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THE EFFECT OF SALT STRESS ON THE NUTRACEUTICAL, PHYSIOLOGICAL AND PHYTOCHEMICAL PROPERTIES OF *TRACHYANDRA CILIATA* (L.f.) Kunth: AN EDIBLE HALOPHYTE FROM THE WESTERN CAPE, SOUTH AFRICA

by

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Thesis submitted in fulfilment of the requirements for the degree

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Bellville

2025

DECLARATION

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

Sihle Ngxabi	**19 May 2025**
Signed	**Date**

ABSTRACT

Water scarcity and high salinity in agricultural lands pose serious threats to food security amid exponential population growth. This necessitates the cultivation of salt and drought tolerant plant species as alternatives to mainstream vegetable crops. *Trachyandra ciliata* (L.f.) Kunth is a wild underutilized halophyte that is endemic to the Western Cape coastal dunes in South Africa. *Trachyandra* genus belongs to Asphodelaceae (Aloe) family, which is widely known for its pharmaceutical importance. The literature on this plant is limited to non-existent, and there are currently no *Trachyandra* species in commercial cultivation. However, it is known that this plant is edible and that Khoe-San people who lived around the South African Cape coast utilized it as food, although its edibility and nutritional composition remain undocumented. The only study on this plant focused on the vegetative growth of *T. ciliata* in response to different growth media and salinity to develop its growth protocol. However, its precise salt tolerance mechanisms are unexplored. Furthermore, the presence of some antioxidants in the plant especially when subjected to salt stress has been reported. This prompts studies on the pharmacological potential of this plant amid global quest to discover more plant-based pharmaceuticals for the treatment of chronic diseases. Thus, this study was carried out to evaluate the nutraceutical, phytochemical, and physiological properties of *T. ciliata* under varying degrees of salinity to promote its consumption, therapeutic use, and commercialization.

The propagation experiment was carried out at the greenhouse nursery of the Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa. Plants were subjected to five salinity (NaCl) concentrations (0, 50, 100, 150, and 200 mM). After 15 weeks of salinity treatments, plants were harvested, dried, and pulverized for extraction and further analyses.

Chapter One introduces the general overview of the research, which includes significance of the research, its aims, and the overall list of objectives, which guided the study. On the other hand, Chapter Two explored nutraceutical, agricultural, and economic potential of *Trachyandra ciliata* through extensive review of literature. This chapter unravels the overall potential of *T. ciliata* as a food source and a therapeutic agent for the treatment of chronic diseases. It further discusses the potential salt tolerance mechanisms and the potential of *T. ciliata* in desalination and phytoremediation.

Chapter three explored the effect of salinity stress on growth parameters, leaf hydration, photosynthetic pigmentation, cation content, oxidative stress markers and concentration of antioxidant enzymes to understand precise mechanisms involved in its salt tolerance. Results revealed that salinity positively influenced growth parameters in all plant parts at low concentration (50 mM NaCl) compared to the control. Moreover, the total chlorophyll content negatively correlated with increasing salinity. *T. ciliata* maintained equivalent Relative Water Content (RWC) from control to 100 mM treatment, which then efficiently decreased with

increasing salinity, while the plant maintained an unchanged leaf succulence among all treatments. On the other hand, high salinity stimulated oxidative stress as indicated by high MDA, cell death, and superoxide radicals, which were more expressive under 200 mM treatment. To counter the catastrophic effects of excessive ROS, *T. ciliata* activated antioxidative defence mechanisms as indicated by high SOD and CAT antioxidative enzymes which were more prevalent at high salinity (200 mM). Furthermore, the high proline content under higher salinity treatments ensured further scavenging of ROS.

Chapter four examined the leaf surface and cross-sectional properties, elemental composition and anatomical responses in *T. ciliata* using Scanning Electron microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) to clarify salt tolerance mechanisms in *T. ciliata*. From the SEM micrographs, salt glands were observed protruding from the epidermis along the vascular system under low salinity and salt crystals appeared under higher concentrations, which makes this plant maintain cellular homeostasis under high salinity, and the plant can be classified as a recretohalophyte. In addition, stomatal distribution, stomatal density and the number of open stomata decreased with increasing salinity. EDX revealed the presence of some important elements such as Potassium, Magnesium, Phosphorus, Calcium and more in the leaves. The results showed that increased salinity led to a decrease in the percentage composition of P, K and Ca^{2+}, while Mg^{2+} was high under control and low salinity (50 mM), decreased under 100 mM and increased again with increasing salinity. On the contrary, increasing salinity caused an increase in Na^{+} and Cl^{-} in a stable manner.

Chapter five investigated the edibility of *T. ciliata* by exploring its proximate, antinutrient, mineral, and phytochemical composition when cultivated under different levels of salinity. Salinity significantly influenced the mineral, proximate, antinutrient, and phytochemical composition of *T. ciliata*. Control and 50 mM treatments recorded significantly higher macro and micronutrient content in the flower buds and leaves, except for heavy metals such as Zn and Cu, which increased with increasing salinity and significantly higher in the roots. Leaves under low salinity treatments recorded higher moisture and protein content, while leaves also recorded higher ash content under high salinity. On the other hand, flower buds under low salinity recorded significantly high fat and NDF composition. Phytochemicals and antinutrients increased with increasing salinity concentrations. The low antinutrient content and high nutritional, mineral and phenolic contents validate the edibility and suitability of *T. ciliata* for human consumption.

Chapter 6 quantified and characterised phytochemicals present in leaves, roots, and flower buds of *T. ciliata* through UHPLC-MS to discover novel bioactive compounds with potential therapeutic applications. The UHPLC-MS identified 71 compounds, which were grouped into flavonoids, anthocyanins, alkaloids, nucleobase, nucleosides/tide, saccharides, fatty acids, amino acids, and coumarins. The diverse identified compounds indicate that the extracts of *T. ciliata* may have biological activities against chronic diseases, including diabetes, oxidative

stress, neurodegenerative disorders, cancer, cardiovascular diseases, and gastrointestinal disorders. Results from this study further show the potential of this plant in the treatment of other ailments such as skin problems, viral diseases, inflammation, oxidative stress, as well as plant plant-based food additives and preservatives.

Chapter 7 evaluated crude extracts of *T. ciliata* as a natural therapeutic agent for the amelioration of cancer, acetylcholinesterase inhibitory activity, and ROS scavenging activity in the liver for the first time. The yellow dye 3-(4,5-dimethyltiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), Ellman's colorimetric method, and the 2',7'-dichlorodihydrofluorescein diacetate assay (H2DCF-DA) were respectively employed to evaluate cytotoxicity, acetylcholinesterase, and ROS scavenging activity of the plant extracts. Results revealed that flower bud extracts prepared from 0 mM and 100 mM salinity treatments at 1mg/mL concentration showed strong cytotoxicity to cancer cells, while they had moderate and weak cytotoxicity to non-cancer cells respectively. All extracts showed high acetylcholinesterase inhibitory activity, except for root and flower bud extracts from 100 mM salinity treatment. Moreover, ROS scavenging activity was mainly observed in the leaf extracts from all treatments, and in the root extract from 0 mM salinity treatment. These findings suggest that *T. ciliata* could be a therapeutic agent for the treatment of cancer, Alzheimer's disease, and liver disorders amidst global quest to develop more plant-based pharmaceuticals for the treatment of chronic diseases.

From the results gathered in this study, *T. ciliata* is recommended for human consumption, domestication and commercial cultivation to mitigate the looming food shortage and hidden hunger due to increasing population and water scarcity. Findings from this study serve as points of reference to commercial farmers, pharmaceutical industries, medical practitioners, communities, scholars, policy makers, and aspiring researchers whose interests are on the potential of easily accessible underutilized wild edible crops to develop new strategies to address global issues concerning human health and wellness.

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This dissertation represents not just years of academic effort, but a deep journey of personal growth, resilience, dedication, and discovery. This work holds a profound meaning to me as it reflects the culmination of countless hours of learning, questioning, and striving against the odds. As I reflect on this milestone, I am reminded of the people whose support, guidance, and belief in me made this achievement possible. I am sincerely grateful to those who stood by me throughout this journey.

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DEDICATION

This work is dedicated to **my younger self:**

Thank you for your strength, your courage, your limitless dreams, and unwavering positive attitude. In a world where many who face the adversities you faced tend to give up too soon, you chose to hold on, to believe more, and to keep going. Your resilience and strong mentality carried us through the darkest days and made this achievement possible. This is for you.

PREFACE

The thesis is drafted differently from the alternative of a conventional format for a thesis. This article-format thesis comprises of published, co-published, and/or “ready-for-publication” articles that were prepared during candidature and applies to the format prescribed by CPUT for 100% doctoral studies. The chapters/articles in this thesis are drafted in accordance with the journals they are published in/intended to be published in.

The thesis comprises of the following chapters, which are concisely discussed as:

Chapter One: This chapter introduces the general overview of the research, which includes significance of the research, its aims, and the overall list of objectives, which guided the study.

Chapter Two: This is a review paper titled “Nutraceutical, agricultural, and economic potential of *Trachyandra ciliata* (Wild cabbage), an under-utilized halophyte from the Western Cape, South Africa”. Through extensive review of literature, this chapter unravels the overall potential of *Trachyandra ciliata* as a food source and a therapeutic agent for the treatment of chronic diseases. It further discusses the potential salt tolerance mechanisms and the potential of *T. ciliata* in desalination and phytoremediation. This chapter has been published as a book chapter in “Food Security and Nutrition: Utilizing Undervalued Food Plants” published by Taylor and Francis (2025).

Chapter Three: This is a research article titled "Salinity Influenced growth, physio-biochemical responses, and antioxidative potential of *Trachyandra ciliata* (L.F) Kunth (Wild cabbage)” which has been submitted for publication in a peer-reviewed journal and is available as a preprint. This chapter provides crucial information on the effect of salinity stress on growth parameters, leaf hydration, photosynthetic pigmentation, cation content, oxidative stress markers and concentration of antioxidant enzymes to understand precise mechanisms involved in its salt tolerance.

Chapter Four: This is a research article titled "Leaf Micromorphological assessment, chemical composition and anatomical responses of *Trachyandra ciliata* (L.F) Kunth to different degrees of salinity” which has been published in “Russian Journal of Plant Physiology”. This chapter examined the leaf surface and cross-sectional properties, elemental composition and anatomical responses using Scanning Electron microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) to clarify salt tolerance mechanisms in *T. ciliata*.

Chapter Five: This is a research article titled "Salinity influenced proximate, minerals, anti-nutrients and phytochemical composition of *Trachyandra ciliata* Kunth (Wild Cabbage): A

promising edible halophyte" which is has been published in "Food Science & Nutrition". This chapter validated the edibility of *T. ciliata* by exploring its proximate, antinutrient, mineral, and phytochemical composition when cultivated under different levels of salinity to promote its cultivation among South African households.

Chapter Six: This is a research article titled "Phytochemical profiling of *Trachyandra ciliata* (L.F) Kunth cultivated under varying degrees of salinity" which was combined with chapter seven and published in "Phytomedicine Plus". This chapter identified and quantified phytochemicals present in leaves, roots, and flower buds of *T. ciliata* to discover novel bioactive compounds with potential therapeutic applications.

Chapter seven: This is a research article titled "Cytotoxicity, Acetylcholinesterase inhibitory activity, and Liver ROS scavenging activity of *Trachyandra ciliata* (Wild cabbage) grown under varying degrees of salinity" which was combined with chapter six and published in "Phytomedicine Plus". For the first time, this chapter evaluated crude extracts of *T. ciliata* as therapeutic agent for the amelioration of cancer, Acetylcholinesterase inhibitory activity, and ROS scavenging activity in the liver.

Chapter eight: General conclusions, recommendations and prospects. This chapter sums up important findings from all other chapters and paves a way for future studies.

RESEARCH OUTPUTS

The following research outputs recognise the candidate's contributions to scientific knowledge and advancement during the PhD program.

Published manuscripts

1. Ngxabi, S., Jimoh, M.O., Sogoni, A., Laubscher, C.P. & Kambizi, L. 2025. Nutraceutical, Agricultural, and Economic Potential of *Trachyandra ciliata* (Wild Cabbage), an Underutilized Halophyte from the Western Cape Province, South Africa. In C. Bvenura & L. Kambizi, eds. ***Food Science and Nutrition: Utilizing Undervalued Food Plants***. Boca Raton: CRC Press: 273–290. https://doi.org/10.1201/9781003469766

2. Ngxabi, S., Jimoh, M.O., Laubscher, C.P. & Kambizi, L. 2024. Leaf Micromorphological Assessment, Chemical Composition and Anatomical Responses of *Trachyandra ciliata* (L.F) Kunth to Different Degrees of Salinity. ***Russian Journal of Plant Physiology***, 71: 112. https://doi.org/10.1134/S1021443723603695

3. Ngxabi, S., Jimoh, M.O., Sogoni, A., Laubscher, C.P., Rautenbach, F. & Kambizi, L. 2025. Salinity Influenced Proximate, Minerals, Anti-Nutrients and Phytochemical Composition of *Trachyandra ciliata* Kunth (Wild Cabbage): A Promising Edible Halophyte. ***Food Science and Nutrition***, 13(1). https://doi.org/10.1002/fsn3.4755

4. Ngxabi, S., Jimoh, M. O., Sogoni, A., Barker, A. M., Keyster, M., Kambizi, L., & Laubscher, C. P. (2025). Salinity Influenced Growth, Physio-Biochemical Responses, and Antioxidative Potential of Trachyandra ciliata Kunth (Wild Cabbage). *Russian Journal of Plant Physiology*, *72*(5), 162. https://doi.org/10.1134/S1021443725602599

5. Ngxabi, S., Sogoni, A., Kerebba, N., Pieters, R., Horn, S., Giesy, J.P., Kambizi, L., Laubscher, C.P. & Jimoh, M.O. 2025. Metabolite profiling, cytotoxicity, liver ROS detoxifiers and acetylcholinesterase inhibitors from Trachyandra ciliata L.F. (Kunth) (wild cabbage). ***Phytomedicine Plus***, 5(4): 100908. https://linkinghub.elsevier.com/retrieve/pii/S2667031325001794

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CHAPTER 5: SALINITY INFLUENCED PROXIMATE, MINERALS, ANTI-NUTRIENTS AND PHYTOCHEMICAL COMPOSITION OF *TRACHYANDRA CILIATA* KUNTH (WILD CABBAGE): A PROMISING EDIBLE HALOPHYTE

CHAPTER 7: CYTOTOXICITY, ACETYLCHOLINESTERASE INHIBITORY ACTIVITY, AND LIVER ROS SCAVENGING ACTIVITY OF *TRACHYANDRA CILIATA* (WILD CABBAGE) GROWN UNDER VARYING DEGREES OF SALINITY

CHAPTER 8: GENERAL CONCLUSIONS, RECOMMENDATIONS AND PROSPECTS

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LIST OF ACRONYMS AND ABBREVIATIONS

ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ACh	Acetylcholine
AChE	Acetylcholinesterase
AChEIs	AChE Inhibitors
AD	Alzheimer's disease
ANOVA	Analysis of variance
CAT	Catalase
DPPH	2,2-diphenyl-1-picrylhydrazyl ethanol
EDX	Energy Dispersive X-Ray Spectroscopy
EtOH	Ethyl Alcohol
FRAP	Ferric Reducing Antioxidant Power
H_2O_2	Hydrogen Peroxide
LSD	Least Significant Difference
MDA	Malondialdehyde
NaCl	Sodium chloride
NDF	Neutral Detergent Fibre
RDA	Recommended Daily Allowance
RT	Retention Time
RWC	Relative Water Content
ROS	Reactive Oxygen Species
SE	Standard Error
SEM	Scanning Electron Microscopy
SOD	Superoxide Dismutase
UHPLC-MS	Ultra-High Performance Liquid Chromatography-Mass Spectrometry

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CHAPTER ONE

GENERAL INTRODUCTION, PROBLEM STATEMENT, AIMS, OBJECTIVES, HYPOTHESIS, AND DELINEATION

Part of this chapter is published in *Food Security and Nutrition* (Taylor and Francis)

Part of this chapter is published in *Food Security and Nutrition*

General Introduction, Problem Statement, Aims, Objectives, Hypothesis, and Delineation

1.1 General introduction

The debate over global water scarcity and food security has recently been more heated, and it's uncertain just how much food and water will be needed in the future (Hanjra & Qureshi, 2010). About 80% of the world's agricultural land, 1.1 billion hectares out of 1.5 billion hectors are rain-fed, producing 60% of the world's staple foods without any irrigation systems (Schultz & De Wrachien, 2002; FAO, 2008). According to reports, 19% of all cultivated land is irrigated, which accounts for around 40% of all agricultural output worldwide. Surprisingly, although contributing relatively little to global food supply, irrigated agricultural land is accountable for 70% of water withdrawals from rivers (Molden et al., 2007).

By 2050, the Sustainable Development Goal 2 (SDG2) aims to eradicate hunger, boost food security and nutrition, and encourage the cultivation of crops sustainably (Hamed & Custódio, 2019). However, food production has been significantly impacted by climate change, increased soil salinization, and a lack of access to fresh water, notably in South Africa, making it challenging to reach the desired goal (Acquaah, 2009; Corwin, 2021). Salinity restricts plant growth and causes a drop in overall yield, decreased leaf formation, a delay in flowering, and the abortion of flower buds. Therefore, ensuring sustainability in terms of the future supply of food for poor communities and the protection of water resources presents a significant problem for scholars. The use of wild salt-tolerant plants (halophytes), which have considerable commercial values such as green vegetables, feed crops, and medicinal properties, is one recently identified method to adapt to these environmental conditions (Ventura & Sagi, 2013).

Water is crucial for future global food security because water shortage lowers agricultural output, which contributes to the rising trend of food security issues (Hamed & Custódio, 2019; Ebert, 2014). This therefore means there is a connection between water scarcity and food poverty. Researchers predict that the world's population will increase by nearly two billion people within the next two to three decades, necessitating the use of more of the water supply for domestic, municipal, industrial, and environmental purposes (Condon et al., 2004; World Health Organization, 2015). It is estimated that agricultural production must rise by 50 to 100% by 2050 in order to meet the demands of the world's constantly expanding population, but in reality, the present increase is just 1 to 5%, making it extremely difficult to ensure sustainable agriculture (Ngxabi et al., 2021b; World Health Organization, 2015). As a result, Government

efforts to achieve high productivity are ongoing in an attempt to boost profitability and satisfy the nation's rising food demand in response to this issue.

The use of salt-tolerant crops can be a useful alternative to investing time and money in remediating the effect of water scarcity and rising soil salinity (Ventura et al., 2011). Halophytes are the ideal crop candidates for salty environments. Recent developments in the food science sector and consumer preferences for varied diets point to the consumption of wild edible plants as diet complements as well as nutritious and useful foods for specific conditions, making commercial cultivation of these plants very crucial (Petropoulos et al., 2018; Nikalje et al., 2019). However, in South Africa most wild species are regarded as weeds for conventional crops, and farmers typically use chemical fertilizers to get rid of them (Łuczaj et al., 2012). Many of these wild edible species have been utilized by rural communities for many years as leafy greens and as the foundation of numerous traditional cuisines and regionally significant local recipes, although they are totally neglected in the commercial vegetable production (Petropoulos et al., 2018; Hamed & Custódio, 2019). Therefore, cultivation of wild halophytes presents a possible solution to support food security, while preserving water resources for domestic, municipal and environmental uses, and these developments need to be implemented at a national level to align the country to sustainable development goals (Ngxabi et al., 2021a).

Trachyandra ciliata is a wild underutilized halophyte that is endemic to the Western Cape coastal dunes in South Africa (Manning & Goldblatt, 2007). *Trachyandra* genus belongs to Asphodelaceae (Aloe) family, which is widely known for its pharmaceutical importance. The literature on this plant is limited to non-existent, and there are currently no *trachyandra* species in commercial cultivation (Manning & Goldblatt, 2007). However, it is known that this plant is edible and that Khoe-san people who lived around the South African cape coast utilized it as food, although its edibility and nutritional composition remain undocumented (De Vynck et al., 2016). The only available scientific study was undertaken by Sihle Ngxabi et al., (2021b) and it was only focused on the vegetative growth of *T. ciliata* in response to different growth media and saline conditions to develop its growth protocol. However, its precise salt tolerance mechanism is unexplored. Furthermore, they also reported the presence of some antioxidants in the plant especially when subjected to salt stress. This prompts studies on the pharmacological potential of this plant amid global quest to discover more plant-based pharmaceuticals for the treatment of chronic diseases. As such, this study was carried out to evaluate the nutraceutical, phytochemical, and physiological properties of *Trachyandra ciliata* under varying degrees of salinity to promote its consumption and commercialization.

1.2 Research problem statement

Water scarcity and high salinity in agricultural lands cause restrictions in plants, which decrease overall output, leaf production, delayed flowering, and flower bud abortion. To cater for the constantly increasing population, researchers predict that overall global food production needs to increase by 50 to 100% by 2050, although the current increase is around 1 to 1.5% annually. This then necessitates the cultivation of more salt and drought tolerant plant species (halophytes) for food and medicine to substitute the traditional vegetable crops that are currently under cultivation. In South Africa, native wild halophytes have been neglected and under-researched, although they have been gaining increasing attention in the past decade. Previous studies have identified *T. ciliata* as an edible wild halophyte that was used for food by the Khoisan people that used to live in the western Cape coastal areas before colonization and massive industrialization of the province. Previous research on this plant focused on determining its cultivation protocol also showed that indeed the plant is more productive under saline conditions, although its anatomic salt tolerance mechanisms, nutritional profile, biochemical and physiological properties remain unknown.

Chronic diseases are accountable for over 41 million deaths annually, which constitutes 74% of all fatalities worldwide, while millions more people live with chronic diseases and have a reduced standard of life. Chronic diseases that cause significant death and disability include neurological and mental health disorders, cancer, cardiovascular diseases, diabetes, substance use disorders (liver disorders), and gastrointestinal disorders, among others. Recently, there is a growing focus on these species due to their rich content of bioactive compounds, including primary and secondary metabolites like polyunsaturated fatty acids, vitamins, flavonoids, anthocyanins, alkaloids, nucleobases, nucleosides/tide, saccharides, amino acids, and coumarins amongst others. Despite the increasing global interest in exploring the medicinal properties of halophytes in recent years, South African halophytes have largely been overlooked as potential agents in the amelioration of chronic diseases.

1.3 Research aim

The aim of this research is to evaluate the nutraceutical, phytochemical, and physiological properties of *T. ciliata* under varying degrees of salinity to promote its consumption, pharmacological use, and commercialization.

1.4 Hypothesis

It is hypothesised that from the results gathered from all experiments, salinity will significantly influence the nutraceutical, phytochemical, and physiological properties of *Trachyandra ciliata* to support the cultivation, consumption, and commercialization of *T. ciliata*.

Null hypothesis (H_0): varying Salinity concentrations will not influence nutraceutical, phytochemical, and physiological properties of *Trachyandra ciliata.*

1.5 Objectives

1.5.1 Main objective

The aim of this research is to evaluate the nutraceutical, phytochemical, and physiological properties of *T. ciliata* under varying degrees of salinity to promote its consumption and commercialization.

1.5.2 Specific objectives

1. To provide the necessary insight on the Nutraceutical, agricultural, and economic potential of *T. ciliata* (Wild cabbage) through extensive review of literature.

2. To determine the influence of salinity on the growth parameters, physio-biochemical responses, and antioxidative potential of *T. ciliata*

3. To conduct leaf Micromorphological assessment, determine chemical composition and anatomical responses of *T. ciliata* to different degrees of salinity

4. To determine the influence of salinity on proximate, minerals, anti-nutrients and phytochemical composition of *T. ciliata*

5. To conduct phytochemical profiling of *T. ciliata* cultivated under varying degrees of salinity through UHPLC-MS

6. To determine Cytotoxicity, Acetylcholinesterase inhibitory activity, and Liver ROS scavenging activity of *T. ciliata* grown under varying degrees of salinity

1.6 Delineation of the research

NUTRIFEED™ fertiliser was used to prepare aqueous nutrient solution during greenhouse experiment. Salinity was applied in the form of Sodium Chloride (NaCl) and five concentrations (0, 50, 100, 150, and 200 mM) were tested.

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CHAPTER TWO

NUTRACEUTICAL, AGRICULTURAL, AND ECONOMIC POTENTIAL OF *TRACHYANDRA CILIATA* (WILD CABBAGE), AN UNDER-UTILIZED HALOPHYTE FROM THE WESTERN CAPE, SOUTH AFRICA

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Nutraceutical, agricultural, and economic potential of *Trachyandra ciliata* (Wild cabbage), an under-utilized halophyte from the Western Cape, South Africa

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Abstract

Water scarcity and high salinity in agricultural lands pose serious threats to food security amid exponential population growth. This then necessitates the cultivation of salt and drought tolerant plant species as alternatives to mainstream vegetable crops. Previous studies have identified *Trachyandra ciliata* (Wild cabbage) and other wild edible halophytes from Southern Africa as potential candidates for commercial vegetable cultivation as they are adapted to drought and salinity. These plants have been used in the past as major food sources. However, these plants have been neglected and are under-researched, with little to no literature at all. Thus, this review was carried out to explore nutraceutical, agricultural, and economic potential of *T. ciliata* to support its domestication, cultivation, and commercialization. This review provides the necessary insight on the economic potential of halophytes and *T. ciliata*, the potential to promote sustainable agricultural production, culinary innovations, preservation of traditional heritage, increase production in regions vulnerable to climate change, maximize production in contaminated soils, maintenance of biological diversity, and their potential in ethnomedicine. The study recommends that more resources should be made available for marketing, capacity building and awareness campaigns to farmers and communities to promote cultivation of these plants, which will lead to more income streams, job creation, and rural development. The study serves as a point of reference to commercial farmers, communities, scholars, policy makers, and aspiring researchers whose interests are on the potential of easily accessible underutilized wild edible crops to develop new strategies to address global issues.

Keywords: *Trachyandra ciliata*, Asphodelaceae, food security, sustainable agriculture, Salinity, climate change, halophytes, phytochemicals, chronic diseases

2.1 Introduction

The discussion over global water scarcity and food security has recently intensified, with uncertainties looming over the future demand for food and water (Hanjra & Qureshi, 2010). About 80% of the world's agricultural land, 1.1 billion ha out of 1.5 billion ha is rain-fed, producing 60% of the world's staple foods without any irrigation systems (Schultz & De Wrachien, 2002; FAO, 2008). According to reports, 19% of all cultivated land is irrigated, which accounts for around 40% of all agricultural output worldwide. Surprisingly, although contributing relatively little to global food supply, irrigated agricultural land is accountable for 70% of water withdrawals from rivers (Molden et al., 2007).

By 2050, the Sustainable Development Goal 2 (SDG2) aims to eradicate hunger, boost food security and nutrition, and encourage the cultivation of crops sustainably. However, food production has been significantly impacted by climate change, increased soil salinization, and a lack of access to fresh water, notably in South Africa (Acquaah, 2009; Corwin, 2021). This makes it a challenge to reach the desired goal. Salinity restricts plant growth and causes a drop in overall yield, decreased leaf formation, a delay in flowering, and the abortion of flower buds. Therefore, ensuring sustainability in terms of the future supply of food for poor communities and the protection of water resources presents a significant problem for scholars. The use of wild salt-tolerant plants (halophytes), which possess considerable commercial value such as green vegetables, feed crops, and medicinal properties is one recently identified method to adapt to these ever-changing environmental conditions (Ventura & Sagi, 2013).

Water is crucial for future global food security because water shortage lowers agricultural output, which contributes to the rising trend of food security issues. Therefore, this means that there is a connection between water scarcity and food poverty. Researchers predict that the world's population will increase by nearly two billion people within the next two to three decades, necessitating an increased water supply for domestic, municipal, industrial, and environmental purposes (Condon et al., 2004; Colvin & Muruven, 2017). It is estimated that agricultural production must rise by 50 to 100% by 2050 to meet the demands of the world's constantly expanding population, but in reality, the present increase is just 1 to 5%, making it extremely difficult to ensure sustainable agriculture (Colvin and Muruven, 2017; Ngxabi et al., 2021b). As a result, government efforts to achieve high productivity are ongoing in an attempt to boost profitability and satisfy the nation's rising food demand in response to this issue.

The use of salt-tolerant crops can be a useful alternative to investing time and money in remediating the effect of water scarcity and rising soil salinity (Ventura et al., 2011). Halophytes are the ideal crop candidates for salty environments. Recent developments in the food science sector and consumer preferences for varied diets point to the consumption of

wild edible plants as diet complements as well as nutritious and useful foods for specific conditions, making commercial cultivation of these plants very crucial (Petropoulos et al., 2018a; Nikalje et al., 2019). However, in South Africa most wild species are regarded as weeds for conventional crops, and farmers typically use chemical fertilizers to get rid of them (Tardío et al., 2006). Many of these wild edible species have been utilized by rural communities for many years as leafy greens and as the foundation of numerous traditional cuisines and regionally significant local recipes but neglected in the commercial vegetable production (Petropoulos et al., 2018a; Hamed & Custódio, 2019). Therefore, the cultivation of wild halophytes presents a possible solution to support food and nutrition security, while preserving water resources for domestic, municipal, and environmental uses. These developments need to be implemented at national level to align countries to sustainable development goals (Ngxabi et al., 2021b).

Trachyandra ciliata is a wild underutilized halophyte that is endemic to the Western Cape coastal dunes in South Africa (Ngxabi et al., 2021a). The genus *Trachyandra* belongs to the Asphodelaceae (Aloe) family, which is widely known for its pharmaceutical importance. Studies on this plant are scarce, and currently, there are no *Trachyandra* species in commercial cultivation (Manning, 2007). However, it is known that this plant is edible and that Khoisan people who lived around the South African cape coast utilized it as food (De Vynck et al., 2016; Ngxabi et al., 2021a). We previously conducted the only study (Ngxabi 2020) and focused on the vegetative growth of *T. ciliata* in response to different growth media and salinity to develop its cultivation protocol. Furthermore, we reported the presence of some antioxidants and phytochemicals in the plant, especially when subjected to salt stress. As such, the present study was conducted to explore the nutraceutical, agricultural, and economic potential of *Trachyandra ciliata* to support its cultivation and commercialization.

2.2 Accumulation of salts on agricultural lands

One of the main abiotic factors in agriculture that promotes deficiency, physiological abnormalities, and lower output of field crops around the world is salinity, along with drought (Ahanger and Agarwal, 2017; Ahmad et al., 2018). Salt stress has over the years become one of the most limiting factors in plant production especially in arid or semi-arid zones (Silveira et al., 2009). The issue arises when soil ion concentrations (Na+, Cl, and other related salts) are higher than the normal levels. This disrupts osmotic processes first, which subsequently changes a number of metabolic processes necessary for healthy plant growth and development (Ahmad et al., 2018; Ho and Adams, 1995).

Although the sensitivity of different plants to soil salinity varies, problems with respiration, photosynthesis, mineral uptake, and oxidative stress have a negative impact on most of their

growth and production attributes (Ho & Adams, 1995; Petropoulos et al., 2017). Some plants, like halophytes are not vulnerable to certain amounts of salts in their root zone, in fact, they prefer saline conditions for optimum growth (Ngxabi et al., 2021b). This therefore necessitates thorough research to determine the precise salt tolerance per species. If the problem is not properly addressed, decreased agricultural output due to salinity could pose serious concerns to global food security in the coming decades. This then calls for the cultivation of plants with high salt tolerance to mitigate the effect of salt toxicity in agricultural lands (Ngxabi et al., 2021a).

2.3 Adaptation of halophytes to saline conditions

The physiological processes within plants can often be substantially altered by unfavourable circumstances like abiotic stressors (drought and salinity), which often reduce growth and production in plants (Ngxabi et al., 2021b). Plants known as halophytes are those that naturally thrive in salty areas and benefit from excess salt in their growing medium (Shabala, 2013). From coastal areas, salt marshes, and mudflats to inland deserts, salt flats, and steppes, halophytes can thrive in a range of saline habitats (Flowers & Colmer, 2008). Although wild cabbage is also classified as a halophyte, its adaptation mechanism to salinity is undocumented.

Halophytes have developed a number of adaptations to withstand seawater and increased salinity concentrations. According to Shabala (2013) , “These include adjustment of their internal water relations through ion compartmentation in cell vacuoles, the accumulation of compatible organic solutes, succulence, and salt secreting glands and bladders”. Shabala (2013) argues that 50 mm of salt concentration is best to achieve optimum growth for monocotyledonous halophytes, and 100 mm for dicots. This author further reported that some halophytes do not show a significant yield decrease when irrigated with sea water. However, Ngxabi et al., (2021b) reported that 100 mm treatment recorded the highest values for vegetative growth in the cultivation of wild cabbage under hydroponics (Figure 2.1). This then makes halophytes the appropriate plants to be cultivated in land affected by salt toxicity.

Figure 2.1: An experiment showing the response of *T. ciliata* to different salinity levels by Ngxabi et al., (2021b).

Most halophytes have been discovered to use ion transport (Na+ and Cl-) as a salt-mitigation technique from roots to leaves where they are contained in vacuoles (Munns & Tester, 2008). Studies by Souid et al., (2016); Al Hassan et al., (2017) reported that ion exportation from roots to shoots keeps NaCl levels stable and minimizes the damaging effects of salt at the root level. González-Orenga et al. (2021) showed that excessive accumulation of inorganic ions in the vacuole is neutralized by the production of osmolytes or solutes in the cytoplasm. They further argued that even at high intracellular levels, these organic non-toxic osmolytes do not affect cellular metabolism, but they serve as "reactive oxygen species" (ROS) scavengers and play critical roles in the osmotic adjustment and protein stabilization processes that occur in plants in response to abiotic stresses.

Plants benefit a lot from reactive oxygen species (ROS) because they promote cellular growth, biological processes, and stability. Even though ROS are necessary for life, an excessive amount in a cell can harm lipids, proteins, DNA, and nucleic acids (Mittler, 2017; Ngxabi et al., 2021b). In case of excessive concentration of ROS, plants address this issue by the production of antioxidant enzymes. These antioxidant enzymes aid in the removal of excess ROS and contribute to the preservation of healthy cellular pH (Souid et al., 2016). Furthermore, halophytes' adaptive defense mechanism against oxidative stress is said to be highly dependent on antioxidant enzymes (Mittler, 2017). This is supported by the study of Jeeva (2020) who reported that *Tetragonia tetragoniodes* (a well-recognized halophyte) stimulated

Superoxide Dismutase (SOD) enzyme's activity to reduce cell membrane damage during saline conditions.

It is also reported that succulents and other salt-tolerant species have developed a system for excreting salt through specialized organs such as salt glands, salt hairs, or salt bladders, which are primarily located on the leaf and epidermis. These specific structures move ions from the leaf tissues to the surface of the leaf, where the saline solution crystallizes and is quickly blown or washed away by wind or rain (Caperta et al., 2020). The wild cabbage's tolerance mechanisms to salinity remain largely unknown, unlike the wealth of information available for other halophytes. This underscores the necessity to unravel the precise mechanisms enabling these plants to thrive in saline environments.

2.4 The significance of halophytes in food security

Water scarcity and rising soil salinity are significant global issues, particularly in agricultural regions (Hanjra & Qureshi, 2010; Tug & Yaprak, 2019). Therefore, using salt-tolerant crops can be a useful alternative to investing time and money in remediating such areas (Ventura et al., 2011). Halophytes are the ideal crop candidates for salty environments. Recent developments in the food science sector and consumer preferences for varied diets point to the consumption of wild edible plants as diet complements as well as nutritious and useful foods for specific conditions, making commercial cultivation of these plants crucial to preventing threats from irrational gathering and genetic erosion (Petropoulos et al., 2018a; Nikalje et al., 2019). Many of these wild edible species have been utilized by rural communities for generations as leafy greens and as the foundation of numerous traditional cuisines and regionally significant local recipes, and they still exist throughout Southern Africa, and indeed the world at large (Petropoulos et al., 2018a).

Wild halophytes typically possess higher nutritional and bioactive contents and flavours like those of common salad crops (Petropoulos et al., 2016). In addition to hand-harvested wild greens, modern human lifestyles and these species' seasonality have created a niche in the market for commercialized production of several species (Ruiz-Rodríguez et al., 2011). With wild plants becoming an essential part of the food supply on a worldwide scale, in both developed and developing countries, recent trends in food science and technology mandate the manufacture of unique and nutritious food products and the integration of creative processing techniques (Petropoulos et al., 2018b). In addition, many halophytes have been confirmed to possess properties that treat many chronic diseases (Ksouri et al., 2011). Therefore, consumers' newfound interests compel a return to previous eating patterns, with a focus on balanced nutrition and self-medication with healthy foods, nutritional supplements, herbal medicines, and functional foods (Łuczaj et al., 2012).

Most wild species are regarded as weeds for conventional crops, and farmers typically use chemical fertilizers to get rid of them. However, this practice has the potential for long-term negative effects, including the extinction of threatened species, resulting genetic erosion, as well as drastic changes in the biodiversity of ecosystems (Tardío et al., 2006). Hence, it is crucial to suggest substitute crop species within the context of saline agriculture that can adapt under difficult conditions and are suitable candidates as prospective cash food and therapeutic crops (Panta et al., 2014). This therefore challenges researchers to conduct extensive studies on the edibility of halophytes and present them for commercial vegetable production, especially in areas that are struggling with salinization of agricultural lands.

2.5 Health benefits of plant-based foods

The food industry has encountered new obstacles in the last few decades, forcing it to create new food products and diets that can halt the progression of lifestyle and/or chronic illnesses (Fehér et al., 2020). Additionally, the health of people has deteriorated in recent decades, a trend that can be linked to bad lifestyle choices, an imbalanced diet, and excessive intake of indulgent foods and beverages (Mariotti, 2023). Consequently, each year, 63% of all deaths worldwide are caused by malignant tumors, diabetes, cardiovascular illnesses, respiratory conditions, and obesity, all of which are lifestyle linked (Fehér et al., 2020). In conjunction with the swift spread of chronic illnesses, the population in developed and semi-developed nations is aging at a rapid pace; as a result, the number of individuals who are inactive and in need of medical attention is rising (Mullins & Arjmandi, 2021). On the other hand, some regions like Asia still boast aging populations and that is partly related to the lifestyle and dietary choices of consuming plant-based foods (Tokudome et al., 2016). Studies on selected Asian countries revealed that people who followed a prudent diet, which is characterized by consuming a lot of whole grains, legumes, vegetables, seafood, and seaweed, had longer life expectancy, while no significant correlation was found between western diet (which is characterized by consuming a lot of refined grains, red or processed meat, and sweetened carbonated drinks) and prolonged life expectancy(Gong et al., 2018; Crous-Bou et al., 2019).

A plant-based diet is a dietary trend in which animal products including eggs, meat, dairy products, and eggs are consumed in moderation, while highly processed foods like sugar, wheat flour, and oil are kept to a minimum. Most of these diets consist mostly of plant-based foods such cereals, nuts, seeds, tubers, legumes, vegetables grains, and fruit that are raw, unprocessed, or just lightly processed foods (Leitzmann, 2014; Bvenura & Afolayan, 2015). Lichtenstein et al., (2021) linked predominantly plant-based diets to improved cardiovascular risk factors, decreased incidence, and slower progression of coronary heart disease (CHD). Randomized controlled trials and epidemiological research have demonstrated that plant-

based diets lead to a considerable reduction in CHD occurrences, lowering risk factors like diabetes and hypertension as well as symptomatic and scintigraphic myocardial ischemia and coronary artery disease (Fraser, 2009; Israeli, 2013; Brennan et al., 2012). Research shows that a plant-based diet can potentially reduce blood pressure, cholesterol, HbA1C, body mass index, and consequently costs associated with healthcare (Fraser, 2009; Fehér et al., 2020). Additionally, such a diet may lessen mortality rates from ischemic heart disease and decrease the amount of medication required to manage chronic illnesses (Israeli, 2013). Furthermore, plants are the only source of phytochemicals like carotenoids, glucosinolates, and flavonoids which perform multiple health functions in the human body including neutralizing free radicals, anti-inflammation, immunity enhancement, optimization of serum cholesterol and protection against cancer (Hever, 2016). Although studies on plant-based diets have been an area of interest globally, South African wild edible plants continue to be foraged without sufficient knowledge on their nutritional and medicinal benefits. This then necessitates extensive studies on the nutritional and medicinal properties of these plants to support their consumption and commercialization.

Table 2.1: Some popular wild edible plants used in ethnomedicine that thrive in similar conditions as *T. ciliata*.

Species	Part used	Mode of application	References
Tulbaghia violacea Harv.	Leaves and bulb	Decoctions of leaves are used to treat cancer of the esophagus. Bulb is used as a remedy for pulmonary tuberculosis and to destroy intestinal worms.	(Amoo et al., 2014)
Perlagonium culallatum (L) L'Hér.	Leaves	For stomach disorders, sores, and wounds	(Amoo et al., 2014)
Aloe cooperi Baker subsp. Cooperi Bak.	roots, leaves, flowers	Roots infusion for easy delivery, flowers cooked and eaten as vegetable.	(Malmir et al., 2018)
Aloe ecklonis Salm-Dyck	Flowers	Flowers cooked and eaten as vegetable.	(Amoo et al., 2014)
Agathosma apiculata G.Mey.	Leaves	Source of essential oils and flavour. Leaf	(Amoo et al., 2014))

		decoctions are used for colds and flu.	
Coleonema pulchellum	Leaves	Rich source of essential oils and flavour. Can treat colds and flu.	(Bodede & Prinsloo, 2020)
Artemesia afra	Leaves	An addition in herbal drinks and iced tea. It is used to treat colds, sore throat, coughs, asthma and headaches.	(Bodede & Prinsloo, 2020)
Carissa macrocarpa.	fruits	Rich source of antioxidants, vitamin C, and pectin.	(Asong et al., 2019)
Carpobrotus edulis	Leaves and fruits	Leaves are used to treat cold sores, bruises, Bee stings, rashes, and sunburns.	(Asong et al., 2019)
Mentha longifolia	Leaves	Rich source of essential oils. Tea made from leaves used for stomach disorders.	(Rankoana, 2021)
Salvia africana lutea	Leaves	Tea made from the leaves good for colds, coughs, and stomach ailments.	
Portulacaria afra Jacq.		Leaves chewed lactating mothers to stimulate production of milk. Also, it is high in phytochemicals.	(Dlamini et al., 2010)
Trachyandra falcata Kunth.	Flowers	Cooked as vegetable	(Tshayingwe et al., 2023)
Trachyandra divaricata Kunth.	Flowers	Cooked as vegetable	(Bulawa et al., 2022)

Table 2.1 shows the ethnomedicinal uses of some wild edible species that thrive in similar conditions as *T. ciliata* and some species in the Asphodelaceae family, which are mostly indigenous to Southern Africa, with numerous species traditional used as laxatives or analgesics for pain relief. Many of these species are widely used by humans for treatment of skin problems, cancer, kidney, bladder, stomach problems (Malmir et al., 2018). Furthermore, some well-known and unspecified species have been used by women as oral contraceptives and during birth, while some edible *Portularcaria* species have been traditionally by lactating mothers to stimulate milk production. Some of the species are consumed as vegetables, fruits, added in salads, and some are used to make tea, although their edibility and nutrition are yet to be recorded (Amoo et al., 2014). Some species are widely used to treat skin related problems, while they are also edible (Asong et al., 2019). Few *Trachyandra* species have been reported only as edible vegetable plants, although thorough research is still needed on this genus (Bulawa et al., 2022; Nortje & van Wyk, 2019).

2.6 Asphodelaceae family

Asphodelaceae is a family of flowering plants, which includes 12 genera and over 1060 species, and is indigenous to Africa, the Mediterranean basin, the Arabian Peninsula, west and central Europe, Madagascar, Central Asia, Australia, and New Zealand (S. Ngxabi et al., 2021; Chase et al., 2016). The nomenclature of this family dates back to 1789 when it was first scientifically published in Jussieu's *Genera Plantarum* (Smith & Van Wyk, 1998).

Asphodeloideae and Alooideae are the two sub-families found under this family (Chase et al., 2000). The asphodelaceae family has a highly diverse range of members, however there are a few traits they all share. They can be small to medium-sized plants, which are frequently succulent, herbs, or enormous trees with terminal rosettes of leaves instead of succulent stems (Ngxabi, 2020). According to Smith & Van Wyk, (1998) "Leaves are dorsiventral, lanceolate-acuminate, linear or subulate, terete, often succulent and thickly conical, spirally arranged or distichous as in some species of Alooideae, amplexicaul, margins toothed, serrate or entire, sharply pointed, parallel veins often obscure".

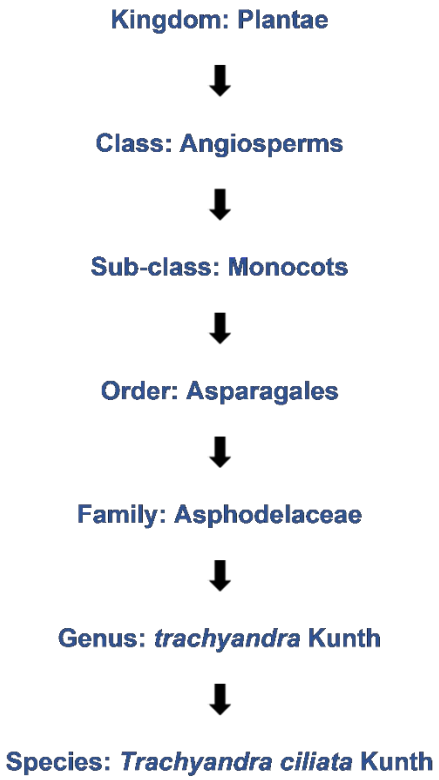

Figure 2.2: Position of *Trachyandra ciliata* in plant classification

Trachyandra is a genus under Asphodelaceae that is made up of more 50 species distributed throughout the sub-Saharan Africa. *Trachyandra* species are distinguished from the species within the family by their soft-textured leaves and white to pink flowers that are short-lived with a deciduous perianth and rough filaments (Ngxabi et al., 2021a; Smith and Van Wyk, 1998). *Trachyandra* species can either be woody or herbaceous and some species can have granular pubescence. They are deciduous plants with fibrous or spindle-shaped root systems that occasionally swell towards the terminals to produce thin tubers (De Vynck et al., 2016).

The upright, woody, or herbaceous stems are typically naked, but occasionally coated with old leaf bases. Rhizomes are most often established as vertical but can also be horizontal. The bottoms of the leaves, which are tube-like and frequently resemble a sheath of fibers, are either homogeneous or have two unique morphologies. On aerial stems, the leaves are grouped in rosette formation, infrequently in alternating form, or in dense spirals. The lamina is flat, cylindrical, sharply three-angled, hairy, or smooth, or glandular pubescent, but occasionally spirally twisted and folded (Obermeyer, 1962). According to our earlier work (Ngxabi et al., 2021a), “The inflorescence is axillary with single or branched raceme unusually sub-umbellate, naked or with sterile bracts. The inflorescence has single pedicels that change their position during the flowering stage and when the capsule ripens. These species have

flowers that are either scentless or characterised by a strong scent that usually open during the day and at night. Plants in this genus have six stamens that are attached to the base of the perianth".

The term *Trachyandra*, which refers to the hairy filaments, is derived from the Greek words '*trachy*', which means rough, and '*andro*', which indicates male. *Trachyandra ciliata* is a spreading perennial herbaceous plant that can reach a height of 0.5 m. The mature leaves are linear, 100 × 4 cm in size, and succulent with hairy margins. The flowers are grouped in crowded racemes and have white tepals with a pink midrib, and they last only a single day. These snake-like flowers distinguish this species from other species in the genus (De Vynck et al., 2016).

The literature on this plant is limited to non-existent, and there are currently no *Trachyandra* species under commercial cultivation (Manning & Goldblatt, 2007). However, it is known that this plant is edible and that Khoisan people who lived around the South African cape coast utilized it as food (De Vynck et al., 2016; Ngxabi et al., 2021a; Ngxabi et al., 2021b). The inflorescence, just like Asparagus, is steamed and eaten as a vegetable, added into a stew, or added into salads. Only two other species in the *Trachyandra* genus are edible (*T. divaricata and T. falcata*) and are also eaten as a vegetable or added into a stew.

F**igure 2.3:** Edible inflorescence of *T. ciliata* (Ngxabi et al., 2021a)

Figure 2.4: Veldkool cooked with Lamb stew (Ngxabi et al., 2021a)

2.7 Distribution of *Trachyandra* species in Southern Africa

Trachyandra is primarily a South African genus, with majority of species occurring in the south-western Cape (Figure 2.5). *Trachyandra* species can be found all over Southern Africa, with most of them being native to the South-western Cape's winter rainfall region. Others can be found in southern Zimbabwe, Angola, Kenya, with one occurring in Ethiopia (Smith & Van Wyk,

1998). Given that this plant is found naturally on the coastal sand dunes of the Western Cape, it suggests a strong tolerance to salinity, making it a potentially beneficial addition to coastal gardens (Ngxabi et al., 2021a).

