported to have higher TFC under elevated CO_2 and high temperatures (800ppm & 45/35 ˚C; $p < 0.05$), in contrast to the control, namely at 48 hours, 96 hours, and the highest at 192 hours (462.2 ± 15.76 ng/100g QE; Figure 4.12 B). The TFC in the underground stems showed a recurrent increase form the control to 96 hours (533.9 ± 5.47 mg/100g QE; $p < 0.05$). The concentration moderately decreased at 144 hours and elevated again at 192 hours ($p < 0.05$). The roots recorded the most significant increase in the concentration of total flavonoids at 48 hours (388.1 ± 6.46 mg/100g QE; $p < 0.05$) followed by 192 hours, 144 hours and 96 hours. Similar to the underground stems, the control had the lowest TFC compared to the samples harvested under 800ppm & 45/35 ˚C. The plants exposed to 800ppm & 45/35 ˚C showed the underground stems to have the highest TFC between 96 and 192 hours, compared to the leaves and roots. The leaves, however, yielded a higher TFC at 48 hours.

The highest concentration recorded, compared to all other elevated CO_2 and high temperature treatments was reported in the leaves at 48 hours, under 800ppm & 45/35 ˚C (4642.03 ± 38.34

mg/100g GAE; $p < 0.05$; Figure 4.12 C). The TTC decreased from 96 to 192 hours. Only the first two harvest periods showed greater tannin content than the control. Following each harvest period, there was an increase in the TTC in the underground stems ($p < 0.05$), with the highest concentration recorded at 192 hours (8333.69 ± 3.14 mg/100g GAE). There were slight differences in the TTC in the roots, showing consistency in the concentrations throughout this treatment. The roots had higher content at 96 hours (3313.77 ± 26.68 mg/100g GAE) followed by 48 hours, 144 hours, and 192 hours. In comparison to the control, the roots under treatment had greater TTC ($p < 0.05$). The underground stems contained greater concentrations of total tannins than the other plant organs.

A higher TPAC was reported at 48 hours (367.1 ± 0.23 mg/100g CE; $p < 0.05$) with the control sample trailing closely behind, and 144 hours (Figure 4.12 D). These were the only leaf extracts to have a concentration above 300 mg/100g CE. There was a slight decrease in the TPAC in the underground stems between the control ($p < 0.05$) and 48 hours (849.5 ± 0.91 mg/100g CE), which continued to 96 hours. It was from here where the concentration increased until 192 hours. The roots contained a greater TPAC at 144 hours (430.5 ± 0.46 mg/100g CE; $p < 0.05$), which was by comparison, higher than the control. *B. natalensis* underground stems displayed the highest concentration of total proanthocyanidins, followed by the roots and leaves, under 800ppm & 45/35 ˚C.

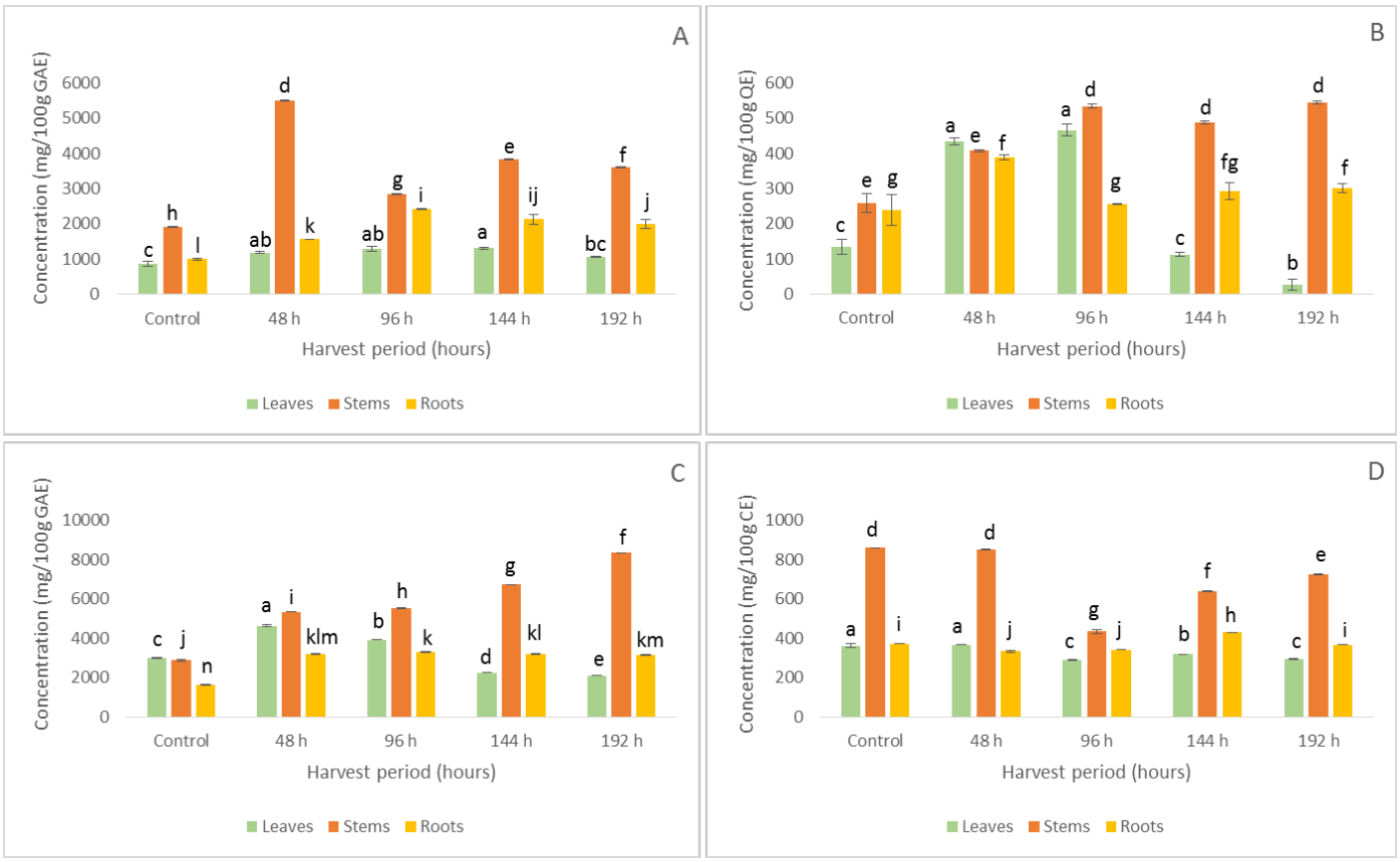

Figure 4.12: Total phenolic (A), flavonoid (B), tannin (C), and proanthocyanidin (D) contents in *B. natalensis* leaves, corms, and roots under 800ppm & 45/35 ˚C. The values were illustrated as mean ± SE. Significance is indicated by different superscript letters ($p < 0.05$).

4.3.2 Qualitative phytochemical screening

a) *Bulbine frutescens*

Seventy percent of the tested phytochemical groups were detected in the control leaf extract, with phenolics and coumarins showing the highest presence, followed by tannins, glycosides and volatile oils, and lastly, the flavonoids and steroids (Figure 4.13). Phenolics, glycosides and coumarins had the greatest occurrence of the respective compounds displaying a higher presence under concurrent elevated CO_2 and high temperature treatments. At least 60% of the

tested compounds showed consistency in their presence in both control and elevated conditions (Figure 4.13). Fewer compound groups were identified in the control root extract compared to the leaves (Figure 4.14). Saponins, steroids, terpenoids, coumarins and phlobatannins were the only compounds present in *B. frutescens* roots. Coumarins, terpenoids and flavonoids showed a higher presence in select treatments of concurrent elevated CO_2 and high temperatures (Figure 4.14).

Treatment	Harvest period	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Terpenoids	Glycosides	Coumarins	Phlobatannins	Volatile oils
Control											
600ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
600ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										

Key: Undetected Low Moderate High

Figure 4.13: Phytochemical screening of *B. frutescens* leaves under various treatments of concurrent elevated CO_2 and temperatures.

Treatment	Harvest period	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Terpenoids	Glycosides	Coumarins	Phlobatannins	Volatile oils
Control											
600ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
600ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										

Key: Undetected Low Moderate High

Figure 4.14: Phytochemical screening of *B. frutescens* roots under various treatments of concurrent elevated CO_2 and temperatures.

b) *Bulbine abyssinica*

The control leaf samples displayed a low presence of saponins and glycosides, and a moderate presence of phenolics, flavonoids, tannins, steroids and terpenoids (Figure 4.15). Coumarins, phlobatannins and volatile oils were not detected in the methanolic leaf extract. The greatest variation in the presence of the tested phytochemical compounds from the elevated conditions was noted from phenolics, with both the 600ppm & 40/30 ˚C and 800ppm & 40/30 ˚C treatments showing a higher presence from the compound group, compared to the control (Figure 4.15). This was followed by the 600ppm & 45/35 ˚C treatment only having a higher presence at the 48- and 96-hour harvest periods. Terpenoids had shown a higher presence in the 800ppm & 40/30 ˚C (96 and 192 hours) and 800ppm & 45/35 ˚C treatments. Glycosides, steroids, flavonoids, and tannins all followed with less recurrent periods where a high presence was reported (in descending order). Interestingly, coumarins and volatile oils were not detected in all treatments, like the control.

Treatment	Harvest period	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Terpenoids	Glycosides	Coumarins	Phlobatannins	Volatile oils
Control											
600ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
600ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										

Key: No detection Low Average High

Figure 4.15: Phytochemical screening of *B. abyssinica* leaves under various treatments of concurrent elevated CO_2 and temperatures.

Only tannins, saponins, steroids, terpenoids and glycosides were detected in the control extract of the underground stems (Figure 4.16). A high presence of tannins, steroids, terpenoids, flavonoids, phlobatannins and volatile oils were reported in selective treatments. Of all the groups assessed, only tannins, saponins, terpenoids and glycosides showed a consistent presence in both control and elevated CO_2 and high temperature treatments. Similar to *B. abyssinica* leaves, coumarins were absent in all treatments in the underground stems.

Treatment	Harvest period	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Terpenoids	Glycosides	Coumarins	Phlobatannins	Volatile oils
Control											
600ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
600ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										

Key: No detection Low Average High

Figure 4.16: Phytochemical screening of *B. abyssinica* underground stems under various treatments of concurrent elevated CO_2 and temperatures.

In the control root extract, only saponins, steroids, and terpenoids were present. Of the three plant parts, the roots showed a high presence in phenolics after 192 hours in the 800ppm & 45/35 ˚C treatment (Figure 4.17). Saponins and steroids were the only compound groups to remain unchanged under elevated conditions, similar to the control. Much like in the leaves, the roots also showed no detection of coumarins and volatile oils. Coumarins were the only phytochemical group not detected in *B. abyssinica* plant parts, both in the control and elevated CO_2 and high temperature treatments.

Treatment	Harvest period	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Terpenoids	Glycosides	Coumarins	Phlobatannins	Volatile oils
Control											
600ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
600ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										

Key: No detection Low Average High

Figure 4.17: Phytochemical screening of *B. abyssinica* roots under various treatments of concurrent elevated CO_2 and temperatures.

c) *Bulbine natalensis*

Eight out of 10 compound groups were detected in the control leaf extract of *B. natalensis*, from which phenolics, tannins, and coumarins had the highest presence (Figure 4.18). In selective elevated CO_2 and high temperature treatments, coumarins, tannins, steroids and phenolics showed a higher presence of the respective compounds, although there was no difference when compared to the control with regards to phenolics, tannins and coumarins. About 70% of the phytochemical compounds were present in all treatments, including the control, whereas saponins were only present at 800ppm & 40/30 ˚C after the 48-hour harvest (Figure 4.18)

Treatment	Harvest period	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Terpenoids	Glycosides	Coumarins	Phlobatannins	Volatile oils
Control											
600ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
600ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										

Key: Undetected Low Moderate High

Figure 4.18: Phytochemical screening of *B. natalensis* leaves under various treatments of concurrent elevated CO_2 and temperatures.

Four compound groups, namely saponins, steroids, terpenoids and glycosides, were noted in the control corm extract. Phlobatannins were the only phytochemical group to show a high presence at 600ppm & 45/35 ˚C (192 hours), 800ppm & 40/30 ˚C (144 hours and 192 hours) and 800ppm & 45/35 ˚C (48 hours) treatments, respectively (Figure 4.19). Saponins and terpenoids were the only compounds to maintain consistency in all treatments. Contrastingly, both coumarins and volatile oils were undetected in the underground stems.

Treatment	Harvest period	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Terpenoids	Glycosides	Coumarins	Phlobatannins	Volatile oils
Control											
600ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
600ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 40/30 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 45/35 ˚C	48 hours										
	96 hours										
	144 hours										
	192 hours										

Key: No detection Low Moderate High

Figure 4.19: Phytochemical screening of *B. natalensis* corms under various treatments of concurrent elevated CO_2 and temperatures.

In the control root extract of *B. natalensis*, half of the tested phytochemical groups were present. Similar to the corms, only one group was reported to have a higher presence. However, in the case of the roots, it was saponins, in three separate treatments: 600ppm & 45/35 ˚C (48 hours), 800ppm & 40/30 ˚C (192 hours) and 800ppm & 45/35 ˚C (48 hours and 96 hours) respectively (Figure 4.20). Five compound groups retained a consistent presence whereas coumarins and volatile oils were not detected.

Treatment	Harvest period	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Terpenoids	Glycosides	Coumarins	Phlobatannins	Volatile oils
Control											
600ppm & 40/30 °C	48 hours										
	96 hours										
	144 hours										
	192 hours										
600ppm & 45/35 °C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 40/30 °C	48 hours										
	96 hours										
	144 hours										
	192 hours										
800ppm & 45/35 °C	48 hours										
	96 hours										
	144 hours										
	192 hours										

Key: No detection Low Moderate High

Figure 4.20: Phytochemical screening of *B. natalensis* roots under various treatments of concurrent elevated CO_2 and temperatures.

4.3.3 In-vitro antioxidant activity

4.3.3.1 Bulbine frutescens

a) DPPH

The DPPH radicals were excellently scavenged at 48 hours (0.85 ± 0.23 mg/ml) in the leaves, compared to all other harvest periods under 600ppm & 40/30 ˚C and the control, which exhibited a good AA against DPPH (Table 4.1). The roots have also shown a good DPPH scavenging activity from the control, to 144 hours, however, following the harvest at 192 hours, the roots had an excellent scavenging activity of 0.64 ± 0.29 mg/ml. About 75% of the harvest periods (48 to 144 hours) revealed a slightly greater DPPH scavenging power in the leaves compared to the roots. Overall, both plant parts showed a significant difference when compared to their respective controls ($p < 0.05$).

The leaves harvested after 48 and 96 hours displayed excellent scavenging abilities against DPPH radicals, with concentrations of 1.37 ± 0.19 mg/ml and 1.87 ± 0.29 mg/ml respectively. The AA slowly decreased from an excellent activity at 48 hours to a weak scavenging activity at 192 hours (Table 4.1). The roots contrastingly had greater scavenging power at 144 hours (1.34 ± 0.17 mg/ml) and 192 hours (0.53 ± 0.07 mg/ml). Both plant parts each had 50% greater activity where a trade-off was observed at 144 hours. Each of the plant parts also showed significant differences overall ($p < 0.05$).

Overall, the leaves had a good DPPH scavenging activity, corresponding with the control under 800ppm & 40/30 ˚C. However, at 144 hours, the AA was slightly stronger (1.66 ± 0.23 mg/ml). The roots showed a decrease in AA from the control, and was evident at 48 hours and 96 hours. This activity greatly improved at 144 hours, before slightly decreasing again at 192 hours, although a good activity was reported. The plants under this treatment showed that the leaves were comparatively better at scavenging DPPH radicals than the roots, and each plant part had a significant difference overall ($p < 0.05$).

The greatest scavenging activity in the leaves was reported following the 48 hour harvest, with an IC_{50} concentration of 0.19 ± 0.04 mg/ml. For the rest of the harvest periods, the leaves adequately reduced DPPH radicals, indicating a good activity in comparison to 48 hours. All of the root extracts showed a good scavenging activity, with roots harvested at 48 hours and 192 hours performing slightly better. Similar to the leaves, the roots harvested under 800ppm & 45/35 ˚C had slightly higher DPPH radical quenching activity than the control. An overall significant difference was reported for both leaves and roots of *B. frutescens* ($p < 0.05$).

Table 4.1: The scavenging activity of *B. frutescens* against DPPH radicals under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Roots
Control		$4.55^{a} \pm 0.21$	$3.43^{b} \pm 0.16$
600ppm & 40/30 ˚C	48 h	$0.85^{c} \pm 0.23$	$3.91^{b} \pm 0.25$
	96 h	$2.33^{b} \pm 0.23$	$3.05^{b} \pm 0.31$
	144 h	$4.33^{a} \pm 0.29$	$6.01^{a} \pm 0.21$
	192 h	$5.26^{a} \pm 0.32$	$0.64^{c} \pm 0.29$
Control		$4.55^{b} \pm 0.21$	$3.43^{c} \pm 0.16$
600ppm & 45/35 ˚C	48 h	$1.37^{c} \pm 0.19$	$5.51^{b} \pm 0.21$
	96 h	$1.87^{c} \pm 0.29$	$22.26^{a} \pm 0.37$
	144 h	$3.56^{b} \pm 0.61$	$1.34^{d} \pm 0.17$
	192 h	$28.78^{a} \pm 0.17$	$0.53^{d} \pm 0.07$
Control		$4.55^{ab} \pm 0.21$	$3.43^{c} \pm 0.16$
	48 h	$5.09^{a} \pm 0.46$	$12.20^{a} \pm 0.04$

800ppm & 40/30 ˚C	96 h	$2.63^{c} \pm 0.19$	$13.50^{b} \pm 0.08$
	144 h	$1.66^{c} \pm 0.23$	$2.75^{c} \pm 0.40$
	192 h	$2.67^{bc} \pm 0.43$	$4.27^{c} \pm 0.32$
Control		$4.55^{a} \pm 0.21$	$3.43^{a} \pm 0.16$
800ppm & 45/35 ˚C	48 h	$0.19^{d} \pm 0.04$	$2.04^{b} \pm 0.25$
	96 h	$2.03^{c} \pm 0.18$	$2.70^{a} \pm 0.27$
	144 h	$3.05^{b} \pm 0.18$	$3.26^{a} \pm 0.29$
	192 h	$2.94^{b} \pm 0.16$	$2.07^{a} \pm 0.47$

b) Hydrogen peroxide

Although the strongest activity against radicals was recorded at 48 hours from the leaves, indicated by the low IC_{50} concentration of 0.16 ± 0.03 mg/ml, all of the successive harvest periods displayed excellent scavenging activity, compared to the control, which also had a great activity (Table 4.2). The roots had also shown excellent activity between 48 hours and 192 hours, with the former having the IC_{50} concentration of 0.37 ± 0.03 mg/ml. The leaves had an overall greater OH^{-} scavenging activity than the roots, though the difference was only moderate. The leaves showed no significant difference ($p > 0.05$) whereas the roots showed significance ($p < 0.05$).

The IC_{50} concentration of the leaf extracts scavenging H_2O_2 radicals continually decreased from the control, 48 hours, 96 hours and had the strongest activity at 144 hours (0.81 ± 0.08 mg/ml). The concentration slightly increased at 192 hours, however, retained an excellent scavenging activity. No significance was observed in this treatment for the leaves, as confirmed by one-way ANOVA and Tukey HSD post-hoc tests ($p > 0.05$; Table 4.2). The roots showed an overall significant difference ($p < 0.05$), with strong radical quenching potential at 48 hours (0.28 ± 0.05 mg/ml), followed by 96 hours, 144 hours, 192 hours, and the control. Both plant

parts each had a 50% greater scavenging power, with the roots performing better 48 and 96 hours, and the leaves at 144 and 192 hours.

The greatest scavenging concentration of H_2O_2 radicals from the leaves was recorded at 144 hours (0.38 ± 0.07 mg/ml). It was only at 48 hours where the IC_{50} concentration was greater than 1 mg/ml, other than the control. Three-quarters of the roots harvested roots had IC_{50} concentrations lower than 1 mg/ml, a similar trend observed from the leaves. The lowest concentration was recorded at 96 hours (0.23 ± 0.02 mg/ml) and the highest concentration at 192 hours (1.18 ± 0.23 mg/ml), however, all the values recorded from the elevated treatment conditions were lower that the control. Both leaves and roots had no significant differences ($p > 0.05$).

The H_2O_2 scavenging potential of *B. frutescens* leaves was highest after at 96 hours (0.09 ± 0.02 mg/ml) followed by 48 hours (0.25 ± 0.01 mg/ml), although an excellent activity was constant at 144 and 192 hours. There was no significant difference observed from the leaves under 800ppm & 45/35 ˚C. Similarly, the roots followed the pattern, by showing an increase in activity from 48 hours to 96 hours, followed by a slight decrease at 144 hours, before yielding the strongest activity at 192 hours (0.79 ± 0.07 mg/ml). Much like the leaves, no significant difference was reported for the roots ($p < 0.05$). The leaves seem to scavenge OH^- radicals better than the roots in ¾ of the harvest periods, namely, 48 hours, 96 hours and 144 hours. The commonality observed from the leaves and roots is that OH^- radicals are quenched comparatively better under the concurrent elevated CO_2 and high temperature treatments than their respective controls.