Figure 2.5: Distribution of *T. ciliata* in South Africa

(Source: http://redlist.sanbi.org/species.php?species=2203-18)

2.8 Significance and potential of Asphodelaceae

Recent research examining the potential of indigenous South African species as food crops has revealed that several species could be profitable products for the food and beverage industries, although Asphodelaceae plants have always been known for their effective medicinal capacity (Van Wyk, 2011). The use of the inner, non-bitter part of some Aloe species as food supplements is a potentially new frontier in research. The presence of flavonoids in some Aloe species have been reported, while some species in this family are used as food preservatives by local Cape farmers (Cock, 2015). Ngxabi (2020) and Ngxabi et al. (2021b) also reported the presence of antioxidants and phytochemicals in *T. ciliata*, especially when subjected to salt stress.

Researchers have identified the health advantages of food and drink items made from the leaf parenchyma of Aloe species and the use of Aloe extracts as direct food additives for human consumption has been approved by the Food and Drug Administration (Salehi et al., 2018). Some Aloe leaf gels have been found to include flavonols as well as some vital vitamins such

as vitamins C, B1, B2, B6, B12, and E that are essential for the effectiveness of the human body (Cock, 2015). These authors also reported iron, calcium, manganese, potassium, phosphorous, copper, magnesium, zinc, molybdenum, and sodium as essential minerals present on the gel of Aloe species. Ngxabi et al., (2021a) reported that *Trachyandra ciliata* together with two other species in the genus *Trachyandra* (*T. Divaricata and T. falcata)* is edible and was used by original Khoisan people who used to live in the Western Cape coastal areas. With little to no literature available about this plant, more research should be conducted to evaluate its nutrition.

2.9 Potential value of *T. ciliata*

2.9.1 Economic potential as a commercial edible vegetable

Due to their saltiness and crispiness, edible halophytes are used in a variety of green foods served in restaurants and have become popular in several cultures worldwide, contributing to the preservation of traditional food systems and culinary heritage (S. Ngxabi et al., 2021; Bvenura & Afolayan, 2015). Many underutilized vegetables have been reported to be rich in phytochemicals and essential nutrients such as vitamins, dietary fibre, proteins, calcium, iron, and magnesium, all of which are essential for human and animal health and nutrition. Therefore, promoting their consumption can contribute to improved nutrition and public health outcomes, potentially reducing healthcare costs related to diet-related illnesses (Bvenura & Sivakumar, 2017; Bvenura & Afolayan, 2015; Gong et al., 2018).

Compared to traditional green leafy vegetable crops, these species' climate adaptability to high temperatures, drought, and saline environments makes them excellent options in some regions that are vulnerable to the effects of climate change (Agudelo et al., 2021; Ebert, 2014). Additionally, compared to conventional crops, underutilized halophytes frequently demand less inputs, such as water, fertilizer, and pesticides. This then calls for the cultivation of these crops to promote more sustainable agricultural practices, reducing environmental impact and promoting ecological resilience (Zuccarini, 2008; Bvenura & Afolayan, 2015). Furthermore, commercial cultivation of underutilized crops by farmers contributes to the conservation of genetic diversity within crop species, which is crucial for breeding programs and maintaining resilience amid environmental challenges such as climate change, drought, and salinization of agricultural lands (Galluzzi & Noriega, 2014).

Due to increasing global population and threatened food security in the future, Recent studies have reported the necessity to enhance agricultural production, with one of the approaches being the introduction of underutilized edible plant species(Bulawa et al., 2022; S. Ngxabi et al., 2021b; Sogoni et al., 2021). This approach has the potential to reduce the dependency on the mainstream crops, diversify farmers' income streams and create resilience to market

fluctuations especially in developing countries (Bvenura & Afolayan, 2015; Ngxabi, 2020). In addition, Aderibigbe et al., (2022) highlighted the potential of exploring wild edible crops to open up new market opportunities that may lead to more export opportunities since these species usually possess distinct characteristics and are mostly endemic to specific regions, which may be profitable to the farmers and contribute to job creation especially in disadvantaged and rural communities. This will contribute to poverty alleviation and rural development. Other market opportunities for wild edible crops may include tapping into processed value-added products such as jams, pickles, sauces, and beverages (Bvenura & Sivakumar, 2017).

Trachyandra ciliata is less studied with the only existing literature mainly based on its taxonomy. However, it has been documented that it was traditionally eaten as a vegetable by Khoisan people in the past, but that information was eradicated due to colonization and migration. Our previous study (Ngxabi et al., 2021b) reported that maximum yield was achieved when irrigated with saline water. We further reported increased phytochemical contents under saline conditions. Further studies on closely related species (*Trachyandra divaricata* Kunth) reported the presence of some significant nutrients such as Ca, Mg, P, N, K, Na, Cu, Zn, and Fe; which support the need for more studies on this genus (Tshayingwe et al., 2023; Bulawa et al., 2022).

Trachyandra remains an under exploited genus with none of its species under known cultivation. People using this plant as food access it through foraging, which is not a recommended practice because it tempers with diversity and sustainability of wild ecosystems (Ngxabi et al., 2021a). This therefore makes *T. ciliata* an appropriate candidate as a commercial vegetable crop. This means there is a need to further study its nutritional contents and attempt to introduce it in the commercial vegetable market.

2.9.2 Potential medicinal value of halophytes

Halophytes have been used medicinally in various cultures around the world and have been shown to be beneficial in treating and managing chronic diseases that trouble modern worlds (Ksouri et al., 2011). According to Ksouri et al. (2011), “Currently, an increasing interest is granted to these species because of their high content in bioactive compounds (primary and secondary metabolites) such as polyunsaturated fatty acids, carotenoids, vitamins, sterols, essential oils (terpenes), polysaccharides, glycosides, and phenolic compounds”. These authors further alluded that these bioactive chemicals exhibit strong antioxidant, anti-microbial, anti-inflammatory, and anti-tumor properties, and as a result, they serve as important anti-aging and disease-prevention agents. This phenomenon in South Africa is however underexplored, with very few halophytes having been studied for medicinal purposes

(Ngxabi et al., 2021b). *T. ciliata*, is therefore no exception and its medicinal potential can be inferred from its relatives in the Asphodelaceae family.

The demand for new drugs with robust activity against these eukaryotic microorganisms has significantly increased because of a rise in the number of immune deficient and immunocompromised individuals suffering from parasitic illnesses and fungi (Era et al., 2014). In southern Africa, the vast majority (85%) of usage records pertaining to Aloe species highlight their pharmaceutical benefits (Grace et al., 2008). The use of species of *Bulbine* (Asphodelaceae) dates to the eighteenth century, when British and Dutch settlers in South Africa used the species to treat a variety of illnesses, including parasitic infections, diarrhoea as well as stomach aches, urinary tract infections, viral infections like HIV, chicken pox, shingles, and sexually transmitted diseases (Bodede & Prinsloo, 2020; van Wyk, 2008). Halophytes are useful for boosting the immune system, lowering inflammation, and guarding against cancers, hypertension, and atherosclerosis (Im et al., 2003). In recent years, there has been a rising research interest worldwide in investigating the medicinal properties of halophytes, as they demonstrate significant potential as a source of secondary metabolites. However, South African halophytes including *T. ciliata* remain undervalued as potential sources of useful bioactive compounds.

2.9.3 Desalinization

Around 80 million hectares of agricultural lands are affected by soil salinity worldwide and this will potentially increase (Flowers et al., 2010; Rabhi et al., 2009). Such soils might be recovered using a variety of techniques, including hydraulic, physical, chemical, and biological approaches (Rabhi et al., 2009; Shiyab et al., 2003). However, in developing and semi-developed countries, hydraulic, physical, and chemical approaches make it difficult to explore these measures due to high costs involved (Pinto & Marques, 2017; Flowers et al., 2010). This then calls for more biological approaches to be adopted as sustainable and environmentally friendly measures for desalinization.

Crop rotation and the use of salt tolerant crops to absorb excess salts in agricultural lands is gaining momentum worldwide. Particularly for some poor nations where chemical additives are becoming increasingly expensive, this plant-based approach is of paramount importance. Shabala (2013) stated that for revegetation and rehabilitation of salt-affected land, halophytes are the best plant species. The study of Watson and O'leary (1993) showed that *Atriplex nummularia* (oldman saltbush) produced 20 to 30 t/ha of biomass per year and accumulated between 20 and 40% NaCl in its dry matter when irrigated with saline water. *Suaeda fruticose* (seablite), on the other hand, may remove more than 2.5 t of salt per hectare in a single harvest of the plant's aerial portions each year (Shabala, 2013). In the context of African halophytes,

this phenomenon is under explored and more studies should be conducted to explore such avenues.

2.9.4 Phytoremediation using halophytes

Global industrialization over the past 200 years has significantly increased the production, usage, and environmental release of heavy metals (Flowers & Colmer, 2015). Due to the build-up of harmful ions brought by using chemical fertilizer in agricultural fields as well as climate change, soil fertility has been lost, and it has also caused salinization and desertification, making it difficult to cultivate crops (Yasin et al., 2019). Farmers have mainly been adopting chemical amendments for the improvement of salty and sodic soils to combat this devastating situation. Nonetheless, this use of chemicals has become expensive especially for developing countries and it can also exert adverse effects on the environment (Hasanuzzaman et al., 2014). This therefore challenges researchers to find cost effective and environmentally friendly methods to reduce the catastrophic effects of salinity in agricultural lands. Halophytes are frequently recommended for phytoremediation (phytostabilization and phytoextraction) because tolerance to salt and heavy metals depends, at least in part, on similar physiological mechanisms (Shabala, 2013; Thomas et al., 1998; Manousaki & Kalogerakis, 2011). Phytostabilization occurs when a metal-tolerant non-accumulator plant can withstand metals in its aboveground parts without transferring or accumulating them. These plants offer soil stabilization and prevent metals from being mobilized or leached into the groundwater, and they also provide vegetation cover (Manousaki & Kalogerakis, 2011). Phytoextraction is the process of removing harmful metals from the soil by building up in the plant's top layers so they can be collected and removed. *Trachyandra ciliata* is one of the unexplored halophytes from South Africa, which makes it one of the options to address the issue contaminated soils.

2.9.5 Companion planting (intercropping)

Utilizing halophytic plants in crop rotations or mixed cropping systems may be a promising management strategy to reduce salt stress-related yield losses because halophytes are able to accumulate high quantities of NaCl in their tissues (Karakas et al., 2016). Intercropping is mostly used in developing nations to increase crop productivity (De La Fuente et al., 2014; Karakas et al., 2016). In South Africa, crop rotation stands out as the most commonly adopted practice, particularly among small-scale farmers, aimed at averting the risk of crop failure by alternating different plant species. Intercropping using halophytic plants in south Africa remains unexplored, although the country is one of the countries threatened by soil salinization and drought (Bantie et al., 2014). In other countries, there are methods that have been adopted to reduce salinity in the soil such as physical reclamation (deep ploughing), chemical reclamation, plant control and water-based approaches. However, these methods are cost

and labor intensive, and therefore not sustainable or feasible for many developing countries (Qadir et al., 2007; Singh et al., 2012; Karakas et al., 2016).

In other countries there are some studies that have been conducted to test the performance of normal cash crops when cultivated with halophytes under saline conditions. Halophytes take up salt from the soil and store it in their tissues, preventing crop plants from getting to harmful ions (Zuccarini, 2008). The study of Colla et al., (2006) investigated the effectiveness of *Salsola soda* (halophyte) as a companion plant for peppers. According to these authors, the presence of *S. soda* decreased the EC value by 43% and increased total crop, marketable crop, and total biomass values by 26, 32, and 22%, respectively. In addition, a study conducted by Zuccarini (2008) found that tomato plants intercropped with halophytes (purslane and garden orache) had lower NaCl concentration and a yield increase of 44% compared to those that were not intercropped. These studies reveal that there is a potential for cultivating traditional food crops in saline soils by intercropping with halophytes. In the case of *T. ciliata*, there is a need to examine its potential as a companion plant because of its edibility. Studies to examine the potential of South African halophytes as companion plants to traditional food crops are desperately needed.

2.10 Conclusion and prospects

Water scarcity and high salinity in agricultural lands cause restrictions in plants, which decrease overall output, leaf production, delayed flowering, and flower bud abortion. To cater for the constantly increasing population, researchers predict that overall global food production needs to increase by 50 to 100% by 2050, although the current increase is around 1 to 1.5% annually. This then necessitates the cultivation of more salt and drought tolerant plant species (halophytes) for food and medicine to substitute the traditional vegetable crops that are currently under cultivation. In South Africa, native wild halophytes have been neglected and under-researched, although they have been gaining increasing attention in the past decade. Previous studies have identified *T. ciliata* as an edible wild halophyte that was used for food by the Khoisan people that used to live in the Western Cape coastal areas before colonization and massive industrialization of the province. Previous research on this plant that focused on determining its cultivation protocol also showed that indeed the plant is more productive under saline conditions, although its anatomic salt tolerance mechanisms, nutritional profile, seed germination protocol, medicinal properties, biochemical and physiological properties remain unknown. Further research is recommended to study its leaf micromorphological properties and chemical composition to understand its precise salt tolerance and changes in its anatomical structures that are vital for biochemical processes. Further studies are recommended to determine the plant's nutritional profile in the form of macronutrients,

micronutrients, and antinutrients to promote its consumption and commercialization. Recent studies including the current research recommend commercial production and promotion of wild edible crops to keep up with the increasing demand for food due to population growth. Other production techniques like hydroponics and vertical cultivation are also recommended to grow these plants, especially in urban areas and private homes to promote healthy dietary lifestyles, more especially that these techniques use less water than field cultivation, contributing to sustainable water usage. To promote the commercial cultivation and popularity of these crops, more resources need to be made available for marketing in the form of education and awareness campaigns to capacitate the farmers and communities about the economic potential of these crops. Capacity building seminars and market days in local and rural communities should also include the benefits of cultivating wild edible crops, which may lead to the emergence of more culinary innovations that can be sold to prominent food markets and wholesalers. Findings from this study serve as points of reference to commercial farmers, communities, scholars, policy makers, and aspiring researchers whose interests are on the potential of easily accessible underutilized wild edible crops to develop new strategies to address global issues.

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CHAPTER THREE

SALINITY INFLUENCED GROWTH, PHYSIO-BIOCHEMICAL RESPONSES, AND ANTIOXIDATIVE POTENTIAL OF *TRACHYANDRA CILIATA* (L.F) KUNTH (WILD CABBAGE)

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Salinity Influenced growth, physio-biochemical responses, and antioxidative potential of *Trachyandra ciliata* (L.F) Kunth (Wild cabbage)

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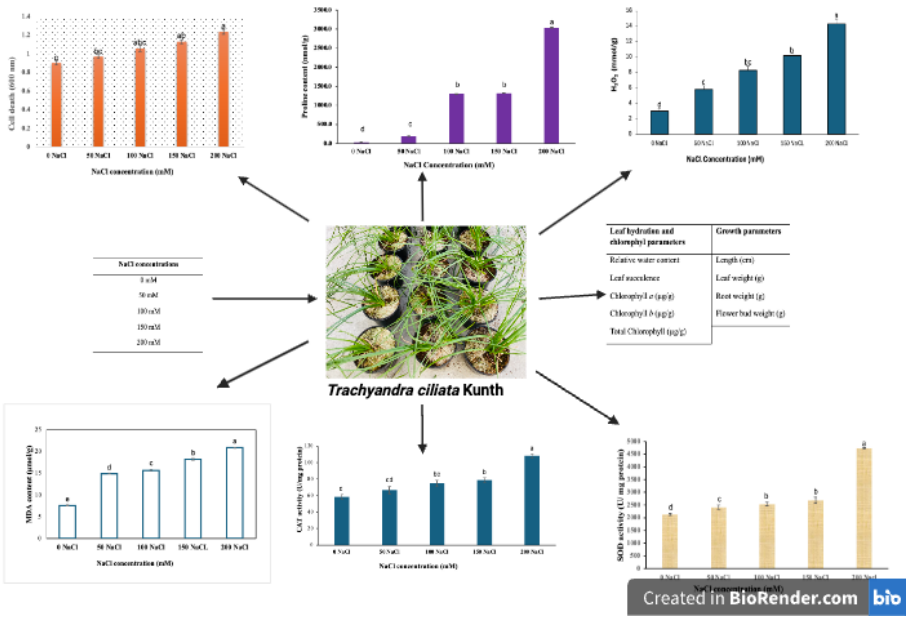
Abstract

Trachyandra ciliata is a wild edible halophyte endemic to the saline Western Cape coastal region. However, its tolerance mechanisms and physio-biochemical responses under salinity stress are undocumented. As such, this research was conducted to investigate the effect of salinity stress on growth parameters, leaf hydration, photosynthetic pigmentation, oxidative stress markers and concentration of antioxidative enzymes to further understand mechanisms involved in its salt tolerance. Four salinity treatments (50, 100, 150, and 200 mM) were assessed by adding NaCl into the aqueous nutrient solution, while control was solely received the nutrient solution. Results revealed that salinity positively influenced growth parameters in all plant parts at low concentration (50 mM NaCl) compared to the control. Then growth parameters substantially decreased as salinity intensified from 100 to 200 mM treatments. Moreover, the total chlorophyll content negatively correlated with increasing salinity. *Trachyandra ciliata* maintained equivalent Relative Water Content (RWC) from control to 100 mM treatment, which then efficiently decreased as with increasing salinity, while the plant maintained an unchanged leaf succulence among all treatments. On the other hand, high salinity stimulated oxidative stress as indicated by high MDA, cell death, and superoxide radicals, which were more expressive under 200 mM treatment. To counter the catastrophic effects of excessive ROS, *T. ciliata* activated antioxidative defence mechanisms as indicated by high SOD and CAT antioxidative enzymes which were more prevalent at high salinity (200 mM). Furthermore, the high proline content under higher salinity treatments ensured further scavenging of ROS. These findings validate that *T. ciliata* is able to tolerate salinity by modulating bio-physiological processes to manage oxidative stress and achieve cellular homeostasis under salinity stress. This implies that *T. ciliata* can be an excellent candidate in bio-saline agriculture and semi-arid areas that are faced with saline lands and shortage of fresh water for agricultural production.

Key words: Antioxidant enzymes, Bio-physiological processes, Catalase, Food security, Oxidative stress, Proline, Reactive Oxygen Species, Superoxide Dismutase.



Graphical abstract

3.1 Introduction

Concerns to end hunger, stabilize food security and nutrition, and encourage sustainable crop development have intensified over the years amid the constantly increasing global population (Hanjra & Qureshi, 2010; Bvenura & Afolayan, 2015; Aderibigbe et al., 2022). However, increasing soil salinity and a lack of availability of fresh water, particularly in South Africa, have severely impaired food production, making it difficult to attain the intended target amid the constant global population growth (Corwin, 2021a; S. Ngxabi et al., 2021). Soil salinization, one of the major abiotic factors linked to climate change, has a negative impact on world food supply and land productivity (Corwin, 2021a; Russo, 2023). Salinity stress leads to several physiological abnormalities such as slower leaf growth, reduced cell elongation and division, decreased photosynthetic rate, reduced cell hydration, membrane damage, and activation of oxidative stress, leading to reduced total biomass (Orzechowska et al., 2021; Balasubramaniam et al., 2023; Anami et al., 2020). Research indicates that the increasing soil salinization and climate change will certainly affect agricultural production and make it impossible to match the demand in the coming years (van Dijk et al., 2021; Alkharabsheh et al., 2021). Consequently, maintaining sustainability in terms of the future supply of food for poor populations and the protection of water resources will become more difficult (Corwin, 2021a; Ventura & Sagi, 2013). This then necessitates the alternative cultivation of salt tolerant vegetable crops (halophytes) to match the food demand and avoid malnutrition in regions affected by salinity and climate change mostly (Nikalje et al., 2019; Colvin & Muruven, 2017; Ngxabi, 2020). Wild edible halophytes are known to be the ideal crop options for saline

environments (Tshayingwe et al., 2023; Mndi et al., 2023). Nonetheless, these plants are still understudied, underutilized, and sometimes considered as weeds, especially in developing countries that are likely to be mostly affected by the looming food shortage soon (Bvenura & Sivakumar, 2017; Hamed & Custódio, 2019).

It is well known that halophytic plants allocate adequate energy to different metabolic and physiological processes to prevent ion toxicity, osmotic stress, nutritional imbalance, and oxidative damage under salinity stress (Azeem et al., 2023). In some species, an increase in root and shoot biomass aids in the regulation of Sodium (Na^+) access to the xylem or reduction in its transport to the shoots, while eliminating and minimizing toxic ions during the process (Salim Akhter et al., 2021; Roy & Chakraborty, 2015). However, under high salinity, distractions in ion homeostasis can cause ion toxicity, which interferes with the photosynthetic process by damaging photosynthetic pigments or interfering with their biosynthesis, resulting in reduced plant biomass (Ali et al., 2022; Orzechowska et al., 2021). Furthermore, excessive presence of Na^+ in plant cells and tissues leads to the plant dedicating more energy for molecular oxygen, consequently causing the overproduction of singlet oxygen, superoxide ion, hydrogen peroxide and other free oxygen radicals (Faryal et al., 2022). It has been reported that a low amount of these radicals can have a positive effect on plants under stress by operating as signalling molecules to regulate growth and adaptation to stress (Khan et al., 2023; Kesawat et al., 2023). However, large amounts of these radicals may be toxic to plants, causing cellular damage and destructive processes such as oxidation of proteins, nucleic acids, and lipids, resulting in cell death (Choudhury et al., 2017; Hsouna et al., 2020).

To prevent the disastrous effects of oxidative stress and maintain cellular homeostasis, halophytes activate different defence mechanisms such as antioxidant enzymes that scavenge the excessive Reactive Oxygen Species (ROS). Superoxide Dismutase (SOD) Catalase (CAT) are among the antioxidative enzymes that play a crucial role in the antioxidant defence system (Zhou et al., 2018; Rajput et al., 2021). During salinity stress, SOD catalyses the removal of superoxide by disproportioning it into Oxygen and Hydrogen Peroxide (H_2O_2), while CAT converts the H_2O_2 into water and molecular oxygen (Rajput et al., 2021; Azeem et al., 2023). It has been reported that halophytes produce more of these enzymes under severe stress, a phenomenon that provides adaptive advantage over salt-sensitive species (González-Orenga, Grigore, et al., 2021). Moreover, plants produce proline, which also scavenges ROS generated under severe stress, stabilizes protein structure, and regulates cytosolic pH and cellular redox homeostasis, thereby strengthening plant resilience to drought stress during growth (Lee et al., 2018; Hnilickova et al., 2021).

Trachyandra ciliata also known as “Wild cabbage” is an underutilized and understudied wild edible halophyte endemic to the winter rainfall Western Cape coastal dunes in South Africa. It belongs to the famous Asphodelaceae (Aloe) family, whose species are known worldwide for their importance in the pharmaceutical industry (Van Wyk, 2011). The plant used to be eaten by indigenous people in the area, whose displacement due to industrialization led to the loss of knowledge about plants that grow in area (Manning & Goldblatt, 2007). The literature on this plant is minimal and there are currently no species of this genus under commercial cultivation (Tshayingwe et al., 2023; S. Ngxabi et al., 2021). However, a previous study on this plant reported that *T. ciliata* can withstand salinity stress and that low to moderate salinity improved growth parameters (Ngxabi, 2020). The study also reported increased phytochemical properties (polyphenols, favonols, FRAP, ABTS, and DPPH) under high salinity stress (150 and 200 mM NaCl), which provided a peripheral insight into defence mechanism of *T. ciliata* (Sihle Ngxabi et al., 2021). However, in-depth studies on the molecular and bio-physiological processes responsible for its ability to withstand salinity are non-existent. This therefore necessitates the assessment of physio-biochemical properties (relative water content, leaf succulence, and changes in photosynthetic pigment), oxidative stress markers (cell viability, Superoxide and MDA), antioxidant enzymes, and proline content. Similar studies have been conducted and documented in other halophytic species to thoroughly understand their salt tolerance mechanisms (Azeem et al., 2023; Hnilickova et al., 2021; Rajput et al., 2021). Thus, this study was carried out to assess the influence of salinity stress on growth parameters, physio-biochemical properties, proline content, and antioxidative enzyme potential in *T. ciliata* to thoroughly understand its salt tolerance mechanisms.

3.2 Materials and Methods

3.2.1 Plant material and experimental design

The growth aspect of the experiment was conducted in the research greenhouse of the Cape Peninsula University of Technology, Bellville campus, South Africa, at coordinates 33.55048.800 S and 18.38032.700 E. With the aid of environmental controller, the study's experimental greenhouse was kept at a steady temperature of between 21 and 26°C during the day and between 12 and 18°C at night, while the Relative humidity was maintained at 60%.

Trachyandra ciliata plant material was propagated using a method described by Ngxabi et al., (2024). A total of 150 uniformly sized cuttings were subsequently transplanted into pots measuring 12.5 cm in height, length, and width, filled with a mixture of peat, perlite, and vermiculite in a ratio of 1:1:1. The plants were allocated into five treatments, each comprising

30 replicates, arranged in a block design. During the initial four weeks, the plants were irrigated exclusively with NUTRIFEED™ water-soluble fertilizer (manufactured by Starke Ayres Pty. Ltd. Bredell Rd, Kaalfontein, Gauteng, South Africa) dissolved in tap water at 10 g per 5 L ratio for plants to adapt in the experimental setup. The aqueous solution comprised the following constituents: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Fe (1500 mg/kg), Cu (20 mg/kg), Mg (22 mg/kg), Mo (10 mg/kg) , Mn (240 mg/kg), B (240 mg/kg) S (75 mg/kg), and Zn (240 mg/kg).

The plants were subsequently irrigated with reverse osmosis (RO) water for five days to drain all the salt residues before being exposed to salinity treatments. Different salt concentrations were modified in the nutritional solutions using sodium chloride (NaCl) as described by (Ngxabi et al., 2024). In this experiment, four salt concentrations (50 mM, 100 mM, 150 mM, and 200 mM of NaCl) were examined, with the control receiving simply nutritional solution irrigation. Each plant was watered at a three-day interval with graded NaCl, while control was watered solely with nutrient solution without NaCl. Drain water from the pots was collected and electrical conductivity was measured to ascertain that accurate salinity levels were maintained. With the help of a calibrated hand-held digital pH meter (Eurotech®TM pH 2 pen), the pH of the solution was kept at 6.0. The plant material was harvested after 15 weeks of salinity treatment and utilized for further analyses.

3.2.2 Determination of plant growth parameters

3.2.2.1 Plant height

Plant height and shoot length were determined using a method described by Nomnqophiso et al., (2024). A standard measuring tape was used to manually measure the shoot length (cm) every week and recorded on a data sheet.

3.2.2.2 Plant weight

A RADWAG® laboratory scale was utilized to measure the plant mass before planting to ensure consistency of the cuttings. After 15 weeks of salinity treatment, leaf, root, and flower buds were harvested and separated using sterile secateurs, and their weights were measured. Subsequently, the plant material was air dried at 45 °C using a LABTECH™ model LDO 150F (Daihan Labtech New Dehli, India.) oven until they were completely dry, and dry weights were then measured (Sihle Ngxabi et al., 2021).

3.2.3 Leaf hydration

3.2.3.1 Relative Water Content (RWC)

Three fresh leaves were removed from three different plants in each of the five treatments and weighed (FW) in order to assess the variability in leaf RWC. They were then submerged in distilled water and kept in the dark for four hours in order to determine the turgid weight (TW). In order to determine the dry weight (DW), the leaves were air dried for 24 hours at 56 °C in an oven. The RWC (%) was calculated using the following equation as described by Bistgani et al., (2019).

$$\text{RWC \%} = \frac{(\text{FW}-\text{DW})}{(\text{TW}-\text{DW})} \times 100$$

3.2.3.2 Leaf succulence

The leaf succulence was calculated using the following equation as described by (Mantovani, 2011).

$$\text{LS} = \frac{(\text{LFW}-\text{LDW})}{(\text{LA})}$$

Where LS is the leaf succulence (mg H_2O cm^2); LFW is the leaf fresh weight (mg); LDW is the leaf dry weight (mg); and LA is the leaf area (cm^2). The leaf area was measured using a portable AM350 leaf area meter produced by Bio-scientific limited, London, United Kingdom.

3.2.3.3 Chlorophyll

The amount of chlorophyll *a* and chlorophyll *b* were determined using spectrophotometric techniques as described by Wang et al., (2023). A 5 ml of 99.5% dimethyl sulfoxide (DMSO; Sigma) was used to extract 100 mg of finely powdered fresh leaves. The absorbance of the supernatants evaluated at 649 and 665 nm was used to determine chlorophyll (Lichtenthaler & Wellburn, 1983).

3.2.4 Determination of oxidative stress markers

3.2.4.1 Evaluation of Cell viability

Cell viability was measured using the method as previously explained by Gokul et al., (2016). The intact undamaged leaf was submerged in 0.25% (m/v) Evans Blue (Sigma; dye concentration ≥75%). Dye absorption was assessed using spectrophotometry at 600 nm, and calculations were conducted according to the approach as outlined by Sanevas et al., (2007).

3.2.4.2 Superoxide Radical and Malondialdehyde (MDA)

The superoxide radical concentration in the tested Wild cabbage leaves was quantified following the method described by Gokul et al., (2018), where an extinction coefficient of 12.8 mM cm^{-1} was utilized for the calculation of superoxide radical levels in the leaves. The MDA content of the investigated samples was quantified following the method as alluded to by González-Orenga et al., (2021).

3.2.5 Activity of Antioxidant Enzymes

3.2.5.1 Determination of protein content

Protein from the leaf samples was extracted using a method described by Ali & Ludidi, (2021). Thereafter, a 200 mg of plant tissue taken from each treatment was homogenized in 400 µL of a protein extraction solution, comprising of 5% (w/v) polyvinylpolypyrrolidone (PVPP) and 40 mM phosphate buffer at pH 7.4, 1 mM ethylenediaminetetraacetic acid (EDTA). The mixture was centrifuged for 20 minutes at 4 °C at 13,000 rpm, after which the supernatant was utilized to conduct different enzymatic assays. Using the Bio-Rad reagent and bovine serum albumin (BSA) as a standard, the protein content of the extracts was determined.

3.2.5.2 Analysis of superoxide dismutase (SOD)

The method explained by Gokul et al., (2016) was utilized to determine the enzymatic activity of SOD. Briefly, a reaction mixture of 1 ml was prepared in 50 mM potassium phosphate buffer using 75 µM Nitrotetrazolium blue (NBT), 2 µM riboflavin, 13 mM DL-methionine, 100 µM EDTA, and 50 µl of enzyme extract. The absorbance was measured at 560 nm.

3.2.5.3 Determination of Catalase (CAT) activity

Catalase activity was determined by monitoring a reduction in absorbance at 240 nm following the consumption of H_2O_2 added to the extracts. One CAT unit is defined as the quantity of enzyme disintegrating one mmol of H_2O_2 at 25 °C per minute.

3.2.6 Statistical analysis

The data obtained from the treatments was expressed as means ± standard errors (SE) at the end of the experiment, and Fisher's least significant difference (LSD) was used to compare the significant differences between treatment means ($p \leq 0.05$). A MINITAB 17 statistical package was used to determine the statistical significance of means of different treatments.

3.3 Results

3.3.1 Plant growth responses

The results obtained from this study revealed that varying salinity concentrations significantly ($p \leq 0.05$) influenced the plant growth responses of *T. ciliata* as displayed in Table 3.1. Shoot length and root weight (wet and dry) were significantly higher on the treatment irrigated with the lowest salinity concentration (50 mM), followed by the control treatment (0 mM) before decreasing with increasing salinity. Interestingly, the highest mean values for leaf weight (Fresh and dry) were comparable between control and 50 mM treatments, while the lowest mean value were recorded under 200 mM, although they were comparable with values recorded in 100 and 150 mM treatments. Moreover, the flower buds' fresh and dry weights were significantly higher under 50 mM treatment followed by the control and 100 mM treatments respectively, while high salinity treatments (150 and 200 mM) did not produce any flower buds.

Table 3.1: Growth parameters of *T. ciliata* in response to salinity.

NaCl conc.	Length (cm)	Leaf FW (g)	Leaf DW (g)	Root FW (g)	Root DW (g)	Flower bud FW (g)	Flower bud DW (g)
0 mM	81.7±1.67b	163.5±11a	10.15±0.92a	26.66±3.37b	3.28±0.46b	82.2±2.48b	7.38±0.16b
50 mM	89.8±2.43a	166.3±15.1a	10.08±0.7a	37.95±4.23a	4.66±0.55a	106.2±4.58a	9.8±0.65a
100 mM	59.6±2.26c	54.5±3.37b	5.16±0.42b	19.21±2.69bc	2.35±0.33bc	40.4±2.14c	3.8±1.16c
150 mM	47.9±1.43d	44.09±4.1b	3.88±0.5b	16.32±1.74c	2.11±0.15c	-	-
200 mM	31.1±1.72e	34.9±2.66b	3.78±0.25b	13.07±1.39c	1.64±0.14c	-	-

FW= Fresh Weight, DW= Dry Weight. The values (mean ± SE) in each column denoted by different letters show significant differences at ($p \leq 0.05$).

3.3.2 Leaf hydration parameters

3.3.2.1 Relative Water Content and leaf succulence

Table 3.2 reveals that varying salinity concentrations significantly influenced the relative water content at $p \leq 0.05$. The highest RWC was obtained from the control treatment, although it was comparable to the values obtained from 50- and 100 mM treatments. The lowest RWC was recorded from 200 mM treatment. On the other hand, varying NaCl concentrations had no significant influence on the leaf succulence. However, leaf succulence marginally increased in direct proportion to increasing salinity concentrations.

3.3.2.2 Chlorophyll

The results obtained from photosynthetic pigment analysis revealed that salinity significantly affected both chlorophyll *a and b* content as shown in Table 3.2. Both chlorophyll *a and b* significantly decreased with increasing salinity, although chlorophyll b was comparable between plants treated with 50 and 100 mM of NaCl. In addition, total chlorophyll significantly decreased with increasing NaCl concentrations.

Table 3.2: Leaf hydration parameters of *T. ciliata* in response to increasing salinity.

NaCl conc.	Relative water content	Leaf succulence	Chlorophyll *a* (µg/g)	Chlorophyll *b* (µg/g)	Total Chlorophyll (µg/g)
0 mM	82.54±1.66a	0.17±0.01a	322.38±1.72a	167.07±0.84a	467.73±1.94a
50 mM	78.45±0.9ab	0.19±0.06a	305.75±1.02b	139.12±0.21b	423.45±1.03b
100 mM	74.76±2.03ab	0.21±0.02a	231.39±2.91c	139.04±2.79b	412.27±4.79c
150 mM	70.22±3.31b	0.24±0.05a	305.75±1.02b	125.95±1.84c	383.91±5.06d
200 mM	71.57±3.71b	0.28±0.03a	231.12±0.31d	101.46±0.16d	327.83±0.26e

The values (mean ± SE) in each column denoted by distinct letters show significant differences at ($p \leq 0.05$).

3.3.3 Oxidative stress markers

3.3.3.1 Cell viability, Superoxide and Malondialdehyde (MDA)

Varying salinity concentrations significantly influenced cell death, superoxide radical and MDA content of *T. ciliata* leaves. A common trend was observed between cell death, superoxide and MDA where mean values were directly proportional to increasing salinity concentrations (Figure 3.1, 3.2, and 3.3). Interestingly, cell viability was comparable between the leaves harvested from 50, 100, and 150 mM treatments (Figure 3.1). In addition, the highest cell death was recorded under the highest salinity treatment (200 mM), although this was comparable to 100 and 150 mM treatments. On the contrary, superoxide radical and MDA

content were significantly higher under 200 mM and low under control treatment (Figure 3.2 and 3.3).

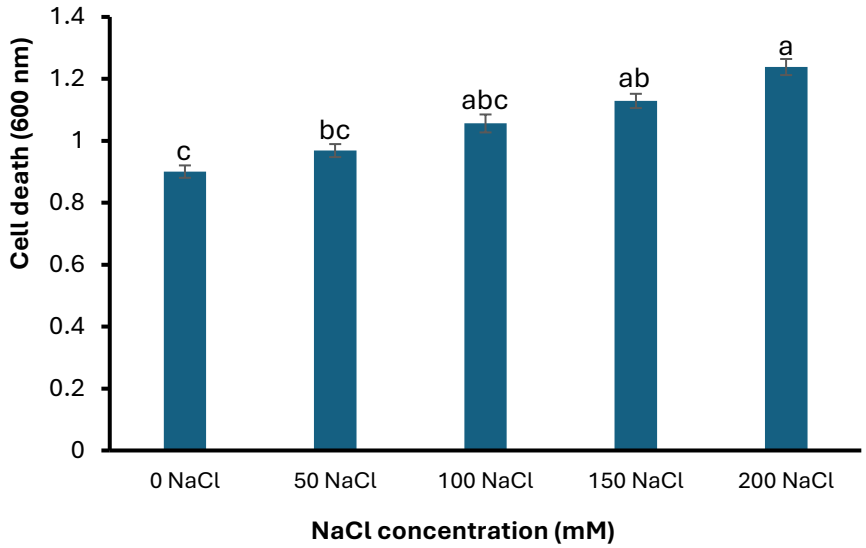


Figure 3.1: Cell viability of *T. ciliata* leaves in response to varying salinity levels. Means denoted with distinct letters are statistically different at $p \leq 0.05$.

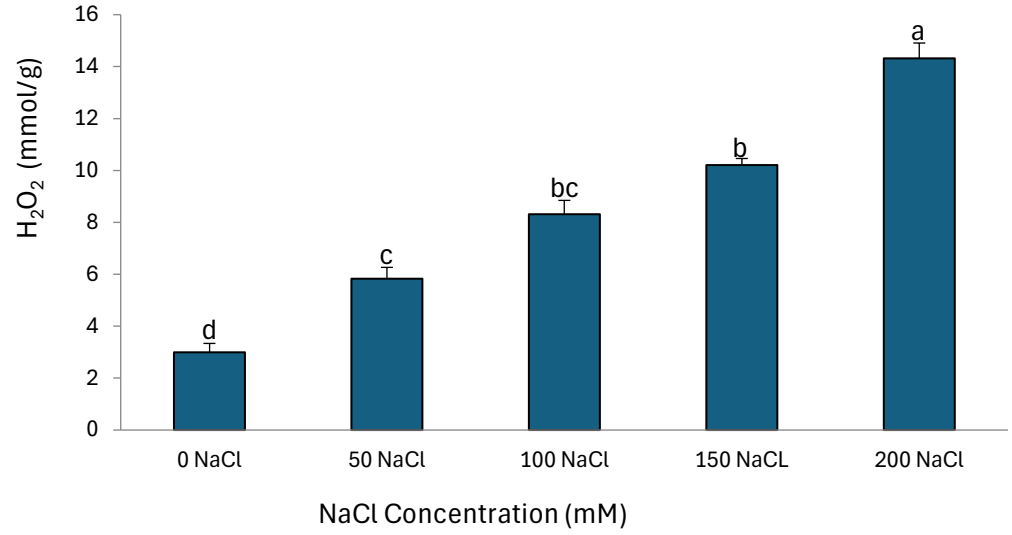


Figure 3.2: Superoxide radical concentration of *T. ciliata* leaves in response to salinity. Means denoted with distinct letters are statistically different at $p \leq 0.05$.

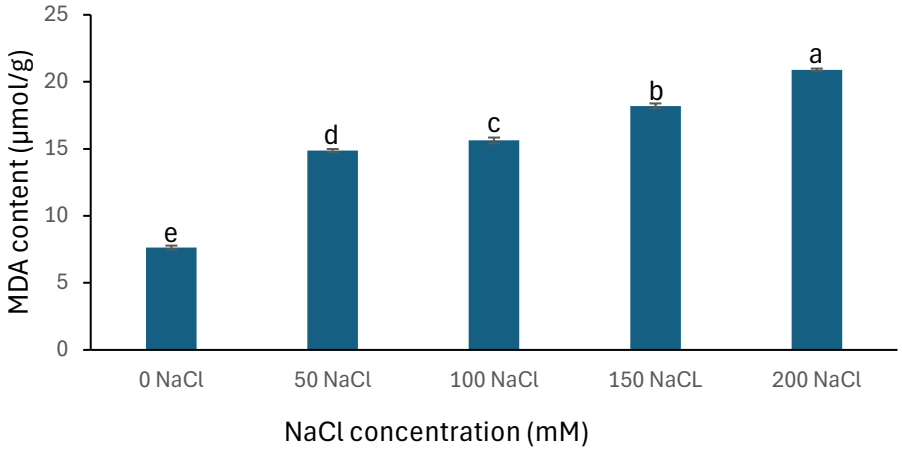


Figure 3.3: Malondialdehyde (MDA) content of *T. ciliata* in response to salinity. Means denoted with distinct letters are statistically different at $p \leq 0.05$.

3.3.4 Antioxidative enzymes activity

3.3.4.1 Superoxide Dismutase (SOD) and Catalase (CAT)

The effect of salinity on the antioxidative enzyme activity is displayed in Figures 3.4 and 3.5 respectively. Both SOD and CAT activities substantially increased in direct proportion to increasing salinity concentrations. The highest activity of both enzymes was recorded in samples collected from 200 mM NaCl treatment, while the lowest activity was recorded under control.

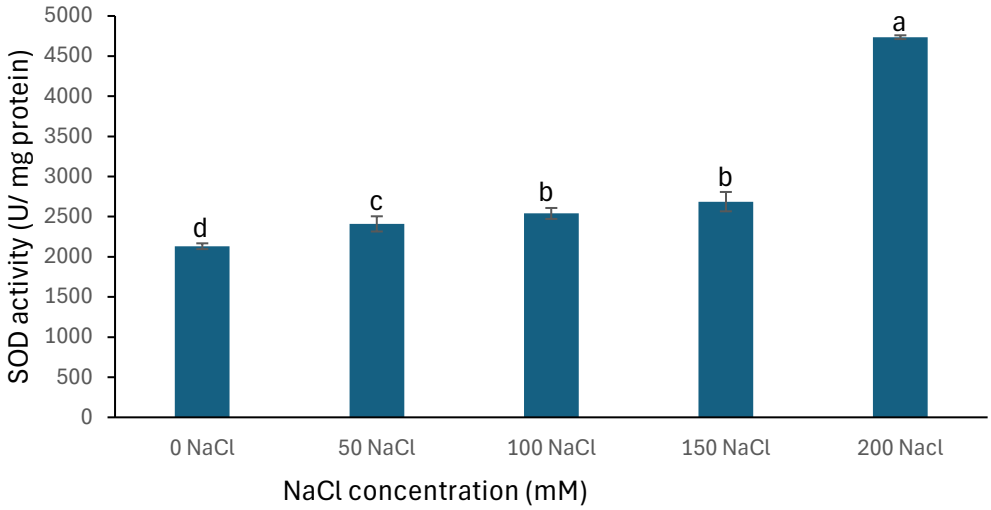


Figure 3.4: Superoxide Dismutase (SOD) activity of *T. ciliata* in response to salinity. Means denoted with distinct letters are statistically different at $p \leq 0.05$.

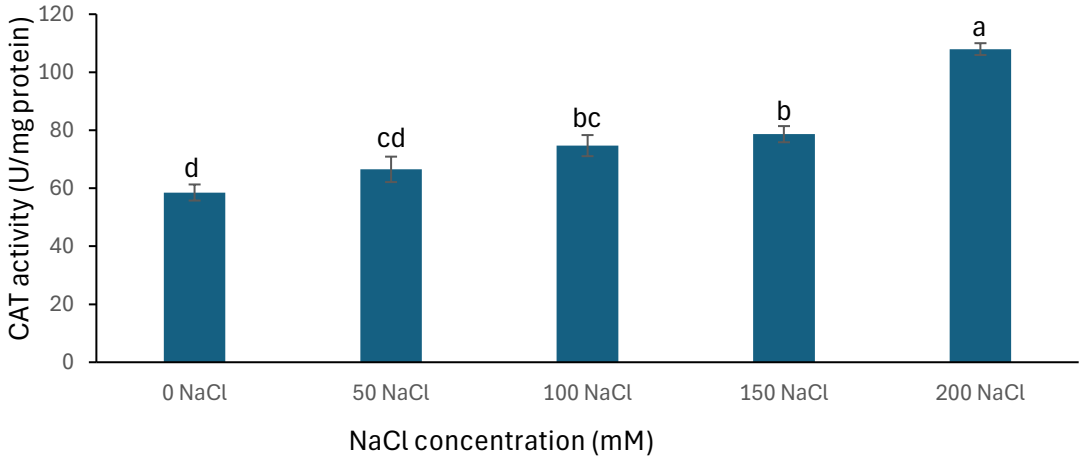


Figure 3.5: Catalase (CAT) activity of *T. ciliata* in response to salinity. Means denoted with distinct letters are statistically different at $p \leq 0.05$.

3.3.5 Proline content

In the tested samples of *T. ciliata*, the proline content significantly increased with increasing salinity (Figure 3.6). Proline content was extremely high under high salinity treatment (200 mM), while it was significantly low under control. Interestingly, proline content was comparable between samples treated with 100 mM and 150 mM salinity.

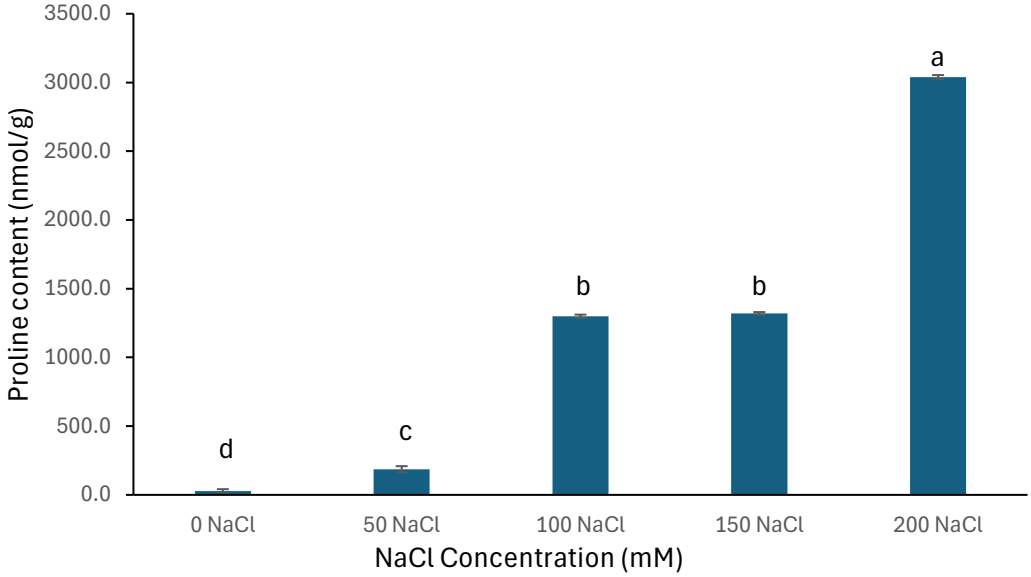


Figure 3.6: Proline content of *T. ciliata* in response to salinity. Means denoted with distinct letters are statistically different at $p \leq 0.05$.

3.4 Discussion

It has been previously reported that *Trachyandra ciliata* can tolerate salinity and that low to moderate salinity improved growth parameters in hydroponics (Ngxabi et al., 2021). However, the mechanisms of its tolerance and physio-biochemical responses under salinity stress are undocumented. Hence, it was necessary to investigate the influence of salinity stress on growth parameters, leaf hydration, photosynthetic pigmentation, cation content, oxidative stress markers and concentration of antioxidant enzymes to further understand mechanisms involved in its salt tolerance.

3.4.1 Growth responses under varying salinity treatments

Salinity is one of the abiotic factors that are known to negatively affect plant growth through interfering with respiration, photosynthetic rates and mineral translocation (Corwin, 2021b; Botella et al., 1997). Nonetheless, halophytes have been reported to tolerate salinity stress and maintain cell homeostasis through osmotic adjustment and mineral translocation, and thus able to complete their life cycles under salinity stress (Nikalje et al., 2019; Panta et al., 2014; Meng et al., 2018). In the current study, salinity positively influenced growth parameters of all plant parts at low concentration (50 mM NaCl) compared to the control. The same trend has been reported on other halophytes such as *Tetragonia decumbens*, *Chenopodium album* and *Portulaca oleraceae* cultivated under low salinity (Jeeva, 2020; Sogoni et al., 2021). Nevertheless, growth parameters expressively decreased with increasing salinity, with the lowest mean values all recorded under 200 mM treatment. It has been reported that plants under salt stress redirect a significant portion of energy towards stress regulation mechanisms instead of growth (Azeem et al., 2023), as demonstrated in this study.