Table 4.2: The scavenging activity of *B. frutescens* against H_2O_2 radicals under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Roots
Control		$1.48^{a} \pm 0.22$	$2.05^{a} \pm 0.21$
600ppm & 40/30 ˚C	48 h	$0.16^{a} \pm 0.03$	$1.29^{a} \pm 0.19$
	96 h	$0.28^{a} \pm 0.02$	$0.37^{b} \pm 0.03$
	144 h	$0.86^{a} \pm 0.07$	$0.40^{a} \pm 0.03$
	192 h	$0.52^{a} \pm 0.04$	$0.60^{a} \pm 0.03$
Control		$1.48^{a} \pm 0.22$	$2.05^{a} \pm 0.21$
600ppm & 45/35 ˚C	48 h	$1.37^{a} \pm 0.19$	$0.28^{b} \pm 0.05$
	96 h	$1.23^{a} \pm 0.17$	$1.15^{a} \pm 0.16$
	144 h	$0.81^{a} \pm 0.08$	$1.43^{a} \pm 0.20$
	192 h	$1.09^{a} \pm 0.15$	$1.34^{a} \pm 0.15$
Control		$1.48^{a} \pm 0.22$	$2.05^{a} \pm 0.21$
800ppm & 40/30 ˚C	48 h	$1.45^{a} \pm 0.16$	$0.62^{a} \pm 0.09$
	96 h	$0.77^{a} \pm 0.07$	$0.23^{a} \pm 0.02$
	144 h	$0.38^{a} \pm 0.07$	$0.24^{a} \pm 0.03$
	192 h	$0.60^{a} \pm 0.12$	$1.18^{a} \pm 0.23$
Control		$1.48^{a} \pm 0.22$	$2.05^{a} \pm 0.21$
800ppm & 45/35 ˚C	48 h	$0.25^{a} \pm 0.01$	$1.52^{a} \pm 0.24$
	96 h	$0.09^{a} \pm 0.02$	$1.19^{a} \pm 0.17$
	144 h	$1.19^{a} \pm 0.17$	$1.26^{a} \pm 0.19$
	192 h	$1.14^{a} \pm 0.11$	$0.79^{a} \pm 0.07$

c) Metal chelating

The iron chelating potential of *B. frutescens* continued to increase following the 48 hour period harvest, compared to the control (1.35 ± 0.09 mg/ml; Table 4.3). From 96 hours, the AA grew stronger, exhibiting an excellent potential until 192 hours (0.65 ± 0.03 mg/ml). The roots on the other hand, showed much stronger scavenging activity than the leaves, from the control (0.37 ± 0.06 mg/ml) as well as at 48 to 144 hours, where the lowest concentration was recorded (0.06 ± 0.01 mg/ml). Both plant parts were significantly different ($p < 0.05$; ANOVA) however, only one of the harvest periods was significant against the control (48 hours for leaves and 192 hours for roots- Tukey's test).

An excellent iron chelating activity was noted between 48 and 144 hours in the leaf extracts, where concentrations were lower than 1 mg/ml. The activity was slightly greater at 96 hours (0.63 ± 0.05 mg/ml), however this difference was minute when compared to 48 and 144 hours (Table 4.3). Each of the leaves harvested was significantly different to the control ($p < 0.05$). The roots had a great activity at 96 hours (0.60 ± 0.03 mg/ml) and 144 hours (0.49 ± 0.04 mg/ml), compared to 48 hours and 192 hours, where a weak activity was reported. Only the root samples harvested at 48 and 192 hours were significantly different to the control ($p < 0.05$; Tukey's test).

The leaves showed an excellent AA between the 48 and 144 hour harvest periods, with the strongest ability to scavenge the iron radicals recorded at 96 hours (1.18 ± 0.09 mg/ml).The weakest activity was reported at 192 hours. Each of the harvested roots displayed excellent radical quenching, with the greatest activity noted at 96 hours (0.07 ± 0.01 mg/ml). The roots outperformed the leaves in reducing the accumulation of iron radicals in 75% of the harvest periods under 800ppm & 40/30 ˚C. Although the ANOVA results showed a significant difference in the leaves and roots ($p < 0.05$), the Tukey's test revealed significance between 48 and 192 hours (leaves) and 144 and 192 hours (roots) against their respective controls.

Each of the leaf extracts recorded IC_{50} concentrations lower than 2 mg/ml, showing great iron radical scavenging. The lowest concentration was recorded at 144 hours (0.52 ± 0.04 mg/ml), followed by 192 hours, 48 hours and 96 hours. The last three periods had moderately lower AA in comparison to the control. Significant difference was noted between the control and 96 and 144 hours respectively ($p < 0.05$; Tukey's test). Only three harvest periods yielded concentrations lower than 1 mg/ml from the root extracts, indicating an excellent scavenging activity. The strongest potential was reported at 192 hours (0.41 ± 0.02 mg/ml), which was proceeded by 48 hours, 144 hours and 96 hours, each of them having a good activity. The control root extract however, had a lower concentration, which indicates a slightly greater scavenging activity. A strong significant difference was reported between the control and the 96-hour harvest ($p < 0.05$; Tukey's test).

Table 4.3: The metal chelating activity of *B. frutescens* under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Roots
Control		$1.35^{bc} \pm 0.09$	$0.37^{b} \pm 0.06$
600ppm & 40/30 ˚C	48 h	$2.11^{a} \pm 0.22$	$0.37^{b} \pm 0.06$
	96 h	$1.83^{ab} \pm 0.12$	$0.49^{b} \pm 0.02$
	144 h	$0.81^{c} \pm 0.11$	$0.06^{b} \pm 0.01$
	192 h	$0.65^{c} \pm 0.03$	$13.76^{a} \pm 1.37$
Control		$1.35^{b} \pm 0.09$	$0.37^{b} \pm 0.06$
600ppm & 45/35 ˚C	48 h	$0.65^{c} \pm 0.03$	$7.88^{a} \pm 1.09$
	96 h	$0.63^{c} \pm 0.05$	$0.60^{b} \pm 0.03$
	144 h	$0.72^{c} \pm 0.04$	$0.49^{b} \pm 0.04$

	192 h	$1.95^{a} \pm 0.18$	$8.08^{a} \pm 0.92$
Control		$1.35^{b} \pm 0.09$	$0.37^{c} \pm 0.06$
800ppm & 40/30 ˚C	48 h	$1.84^{b} \pm 0.17$	$0.74^{bc} \pm 0.04$
	96 h	$1.18^{b} \pm 0.09$	$0.07^{c} \pm 0.01$
	144 h	$1.23^{b} \pm 0.09$	$1.45^{a} \pm 0.23$
	192 h	$13.00^{a} \pm 1.15$	$1.39^{ab} \pm 0.24$
Control		$1.35^{b} \pm 0.09$	$0.37^{b} \pm 0.06$
800ppm & 45/35 ˚C	48 h	$1.97^{ab} \pm 0.20$	$0.67^{b} \pm 0.09$
	96 h	$1.99^{a} \pm 0.15$	$2.74^{a} \pm 0.25$
	144 h	$0.52^{c} \pm 0.04$	$0.70^{b} \pm 0.14$
	192 h	$1.47^{ab} \pm 0.14$	$0.41^{b} \pm 0.02$

4.3.3.2 Bulbine abyssinica

a) DPPH

Table 4.4 summarises the DPPH scavenging properties of the crude leaf, underground stem and root methanolic extracts of *B. abyssinica*. The leaves showed an increase in the scavenging activity between the control and at 48 hours, of which the latter also had the greatest activity as indicated by the low concentration (1.13 ± 0.48 mg/ml). At 96 hours and 192 hours, an excellent AA was noted, and a good activity was reported at 144 hours. The control leaves were determined to be significantly different to each of the harvest periods under 600ppm & 40/30 ˚C ($p < 0.05$). The underground stems only displayed stronger DPPH radical scavenging from the control (0.46 ± 0.04 mg/ml) to 48 hours (1.50 ± 0.38 mg/ml), and the weakest activity at 96 hours and 192 hours, with concentrations being higher than 10 mg/ml. Significant differences were observed between the control and 96 hours, 144 hours, and 192 hours respectively ($p < 0.05$). The control root extract recorded the strongest scavenging potential

against DPPH radicals (0.11 ± 0.01 mg/ml), followed by the roots harvested at 192 hours (1.17 ± 0.30 mg/ml). At 48 and 144 hours, the roots showed a good antioxidant activity, whereas at 96 hours, the scavenging activity slightly weakened. Overall, a significant difference was observed ($p < 0.05$), and the control showed significance between each harvest period (Tukey post-hoc HSD). Only the leaves exhibited a stronger scavenging activity under 600ppm & 40/30 ˚C compared to the control, in contrast to the underground stems and roots, which had greater DPPH radical scavenging properties in their respective controls.

The antioxidant activity in the leaves became weaker after 48 hours, but showed improvement between 96 hours and 192 hours, with the lowest concentration recorded at 144 hours (1.05 ± 0.12 mg/ml). Significance was noted between the control and 48 hours, 144 hours and 192 hours ($p < 0.05$). In the underground stems, the DPPH scavenging activity ranged between excellent (control and 96 hours) to weak (48 hours and 144 hours). All of the harvest periods showed a significant difference against the control ($p < 0.05$). About 50% of the root extracts under 600ppm & 45/35 ˚C had excellent DPPH scavenging activity, namely at 96 hours (1.53 ± 0.16 mg/ml) and 144 hours (1.62 ± 0.24 mg/ml), with the remaining harvest periods showing a good activity. The concentration of control extract was significantly different to each of the root extracts from the treatment ($p < 0.05$). The leaves showed better scavenging activity at 144 hours and 192 hours, whereas the underground stems and roots had lower concentrations at 96 hours.

The leaves harvested under 800ppm & 40/30 ˚C displayed an overall good antioxidant activity against DPPH radicals, with an excellent scavenging activity reported at 192 hours (0.97 ± 0.18 mg/ml). An overall significant difference was observed from the one-way ANOVA ($p < 0.05$), with the control further showing significance against 48, 96 and 192 hours respectively

(Tukey's post-hoc). A similar trend was observed with the underground stems, where a good AA was reported, and the greatest activity recorded at 192 hours (1.26 ± 0.19 mg/ml) ($p < 0.05$). At least 75% of the root extracts had a good scavenging activity, namely at 48, 144 and 192 hours respectively, but all harvested under elevated conditions were weaker than the control ($p < 0.05$).

Comparatively lower concentrations of leaf extracts were reported at 48, 96, and 192 hours, exhibiting a fairly better AA than the control ($p < 0.05$). The underground stems had the weakest scavenging power under 800ppm & 45/35 ˚C, with three-quarters of the harvested material (48, 96 and 192 hours) having IC_{50} concentrations greater than 10 mg/ml ($p < 0.05$). The roots had a better AA in comparison to the leaves and the underground stems, with half of the extracts yielding an excellent activity, namely at 144 (1.53 ± 0.24 mg/ml) and 192 hours (0.78 ± 0.11 mg/ml) respectively. Most of the leaves harvested under the various concurrent elevated CO_2 and high temperature treatments had stronger scavenging power against DPPH radicals, compared to the control. The underground stems and roots mostly showed a good activity under various treatments than their respective controls, which had excellent scavenging potential.

Table 4.4: The scavenging activity of *B. abyssinica* against DPPH radicals under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		$4.79^{a} \pm 0.35$	$0.46^{d} \pm 0.04$	$0.11^{d} \pm 0.01$
	48 h	$1.13^{b} \pm 0.48$	$1.50^{d} \pm 0.38$	$2.40^{b} \pm 0.16$
	96 h	$1.29^{b} \pm 0.48$	$26.62^{a} \pm 0.25$	$12.01^{a} \pm 0.23$

600ppm & 40/30 ˚C	144 h	$2.30^{b} \pm 0.39$	$9.37^{c} \pm 0.27$	$2.88^{b} \pm 0.19$
	192 h	$1.58^{b} \pm 0.47$	$48.52^{a} \pm 0.19$	$1.17^{c} \pm 0.30$
Control		$4.79^{b} \pm 0.35$	$0.46^{d} \pm 0.04$	$0.11^{c} \pm 0.01$
600ppm & 45/35 ˚C	48 h	$11.49^{a} \pm 0.20$	$10.66^{b} \pm 0.39$	$10.06^{a} \pm 0.22$
	96 h	$4.40^{b} \pm 0.21$	$0.97^{cd} \pm 0.09$	$1.53^{b} \pm 0.16$
	144 h	$1.05^{c} \pm 0.12$	$33.31^{a} \pm 0.11$	$1.62^{b} \pm 0.24$
	192 h	$1.46^{c} \pm 0.21$	$2.28^{c} \pm 0.34$	$9.23^{a} \pm 0.39$
Control		$4.79^{b} \pm 0.35$	$0.46^{d} \pm 0.04$	$0.11^{d} \pm 0.01$
800ppm & 40/30 ˚C	48 h	$2.07^{c} \pm 0.48$	$9.97^{a} \pm 0.21$	$4.50^{b} \pm 0.27$
	96 h	$12.58^{a} \pm 0.49$	$2.23^{bc} \pm 0.57$	$16.47^{a} \pm 0.24$
	144 h	$4.86^{b} \pm 0.44$	$3.08^{b} \pm 0.82$	$3.02^{c} \pm 0.29$
	192 h	$0.97^{c} \pm 0.18$	$1.26^{c} \pm 0.19$	$4.05^{bc} \pm 0.23$
Control		$4.79^{bc} \pm 0.35$	$0.46^{a} \pm 0.04$	$0.11^{d} \pm 0.01$
800ppm & 45/35 ˚C	48 h	$3.85^{cd} \pm 0.31$	$17.64^{a} \pm 0.38$	$2.05^{b} \pm 0.28$
	96 h	$3.61^{b} \pm 0.59$	$23.23^{a} \pm 0.16$	$5.24^{a} \pm 0.27$
	144 h	$15.62^{a} \pm 0.05$	$5.61^{a} \pm 0.33$	$1.53^{bc} \pm 0.24$
	192 h	$2.88^{d} \pm 0.37$	$11.50^{a} \pm 0.38$	$0.78^{cd} \pm 0.11$

b) Hydrogen peroxide

An excellent AA against hydroxyl radicals was reported in *B. abyssinica* leaves, from the control to 144 hours, where the lowest concentration was reported (0.09 ± 0.01 mg/ml) ($p < 0.05$; Table 4.5). All of the underground stems harvested under 600ppm & 40/30 ˚C showed a great hydroxyl ion scavenging activity, with all concentrations falling under 2 mg/ml. The strongest activity was recorded at 144 hours (0.77 ± 0.06 mg/ml) which was slightly lower in concentration than the control (0.82 ± 0.05 mg/ml). Both the one-way ANOVA and Tukey's post-hoc tests revealed no significant difference across all treatments and the control ($p > 0.05$).

The root extracts showed a similar pattern as the leaves, with an excellent AA reported between the control (0.73 ± 0.07 mg/ml) through 144 hours (1.60 ± 0.02 mg/ml) ($p < 0.05$). Although the underground stems displayed an excellent OH^- radical quenching activity under 600ppm & 40/30 ˚C, the leaves had a much greater AA in 75% of the harvest periods (48 to 144 hours) compared to *B. abyssinica*'s subterranean counterparts.

All of the IC_{50} concentrations from the leaf extracts were lower than 1 mg/ml, indicating an excellent H_2O_2 reducing power. The greatest activities were reported at 48 hours and 96 hours respectively ($p > 0.05$). Fifty percent of the underground stem extracts were much better at reducing OH^- radicals, with IC_{50} concentrations lower than the control, namely at 48 hours (0.10 ± 0.04 mg/ml) and 144 hours (0.79 ± 0.19 mg/ml) respectively. A good activity was reported at 96 and 192 hours ($p < 0.05$). Much like the leaves, *B. abyssinica* roots also had an excellent AA in all harvest periods. Lower IC_{50} values were recorded from 96 hours to 192 hours ($p > 0.05$). The roots outperformed the leaves and underground stems in reducing the accumulation of H_2O_2 radicals under 600ppm & 45/35 ˚C.

The IC_{50} values obtained from the leaf extracts gradually increased from the control until 144 hours, yet still retained an excellent scavenging activity, before weakening to a value of 27.79 ± 0.85 mg/ml at 192 hours ($p > 0.05$). The underground stems maintained a great AA under 800ppm & 40/30 ˚C, with periods of 144 and 192 hours yielding concentrations lower than 1 mg/ml ($p > 0.05$). A moderately good AA was observed from the roots, with the greatest IC_{50} concentration recorded at 48 hours (0.60 ± 0.06 mg/ml) ($p < 0.05$). Despite the fact that the roots and leaves had comparatively greater scavenging power than the underground stems at 48 and 96 hours respectively, the underground stems quenched H_2O_2 radicals more successfully overall.

The leaves showed a great hydrogen peroxide radical scavenging activity under 800ppm & 45/35 ˚C, with all the extracts having lower IC_{50} values than the control. The strongest activity was recorded at 96 hours (0.19 ± 0.09 mg/ml) and 192 hours (0.28 ± 0.02 mg/ml) ($p > 0.05$). Similarly, the underground stems also exhibited excellent hydroxyl quenching, with parts harvested at 144 and 192 hours having greater activity than the control with IC_{50} values of 0.26 ± 0.02 mg/ml and 0.30 ± 0.04 mg/ml respectively ($p > 0.05$). The roots only had stronger AA at 96 and 144 hours, of which the latter recorded a lower IC_{50} concentration compared to the control (0.31 ± 0.02 mg/ml). Overall, the roots showed a good AA under 800ppm & 45/35 ˚C ($p < 0.05$). All three plant parts showed an excellent H_2O radical scavenging power at 96 and 144 hours, with the latter having the strongest potential. Comparatively, the leaves showed the greatest potential to reduce H_2O_2 radicals produced under elevated CO_2 and high temperatures, followed by the underground stems and roots.

Table 4.5: The scavenging activity of *B. abyssinica* against H_2O_2 radicals under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		$0.66^{b} \pm 0.07$	$0.82^{a} \pm 0.05$	$0.73^{b} \pm 0.07$
600ppm & 40/30 ˚C	48 h	$0.79^{b} \pm 0.05$	$1.49^{a} \pm 0.14$	$1.47^{b} \pm 0.07$
	96 h	$0.28^{b} \pm 0.05$	$1.04^{a} \pm 0.02$	$1.53^{b} \pm 0.08$
	144 h	$0.09^{c} \pm 0.01$	$0.77^{a} \pm 0.06$	$1.60^{b} \pm 0.02$
	192 h	$16.09^{a} \pm 0.09$	$1.35^{a} \pm 0.14$	$2.04^{a} \pm 0.18$
Control		$0.66^{a} \pm 0.07$	$0.82^{b} \pm 0.05$	$0.73^{a} \pm 0.07$
	48 h	$0.17^{a} \pm 0.02$	$0.10^{a} \pm 0.04$	$0.75^{a} \pm 0.09$

600ppm & 45/35 ˚C	96 h	$0.25^{a} \pm 0.03$	$2.31^{a} \pm 0.13$	$0.17^{a} \pm 0.02$
	144 h	$0.98^{a} \pm 0.12$	$0.79^{a} \pm 0.19$	$0.35^{a} \pm 0.03$
	192 h	$0.51^{a} \pm 0.03$	$2.56^{a} \pm 0.39$	$0.20^{a} \pm 0.01$
Control		$0.66^{b} \pm 0.07$	$0.82^{a} \pm 0.05$	$0.73^{c} \pm 0.07$
800ppm & 40/30 ˚C	48 h	$0.72^{b} \pm 0.10$	$1.36^{a} \pm 0.19$	$0.60^{bc} \pm 0.06$
	96 h	$0.89^{b} \pm 0.13$	$1.32^{a} \pm 0.21$	$8.80^{a} \pm 0.01$
	144 h	$1.40^{b} \pm 0.18$	$0.58^{a} \pm 0.03$	$1.71^{b} \pm 0.16$
	192 h	$27.79^{a} \pm 0.85$	$0.83^{a} \pm 0.08$	$2.14^{bc} \pm 0.06$
Control		$0.66^{a} \pm 0.07$	$0.82^{a} \pm 0.05$	$0.73^{cd} \pm 0.07$
800ppm & 45/35 ˚C	48 h	$0.44^{a} \pm 0.02$	$1.62^{a} \pm 0.23$	$2.54^{b} \pm 0.03$
	96 h	$0.19^{a} \pm 0.01$	$1.59^{a} \pm 0.23$	$1.64^{bc} \pm 0.24$
	144 h	$0.41^{a} \pm 0.01$	$0.26^{a} \pm 0.02$	$0.31^{cd} \pm 0.02$
	192 h	$0.28^{a} \pm 0.02$	$0.30^{a} \pm 0.04$	$6.35^{a} \pm 0.15$

c) Metal chelating

The metal chelating activity of *B. abyssinica* leaves mostly showed good iron radical quenching under 600ppm & 40/30 ˚C, however, the IC_{50} concentration at 48 hours indicated an excellent activity (1.70 ± 0.71 mg/ml; Table 4.6). The underground stems had a much greater iron radical scavenging activity under elevated CO_2 and high temperatures compared to the control, with the strongest scavenging power reported at 192 hours (0.09 ± 0.01 mg/ml). At harvest periods between 48 and 144 hours, the roots had lower IC_{50} concentrations than the control and at 192 hours implying an excellent activity. The iron radicals were greatly reduced by the root extract at 96 hours (0.60 ± 0.03 mg/ml). The subterranean plant organs of *B. abyssinica* displayed a comparatively better iron radical reducing power than the leaves.

An overall good AA was exhibited from the leaves with both 96 and 192 hour periods respectively having stronger iron radical scavenging. The higher IC_{50} concentration recorded at 144 hours indicated a poor scavenging activity. A moderately good iron chelating activity was reported from the underground stems. The IC_{50} concentrations gradually increased from 48 hours to 192 hours, showing a shift from excellent to good AA. Much like the underground stems, the roots also showed a good iron chelating activity with stronger chelation reported at 48 hours (0.03 ± 0.01 mg/ml) and 144 hours (0.69 ± 0.05 mg/ml). Although the leaves and roots had moderately better chelating power 96 and 192 hours as well as 48 and 144 hours respectively, the underground stems showed the smallest increase in IC_{50} concentration, indicating the strongest iron reducing power in comparison.

All of the leaves harvested under 800ppm & 40/30 ˚C displayed an adequate AA with IC_{50} values ranging between 2 and 7 mg/ml. Three of the harvest periods (48 to 144 hours) had greater iron chelating activity in the underground stems, as the IC_{50} concentrations increased up to 192 hours, where a good AA was recorded. The roots showed the greatest scavenging activity overall, in comparison to the leaves and underground stems. The lowest IC_{50} concentrations were recorded at 144 hours (0.04 ± 0.02 mg/ml) and 96 hours (0.31 ± 0.04 mg/ml) respectively.