Furthermore, a similar trend was noted in the flower buds, where 50 mM treatment recorded significantly higher values followed by control and 100 mM respectively, while no flower buds emerged from high salinity treatments (150 and 200 mM NaCl). Similar results have been reported on edible flowers of *Tagetes erecta* and *Crithmum maritimum,* where inflorescence yield decreased due to the reduction of MADS-box transcription factors (Proteins) which regulate many aspects of plant development such as flowering and fruit ripening (Guzman & Marques, 2023; Ventura et al., 2014).

3.4.2 Leaf hydration in response to salinity

The relative water content (RWC) of a leaf serves as a crucial measure of a plant's water status, as it represents the equilibrium between water supply to the leaf tissue and the rate of transpiration (Hand et al., 2017; Khatami et al., 2022). In the present study, the highest RWC mean value was comparable between control, 50 mM, and 100 mM treatments, then efficiently

declined under higher salinity treatments (150 and 200 mM). However, the lowest RWC was still comparable from 50 mM to 200 mM salinity treatments. The ability to maintain uniform RWC under low salinity to high salinity concentrations is a clear indication that *T. ciliata* has indeed developed mechanisms to maintain optimum water uptake under salinity stress. The efficient RWC across all treatments may be attributed to the large accumulation of key osmolytes which make uptake of surplus water possible under salinity as previously reported on other salt tolerant species (Hand et al., 2017; Ben Amor et al., 2020). It has also been reported that halophytes have the ability to maintain cellular homeostasis through osmotic adjustment (Ben Amor et al., 2020).

Furthermore, Results obtained from the current study revealed that salinity had no statistical difference on the leaf succulence of Wild cabbage at $p \leq 0.05$. Nevertheless, it is worth noting that leaf succulence increased in direct proportion to increasing salinity with the lowest recorded under control and highest leaf succulence obtained from 200 mM salinity treatment. It has been reported that leaf succulence may not drastically increase in some halophytic plants because of the mechanisms they have developed such as salt exclusion, salt elimination, salt redistribution, osmotic adjustments, and increased proline content amongst others (Guo et al., 2023; Yuan et al., 2016). The results in obtained from this study suggest that *T. ciliata* may be using salt exclusion mechanism to maintain cellular homeostasis to avoid salt toxicity at higher salinity concentrations.

3.4.3 Accumulation of chlorophyll in response to salinity

Total Chlorophyll content has been reported as an effective method for assessing the photosynthetic capacity and health of plants under both normal and stress conditions, including salinity stress (Guo et al., 2023). In this study, total chlorophyll negatively correlated with increasing salinity. Salinity stress is known to reduce leaf osmotic potential with a simultaneous increase oxidative stress, which may result in the production of Reactive Oxygen Species (ROS) (Salim Akhter et al., 2021; Siddiqui et al., 2020). Excessive presence of these ROS in the chloroplast may break the double bonds of unsaturated fatty acids, which can cause chloroplast membrane damage and chlorophyll leakage from thylakoids (Guo et al., 2023). This salinity induced oxidative stress leads to the reduction of photosynthetic activity by disrupting the reaction centres of photosystem and complicating the oxygen evolution (Salim Akhter et al., 2021). Similar trends of reduced chlorophyll content with increasing salinity have been reported in other halophytes such as *Nitraria schoberi*, *Lobularia maritima* and *Hordeum vulgare* (Hsouna et al., 2020; Zilaie et al., 2022; Salim Akhter et al., 2021).

3.4.4 Oxidative stress markers

Various biochemical indicators, including cell viability, superoxide, and lipid peroxidation, are frequently utilized to measure oxidative stress levels in plants growing under unfavourable conditions (Azeem et al., 2023). Damage markers such as Superoxide radicals and MDA are linked to free radical production, which can harm cellular structures and macromolecules, disrupt the oxidation-reduction potential, and reduce membrane fluidity, resulting in leakage of electrolytes and rapid dehydration (Azeem et al., 2023; Khatami et al., 2022). In the present study, cell death, superoxide radicals, and MDA (biomarker for measuring lipid peroxidation) all increased in direct proportion to increasing salinity concentration. The increase in superoxide and lipid peroxidation was significantly higher under 200 mM NaCl treatment, while cell death was consistent when salinity intensified from 100 to 200 mM salinity treatments. It has been reported that a reasonable amount of superoxide can have a positive effect on plants under stress by operating as signalling molecules to regulate growth and adaptation to stress (Khan et al., 2023; Kesawat et al., 2023). However, large amounts of these radicals may be toxic to plants, causing cellular damage and destructive processes such as oxidation of proteins, nucleic acids, and lipids, resulting in cell death (Choudhury et al., 2017; Hsouna et al., 2020). From these results, the significant increase and unchanged growth parameters of *T. ciliata* at 50 mM NaCl indicate that superoxide radicals were used for managing salt stress, which may have led to the activation of antioxidative enzymes (CAT and SOD) and stress adopter molecules such proline and soluble sugars, indicating the positive influence of superoxide radicals in salt tolerance of the plant. These findings are in consistence with Azeem et al., (2023) who reported an increase in oxidative stress markers as salinity increased, with reference to the positive influence of superoxide radicals under low salinity in *Moringa oleifera*.

3.4.5 Antioxidant enzymes activity

The immobile existence of plants has resulted in the development of a sophisticated, grid-like antioxidant defence system composed of many enzyme elements, which are essential for mitigating diverse stress conditions, including salinity (Rajput et al., 2021). The capacity of plants to mitigate the detrimental effects of ROS appears to be one of the most important variables influencing their resilience to salinity stress. Catalase (CAT) and Superoxide Dismutase (SOD) are among the most influential antioxidant enzymes that play a crucial role in mitigating the catastrophic effects of stress as part of the antioxidant defence system (Zhou et al., 2018; Rajput et al., 2021). During salinity stress, SOD catalyses the elimination of superoxide by disproportioning it into Oxygen and Hydrogen Peroxide (H_2O_2), while CAT converts the H_2O_2 into water and molecular oxygen (Rajput et al., 2021; Azeem et al., 2023). In the current study, both SOD and CAT increased with increasing salinity with a more

noticeable content recorded under high salinity treatment (200 mM NaCl). It has been reported that halophytes produce more of these enzymes under severe stress, a phenomenon that provides adaptive advantage over salt-sensitive species (González-Orenga, Grigore, et al., 2021). These findings indicate that *T. ciliata* produce high amounts of these enzymes as a first line of defence against ROS caused by salinity stress. These findings are in consistence with earlier observations on other salt tolerant species such as *Limonium* species, Bupleurum species, and *Moringa oleifera* (Azeem et al., 2023; González-Orenga et al., 2021a; González-Orenga, et al., 2021b).

3.4.6 Proline content

Accumulation of proline serves as a key indicator of salinity stress amongst other metabolites such as amino acids and peptides that are produced by plants under stress (Hnilickova et al., 2021). Studies indicate that proline scavenges ROS generated under severe stress, stabilizes protein structure, and regulates cytosolic pH and cellular redox homeostasis, hence strengthening plant resilience to drought stress during growth (Lee et al., 2018; Hnilickova et al., 2021). In this study, Proline content increased in direct proportion to increasing salinity concentration, with a significantly high amount observed under 200 mM NaCl treatment. This suggest that *T. ciliata* produced high proline content to maintain cellular homeostasis and growth under salinity stress, hence the plant remained alive in all saline treatments as observed in other halophytes such as *Salicornia prostrata*, *Suaeda prostrata*, *portulaca oleracea*, and *Bassia sedoides* (Hnilickova et al., 2021; Lee et al., 2018; Akcin & Yalcin, 2016).

3.5 Conclusion

Results from this study reveal that low salinity (50 mM NaCl) enhances growth parameters when compared to the control while high salinity concentration significantly decreases growth in *T. ciliata*. The efficient RWC across all treatments is attributed to the large accumulation of key osmolytes which makes surplus of water uptake under salinity possible, while the constant leaf succulence indicates salt exclusion, salt elimination, salt redistribution, and osmotic adjustment ability that has been reported earlier on this plant. To mitigate the catastrophic effect of salinity, *T. ciliata* produces high amounts of antioxidant enzymes (SOD and CAT) which catalyse superoxide and convert it to water and molecular oxygen, while high proline content ensures further scavenging of ROS, hence stabilizing protein structure and maintaining cellular homeostasis under high salinity. These findings indicate that *T. ciliata* can tolerate salinity by modulating bio-physiological processes to manage oxidative stress successfully and this potential may be exploited in bio-saline agriculture. Further studies on the leaf ultrastructure and micromorphological parameters are recommended to validate the salt exclusion suggested by these results.

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3.7 Ethical declaration

This work does not include any animal or human participants.

3.8 Conflict of interest

The authors declare that they do not have any conflicts of interest

3.9 References

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CHAPTER FOUR

LEAF MICROMORPHOLOGICAL ASSESSMENT, CHEMICAL COMPOSITION AND ANATOMICAL RESPONSES OF *TRACHYANDRA CILIATA* (L.F) KUNTH TO DIFFERENT DEGREES OF SALINITY

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Leaf Micromorphological assessment, chemical composition and anatomical responses of *Trachyandra ciliata* (L.F) Kunth to different degrees of salinity

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Abstract

Many studies have examined the morphological and micromorphological responses of different halophytes to determine their salt tolerance mechanisms. However, few studies have focused on the South African edible halophytes. This study examined the leaf micromorphology, elemental composition, and anatomical responses using the Scanning Electron microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) to examine salt tolerance levels in *T. ciliata*. The treatments included varying sodium chloride (NaCl) concentrations: 50 mM, 100 mM, 150 mM and 200 mM, while control (0 mM) was watered with nutrient solution only. From the SEM micrographs, salt glands were observed protruding from the epidermis along the vascular system under low salinity and salt crystals appeared under higher concentrations, which makes this plant maintain cellular homeostasis under high salinity, and the plant can be classified as a recretohalophyte. Stomatal distribution, stomatal density and the number of open stomata decreased with increasing salinity. EDX revealed the presence of some important elements such as Potassium, Magnesium, Phosphorus, Calcium and more in the leaves. The results showed that increased salinity led to a decrease in the percentage composition of P, K and Ca^{2+}, while Mg^{2+} was high under control and low salinity (50 mM), decreased under 100 mM and increased again with increasing salinity. On the contrary, increasing salinity caused an increase in Na^{+} and Cl^{-} in a stable manner. These findings reveal that *T. ciliata* acquires salt tolerance through changes to its leaf surface properties, osmotic adjustment, and the regulation of Na^{+} uptake and distribution in the leaves.

Keywords: Asphodelaceae, recretohalophytes, micromorphology, salt glands, *Tachyandra ciliata*, Wild cabbage, salt stress

Abbreviations:

SEM- Scanning Electron Microscopy

EDX- Energy Dispersive X-Ray Spectroscopy

LSD- Least Significant Difference

Na- Sodium

Ca- Calcium

Mg- Magnesium

NaCl- Sodium Chloride

P- Phosphorus

K- Potassium

4.1 Introduction

One of the main abiotic factors in agriculture that promotes deficiency symptoms, physiological abnormalities, and lower output of field crops around the world is salinity, along with drought (Ahmad et al., 2018). However, halophytes have developed several adaptations to withstand seawater and increased salinity concentrations, such as accumulation of suitable organic solutes, succulence, salt-secreting glands and bladders, ion compartmentalization in cell vacuoles, and adjustment of their internal water relations (Shabala, 2013; Mishra & Tanna, 2017). It is stated that the sensitivity of different plants to soil salinity varies. Hence it is important to study anatomical responses to salinity for different halophytes (Chavarria et al., 2020).

Many studies have been conducted to examine the morphological and micromorphological responses of different halophytes to determine their salt tolerance mechanisms (Jimoh et al., 2019; Sogoni et al., 2023). However, few studies have focused on the South African edible halophytes. *Trachyandra ciliata*, sometimes known as wild cabbage or Veldkool (Afrikaans), is a halophytic plant in the Asphodelaceae family, endemic to the coastal winter rainfall southwestern Cape in South Africa (Manning & Goldblatt, 2007; S. Ngxabi et al., 2021). The therapeutic characteristics of the Asphodelaceae family have been thoroughly investigated throughout the years, and they are frequently employed in the beverage and pharmaceutical sectors(Grace et al., 2008; Bodede & Prinsloo, 2020). However, the *Trachyandra* genus is underexploited with little to no literature (De Vynck et al., 2016). Ngxabi et al., (2021b) reported that the inflorescence of *T.*

ciliata is edible and was used as a vegetable by the native people that lived in the area before colonization and removal of people from the area. They further studied its salt tolerance and phytochemicals in response in response to salinity stress (Sihle Ngxabi et al., 2021). As such, there is a need to further examine its anatomical and micromorphological responses to understand the internal mechanisms it employs to tolerate high levels of salinity.

For morphological microanalysis of plant tissues, scanning electron microscopy (SEM) has been utilized in the past (Sharaibi & Afolayan, 2017; Fank-De-Carvalho et al., 2010). Great magnifications are used in SEM, which results in highly precise images with great resolution (Chavarria et al., 2020). Several studies have been conducted to examine salt gland shapes, stomatal distribution and density in different halophytes (Pompelli et al., 2021; Sogoni et al., 2023). However, there have not been many of these studies regarding South African halophytes and *T. ciliata* is no exception (Sihle Ngxabi et al., 2021). Furthermore, with methods like energy-dispersive X-ray spectroscopy, the difficulty of getting precise biological information on the classical ultrastructure of plants has been substantially reduced in recent years (Sogoni et al., 2023; Jimoh et al., 2019). Energy Dispersive X-ray spectroscopy, a chemical microanalysis, has been used to ascertain the elemental composition of plant tissue. Additionally, silica body quantity and presence in the foliar epidermis of various plant species have been evaluated using EDX (Ushilo et al., 2017; Chavarria et al., 2020). The main aim of this study was to examine leaf micromorphology, elemental compartmentalization and anatomical responses using Scanning Electron microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) to clarify salt tolerance mechanisms in *T. ciliata*.

4.2 Material and Methods

This study was conducted at the Bellville campus of the Cape Peninsula University of Technology in Cape Town, South Africa, at coordinates 33.55048.800 S and 18.38032.700 E. With the aid of environmental control, the study's experimental greenhouse was maintained at a constant temperature of between 12 and 18 °C at night and between 21 and 26 °C during the day. 60% was the average relative humidity.

4.2.1 Plant material and experimental design

Healthy stalks of *T. ciliata* were acquired from a local nursery. Due to the presence of rhizomes, the plant material was propagated through the division technique. A total of 150 plants of uniform size were then transplanted into pots (12.5 cm height × 12.5 cm length × 12.5 cm width) containing a mixture of Peat: perlite: vermiculite (PPV) at (1:1:1). Cuttings were obtained from the same

mother stock population to ensure they are as genetically identical as possible. The plantlets were divided into 5 treatments of 30 replicates each in a block arrangement. At the beginning of the greenhouse experiment, for four weeks plants were watered with only NUTRIFEED™ complete water-soluble fertilizer manufactured by (STARKE AYRES Pty. Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, South Africa, 1619) dissolved in municipal water at 10g per 5L ratio for plants to acclimatize in the experimental setup. The aqueous solution contained the following ingredients: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg). Using sodium chloride (NaCl) in the nutrient solutions, various salt concentrations were adjusted. Four salt concentrations (50 mM, 100 mM, 150 mM, and 200 mM of NaCl) were tested in this experiment, while the control was watered only with the nutrient solution. Plants were equally watered at a three-day interval with 300 mL of the nutrient solution with and/or without NaCl. To ascertain that proper salinity levels were maintained, drain water from every pot was collected, and the electrical conductivity was measured. The pH of the solution was maintained at 6.0 with the aid of a calibrated hand-held digital pH meter (Eurotech®TM pH 2 pen). Potassium hydroxide was used to elevate pH, while phosphoric acid was used to decrease the pH of the nutrient solution (Sihle Ngxabi et al., 2021). A calibrated hand-held digital EC meter (Hanna instruments®TM HI 98312) was utilized to closely monitor the electric conductivity of the nutrient solution. The plants were harvested after 15 weeks of salinity treatments to conduct micromorphological assessments.

4.2.2 Sample preparation for SEM and EDX analyses

A minora blade was used to cut the samples into 1 cm by 1 cm squares, and they were then immediately submerged in 2.4% glutaraldehyde (GLA) for four hours. Following the fixing procedure, the samples were dehydrated using 50% -70% - 90% EtOH for 15 seconds, followed by 100% EtOH for 2 seconds on each sample. The samples were mounted on aluminium stubs with double-sided carbon tape. The samples were subsequently covered in a thin (10 nm) and thick (15 nm) layer of carbon using the Quorum Q150TE carbon coater to make the sample surface electrically conductive to avoid electron build-up on the sample surface, which may cause electron charge. This procedure was followed as suggested by Sogoni et al., (2023).

4.2.3 Laboratory SEM and EDX examination of leaves

SEM and EDX examination were conducted following a procedure as described by Sogoni et al., (2023). The back Scattered Electron images of treated samples were examined in A Zeiss 5-diode

Back Scattered Electron (BSE) Detector (Zeiss NTS BSD) and Zeiss Smart SEM software at different magnifications to provide surface topography and morphological information of the sample. The samples' chemical composition was established by using semi-quantitative/full quantitative Energy Dispersive X-Ray Spectrometry using an Oxford Instruments® X-Max 20 mm2 detector and Oxford Aztec software/INCA Oxford software. Beam conditions included a 20 kV accelerating voltage, 1.4 nA probe current, a working distance of 9.5 mm, and a beam current of 11 nA for the quantitative analysis and backscattered electron image analysis on the Zeiss MERLIN. The counting time was 10 seconds live-time/ Zeiss EVO MA15 accelerating voltage 20kV, IProbe 1.1nA, and specimen current of 19nA and 8.5 mm working distance. Stomata density, distance between two stomata, and percentage of open and closed stomata were all considered as quantitative parameters. The qualitative parameters measured were stomata distribution, salt glands, and salt crystals.

4.2.4Statistical analysis

At the end of the experiments, the data obtained from the treatments was expressed as means ± standard errors (SE) of two replicates, and Fisher's least significant difference (LSD) was used to compare the significant differences between treatment means at $p \leq 0.05$. A MINITAB 17 statistical package was used to determine the statistical significance of means of different treatments for EDX chemical composition, stomata density, and percentage of open and closed stomata.

4.3 Results

4.3.1 Electron microscopy analysis of leaf surfaces

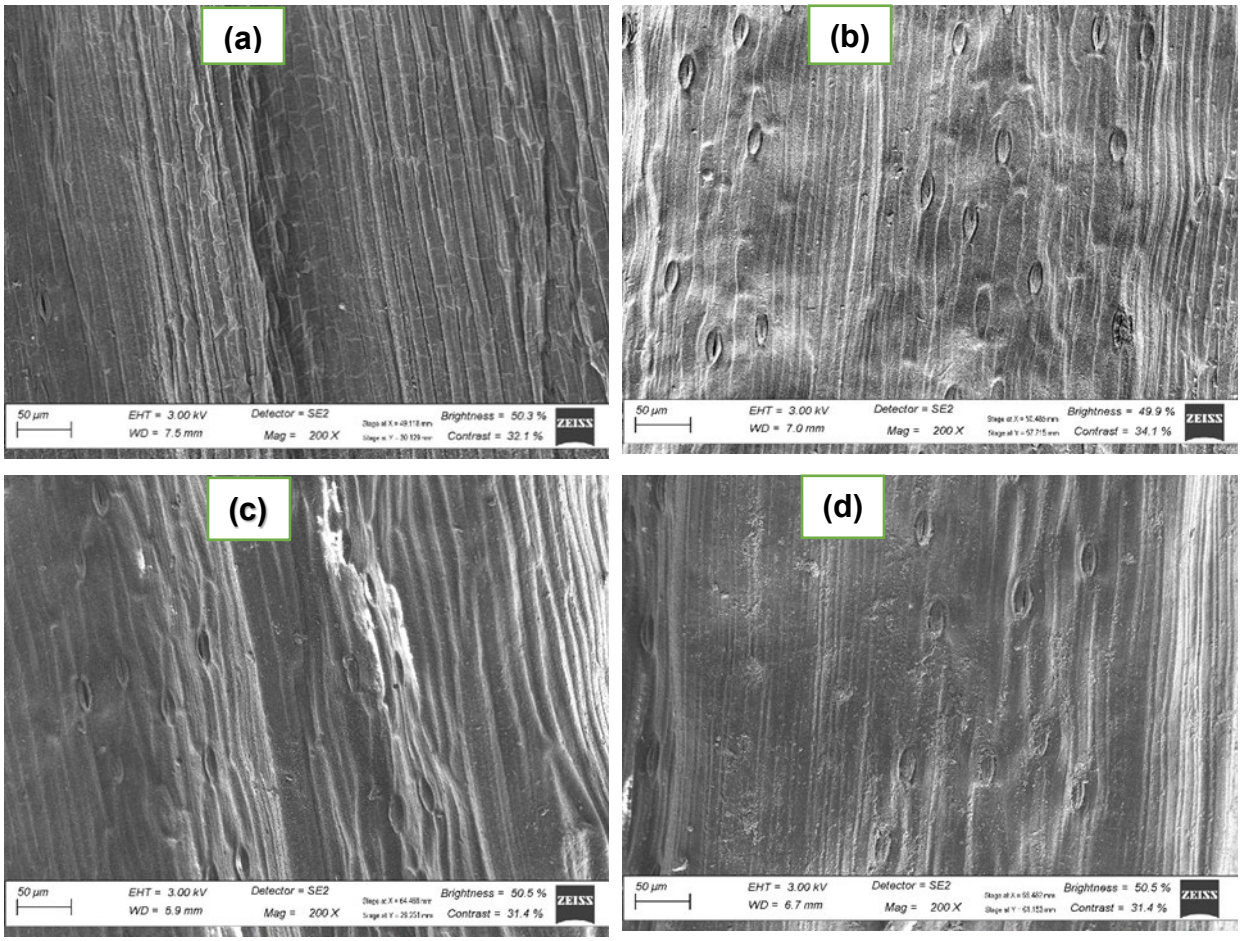
4.3.1.1 Stomatal Density and distribution

Based on stomatal distribution observed in SEM micrographs under 200 x magnification across all treatments, the leaves are characterized by sunken, anomocytic stomata that lack subsidiary cells (Figure 4.1). Stomatal density was calculated as the stomatal count per unit area (Jimoh & Olowokudejo, 2017). Results gathered from the experiment reveal that salinity treatments significantly influenced stomatal density and distribution. The highest stomatal density mean value was recorded under low salinity treatment (50 mM) followed by moderate salinity treatment (100 mM), while control and 150 mM recorded equivalent stomatal density (Table 4.1). In contrast, a significantly low stomatal density mean value was recorded under the highest salinity treatment of 200 mM. Stomatal distribution was extracted from SEM micrographs under 200 x magnification across all treatments (Figure 4.1).

Table 4.1: Effect of salinity treatments on stomatal density

NaCl treatments	Control	50 mM	100 mM	150 mM	200 mM
Stomatal density, mm2	11.2 ± 0.86bc	17.9 ± 1.29a	13.9 ± 1.08b	11.2 ± 1.29bc	7.9 ± 0.9c

Mean values that do not share the same letters are statistically different at P ≤ 0.001



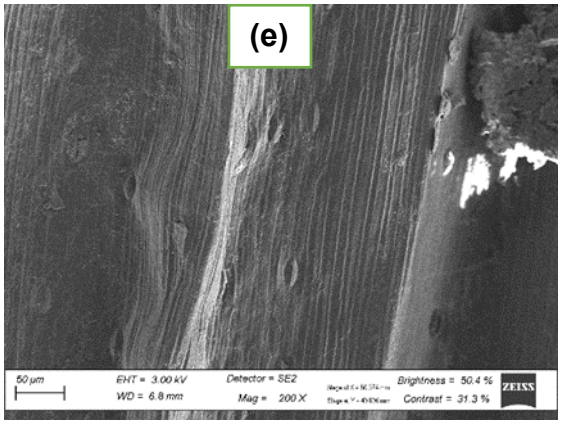


Figure 4.1: (a) Control, (b) 50 mM NaCl, (c) 100 mM NaCl, (d) 150 mM NaCl, and (e) 200 mM SEM images demonstrating stomatal density and distribution on the leaf surface of wild cabbage.

4.3.1.2 Open and closed stomata

A microscopic analysis demonstrated clear changes between the leaf surfaces of the five treatments due to increasing salinity including the number of open and closed stomata. Statistical analysis revealed that salinity significantly influenced the percentage of open and closed stomata among the treatments. Plants cultivated under control (0mM) and low salinity (50mM) treatment recorded significantly high percentages of open stomata compared to all other treatments, with control recording the highest percentage (71%) (Figure 4.2). As salinity increased, the percentage of open stomata reduced significantly, with the lowest percentage (28%) recorded under the highest salt treatment (200mM). On the other hand, the highest percentage of closed stomata (72%) was observed under the highest salt treatment followed by 150mM, which recorded 61.67% (Figure 4.2). The lowest percentages of closed stomata were observed under control treatment followed by low salinity levels, which recorded 29 and 34.33% respectively. Under moderate salinity (100 mM) the percentage of closed stomata was higher than that of open stomata, but the numbers were statistically different.

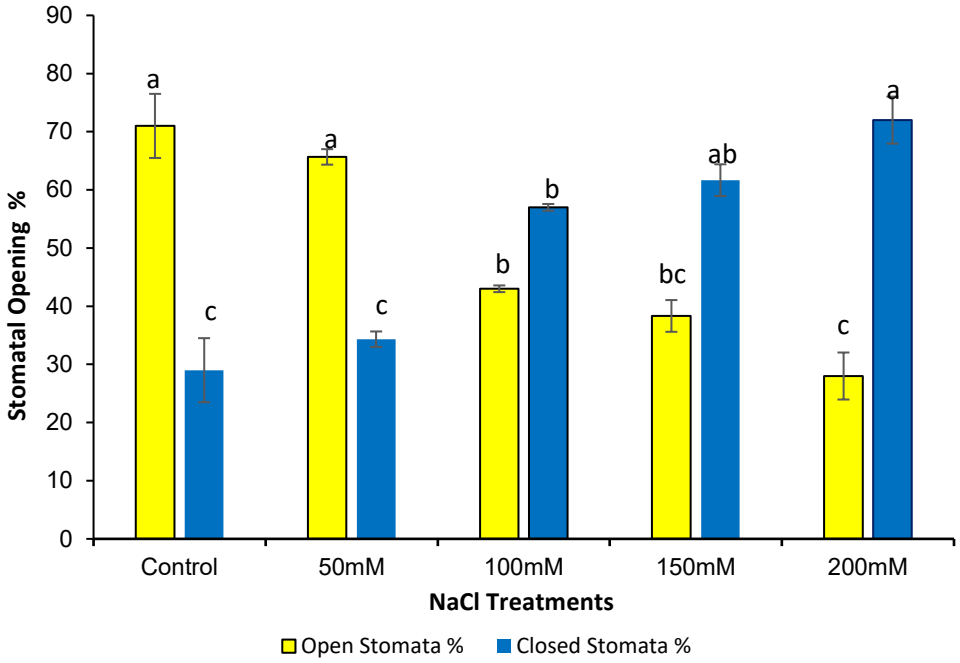


Figure 4.2: The influence of different NaCl treatments on the stomatal opening.

4.3.1.3 Salt glands and salt crystals

Salt glands were observed protruding from the epidermis along the vascular system under control, low (50 mM), and moderate salinity levels (100 mM). The oval-shaped salt glands are smaller under control, bigger and well-defined under low salinity, and smaller again under moderate salinity (Figure 4.3). Salt crystals were observed on the leaf surface of wild cabbage under higher salinity concentrations (150 mM and 200 mM). It was observed that the plant keeps salt in salt glands under low salt concentrations, while glands get ruptured by the emergence of salt crystals under higher salt concentrations (Figure 4.3) as the plant excludes salts to avoid cell damage by sodium ions. It was also observed that as the salt glands get ruptured by salt crystals, the surface becomes flaccid demonstrating loss of water and cell damage under high salt concentration (Figure 4.3, (e)) compared to the turgid surface observed in control (Figure 4.3, (a)).

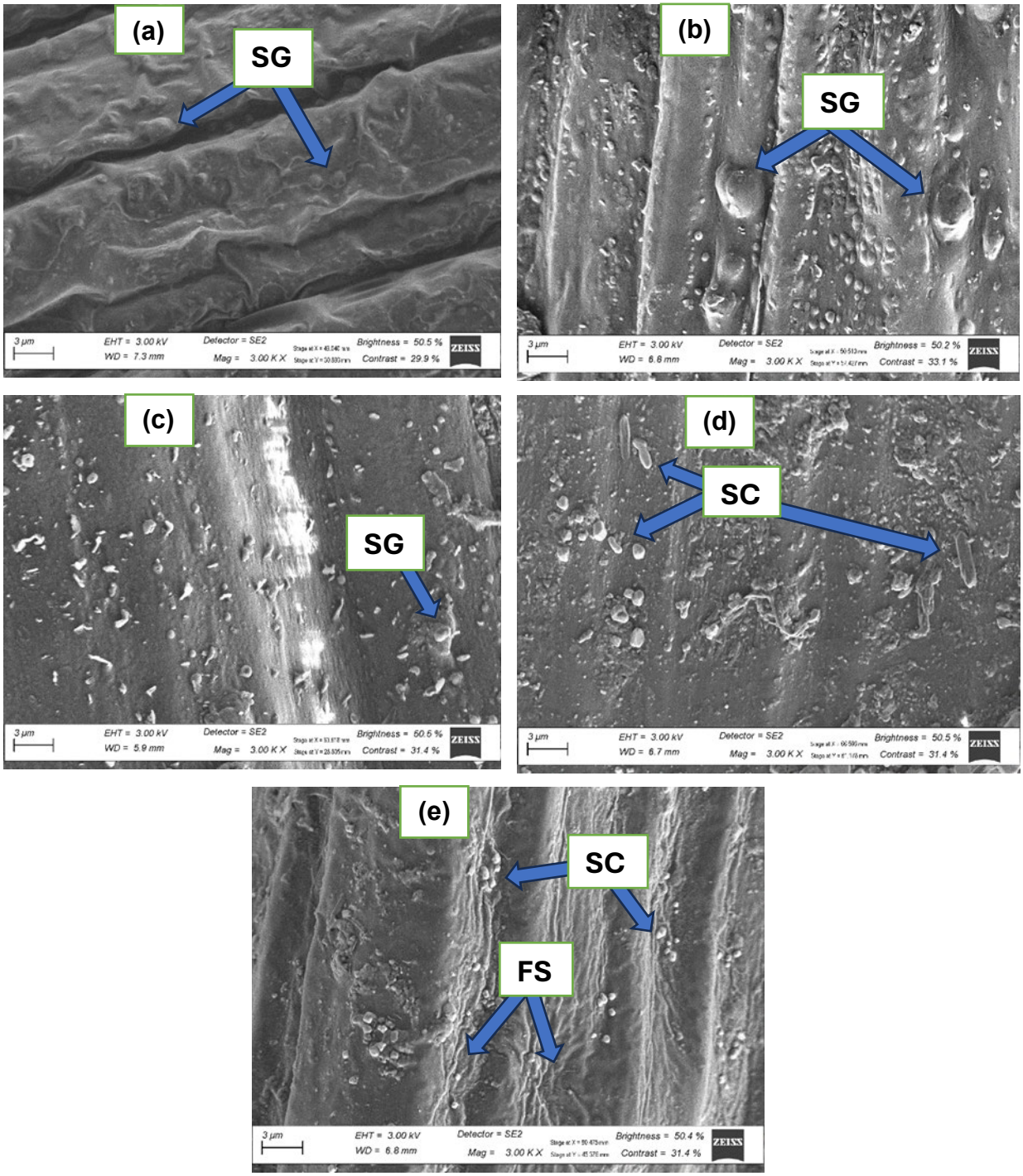


Figure 4.3: (a) Control, (b) 50 mM NaCl, (c) 100 mM NaCl, (d) 150 mM NaCl, and (e) 200 mM.SEM images demonstrating the appearance of salt glands and salt crystals on the leaf surface of Wild cabbage under different levels of salinity. SG= salt glands; SC= salt crystals; FS= flaccid surface.

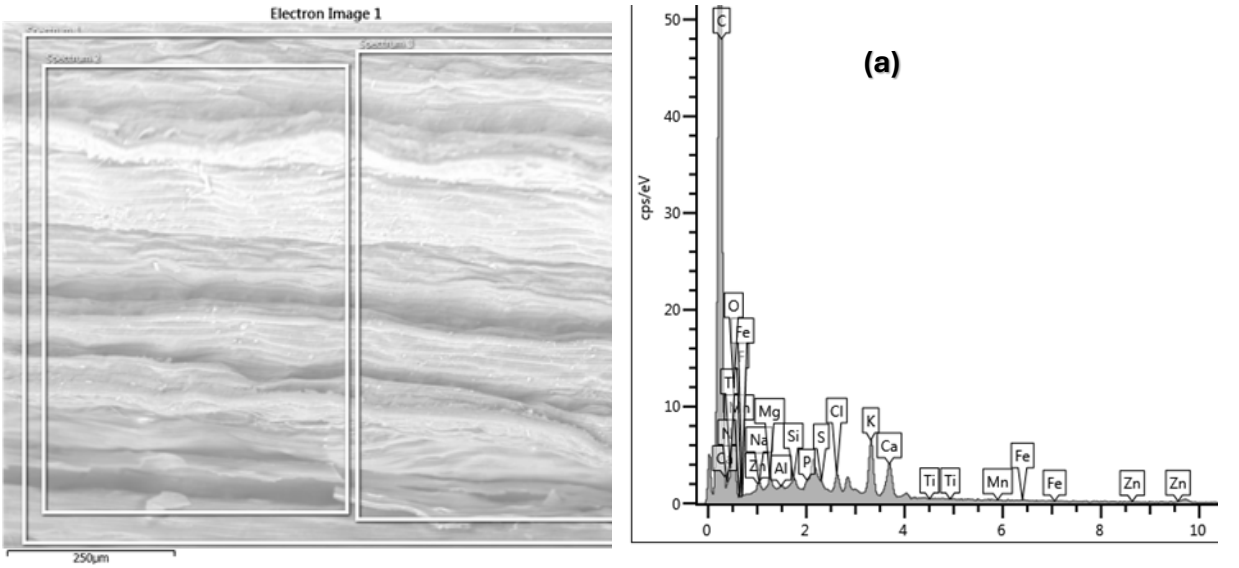
4.3.2 Energy dispersive spectroscopy (EDX) analysis of leaf surface

The percentage of chemical atomic composition in the leaf epidermal layer of *T. ciliata* was subsequently determined by using energy dispersive spectroscopy. The EDX analysis confirmed that increasing salinity significantly modulated the distribution of chemical elements on the leaf surface of *T. ciliata* as presented in Table 4.2 and Figure 4.4. The concentration of Sodium (Na) increased in direct proportion with increasing salinity levels, with the lowest concentration (0.6±0.07%) detected under the control treatment and the highest concentration (3.9±0.22%) detected under the highest salt concentration. Control and low salinity concentrations produced the highest levels of Magnesium (Mg) with 0.65±0.01 and 0.59±0.04% respectively, while the lowest atomic mass (0.12±0.02%) was found under moderate salinity level (100 mM) and surprisingly increasing again under 150mM salinity. An equally higher amount of Phosphorus (P) was found under control and 50mM salinity, decreased under moderate (100mM) salinity, and surprisingly increased again as salinity increased. Likewise with Potassium (K), an equally high composition (3.9%) was detected under control and 50mM salinity levels exponentially declined with increasing salinity. Chlorine (Cl) composition was directly proportional to the increasing salinity as described for sodium. However, Calcium (Ca) composition was significantly higher (3.56±0.21%) under 50mM salinity, followed by control (2.65±0.06%), and then gradually decreased as salinity increased.

Table 4.2: Main chemical components detected on the surface and subsurface of leaves using energy dispersive spectroscopic analysis.

Treatments	Na%	Mg%	P%	K%	Cl%	Ca%
0 mM	0.6±0.07d	0.65±0.01a	0.35±0.03a	3.9±0.09a	0.2±0.01e	2.65±0.06b
50 mM	2.2±0.08c	0.59±0.04a	0.35±0.03a	3.9±0.03a	1.97±0.08d	3.56±0.21a
100 mM	3.3±0.06b	0.12±0.02d	0.21±0.03b	2.8±0.11b	4±0.15c	1.59±0.01c
150 mM	3.5±0.12ab	0.4±0.03b	0.17±0.03b	2.7±0.05b	5.6±0.16b	1.44±0.06c
200 mM	3.9±0.22a	0.3±0.01c	0.18±0.04b	1.7±0.07c	6.09±0.23a	1.3±0.06c
			F- Statistic			
F- Value	155.9**	74.3**	7.6*	44.1**	288**	85.9**

Values (Mean ± SE) followed by different letters are statistically different from each other as determined by Fisher's least significant difference at P ≤0.05 (*) and P ≤0.001 (**) probability levels.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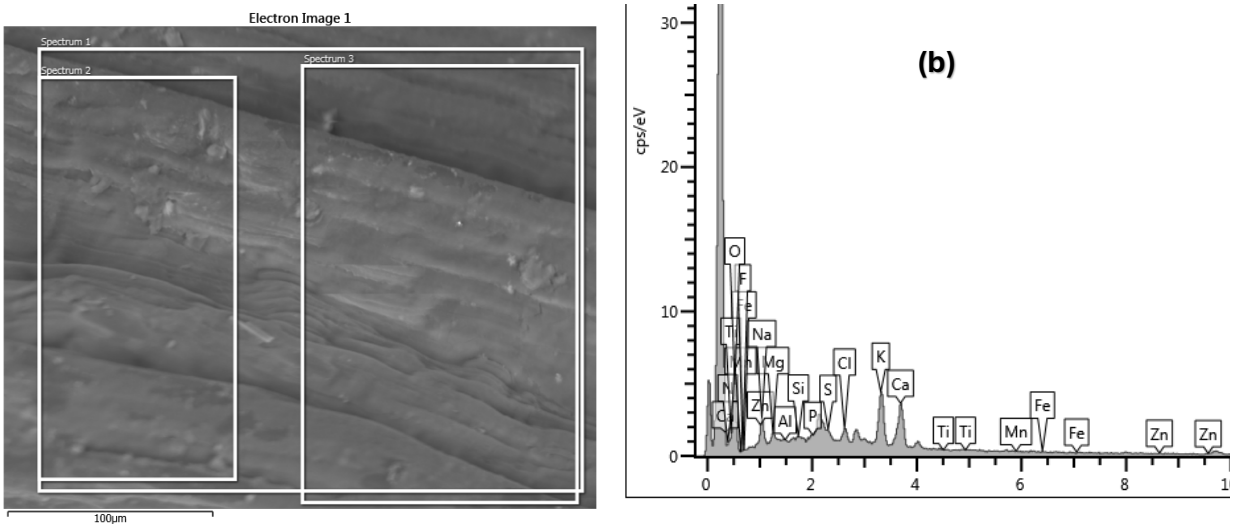
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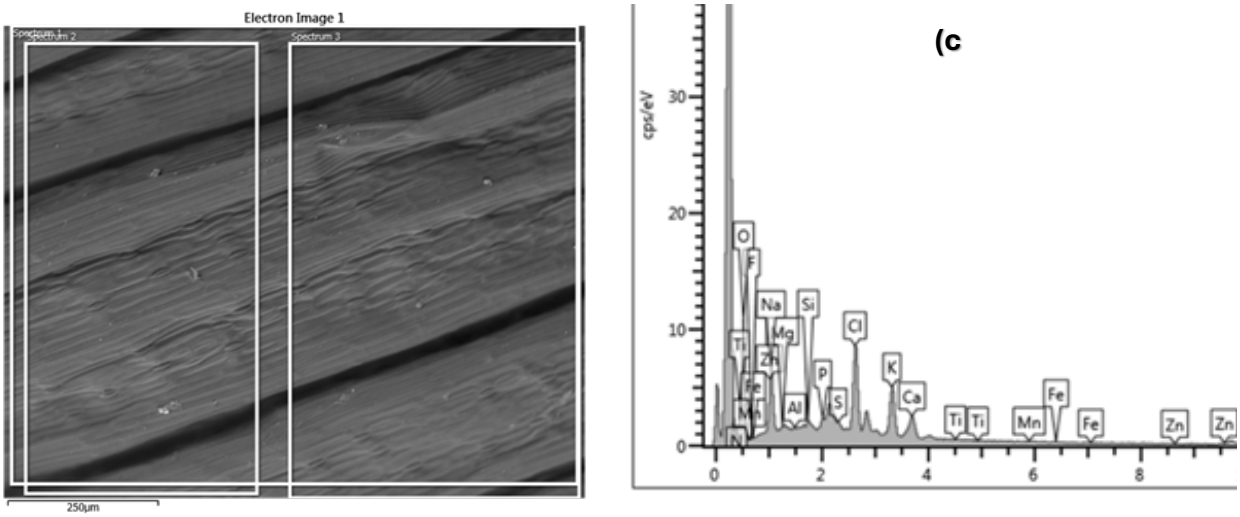
Electron Image 1
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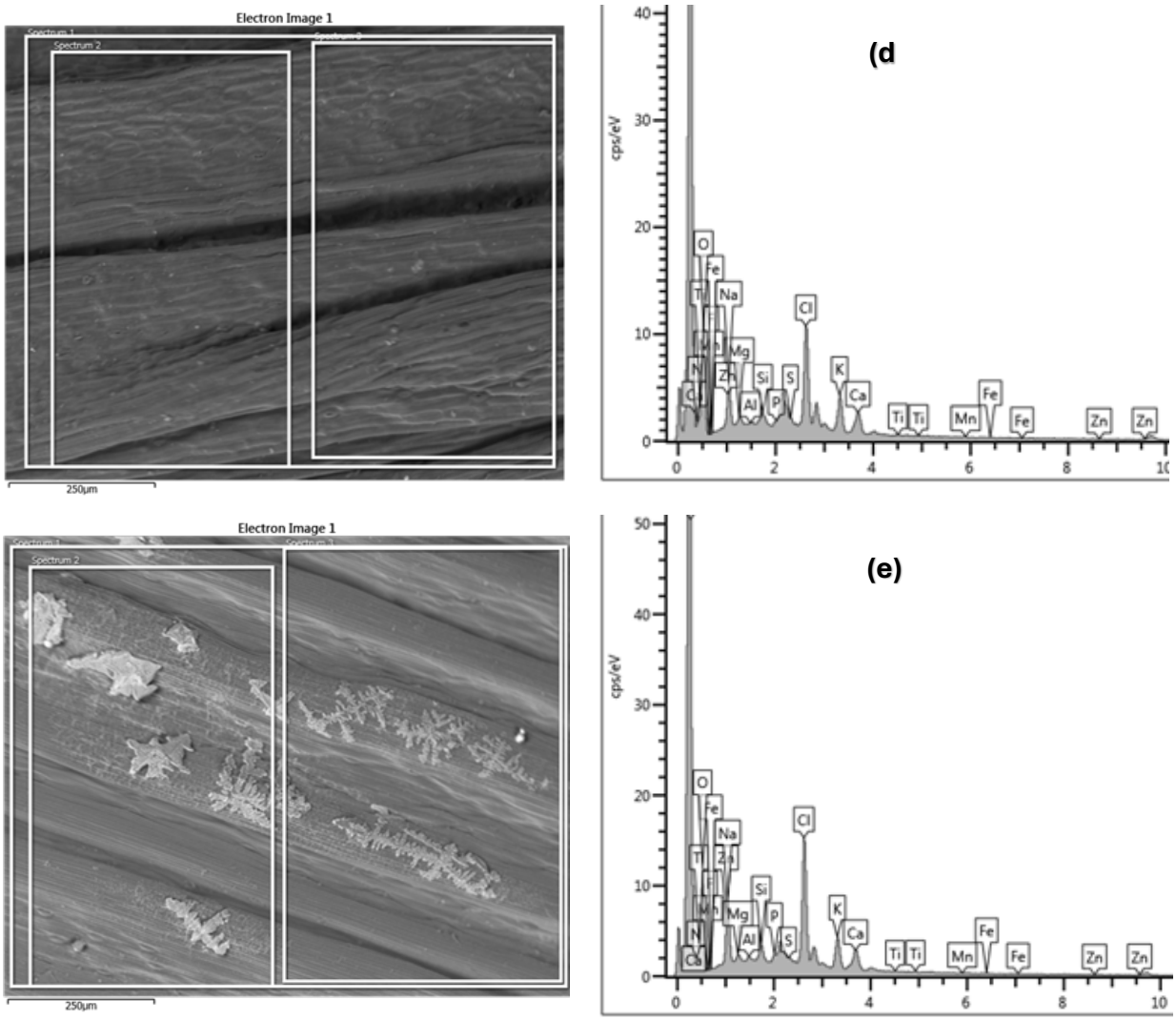

Figure 4.4: (a) Control, (b) 50 mM NaCl, (c) 100 mM NaCl, (d) 150 mM NaCl, and (e) 200 mM Randomly picked plates demonstrating the main elemental components on the leaf epidermis of *T. ciliata* by energy dispersive spectroscopic analysis.

4.4 Discussion

This study examined the leaf micromorphology, elemental composition and anatomical responses using Scanning Electron microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) to clarify salt tolerance mechanisms in *T. ciliata*. The results revealed that salinity induced stomatal density and the ratio of open and closed stomata, which are important factors in regulating gaseous exchange and water loss. The highest stomatal density was recorded under low salinity (50 mM) followed by moderate salinity (100 mM), while control had the same stomatal density as 150 mM, and the lowest stomatal density was recorded under high salinity treatment (200 mM). Control and low salinity treatment had a significantly higher ratio of open stomata, while high

salinity treatment resulted in a significantly low percentage of open stomata. The reduction in stomatal density and reduced percentage of open stomata under salt stress has also been recorded in other halophytic species such as *Chenopodium quinoa, Tetragonia decumbens,* and *Chenopodium album* (Sogoni et al., 2023; Rasouli et al., 2021). These findings suggest that halophytes can regulate stomatal number in response to salinity stress. It has also been reported in another study that halophytes typically utilize this strategy to prevent dehydration and perhaps slow down the rate at which transpiration water fluxes supply salt to the leaves (Lawson & Vialet-Chabrand, 2019).

From microscopic analysis, the oval-shaped salt glands were observed protruding from the epidermis along the vascular system. These glands enable plants to store and secrete excess salts to maintain osmotic balance and prevent damage to the cells (Gao et al., 2015; Yuan & Wang, 2020). The salt glands were observed under control where they were relatively small, 50 mM where they appeared bigger and well defined, and at 100 mM where they appeared small again, while there was no sign of the glands under higher salt concentrations. Salt crystals were observed under 150 mM and 200 mM, while none were observed under lower concentrations. This suggests that salt glands may have been ruptured by the emergence of salt crystals which appeared at high concentrations as observed in *Sporobolus virginicus* (Naidoo & Naidoo, 1998). The disappearance of salt glands and observation of salt crystals on the leaf surface of Wild cabbage under higher concentrations is a clear indication of ion secretion, which implies that the plant can be categorized under halophytes that are salt secretors (exo-recretohalophytes) (Ding et al., 2010; Dassanayake & Larkin, 2017; Yuan & Wang, 2020). This is supported by reports suggesting that certain halophytes (recretohalophytes) could excrete excess salt as a liquid that crystallizes and appears apparent on the surface of plant leaves when exposed to air (Yuan et al., 2016; Shabala, 2013; Chavarria et al., 2020). It was also observed at the highest concentration (200 mM) that the surface became flaccid, which demonstrates water loss and cell damage. This is consistent with the reports that extreme salinity levels decrease leaf growth due to the osmotic and ionic stress caused by inadequate water absorption, which causes cell shrinking (Alshiekheid et al., 2023; Rahman et al., 2021).

The percentage of elemental composition of *T. ciliata* varied with regard to the nutrient ions acquired from various salinity treatments, as revealed by EDX spectroscopy of the leaf epidermis. As anticipated, a significantly high amount of Carbon was due to carbon coating. (Jimoh et al., 2019) also reported high gold content due to gold coating. The EDX analysis illustrated the increase of Na^+ and Cl^- in direct proportion to increasing salinity, with the lowest

amounts found at 0 mM and the highest amounts detected under high salinity treatments. However, the increase in Na^+ was not statistically different between 100 mM, 150 mM and 200 mM salinity treatments, which shows the ability of *T. ciliata* to maintain chemical balance under high salinity (Rahman et al., 2021). These findings are consistence with previous studies, which reported the capacity of halophytes to maintain osmotic balance in cells by storing excess salts in salt glands (Chavarria et al., 2020; Flowers & Colmer, 2015; Meng et al., 2018). Furthermore, other studies reported the presence of salt crystals at high concentrations as a clear indication of ion secretion for the maintenance of cellular ion homeostasis (Flowers & Colmer, 2008; Alshiekheid et al., 2023; Yuan et al., 2016).