The greatest activity in *B. abyssinica* leaves was reported at 96 hours (3.06 ± 0.65 mg/ml), followed by 144 hours, 96 hours, and 192 hours. Although the control had a slightly better scavenging activity, all of the IC_{50} values indicated a good AA. Low concentrations ranging between 0.50 and 2 mg/ml were recorded between 96 and 192 hours from the underground stems, with the strongest chelating activity reported at 96 hours (0.53 ± 0.03 mg/ml). All of the

roots harvested under 800ppm & 45/35 ˚C exhibited better iron radical chelation than the control, especially after 144 and 192 hours, where the IC_{50} values had concentrations lower than 1 mg/ml. Overall, both underground plant parts, more especially the roots, had a comparatively stronger iron chelating activity than the leaves. All treatments were significantly different overall ($p < 0.05$).

Table 4.6: The metal chelating activity of *B. abyssinica* under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		$2.68^{b} \pm 0.16$	$2.69^{a} \pm 0.13$	$3.02^{a} \pm 0.13$
600ppm & 40/30 ˚C	48 h	$1.70^{b} \pm 0.71$	$0.41^{c} \pm 0.07$	$0.94^{bc} \pm 0.07$
	96 h	$7.86^{a} \pm 0.98$	$0.19^{c} \pm 0.01$	$0.60^{c} \pm 0.03$
	144 h	$8.46^{a} \pm 1.02$	$1.68^{b} \pm 0.24$	$1.22^{b} \pm 0.09$
	192 h	$9.87^{a} \pm 1.04$	$0.09^{c} \pm 0.01$	$2.53^{a} \pm 0.23$
Control		$2.68^{c} \pm 0.16$	$2.69^{a} \pm 0.13$	$3.02^{b} \pm 0.13$
600ppm & 45/35 ˚C	48 h	$5.69^{b} \pm 0.68$	$0.58^{d} \pm 0.03$	$0.03^{c} \pm 0.01$
	96 h	$1.29^{c} \pm 0.62$	$1.51^{c} \pm 0.21$	$8.76^{a} \pm 1.28$
	144 h	$39.99^{a} \pm 0.73$	$2.08^{b} \pm 0.02$	$0.69^{bc} \pm 0.05$
	192 h	$1.46^{c} \pm 0.68$	$2.27^{ab} \pm 0.08$	$2.24^{bc} \pm 0.08$
Control		$2.68^{bc} \pm 0.16$	$2.69^{a} \pm 0.13$	$3.02^{a} \pm 0.13$
800ppm & 40/30 ˚C	48 h	$4.14^{abc} \pm 0.83$	$0.08^{c} \pm 0.02$	$1.29^{c} \pm 0.09$
	96 h	$6.51^{a} \pm 1.14$	$0.54^{c} \pm 0.08$	$0.31^{d} \pm 0.04$
	144 h	$5.95^{ab} \pm 0.64$	$1.21^{bc} \pm 0.09$	$0.04^{d} \pm 0.02$

	192 h	$2.26^{c} \pm 0.83$	$2.61^{ab} \pm 0.55$	$1.99^{b} \pm 0.03$
Control		$2.68^{b} \pm 0.16$	$2.69^{b} \pm 0.13$	$3.02^{a} \pm 0.13$
800ppm & 45/35 ˚C	48 h	$3.36^{ab} \pm 0.93$	$3.97^{a} \pm 0.40$	$1.11^{b} \pm 0.06$
	96 h	$3.05^{b} \pm 0.65$	$0.53^{d} \pm 0.03$	$1.14^{b} \pm 0.07$
	144 h	$3.12^{b} \pm 0.80$	$1.08^{cd} \pm 0.08$	$0.10^{c} \pm 0.01$
	192 h	$6.49^{a} \pm 0.64$	$1.57^{c} \pm 0.22$	$0.35^{c} \pm 0.03$

4.3.3.3 Bulbine natalensis

a) DPPH scavenging activity

B. natalensis leaves fairly reduced the production of DPPH radicals at 48, 144, and 192 hours, and a weaker activity reported at 96 hours (Table 4.7). All the harvested leaves under 600ppm & 40/30 ˚C had slightly higher IC_{50} concentrations than the control (3.88 ± 0.16 mg/ml). About half of the underground stems showed a good AA at 96 hours (3.00 ± 0.32 mg/ml) and 144 hours (3.49 ± 0.31 mg/ml) respectively. The poorest activity was reported at 48 hours, 192 hours, and the control, of which the latter displayed the weakest DPPH scavenging activity (35.86 ± 0.29 mg/ml). Most of the roots harvested under elevated CO_2 and high temperatures had weaker scavenging activities, in comparison to the control, which had excellent AA (0.36 ± 0.02 mg/ml). The leaves and underground stems each had better DPPH radical quenching at 48 and 192 hours, as well as 96 and 144 hours respectively, compared to the roots. However, all three plant parts under 600ppm & 40/30 ˚C were significantly different to their respective controls ($p < 0.05$).

Three of the harvest periods (48, 96 and 192 hours) had lower IC_{50} concentrations from the leaf extracts than the control ($p < 0.05$; Table 4.7). Each of the values indicated an excellent potential to reduce DPPH radicals, especially at 48 hours (0.27 ± 0.05 mg/ml). All the underground stem extracts showed significantly greater DPPH scavenging activity than the control ($p < 0.05$), with values ranging between 0.24 to 0.67 mg/ml. An adequate radical

quenching activity was reported from the roots. Although a good AA was recorded at 48 and 96 hours respectively, the control still exceeded their potential. Overall, the underground stems, followed by the leaves, greatly outperformed the roots in reducing DPPH radicals under 600ppm & 45/35 ˚C.

The leaves harvested at 96 and 192 hours under 800ppm & 40/30 ˚C showed excellent AA (1.64 ± 0.11 mg/ml and 0.61 ± 0.09 mg/ml respectively) compared to 48 and 144 hours, which displayed a good DPPH scavenging activity (Table 4.7). The control, however, was slightly weaker in activity than the leaves harvested under 800ppm & 40/30 ˚C ($p < 0.05$). The IC_{50} values of the underground stems significantly decreased from the control to 144 hours where the greatest activity was recorded (0.76 ± 0.09 mg/ml). Similar to the leaves, all of the underground stems under this treatment scavenged DPPH radicals much better than the control ($p < 0.05$). The strongest AA from the roots harvested under 800ppm & 40/30 ˚C, was reported at 192 hours (0.51 ± 0.05 mg/ml) which was indifferently higher in concentration than the control. A good activity was observed at 48 and 144 hours, and the weakest activity was reported at 96 hours.

The IC_{50} concentrations of the leaf extracts had decreased from 9.78 ± 0.44 mg/ml at 48 hours (good AA) to 1.30 ± 0.12 mg/ml at 96 hours (excellent AA) and the lowest concentration at 144 hours (0.49 ± 0.04 mg/ml; Table 4.7). Only the latter two harvest periods had a higher scavenging potential than the control ($p < 0.05$). A positive activity was observed in the underground stems, with 50% of the harvest periods exhibiting an excellent radical quenching activity (96 hours – 0.82 ± 0.07 mg/ml and 192 hours – 1.58 ± 0.17 mg/ml, respectively) while the other half had a good AA (48 and 144 hours). The roots displayed a polarizing DPPH radical scavenging activity, with a good AA reported at 48 and 144 hours, and a weak AA noted at 96 and 192 hours. All the recorded IC_{50} concentrations of the roots under 800ppm &

45/35 ˚C were significantly higher than the control ($p < 0.05$). The greatest radical scavenging activity was found to come from the underground stems, specifically at 48, 96, and 192 hours.

Table 4.7: The scavenging activity of *B. natalensis* against DPPH radicals under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Corms	Roots
Control		$3.87^{d} \pm 0.16$	$35.86^{a} \pm 0.29$	$0.36^{e} \pm 0.02$
600ppm & 40/30 ˚C	48 h	$5.50^{c} \pm 0.27$	$22.4^{b} \pm 0.23$	$11.41^{c} \pm 0.32$
	96 h	$13.74^{a} \pm 0.32$	$3.00^{c} \pm 0.33$	$8.65^{d} \pm 0.17$
	144 h	$9.11^{b} \pm 0.20$	$3.49^{c} \pm 0.31$	$38.15^{a} \pm 0.40$
	192 h	$6.18^{c} \pm 0.21$	$22.89^{b} \pm 0.44$	$13.49^{b} \pm 0.49$
Control		$3.87^{b} \pm 0.16$	$35.86^{a} \pm 0.29$	$0.36^{e} \pm 0.02$
600ppm & 45/35 ˚C	48 h	$0.27^{b} \pm 0.05$	$0.51^{b} \pm 0.06$	$2.89^{d} \pm 0.42$
	96 h	$1.77^{c} \pm 0.13$	$0.67^{b} \pm 0.06$	$7.09^{c} \pm 0.35$
	144 h	$25.42^{a} \pm 0.06$	$0.42^{b} \pm 0.03$	$16.5^{b} \pm 0.37$
	192 h	$1.91^{c} \pm 0.29$	$0.24^{b} \pm 0.01$	$20.5^{a} \pm 0.42$
Control		$3.87^{a} \pm 0.16$	$35.86^{a} \pm 0.29$	$0.36^{d} \pm 0.02$
800ppm & 40/30 ˚C	48 h	$2.39^{b} \pm 0.12$	$11.95^{b} \pm 0.41$	$3.77^{b} \pm 0.55$
	96 h	$1.64^{c} \pm 0.11$	$3.13^{d} \pm 0.44$	$29.99^{a} \pm 0.19$
	144 h	$2.55^{b} \pm 0.12$	$0.76^{e} \pm 0.09$	$2.29^{c} \pm 0.33$
	192 h	$0.61^{d} \pm 0.09$	$5.17^{c} \pm 0.85$	$0.51^{cd} \pm 0.05$
Control		$3.87^{c} \pm 0.16$	$35.86^{a} \pm 0.29$	$0.36^{e} \pm 0.02$
	48 h	$9.78^{b} \pm 0.44$	$2.86^{c} \pm 0.44$	$3.13^{d} \pm 0.35$
	96 h	$1.30^{d} \pm 0.12$	$0.82^{d} \pm 0.07$	$20.14^{b} \pm 0.24$

800ppm & 45/35 ˚C	144 h	$0.49^{d} \pm 0.04$	$7.24^{b} \pm 0.22$	$8.35^{c} \pm 0.23$
	192 h	$27.14^{a} \pm 0.35$	$1.58^{cd} \pm 0.17$	$24.55^{a} \pm 0.20$

b) Hydrogen peroxide scavenging activity

Of the three harvest periods where an excellent H_2O_2 reducing power was reported in the leaves under 600ppm & 40/30 ˚C (IC_{50} values lower than 1 mg/ml) namely at 48, 96 and 192 hours, the latter two had the strongest activity (0.19 ± 0.04 mg/ml and 0.18 ± 0.04 mg/ml respectively; Table 4.8). The control, however, had the greatest scavenging power ($p < 0.05$). A gradual decrease in the IC_{50} concentrations of the underground stems was observed from the control (0.61 ± 0.05 mg/ml) to 144 hours (0.22 ± 0.03 mg/ml; $p < 0.05$), indicating greater AA compared to the stems harvested at 192 hours. The roots showed an excellent (96 and 192 hours) to good (48 and 144 hours) H_2O_2 scavenging activity under 600ppm & 40/30 ˚C, however, none of these were stronger at hydroxyl ion reduction than the control (0.24 ± 0.04 mg/ml). The leaves and underground stems both had scavenged H_2O_2 ions much better than the roots.