Results from EDX analysis revealed an equally high composition of K^+ in 0 mM and 50 mM salinity levels, which then significantly decreased with increasing salinity. Potassium ions function as a prerequisite feature to build salt-tolerance and play a critical role in mitigating the effects of salinity stress on plants by recasting essential plant processes (Rahman et al., 2021). In addition, salinity negatively affected the composition of Mg^{2+}, which is primarily required for the formation of chlorophyll, its formation, movement, and use of photo-assimilates, as well as the activation of enzymes and proteins (Rahman et al., 2021). Interestingly, 50 mM recorded a significantly higher accumulation of Ca^{2+} than the control, while there was no significant difference from 100 mM to 200 mM. Ca^{2+} is reported to be the requirement for structural functions in the membranes and cell walls, and in the vacuole as a counter-cation for both inorganic and organic anions (Rahman et al., 2021). The high accumulation of Ca^{2+} under low salinity and its stability under high salinity may be related to the reports stating that *T. ciliata* achieves maximum growth and chemical balance under low salinity and the secretion of Na^+ under high salinity (Sihle Ngxabi et al., 2021; Shabala, 2013). A similar trend was found in a halophyte, *Tetragonia decumbens* where salinity did not have a significant influence on the accumulation of Ca^{2+} (Sogoni et al., 2023). Similarly, leaves under control and 50 mM treatments accumulated an equally high amount of Phosphorus (P), which lowered under 100 mM and increased in direct proportion to increasing salinity. Phosphorus plays a role in many plant processes which include the transfer of energy, photosynthesis, the processing of sugars and starches, the flow of nutrients throughout the plant, and the genetic transfer (Haider et al., 2023). The stability of P in this plant may be related to its ability to maintain ion homeostasis and osmotic adjustment under high salinity.

4.5 Conclusion

For the first time, the leaf surface and cross-sectional properties of *T. ciliata* were examined using SEM and EDX in this study. The results obtained from the analyses confirmed that salinity had a significant influence on the leaf surface characteristics and percentage chemical composition of Wild cabbage. The observation of salt glands protruding from the epidermis along the vascular system under low salinity and the emergence of salt crystals under higher concentrations makes this plant superior compared to other plants in the maintenance of cellular homeostasis under high salinity, and the plant can be classified as recretohalophytes. Increased salinity led to a decrease in the percentage composition of some important chemical elements such as P, K and Ca^{2+}, while Na^{+} and Cl^{-} increased in a stable manner. The results of this study demonstrate the complexity of Wild cabbage's reaction to salinity, which includes a variety of physiological mechanisms such as ion exchange and excretion in a form of salt crystals through salt glands, a decrease in stomatal density, and the distribution of Na^{+} and Cl^{-} inside the plant. The findings from this study confirm that *T. ciliata* achieves salt tolerance by modifying leaf surface characteristics, osmotic adjustment, and the regulation of Na^{+} uptake and distribution in the leaves, and the results may be used to update the existing taxonomic information on leaf ultrastructure of the species. Further studies are recommended to unravel the mechanism of ion exchange and determine candidate genes controlling salt tolerance in *T. ciliata*.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants as objects of research.

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CHAPTER 5

SALINITY INFLUENCED PROXIMATE, MINERALS, ANTI-NUTRIENTS AND PHYTOCHEMICAL COMPOSITION OF *TRACHYANDRA CILIATA* KUNTH (WILD CABBAGE): A PROMISING EDIBLE HALOPHYTE

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Salinity influenced proximate, minerals, anti-nutrients and phytochemical composition of *Trachyandra ciliata* Kunth (Wild Cabbage): A promising edible halophyte

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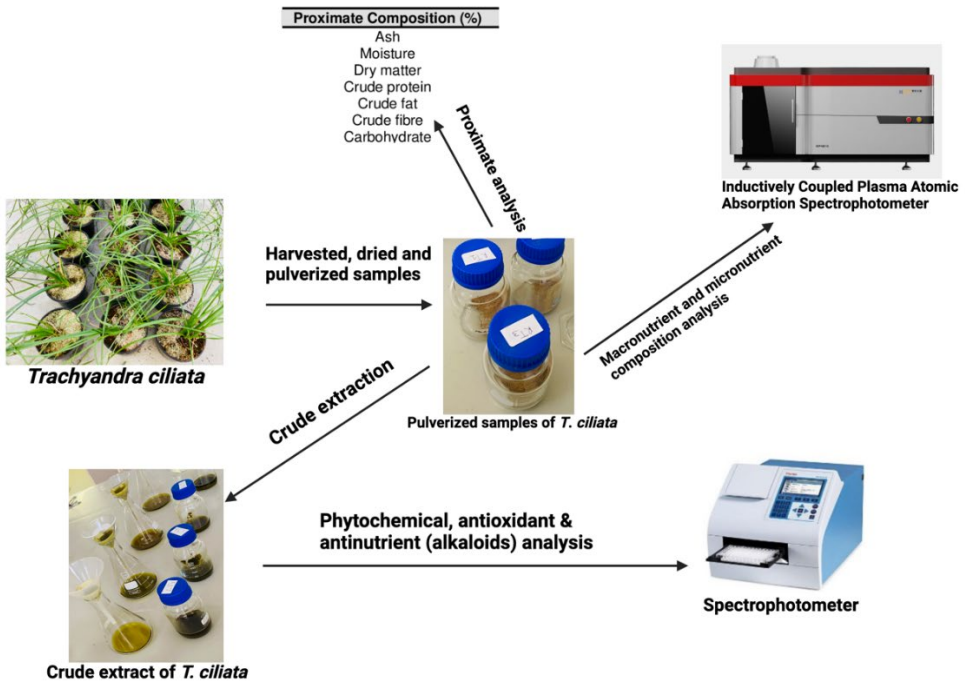
Abstract

Climate change, drought, and soil salinization present huge limitations to global agricultural output, which threatens food security. This necessitates the cultivation and domestication of wild edible halophytes as alternatives to mainstream food crops, especially in arid and semi-arid regions. *Trachyandra ciliata* is one of the under-researched and underutilized edible halophytes native to South Africa. The plant was used as a food source by Khoisan people in the past although its edibility and nutritional capacity are undocumented. Thus, the current study explored the effect of varying salinity concentrations on minerals, proximate, phytochemical, and anti-nutrient composition of *T. ciliata* to evaluate its edibility and promote its cultivation among South African households. Plants were subjected to varying salinity treatments from 0 mM, 50 mM, 100 mM,150 mM, and 200 mM prepared by adding sodium chloride (NaCl) to the nutrient solution. Salinity significantly influenced the mineral, proximate, antinutrient, and phytochemical composition of *T. ciliata*. Control and 50 mM treatments recorded significantly higher macro and micronutrient content in the flower buds and leaves, except for heavy metals such as Zn and Cu, which were significantly higher in the roots and were found to increase with increasing salinity. Leaves under low salinity treatments recorded higher moisture and protein content, while under high salinity treatments they recorded higher ash content. On the other hand, flower buds under low salinity recorded significantly high fat and NDF composition. Phytochemicals and antinutrients increased with increasing salinity concentrations. The low antinutrient content and high nutritional, mineral and phenolic contents validate the edibility and suitability of *T. ciliata* for human consumption.

Keywords: Asphodelaceae, halophytes, nutraceuticals, phenolic compounds, Recommended Daily Allowance, Wild cabbage



Graphical abstract

5.1 Introduction

The World Health Organization has estimated that agricultural production must rise by 50 to 60% by 2050 to meet the demands of the world's constantly expanding population (Falcon et al., 2022; World Health Organization, 2015). However, salinity, along with drought and other stresses, is one of the primary abiotic factors in agriculture that lead to reduced yields of edible crops worldwide (Ahanger and Agarwal, 2017; Ahmad et al., 2018). Recent studies suggest that edible halophytes are excellent alternatives in some areas that are susceptible to the adverse effects of climate change due to their high tolerance to extreme temperatures, droughts, and salinity than traditional green leafy vegetable crops (Agudelo et al., 2021; Ebert, 2014). Edible halophytes have developed numerous adaptation mechanisms to withstand high temperatures and salinity, and maintain nutrient and osmotic balance (Shabala, 2013a). In addition, developments in the food science sector and consumer preferences for varied diets point to the consumption of wild edible halophytic plants as diet complements as well as nutritious and useful foods for specific conditions. This makes commercial cultivation of these plants crucial to preventing threats from genetic erosion (Petropoulos et al., 2018).

Halophytes have also been used medicinally in various nations and are beneficial in treating and managing chronic diseases due to their high concentrations of bioactive compounds (Sihle Ngxabi et al., 2021; Petropoulos et al., 2016). Recently, these plants have been gaining momentum in Europe and Asia and are being included in restaurant menus, processed food, and beverage industries (Bvenura and Sivakumar, 2017; Sogoni et al., 2021). Moreover, many of Wild edible halophytes in Asia and Europe have been utilized by rural communities as the foundation of numerous traditional cuisines and regionally significant local recipes. In contrast, in many African countries, these plants are still neglected and understudied with no scientific justification on their nutrition and edibility (Bvenura and Afolayan, 2015; Petropoulos et al., 2018). Previous studies on other halophytes (*Chenopodium quinoa* Willd*, Tetragonia decumbens* Mill*, and Chenopodium album* L.) reported that moderate salinity improved the accumulation of some essential minerals and proximate content (Rasouli et al., 2021; Sogoni et al., 2023, 2021). Sogoni et al., (2021) further reported that high salinity levels led to increased phytochemical contents of *Tetragonia decumbens*. However, most of these wild edible halophytes are underutilized and understudied especially in many African countries although they have potential as alternatives to mainstream vegetable crops (Bvenura and Afolayan, 2015; Ogundola et al., 2018).

Trachyandra ciliata (wild cabbage) is one of the under-researched and underutilized halophytes native to the Western Cape coastal dunes, South Africa. It is classified under Asphodelaceae (Aloe) family, which is well known worldwide for its importance in the pharmaceutical industry (Manning and Goldblatt, 2007). Interestingly, this plant is reported to have been used as a food source by the Khoisan people who resided in the coastal areas of the Western Cape before colonization and massive industrialization of the province. The flower buds (which resemble the edible shoots of *Asparagus officinalis*) were steamed and cooked as vegetable or added into stews and salads (De Vynck et al., 2016). However, its edibility, nutritional capacity, and pharmacological properties are undocumented and yet to be explored. A previous study by Ngxabi, (2020) on *T. ciliata* reported that moderate salinity promoted growth, while recent studies on a closely related species (*T. divaricata*) reported the presence of essential minerals above the Recommended Daily Allowance (RDA) and high phenolic compounds (Bulawa et al., 2022; Tshayingwe et al., 2023). It is therefore imperative to study the nutritional value, phytochemical, and anti-nutrient composition of *T. ciliata* to investigate its edibility to promote its consumption and commercialization. Findings from this study are anticipated to serve as points of reference for commercial growers, households, public, scholars, and aspiring researchers whose interests are on the potential of easily accessible underutilized vegetable crops and medicinal plants.

5.2 Materials and methods

This research was carried out at the Cape Peninsula University of Technology's Bellville campus in Cape Town, South Africa, at coordinates 33.55048.800 S and 18.38032.700 E. Using environmental control, the study's experimental greenhouse temperatures were maintained between 21 and 26 ˚C during the day and between 12 and 18 ˚C at night, while average relative humidity was maintained at 60%.

5.2.1 Experimental design

5.2.1.1 Plant material

The Cape Peninsula University of Technology nursery, Bellville campus provided the *Trachyandra ciliata* plant material. Due to the presence of rhizomes, the plant material was propagated through division technique (Bulawa et al., 2022). Once established about 150 plants were transferred in 12.5 cm pots containing a mixture of peat, perlite and vermiculite at 1:1:1 ratio. To guarantee maximum genetic homology, cuttings were taken from the same mother stock population. The plantlets were split into five treatments, each consisting of thirty duplicates, and arranged in blocks.

5.2.1.2 Nutrient solutions

Nutrifeed™ fertilizer supplied by (STARKE AYRES Pty. Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, South Africa, 1619) has been certified as containing all the essential nutrients necessary for healthy and vigorous plant growth and is now widely used to make aqueous solutions that promote maximum production of plants. The specifications are as follows: 75 mg/kg S, 240 mg/kg B, 240 mg/kg Ca, 10 mg/kg Mo, 22 mg/kg Mg, 240 mg/kg Mn, 65 g/kg N, 27 g/kg P, 130 g/kg K, 70 mg/kg Ca, 20 mg/kg Cu, 1500 mg/kg Fe, and 240 mg/kg Zn. In this experiment, fertilizer group 1 Reg No: K2025 (Act 36/1947) was used in all the blocks to deliver the same amount of nutrients to the nutrient solution. For the first four weeks, the plants were irrigated solely with this solution before salinity was gradually increased to minimize transplant shock.

5.2.1.3 Salinity treatments

Different salt concentrations were established by adding sodium chloride (NaCl) to the nutritional solutions. This experiment examined four salt concentrations: 50 mM, 100 mM, 150 mM, and 200 mM of NaCl. The control group (0 mM) received only nutrient solution irrigation. NaCl was gradually added to other treatments after four weeks of the plants being irrigated with Nutrifeed solution. Plants received 300 mL of nutrient solutions, either with or without NaCl, at equal intervals every three days. Drain water from each pot was gathered, and the electrical conductivity was measured to ascertain that the right salinity levels were maintained. Using a calibrated hand-held digital pH meter (Eurotech®TM pH 2 pen), the solution's pH was kept at 6.0. The nutrition solution's pH was raised with potassium hydroxide and lowered with phosphoric acid. A calibrated hand-held digital electrical conductivity (EC) meter (Hanna Instruments®TM HI 98312) was used to measure the EC in the nutrient solution every day. The plants were harvested after 15 weeks of salinity treatment to conduct the analyses.

5.2.2 Mineral analysis of leaves, roots, and flower buds

With an Inductively Coupled Plasma-Optical Emission Spectrometer (Varian Vista-MPX, Victoria 3170, Australia), the mineral content of leaves, roots, and flower buds harvested from each replicate treatment was examined (Bulawa et al., 2022; Tshayingwe et al., 2023). The analyses were conducted at the KwaZulu Natal Department of Agriculture and Rural Development's mineral analysis laboratory.

5.2.2.1 Proximate analysis of leaves, roots, and flower buds

5.2.2.1.1 Moisture content

To ascertain the moisture content of the plant samples studied, a method outlined by Jimoh et al., (2020) was followed, however with slight alterations. After being dried in an oven at 105 °C for an hour, empty ceramic vessels were weighed and labelled W1, then allowed to cool. After being weighed out, 1 g samples of *Trachyandra ciliata* (W2) were placed in a container and dried at 105 °C in an oven until a consistent weight was achieved. After being chilled in a desiccator, the container together with the plant material were weighed again (W3). The % moisture content was determined using the formula below:

$$\%\ \text{moisture content} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

5.2.2.1.2 Crude fibre content

The pulverized and air-dried sample (1 g) was weighed in an empty crucible (W1). At room temperature, 100 mL of neutral detergent solution and 0.5 g of Na_2SO_3 was added to the crucible, followed by a few drops of octanol. Following 60 minutes of boiling, the mixture was filtered, and the residue was washed two times in cold acetone and hot water. The residue was then oven-dried for eight hours at 105 °C before being cooled in a desiccator and weighed (W2). The following formula was applied to calculate the Neutral Detergent Fibre (NFD) content as reported by Tshayingwe et al., (2023).

$$\%\ \text{Neutral Detergent Fibre (NDF)} = \frac{(\text{W1} + \text{W2}) - \text{W1}}{\text{Weight of the sample}} \times 100$$

5.2.2.1.3 Crude fat content

The composition of fat content of the leaves, roots, and flower buds of Wild cabbage was estimated following a laboratory method described by Cebani et al., (2024). An orbital shaker was used to agitate one gram of the ground plant with 100 milliliters of diethyl ether for twenty four hours. Following extraction, the mixture was strained, and the resulting filtrate was then poured into a freshly weighed beaker. After homogenizing the ether extract with 100 mL diethyl ether and shaking it for a further 24 hours on an orbital shaker, the filtrate was collected in a beaker labeled W1. Prior to being reweighed in the beaker (W2), the ether filtrate was oven-dried at 55°C after being dried in a steam bath to concentrate. The following equation was used to calculate the crude fat of the extracts:

$$\%\ \text{fat content} = \frac{\text{W2} - \text{W1}}{\text{original weight of the pulverised sample}} \times 100$$

5.2.2.1.4 Crude ash

A procedure outlined by Tshayingwe et al., (2023) was used to assess the percentage ash content of the examined *Trachyandra ciliata* leaves, roots, and flower buds. After being labelled with sample codes using a heat-resistant marker, porcelain crucibles were oven-dried for one hour at 105 °C. Once the crucibles were cooled in a desiccator, they were weighed (W1). Subsequently, 1 g of plant powder was weighed and added to ceramic crucibles that had been previously pre-weighed (W2). In order to completely ash the samples, the crucibles and their contents were heated in a muffle furnace to 250 °C for an hour followed by heating to 550 °C for five hours. The samples were weighed following their cooling in a desiccator (W3).

$$\%\ \text{ash content} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

5.2.2.1.5 Crude protein

Using a digestion tablet as a catalyst, 2 g of each pulverized sample was boiled in 20 mL of concentrated H_2SO_4 in a Kjeldahl flask to produce a clear liquid, which was used to quantify crude protein. After being filtered and diluted in 250 mL, the digested extracts were then distilled. In a 500 mL round-bottomed flask, an aliquot containing 50 mL of 45% NaOH was further distilled, and 150 mL of the distillate was put into a flask containing 100 mL of 0.1 M HCl. After that, methyl orange was used to titrate this against 2.0 mol/L NaOH. A yellow color shift signalled the titration's endpoint, and the percentage of nitrogen content was assessed using the following equation.

$$\text{Crude protein} =$$

$$\frac{[(\text{mlstd acid} \times \text{N of acid}) - (\text{ml bank} \times \text{N of base})] - (\text{mlstd base} \times \text{N of base}) \times 1.4007}{\text{original weight of the pulverised sample}} \times 100$$

Where N = normality and the percentage of crude protein were obtained by multiplying the nitrogen value by a constant factor of 6.25 (Mndi et al., 2023).

5.2.3 Antioxidant and Phytochemical Assays

Phytochemical content and antioxidant capacity of metabolites in the plant extract were assayed for total flavonols, flavonoids, total polyphenols, ferric reducing antioxidant power (FRAP), ABTS, and DPPH.

5.2.3.1 Formulation of crude extracts

The crude extracts were formulated by applying a protocol as outlined by Faber et al., (2020). The finely crushed leaves, roots, and flower buds harvested at week 15 were stirred in ethyl alcohol (EtOH) and centrifuged for five minutes at 4000 rpm to obtain crude extracts. A Buchner funnel with an electric vacuum pump attached to it held Whatman No. 1 filter paper, through which the supernatant was filtered. Unmacerated tissue and other debris were removed using this procedure. Phytochemical and antioxidant assays were conducted using the crude extracts that were obtained from all of the treated plant samples.

5.2.3.2 Total Polyphenol Assay

The Folin–Ciocalteu technique was used to analyze the extracts' total polyphenol content, as detailed by Sogoni et al., (2021). About 125 µL of Folin-Ciocalteu reagent (Merck, Johannesburg,

South Africa) was diluted ten times with distilled water and combined with about 25 µL each of the crude extract. Subsequently, a 96-well microplate containing the extract was filled with a 7.5% sodium carbonate (Sigma, Alberton, South Africa) solution. The absorbance of the plate was measured at 765 nm using a Multiskan spectrum plate reader (Thermo Electron Corporation, Waltham, MA, USA) after it was incubated for two hours at room temperature. The results were represented as milligrams gallic acid equals per g dry weight (mg GAE/g DW), and the standard curve was created using 0, 20, 50, 100, 250, and 500 mg/L gallic acid (Sigma, South Africa) in 10% EtOH.

5.2.3.3 Determination of Flavonol Content

The method to quantify flavonol composition was derived from a study by Faber et al., (2020). Quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, Johannesburg, South Africa) was used as a baseline to calculate the flavonol concentration of the extracts. Quercetin equivalent milligrams per gram of dry weight, or mg QE/g DW, was used to express the results.

5.2.3.4 Estimation of flavonoid content

By following the aluminium chloride spectrophotometric assay with slight modification as reported by Jimoh et al., (2023), the total flavonoid in the crude extracts and standard was determined. The assay measures the yellow-orange colouring of the flavonoid-$AlCl_3$ complex, which results from the interaction of flavonoids with $AlCl_3$. In 1 mg mL^{-1} of methanol, a stock of the plant extracts and standard (quercetin) was made. Quercetin extract solutions (0.5 mL) and graded concentrations (0.2-1.0 mg mL^{-1}) were prepared and then put into different test tubes. The test tubes were then filled with 2 mL of distilled water and 0.15 mL of 5% $NaNO_2$. After completely vortexing the mixture, it was let to stand at room temperature for six minutes. After that, 0.15 mL of 10% $AlCl_3$ and 1 mL of 1 M NaOH were added to the mixture and allowed to sit for 5 minutes. The solution was diluted with distilled water to a volume of 5 mL, and it was then incubated for 30 minutes at 40°C in a water bath. After incubation, the solution was transferred into a glass cuvette, and the spectrophotometer was used to detect absorbance at 430 nm. The standard calibration curve was used to quantify the total flavonoid content of the crude extract as mg g^{-1} of quercetin equivalent (QE g^{-1}).

5.2.3.5 Determination of Ferric Reducing Antioxidant Power (FRAP)

A 30 mL volume of acetate buffer (0.3 M, pH 3.6) (Merck, South Africa) was combined with 3 mL of 2,4,6-tripyridyl-s triazine (10 mM in 0.1M hydrochloric acid) (Sigma, South Africa), 3 mL of iron

(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) (Sigma, South Africa), and 6 mL of distilled water to create the FRAP reagent. The mixture was then incubated for 30 minutes at 37 ◦C. Subsequently, 300 µL of the FRAP reagent and 10 µL of the crude sample extract were combined in a 96-well plate. After that, the absorbance was determined using a Multiskan spectrum plate reader at 593 nm. To determine the FRAP sample values, L-ascorbic acid (Sigma-Aldrich, South Africa) was utilized as a standard, with a concentration curve ranging from 0 to 100 M. The results were expressed as µM ascorbic acid equivalents (AAE) per g dry weight (µM AAE/g DW) (Ngxabi et. al., 2021b).

5.2.3.1.6 Determination of ABTS Antioxidant Capacity

Using a technique outlined by Jimoh et al., (2023) with slight adjustments, the ABTS antioxidant capacity was measured. A 140 mM of potassium peroxide ($K_2S_2O_8$) (Merck, South Africa) and 7 mM of ABTS were prepared as stock solutions. Following that, 5 mL of ABTS solution was mixed with 88 µL of $K_2S_2O_8$to create the experiment's solution. These two solutions were combined, and they were allowed to react at room temperature for 24 hours in the dark. The standard utilized in the experiment was Trolox (6-Hydrox-2,5,7,8- tetramethylchroman-2-20 carboxylic acid), which was present in quantities ranging from 0 to 500 µM. The absorbance was measured at 734 nm at 25 ◦C in a microplate reader after crude sample extracts (25 µL) were allowed to react with 300 µL ABTS in the dark at room temperature for five minutes. Trolox equivalent per gram of dry weight (µM TE/g DW) was used to express the results.

5.2.3.7 Antioxidant Capacity of DPPH free Radicals

The DPPH free radical antioxidant capacity was measured by employing a protocol as outlined by Unuofin et al., (2017). The 0.135 mM DPPH solution, which was made in a dark bottle, produced the DPPH radical. A reaction was conducted using approximately 300 µL of DPPH solution, 25 µL of crude extract, and graded concentrations (0 and 500 µM) of Trolox standard (6-Hydrox-2,5,7,8-tetramethylchroman-2- 20 carboxylic acid) solution. After 30 minutes of incubation, the integrates' absorbance at 517 nM was measured. The results were presented as µM/Trolox equivalent per g dry weight (µM TE/g DW).

5.2.4 Determination of anti-nutrients

5.2.4.1 Quantification of Alkaloids

The total alkaloid composition of leaves, roots, and flower buds was quantified using the Bromo cresol green method based on an atropine standard as described by Faber et al., (2020). In order to extract the plant material, 100 mg was placed in 10 mL of methanol inside a 15 mL lab-grade

plastic tube. The tubes were then centrifuged for two minutes at 4000 rpm. A 1 mL of methanol extract, 5 mL of buffered solution (pH 4.7), 12 mL of bromocresol green, and 12 mL of chloroform were added to a fifty millilitre lab-grade plastic tube. When the chloroform separates from the buffering solution and Bromo cresol green, alkaloids are present in the extract if a yellow color is recognized. After that, the yellow chloroform liquid would be put in a multi-scan spectrum scanner by pipetting it into 96-well plates, three cells per extraction at 300 µL. The software application, SkanIt™ was used to analyze the extracted materials at 417 nm, which is the wavelength of the colour. This allowed for the measurement of the total amount of alkaloids in the sample, or the number of yellow pigments.

5.2.4.2 Determination of oxalate content

The modified titration method outlined by (Jimoh et al., 2020) was used to determine the oxalate content. About 1 g of each plant sample was diluted into 75 mL of 3 M H_2SO_4. The mixture was then stirred for an hour using a magnetic stirrer. After the mixture was filtered, 25 mL of the filtrate was heated to roughly 90 degrees Celsius. The hot aliquot was continuously titrated against 0.05 M KMnO4 until a 15-second period of bright pink color shift was noticed, which marked the endpoint of titration. Each plant sample extract's titre value was multiplied by 2.2 mg of oxalate, which was calculated to be the equivalent of 0.05 M of KMnO4 utilized in the titration.

5.2.4.3 Determination of saponin concentration

A 50 mL of 20% aqueous ethanol was mixed with five grams of powdered plant samples each to assess the percentage saponin content. Following a 30-minute orbital shaker, the mixture was heated for four hours at 55 degrees Celsius in a water bath. Following filtering, the mixture was again extracted with residue using 200 mL of ethanol. In a water bath set at 90 degrees Celsius, the filtrates from both extractions were collected in a calibrated beaker and concentrated to roughly 40 mL. After shaking and decanting the concentrated filtrate into a separating funnel, 20 mL of diethyl ether was added and vigorously shaken. In the separating funnel, the mixture was let to settle until the ether and aqueous layers separated. The aqueous (bottom) layer was retained in the collecting beaker while the ether (upper) layer was disposed of. After reintroducing the set-aside layer into the separating funnel, 60 mL of n-butanol was added. After giving the mixture a good shake, it was allowed to settle until two different layers were visible. The top layer, which included butanol extract was collected and evaporated to a constant dry weight in an oven preheated to 40 degrees Celsius, while the lower layer was disposed away. The following equation was used to calculate the percentage saponin content of the sample.

$$\%\ \text{saponin content} = \frac{\text{Weight of concentrated residue}}{\text{original weight of the sample}} \times 100$$

5.2.5 Statistical analysis

Upon the conclusion of the experiments, the data acquired from the treatments was expressed as means ± standard errors (SE) of three replicates. One way ANOVA and Fisher's least significant difference (LSD) were employed to compare the significant differences between treatment means at $p < 0.05$ using the MINITAB 17 statistical software.

5.3 Results

5.3.1 Effects of salinity on mineral composition

5.3.1.1 Macronutrients

The results obtained from this study revealed that salinity concentrations significantly ($p \leq 0.05$) influenced the macronutrient composition of flower buds, leaves, and roots of *Trachyandra ciliata*. From the results displayed in table 5.1, the highest nitrogen yield (3890 mg/100g) was recorded in the leaves under the control treatment, while the lowest mean values were recorded in the roots under high salinity treatments (150 and 200 mM). The highest Phosphorus content (450 mg/100g) was recorded in both flower buds and the roots under control and 50 mM treatments respectively, while the lowest Phosphorus yield was obtained in the samples collected from the leaves under high salinity treatment. Potassium content decreased indirectly was proportional to increasing salinity among all plant parts. However, leaves followed by flower buds recorded considerably higher values compared to the roots. Interestingly Calcium and magnesium recorded the highest mean values under 50 mM salinity in the flower buds, while low values were recorded under high salinity concentrations among all plant parts. Unsurprisingly, the Sodium content was recorded under high NaCl treatments, while control recorded the lowest Sodium content in all plant parts.

Table 5.1: The influence of varying salinity levels on the macronutrient composition of flower buds, leaves, and roots of *T. ciliata*.

Plant parts	NaCl conc.	N (mg/100g)	P (mg/100g)	K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	Na (mg/100g)
Flower buds	0 mM	2700±0.11d	450±0.05a	3150±0.05c	2620±0.01cd	350±0.01bc	220±0.01gh
	50 mM	3320±0.14b	390±0.02b	2640±0.14d	3680±0.3a	550±0.05a	2300±0.2ef
	100 mM	2440±0.06de	360±0.01b	2150±0.05ef	2410±0.1de	270±0.01def	2800±0.3de
	150 mM	-	-	-	-	-	-
	200 mM	-	-	-	-	-	-
Leaves	0 mM	3890±0.16a	380±0.03b	6770±0.27a	2530±0.46d	320±0.02bcde	720±0.17g
	50 mM	3010±0.07c	360±0.02b	4840±0.08b	3210±0.11ab	370±0.02b	3730±0.1c
	100 mM	3000±0.23c	250±0.01c	3420±0.19c	1790±0.13fg	310±0.01cde	4830±0.71b
	150 mM	2170±0.07efg	180±0.01d	2470±0.13de	1600±0.01fg	270±0.02efg	7910±0.17a
	200 mM	2050±0.05gh	120±0.01e	1810±0.12fg	1310±0.02g	210±0.01h	8530±0.22a
Roots	0 mM	2070±0.06fgh	380±0.02b	1700±0.18g	3150±0.33abc	330±0.03bcd	450±0.05gh
	50 mM	2340±0.04ef	450±0.02a	1590±0.03g	3210±0.05ab	270±0.03efg	1760±0.23f
	100 mM	2040±0.02gh	340±0.01b	930±0.02hi	2790±0.08bcd	220±0.01fgh	2690±0.03e
	150 mM	1830±0.03hi	280±0.01c	620±0.03i	1920±0.03ef	210±0.02gh	3010±0.1de
	200 mM	1610±0i	230±0.02cd	1030±0.01h	1890±0.2ef	120±0.01i	3490±0.18cd

Values (mean ± SE) in each column followed by the different letters are significantly different at $p \leq 0.05$ (*). ns= Not significantly different. Dash (-) = No flower buds produced.

5.3.1.2 Micronutrients

Table 5.2 shows that varying NaCl concentrations significantly affected the micronutrient contents of flower buds, leaves, and roots at $p \leq 0.05$. Surprisingly, the flower buds recorded the lowest Manganese (Mn) content when compared to other parts of the plant. The highest composition was obtained in the leaves under 100 mM treatment, while roots maintained an average Mn content. On the contrary, the highest Iron (Fe) mean values were recorded in the roots, with control treatment recording the highest Fe content (46.5 mg/100g), while the lowest values were recorded in the in the flower buds. The accumulation of other heavy metals (Copper and Zinc) was directly proportional to increasing salinity in all plant parts. The highest Cu mean values (7.9 and 3.15 mg/100g) were recorded in the roots under 200 and 100 mM treatments respectively, while the lowest value was recorded in the flower buds under control treatment. Similarly, the highest Zn content (70.05 and 51.9 mg/100g) was recorded in the roots under 200 mM and 100 mM salinity concentrations respectively, while control treatment in the flower buds recorded the lowest Zn content (2.85 mg/100g).

Table 5.2: The influence of varying salinity levels on the micronutrient composition of flower buds, leaves, and roots of *T. ciliate*.

Plant parts	NaCl conc.	Mn (mg/100g)	Fe (mg/100g)	Cu (mg/100g)	Zn (mg/100g)
Flower buds	0 mM	1.95±0.05g	10.8±0.6efg	0.25±0.05hij	2.3±0.1gh
	50 mM	2.2±0.1g	9.4±0.5fg	0.35±0.0.5ghi	2.55±0.05gh
	100 mM	2.35±0.05g	6.25±0.35gh	0.55±0.05fg	2.85±0.05g
	150 mM	-	-	-	-
	200 mM	-	-	-	-
Leaves	0 mM	8.8±0.1b	10.5±1.7efg	0.1±0.02ij	2.7±0.3gh
	50 mM	9.2±0.3b	12.6±2.3ef	0.15±0.03hij	3.25±0.35g
	100 mM	11.7±0.2a	30.4±1.8c	0.25±0.05hij	4.05±0.15g
	150 mM	8.6.5±0.15b	22.7±0.6d	0.4±0.06fgh	7.45±1.25f
	200 mM	5.85±0.25d	16.7±0.3de	0.65±0.05f	23.9±0.3e
Roots	0 mM	4.55±0.75e	46.5±7a	1.6.5±0.25e	27.6±0.15d
	50 mM	4.75±0.55e	30.15±1.05c	2±0.05d	37.8±2.8c
	100 mM	7.1±0.2c	37.4±1.4b	3.15±0.05b	51.9±1.1b
	150 mM	5.2±0.1de	32.65±0.65bc	2.75±0.05c	37.45±0.45c
	200 mM	3.7±0.1f	38.9±1b	7.9±0.1a	70.05±1.05a

Values (mean ± SE) in each column followed by the different letters are significantly different at $p \leq 0.05$ (*). ns= Not significantly different. Dash (-) = No flower buds produced

5.3.2 Influence of salinity on the proximate content

This study discovered that varying salinity led to significant variations on the nutritional contents of flower buds, leaves, and roots of *Trachyandra ciliata* with regards to Neutral Detergent Fibre (NDF), moisture, fat, ash, and protein (Table 5.3). From the results gathered in this study, moisture content decreased with increasing salinity in all plant parts. The highest moisture content was recorded on the samples collected from the leaves under control treatment, 50 mM and 100 mM respectively, followed by the roots under control and low salinity, while the lowest moisture content

was recorded in the flower buds under high salinity treatment. On the other hand, the ash content increased with increasing salinity with the highest comparable values (36.31% and 33.67%) obtained in the leaves under 200 and 150 mM treatments respectively. Similarly, flower buds recorded the lowest ash content compared to other plant parts, with the lowest mean value (9.4) recorded under control treatment. The highest protein content (24.34%) was recorded in the leaves under control treatment followed by 20.73% recorded in the flower buds under 50 mM salinity. The lowest protein content (10.08%) was obtained from the samples collected from the roots under 200 mM, although value was comparable to 11.46% recorded in the roots under 150 mM salinity treatment. Interestingly, the highest Neutral Detergent Fibre (NDF) content (39.45%) was recorded on the samples collected from the flower buds under low salinity treatment, although this was comparable to the mean value obtained the flower buds under control treatment. The lowest NDF content was recorded in the roots under 200 mM salinity treatment, although it was comparable to the values obtained from 100 and 150 mM in the roots, 100 mM in the flower buds, and all values obtained from the leaves.

Table 5.3: The influence of varying salinity levels on the proximate content of flower buds, leaves, and roots of *T. ciliata*

Plant Parts	NaCl conc.	Moisture %	Ash %	Fat %	Protein %	NDF %
Flower buds	0 mM	5.24±0.09cde	9.4±0.7g	2.75±0.05b	16.87±0.67d	35.72±0.02ab
	50 mM	4.67±0.06ef	11.37±0.47fg	3.1±0.11a	20.73±0.88b	39.45±0.95a
	100 mM	3.3±0.1g	12.83±0.28f	2.05±0.15de	15.25±0.35de	29.66±1.32de
	150 mM	-	-	-	-	-
	200 mM	-	-	-	-	-
Leaves	0 mM	7.15±0.25a	28.06±0.1bc	2.46±0.04c	24.34±0.97a	30.39±2.26de
	50 mM	6.16±0.26b	30.02±0.82b	2.77±0.03b	18.84±0.46c	28.51±0.34de
	100 mM	6.12±0.25b	29.96±1.24b	2.34±0.03c	18.77±1.46c	29.39±0.19de
	150 mM	5.14±0.24cde	33.67±0.43a	2.34±0.14c	13.55±0.45efg	29.33±0.43de
	200 mM	4.35±0.59f	36.34±0.96a	2.26±0.11cd	12.83±0.31gh	27.62±0.32de
Roots	0 mM	5.75±0.18bc	24.06±3.5d	1.72±0.08f	12.94±0.35fgh	35.22±2.07abc
	50 mM	5.4±0.17cd	18.77±1.55e	1.8±0.2ef	14.65±0.27ef	31.57±0.83bcd
	100 mM	4.82±0.05def	24.76±0.06cd	1.62±0.07f	12.77±0.14gh	28.87±4.67de
	150 mM	4.75±0.05def	25.96±0.66cd	1.6±0.08f	11.46±0.16hi	30.66±1.36cde
	200 mM	4.3±0.38f	30.06±0.15b	1.29±0.08g	10.08±0.01i	26.55±0.35e

Values (mean ± SE) in each column followed by the different letters are significantly different at $p \leq 0.05$ (*). ns= Not significantly different. Dash (-) = No flower buds produced.

5.3.3 Effects of salinity on the antinutrient content

The antinutrient composition of flower buds, leaves, and roots of *T. ciliata* cultivated under varying salinity concentrations is presented in Table 5.4 as a percentage of oxalate, saponin, and alkaloid in the crude extract. The study discovered that salinity has a significant influence on the antinutrient composition of the flower buds, leaves, and roots of wild cabbage. All antinutrient parameters increased with increasing salinity across all plant parts. The roots under 200 mM salinity treatment recorded the highest oxalate content (4.02%) followed by 3.59% which was also obtained in the roots under 150 mM salinity treatment, although this value was statistically comparable to 3.55% that was obtained in the flower buds under 100 mM salinity treatment. The lowest oxalate content (1.83%) was obtained in the leaves under 50 mM salinity level. On the other hand, the highest saponin content (5.7%) was recorded in the samples obtained from the leaves under 200 mM salinity followed by 5.07% which was also recorded in the leaves under 150 mM, although this value was statistically comparable to 4.77% that was obtained in the flower buds under 100 mM salinity treatment. The lowest saponin content (0.91%) was recorded in the samples collected from the leaves under control treatment. Alkaloid composition of wild cabbage was relatively lower compared to other parameters, with the highest value (0.41) obtained in the roots under 200 mM, while the lowest value (0.02%) was recorded in the flower buds under control treatment.

Table 5.4: The influence of varying salinity levels on the antinutrient composition of flower buds, leaves, and roots of *T. ciliata*

Plant parts	NaCl conc.	Oxalate %	Saponin %	Alkaloids %
Flower buds	0 mM	2.12±0.06fgh	2.28±0.08g	0.02±0.005ef
	50 mM	2.67±0.25d	3.29±0.67f	0.03±0.005ef
	100 mM	3.55±0.11b	4.77±0.1bc	0.08±0.01de
	150 mM	-	-	-
	200mM	-	-	-
Leaves	0 mM	1.86±0.05h	3.89±0.01e	0.04±0.02def
	50 mM	1.83±0.12h	4.16±0.03de	0.03±0.001ef
	100 mM	2.1±0.11gh	4.39±0.02cde	0.07±0.01de
	150 mM	2.49±0.02de	5.07±0.13b	0.1±0.01d
	200 mM	2.43±0.21def	5.7±0.1a	0.18±0.03c
Roots	0 mM	2.19±0.05efg	0.91±0.61h	0.05±0.01def
	50 mM	3.07±0.05c	2.07±0.04g	0.05±0.005def
	100 mM	3.37±0.08bc	3.13±0.02f	0.11±0.03d
	150 mM	3.59±0.08b	3.2±0.01f	0.28±0.07b
	200 mM	4.02±0.06a	4.51±0.16cd	0.41±0.02a

Values (mean ± SE) in each column followed by the different letters are significantly different at $p \leq 0.05$ (*). ns= Not significantly different. Dash (-) = No flower buds produced.

5.3.4 Effects of salinity on the phytochemical and antioxidant composition

The phytochemical and antioxidant capacity of flower buds, leaves, and roots of wild cabbage grown under different salinity levels is displayed in Table 5.5 in the form of polyphenols, flavonols, Ferric Reducing Ability of Plasma (FRAP), 2,2′-Azinobis (3-ethylbenzothiazoline-6-Sulfonic Acid (ABTS), and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH). The results obtained from this study reveal that different salinity concentrations had a significant influence on the phytochemical and antioxidant capacity of flower buds, leaves, roots of wild cabbage at $p \leq 0.05$. Polyphenolic content increased in direct proportion to increasing salinity across all plant parts. The highest polyphenol (15.37 mg QE/L) was recorded in the roots under 150 mM salinity level followed by 13.32 mg QE/L recorded in the roots under 200 mM salinity, although this amount was not significantly different from 12.93 mg QE/L that was recorded in the flower buds under 100 mM salinity treatment. The lowest polyphenol content was recorded in the samples cultivated under control and low salinity in the leaves. On the other hand, the highest flavonol composition (3.78 and 3.69 mg QE/L) was recorded in the leaves under 200 mM and 150 mM respectively, while the lowest value (1.56 mg QE/L) was recorded in the samples collected from the roots under control treatment. The highest FRAP activity was observed in the roots followed by flower buds, while leaves recorded the lowest FRAP activity. The highest FRAP activity (105.41 µmol AAE/g) was recorded on the samples cultivated under 150 mM in the roots, while the lowest activity (40.65 µmol AAE/g) was observed under control treatment in the leaves. Interestingly, the highest ABTS and DPPH scavenging activities were both recorded in the samples cultivated under 200 mM salinity in the roots, followed by 150 mM salinity in the roots, while they both had the lowest antioxidant activities under samples collected from the leaves under control treatment.

Table 5.5: The influence of varying salinity levels on the phytochemical and antioxidant capacity of flower buds, leaves, and roots of *T. ciliata*.

Plant parts	NaCl conc.	Polyphenols (mg GAE/g)	Flavonols (mg QE/L)	FRAP (µmol AAE/g)	ABTS (µmoL TE/g)	DPPH (µmoL TE/g)
Flower buds	0 mM	9.57±0.59f	2.3±0.06d	61.78±1.25e	38.54±1.32e	25.05±0.67e
	50 mM	12.07±0.27d	2.77±0.04c	65.49±2.25e	39.43±1.04de	29.95±0.99d
	100 mM	12.93±0.15bc	3.3±0.14b	73.91±1.41d	47.96±0.24b	30.18±1.37d
	150 mM	-	-	-	-	-
	200 mM	-	-	-	-	-
Leaves	0 mM	8.18±0.18g	2.17±0.13de	40.66±0.34h	31.56±0.32h	12.86±0.9h
	50 mM	8.67±0.02g	2.92±0.04c	43.52±0.29gh	32.17±0.3h	15.86±0.71gh
	100 mM	9.79±0.08f	3.32±0.07b	45.52±0.31g	32.93±0.12gh	17.09±0.86g
	150 mM	10.18±0.18f	3.69±0.06a	47.1±0.37g	33.96±0.09g	19.05±0.33fg
	200 mM	11.43±0.29e	3.78±0.15a	54.4±1.07f	36.38±0.67f	21.53±0.27f
Roots	0 mM	8.38±0.16g	1.56±0.09g	66.11±2.05e	36.48±0.71f	32.78±0.36cd
	50 mM	12.39±0.13cd	1.96±0.03ef	79.8±2.99c	40.59±0.7d	34.88±1.34c
	100 mM	13±0.1bc	2.37±0.09d	86.8±0.9b	42.8±0.54c	35.74±0.62c
	150 mM	15.37±0.08a	2.75±0.07c	105.41±0.69a	47.03±0.59b	41.52±2.86b
	200 mM	13.32±0.24b	1.82±0.08f	83.64±3.22bc	61.93±0.35a	56.48±1.78a

Values (mean ± SE) in each column followed by the different letters are significantly different at $p \leq 0.05$ (*). ns= Not significantly different. Dash (-) = No flower buds produced.

5.4 Discussion

Several studies have been conducted on the effects of salinity on halophytes, with results suggesting that salinity modulates growth, phytochemical, proximate, and mineral composition of different halophyte species (Bulawa et al., 2022; Mndi et al., 2023; Sogoni et al., 2021; Tshayingwe et al., 2023). However, developments in the food science sector and consumer preferences for varied diets also corroborate the consumption of wild edible halophytic plants as diet complements as well as nutritious and useful foods for specific conditions (Petropoulos et al., 2018). *Trachyandra ciliata* is one of the underutilized halophytes from South Africa. There is paucity of information on its nutrition and edibility even though its flower buds were reportedly consumed by the Khoisan people that lived in the western Cape coast of South Africa (Smith and Van Wyk, 1998). Investigating the proximate, antinutrient, mineral, and phytochemical composition of *T. ciliata* cultivated under different levels of salinity became crucial to validate its edibility and promote its consumption and commercialization.

In this study, varying salinity concentrations caused significant variations in the mineral, proximate, antinutrient, and phytochemical composition of *T. ciliata*. A significantly higher macro and micronutrient content was recorded in the flower buds and leaves of the untreated (control) plants and those cultivated with 50 mM treatments although heavy metals such as Zn and Cu increased with increasing salinity and significantly higher in the roots. The decrease in macro and micronutrient content with increasing salinity can be attributed to osmotic stress, decreased water availability, changes in the cellular ionic balance, and excessive presence of Na^+ and Cl^- that are caused by salinity, which may lead to deficiency and or toxicity of some nutrients (Ehtaiwesh, 2022). The stability of nutrients under low salinity treatments is caused by the ability of halophytes to maintain osmotic balance, cell homeostasis, and nutrient balance under salinity stress (Corwin, 2021; Shabala and Pottosin, 2014). The increase and stability in some micronutrients may be caused by the ability of halophytic plants to employ morphophysiological strategies such as salt exclusion, salt excretion via specialized organs like salt glands or salt bladders, and by diluting salt ions through succulence (Rasouli et al., 2021; Shabala, 2013b). These strategies contribute to the ability of halophytes to maintain cell balance under salinity stress.

Minerals are essential parts of the human diet. They sustain life by supplying the essential nutrients required for the body's psychophysical well-being (Jimoh et al., 2020). Most of the mineral composition of the investigated parts of *T. ciliata* are similar to that of the flower buds of a closely related species *T. divaricata*, (Bulawa et al., 2022; Tshayingwe et al., 2023) although they exceeded the Recommended Daily Allowance (RDA). For instance, the calcium content of

T. ciliata is well above the amounts recorded previously on the flower buds of a closely related *T. divaricata* that had the highest value of 1101 mg/100g and 700 mg/100g (Bulawa et al., 2022; Tshayingwe et al., 2023). Calcium has an RDA of 1000 mg for an adult (Vannucci et al., 2017), which was exceeded in all examined samples regardless of plant part or salinity treatment. This suggests that frequent consumption of wild cabbage will promote healthy bones, muscles, and may limit the chance of osteopenia and osteoporosis in cancer patients as calcium is essential for the development and maintenance of bone and muscle (Tu et al., 2016).

Potassium (K) is a necessary ingredient for physiological function because extracellular and intracellular cations are needed to sustain blood pressure, nerve impulse conduction, and muscle contraction (Udensi and Tchounwou, 2017). The recommended consumption of K is approximately 2000 mg for an adult, which is widely present in wild cabbage. In the current study, all the samples collected from flower buds and leaves exceeded the minimum RDA of K except the roots samples which did not meet the RDA. These results corroborate earlier findings on other halophytes such as *T. divaricata, Tetragonia decumbens*, and *Mesembryanthemum crystallinum* where the potassium content exceeded the RDA values (Bulawa et al., 2022; Mndi et al., 2023; Sogoni et al., 2021). In addition, most of the samples examined in this study have higher K content than the edible part of *Asparagus officinalis* (2760 mg/100g) which resembles the flower bud of *T. ciliata* (Redondo-Cuenca et al., 2023). The reduction in K content of the root samples may be related to high ionic imbalances caused by an excess of Na^+ and Cl^- that may reduce the selectivity of root membranes (Avarseji et al., 2013; Botella et al., 1997; Nieves-Cordones et al., 2016).

Magnesium (Mg) performs several essential functions, such as stabilizing Ca/K homeostasis, releasing neurotransmitters, playing structural roles in proteins and polyribosomes, cell adhesion, nucleic acids, and enzyme cofactors(Cebani et al., 2024; Ksouri et al., 2011). In the present study, Mg content was found to be abundant in wild cabbage and well above the RDA. For an example, the USDA recommended 12 mg for cooked broccoli and 55 mg for amaranth food as standard magnesium content for 100 mg, which is far below the findings of this study (Drewnowski et al., 2022). The Mg content in tested plant samples ranged from 120 and 550 mg/100g, suggesting that wild cabbage is very rich in Mg when subjected to salinity. These findings concur with the results of Tshayingwe et al., (2023) on *T. divaricata* where Mg content ranged from 249.00 to 478.50 mg/100g. Similarly, findings from this study are also comparable to the data provided by Redondo-Cuenca et al., (2023) on the edible portion of *Asparagus* in which a magnesium level of 210 mg/100g was reported. In contrast to the Mg content, the phosphorus content of all the

samples was below the RDA of 700 mg. The highest phosphorus content (450 mg/100g) was recorded in the flower buds under the control treatment. Phosphorus is necessary for human nutrition since it serves as a physiological buffer, a substrate for vital cellular processes, and a part of bone mineral in the skeleton (Chang and Anderson, 2024). These results are in contradiction with the reports on *T. divaricata,* a closely related species of *T. ciliata*, in which flower buds met the RDA (Bulawa et al., 2022; Tshayingwe et al., 2023). The reduction in phosphorus may be related to findings from studies of Benito et al., (2014) and Serna and Bergwitz, (2020) which suggest that excessive presence of Na^+ in the plant cells negatively affects plant absorption, mobility, and bioavailability of phosphorus. Furthermore, this reduction in phosphorus level corresponds to the findings on a South African halophyte *T. decumbens*, in which increasing NaCl levels reduced the phosphorus and other macronutrient composition in a stable manner. Notably, Na^+ increased with increasing salinity due to NaCl application, although it was notably higher in the leaves than other plant parts tested. The high Na content in the leaves is consistent with recent findings that *T. ciliata* employs different physiological mechanisms in the leaves such as excretion in the form of salt crystals and the formation of salt-secreting glands that aid bioaccumulation and storage of excessive salts (Ngxabi et al., 2024). These results on the macronutrient composition suggest that wild cabbage can maintain cell homeostasis and optimum biochemical processes under low salinity compared to higher salinity levels (Shabala, 2013b).

The critical micronutrients such as Iron (Fe), Manganese (Mn), Copper (Cu), and Zinc (Zn) are all required by the body in smaller quantities (less than 20 mg per day) and constitute less than 0.01% of the total body weight (National Research Council, 1989; Ogundola et al., 2018). Most of the samples examined in this study recorded micronutrient contents far less than the RDA, except for zinc in the roots where all treatments recorded higher values than the RDA. These results agree with the findings of Bulawa et al., (2022) and Sogoni et al., (2021) who respectively reported micronutrients far below the RDA in *T. divaricata* and *Tetragonia decumbens* both of which are related edible halophytes from the same region. Nevertheless, these findings support the reports on edible *Asparagus officinalis* shoots which resembles the edible flower bud of *T. ciliata* (Redondo-Cuenca et al., 2023). Moreover, the low accumulation of heavy metal by wild cabbage is in line with earlier reports which suggested that sufficient uptake and transportation of K^+ under saline conditions contribute to mitigating ion toxicity in plant cells (Benito et al., 2014; Sogoni et al., 2021; Wang et al., 2023). It could be inferred from this study that *T. ciliata* can maintain cell balance and optimum micronutrient ratios under high salinity conditions. Hence, it can be assumed that wild cabbage is safe for consumption and may be used as an alternative to the well-known *Asparagus*.

Increasing salinity caused variations in the proximate content (Moisture, ash, protein, NDF, and fat) of wild cabbage. The moisture content was significantly higher in all plant parts cultivated under low salinity treatments while higher concentrations recorded lower moisture contents. However, the moisture content of the tested samples ranged from 3.3 to 7.15%, implying that this species may have lower microbial contamination and chemical degradation, which is usually related to high moisture content (Mndi et al., 2023; Ooi et al., 2012). On the contrary, higher salinity concentrations increased ash content in all plant parts. One of the main characteristics of halophytes is their high ash concentration. Therefore, the primary factor that limits the use of halophytes in uncooked dishes may be their high ash concentration (Wang et al., 2023). Ash content in the present study ranged from 9.4 to 36.34%, which is relatively comparable to the composition of most processed foods (Redondo-Cuenca et al., 2023). However, the ash content of the edible flower bud of *T. ciliata* ranged from 9.4 to 12.83%, which is significantly lower than processed foods. Tshayingwe et al., (2023) reported similar data on the inflorescence of a closely related species (*T. divaricata*). A similar trend was reported by Hessini et al., (2020) on Chenopodiaceae species where increasing salinity led to increased ash content and further suggested that high ash content in halophytes is a major limiting factor on nutrition and use. However, a high ash value suggests that the plant contains significant quantities of dietary fiber which harbours and protects digestive organisms in the gastrointestinal tract (Hessini et al., 2020; Redondo-Cuenca et al., 2023).

Along with other nutrients, the protein content plays a significant role in a variety of processes, including signal transduction, osmotic and ionic homeostasis, photosynthesis, and reactive oxygen species-scavenging systems (Cebani et al., 2024; Mndi et al., 2023; Wang et al., 2023). In the present study, crude protein was significantly higher under low salinity treatments and decreased with increasing salinity. These results are in correspondence with earlier studies on other halophytes, where protein content increased under low salinity and decreased with increasing salinity in the irrigation water (Sogoni et al., 2021; Wang et al., 2023). The initial crude protein increase is an important component of salt tolerance. As such, there is a general trend for transporter protein activity and abundance to increase under hyperosmotic salinity at first and then tend to decrease as the concentration of salinity in the soil and irrigation water increases (Koyro et al., 2013). According to Koyro et al., (2013), a decrease in crude protein content is a sign of extreme stress and eventually lead to plant death, which is in line with the current findings. Low availability of amino acids, denaturation of enzymes involved in protein synthesis, and decreased nutrient uptake from saline soils are indicators and potential causes of decreased

crude protein concentration in saline environments (Hedayati-Firoozabadi et al., 2020; Wang et al., 2023).

In most instances, wild vegetables including edible halophytes have previously been reported to contain relatively low unsaturated fat content, ranging from 2 to 4% (Mndi et al., 2023). The crude fat content of the samples in the present study concurs with previous studies on South African edible halophyte species, *Mesembryanthemum crystallinum* and *Trachyandra divaricata*, where crude fat did not exceed 4% (Bulawa et al., 2022; Mndi et al., 2023). Foods high in fat can raise cholesterol, which is a major contributor to cardiovascular diseases(Wang et al., 2023). As a result, eating this green vegetable will help manage weight loss and ailments brought on by an excess of fat. Dietary fibre is necessary to reduce the absorption of cholesterol, prevent cardiovascular disease, and regulate bowel motions (Carlsen and Pajari, 2023; Nie and Luo, 2021). In the present study, the Neutral Detergent Fibre (NDF) ranged from 26.55 to 39.45%. This was higher than NDF values that have been reported previously in other underutilized vegetables such as *M. crystallinum* (29%), Solanum nigrum (9.56%), *Amaranthus cruentus* (8.45%), and Celosia argentea (23%) (Adegbaju et al., 2019; Ajayi et al., 2018; Mndi et al., 2023). The high NDF concentration in *T. ciliata* suggests that the species may help control intestinal transit, boost dietary bulk, and reduce the risk of numerous metabolic diseases orchestrated by inadequate consumption of crude fiber, including colon cancer, obesity, and diabetes (Adegbaju et al., 2019).

Contrary to nutritious elements, antinutrients temper with the absorption of some essential minerals and micronutrients in the digestive system which might have a harmful impact on the functioning of certain organs (Adegbaju et al., 2019; Jimoh et al., 2020). In this study, increasing salinity caused significant variations on the accumulation of three antinutrients namely alkaloids, oxalic acids, and saponins in all plant parts. Alkaloids are active substances in many medicinal plants, which are naturally occurring chemical compounds containing basic nitrogen atoms (Gemede and Ratta, 2014). However, a high alkaloid accumulation is poisonous to both people and animals (Matsuura and Fett-Neto, 2017). The alkaloid concentration recorded in the current study is far below the concentration that is perceived to be toxic, making the alkaloid content of the plant more of pharmacological importance than toxicity (Ajayi et al., 2018). Reports from previous studies suggest that excessive levels of saponins can inhibit digestive and metabolic enzymes and bind with minerals like zinc, which can impact nutritional absorption (Ajayi et al., 2018; Wang et al., 2023). The saponin concentrations recorded in the current study ranged from 2.28 to 5.7%, which is comparable to the results reported on *Celosia argentea* with saponin content of 5.3% (Adegbaju et al., 2019). Notwithstanding, it is believed that saponin content lower

than 10% in a diet is harmless to the human body (Adegbaju et al., 2019; Gemede and Ratta, 2014). This suggests that saponin level of *T. ciliata* is within the harmless concentration and may be considered safe for consumption. Oxalate is an antinutrient that is contained in the sap of many green leafy vegetable crops. It works by creating a powerful chelate with calcium and other minerals found in food, which prevents the body from absorbing and assimilating those nutrients (Gemede and Ratta, 2014; Natesh et al., 2017). Kidney stones are a major health issue caused by the accumulation of this insoluble calcium oxalate in crystal form within the kidney (Natesh N et al., 2017). In the present study, the highest oxalate concentration was recorded under high salinity treatment in all plant parts, with the highest value (4.02%) recorded in the root samples. However, the highest values for oxalate composition of *T. ciliata* in all plant parts are lower than what was recorded in the previous studies for *Celosia argentea* (4.85%), *Amaranthus cruentus* (5.08%), *Solanum nigrum* (5.88%) (Adegbaju et al., 2019; Ajayi et al., 2018). The low antinutrient composition of wild cabbage further confirms its edibility and safety for human consumption.

To mitigate the consequences of oxidative stress, plants accumulate secondary metabolites, including phenolic compounds that function as hydrogen donors, reducing agents and singlet oxygen quenchers (Ogbe et al., 2020). A plant-based diet rich in phenolic content can prevent oxidative damage to human tissue by scavenging free radicals and preventing lipid peroxidation. This improves nutritional quality and helps prevent issues that may arise from consuming too many synthetic additives (Tshayingwe et al., 2023). Therefore, it is important to apply techniques that improve phytochemical properties of vegetables during cultivation. Furthermore, phenolic compounds are of great importance due to their significant impact on the flavor and taste of food products, which has given high interest to the screening of plants for these compounds (Cebani et al., 2024; Mndi et al., 2023). In the current study, phenolic compounds significantly increased in direct proportion with increasing salinity concentration. The flower buds accumulated the highest phenolic compounds under moderate salinity (100 mM). On the other hand, leaves and roots accumulated highest phenolic content under high salinity treatments (150 mM and 200mM). These findings correspond to recent studies on other halophytes (*Schizonepeta tenuifolia* and *Tetragonia decumbens*), which reported that increasing salinity enhanced the accumulation of phenolic compounds (Sogoni et al., 2021; Zhou et al., 2018). A similar trend was reported for other species cultivated under varying salinity, where increasing salinity significantly increased the accumulation of phenolic compounds and antioxidant capacities of *Cakile maritima*, *Cynara scolymus* and *Matthiola incana* (Jafari and Garmdareh, 2019; Ksouri et al., 2007; Rezazadeh et al., 2012).

5.5 Conclusions

For the first time, this study validates the edibility of *T. ciliata* based on the high accumulation of proximate and mineral contents of the plant as stated in earlier reports that lacked scientific justification. Its high fiber, protein and energy content, particularly in the flower buds, confirms that it is effective for human digestion, and could serve as an immune system booster, a significant source of nutrition, and as a functional food. From the results obtained in this study, low salinity has shown to be more effective for maximum production of nutritional content. This opens the potential of wild cabbage to be cultivated in abandoned saline soils or diluted seawater, which is a water-saving approach. The low antinutrient composition and high phenolic content of the plant reflect its nutraceutical potential and safety to be consumed by humans. Therefore, *T. ciliata* is recommended for domestication and commercial cultivation. Similar studies are recommended on other wild edible plants to mitigate the looming food shortage and hidden hunger due to increasing population and water scarcity.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL REVIEW

This study does not involve any human or animal testing

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CHAPTER 6

PHYTOCHEMICAL PROFILING OF *TRACHYANDRA CILIATA* (L.F) KUNTH CULTIVATED UNDER VARYING DEGREES OF SALINITY

This chapter is in production in *Phytomedicine Plus* (ScienceDirect)

Phytochemical profiling of *Trachyandra ciliata* (L.F) Kunth cultivated under varying degrees of salinity

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Abstract

Although halophytes have started to gain global interest amid quest to discover more plant-based pharmaceuticals to treat chronic diseases, Wild edible halophytes remain underexplored in many African countries, including South Africa. *Trachyandra ciliata* is an underutilized edible halophyte from South Africa, whose edibility has been recently validated. However, its therapeutic value is yet to be explored. Thus, the present study was carried out to identify and quantify phytochemicals present in leaves, roots, and flower buds of *T. ciliata* to discover novel bioactive compounds with potential therapeutic applications. Plants were cultivated under four saline (NaCl) concentrations (0, 50, 100, 150, and 200 mM). The Ultra-High Performance Liquid Chromatography Mass Spectroscopy (UHP-LCMS) was employed to quantify and characterize metabolites in leaves, roots, and flower buds of *T. ciliata*. The UHPLC-MS identified 71 compounds, which were grouped into flavonoids, anthocyanins, alkaloids, nucleobase, nucleosides/tide, saccharides, fatty acids, amino acids, and coumarins. The diverse compounds identified shows the extracts of *T. ciliata* could have biological activities against chronic diseases such as diabetes, neurodegenerative disorders, cancer, cardiovascular diseases, and gastrointestinal disorders. Results from this study further show the potential of this plant in the treatment of other ailments such as skin problems, viral diseases, inflammation, oxidative stress, as well as plant plant-based food additives and preservatives. These results support the use of *T. ciliata* as a therapeutic agent in the treatment of various ailments.

Keywords: Antioxidants, Anti-inflammatory properties, Base peak chromatograms, Bioactive compounds, Chronic diseases, cytotoxicity.

6.1 Introduction

Plants are a major source of natural compounds that are essential for human health (Samtiya et al., 2021). They produce beneficial secondary metabolites such as alkaloids, amino acids, fatty acids, saccharides, anthocyanins, coumarins, among others, which are all known for their biological activities in the treatment of chronic diseases such as cancer, neurodegenerative disorders, diabetes, gastrointestinal, cardiovascular diseases, and obesity (Miles & Calder,

2021). Furthermore, guidelines for a healthy diet include high intake of plant-based foods such as fruits and vegetables on a regular basis to minimize the risk of chronic diseases (Mariotti, 2023; Mullins & Arjmandi, 2021; Fehér et al., 2020).

It is evident from previous studies that the nutritional and phytochemical content of plants correlates to the environmental conditions in which plants grow (Meng et al., 2018; Sogoni et al., 2024). It has been reported that drought and salinity induced oxidative stress in plants lead to increased concentrations of phytochemicals and secondary metabolites (Azeem et al., 2023; Ngxabi et al., 2025). Therefore, plants that are adapted to saline conditions (halophytes) are often inferred to produce more secondary metabolites than other vegetable crops (Sunita et al., 2020). Previous studies on other halophytes such as *Chenopodium album, Tetragonia decumbens,* and *Portulaca oleraceae* reported higher mineral content, phytochemicals, and antioxidant capacity under salinity stress (Jeeva, 2020; Sogoni, Jimoh, et al., 2024).

In addition, recent developments in the food science and pharmaceutical sectors recommend the incorporation of halophytes as diet supplements as well as healthy and functional foods to minimize the occurrence of chronic disorders (Godswill, 2019; Bacchetta et al., 2016; Nikalje et al., 2019). However, in many African countries including South Africa, most wild species are under explored and are regarded as weeds for conventional crops (León-Osper et al., 2020). Numerous studies have been conducted on the phytochemical profiling of many halophytes to maximize their pharmacological uses, but little attention has been given to South African wild edible halophytes (Hawas et al., 2022; Rangani et al., 2019; Mohammed et al., 2021). This necessitates more studies to be conducted on the pharmacological potential of South African halophytes.

Trachyandra ciliata (wild cabbage) is an underexplored South African wild edible halophyte endemic to the Western Cape coastal sand dunes. It belongs to the well-known *Aloe* family (Asphodelaceae) that has been widely studied and utilized for therapeutic purposes (Ngxabi et al., 2021; Manning & Goldblatt, 2007). The flower buds are edible and were eaten as vegetable by Khoi-San people who inhabited the coastal areas of the Western Cape (De Vynck et al., 2016a). A recent study by Ngxabi et al., (2025) reported more phenolic compounds (flavonols and polyphenols), mineral (Ca^{+}, K, Fe, P and N), proximate (Fibre and protein) and lower antinutrient (oxalate and saponin) concentrations in *T. ciliata* grown under saline conditions compared to mainstream vegetables such as Asparagus. Although the edibility of flower buds has recently been scientifically proven (Ngxabi et al., 2025), it is crucial to explore other plant parts for potential therapeutic properties to maximize its use and importance. There are currently no studies in the literature that have explored individual compounds in this plant. Thus, this research was carried out to profile phytochemical content of root, leaves, and flower

bud extracts of *T. ciliata* using UHPLC-MS to explore its possible medicinal and therapeutic uses.

6.2 Material and Methods

6.2.1 Experimental design

The cultivation experiment was carried out at the greenhouse nursery of the Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa. The greenhouse conditions and the propagation methods used were similar to those reported by Ngxabi et al., (2024). Plants were then subjected to four NaCl concentrations (50, 100, 150, and 200 mM), whereas the control was irrigated with the nutritional solution only. After 15 weeks of salinity treatments, plants were harvested, dried, and pulverized for extraction.

6.2.2 Extraction protocol

The experiment was conducted at the greenhouse nursery of the Cape Peninsula University of Technology's Bellville campus in Cape Town, South Africa. The greenhouse conditions and propagation procedures utilized were identical to those described by Ngxabi et al., (2025). Plants were subsequently exposed to five salinity concentrations (0, 50, 100, 150, and 200 mM of NaCl). Following 15 weeks of salinity treatment, the plant material (leaves, roots, and flower buds) was harvested, dried, and ground for extraction and further analyses. Higher salinity concentrations did not produce any flower buds due to oxidative stress caused by salinity.

6.2.3 LC-MS analysis of phytochemicals

High-resolution ultra-performance liquid chromatograph mass spectrometry (UPLC-MS) analysis was performed using a Waters Cyclic select IMS quadrupole time-of-flight (QTOF) MS connected to a Waters (UPLC) (Waters, Milford, MA, USA). Electrospray ionisation was used in both negative and positive ion modes, with a cone voltage of 15 V, a desolvation temperature of 275°C, a desolvation gas flow rate of 650 L/h, and the remaining MS settings optimised for maximum resolution and sensitivity. Data were collected by scanning from 150 to 1500 m/z in both resolution and MSE modes. In MSE mode, two channels of MS data were acquired: one at low collision energy (4 V) and the second using a collision energy ramp (40–100 V) to obtain fragmentation data as well. Leucine enkephalin was utilised as the lock mass (reference mass) for precise mass determination, and the device was calibrated with sodium formate. A Waters HSS T3 column (2.1 × 150 mm) was used for separation. The injection volume was 2 µL, with the mobile phase consisting of 0.1% formic acid in water (solvent A) and acetonitrile containing 0.1% formic acid (solvent B). The gradient began at 100% solvent A for 0.5 minutes and progressed to 100% B over 12 minutes in a linear fashion. It then maintained 100% B for 1 minute, followed by a 0.1-minute wash step at 100% B, before re-

equilibrating to initial conditions until the 15th minute. The flow rate was 0.35 mL/min, with the column temperature kept at 55°C.

MassLynx™ software version 4.1 (Waters, Milford, MA, USA) was used to collect and process data. Data were converted from project files (.PRO) to NetCDF files (CDF) in MassLynx (Waters, Milford, MA) using Databridge before being imported into MZmine for analysis. To process the MS data, the following variables were changed: m/z tolerance to 0.01 or 10 ppm, and retention time tolerance to 0.1 minute. The data matrix was analysed to extract and analyse peak regions for individual ions. The retention time (RT), mass-to-charge ratio (m/z), and MS/MS data were combined to forecast new chemicals (Figure 6.1).

6.2.4 Validation of analytical protocols for UHPLC-MS method and quantification of samples using UPLC-QTOF-MS and HPLC-DAD

The developed UHPLC-MS technique was validated using the linearity curve, limit of detection (LOD), limit of quantification (LOQ), precision, and recovery recommendations from the International Conference on Harmonisation (ICH) (Zhao et al., 2020). The standard combination was injected at various concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 ppm) for measurement purposes. The linearity of the calibration curve was tested by plotting the peak areas against a series of standard solution concentrations (mg/L) and evaluating the correlation coefficient with a linear regression model. When $R^2 > 0.99$, the system was always linear. LOD and LOQ were determined using the analytical curve parameters (standard deviation of response and slope).

The repeatability of samples was tested using intraday and interday variations, and the relative standard deviation (RSD) was used to calculate precision. The intraday and interday variations were evaluated using six replicates on the same day and three successive days. For each detected peak, the % RSD of peak regions (UV detection) and retention times were calculated. The intraday repeatability (provided as % RSDs) of the RTs ranged from 0.25 to 4.14%, while the interday repeatability ranged from 1.5 to 3.0%. The intraday repeatability (provided as % RSDs) of the total peak area was 0.4-0.9%, while the interday repeatability was 1.5–1.5%.

Assessing the method accuracy, known amounts of standards were added to samples at three levels: low (50% of the known amounts), medium (the same as the known amounts), and high (150% of the known amounts). This procedure was applied in triplicate. The methodologies show that the chemicals were detectable and quantifiable in the extract sampled with high sensitivity, as demonstrated by their limits of quantification and detection (LOQs and LODs, respectively). The observed LODs and LOQs as well as the predicted recoveries, show that

this approach is suitable for profiling chemicals from the samples in the current study (Table 6.1).

Table 6.1: Measures of analytical protocol used in the quantification of phenolic bioactive compounds.

No	Name	t_R (min)	$UV_{\lambda max}$ (nm)	*m/z* $(M-H)^-$	*m/z* MS/MS	Regression equation	Linear range mg/L	R^2	LOD mg/L	LOQ mg/L
1	Citric acid	1.25	278	191	111	y = 11023x-2005	0.5-10.0	0.999	1.1	3.6
2	Galic acid	3.03	278	169	125	y = 1553.3x + 938.1	0.5-10.0	0.998	7.7	25.8
3	Glucose	3.71		161	111	y = 4091x + 378	0.5-10.0	0.999	2.9	9.8
4	neochlorogenic acid	3.61	300, 309	353	191, 179	y = 29803x + 1930	0.5-10.0	1.000	0.4	1.3
2	Catechin	3.99	279	289	289, 245, 204	y = 22713x-1423	0.5-10.0	1.000	0.5	1.8
6	Rutin	4.74	254, 255, 354	609	609, 301	y = 48655x – 4425	0.5-10.0	0.997	0.2	0.8
7	Pelargonidin 3-rutinoside	5.18	284, 330	579	579, 271	y = 43767x-3738	0.5-10.0	0.997	0.3	0.9
8	Peonidin-3-O-rutinoside	5.29	284	609	609,299	y = 28723x + 4124	0.5-10.0	1.000	0.4	1.4
	Kaempferol	6.95		285	285	y = 37364x - 7847				

6.3 Results.

Identification of phytochemicals

6.3.1 Characterisation of flavonoids

6.3.1.1 Flavonols

Peak 14 was identified as quercetin-3-O-(6-O-rhamnosylglucoside) (rutin), by comparing with the reported data and database (Table 6.2) (González-Barrio et al., 2018). This is due to conjugation with one or several sugar molecules, as indicated in the fragment ion at *m/z* 447 $[M-H-162]^-$. Fragmentation would produce the aglycone ion of quercetin, *m/z* 301 in negative ion mode. This compound was also identified in positive ion mode (Table 3). Peaks 19 and 20 were identified as kaempferol-3-O-rutinoside and kaempferol-7-neohesperidoside, respectively, after sugar conjugation or malonyl esterification. Upon Collision-Induced Dissociation CID, after losing glucose or any other sugar moiety, main ions at *m/z* 285 and 284 were observed, originating from a hemolytic and heterolytic cleavage of the O-glycosidic bond, which is consistent with the kaempferol CID spectrum as reported previously (Ye et al., 2005). Peak 23 was identified as isorhamnetin-3-O-rutinoside due to the fragmentation that could lead to isorhamnetin aglycone (315 Da) and ions and UV absorbances consistent with previous reports (Rivera-Pastrana et al., 2010). The fragment ions are at *m/z* 323, 443, 447.

6.3.1.2 Flavone

Peaks 21 and 22 were identified as luteolin-6-C-glucoside (isoorientin) and luteolin-8-glucoside (homorientin), respectively. The UHPLC-TOF/MS in the negative ion mode of peak 16 showed the molecular ion $[M-H]^-$ at *m*/*z* 609.1479 and fragment ions at *m*/*z* 327. The fragment ions at *m*/*z* 327 $[(M-H)-(90+30+162)]^-$ were typically characterised as the *C*-glycosylflavone group, based on a previous literature (Ferreres et al., 2008). Moreover, this ion *m*/*z* 327.0515 was formed by mono glycosylflavone with the loss of 120 amu. Accordingly, the fragment ion fragmentation pattern is characteristic of luteolin. Therefore, this peak was elucidated as luteolin-6,8-di-*C*-glucoside (lutonarin) (Lee et al., 2016; Ferreres et al., 2008). Luteolin-3',7-di-O-glucoside (peak 59) was identified at RT of 4.30 in the negative ion mode from databases (MassBank, ResPect).

6.3.1.3 Flavanones

Chromatographic peak 16 was identified as 3',4',7-trihydroxyflavanone, which is a flavanone (Shen et al., 2017). It generated fragment ions at *m/z* 243 $[M-H-CO]^-$ and 227 $[M-H-CO_2]^-$. Apigenin-6-C-glucoside-7-O-glucoside was eluted at RT 4.64 minutes and exhibited the characteristic ion m/z 271 of the apigenin aglycone.

6.3.2 Identification of anthocyanins

Anthocyanins are a group of O-glycosides of 3,5,7,3-tetrahydroxyflavylium cation responsible for the red, blue, and violet colours of most berries and other fruits and vegetables. The two anthocyanin monoglycosides (*m/z* 449 and 433) produced their corresponding aglycones (cyanidin and pelargonidin) like peonidin-3-O-beta-galactopyranoside with the aglycone cation (*m/z* 271) and a fragment at *m/z* 595during product-ion analysis which matched previously reported. Peak 33 represented peonidin aglycone with 279, 284, and 265. The desugar conjugates such as cyanidin-3-O-(2''-O-beta-glucopyranosyl-beta-glucopyranoside), cyanidin-3-O-rutinoside, delphinidin-3-O-(6''-O-alpha-rhamnopyranosyl-beta-glucopyranoside) and correspond to loss of rhamnose from the molecular cation.

6.3.3 Identification of alkaloids, nucleobase, nucleosides/tide.

Peak 28 was tentatively identified as N-trans-coumaroyl-tyramine in both positive and negative ion modes. It showed $[M-H]^-$ at *m/z* 282 and major MS^2 fragments *m/z* 119 ([coumaric acid-CO_2]$^-$ and *m/z* 173 [M-H- coumaric acid -CO_2]$^-$. Compound 29 was identified as N-trans-feruloyl-tyramine with $[M-H]^-$ ion at *m/z* 312 and *m/z* 173 [M-2H- tyramine]$^-$, 145 [M-H-tyramine-2CH_3]$^-$. Compounds showed an $[M+H]^+$ ion at *m/z* 188 with the same formula, $C_{11}H_9O_2N$. The fragment ions at *m/z* 170 $[M+H-H_2O]^+$, 143 $[M+H-COOH]^+$, 118 $[M+H-C_2H_2COOH]^+$, and 91 $[M+H-C_4H_5COOH]^+$ were produced in the MS^2 spectrum. They were tentatively identified as trans-3-indoleacrylic acid and indole-3-acrylic acid, respectively generated an $[M+H]^+$ ion at *m/z* 247.1434, and it further produced fragment ions at *m/z* 188.0701 $[M+H-NCH_3]^+$ and 146.0598 $[M+H-NCH_3-CO_2]^+$. It was unambiguously identified as hypaphorine (Zhang et al., 2011). In addition, N-palmitoyl-D-erythro-sphingosine fatty acid derivative was identified in positive ion mode.

Additionally, nucleoside; adenosine (53, *m/z* 268.1054 $[M+H]^+$) was identified alongside purine nucleobase; adenine ion *m/z* 136 was identified along with its respective. Likewise, pyrimidine nucleoside cytidine (63, *m/z* 242.12854 $[M+H]^+$). The nucleoside fragmentation pattern involved the cleavage of the glycosidic linkage to give the respective nucleobase at *m/z* 136 (adenine) and 112 (cytosine).

6.3.4 Identification of saccharides

Non-reducing disaccharides; sucrose and its isomers (1,2,5,40), and trehalose and its isomers (3, 6, 13, 48, 49), melibiose (38, 47) and gentiobose (39), were differentiated through their elution time, and accurate masses. Reducing ones which displayed *m/z* 341 $[M-H]^-$ and produced *m/z* 179 as the base peak by glycosidic link cleavage (Calvano et al., 2017). The

neutral loss of one and two hexose units through oxygen linkages was observed at *m/z* 325. The ion 179 and 161 due to loss of water.

6.3.5 Identification of Fatty Acids

Fatty acids were identified at peaks 36, 37, 41 and 64 as polyunsaturated and hydroxylated fatty acid forms. Peak 64 was identified as linoleic acid. Its fragmentation pattern is similar to previous work by Serag et al., (2019). Its hydroxylated form was represented at peak 41. Linoleic acid is an essential fatty acid that possesses anti-inflammatory properties by providing the building blocks for prostaglandins. Several other hydroxylated fatty acids were also detected as major peaks, and they showed an extra loss of water molecules (Serag et al., 2019). The polyunsaturated fatty acids included dihydroxy-octadecadienoic acid and methyl-13-hydroperoxy-delta9Z,11E-octadecadienoic acid. Dihydroxy-octadecadienoic acid showed main MS/MS fragments at *m/z* 311 and 293 due to the subsequent loss of two water molecules and the main fragment at *m/z* 211 due to the C15\ C16 bond cleavage (MassBank, ResPect) (Serag et al., 2019).

6.3.6 Identification of Organic and carboxylic acids.

There are eight carboxylic acids identified in the extract, namely citric acid (7), iso citric acid (8), 2-isopropylmalic acid (4), cis-aconitic acid (11), 6,8-thioctic acid (12) and suberic acid (24). Fragmentation of the carboxylic acids occurred mostly by releasing one or two H_2O molecules, which are characterised by the ion fragments $[M-H-18]^-$ and CO_2, at *m/z* 129 $[M-H-CO_2]^-$ and 111 $[M-H-CO_2-H_2O]^-$ for trans-aconitic acid and citric acid.

6.3.7 Identification of coumarins

Coumarins were detected at peaks 9, 61 and 62, most of which exhibited characteristic UV λmax at about 274 nm. Peak 1 belonged to a dihydroxyl derivative of coumarin through addition of more of the 16 mass units to m/z 147 for coumarin. Free coumarin was identified at peak 2 with characteristic MS/MS fragment ion, *m/z* 103 after retro Diels Alder cleavage through the lactone ring of coumarin. Peak 9 was tentatively identified as scopoletin via its lithium adduct in the positive ion mode with MS^2 of characteristic ion, m/z 191 $[M-H]^-$. Peaks 61 and 62 were identified as ethoxy-methoxy coumarin derivatives after adding methoxy or ethoxy units of 31 or 45, respectively on coumarin, *m/z* 147.

Table 6.2: Analysis of compounds in the negative ion mode

						Amount in the sample												
No	**RT**	**Precursor ion** M-H]-	**MS/MS**	**Annotation**	**Identification (Database)**	**RC**	**RT1**	**RT2**	**RT3**	**RT4**	**FC**	**FT1**	**FT2**	**LC**	**LT1**	**LT2**	**LT3**	**LT4**
1	1.59	341.1082	313, 159, 133	Sucrose	MassBank	57.4						47.8					6.3	
2	1.64	341.1086	133	Sucrose Isomer I	ResPect						78.4	47.8						
3	1.64	341.1078	179, 133	D-(+)-Trehalose	ResPect		91.9	63.9	94.3	44.7			65.8	65.8				
4	1.79	191.0193	111	Isocitric acid	ResPect													
5	2.88	341.1081	133	Sucrose isomer II	MassBank, ResPect	14.1				14.8			17.4	14.0	1.0	14.6	13.7	13.9
6	2.88	341.1086	179, 133	D- (+)-Trehalose Isomer I	MassBank		17.2	17.4	13.9		14.8	14.0	17.4	17.4				
7	2.93	191.0185	111, 139	Citric acid/Citrate	MassBank, ResPect	7.2	5.5	3.8	3.1	3.7	10.1	7.6	7.4	10.3	10.4	7.4		4.2
8	2.93	191.0182	111	DL-Isocitric acid	ResPect										6.8			
9	2.93	191.019	111, 128	Scopoletin	ResPect								-	-		-		
10	3.04	282.0852	267	Guanosine	MassBank											-		
11	3.29	173.0075	147	cis-Aconitic acid	ResPect											2.0		
12	3.34	205.0346	173, 147, 111	DL-6,8-Thioctic acid	MassBank, ResPect						1.4		1.6	3.0	3.0	2.1		
13	3.97	341.0876	179, 203, 323	D-(+)-Trehalose isomer II	MassBank							10.0						
14	4.33	609.1479	447, 327	Rutin	ResPect										0.9			0.4
15	4.33	609.1461	298, 489, 327	Delphinidin-3-O-(6"-O-alpha-rhamnopyranosyl-beta-glucopyranoside)	MassBank						1.4	0.6	0.8	0.8		0.6	0.6	
16	4.33	609.1479	447, 327	luteolin-6,8-di-C-glucoside	MassBank								1.5	0.9	0.9			0.4
17	4.58	229.0086		D-Ribose 5-phosphate	MassBank				5.2									
18	4.62	593.1541	473, 298, 357	Keracyanin (cyanidin-3-O-rutinoside)	MassBank, ResPect						4.6						2.2	1.9
19	4.62	593.1514	473, 298	Kaempferol-3-O-rutinoside	MassBank, ResPect								1.5	3.1	3.1		2.0	
20	4.62	593.1514	473, 298	Kaempferol-7-neohesperidoside	ResPect										3.5	1.9		

21	4.72	447.0941	329, 215	Luteolin-6-C-glucoside (isoorientin)	MassBank, ResPect						-		-	-		-	-	-
22	4.72	447.0931	329	Homoorientin	MassBank, ResPect							-		-	-			
23	4.87	623.1618	323, 443, 447	Isorhamnetin-3-O-rutinoside	MassBank, ResPect						0.5		0.4	0.4				
24	5.23	173.0811	147	Suberic acid	MassBank, ResPect				2.0	2.0		2.2	1.8	2.1	2.1	1.9	2.11	1.8
25	5.13	533.0900	489								-							
26	5.91	461.1075	187, 209	Peonidin-3-O-beta-galactopyranoside	MassBank		0.5											
27	5.91	461.1082	187, 209	Peonidine-3-O-glucoside chloride	MassBank			0.5	0.7									
28	6.33	282.1125	187	N-trans-coumaroyl-tyramine	MassBank, ResPect			-										
29	6.42	312.1232	297	N-trans-feruloyl-tyramine	MassBank, ResPect			-										
30	7.28	431.2224	329,311, 325, 298	apigenin-8-C-glucoside	MassBank, ResPect													
31	7.53	431.2292	325, 311, 339, 297	apigenin-6-C-glucoside	MassBank, ResPect													
32	7.91	299.0557	279, 284, 265	Kaempferide	MassBank, ResPect	1.0	1.5	1.3	1.4									
33	7.96	299.0541	279, 284, 265	Peonidin						1.4								
34	7.96	299.0553	265, 297	3 5 7-trihydroxy-4'-methoxyflavone	MassBank	1.0		1.3	1.4									
35	9.22	343.2128		17-HDoHE	ResPect		-								-	-	-	
36	11.51	311.2004	293, 265, 211	Dihydroxyoctadecadienoic acid	MassBank													
37	11.71	311.2007	183, 211	Methyl-13-hydroperoxy-delta9Z,11E-octadecadienoic acid	ResPect		-					-						
38	12.26	341.1126	325	Melibiose	MassBank								36.0			31.0		
39	12.26	341.1098	325	Gentiobiose	ResPect					25.2								
40	12.31	341.1094	325	Sucrose isomer III	MassBank					25.6			36.1	36.0	29.8	31.0		
41	13.11	293.1773	265	Hydroxylinolenic acid	ResPect													
42	13.45	149.9916		Benzisothiazolone (BIT)	MassBank, ResPect	-	-					-	-	-	-	-	-	-

43	13.99	506.3192		Phosphatidylethanolamine lyso 20	MassBank	-	-											
44	14.06	806.63		Phosphatidylethanolamine alkenyl 18	ResPect													-
45	14.06	423.2309		Pravastatin	ResPect											-		
46	14.25	455.1045		Riboflavin-5'-monophosphate	ResPect							-						-
47	14.30	341.1082	325	Melibiose isomer I	ResPect								92.7					
48	14.25	341.1104	179, 325	D-(+)-Trehalose isomer IV	MassBank				98.7	98.7						82.3		108.0
49	14.30	341.1079	179, 325	D-(+)-Trehalose isomer V	MassBank, ResPect	83.1	89.5	118.0	98.7	85.9	90.0	85.6	17.4	167.4	167.4	82.3	83.6	109.0
50	14.30	455.101		Riboflavin-5'-monophosphate isomer	MassBank, ResPect	-	-	-	-	-	-	-	-	-	-	-	-	-

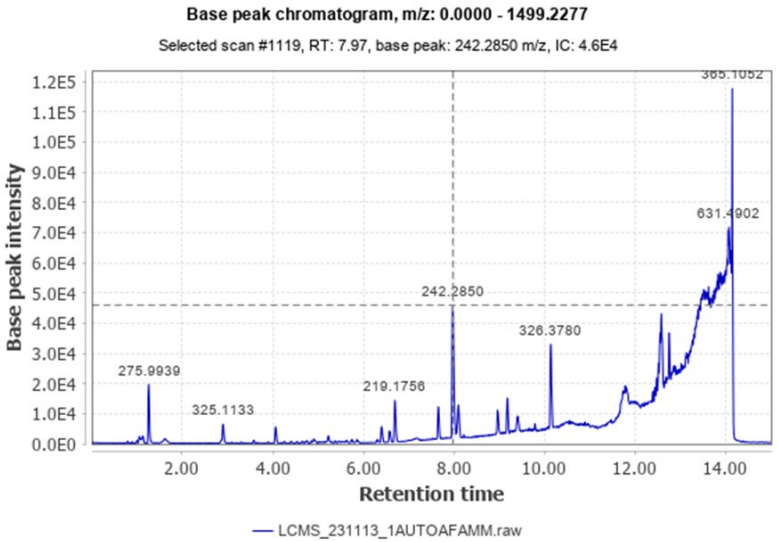
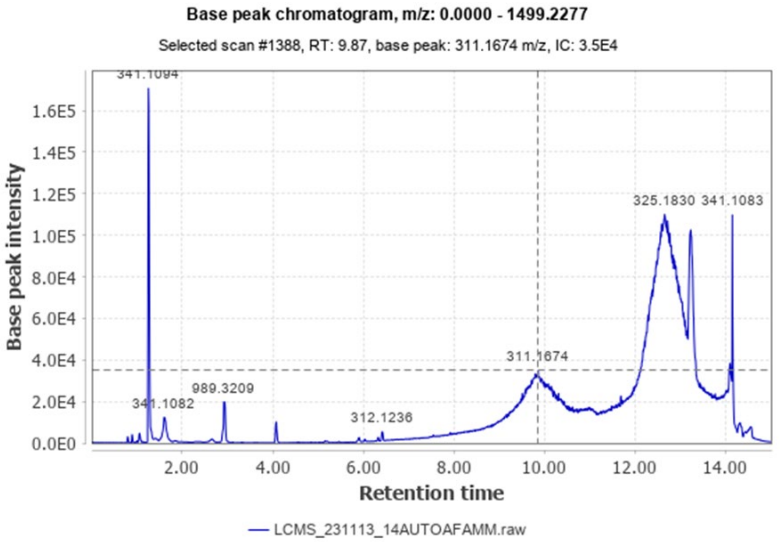
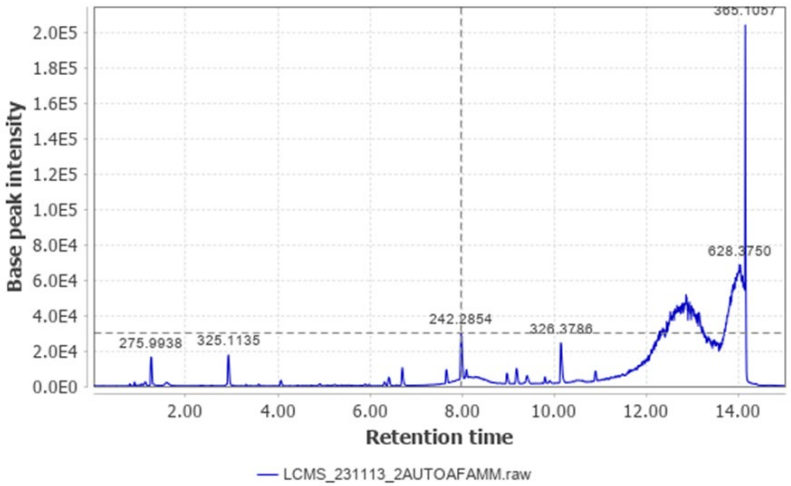
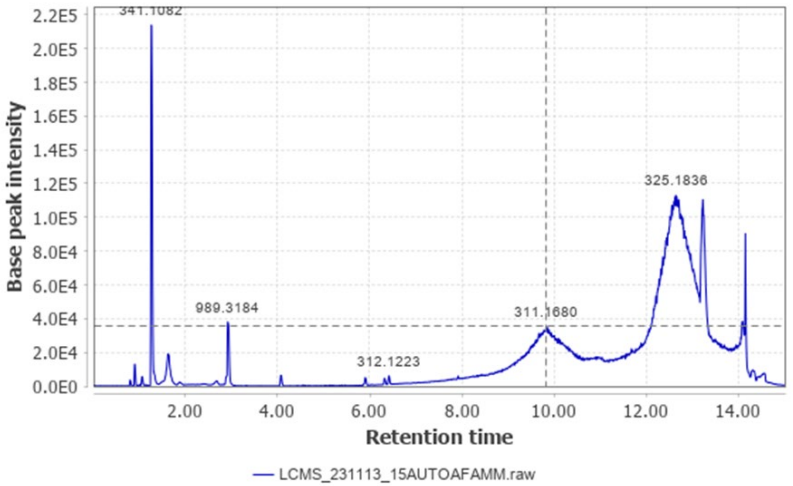
For sample codes RC=1= Root Control, RT1=2=Root T1, RT2=3=Root T2, RT3=4=Root T3, RT4=5=Root T4, FC=6= Flower Control, FTI=7=Flower T1, FT2=8=Flower T2, LC=9=Leaves control, LT1=10=Leaves T1, LT2=11=Leaves T2, LT3=12=Leaves T3, LT4=13=Leaves T4. For dash means identified but not quantified due to absence of a standard

Table 6.3: Analysis of compounds in the positive ion mode

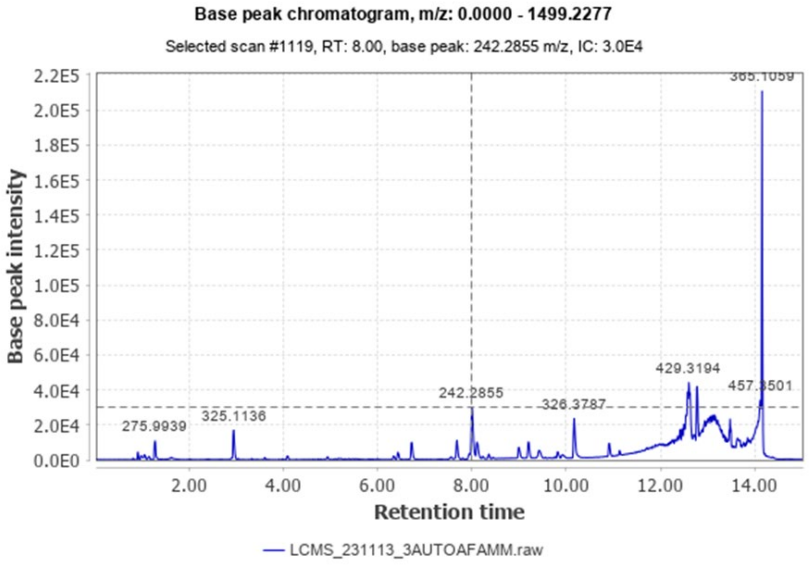
No	RT	Precursor ion$[M+H]^+$	MS/MS	Metabolite name	Sample
51	2.94	325.1135	185, 137	p-Coumaric acid glucoside	RC, RT1,
52	2.94	224.1295		Cerulenin	RC, RT1, LTI, LT2, LT3, LT4
53	2.94	268.1054	152, 136, 204, 231	Adenosine	LTI, LT2,
54	3.10	247.1678	124, 142	Hypaphorine	
55	3.30	229.0333		Not identified	
56	3.39	120.0847		Not identified	
57	3.40	166.0890	120,103	L-(-)-Phenylalanine	LTI, LT2, LT3, LT4
58	3.41	395.1344	188	Hypaphorine	RC, LT1
59	4.30	611.1634	499, 299	Luteolin-3', 7-di-O-glucoside	LT1
14	4.34	611.1625	303	Rutin	LT2
19	4.63	595.1668	287	Kaempferol-3-O-rutinoside	LT2, LT3, LT4
60	4.64	595.1674	271	Apigenin-6-C-glucoside -7-O-glucoside	LT1
21	4.74	449.1095	287	Luteolin-6-C-glucoside	LTI, LT2, LT4
28	6.33	284.1125	187	N-trans-coumaroyl-tyramine	RC, RT1
29	6.42	314.1232	297	N-trans-feruloyl-tyramine	RC, RT1, RT2, RT3, RT4, FC, FT1, FT2, LC, LT1
61	6.74	219.1759	147	Ethoxy-methoxycoumarin	RC
62	7.66	219.1756	147	Ethoxy-methoxycoumarin isomer	RC
63	7.98	242.2854	112	cytidine	RC, RT1, RT2, RT3, RT4, FC, FT1, FT2, LC, LT1
64	8.44	281.2484^K	275	Linoleic acid	
65	11.58	149.0264		Citramalate	LT2
66	12.42	223.2074		alpha-bisabolol	LT3
67	12.45	223.2069	207, 73	Farnesol (mixture of isomers)	RC, RT1, LT4
68	12.77	149.0267	149	Citramalate isomer	RC, RT1, LTI, LT2, LT3, LT4

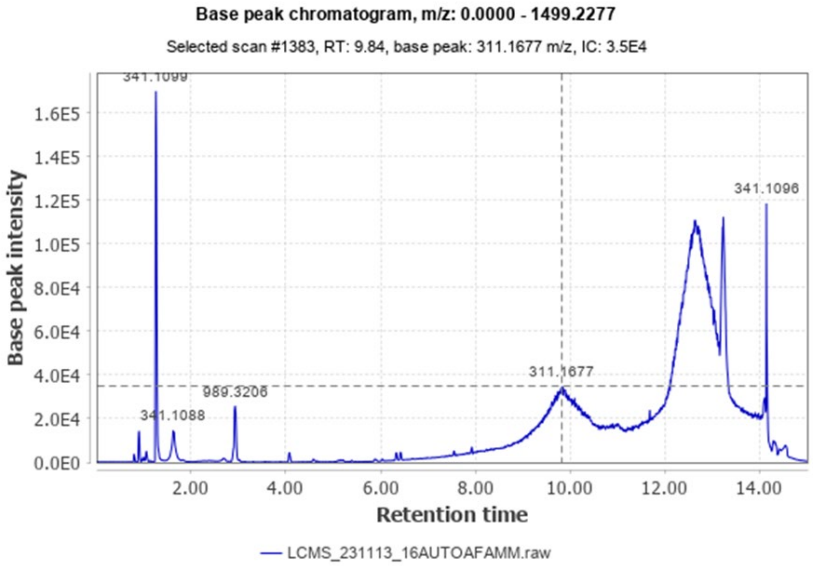
69	12.77	398.2331	149	Citramalate isomer II	RC, RT1, LTI, LT2, LT3, LT4
70	12.90	538.5190	264	N-Palmitoyl-D-erythro-Sphingosine	RC
71	13.5	393.3014		Sodium Deoxycholate	RT1, LTI, LT2, LT3, LT4

For sample codes RC= Root Control, RT1=Root T1, RT2=Root T2, RT3=Root T3, RT4=Root T4, FC= Flower Control, FTI=Flower T1, FT2=Flower T2, LC=Leaves control, LT1=Leaves T1, LT2=Leaves T2, LT3=Leaves T3, LT4=Leaves T4. Note: The quantification was done in negative mode since standards and many compounds were better analysed in the negative mode.