Although each of the harvested leaves under 600ppm & 45/35 ˚C had excellent activity, with the greatest H_2O_2 quenching reported at 48 hours (0.46 ± 0.14 mg/ml), the control still showed a slight but significantly stronger AA (Table 4.8). The underground stems had a fairly good AA. The scavenging potential was better at 96 and 192 hours, where IC_{50} values were lower (0.36 ± 0.02 mg/ml and 1.17 mg/ml respectively). All the root extracts displayed excellent H_2O_2 radical scavenging activity, with the IC_{50} values being lower than 1 mg/ml. The roots harvested after 144 hours had the strongest AA with an IC_{50} concentration of 0.15 ± 0.03 mg/ml.

A slightly stronger AA in the leaves was discovered at 96 hours (0.25 ± 0.03 mg/ml), followed by 192 hours and 48 hours under 800ppm 40/30 ˚C, of which both had higher IC_{50} concentrations than the control (Table 4.8). A comparatively weaker, but good AA was reported at 144 hours. The AA in the underground stems greatly improved from 48 hours to 192 hours under elevated CO_2 and high temperature conditions. This was evident from the continual decrease in the IC_{50} concentrations, with a better scavenging power recorded at 144 hours (0.41 ± 0.02 mg/ml) and 192 hours (0.17 ± 0.04 mg/ml) respectively. An excellent H_2O_2 radical scavenging activity was reported in all root extracts. The greatest activity was noted at 96 hours (0.44 ± 0.03 mg/ml), which is approximately double the concentration of the control. All plant parts had excellent H_2O_2 scavenging activity at 96 and 192 hours. The ANOVA results revealed that only the leaves showed an overall significant difference ($p < 0.05$), whereas the underground stems and roots were insignificant.

The leaves had a great H_2O_2 reducing power in all harvest periods from the 800ppm & 45/35 ˚C, with the IC_{50} concentrations being lower than 1 mg/ml (Table 4.8). Although there was no significant difference between the concentrations recorded for each harvest period and the control, the latter had the lowest concentration. A much better scavenging activity from the underground stems was reported at 96 (0.10 ± 0.02 mg/ml) and 144 hours (0.15 ± 0.01 mg/ml) in comparison to the control ($p < 0.05$), 48 hours and 192 hours, of which the latter had a good activity. The roots showed a similar trend in the excellent scavenging potential against H_2O_2 radicals as leaves, however in contrast, the roots slightly reduced the production of radicals better than the leaves. Only two harvest periods had lower IC_{50} concentrations, particularly at 144 hours (0.18 ± 0.04 mg/ml) and 192 hours (0.21 ± 0.03 mg/ml) than the control. Both the underground stems and roots showed stronger H_2O_2 scavenging activity than the leaves at 96 and 144 hours, as well as 48 and 192 hours respectively.

Table 4.8: The scavenging activity of *B. abyssinica* against H_2O_2 radicals under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		$0.29^{b} \pm 0.05$	$0.61^{b} \pm 0.05$	$0.24^{b} \pm 0.04$
600ppm & 40/30 ˚C	48 h	$0.71^{s} \pm 0.05$	$0.38^{ab} \pm 0.06$	$3.24^{a} \pm 0.30$
	96 h	$0.19^{s} \pm 0.04$	$0.27^{b} \pm 0.02$	$1.36^{ab} \pm 0.10$
	144 h	$2.31^{s} \pm 0.32$	$0.22^{b} \pm 0.03$	$4.64^{a} \pm 0.65$
	192 h	$0.18^{s} \pm 0.04$	$2.94^{a} \pm 0.41$	$1.94^{ab} \pm 0.08$
Control		$0.29^{b} \pm 0.05$	$0.61^{b} \pm 0.05$	$0.24^{a} \pm 0.04$
600ppm & 45/35 ˚C	48 h	$0.46^{ab} \pm 0.14$	$7.06^{a} \pm 1.08$	$0.45^{a} \pm 0.09$
	96 h	$1.16^{a} \pm 0.34$	$0.36^{b} \pm 0.02$	$0.51^{a} \pm 0.04$
	144 h	$0.62^{ab} \pm 0.06$	$2.51^{b} \pm 0.24$	$0.15^{a} \pm 0.03$
	192 h	$0.83^{a} \pm 0.07$	$1.17^{b} \pm 0.09$	$0.82^{a} \pm 0.08$
Control		$0.29^{b} \pm 0.05$	$0.61^{a} \pm 0.05$	$0.24^{a} \pm 0.04$
800ppm & 40/30 ˚C	48 h	$0.78^{b} \pm 0.07$	$2.67^{a} \pm 1.92$	$1.20^{a} \pm 0.12$
	96 h	$0.25^{b} \pm 0.03$	$0.68^{a} \pm 0.05$	$0.44^{a} \pm 0.03$
	144 h	$6.94^{a} \pm 0.31$	$0.41^{a} \pm 0.02$	$0.85^{a} \pm 0.07$
	192 h	$0.77^{b} \pm 0.07$	$0.17^{a} \pm 0.04$	$0.86^{a} \pm 0.06$
Control		$0.29^{a} \pm 0.05$	$0.61^{b} \pm 0.05$	$0.24^{a} \pm 0.04$
800ppm & 45/35 ˚C	48 h	$0.73^{a} \pm 0.10$	$0.72^{a} \pm 0.08$	$0.56^{a} \pm 0.09$
	96 h	$0.35^{a} \pm 0.07$	$0.10^{a} \pm 0.02$	$0.59^{a} \pm 0.06$
	144 h	$0.83^{a} \pm 0.08$	$0.15^{a} \pm 0.01$	$0.18^{a} \pm 0.04$
	192 h	$0.57^{d} \pm 0.04$	$2.82^{a} \pm 0.26$	$0.21^{a} \pm 0.03$

c) Metal chelating activity

The leaves displayed a good iron chelating activity under 600ppm & 40/30 ˚C, however, the control excellently quenched the radicals in comparison (0.19 ± 0.01 mg/ml; Table 4.9). All the underground stem extracts yielded great iron quenching activity, with the 48 hour (0.69 ± 0.04 mg/ml) and 144 hour (0.71 ± 0.05 mg/ml) harvest periods having comparatively lower IC_{50} values than the control (0.89 ± 0.02 mg/ml). The roots showed the best iron radical scavenging activity compared to the underground stems and leaves. All of the IC_{50} values recorded under 600ppm & 40/30 ˚C were lower than 1 mg/ml. The control however, was significantly stronger (0.33 ± 0.01 mg/ml).

The lowest IC_{50} concentrations from the methanolic leaf extracts were reported at 96 hours (0.40 ± 0.09 mg/ml) and 144 hours (0.41 ± 0.09 mg/ml) respectively (Table 4.9). Overall, the leaves showed an excellent iron reducing activity. The underground stems showed the strongest reducing power at 144 hours (0.07 ± 0.02 mg/ml), followed by the 48 hours and the control. A good AA was reported at 96 and 192 hours. An overall good AA was observed for the roots, showing significantly higher IC_{50} concentrations than the control. Of the three plant parts, the leaves had stronger iron radical scavenging activity under 600ppm & 45/35 ˚C, followed by the underground stems and the roots.

The iron chelating activity of the leaves slowly decreased at each of the harvest periods, with the lowest IC_{50} concentration at 48 hours (0.36 ± 0.02 mg/ml) to the highest at 192 hours (1.63 ± 0.18 mg/ml; Table 4.9). At least 75% of the harvest periods showed greater iron chelating power at 48 hours (0.99 ± 0.03 mg/ml), 144 hours (1.99 ± 0.07 mg/ml) and 192 hours (0.87 ± 0.20 mg/ml) respectively for the underground stems. The roots exhibited an excellent scavenging activity between 48 hours and 144 hours. The greatest activity was reported at 96 hours (0.66 ± 0.06 mg/ml), which was moderately stronger than the control. All the plant organs

displayed excellent iron chelating activity at 48 hours and 144 hours, with the harvested material from the latter period having a stronger antioxidant power.

The leaves showed the greatest iron radical reducing power at 96 hours (0.14 ± 0.02 mg/ml), followed by the control, 144 hours, 192 hours, and 48 hours. Half of the underground stem extracts had more powerful iron radical quenching than the control, particularly at 48 hours (0.07 ± 0.01 mg/ml) and 144 hours (0.07 ± 0.02 mg/ml). The IC_{50} concentrations recorded from the root extracts decreased continually, indicating a stronger AA with each harvest period. The roots harvested at 144 and 192 hours had the best scavenging activity (0.80 ± 0.36 mg/ml and 0.80 ± 0.11 mg/ml respectively). *B. natalensis* was overall, significantly different ($p < 0.05$; ANOVA) in the metal chelating activity, however, the control was found to be significantly different to each of the harvest periods in several treatments (600ppm & 40/30 ˚C- leaves and underground stems and 600ppm & 45/35 ˚C- roots) respectively.

Table 4.9: The metal chelating activity of *B. abyssinica* under concurrent elevated CO_2 and temperatures (mg/ml). Values are expressed as mean ± SE. Mean values of different superscript letters in the same column indicates significance ($p < 0.05$).

Treatment	Harvest period	Leaves	Underground stems	Roots
Control		$0.19^{b} \pm 0.01$	$0.89^{b} \pm 0.02$	$0.33^{b} \pm 0.01$
600ppm & 40/30 ˚C	48 h	$1.54^{a} \pm 0.14$	$0.69^{c} \pm 0.04$	$0.84^{a} \pm 0.10$
	96 h	$1.95^{a} \pm 0.17$	$1.07^{a} \pm 0.01$	$0.58^{ab} \pm 0.02$
	144 h	$2.62^{a} \pm 0.37$	$0.71^{c} \pm 0.05$	$0.74^{a} \pm 0.03$
	192 h	$2.39^{a} \pm 0.33$	$1.19^{a} \pm 0.05$	$0.83^{a} \pm 0.09$
Control		$0.19^{b} \pm 0.01$	$0.89^{c} \pm 0.02$	$0.33^{b} \pm 0.01$
600ppm & 45/35 ˚C	48 h	$0.57^{a} \pm 0.03$	$0.80^{c} \pm 0.07$	$3.08^{a} \pm 0.13$
	96 h	$0.40^{ab} \pm 0.09$	$2.48^{b} \pm 0.21$	$3.42^{a} \pm 0.43$
	144 h	$0.41^{ab} \pm 0.09$	$0.07^{c} \pm 0.02$	$3.81^{a} \pm 0.47$
	192 h	$0.57^{a} \pm 0.02$	$4.96^{a} \pm 0.46$	$3.19^{a} \pm 0.09$
Control		$0.19^{b} \pm 0.01$	$0.89^{b} \pm 0.02$	$0.33^{b} \pm 0.01$
800ppm & 40/30 ˚C	48 h	$0.36^{b} \pm 0.02$	$0.99^{b} \pm 0.03$	$1.19^{b} \pm 0.06$
	96 h	$0.57^{b} \pm 0.07$	$2.18^{a} \pm 0.38$	$0.66^{b} \pm 0.06$
	144 h	$1.57^{a} \pm 0.23$	$1.99^{a} \pm 0.07$	$1.36^{b} \pm 0.08$
	192 h	$1.63^{a} \pm 0.18$	$0.87^{b} \pm 0.20$	$4.69^{a} \pm 0.44$
Control		$0.19^{b} \pm 0.01$	$0.89^{b} \pm 0.02$	$0.33^{b} \pm 0.01$
800ppm & 45/35 ˚C	48 h	$0.57^{a} \pm 0.02$	$0.07^{b} \pm 0.01$	$2.10^{a} \pm 0.30$
	96 h	$0.14^{b} \pm 0.02$	$3.62^{a} \pm 0.16$	$1.21^{ab} \pm 0.08$
	144 h	$0.50^{a} \pm 0.03$	$0.07^{b} \pm 0.02$	$0.80^{b} \pm 0.36$
	192 h	$0.55^{a} \pm 0.04$	$3.96^{a} \pm 0.37$	$0.80^{b} \pm 0.11$