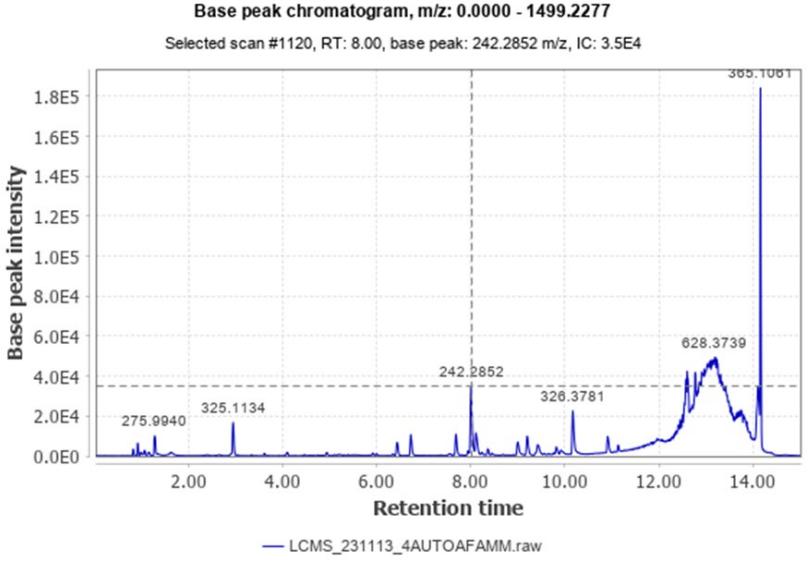
	Positive ionization mode	Negative ionization mode
RC		
RT1		

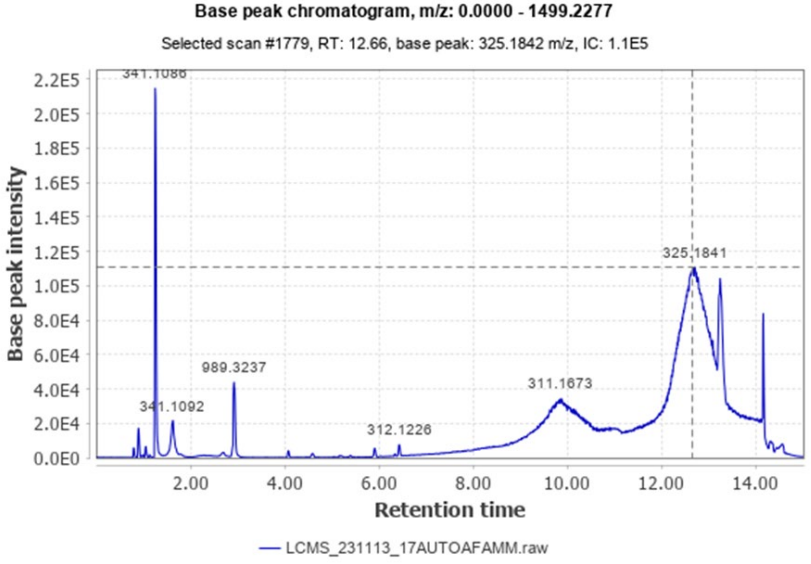
RT2


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1119, RT: 8.00, base peak: 242.2855 m/z, IC: 3.0E4
Base peak intensity
Retention time
275.9939
325.1136
242.2855
326.3787
429.3194
LCMS_231113_3AUTOAFAMM.raw

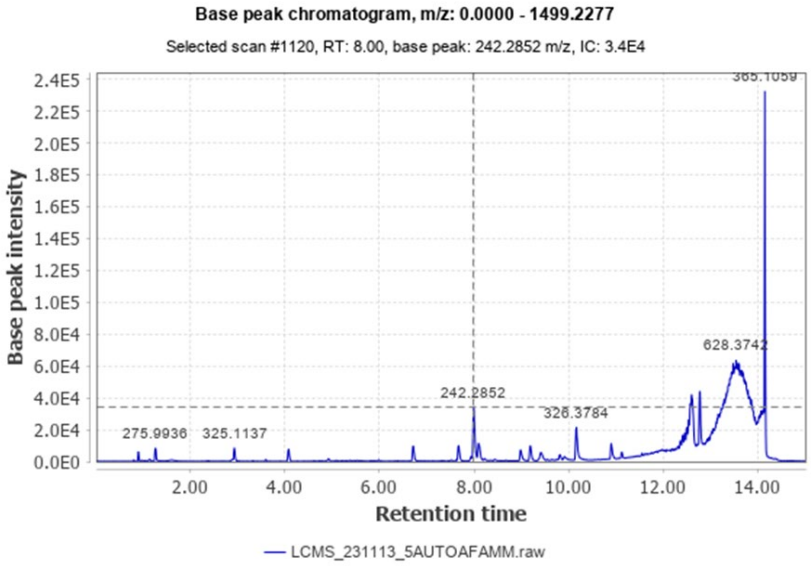

Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1383, RT: 9.84, base peak: 311.1677 m/z, IC: 3.5E4
Base peak intensity
Retention time
341.1088
989.3206
311.1677
341.1096
LCMS_231113_16AUTOAFAMM.raw

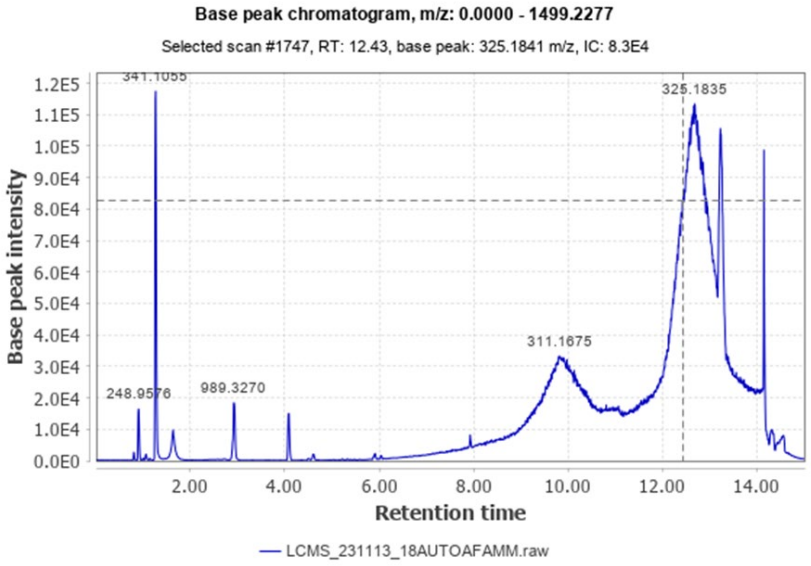
RT3


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1120, RT: 8.00, base peak: 242.2852 m/z, IC: 3.5E4
Base peak intensity
Retention time
275.9940
325.1134
242.2852
326.3781
628.3739
LCMS_231113_4AUTOAFAMM.raw

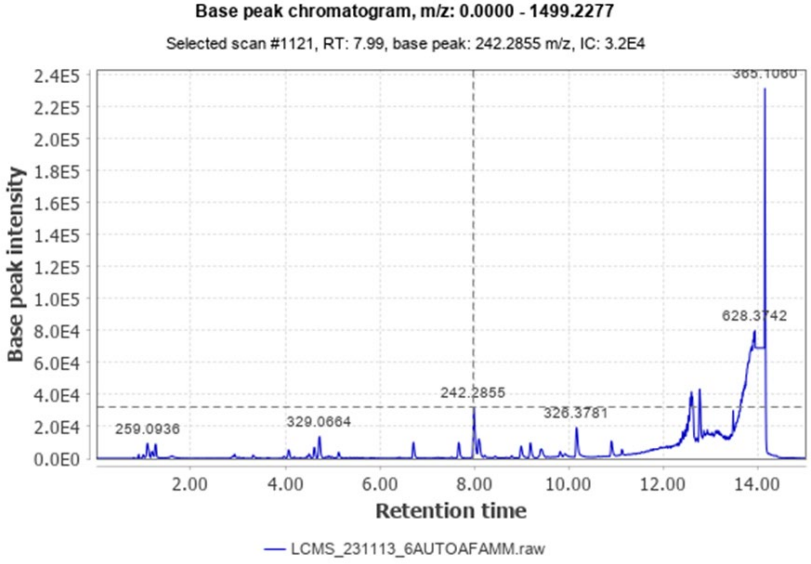

Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1779, RT: 12.66, base peak: 325.1842 m/z, IC: 1.1E5
Base peak intensity
Retention time
341.1092
989.3237
312.1226
311.1673
325.1841
LCMS_231113_17AUTOAFAMM.raw

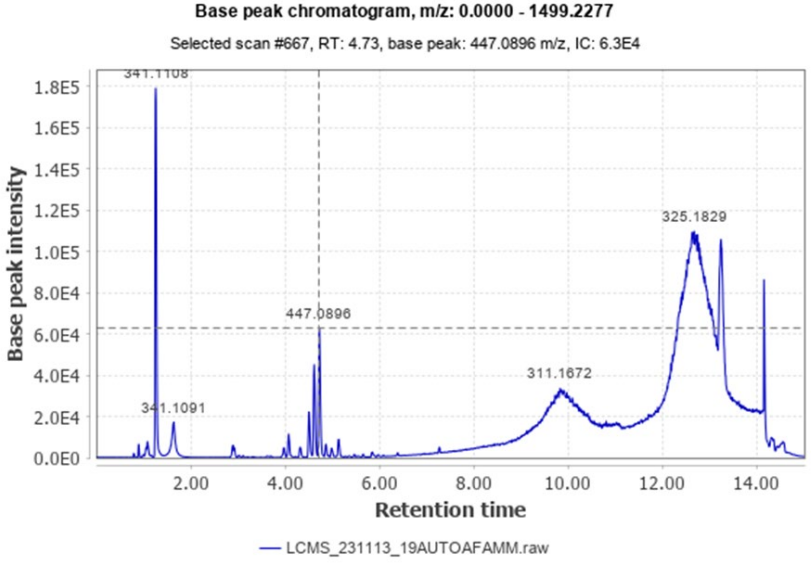
RT4


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1120, RT: 8.00, base peak: 242.2852 m/z, IC: 3.4E4
Base peak intensity
Retention time
365.1059
628.3742
242.2852
326.3784
275.9936
325.1137
LCMS_231113_5AUTOAFAMM.raw

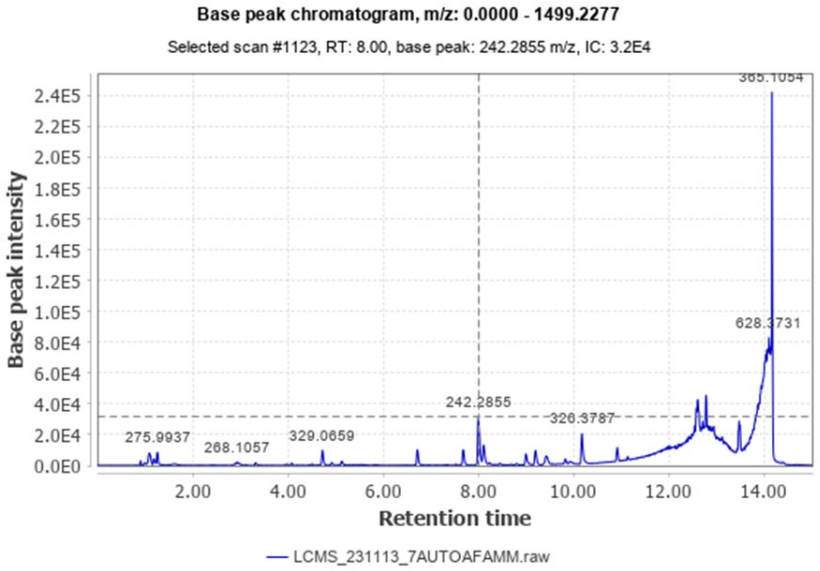

Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1747, RT: 12.43, base peak: 325.1841 m/z, IC: 8.3E4
Base peak intensity
Retention time
341.1055
325.1835
311.1675
248.9576
989.3270
LCMS_231113_18AUTOAFAMM.raw

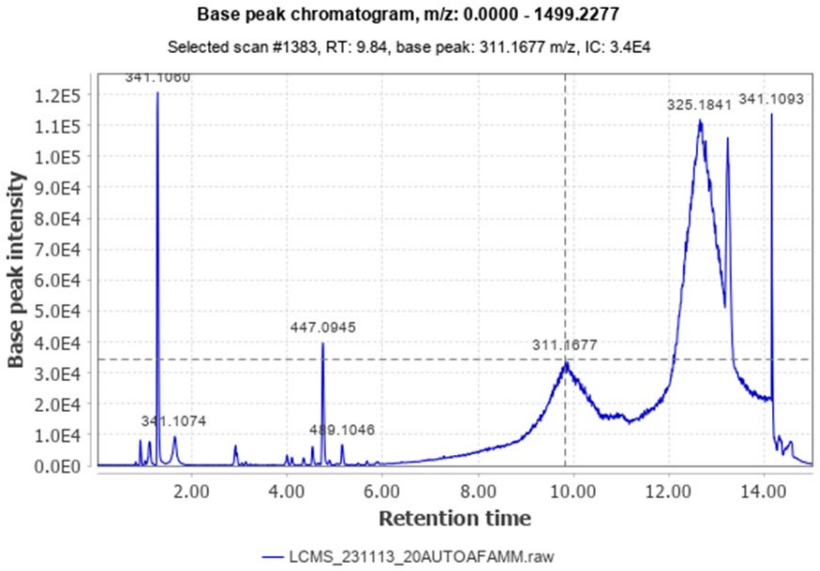
FC


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1121, RT: 7.99, base peak: 242.2855 m/z, IC: 3.2E4
Base peak intensity
Retention time
365.1060
628.3742
242.2855
326.3781
259.0936
329.0664
LCMS_231113_6AUTOAFAMM.raw

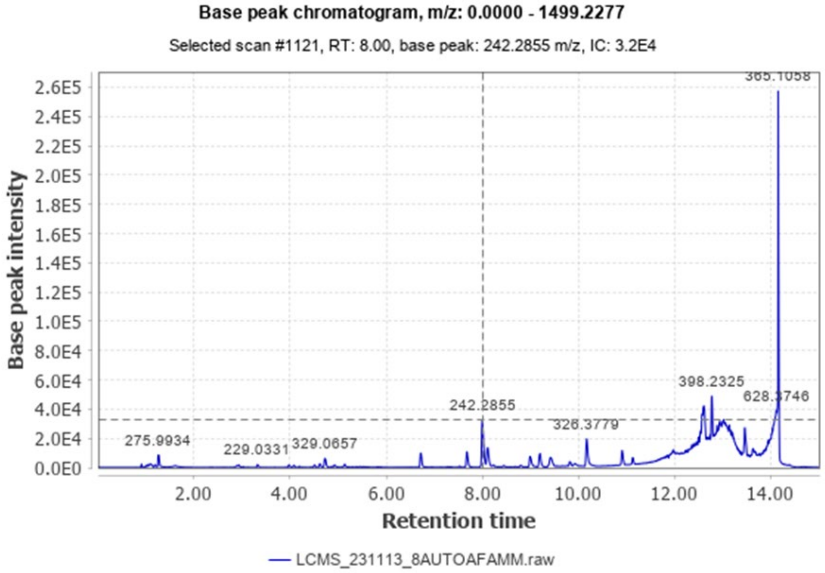

Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #667, RT: 4.73, base peak: 447.0896 m/z, IC: 6.3E4
Base peak intensity
Retention time
341.1108
325.1829
447.0896
311.1672
341.1091
LCMS_231113_19AUTOAFAMM.raw

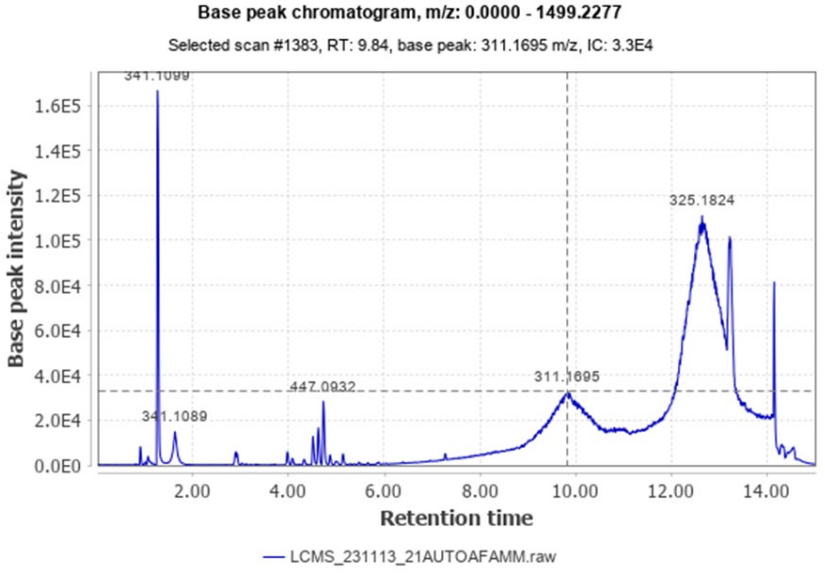
FT1


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1123, RT: 8.00, base peak: 242.2855 m/z, IC: 3.2E4
Base peak intensity
Retention time
365.1054
628.3731
242.2855
326.3787
275.9937
268.1057
329.0659
LCMS_231113_7AUTOAFAMM.raw

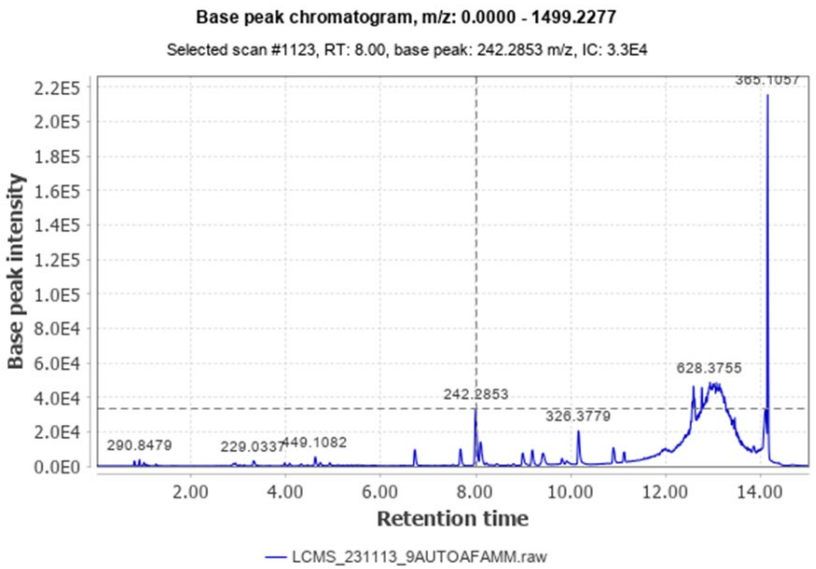

Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1383, RT: 9.84, base peak: 311.1677 m/z, IC: 3.4E4
Base peak intensity
Retention time
341.1060
325.1841
341.1093
447.0945
311.1677
341.1074
489.1046
LCMS_231113_20AUTOAFAMM.raw

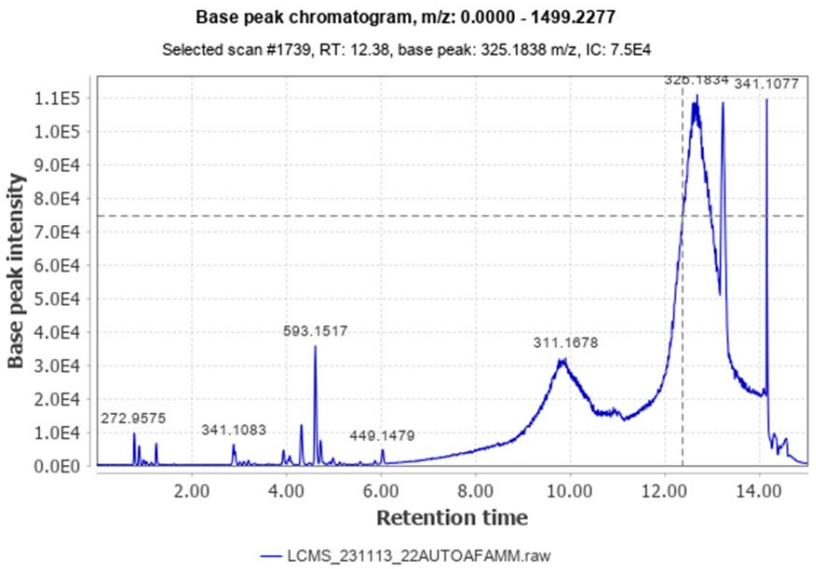
FT2


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1121, RT: 8.00, base peak: 242.2855 m/z, IC: 3.2E4
Base peak intensity
Retention time
365.1058
398.2325
628.3746
242.2855
326.3779
275.9934
229.0331
329.0657
LCMS_231113_8AUTOAFAMM.raw

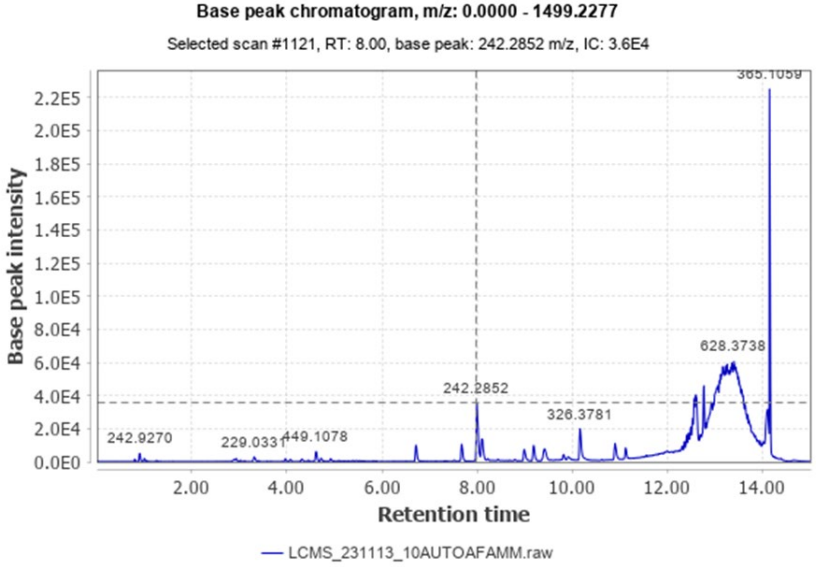

Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1383, RT: 9.84, base peak: 311.1695 m/z, IC: 3.3E4
Base peak intensity
Retention time
341.1099
325.1824
447.0932
311.1695
341.1089
LCMS_231113_21AUTOAFAMM.raw

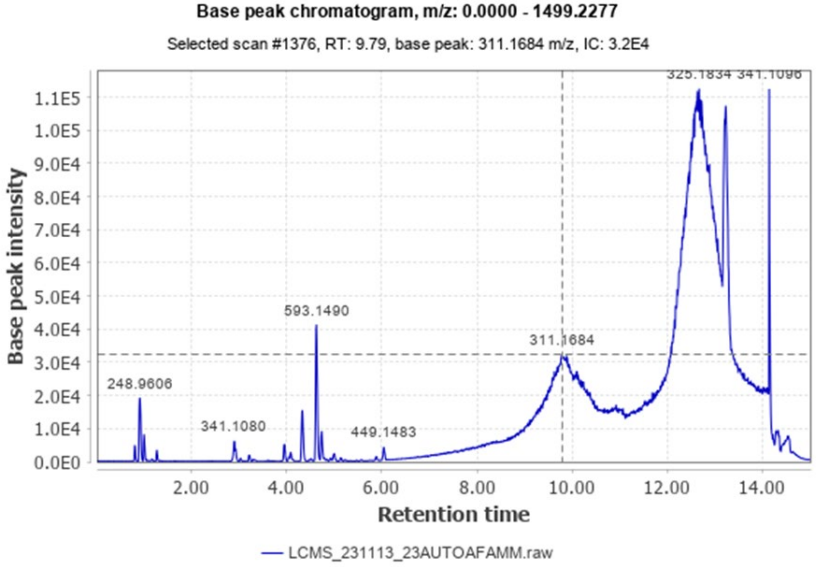
LC


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1123, RT: 8.00, base peak: 242.2853 m/z, IC: 3.3E4
Base peak intensity
Retention time
365.1057
628.3755
242.2853
326.3779
290.8479
229.0337
449.1082
LCMS_231113_9AUTOAFAMM.raw

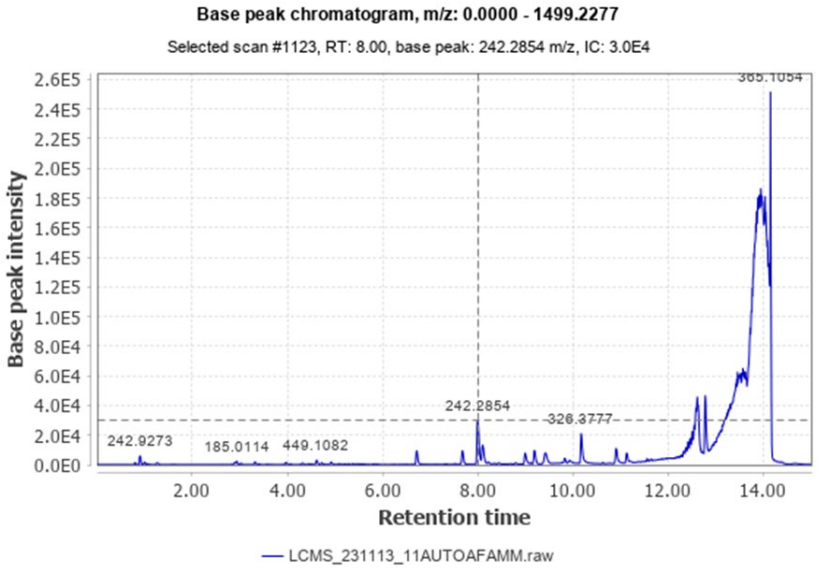

Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1739, RT: 12.38, base peak: 325.1838 m/z, IC: 7.5E4
Base peak intensity
Retention time
325.1834
341.1077
593.1517
311.1678
272.9575
341.1083
449.1479
LCMS_231113_22AUTOAFAMM.raw

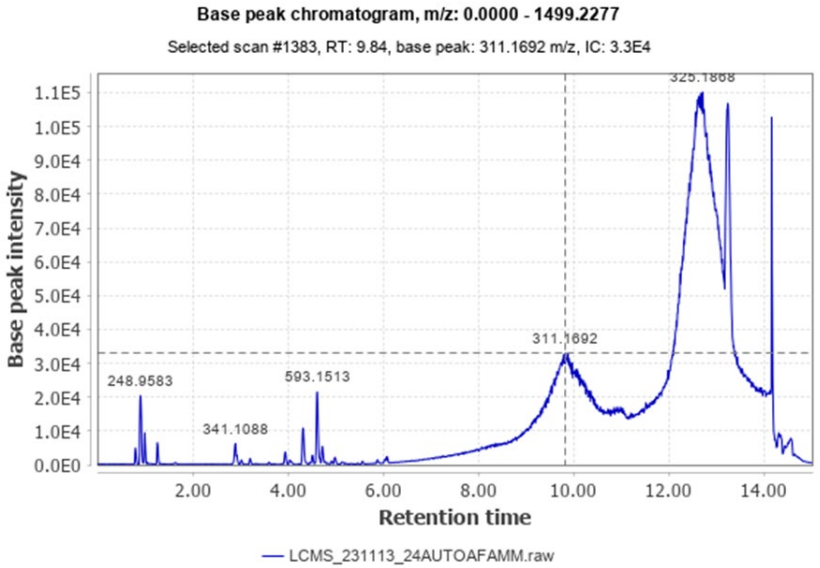
LT1


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1121, RT: 8.00, base peak: 242.2852 m/z, IC: 3.6E4
Base peak intensity
Retention time
365.1059
628.3738
242.2852
326.3781
242.9270
229.0331
449.1078
LCMS_231113_10AUTOAFAMM.raw

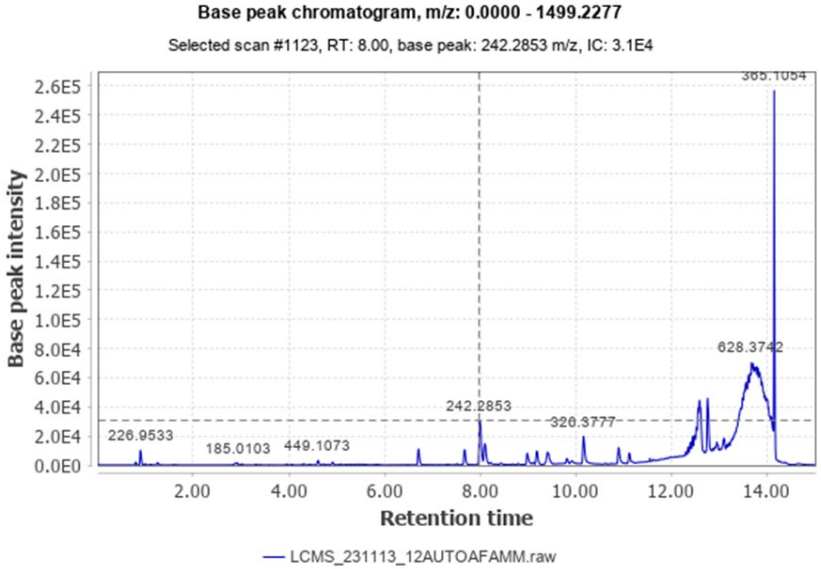

Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1376, RT: 9.79, base peak: 311.1684 m/z, IC: 3.2E4
Base peak intensity
Retention time
325.1834
341.1096
593.1490
311.1684
248.9606
341.1080
449.1483
LCMS_231113_23AUTOAFAMM.raw

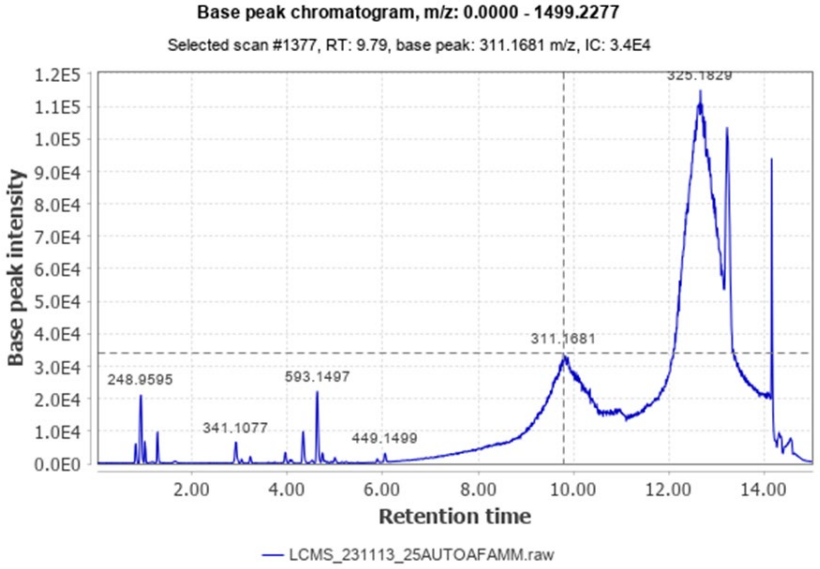
LT2


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1123, RT: 8.00, base peak: 242.2854 m/z, IC: 3.0E4
Base peak intensity
Retention time
242.9273
185.0114
449.1082
242.2854
326.3777
365.1054
LCMS_231113_11AUTOAFAMM.raw


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1383, RT: 9.84, base peak: 311.1692 m/z, IC: 3.3E4
Base peak intensity
Retention time
248.9583
341.1088
593.1513
311.1692
325.1868
LCMS_231113_24AUTOAFAMM.raw

LT3


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1123, RT: 8.00, base peak: 242.2853 m/z, IC: 3.1E4
Base peak intensity
Retention time
226.9533
185.0103
449.1073
242.2853
326.3777
628.3742
365.1054
LCMS_231113_12AUTOAFAMM.raw


Base peak chromatogram, m/z: 0.0000 - 1499.2277
Selected scan #1377, RT: 9.79, base peak: 311.1681 m/z, IC: 3.4E4
Base peak intensity
Retention time
248.9595
341.1077
449.1499
593.1497
311.1681
325.1829
LCMS_231113_25AUTOAFAMM.raw

LT4

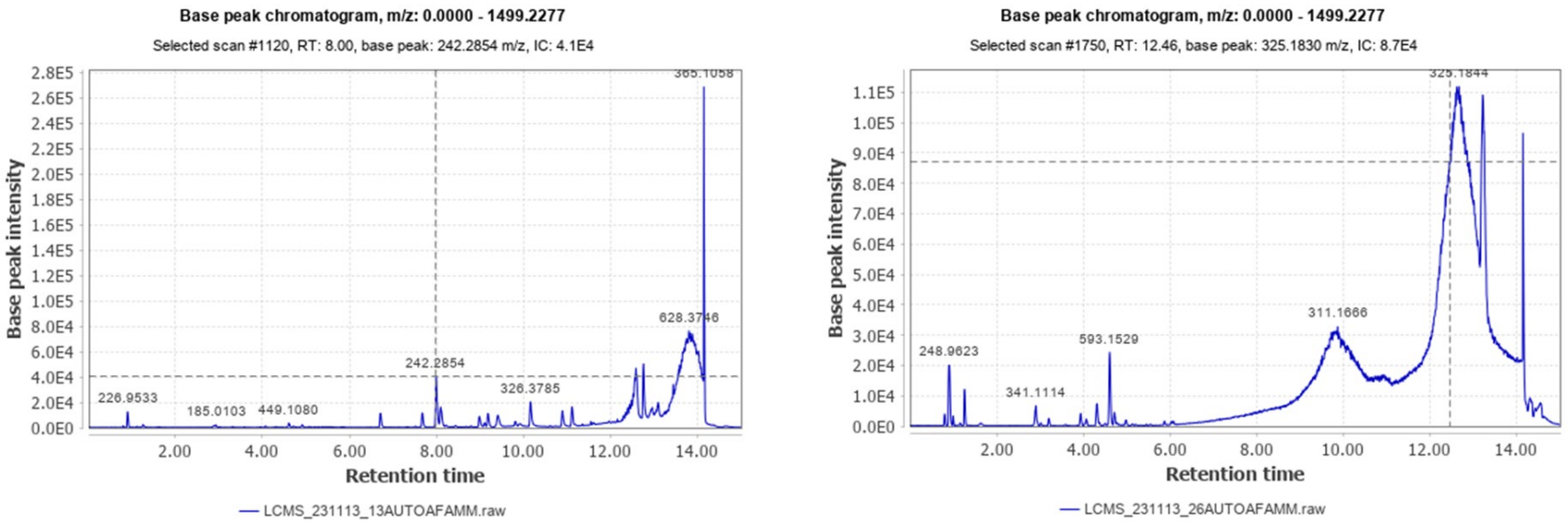


Figure 6.2: UHPLC-ESI-MS base peak chromatogram of *T. ciliata* leaves, roots, and flower buds extracts analysed in the ESI negative and positive modes.

6.4 Discussion

The medicinal benefits of plants are connected to their phytochemical composition (Godswill, 2019). It is evident that the nutritional and phytochemical content of plants correlates to the environmental conditions in which plants grow (Hnilickova et al., 2021; Sunita et al., 2020). It has been reported that drought and salinity induced oxidative stress in plants lead to increased concentrations of phytochemicals and secondary metabolites. As such, halophytes are often inferred to produce more secondary metabolites than many vegetable crops (Sogoni et al., 2023; Hsouna et al., 2020). Besides the environmental factors involved, different plant parts in halophytes may exhibit different medicinal properties due to different metabolic processes and salt tolerance levels, leading to varying phytochemical content (Nemzer et al., 2020). However, wild edible halophytes are understudied in many African countries, including South Africa (Bvenura & Sivakumar, 2017; Unuofin et al., 2017; Ngxabi et al., 2021). Hence, it is crucial to explore these plants also as sources of organic compounds that are beneficial in the pharmacological industry.

Trachyandra ciliata is an underexplored edible halophyte endemic to the Western Cape coastal dunes in South Africa. A previous study by Ngxabi et al., (2025) reported more phenolic compounds (flavonols and polyphenols), mineral (Ca^{+}, K, Fe, P and N), proximate (Fibre and protein) and lower antinutrient (oxalate and saponin) concentrations in *T. ciliata* grown under saline conditions compared to mainstream vegetables such as Asparagus. Although only flower buds of *T. ciliata* have been declared edible based on folkloric record (De Vynck et al., 2016b), it is paramount to explore other parts of the plant to assess their medicinal potential. Thus, the current study was conducted to profile phytochemical content of root, leaves, and flower bud extracts of *T. ciliata* using UHPLC-MS to explore possible medicinal and therapeutic uses of the plant.

In the current study, the UHPLC-MS phytochemical profiling of different parts of *T. ciliata* led to the identification of 71 compounds grouped into flavonoids, anthocyanins, alkaloids, nucleobase, nucleosides/tide, saccharides, fatty acids, amino acids, and coumarins, all of which are known for their vital pharmacological benefits.

6.4.1 Flavonoids

Flavonoids are a broad collection of naturally occurring polyphenolic compounds found in plants, divided into subclasses such as flavonols, flavones, and flavanones, each with unique structure and possible health benefits (Kopustinskiene et al., 2020; Patel & Patel, 2019).

6.4.1.1 Flavonols

These types of flavonoids are well known for their potential medicinal benefit such as antioxidant and anti-inflammatory effects, and they are mostly found in edible plants (Mihanfar et al., 2021). In this study, Rutin in the form of quercetin-3-O-(6-O-rhamnosylglucoside) was identified only in the leaves grown under low and moderate salinity treatments. This compound is linked to a broad range of medicinal benefits. Previous studies have reported antitumor, antibacterial, anti-inflammatory, anti-ulcer, antioxidant, cytoprotective, vasoprotective, cytoprotective, immunomodulatory, and neuroprotective activities of rutin (Mihanfar et al., 2021; Kaur & Muthuraman, 2016; Khajevand-Khazaei et al., 2018; Patel & Patel, 2019). A study conducted by Yuan et al., (2024) on the antidiabetic capacity of quercetin reported that it reduces insulin resistance, repairs the intestinal barrier, alters the intestinal microbiota, and modifies gut metabolites. This discovery therefore implies that although flower buds are the edible part of *T. ciliata*, leaves could be of great importance in the treatments of many chronic diseases such as diabetes, neurological disorders, and gastrointestinal diseases. Another flavonol, isorhamnetin-3-O-rutinoside widely known as narcissin was identified in the extracts of edible flower buds. Numerous studies have demonstrated that this flavonol has exceptional antioxidant activity and inhibiting lipid peroxidation (Kalai et al., 2022; Seo et al., 2016). Further studies proved that isolated isorhamnetin extracts inhibit human breast and cervical cancer cell line HeLa proliferation through cell cycle arrest at the G_2/M stage by the activation of the ATM-Chk2 pathway (Wei et al., 2018; Wu et al., 2018). This suggests that the consumption of *T*. *ciliata* flower buds and isolation of this compound present a huge potential use in the treatment of breast and cervical cancer.

6.4.1.2 Flavones

Flavones have been reported to have wide range of biological activities, including antioxidant, anti-inflammatory, antiallergenic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic properties (Bose et al., 2021; Martens & Mithöfer, 2005). In the present study, luteolin-6-C-glucoside and luteolin-8-glucoside were detected precisely on the flower buds and leaves of *T. ciliata*, and this is in correspondence with previous reports (Rambabu et al., 2016; Kopustinskiene et al., 2020). Luteolin has been reported to have anti-inflammatory, antioxidant, anti-cancer, wound healing, antibacterial, antiviral, and COVID-19 effects (He et al., 2023; Huang et al., 2023; Singh Tuli et al., 2022; Wu et al., 2013; Theoharides et al., 2021). Moreover, its influence has been reported to extend to anti-allergic, anti-aging, renal disorders, eye diseases, arthritis, cardioprotective, obesity, and cytotoxicity (Jiang et al., 2013; Shi et al., 2015; Lin et al., 2016; Zhang et al., 2016). A recent study demonstrated an enhanced would healing process on both diabetic and non-diabetic skin tissues when luteolin ointments were

applied (Ozay et al., 2018). This compound's versatility as a therapeutic agent is accentuated by its capacity to efficiently interact with molecular targets and influence key cellular pathways (Singh et al., 2024). These findings suggest that inclusion of *T. ciliata* in diet and isolation of luteolin from its extracts could contribute to the treatment of many ailments such as cardiovascular diseases, cancer, arthritis, neurological disorders, and wound healing. Furthermore, inclusion of flower buds in daily diet could help prevent obesity and aging.

6.4.1.3 Flavanones

Similar to other subclasses of flavonoids, flavanones are well known for their antioxidant and anti-inflammatory properties and have been widely reported mainly in *citrus* species, although they are also found in other families (Barreca et al., 2017). In this study, Apigenin-6-C-glucoside -7-O-glucoside, also referred to as vicenin-2 was identified in the leaf extract from low salinity treatment (50 mM). Vicenin-2 has been identified as having potential antidiabetic, antioxidant, and anti-inflammatory properties, as well as enhancing cell proliferation and migration effects (Muhammad et al., 2020; Tan et al., 2020). A study by Tan et al., (2020) demonstrated that the vicenin-2 (VCN-2) molecule dramatically boosted the proliferation and migration of Human Dermal Fibroblasts (HDF). It also regulated the synthesis of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α from HDF in wound repair. The presence of apigenin in *T. ciliata* leaves present potential pharmaceutical benefits such as diabetes and healing of chronic wounds.

6.4.2 Anthocyanins

Anthocyanins (ACNs) are a class of O-glycosides of the 3,5,7,3′-tetrahydroxyflavylium cation responsible for the red, blue, and violet colours of most fruits and vegetables (Burton-Freeman et al., 2016). ACNs are natural bioactive chemicals that have a variety of pharmacological effects, including antioxidant and anti-inflammatory properties, as well as the prevention of age-related chronic diseases such as cardiovascular disease (CVD), cancer, neurodegenerative diseases, and eye diseases (Pour et al., 2019; Salehi et al., 2020). A study by Demeilliers et al., (2017) reported that dietary ACNs from blue corn protected the brain from the mitochondrial DNA common deletion (mtDNA CD) caused by moderate ethanol consumption. ACNs have been reported to also possess antiviral effects as recent *in vitro* investigations have revealed that they can prevent the replication of viruses such herpes simplex, parainfluenza, syncytial virus, HIV, rotavirus, and adenovirus (Pour et al., 2019). Moreover, ACNs are often used in the food industry to substitute synthetic colorants (Roy & Rhim, 2021; Iorizzo et al., 2020). In this study Peonidin, an anthocyanidin was detected mainly in extracts derived from the roots of *T. ciliata*. Peonidin imparts a purplish-red color to foods and is extensively used as an organic food colorant (Bakovic et al., 2738; Iorizzo et al.,

2020). Research suggests that peonidin exhibit neuroprotective effects that could help treat neurodegenerative disorders due to its free radical scavenging capacities (Bao et al., 2023; Sun et al., 2018). These findings suggest that peonidin-based anthocyanins derived from *T. ciliata* may be of great use as an organic food colourant and as a nutraceutical to treat neurodegenerative diseases. Furthermore, this plant may contribute to pharmaceutical developments in the treatment and prevention of viruses such as HIV, rotavirus, parainfluenza, and herpes simplex.

6.4.3 Alkaloids, nucleobase, nucleosides/tide

Alkaloids are significant secondary metabolites that are broadly recognized for their therapeutic properties. Alkaloids are produced by many organisms, including bacteria, fungi, plants, and animals. They are among the most versatile, effective, and therapeutically significant plant compounds (Kamarul Zaman & Mohamad Azzeme, 2018; Roy, 2017). Primarily, alkaloids are synthesized by plants to increase survival chances in harsh conditions. Alkaloid-rich plants have many pharmacological effects, including antibacterial, analgesic (pain killer), anti-depressive, and anticancer. Some of them have been shown to have neuroprotective qualities, making them effective in treating Alzheimer's disease (Lu et al., 2012; Wadood, 2013). However, excessive alkaloid accumulation is harmful to both humans and animals due to their high reactive nature (Ngxabi et al., 2025; Matsuura & Fett-Neto, 2017). A recent study by Ngxabi et al., (2025) reported that the alkaloid content in *T. ciliata* is below toxic levels, making the plant suitable even as a nutraceutical. N-trans-coumaroyltyramine and N-trans-feruloyltyramine, which are tyramine (alkaloid) were identified only in the root extracts of *T. ciliata*. Tyramine has been reported to be essential for metabolism, heart and muscle function, brain development, and bone maintenance (Bainbridge et al., 2008; Hajam et al., 2022). A recent study by Bakrim et al., (2024) who explored the chemistry, biological effects, and mechanism insights of natural coumaroyltyramine found that it has antihyperglycemic activity and could have an impact on diabetes and metabolic disorders. They also observed that coumaroyltyramine has hypocholesterolemic and neuroprotective properties, which reduces the incidence of heart and vascular disease incidence and aids in the prevention of neurological disorders. A study that explored Potential of N-trans feruloyltyramine from a halophyte (*Lycium barbarum*) extract on neurogenesis and neurotrophins reported that in the nervous system, it improved neuronal survival and differentiation by enhancing neurotrophic factor secretion in C6 glioma cells (Khan et al., 2021). This then suggest that Tyramine from isolated from root extracts of *T. ciliata* may be beneficial in the treatment of brain, metabolic, and heart disorders due to its inti-inflammatory, antioxidant and neuroprotective properties. In Addition, hypaphorine which

is an alkaloid that has been reported to exhibit anti-inflammatory, sleep-promoting, and neuroprotective properties in *Erythrina velutina* was identified (Braschi et al., 2021).

On the other hand, adenosine and cytidine nucleosides were identified in the leaves and flower bud extracts along with their nucleobases, purine and pyrimidine. These nucleosides are critical for several biological activities, acting as signaling molecules and precursors for nucleotides required for DNA and RNA synthesis, with adenosine also employed therapeutically and diagnostically in cardiology (Phan et al., 2018). Studies have reported that adenosine has significant impacts on the coronary artery and anti-epilepsy, and is frequently utilized to treat cerebrovascular diseases, apoplexy sequelae, coronary deficiency, angina, arteriosclerosis, and primary hypertension (Phan et al., 2018; Liu et al., 2019).

6.4.4 Saccharides

Saccharides, often known as carbohydrates, are essential energy sources, structural components, and building blocks for many biological entities, including proteins and nucleic acids (Feher, 2017). Disaccharides are typical sugar molecules that are important for energy storage and transmission. Made up of two monosaccharide units linked together by glycosidic bonds, disaccharides have properties comparable to their constituents and are mostly referred to as table sugar (Niaz et al., 2020; Selvaraj et al., 2023). Disaccharides: sucrose and its isomers (Sucrose Isomer I, II, and III), and trehalose and its isomers (D- (+)-Trehalose Isomer I, II, IV, and V) melibiose and its isomer (Melibiose isomer I) and gentiobose were identified (in all plant parts) in the present study. In human sperm cryopreservation, it has been discovered that cryoprotectants such as trehalose and gentiobiose can increase sperm motility, viability, and cellular integrity during freezing and thawing by stabilizing proteins and protecting them from denaturation (Selvaraj et al., 2023; Gholami et al., 2023; Chen & Gibney, 2023). Other studies have linked trehalose has neuroprotection (inducing autophagy and clearing protein aggregates), metabolic health (prevention of pathologies related to postprandial hyperglycemia), food preservation, and cosmetics (protecting the skin from free radical damage and oxidative stress (Khalifeh et al., 2019; Yoshizane et al., 2020). On the other hand, Melibiose has been identified as a substrate for certain bacteria and fungi. It also aids in the absorption of quercetin glycosides and may be involved in the generation of MAGE, a glycation product (Staniszewska et al., 2021; Gostomska-Pampuch et al., 2022). The identification of these saccharides in *T. ciliata* suggest that this plant may be on great use in the strives to utilize organic more organic than synthetic compounds in the treatment of some chronic diseases, in sperm cryopreservation, and as food additives.

6.4.5 Fatty Acids

Fatty acids are the building blocks of fats that consist of long chains of carbon atoms with a carboxyl group at one end and a methyl group at the other (Zhang et al., 2023). Fatty acids perform critical roles in the body, serving as both structural components of cell membranes and a major source of energy, as well as signalling molecules and precursors to other essential compounds (De Carvalho & Caramujo, 2018). In the present study dihydroxyoctadecadienoic acid, methyl-13-hydroperoxy-delta9Z,11E-, hydroxylinolenic acid, and linoleic acid were detected. Linoleic acid is an important fatty acid that has anti-inflammatory actions by forming the building blocks for prostaglandins (Serag, Ion-Margineanu, et al., 2019; Whelan & Fritsche, 2013). Moreover, linoleic acid is an essential omega-6 fatty acid that plays vital roles in maintaining skin health, supporting nerve function, and modulating inflammatory responses, as well as being a precursor to other important fatty acids (Whelan & Fritsche, 2013; Wang et al., 2025). Brownlee et al., (2016) reported that linoleic acid is a key component of neuronal membrane phospholipids and a substrate for the synthesis of PGE, which appears to be vital in maintaining nerve blood flow. These findings suggest that *T. ciliata* may be a good candidate for natural fatty acids that may play a role in the treatment of skin ailments and neurodegenerative disorders.

6.4.6 Organic and carboxylic acids

In halophytes, carboxylic acids serve an important role in stress tolerance, particularly against alkali stress, by regulating pH in the rhizosphere and possibly detoxifying toxic substances (Badea & Radu, 2016; Wang et al., 2024). They further assist with nutrient mobilization and participate in numerous physiological processes (Wang et al., 2024). Carboxylic acids have a crucial role in human health, influencing cell membrane function, nutrition metabolism, and serving as food preservatives and antimicrobials (Bensid et al., 2022; Guan & Liu, 2020; Mira et al., 2024). In the current study, eight carboxylic acids were identified in the extract, namely citric acid, iso citric acid, 2-isopropylmalic acid, cis-aconitic acid, 6,8-thioctic acid, and suberic acid. These compounds were mainly identified in the leaves and flower bud extracts, except for citric and suberic acids, which were also found in the root extracts. Citric acid, which is naturally common in citrus fruits, benefits human health by functioning as an antioxidant, potentially protecting against kidney stones, and aiding in nutrient absorption, as well as being a common metabolic intermediate (Hamid et al., 2024). Citric acid has also been reported to possess anti-inflammatory, antiviral, and skin health properties (Miles & Calder, 2021; Amani et al., 2024; Balta et al., 2020). Iso citric acid has medicinal applications, such as treatment of iron-deficient anaemia and metabolic myopathy, stress and is utilized in medical research and diagnostic imaging, specifically in Positron Emission Tomography (PET) (Kamzolova et al.,

2023a). Other carboxylic acids such as isopropylmalic acid and cis-aconitic acid have been used as an internal standard in a diagnostic model for pancreatic cancer, standard for organic acid determination in plant tissues, an intermediate in pharmaceutical production, a reagent in chemical research, and possess antioxidant, antibacterial and bactericidal activity against human pathogens (Fan et al., 2021; Kamzolova et al., 2023b). Furthermore, thioctic acid (also identified in *T. ciliata*) is frequently utilized for its antioxidant properties in the treatment of diabetic neuropathy, and it has also been tested in the treatment of liver failure (Dugbartey et al., 2022; Viana et al., 2022; Mrakic-Sposta et al., 2018). The abundance of these acids in *T. ciliata* reveal that it could be a potential therapeutic agent for the treatment of skin ailments, anaemia, stress related disorders, diabetic neuropathy, to develop standards for different diagnostic models, as well as in the development of organic food preservatives.

6.4.7 Coumarins

Coumarins are a family of natural, organic compounds found in plants that are distinguished by a benzene ring fused to a pyrone ring and have become recognized for their various pharmacological and biological properties (Poumale et al., 2013; Akwu et al., 2023). In the current study scopoletin was detected in the leaf extract under low salinity (50 mM NaCl) treatment, while ethoxy-methoxy coumarin derivatives were identified in the root extract cultivated under control treatment (0 mM NaCl). Scopoletin, a naturally occurring coumarin, has a variety of potential medical applications, including anti-inflammatory, antimicrobial and blood pressure regulation, as well as antioxidant and neuroprotective characteristics (Gao et al., 2024; Das et al., 2020; Molokoane et al., 2023). Gao et al., (2024) and Meilawati et al., (2023) suggested that plant derived scopololetin has the potential to be used as a therapeutic candidate to treat cancer, liver disease, diabetes, neurodegenerative disease, and mental problems due to its desirable pharmacological properties. This makes *T. ciliata* a potential candidate as a source of organic scopoletin for the treatment of various diseases. Furthermore, ethoxy-methoxy coumarin and other coumarins are being studied for the treatment of disorders such as Netherton syndrome, where the goal is to prevent excess kallikrein activity and enable skin to function appropriately (Medicines Agency, 2015; Chiodi & Ishihara, 2024). Other studies have reported ethoxy-methoxy to exhibit anticancer, antioxidant, anti-inflammatory, and antimicrobial activities, as well as uses as a solvent and as fragrance agents in different industries (McKeen, 2012; Stanard, 2005; Chiodi & Ishihara, 2024). This suggests that *T. ciliata* may be a potential candidate as a source of ethoxy-methoxy coumarin with potential use in the treatment of cancer and other chronic diseases, cosmeceutical applications, as well as in the fragrance industry.

6.5 Conclusion

Chemical profile of metabolites aggregated in the crude extract of different plant parts of *T. ciliata* were evaluated for the first time with UHPLC-MS, and 71 compounds were detected. These diverse groups of compounds included flavonoids, anthocyanins, alkaloids, saccharides, fatty acids, amino acids, and coumarins, known for their biological activities in the amelioration of chronic diseases such as diabetes, neurodegenerative disorders, cancer, cardiovascular diseases, and gastrointestinal disorders. Results from this study further show the potential of this plant in the treatment of other ailments such as skin problems, viral diseases, inflammation, oxidative stress, as well as plant plant-based food additives and preservatives. Thus, further studies are recommended on the isolation and characterization of pure compounds from the species using Nuclear Magnetic Resonance (NMR) and evaluate their potential biological activities. Moreover, further studies using different solvents and extending the extraction period could maximize the identification of more compounds.

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CHAPTER 7

CYTOTOXICITY, ACETYLCHOLINESTERASE INHIBITORY ACTIVITY, AND LIVER ROS SCAVENGING ACTIVITY OF *TRACHYANDRA CILIATA* (WILD CABBAGE) GROWN UNDER VARYING DEGREES OF SALINITY

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Cytotoxicity, Acetylcholinesterase inhibitory activity, and Liver ROS scavenging activity of *Trachyandra ciliata* (Wild cabbage) grown under varying degrees of salinity

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Abstract

Despite the increasing global interest in exploring the pharmacological properties of halophytes in recent years, South African halophytes have largely been overlooked as potential agents in the amelioration of chronic diseases. *Trachyandra ciliata* (wild cabbage) is one of the understudied wild edible halophytes from South Africa. Although its edibility has recently been validated, its therapeutic potential is yet to be explored. Thus, the present study investigated the crude extracts of *T. ciliata* as therapeutic agent for cancer, Acetylcholinesterase and ROS production in the liver for the first time. Plants were grown under 0, 50, 100, 150, and 200 mM salinity concentrations. The yellow dye 3-(4,5-dimethyltiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), Ellman's colorimetric method, and the 2',7'-dichlorodihydrofluorescein diacetate assay (H2DCF-DA) were respectively employed to evaluate cytotoxicity, acetylcholinesterase, and ROS scavenging activity of the plant extracts. Results revealed that flower bud extracts prepared from 0 mM and 100 mM salinity treatments at 1mg/mL concentration showed strong cytotoxicity to cancer cells, while they had moderate and weak cytotoxicity to non-cancer cells respectively. All extracts showed high acetylcholinesterase inhibitory activity, except for root and flower bud extracts from 100 mM salinity treatment. Moreover, ROS scavenging activity was mainly observed in the leaf extracts from all treatments, and in the root extract from 0 mM salinity treatment. These findings suggest that *T. ciliata* could be a therapeutic agent for the treatment of cancer, Alzheimer's disease, and liver disorders amidst the quest to develop more plant-based pharmaceuticals for the treatment of chronic diseases.

Key words: Acetylcholine, Alzheimer's disease, Bioactive compounds, Cancer, Halophytes, Liver disorders, MTT colorimetric essay

7.1 Introduction

Chronic diseases are distinguished by their inability to spread, numerous risk factors, extended development period, extended temporal course, functional damage or disability, and incurability (Piovani et al., 2022; Zullaihat Muhammad Abdullahi et al., 2025). They are accountable for over 41 million deaths annually, which constitutes 74% of all fatalities worldwide, while millions more people live with NCDs and have a reduced standard of life (Piovani et al., 2022). Chronic diseases that cause significant death and disability include neurological and mental health disorders, cancer, cardiovascular diseases, diabetes, substance use disorders (liver disorders), and gastrointestinal disorders, among others (GBD 2019 Diseases and Injuries Collaborators, 2020; Babashahi et al., 2021).

Cancer remains a significant global health issue, accounting for the second highest cause of deaths in the current millennium with more than 10 million deaths reported in 2020 (Mohammed et al., 2023; Lucero-Prisno et al., 2023). As a result, one of the SDGs for lowering early mortality from noncommunicable diseases is to reduce cancer mortality (Lucero-Prisno et al., 2023). Conventional cancer treatment includes surgery, chemotherapy, immunotherapy, and radiotherapy, all of which have limitations and accompanying complications such as myelosuppression, gastrointestinal discomfort, liver and kidney toxicity, and heart damage (Venkatachalapathy et al., 2021; Siegel et al., 2019). As such, the use of plant-based approaches is gaining popularity because they offer a potential complementary or alternative option, with some showing promise in lowering cancer risk or adverse effects (Ferreira et al., 2022; Lee et al., 2024).

Alzheimer's disease (AD), the most prevalent neurodegenerative disorder and the cause of adult-onset cognitive impairment, is distinguished by a gradual loss in cognitive abilities, which is accompanied by behavioural symptoms (Marucci et al., 2021). A proper equilibrium of neurotransmitter systems, including acetylcholine (ACh), is necessary for efficient cognitive function and Alzheimer's disease prevention (Yang et al., 2023; Chen et al., 2022). Acetylcholinesterase (AChE) is constantly located in proximity to amyloid deposits in AD and may have a role in the synthesis of amyloid proteins, which are characteristic of AD (Gajendra et al., 2024a; Huang et al., 2022). Thus, AChE inhibitors (AChEIs) have been used as the principal treatment for dementia since the 1990s (Kaushik et al., 2018; Gajendra et al., 2024b). However, synthetic pharmaceuticals that are commonly used to treat Alzheimer's such as donepezil, rivastigmine, and galantamine can induce adverse effects such as reduced heart rate, decreased appetite, and gastrointestinal difficulties (de Oliveira et al., 2024; ALNasser et al., 2025). Hence, natural compounds derived from plant materials, including halophytes have begun to gain recognition globally as promising acetylcholinesterase inhibitors, which could

be employed as a therapeutic alternative for Alzheimer's disease treatment (Islam et al., 2024; Taqui et al., 2022; ALNasser et al., 2025).

The liver is the primary biological producer of immune modulators, plasma proteins, blood coagulation factors, and protease inhibitors, while it also helps in the digestion of nutrients from food, medications, alcohols, and xenobiotics (Schulze et al., 2019). Liver disorders are often associated with excessive production of reactive oxygen species (ROS), which can cause oxidative stress and damage to liver cells, proteins, and DNA (Lee et al., 2022). Therefore, it is crucial for cellular defence systems to always maintain redox homeostasis (Sies, 2015). The medicinal potential of plant extracts with high antioxidant qualities has drawn more attention in recent years due to the adverse effects presented by synthetic pharmaceuticals (Liu et al., 2023). For instance, phytochemical compounds such as Apigenin (Flavanone), Peonidin (anthocyanin), Adenosine (nucleoside), thioctic acid (carboxylic acid), Scopoletin (coumarin) among many other compounds have been reported to possess high ROS scavenging activities that may be useful in the treatment/prevention of liver disorders (Liu et al., 2023).

Natural products derived from herbal remedies, medicinal plants, and functional foods, along with their constituents, have been utilised for the treatment of various diseases, such as cancer, neurodegenerative disorders, liver disorders, diabetes, and gastrointestinal disorders from ancient times to the present day (Kim et al., 2018; Gonfa et al., 2023; Cock et al., 2021; Asong et al., 2019). Halophytes have been utilised for medicinal purposes across different countries and have demonstrated effectiveness in the treatment and management of chronic diseases that afflict modern societies (Mohammed et al., 2023). Recently, there is a growing focus on these species due to their rich content of bioactive compounds, including primary and secondary metabolites like polyunsaturated fatty acids, vitamins, flavonoids, anthocyanins, alkaloids, nucleobases, nucleosides/tide, saccharides, amino acids, and coumarins amongst others (Lee et al., 2024; Ferreira et al., 2022). Despite the increasing global interest in exploring the medicinal properties of halophytes in recent years, South African halophytes have largely been overlooked as potential agents in the amelioration of chronic diseases (Cock, 2015; Sogoni et al., 2025).

Trachyandra ciliata (wild cabbage) is one of the understudied wild edible halophytes from South Africa (Ngxabi et al., 2021). A previous study by (Ngxabi et al., 2025) validated its edibility and reported that salinity improved its nutritional composition and antioxidant capacity. However, its pharmacological potential remains undocumented. In the previous chapter (Chapter 6), the UPHLC-MS identified 71 phytochemical compounds, which have been reported to have biological activities against numerous diseases and ailments. Thus, the

present study investigated the crude extracts of *T. ciliata* as therapeutic agent for cancer, Acetylcholinesterase and ROS production in the liver.

7.2 Materials and Methods

7.2.1 Greenhouse experimental design

The experiment was conducted at the greenhouse nursery of the Cape Peninsula University of Technology's Bellville campus in Cape Town, South Africa. The greenhouse conditions and propagation procedures utilized were identical to those described by Ngxabi et al., (2025). Plants were subsequently exposed to five salinity concentrations (0, 50, 100, 150, and 200 mM of NaCl). Following 15 weeks of salinity treatment, the plant material (leaves, roots, and flower buds) was harvested, dried, and ground for extraction and further analyses.

7.2.2 Extraction protocol

The extraction was carried out using a method outlined by Jimoh et al., (2024). Briefly, 10 grams of pulverized plant material were weighed and put into a round bottom flask holding 200 mL of 70% ethanol. After two days of vigorous shaking at 120 rpm on an orbital shaker (Orbital Incubator Shaker, Gallenkamp), the mixture was filtered through Whatman No. 1 filter paper in a Buchner funnel. A vacuum pump connected to the Buchner funnel produced suction during the filtration process. The crude extract was concentrated and dried by eliminating excess ethanol from the filtrate using a rotary evaporator (Strike-202 Steroglass, Italy) set to 78 °C.

7.2.3 Cytotoxicity

7.2.3.1 Cell culture maintenance

The ROS scavenging capacity and AChE inhibition assays were done in vitro, using two cell lines: the genetically modified rat hepatoma cells (H4IIE-*luc* cell line) (Aarts et al., 1993; Jimoh et al., 2024) and the Vero (non-cancerous) cell line, derived from African green monkey kidney cells. The University of Saskatchewan, Saskatoon, Canada, gifted the H4IIE-*luc* cells. It is presumed that the nature of its genetic modification would not impact the cells' behaviour, given that it is focused on expressing a firefly gene in the presence of aryl-hydrocarbon receptor (AhR) ligands and that AhR is endogenous to this cell line. The unmodified Vero cells were obtained from the American Type Culture Collection (ATCC) (Manassas, Virginia, USA) (HB-8065). Both cell lines were cultivated at 37°C in humidified air supplemented with 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma: D2902; St. Louis, MO, USA) supplemented with 10% foetal bovine serum (Thermo Scientific, USA) (Idris et al., 2023). All tissue culture work was done aseptically and, where applicable, inside a biosafety cabinet.

7.2.3.2 Cell viability assay (MTT)

A colorimetric assay measured cell viability based on metabolic activity using the yellow dye 3-(4,5-dimethyltiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma-Aldrich (Pty) Ltd) (Sogoni et al., 2025). Live cells transform MTT into an insoluble formazan (purple) product using NAD(P)H-dependent cellular oxidoreductase enzymes. A series of six dilutions (0.03, 0.06, 0.13, 0.25, 0.5, and 1 mg/mL) of each plant extract was tested in triplicate in clear 96-well plates. Cells (both Vero and H4IIE-*luc*) were seeded at 80,000 cells/mL and incubated for 24 hours before incubation with the respective plant extracts for another 24 h. Each plate had a triplicate of untreated wells (positive control: plant extracts were diluted with cell culture growth medium) as well as untreated wells that were killed with methanol at the end of the exposure period to create a negative control, i.e. all the cells are dead. At the end of the exposure period, the medium was replaced with 500 µg/mL MTT and incubated for 30 minutes. Purple formazan crystals produced by reduced MTT were dissolved in dimethyl sulphoxide (DMSO), and absorbance was determined spectrophotometrically at 560 nm. The amount of purple formazan produced is associated with the number of viable cells, and the proportion (%) of viable to dead cells was determined by comparing it to the positive control.

Magnitudes of effects of the extracts on cell viability were defined as follows: non-toxic (> 80%); weakly cytotoxic (60–80%); moderately cytotoxic (40–60%); and strongly cytotoxic (< 40%) (International Organisation for Standardisation (ISO), 2009; López-García et al., 2014).

7.2.4 ACHE inhibitory activity

7.2.4.1 Exposure cells and harvesting cell contents

Non-neuronal AChE is produced in liver cells; therefore, only the H4IIE-*luc* cells were used in this assay. The Ellman assay (Ellman, et al., 1961) was adapted for a 12-well format. This colorimetric assay measures the absorbance of yellow 2-nitro-5-thiobenzoate (TNB) after thiocholine reacts with Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid); DTNB) (Patel, 2023). The measured absorbance is proportional to AChE activity (Härtl et al., 2011; Zimmermann et al., 2008). Cells were seeded at 80,000 cells/mL and exposed to plant extracts at 0.03 mg/mL in triplicate for 24 h after an initial 24 h incubation at 37°C. Wells with untreated cells were included as controls. The 0.03 mg/mL concentration was the greatest concentration at which none of the samples were cytotoxic to the H4IIE-*luc* cells and was determined in the viability assay (Sub-section 2.5.2).

The cells were harvested from the 12-well plate by washing with Dulbecco's phosphate-buffered saline (DPBS) and lysing the cells with trypsin. The enzyme activity was

stopped with DPBS, and the cell suspension was centrifuged at 1000 *g* for 4 minutes at room temperature. The supernatant was discarded, and the cell pellet resuspended in 400 µL Tris-HCl/sucrose buffer [12.5 mM Tris-HCl + 125 nM sucrose), vortexed and sonicated at medium intensity for 30 s. This was centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was used for AChE inhibitory determination as well as protein content. AChE activity was expressed in terms of the protein content of the cells.

7.2.4.2 AChE activity assay

This assay was performed in the dark in 96-well white-walled, clear, flat-bottom microplates. Each well received 210 µL ice-cold 0.09 M K_2HPO_4 buffer (pH adjusted to 7.4 by 0.09 M KH_2PO_4), 10 µL each of 30 mM acetylthiocholine iodide and Ellman's reagent (10 mM in methanol). The plates were incubated at 37°C for 5 min. Sample supernatant was added (10 µL) to the plate in triplicate. Experimental blanks containing the potassium phosphate buffer, acetylthiocholine iodide and Ellman's reagent were included on each plate. Absorbance was measured as soon as possible at 412 nm every minute for six minutes, starting at time zero, resulting in seven points. To determine AChE activity, the mean absorbance of each time interval was determined for the samples, untreated cells, and experimental blanks. This was followed by calculating the reaction gradient. The reaction rate (change in absorbance over six minutes) was determined, and the values were normalised against the protein content. Acetyl choline esterase activity is expressed in absorbance/min/mg/protein (Patel, 2023; Ellman et al., 1961).

7.2.4.3 Quantification of Protein

The total protein concentration in each cell lysate that was harvested after the exposure was determined according to Bradford, (1976) using bovine serum albumin (BSA) as a standard (0, 62.5, 125, 250, 500, 1000, 2000 µg/mL) (Hanumanthappa et al., 2024). This determination was also performed in the dark in the same plate format as the AChE determination. The standard or the cell lysate (5 µL) was added in triplicate to the wells, followed by 245 µL Bradford's reagent. The protein content was determined by measuring the absorbance at 590 nm in a microplate reader. The protein concentration in the lysate was determined using the regression line formula created by the BSA concentrations and corresponding absorbances.

7.2.5 Intracellular ROS production and scavenging activity

The 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) assay was used to test the intracellular generation of ROS, in the H4IIE-*luc* cells, as well as the scavenging activity of the *T. ciliata* extracts. Intracellular ROS, including hydrogen peroxide (H_2O_2), can be determined

using small molecule fluorescent sensors (eg, H_2DCF-DA) that react to specific stimuli. During the assay, the fluorogenic dye diffuses through the plasma membrane of cells and is hydrolysed to non-fluorescent 2′,7′-dichlorodihydrofluorescein (DCFH) by intracellular enzymes, where it stays trapped (Lebel et al., 1992; Zhang et al., 2021). Under oxidative stress, DCFH reacts with intracellular ROS, leading to the oxidation of DCFH to its highly fluorescent fluorescein derivative, 2′,7’-dichlorofluorescein (DCF) (Gomes et al., 2005). The DCF fluorescence is proportional to the amount of ROS produced.

The method described by Engelbrecht et al. (2024) was applied to determine the AChE activity. Briefly, 80,000 cells/mL H4IIE-*luc* cells were seeded in a 6-well microplate and incubated for 24 h at 37°C. The cells were exposed to 0.03 mg/mL of *T. ciliata* extract for 24 h in triplicate. A set of three wells received 14.2 ng/mL hydrogen peroxide (H_2O_2) to induce oxidative stress (positive control). The negative control (blank) consisted of cells that had not been treated to H_2O_2 or *T. ciliata* extract. After the 24 h exposure period, the cell medium was replaced with 800 µL 10 µM H_2DCF-DA and incubated for 30 min. The cells were washed, lysed with trypsinand centrifuged at 1 000 g to remove the supernatant. Each cell pellet was resuspended in 800 µL DPBS. The quantification of the fluorescence was measured in black 96-well microplates by transferring 200 µL of the cell suspension into a well (in triplicate for each well on the original 6-well plate resulting in nine fluorescent readings for one sample). Fluorescence was measured as relative fluorescence units (RFUs) at excitation and emission wavelengths of 480 nm and 535 nm, respectively, using a SpectraMax® iD3 multi-mode microplate reader. Since the emitted DCF fluorescence is directly proportional to the amount of ROS produced intracellularly (Engelbrecht et al., 2024; Mattia et al., 1991), the ROS produced by the cells after exposure is calculated based on the difference between the RFUs of treated and untreated cells (blank control) (Yao et al., 2015).

7.2.6 Statistical analysis

A one-way analysis of variance was employed to determine the significance of treatment differences. Non-Parametric statistics' assumptions were validated. The Mann-Whitney U test was used to determine statistical difference ($p \leq 0.05$) between treated cells and untreated cells using IBM SPSS (version 29.0) statistical software**.**

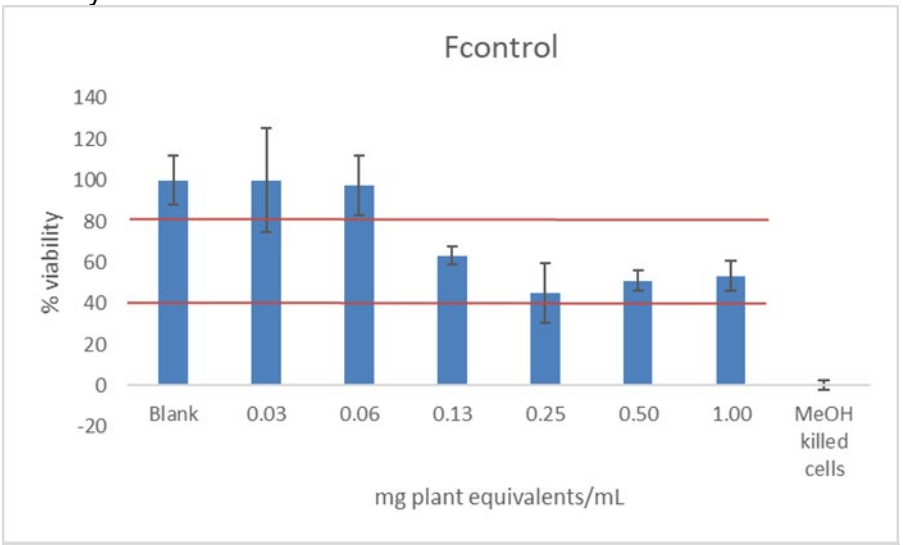
7.3 Results

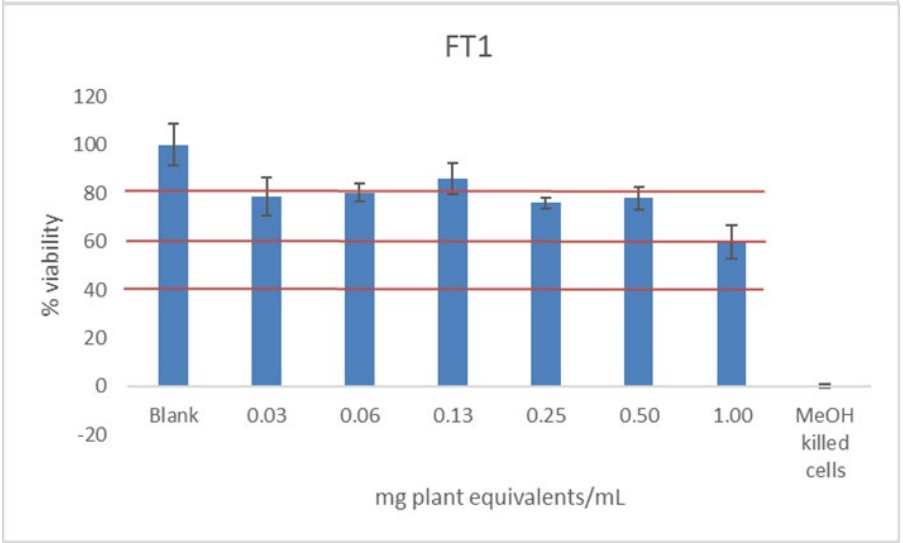
7.3.1 *In Vitro* Cytotoxicity determination

Ethanolic extracts of flower buds, leaves, and roots of *T. ciliata* cultivated under different salinity treatments significantly influenced cell viability of rat hepatoma (H4IIE-*luc*) and African green monkey kidney (Vero) cells (Figure 7.1). Flower bud extracts prepared from 0 mM and

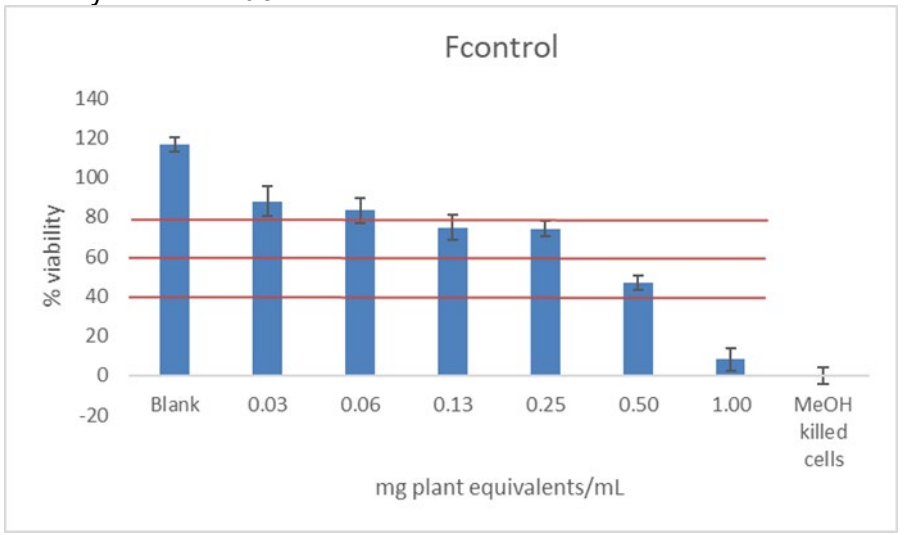
100 mM salinity treatments at 1 mg/mL extract concentration showed strong cytotoxicity (< 40%) to H4IIE-*luc*, while they had moderate and weak cytotoxicity to Vero cells, respectively. Root extract obtained from 0 mM salinity treatment at 0.5 mg/mL extract concentration showed moderate toxicity to H4IIE-*luc* cells, while it was non-cytotoxic (> 80%) to non-cancer cells. Moreover, root extract prepared from 150 mM salinity treatment at 0.13 mg/mL showed moderate cytotoxicity to H4IIE-*luc* cells, while it was not cytotoxic to Vero cells. All other extracts showed mild cytotoxicity to both cancer and non-cancer cells.

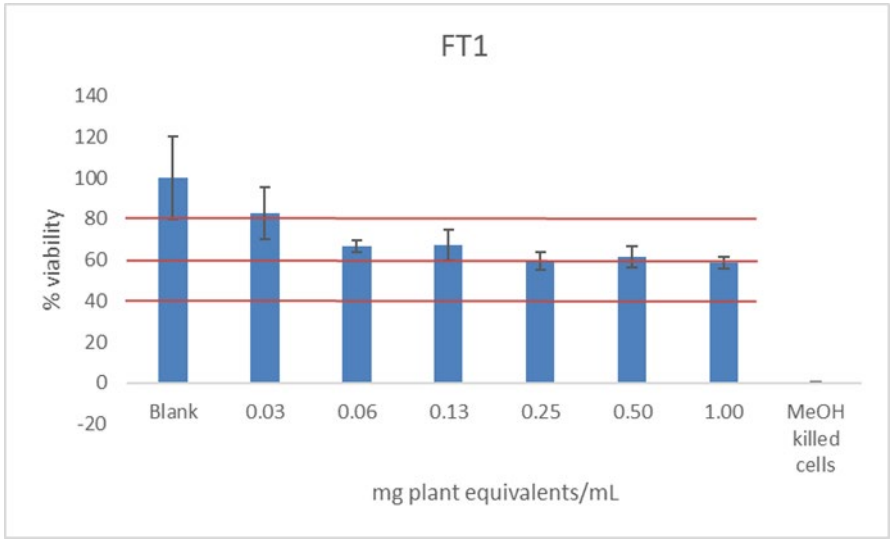
Viability on Vero cells

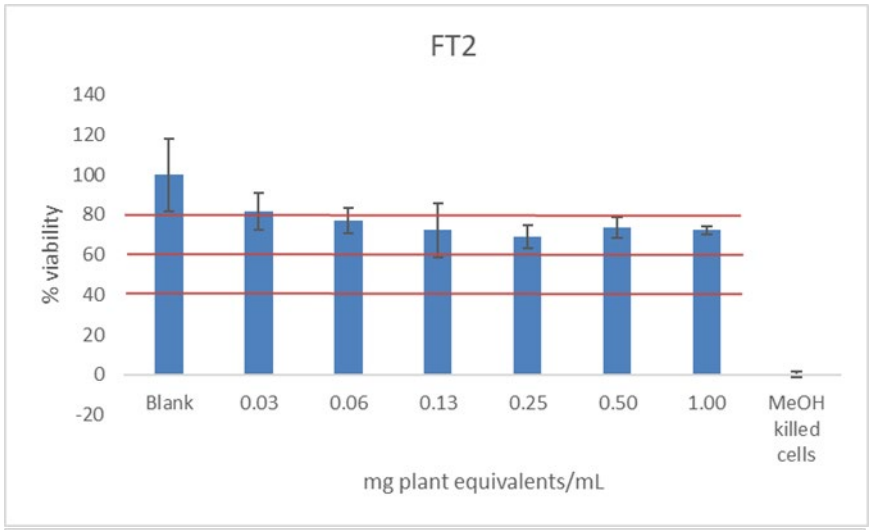




Viability on H4IIE-*luc* cells



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Blank
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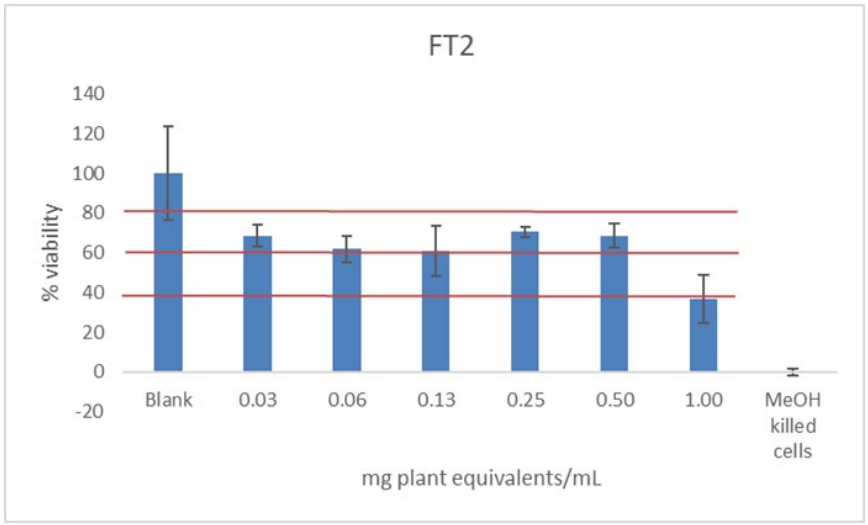
0.50
1.00
MeOH killed cells
mg plant equivalents/mL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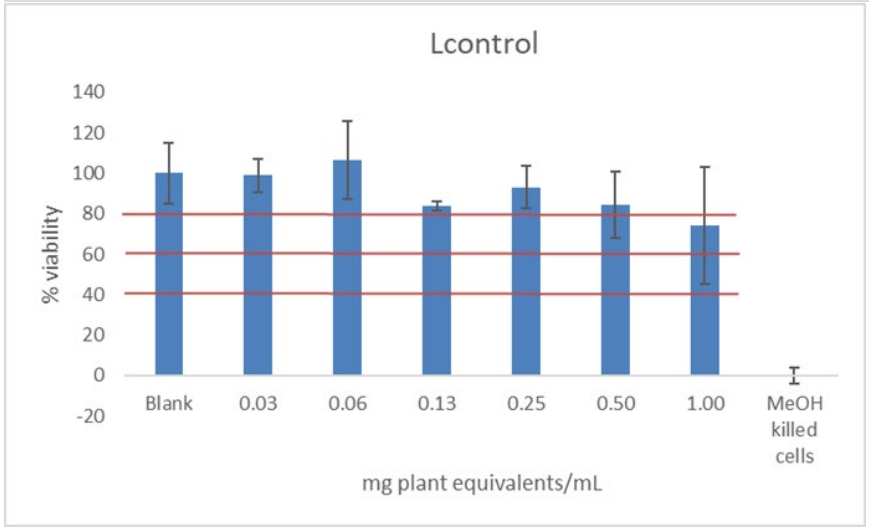
mg plant equivalents/mL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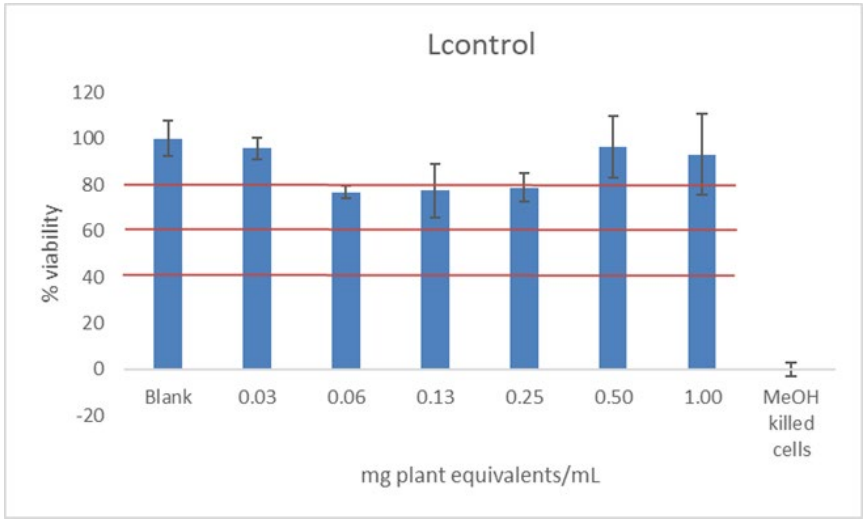
mg plant equivalents/mL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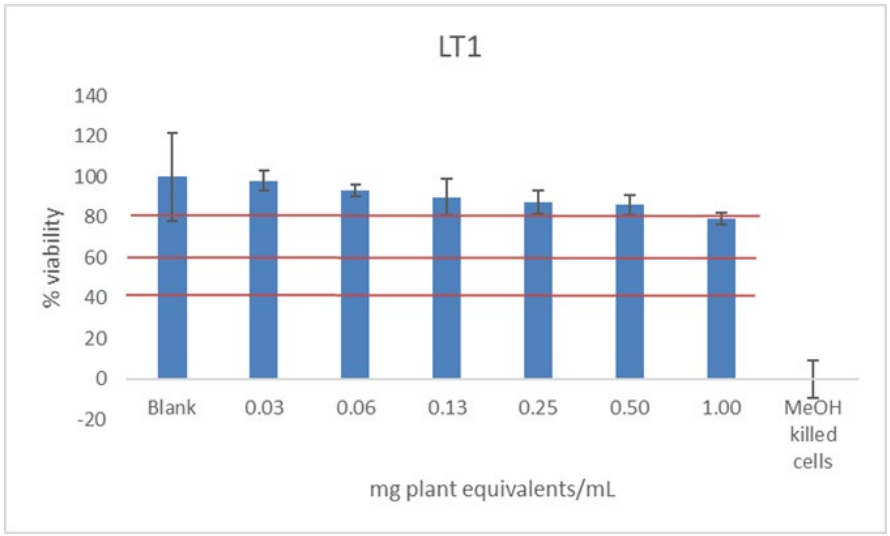
mg plant equivalents/mL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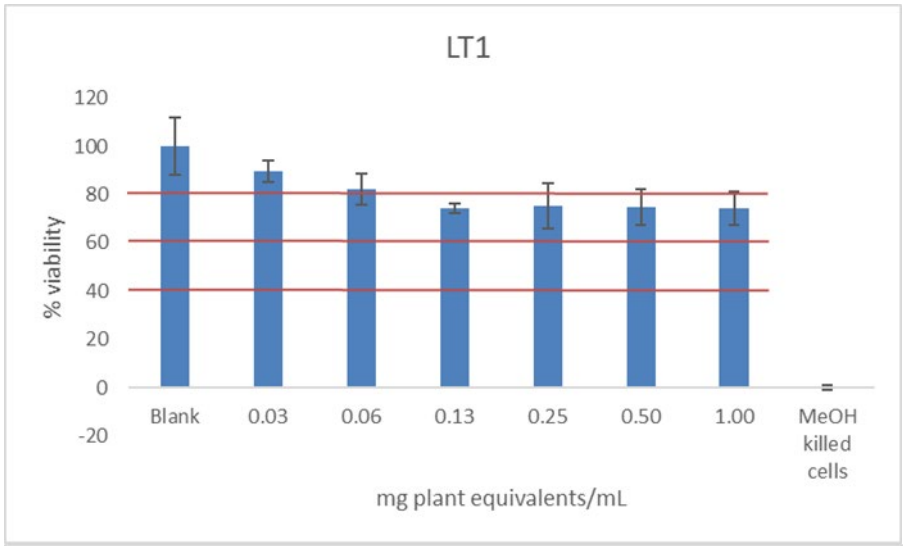
mg plant equivalents/mL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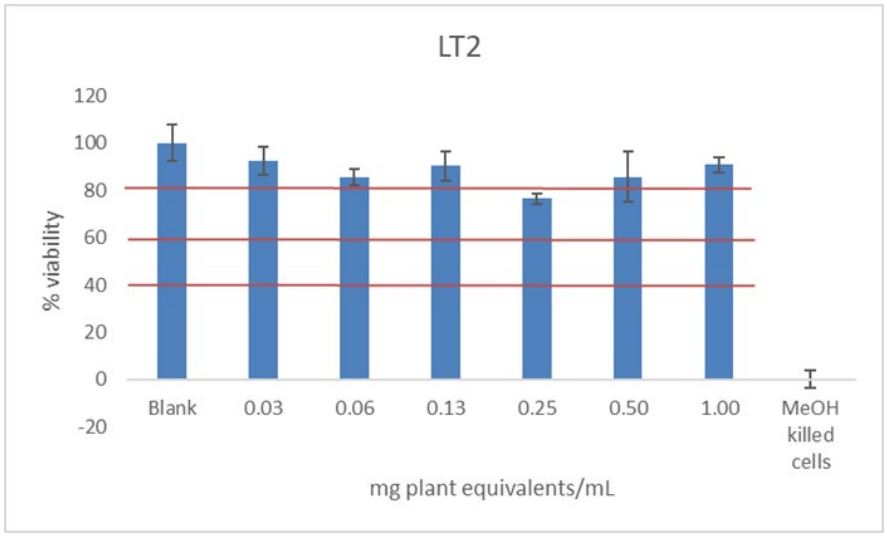
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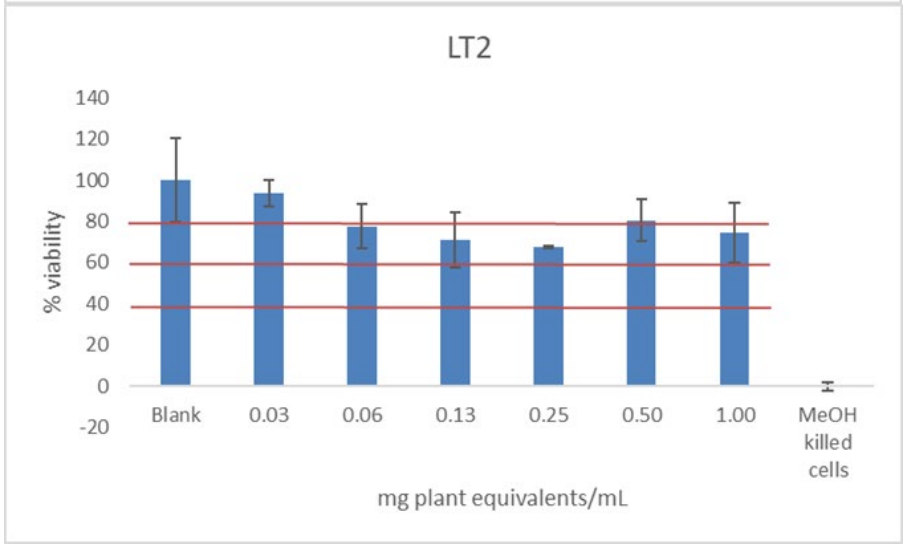
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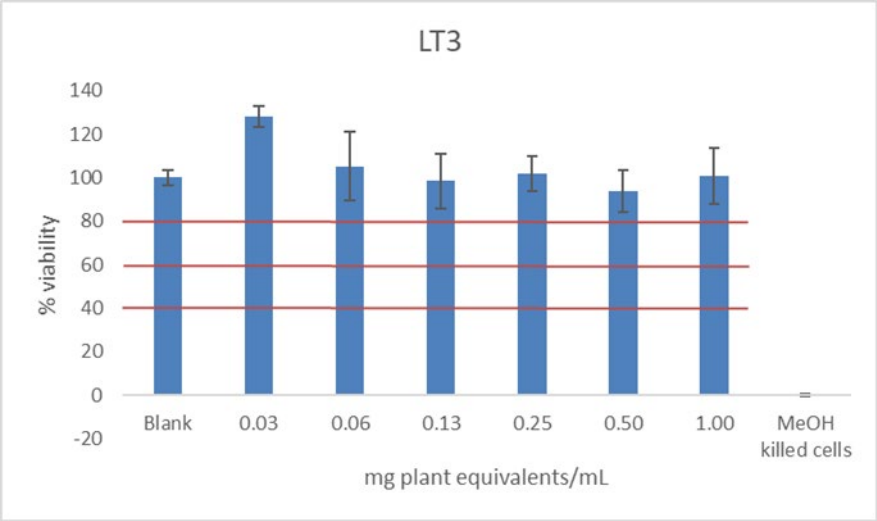
mg plant equivalents/mL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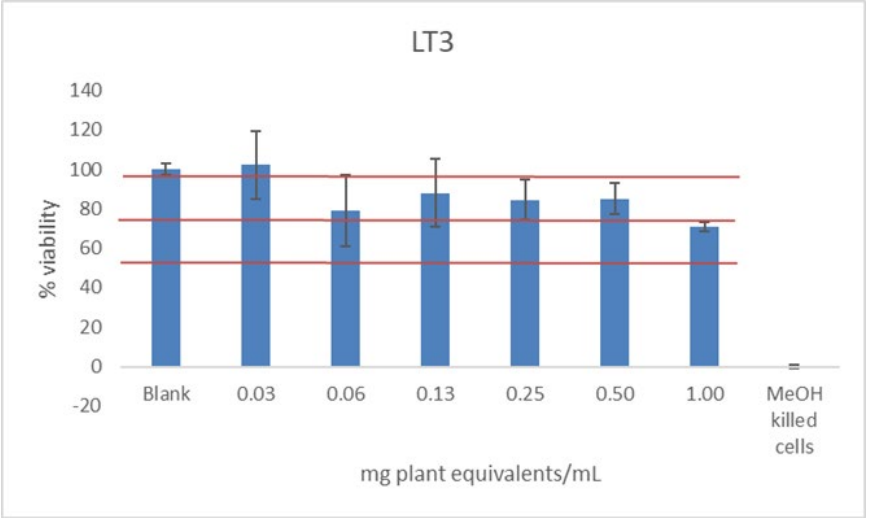
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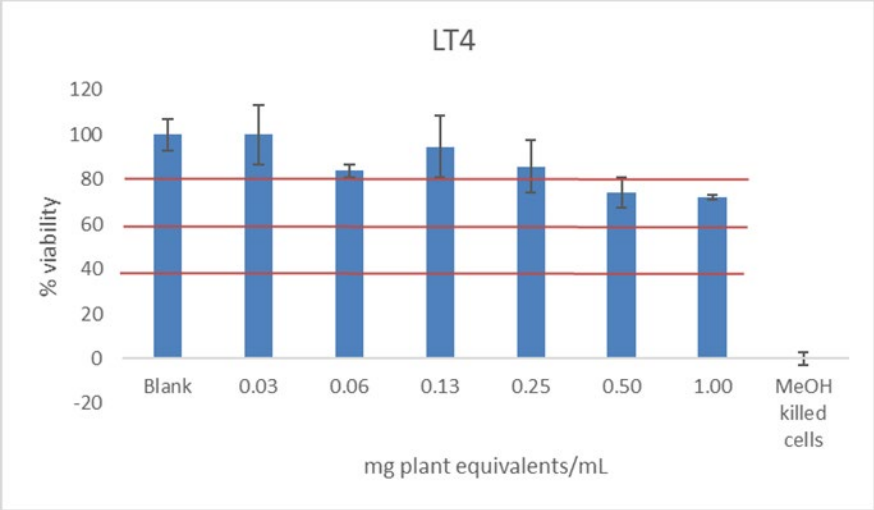
MeOH killed cells
mg plant equivalents/mL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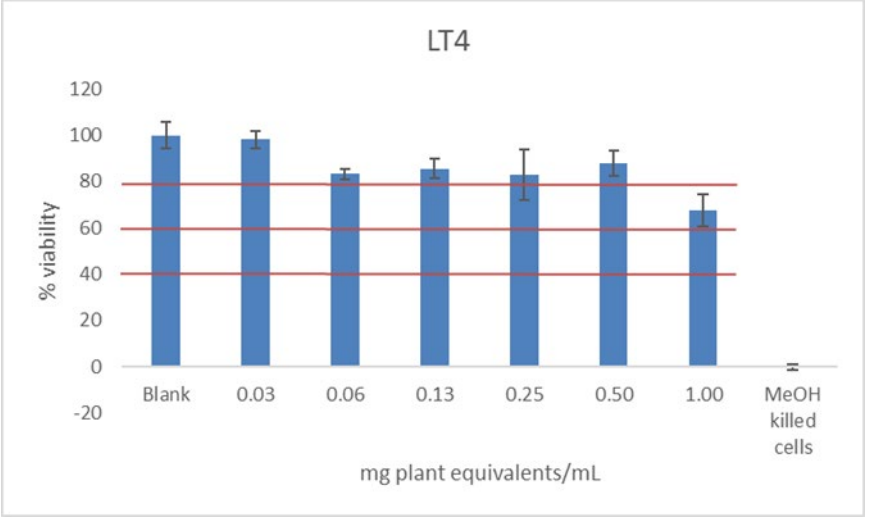
mg plant equivalents/mL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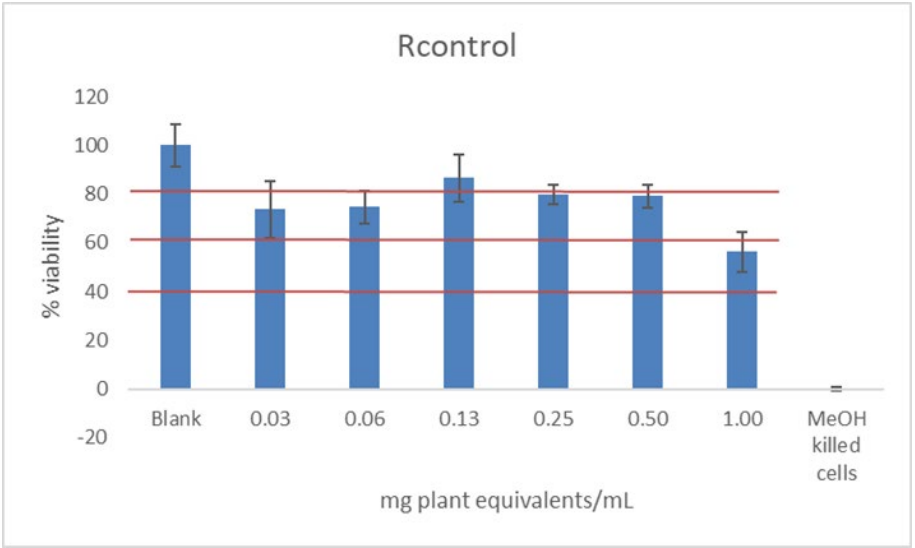
mg plant equivalents/mL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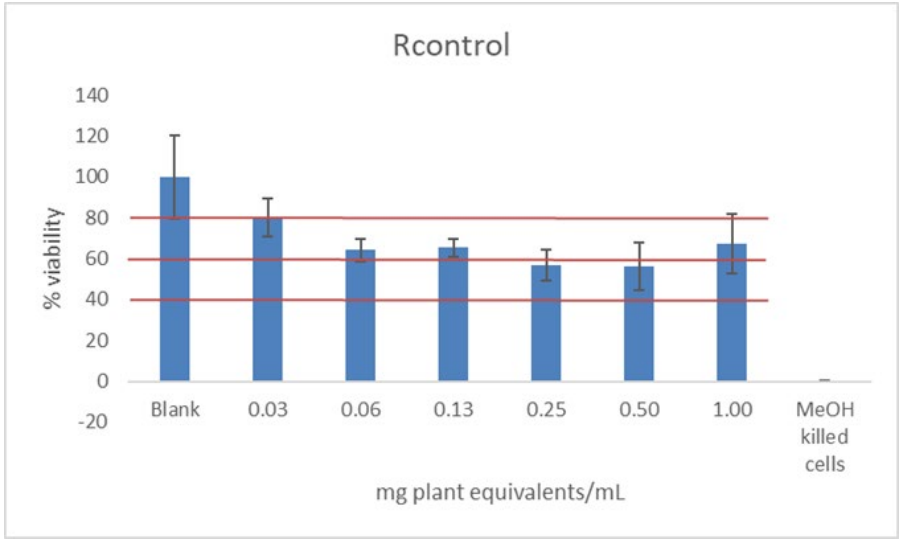
MeOH killed cells
mg plant equivalents/mL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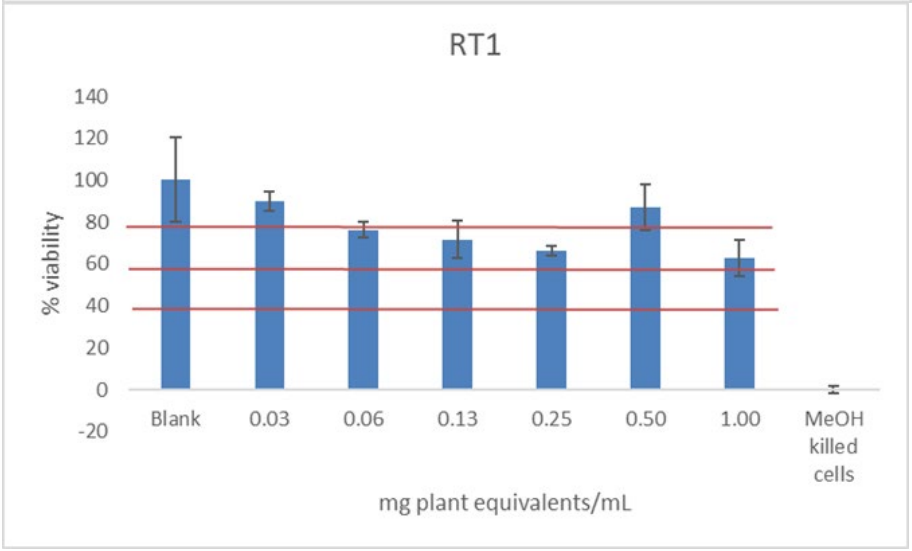
MeOH killed cells
mg plant equivalents/mL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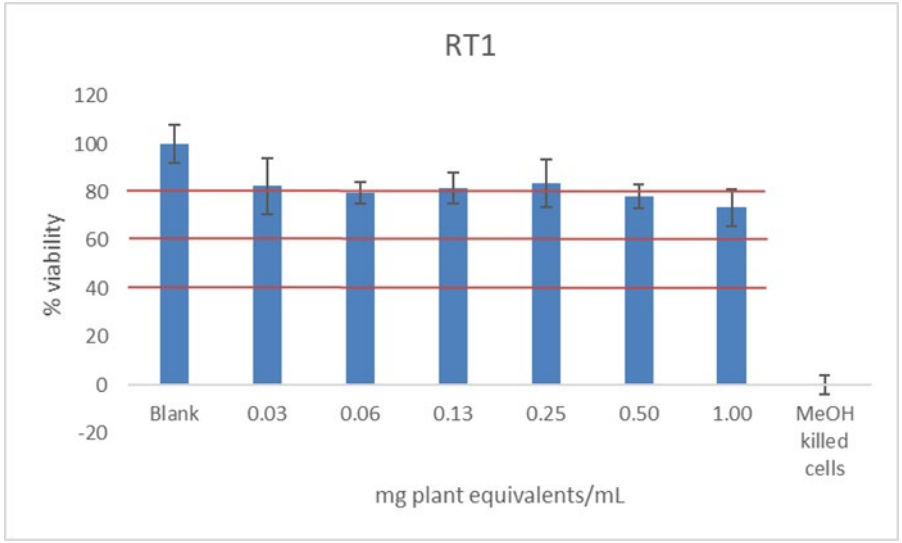
MeOH killed cells
mg plant equivalents/mL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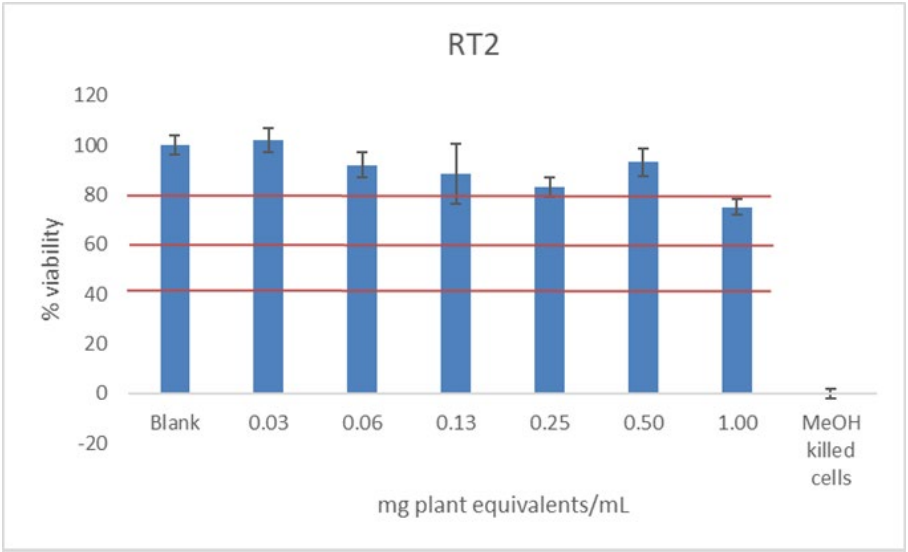
MeOH killed cells
mg plant equivalents/mL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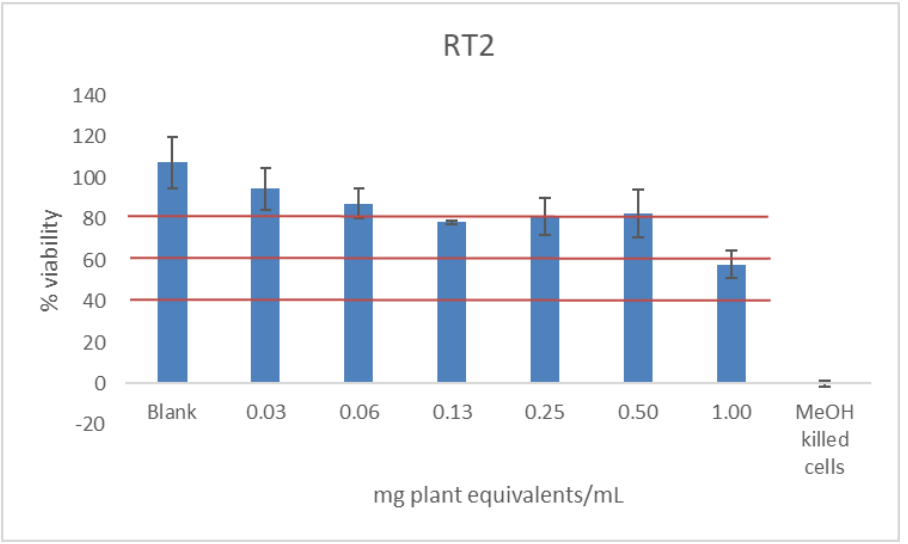
MeOH killed cells
mg plant equivalents/mL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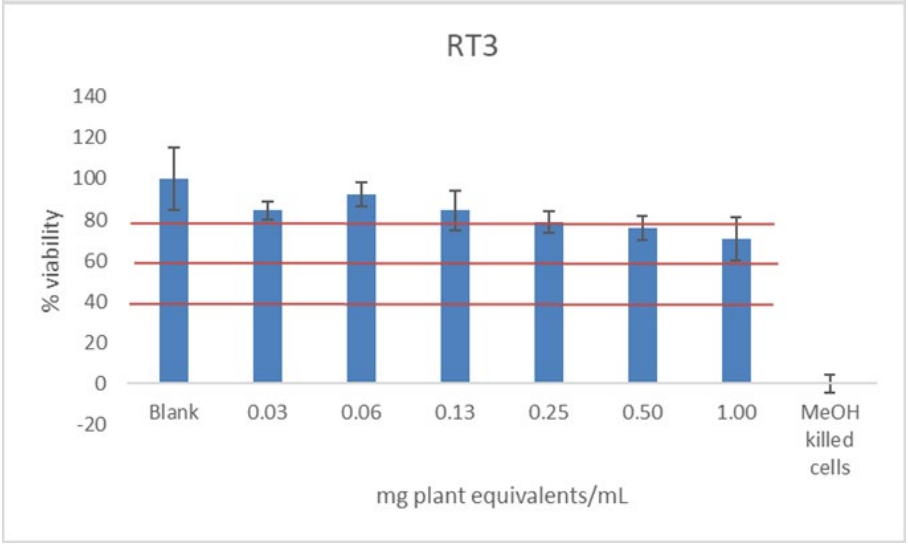
MeOH killed cells
mg plant equivalents/mL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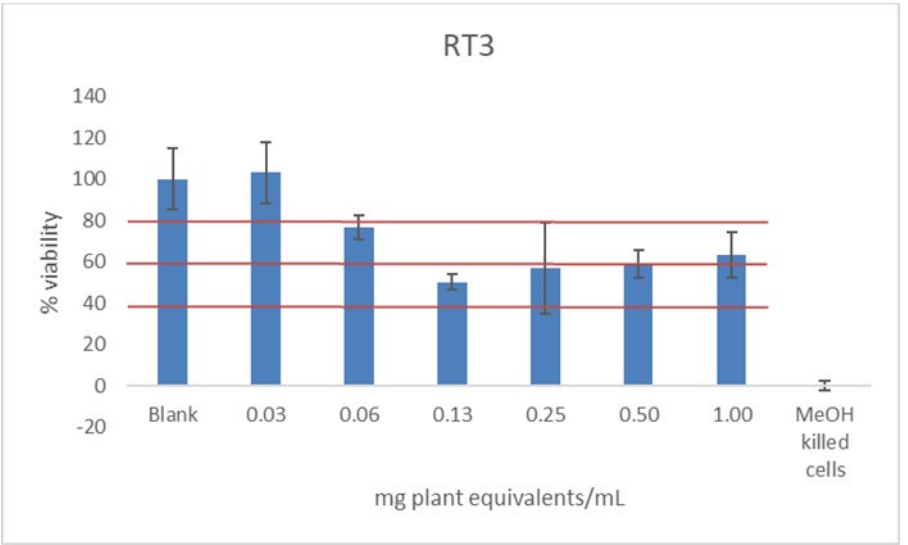
mg plant equivalents/mL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mg plant equivalents/mL