4.4 Discussion

Phytocompounds possess considerable bioactivity which contributes to medicinal plant therapeutic properties. Environmental factors can influence the synthesis and changes in concentration of these compounds, and furthermore, the pharmacological activities (Adeleye and Risenga, 2022). The current study assessed the changes in the phytochemical profile and antioxidant activities of *B. frutescens*, *B. abyssinica*, and *B. natalensis*. The preliminary phytochemical screening showed that the leaves of all three species had the greatest presence of compounds in all treatments, compared to their respective underground organs. There is a great deal of importance in conducting screening assessments, as they provide notable information about the occurrence of different classes of compounds (Sapkota *et al*., 2024). Leaves are the primary organs of plant growth and development, through the process of photosynthesis. Being the most exposed plant organ, leaves also offer temporary storage of secondary metabolites (Li *et al*., 2020). The duration of exposure to abiotic conditions also plays its part in the fluctuation of the metabolite profile across the whole plant. The increase in CO_2 concentrations can result in the uneven accumulation of carbon-based compounds (Basson *et al*., 2024). Temperature also has similar effects. Mashigo *et al* (2023) investigated the effects of high and low temperatures on the qualitative phytochemical screening of *Carpobrotus edulis* leaves over a six-day exposure period. Their findings showed that most compounds, such as phenolics, terpenoids, and steroids, retained a strong presence when *C. edulis* was exposed to low (15/10 ˚C) and high (45/35 ˚C) temperatures. Some compound groups such as tannins and flavonoids showed slight changes in their presence under both conditions.

The quantitative phytochemical content showed an exchangeable quantity of the various tested compounds among the plant parts of *B. abyssinica* and *B. frutescens* under different treatments of concurrent elevated CO_2 and temperatures. *B. natalensis* subterranean organs however, yielded higher phyto-content than its leaves. As a geophyte, *B. natalensis* utilises its corms as

a primary storage unit for C-based compounds such as carbohydrates, and most secondary metabolites (Tribble *et al*., 2021). This would also play a beneficial role in the species' adaptive mechanism against abiotic stressors. Another discovery made was the quantity of total tannins and total phenolics were greater in all three screened *Bulbine* species, compared to total flavonoids and proanthocyanidins. Polyphenols are the largest group of secondary metabolites in plants, and they are involved in the morpho-physiological processes of plant species (Dehghanian *et al*., 2022). Phenolic compounds also contribute to plant defence and protection against the environment, herbivores, and pathogens (Lattanzio, 2021; Pacheco-Ordaz *et al*., 2018; Dehghanain *et al*., 2022). The second most abundant group of polyphenols are the tannins, which also aid in plant defence, especially abiotic factors, including ultra-violet radiation, heat, and drought (Dehghanian *et al*., 2022). Both of these compounds also mitigate oxidative stress by counteract the effects of reactive oxygen species (Mohammed and Manan, 2015). The accumulation of the phytocompounds in *B. frutescens*, *B. abyssinica*, and *B. natalensis* under elevated CO_2 and temperatures alludes to the production of novel compounds which suggest that the pharmacological properties of the species could be greatly enhanced.

Abiotic stress is known to induce oxidation in plants. This is a phenomenon described by Sulaiman *et al* (2013) as "the imbalanced state where excessive quantities of ROS and/or reactive nitrogen species overshadow the endogenous antioxidative capability of the cells which stimulates the oxidation of macromolecules." Reactive free radicals such as hydroxyls, singlet oxygen, superoxide anion, and lipid peroxides, are by-products of the functional activities occurring in aerobic organisms (Lourenco *et al*., 2019). Plants produce antioxidants in the form of secondary metabolites, in order to prevent excessive build-up of oxidative by-products from the metabolic processes. This brings about redox homeostasis in the antioxidant system of plants. In the previous section, it was mentioned that the phytochemical content in *B. frutescens*, *B. abyssinica*, and *B. natalensis* mostly increased higher than their control

samples under the short-term exposure to simultaneous elevated CO_2 and temperature. Phytocompounds are responsible for thee antioxidant potential of medicinal plants (Adeleye and Risenga, 2022), and their accumulation is assumed to improve their radical-quenching abilities, further implicating an improvement in their health-promoting properties. Overall, the results from the in-vitro antioxidant activity assays revealed that H_2O_2 radicals were the most scavenged by all three *Bulbine* species under elevated CO_2 and high temperatures. Hydrogen peroxide is commonly produced in peroxisomes, chloroplasts, mitochondria, and vacuoles (Smirnoff and Arnaud, 2018), especially during photosynthesis. It is said to be the most stable compound in comparison to other radicals (Asaeda *et al*., 2018) that is involved in signal transduction, which is crucial for developmental processes such as plant cell wall reinforcement via lignification, stomatal control, and root hair development (Dempsey and Klessig, 1995; Saxena *et al*., 2016). Abiotic stress has been shown to exacerbate the concentration of H_2O_2 to the point of toxicity, initiating apoptosis in plants (Saxena *et al*., 2016). This indicates a steady improvement in the species' antioxidant activity. Some of the harvest periods did reveal a weaker scavenging activity, especially against DPPH radicals throughout the species plant parts. Some secondary metabolites are precursors to the antioxidant activity of plant species. Their sudden increase in the face of abiotic conditions could also pose negative effects, such as catalysing the oxidative process, putting strain on the antioxidant system (Indumathy, 2024). The study provides evidence on how *B. frutescens*, *B. abyssinica*, and *B. natalensis* adapt to climate change in our near future.

4.5 Conclusion

Climate projections show that the levels of atmospheric CO_2 will increase to nearly 1000 ppm by the end of the 21st century. The rise in CO_2 could also influence the changes in temperatures,

and both factors could affect the phytochemical content and oxidative radical scavenging potential ability of medicinal plant species. The current study investigated the effects of concurrent elevated CO_2 and temperatures on the phytochemical profile and antioxidant activities of *B. frutescens*, *B. abyssinica*, and *B. natalensis* over a 192-hour exposure period. The findings showed that the phytochemical profile had improved from most harvest periods under each treatment, which indicates a positive adaptive response against the environmental conditions. All species were able to excellently scavenge hydrogen peroxide radicals, but a good activity overall against DPPH and iron radicals. The underground organs had greater accumulation of phytocompounds compared to the leaves, as well as a better antioxidant potential. The improvement of both parameters under concurrent elevated CO_2 and temperatures does provide evidence on the possible changes in the defence mechanisms of each species. Considering that all the test subjects are succulents, they already possess morphological modifications enabling them to withstand harsh conditions. Understanding the secondary metabolic system supplements information that contributes to their overall physiology. Furthermore, their beneficial properties could potentially improve in the future, allowing for stronger medicinal activity, with the possibility of healing more ailments outside of their ethnomedicinal uses.

4.6 References

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CHAPTER FIVE

General Discussion

5.1 Discussion

Plant-based traditional medicine involves the application of various plant parts, such as flowers, fruit, leaves, bark, stems, and roots, which are used in homeopathic treatment of ailments (WHO, 2001; Phua et al., 2009; Ozioma and Chinwe, 2019). Traditional health practitioners often prescribe herbal medicine in different forms, including tinctures, infusions, extractions, decoctions, and other miscellaneous method (Ozioma and Chinwe, 2019). There are several reasons in which traditional medicine is used, especially among the Black demographic in South Africa. They are accessible, readily available, and affordable, and are often used for ethnically spiritual reasons (Mothibe and Sibanda, 2019). It is because of ethnomedicine that research on the therapeutic properties of medicinal plants is possible, with the intention of scientifically validating their used. This is done through investigating the action of phytochemicals, which are responsible for carrying out these properties (Dawang *et al.,* 2016; Ozioma and Chinwe, 2019). Phytochemicals consist of various structurally and functionally diverse compounds such as phenols, terpenoids, alkaloids, flavonoids, and tannins (Teoh, 2015; Reshi *et al.,* 2023). Their primary function is to assist plants in adapting to their surrounding environment by protecting them against stressors such as drought, herbivores, pathogens (Xu *et al.,* 2023), salinity, radiation, and temperatures (Reshi *et al.,* 2023).

Extreme temperatures are linked to an increase in the production of ROS, which induces oxidative damage (Shohael *et al,* 2006; Qaderi *et al.,* 2023). This could possibly be related to the accumulation of secondary metabolites as an adaptive response. Elevated CO_2 has been reported to minimize the effects of harsh environmental conditions. Processes such as photosynthesis are enhanced, which produces a higher concentration of carbon-based compounds that are channelled into secondary metabolic pathways (Becker and Klaring, 2016; Qaderi *et al.,* 2023). The exposure period to elevated CO_2 could also affect the response of

secondary metabolism in plants (de Rezende *et al.,* 2015; Qaderi *et al.,* 2023). It has been noted that secondary metabolites decrease under elevated CO_2, inhibiting certain metabolic activities, which could hinder biosynthetic pathways (Buchner *et al.,* 2015). This however, is based on the effect of elevated CO_2 alone, without any pairing of other abiotic factors. In this study, the effects of concurrent elevated CO_2 and temperatures on the phytopharmacological properties of *B. frutescens*, *B. abyssinica*, and *B. natalensis* were investigated. There was a higher presence of phytochemicals detected in the leaves from each species. Under elevated conditions, the quantitative phytochemical content increased at most harvest periods across all plant organs of each species. Abiotic conditions are said to influence the increased production of secondary metabolites, significantly affecting plant metabolism and shifting their chemical profile to favour plant survival over vegetative and reproductive development (Yang *et al.,* 2018).

The increase in the phytochemical content has also shown an improved antioxidant activity in most treatments of elevated CO_2 and temperatures. Secondary metabolites form part of the non-enzymatic antioxidant system, and are responsible for regulating the excessive production of ROS in the face of extreme conditions (Concato *et al.,* 2022). The qualitative agar-well diffusion antibacterial activity results had shown an overall intermediate activity against Gram-positive *S. aureus* and Gram-negative *E. coli*. This provides insight on how crude extracts exhibit antibacterial potential with an unknown concentration, similar to how traditional medicine is administered. Under elevated CO_2 and temperatures, the crude extracts of species' plant parts all had the same inhibitory effects. The MIC antimicrobial assay revealed how the inhibitory activity of the crude extracts vary at different concentrations against several bacterial and fungal pathogens. The evidence can be linked to the changes in the phytochemical profile at various exposure periods, influencing the antimicrobial activity.