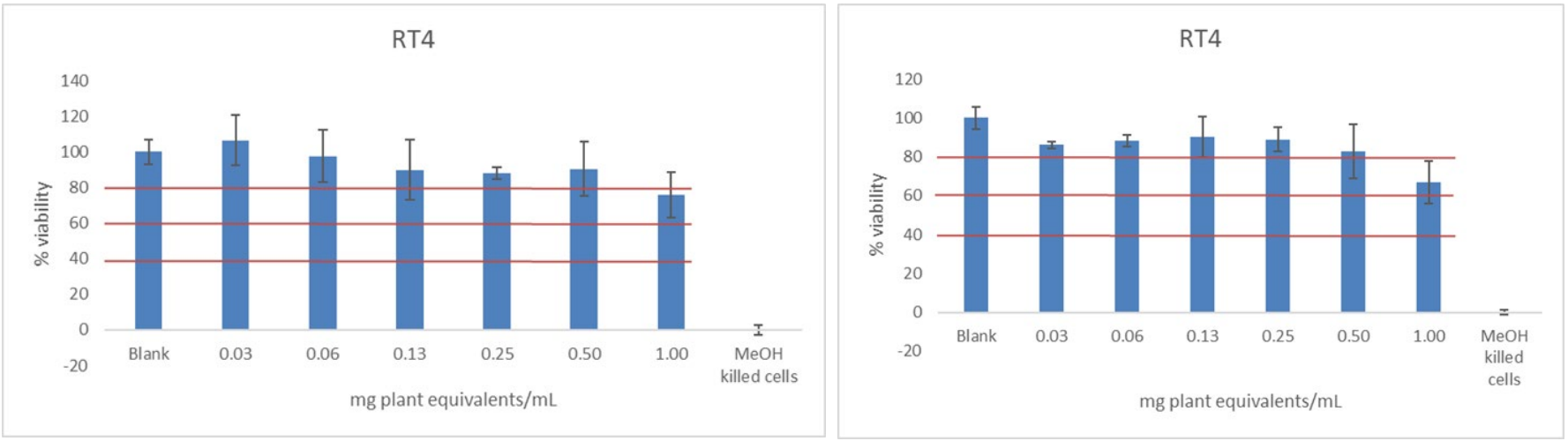

Figure 7.1: Cytotoxicity of the different concentrations of *T. ciliata* Flower buds (F), Leaves (L) and Root (R) extracts in mg/mL against H4IIE-*luc* and Vero cells. Blank represents positive control (untreated cells). F= flower bud extracts, L= Leaf extract, and R= root extracts. Control= 0mM NaCl, T1= 50 mM NaCl, T2= 100 mM NaCl, T3= 150 mM NaCl, T4= 200 mM NaCl. Cell viability: > 80%= non-cytotoxic, 60-80%= weak cytotoxicity, 40-60%= moderate cytotoxicity, < 40%= strong cytotoxicity.

7.3.2 ACHE Inhibitory activity

All ethanolic extracts of *T. ciliata* exhibited anti-AChE activity. The highest AChE inhibitory activity was observed from the cells treated with extract from flower buds at 100 mM salinity concentration, followed by root extract obtained from 100 mM salinity concentration. The lowest activity was exhibited by leaves cultivated under 150 mM salinity treatment.

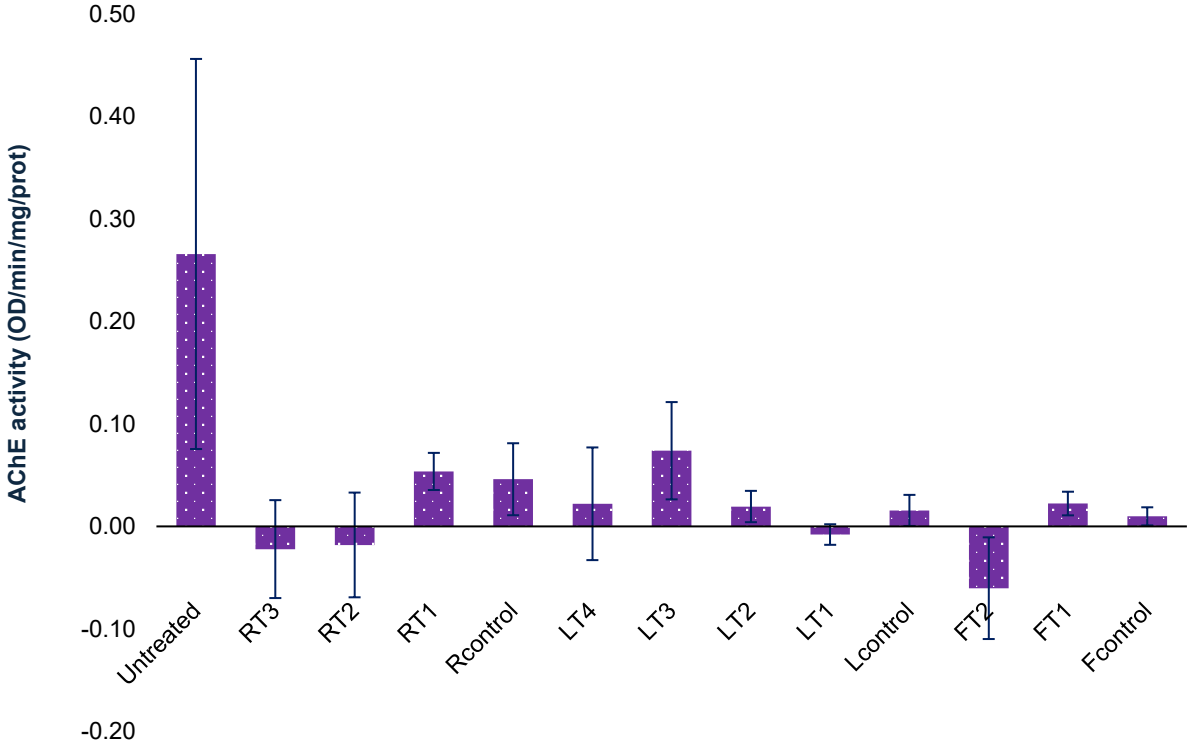

Figure 7.2: AChE activity of the plant extracts (OD/min/mg/prot) compared to the control (untreated cells). F = flower buds extracts, L = Leaf extract, and R = root extracts. Control = 0 mM NaCl, T1 = 50 mM NaCl, T2 = 100 mM NaCl, T3 = 150 mM NaCl, T4 = 200 mM NaCl

3.3.3 Intracellular ROS scavenging activity

Based on the results of the 7'-dichlorodihydrofluorescein diacetate assay (H_2DCF-DA) *in vitro* *T. ciliata* extracts leaf, root, and flower bud of *T. ciliata* cultivated under varying salinity treatments notably influenced the intracellular production of ROS compared to the untreated cells (Figure 7.3). The cells that received the peroxide had a surprisingly low production of ROS. The highest significant ROS scavenging activity was observed in the cells treated with extracts prepared from roots at 0 mM salinity treatment. All leaf extracts showed ROS scavenging activity. The ROS scavenging activity of leaf extracts was indirectly proportional to increasing salinity concentration.

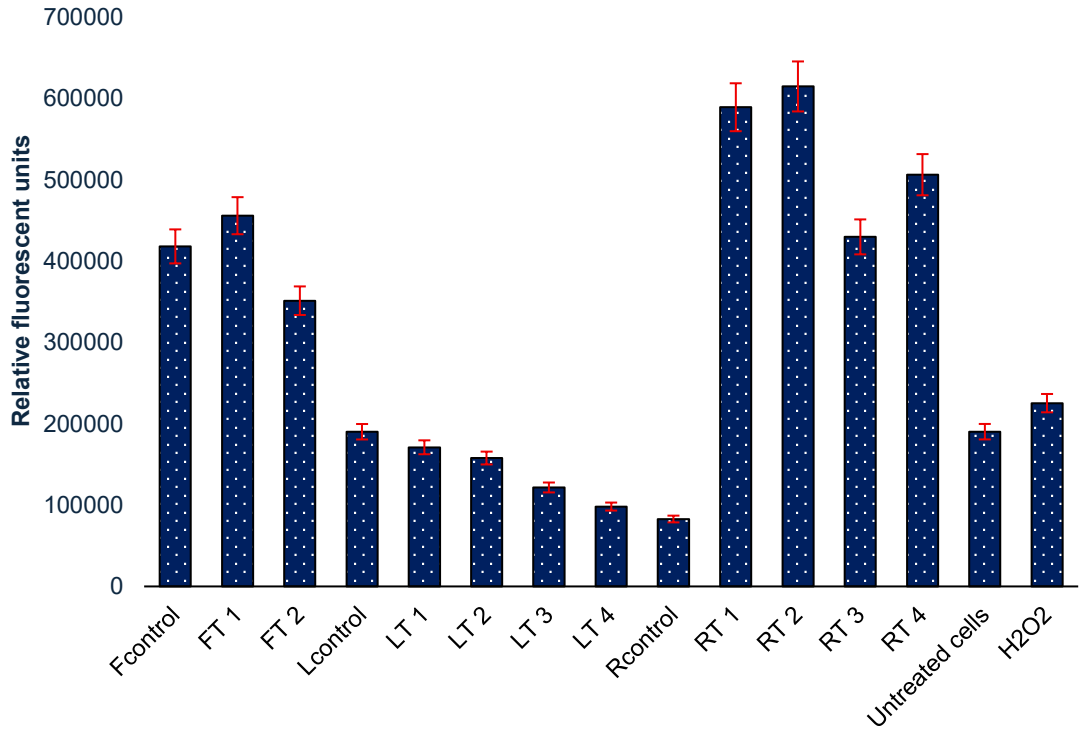

Figure 7.3: Reactive oxygen species production in liver epithelial hepatoma H4IIE-*luc* cells. Data are expressed as mean ± standard deviation. F = flower buds extracts, L = Leaf extract, and R = root extracts. Control = 0 mM NaCl, T1 = 50 mM NaCl, T2 = 100 mM NaCl, T3 = 150 mM NaCl, T4 = 200 mM NaCl, H2O2 = H_2O_2.

7.4 Discussion

In spite of the current surge in interest worldwide in the therapeutic potential of medicinal halophytes, South African halophytes have received little attention as possible agents for the treatment of chronic illnesses (Cock, 2015; Sogoni et al., 2025). *Trachyandra ciliata* (wild cabbage) is one of the understudied wild edible halophytes from South Africa (Ngxabi et al., 2024). A previous study by Ngxabi et al., (2025) validated its edibility and reported that salinity improved its nutritional composition and antioxidant capacity. Ultra-High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS) in the previous chapter (Table 6.2 and 6.3) identified 71 phytochemical compounds, which have been reported to have biological activities against numerous diseases (unpublished). However, the pharmacological potential of the species remains undocumented. Thus, the present study investigated the crude extracts of *T. ciliata* as a therapeutic agent for the treatment of prominent chronic diseases such as cancer, cognitive impairment and ROS production in the liver.

Cancer remains a significant global health issue, accounting for the second highest cause of deaths in the current millennium (Mohammed et al., 2023; Siegel et al., 2019). Due

complications associated with conventional cancer treatments, plant-based approaches are gaining popularity because they offer a potential complementary or alternative option at a lower price and reduced side effects (Ferreira et al., 2022; Lee et al., 2024; Venkatachalapathy et al., 2021). In the present study, ethanolic extracts of flower buds, leaves, and roots of *trachyandra ciliata* cultivated under different salinity treatments significantly influenced the cell viability of cancer and non-cancer cells. Flower bud extracts prepared from 0 mM and 100 mM salinity treatments at 1mg/mL concentration showed strong cytotoxicity to cancer cells, while they had moderate and weak cytotoxicity to non-cancer cells respectively. Root extract obtained from 0 mM salinity treatment at 0.5 mg/mL extract concentration showed moderate toxicity to H4IIE cells, while it was non-cytotoxic to non-cancer cells. This may be attributed to the presence of anti-cancer compounds such as quercetin-3-0-(6-0-rhamnosyglucoside), isorhamnetin-3-O-rutinoside, Luteolin, and scopololetin identified in the previous chapter (Table 6.2 and 6.3). For instance, Luteolin has been shown to have biological actions against cancer, such as promoting apoptosis in lung cancer cells, inhibiting tumour proliferation, invasion, and metastasis, modulating immune responses, and acting as an adjuvant to radio chemotherapy (Çetinkaya & Baran, 2023; Zhang & Ma, 2024). A study by (Li et al., 2015) reported that Scopoletin inhibited the proliferation in LNCaP prostate cancer cells in a dose-dependent manner, and it caused G2/M phase growth halt and an increase in the sub-G0/G1 cell population after treatment with increasing doses, resulting in cell death. Furthermore, it was reported that plant-derived isorhamnetin-3-O-rutinoside inhibited tumour expansion through by increasing cleaved Caspase-9, Hdac11, and Bai1 protein levels in xenografted immunosuppressed mice (Antunes-Ricardo et al., 2021; Wang et al., 2023). On the other hand, Quercetin has been heavily studied over the years as a chemoprevention drug in numerous forms of cancer because of its antioxidant, anti-tumour, and anti-inflammatory properties (Jeong et al., 2009). (Asgharian et al., 2022) reported that Quercetin possesses anti-cancer characteristics that reduce tumour proliferation, invasion, and metastasis while also modulating the activity of oncogenic and tumour suppressor ncRNAs. The presence of these biological compounds and in the extracts of *T. ciliata* suggest that it has a potential as a therapeutic agent in the treatment of cancer, although further isolation of these compounds is recommended for future studies.

Alzheimer's disease (AD), the most prevalent neurodegenerative disorder and the cause of adult-onset cognitive impairment, is distinguished by a gradual loss in cognitive abilities, which is accompanied by behavioural symptoms (Marucci et al., 2021). An increase in acetylcholinesterase plays an important role in acetylcholine (ACh) depletion, leading to Alzheimer's disease (Huang et al., 2022). Thus, AChE inhibitors (AChEIs) have been used as the main treatment for Alzheimer's disease (Kaushik et al., 2018; Gajendra et al., 2024b).

Plant-based compounds, including halophytes have begun to gain recognition globally as promising acetylcholinesterase inhibitors because of the side effects associated with synthetic drugs (Islam et al., 2024; Taqui et al., 2022; ALNasser et al., 2025).

Results from the present study revealed that ethanolic extracts of leaves, roots, and flower buds of *T. ciliata* had high acetylcholinesterase inhibitory activity compared to untreated cells. The highest ACHE inhibitory activity was observed from the cells treated with extract from flower buds at 100 mM salinity concentration, followed by root extract obtained from 100 mM salinity concentration. Chemical profiling through Ultra-High Performance Liquid Chromatography– Mass Spectrometry (UHPLC-MS) identified numerous phytochemicals in *T. ciliata* that are linked to activities against neurodegenerative disorders such as quercetin and kaempferol (flavonols), peonidin (anthocyanins), tyramine (alkaloid), trehalose (disaccharide), linoleic acid (fatty acid), and scopoletin (coumarin), which have all been linked with amelioration of neurodegenerative disorders. The high acetylcholinesterase inhibitory activity of *T. ciliata* extracts in this study can be directly attributed to the presence of theses biological compounds. Flavonols such as quercetin and kaempferol have been reported to exhibit potent inhibition of acetylcholinesterase in previous studies. Recent studies have reported that plant-derived kaempferol demonstrates strong suppression of AChE based on the presence and location of the hydroxyl groups (Adetuyi et al., 2024a; Cichon et al., 2025a). Other studies suggest that it also increases Acetylcholine (Ach) levels in the brain, improves memory function, and mitigates cognitive deficits, suggesting that kaempferol may be useful as a treatment for Alzheimer's disease (Adetuyi et al., 2024b; Kouhestani et al., 2018; Lin et al., 2023). On the other hand, studies have demonstrated quercetin inhibition of AChE by bind binding the enzyme via through hydrogen bonding, by inducing allosterism, and by reducing Aβ-amyloid deposition, thereby disrupting the enzyme's hydrogen bond setup (Liao et al., 2022; Orhan, 2020; Cichon et al., 2025b). Research shown that peonidin exhibit AChE inhibitory activity due to its antioxidant activity (Zavala-Ocampo et al., 2022). Tyramine (alkaloid) derived from the bark *Celtis chinensis In Vitro* demonstrated AChE inhibitory activity in a dose-dependent manner (Kim & Lee, 2003), which is consistent with recent studies on the AChE inhibition of Tyramine (Kim & Lee, 2003). Moreover, other compounds such as Trehalose, Linoleic acid, and Scopoletin have also been reported to exhibit neurogenerative functions and improve cognitive function (Karunakaran et al., 2022; Akay et al., 2023; Pupyshev et al., 2024). These findings infer that *T. ciliata* could be a potential therapeutic agent in the treatment of Alzheimer's disease and other neurodegenerative disorders.

Liver disorders are often associated with excessive production of reactive oxygen species (ROS), which can cause oxidative stress and damage to liver cells, proteins and DNA (Lee et al., 2022). It is therefore important to maintain redox homeostasis to prevent and treat liver

disorders (Sies, 2015; Liu et al., 2023). In the present study, ROS scavenging activity was mainly observed in the leaf extracts from all treatments, and in the root extract from 0 mM salinity treatment. This may be due to the presence of phytochemicals such as Apigenin (Flavanone), Peonidin (anthocyanin), Adenosine (nucleoside), thioctic acid (carboxylic acid), Scopoletin (coumarin), which have been reported to reduce oxidative stress in human cells and ameliorate liver disorders (Gao et al., 2024; Viana et al., 2022; Yoshizane et al., 2020; Phan et al., 2018; Tan et al., 2020). These compounds were identified in *T. ciliata* extracts in the previous chapter (Table 6.2 and 6.3). These results are consistent with the findings of Ejiofor et al., (2022) who reported that leaf extracts of *Amaranthus hybridus* (a semi-halophyte) ameliorated oxidative stress and liver damage caused by thioacetamide. Another recent study revealed that *Echinops* species extract reduced oxidative stress, inflammation, and cell death to prevent liver damage induced by malathion (Eid et al., 2024). This suggest that *T. ciliata* leaves could be a therapeutic agent of phytochemicals in the treatments of liver disorders and oxidative stress.

7.5 Conclusion

For the first time, crude extracts of *T. ciliata* were investigated as therapeutic agents for the amelioration of cancer, Acetylcholinesterase inhibitory activity, and ROS scavenging activity in the liver. Flower bud extracts prepared from 0 mM and 100 mM salinity treatments showed strong cytotoxicity to cancer cells, while they had moderate and weak cytotoxicity to non-cancer cells respectively. Almost all extracts possessed high AChE inhibitory activities. Mainly, leaf extracts and root extracts from 0 mM salinity treatment demonstrated high ROS scavenging activity in liver cells. These findings suggest that *T. ciliata* could be a therapeutic agent for the treatment of cancer, Alzheimer's disease, and liver disorders amidst the quest to develop more plant-based pharmaceuticals for the treatment of chronic diseases. Further studies are recommended on the isolation and characterization of pure compounds from the species using Nuclear Magnetic Resonance (NMR) to increase their potency. Studies that use different solvents other than ethanol are also recommended to maximise the effectiveness of the extracts.

7.6 References

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CHAPTER 8

GENERAL CONCLUSIONS, RECOMMENDATIONS AND PROSPECTS

General Conclusions, Recommendations and Prospects

8.1 General conclusions

To accommodate the consistently increasing world population, scholars recommend that the global food production should increase by 50 to 100% by 2050, while the present increase is thought to be roughly about 1 to 1.5% annually. However, high salinity levels and water shortages in farming lands are among the leading abiotic factors that restrict plant growth, leaf production, flowering, leading to a decrease in in overall agricultural output. This then calls for interventions such as the cultivation of drought and salt tolerant plants such as halophytes for food and medicine to supplement the mainstream vegetables that are presently being cultivated. Moreover, halophytes have been utilised for medicinal purposes across different countries and have demonstrated effectiveness in the treatment and management of chronic diseases that afflict modern societies. In the South African context, wild edible halophytes have been ignored and are under-researched, while they have been gaining more attention in other countries in recent years. *Trachyandra ciliata* is an under-utilized edible wild halophyte that was consumed by the Khoi-San people that used to inhabit the western Cape coastal lands prior to colonization and the massive industrial development of the province. Earlier study on wild cabbage showed that it was more productive under low to moderate saline conditions in hydroponics, although its salt tolerance mechanisms, anatomical response to salt stress, nutritional composition, therapeutic properties, biochemical and physiological properties remained unknown. Thus, this research was carried out to evaluate the nutraceutical, phytochemical, and physiological properties of *T. ciliata* under varying degrees of salinity for the first time to ascertain its consumption, pharmacological use, and promote its commercialization. This is the first study that investigates nutraceutical, phytochemical, and physiological properties of *T. ciliata* under varying degrees of salinity treatments.

Findings from chapter 3 and 4 which focused on growth and salt tolerance mechanisms revealed that low salinity (50 mM NaCl) enhances growth parameters when compared to the control. *Trachyandra ciliata* produces high amounts of antioxidant enzymes (SOD and CAT) which catalyse superoxide and convert it to water and molecular oxygen to mitigate the catastrophic effect of salinity. It also produces high proline that ensures further scavenging of ROS, hence stabilizing protein structure and maintaining cellular homeostasis under high salinity. Furthermore, the salt glands that were observed protruding from the epidermis in the vascular system under low salinity treatments and the appearance of salt crystals under higher concentrations makes this plant superior compared to other plants in the maintenance of cellular homeostasis under high salinity. Due to previously mentioned abilities, this plant can

be classified under recretohalophytes. Increased salinity led to a decrease in the percentage composition of some important chemical elements such as P, K and Ca^{2+}, while Na^{+} and Cl^{-} increased in a stable manner. Findings from these chapters indicate that *T. ciliata* can tolerate salinity by modulating bio-physiological processes to manage oxidative stress successfully and this potential may be exploited in bio-saline agriculture. In addition, these findings suggest that wild cabbage further attains salt tolerance by modulating its leaf surface features, osmotic adjustment, and the regulation of Na^{+} uptake and distribution in the leaves. these results may be used to update the existing taxonomic database on leaf ultrastructure of this species.

Chapter five Investigated the proximate, antinutrient, mineral, and phytochemical composition of *T. ciliata* cultivated under salinity stress in order to determine its suitability for human consumption. This chapter's results substantiate the edibility of wild cabbage, evidenced by its significant concentration of proximate and mineral constituents, as previously reported without scientific validation. The elevated fibre, protein, and energy levels, especially in the flower buds, substantiate its efficacy for human digestion and its potential role as an immune system booster, a vital nutritional source, and a functional food. According to the findings of this study, low salinity treatment (50 mM) is more successful at producing maximal nutritious value. The plant's low antinutrient composition and high phenolic content demonstrate its nutraceutical potential and safety for human consumption.

In chapter six, chemical profile of metabolites aggregated in the crude extract of different plant parts of *T. ciliata* were evaluated for the first time with UHPLC-MS, and 71 compounds were detected. These groups of compounds included flavonoids, anthocyanins, alkaloids, saccharides, fatty acids, amino acids, and coumarins, all of which are known for their biological activities in the amelioration of chronic diseases such as diabetes, neurodegenerative disorders, cancer, cardiovascular diseases, liver disorders and gastrointestinal disorders amongst others. These findings further show the potential of this plant in the treatment of other ailments such as skin problems, viral diseases, inflammation, oxidative stress related disorders, as well as plant plant-based food additives and preservatives.

In chapter seven, crude extracts of *T. ciliata* were investigated as therapeutic agent for the amelioration of cancer, Acetylcholinesterase inhibitory activity, and ROS scavenging activity in the liver. Flower bud extracts prepared from 0 mM and 100 mM salinity treatments showed strong cytotoxicity to cancer cells, while they had moderate and weak cytotoxicity to non-cancer cells respectively. Almost all extracts possessed high AChE inhibitory activities. Mainly, leaf extracts and root extracts from 0 mM salinity treatment demonstrated high ROS scavenging activity in liver cells. These findings suggest that *T. ciliata* could be a therapeutic

agent for the treatment of cancer, Alzheimer's disease, and liver disorders amidst the quest to develop more plant-based pharmaceuticals for the treatment of chronic diseases.

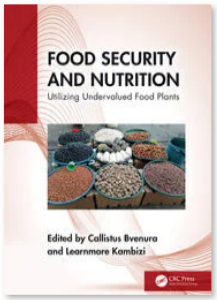
8.2 Recommendations and prospects

Based on the findings of this study, *Trachyandra ciliata* is recommended for human consumption, domestication, and commercial production to alleviate the predicted food crisis and hidden hunger caused by rising population and water shortages. The introduction of wild edible halophytes into the vegetable market would contribute to Sustainable Development Goals 1 and 2 by ensuring that there are more food options available even for the low income households as well as more job opportunities under large scale production. Low salinity of 50 mM of NaCl is recommended to achieve maximum yield and nutrition. *Trachyandra ciliata* is also recommended for the pharmaceutical industry as a therapeutic agent for the treatment of cancer, Alzheimer's disease, and liver disorders amidst the quest to develop more plant-based pharmaceuticals for the treatment of chronic diseases. The results from this study suggest that leaves may be more effective for the ROS scavenging in the liver and all plant parts can be used for Alzheimer's disease, while roots and flower buds may be used for cytotoxicity.

In order to encourage the commercial farming and popularity of wild edible halophytes in South Africa, more resources should be channelled to marketing in the form of awareness campaigns, education, community outreach programs to capacitate relevant stakeholders about the agricultural and economic potential of these plants. Capacity building seminars and market days in remote areas should also include the benefits of cultivating wild edible plants, which can lead to the development of more culinary innovations for prominent food markets and development of new medicines for chronic diseases. Further studies are recommended to explore the usage of diluted sea water to cultivate this plant and other edible halophytes, subsequently reducing the demand for fresh water to cultivate food crops. Further studies are recommended on the isolation and characterization of pure compounds from the species using nuclear magnetic resonance (NMR) to elucidate the chemical nature of isolated compounds and underpin the medicinal utilization of *T. ciliata*. The use of other solvents other than ethanol is recommended in future studies to expand the pharmacological potential of the species. Furthermore, clinical, *in vivo*, and *in silico* studies are also recommended in future research to validate findings from this study. Molecular studies such as gene expression of key ion transporters and genes encoding antioxidant enzymes are recommended in future research. Findings from this research serve as reference points to farmers, pharmaceutical industries, medical practitioners, communities, scholars, policy makers, and aspiring researchers are interested in exploring the potential value of underutilized wild edible crops to develop new strategies to address global issues concerning human health, nutrition, and wellness.

LIST OF APPENDICES

APPENDIX A: Appendix A Book chapter published in Food Security and Nutrition (Tylor & Francis)



Chapter

Nutraceutical, Agricultural, and Economic Potential of Trachyandra ciliata (Wild Cabbage), an Underutilized Halophyte from the Western Cape Province, South Africa

By Sihle Ngxabi, Muhali Olaide Jimoh, Avela Sogoni, Charles Petrus Laubscher, Learnmore Kambizi

Book Food Security and Nutrition

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APPENDIX B: Journal article published in Russian Journal of Plant Physiology (Springer)

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RESEARCH PAPERS

Leaf Micromorphological Assessment, Chemical Composition and Anatomical Responses of *Trachyandra ciliata* (L.F) Kunth to Different Degrees of Salinity

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Abstract—Many studies have examined the morphological and micromorphological responses of different halophytes to determine their salt tolerance mechanisms. However, few studies have focused on the South African edible halophytes. This study examined the leaf micromorphology, elemental composition, and anatomical responses using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) to examine salt tolerance levels in *Trachyandra ciliata* (L.F) Kunth. The treatments included varying sodium chloride (NaCl) concentrations: 50 mM, 100 mM, 150 mM and 200 mM, while control (0 mM) was watered with nutrient solution only. From the SEM micrographs, salt glands were observed protruding from the epidermis along the vascular system under low salinity and salt crystals appeared under higher concentrations, which makes this plant maintain cellular homeostasis under high salinity, and the plant can be classified as a recretohalophyte. Stomatal distribution, stomatal density and the number of open stomata decreased with increasing salinity. EDX revealed the presence of some important elements such as potassium, magnesium, phosphorus, calcium and more in the leaves. The results showed that increased salinity led to a decrease in the percentage composition of P, K and Ca^{2+}, while Mg^{2+} was high under the control and low salinity (50 mM), decreased under 100 mM and increased again with increasing salinity. On the contrary, increasing salinity caused an increase in Na^+ and Cl^- in a stable manner. These findings reveal that *T. ciliata* acquires salt tolerance through changes to its leaf surface properties, osmotic adjustment, and the regulation of Na^+ uptake and distribution in the leaves.

Keywords: Asphodelaceae, recretohalophytes, micromorphology, salt glands, *Trachyandra ciliata*, wild cabbage, salt stress

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APPENDIX C: Journal article published in Food Science and Nutrition

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ORIGINAL ARTICLE

Salinity Influenced Proximate, Minerals, Anti- Nutrients and Phytochemical Composition of *Trachyandra ciliata* Kunth (Wild Cabbage): A Promising Edible Halophyte

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Keywords: Asphodelaceae | halophytes | nutraceuticals | phenolic compounds | recommended daily allowance | wild cabbage

ABSTRACT

Climate change, drought, and soil salinization present huge limitations to global agricultural output, which threatens food security. This necessitates the cultivation and domestication of wild edible halophytes as alternatives to mainstream food crops, especially in arid and semi- arid regions. *Trachyandra ciliata* is one of the under- researched and underutilized edible halophytes native to South Africa. The plant was used as a food source by Khoisan people in the past although its edibility and nutritional capacity are undocumented. Thus, the current study explored the effect of varying salinity concentrations on minerals, proximate, phytochemical, and anti- nutrient composition of *T. ciliata* to evaluate its edibility and promote its cultivation among South African households. Plants were subjected to varying salinity treatments from 0, 50, 100,150, and 200 mM prepared by adding sodium chloride (NaCl) to the nutrient solution. Salinity significantly influenced the mineral, proximate, antinutrient, and phytochemical composition of *T. ciliata*. Control and 50 mM treatments recorded significantly higher macro and micronutrient content in the flower buds and leaves, except for heavy metals such as Zn and Cu, which increased with increasing salinity and significantly higher in the roots. Leaves under low salinity treatments recorded higher moisture and protein content, while leaves also recorded higher ash content under high salinity. On the other hand, flower buds under low salinity recorded significantly high fat and NDF composition. Phytochemicals and antinutrients increased with increasing salinity concentrations. The low antinutrient content and high nutritional, mineral and phenolic contents validate the edibility and suitability of *T. ciliata* for human consumption.

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APPENDIX D: Journal article published in Russian Journal of Plant Physiology

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RESEARCH PAPERS

Salinity Influenced Growth, Physio-Biochemical Responses, and Antioxidative Potential of *Trachyandra ciliata* Kunth (Wild Cabbage)

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Abstract—*Trachyandra ciliata* is a wild edible halophyte endemic to the saline Western Cape coastal region. However, its tolerance mechanisms and physio-biochemical responses under salinity stress are undocumented. Thus, this study was conducted to evaluate the effect of salinity stress on growth parameters, leaf hydration, photosynthetic pigmentation, oxidative stress markers and concentration of antioxidative enzymes to further understand mechanisms involved in its salt tolerance. Four salinity concentrations (50, 100, 150, and 200 mM) were assessed by adding NaCl into the nutrient solution, while control was solely irrigated with nutrient solution. The results revealed that salinity positively influenced growth parameters in all plant parts at low concentration (50 mM NaCl) compared to the control. Moreover, the total chlorophyll content negatively correlated with increasing salinity. *Trachyandra ciliata* maintained equivalent relative water content (RWC) from control to 100 mM treatment, which then efficiently decreased with increasing salinity, while leaf succulence was equivalent among all treatments. On the other hand, high salinity stimulated oxidative stress as indicated by high MDA, cell death, and superoxide radicals, which were more expressive under 200 mM treatment. To counter the catastrophic effects of excessive ROS, *T. ciliata* activated antioxidative defence mechanisms as indicated by high SOD and CAT antioxidative enzymes which were more prevalent at high salinity (200 mM). Furthermore, the high proline content under higher salinity treatments ensured further scavenging of ROS. These findings validate that *T. ciliata* is able to tolerate salinity by modulating bio-physiological processes to manage oxidative stress and achieve cellular homeostasis under salinity stress.

Keywords: antioxidant, catalase, food security, oxidative stress, proline, reactive oxygen species, superoxide dismutase

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APPENDIX E: Journal article published in Phytomedicine Plus

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Contents lists ScienceDi

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Metabolite profiling, cytotoxicity, liver ROS detoxifiers and acetylcholinesterase inhibitors from *Trachyandra ciliata* L.F. (Kunth) (wild cabbage)

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ARTICLEINFO

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Liver disorders
Salinity

ABSTRACT

Introduction: Wild cabbage (*Trachyandra ciliata*) is one of the understudied, wild, edible halophytes from South Africa. Although its edibility has recently been validated, its therapeutic potential was yet to be explored. This research was carried out to profile and characterise the phytochemical content of *T. ciliata* extracts and evaluate its potential as a therapeutic agent for cancer, acetylcholinesterase (AChE) inhibition and reactive oxygen species (ROS) scavenging in the liver.

Methods: Cuttings of *T. ciliata* were grown under 0, 50, 100, 150, and 200 mM salinity concentrations. The ultra- high-performance liquid chromatography mass spectrometry (UHPLC-MS) was employed to quantify and characterise metabolites in leaves, roots, and flower buds of *T. ciliata*. The yellow dye 3-(4,5-dimethyltiazol-2yl)-2,5- diphenyl tetrazolium bromide (MTT), Ellman's colourimetric method, and the 2′,7′-dichlorodihydrofluorescein diacetate assay (H_2DCF-DA) were respectively employed to evaluate cytotoxicity, AChE antagonism, and ROS scavenging of the extracts of *T. ciliata*.

Results: A total of 71 compounds were observed, which were grouped into flavonoids, anthocyanins, alkaloids, nucleobase, nucleosides/tide, saccharides, fatty acids, amino acids, and coumarins. A concentration of 1 mg/mL of extracts of *T. ciliata* flower buds prepared from plants grown at 0 mM and 100 mM salinity, showed strong cytotoxicity to cancer cells, however, the extracts also had moderate and weak cytotoxicity to non-cancer cells. All extracts inhibited AChE activity. Moreover, ROS scavenging was mainly observed primarily in the extracts of leaves from plants grown at all salinities, and in the extract of roots from plants grown at 0 mM salinity treatment.

Conclusion: A maiden documentation of anticancer, acetylcholinesterase inhibition, and ROS scavenging activity of crude extracts of *T. ciliata* was achieved in this study. These findings suggest that *T. ciliata* could be a potential therapeutic agent for the treatment of cancer, Alzheimer's disease, and liver disorders, amidst the quest to develop more plant-based pharmaceuticals for the treatment of chronic diseases.

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