Often, medicinal plant research involves the assessment of aerial plant parts and seldom on other vegetative organs. It is necessary to document the whole plant as the medicinal activity varies with the plant organ utilised. Climate change could also affect the chemical profile of the whole plant, possibly altering the medicinal plant activity of many species. As medicinal plants are more vulnerable to the environment, climate studies, whether simulated or natural, are important to take record of the effects abiotic stress for future reference. This could help prepare alternative methods for sustainable harvesting and cultivation via tissue culture, for the preservation of species biodiversity. Due to the rise in the commercialisation of medicinal plants in South Africa since the early 1990s, many products have been manufactured and distributed in various retail stores and pharmacies nationwide (Semenya and Potgieter, 2014; Mothibe and Sibanda, 2019). This study also reports on how abiotic factors can manipulate the bioactivity of the species. Pharmaceutical industries can utilise the findings from the study to cultivate plants in controlled environments, with the goal to extract desirable compounds of interest and test their pharmacological properties for the production of various medicines. This will also substitute synthetic products which are known to impose a multitude of side effects.

5.2 Conclusion and recommendations

There have been numerous studies reporting on the effects of environmental conditions on medicinal plant species. However, studies involving the combined impact of multiple stressors are limited. Additionally, South African medicinal plants have received little attention in the context of climate change. The current study reported on the influence of concurrent elevated CO_2 and temperatures on the phytopharmacological activities of three Bulbine species. Various plant parts of each species have displayed an altered phytochemical profile as an adaptive response to the combined stressors over an eight-day period. Significant improvements in the

phytochemical profile under elevated conditions has been reported across all species, supporting this hypothesis. This also reflected on their antioxidant and antimicrobial activities. All species had the greatest antioxidant activities against hydrogen peroxide and iron radicals, compared to DPPH, which was moderately scavenged in most treatments. The species' antimicrobial activities showed considerable improvement in a fairly good amount of harvest periods, whereas some exhibited a standard activity. Since most of the pharmacological properties of each species had improved under elevated conditions, the hypothesis based on this factor, was not proven correct. Overall, the findings show that the species would able to retain their properties in a changing environment. Contributions of this study to the Bulbine species have shown how CO_2 and temperatures could affect their phytopharmacological properties, with potential ecological and evolutionary implications for the species' sustainability in the long run.

Future recommendations should aim at the characterization of the phytochemical compounds under abiotic conditions. The accumulation of compounds due to climate change could yield novel compounds which could further explain the quantitative concentrations reported, as well as their influence on the species' ethnopharmacological uses. The discovery of such novel compounds could also lead to their pharmacological evaluation and application in pharmaceutical products. Genetic analysis could also reveal how these phytocompounds accumulate through their biosynthetic pathways. This could further deduce any ecological and evolutionary reasons to the species' resilience in natural environments. Comparisons through long-term exposure and field-based research could lead to more discoveries on Bulbine. The increase in the phytochemical profile may also result in an increase in the toxicity of the species. Some of the compounds induce a high level of toxicity which may go against the beneficial uses of the species. Performing a toxicity assessment would help to find ways to control and regulate toxic levels. Continual research needs to be conducted on all vegetative

plant parts so that more information regarding the species' pharmacological uses are documented.

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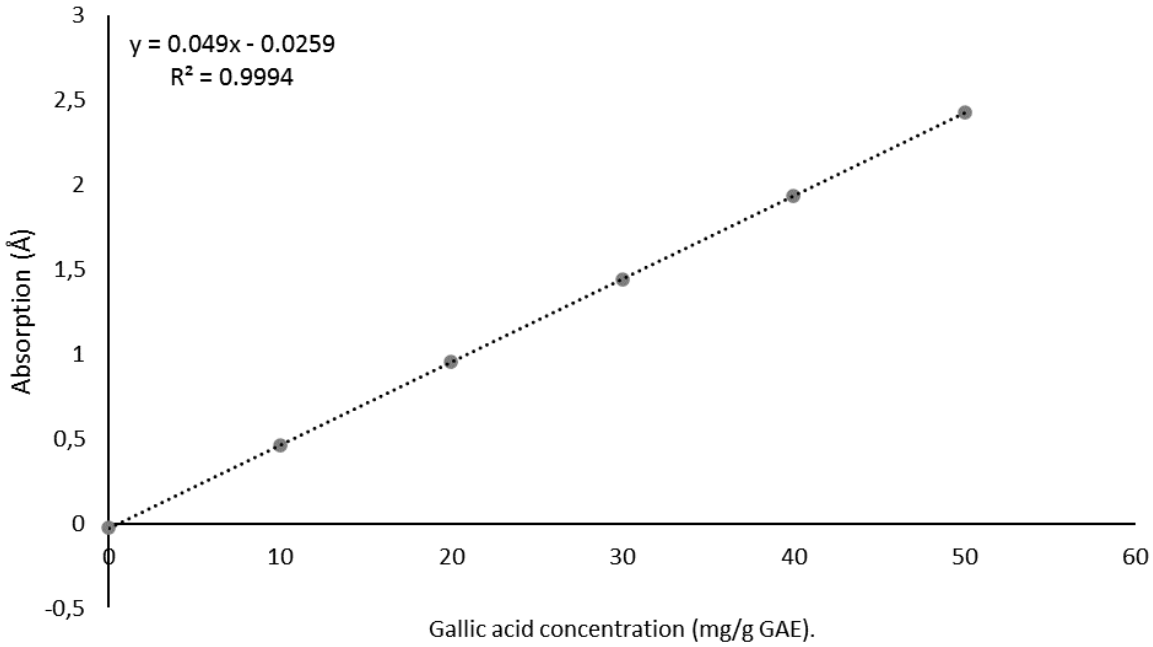
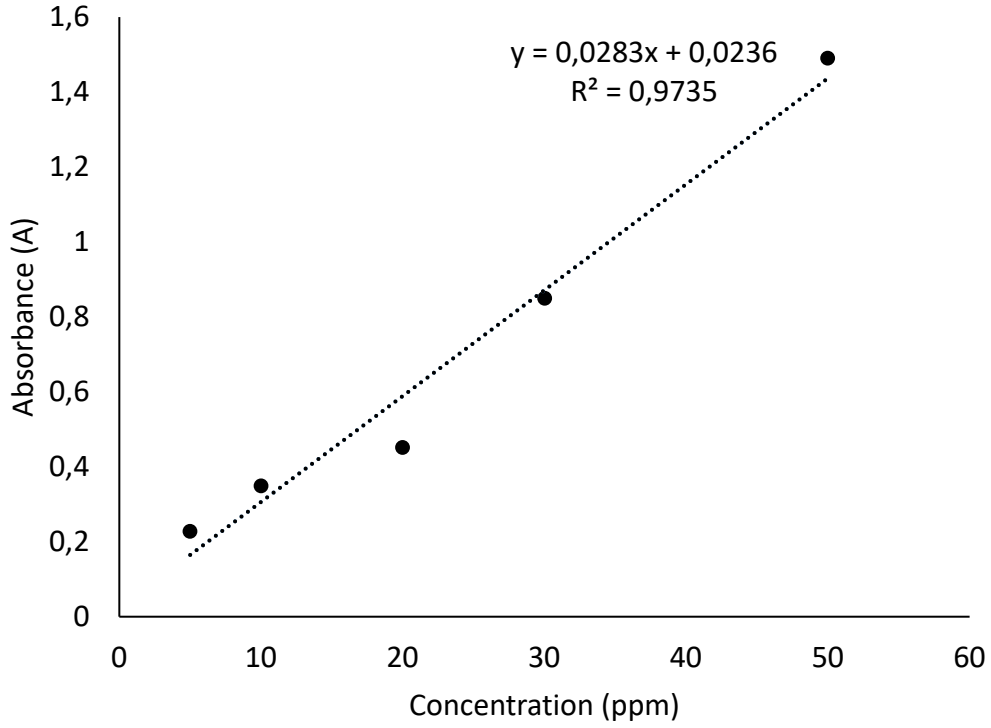
Appendices

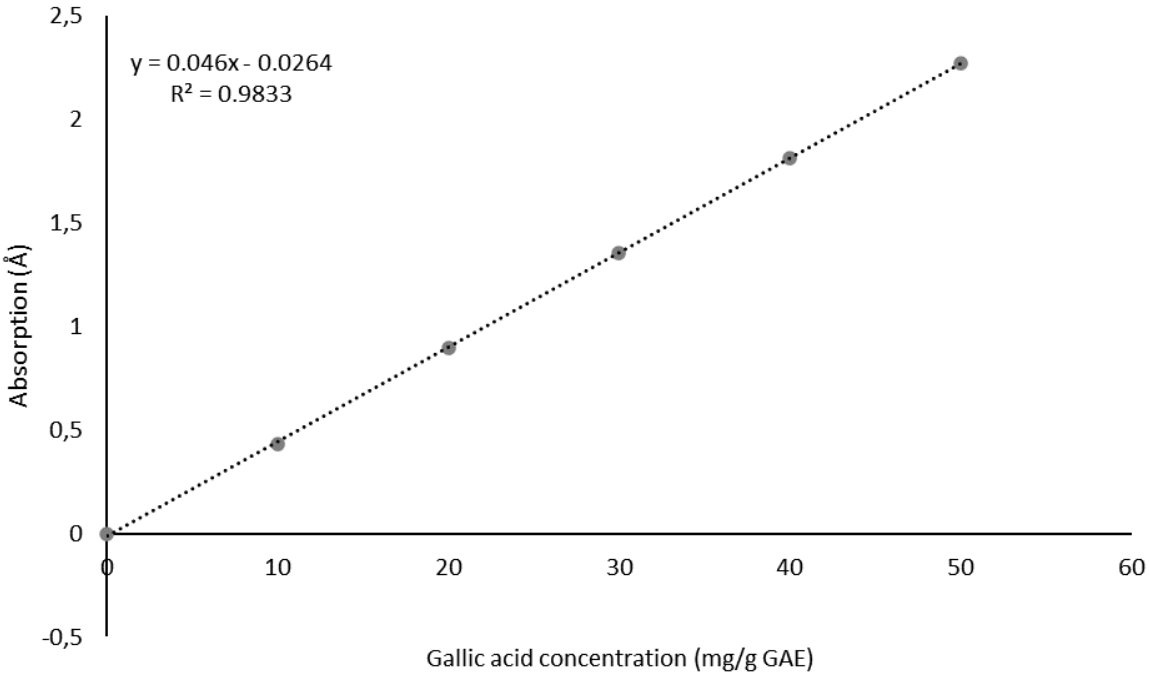
Appendix A: Gallic acid calibration curve for total phenolic content.



Appendix B: Quercetin calibration curve for total flavonoid content.



Appendix C: Gallic acid calibration curve for total tannin content.



Appendix D: Catechin standard calibration curve for total proanthocyanidin content.