Cape Peninsula University of Technology

Nutraceutical, phytochemical, intercropping and morpho-physiological response of *Tetragonia decumbens* Mill. to salt-stress: A promising wild leafy vegetable for bio-saline agriculture in South Africa

By

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DECLARATION

I, Avela Sogoni, 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.

2025/03/25

Signed **Date**

ABSTRACT

Tetragonia decumbens Mill., a neglected South African edible halophyte has shown promising production returns for bio-saline agriculture. However, in-depth studies on the micro-morphological, physiological, biochemical and antioxidative mechanisms of this species to salinity remains underexplored for precise bio-saline agriculture. Furthermore, the lack of scientific knowledge on its nutritional profile, pharmacological activity, and its potential use as a phytoremediator in salt affected soils has contributed to its underutilisation. Therefore, this study examined the nutraceutical, phytochemical, intercropping and morpho-physiological response of *T. decumbens* to salt-stress to further support its domestication, consumption, and cultivation among South African households and in regions affected by salinity.

To elucidate the salt-tolerance mechanisms in *T. decumbens,* the effect of salinity on micro-morphological, physiological, biochemical and antioxidative mechanism was evaluated. Plants were subjected to varying salinity doses (0, 50, 100, 150, 200 and 250 mM NaCl). Results revealed a substantial enhancement in plant growth, relative water content, as well as total fresh weight in plants irrigated with 50 and 100 mM NaCl in comparison to the control, while higher saline concentrations (150-250 mM NaCl) reduced plant growth, chlorophyll content and stomatal density. Similarly, these high salt concentrations induced more severe oxidative stress indicated by high amounts of superoxide, cell death viability and malondialdehyde. Nevertheless, *T. decumbens* modulated various defence mechanisms with increasing salinity stress; these include the upregulation of superoxide dismutase, catalase, polyphenols, flavonoids, proanthocyanidins and the build-up of sodium ions in the leaves. Moreover, micromorphological examination of the adaxial layer of the epidermis with scanning electron microscope revealed distinctive up-rooted glandular peltate trichomes with increasing salinity, thus suggesting this species may have utilized these specialised structures to store or dilute excessive sodium ions. These results showed that *T. decumbens* can withstand salinity by modifying its anatomical features, morpho-physiological traits, antioxidant defence systems, and managing ion toxicity and oxidative stress efficiently.

To justify its nutritional potential for consumption, cultivated and wild collected samples were analysed for nutritional composition, secondary metabolites, and anti-microbial potential. Wild samples were collected from three coastal areas (Strand beach, Muizenberg beach and Blouberg beach) during the dry (summer) and wet (winter) seasons in Cape Town, and the greenhouse cultivated which were subjected to varying salinity doses (0, 50, 100, 150, 200 and 250 mM). Results revealed a considerable increase in minerals (N, P and Mg) and proximate composition (Ash, moisture, and carbohydrates) in greenhouse cultivated plants

subjected to 50 mM of salinity, while the highest crude fat and neutral detergent fibre were recorded in wild samples. Moreover, heavy metal uptake, phytochemicals, anti-nutrients and anti-microbial activities were more pronounced in wild plants than in cultivated samples. These findings validated for the first time, the relevance of nutritional quality of *T. decumbens* in assessing its suitability as a source of nutrients. Moreover, the efficacy of antimicrobial metabolites of the crude extract of wild samples *T. decumbens*, suggest that this halophyte might be suitable for both anti-bacterial and anti-fungal applications. Therefore, for optimal yield of minerals and proximate constituents, it is recommended to cultivate this species under 50 mM of salinity while for antimicrobial purposes, wild samples were recommended.

To validate its phytoremediation potential, an intercropping system under saline condition was conducted. Spinach (*Spinacia oleracea*) seedlings were grown alone and in consociation with this halophyte under various salt stresses (50, 100, 150 and 200 mM NaCl). Results showed that increasing salinity reduced crop growth, relative water content, chlorophyll, and nutritional quality of spinach in monocultured system. Interestingly, intercropped spinach irrigated with 50 and 100 mM of NaCl revealed a substantial enhancement in crop performance, reduction in oxidative stress and had improved nutritional quality depicted by high amounts of minerals, proximate constituents, and vitamins. These results validate its phytoremediation potential of saline soils, support the introduction of *T. decumbens* in vegetable farming systems and highlighted its positive impact on improving the overall crop performance of salt sensitive vegetables under saline condition.

The pharmacological potential including cytotoxicity, acetylcholinesterase inhibitory activity, anti-cancer and anti-inflammatory activity of diverse chemicals aggregated in the crude extracts of *T. decumbens* was evaluated. For the first time, the ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) identified 98 compounds in saline cultivated samples (0, 50, 100, 150, 200 and 250 mM NaCl) most of which were stilbenoids (E-Piceatannol), hydroxybenzoic acid derivatives (6-Gingerol and 6-Gingerol-o- pentose), tannin (3,3'-di-O-methylellagic acid), coumarins (Bergapten and Xanthotoxin), Phenylsulfate (catechol sulphate), Sesquiterpene (Zederone), and alkaloid (Trigonelline) among others. Potent anti-inflammatory activity in lipopolysaccharide (LPS)-induced cells was noted in the dose of 100 µg/mL in crude extract of 100 mM NaCl treatment, and this was significantly higher than cells treated with Aminoguanidine (positive control). There was also a significant inhibitory activity of acetylcholinesterase in Human SH-SY5Y neuroblastoma cells treated with crude extracts of 0 to 100 mM NaCl, when compared with untreated cells. These findings suggest that the identified compounds in *T. decumbens* could have potential anti-inflammatory and acetylcholinesterase inhibitory activities.

Overall, this study has shown the potential value of *T. decumbens* as a nutritious vegetable with numerous pharmacological values for bio-saline agriculture. Moreover, its phytoremediation potential of saline soils supports its introduction in vegetable farming systems in saline affected areas.

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I would like to sincerely thank my supervisors, Prof. Charles Laubscher, Prof. Learnmore Kambizi and Dr Muhali Jimoh, for their tremendous guidance, support, and involvement in successfully overseeing and directing the completion of this study.

I am also thankful to all the staff members of the Horticultural department for their full cooperation and help. My sincere thanks to Mr Terence Mabela and Mr Phumlani Roto. Your willingness to assist where necessary was remarkable.

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The financial assistance of the National Research Foundation (Grant UID: 140847) towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the National Research Foundation.

DEDICATION

This consolidated work is dedicated to my late grandfather Zongezile Mxwebisa

PREFACE

This thesis is prepared in an article format of published, co-published and/or ready-for-publication papers as prescribed by the Cape Peninsula University of Technology. It consists of eight chapters, which are concisely discussed below:

Chapter 1: Provides the significance of the study, its aim and the overall list of specific objectives, which guided the study.

Chapter 2: Consist of a review paper titled "Evaluating the nutritional, therapeutic, and economic potential of *Tetragonia decumbens* Mill.: A promising wild leafy vegetable for bio-saline agriculture in South Africa" which examined the potential of this species as a leafy vegetable, describing its morphology and ecology, its propagation and cultivation requirements as well as its potential use on human health and in phytoremediation of saline soils.

Chapter 3: Investigated the effect of salinity on morpho-physiology, biochemical, and antioxidant defence systems in *T. decumbens*.

Chapter 4: Evaluated the leaf anatomical responses and chemical composition in *T. decumbens*. under saline cultivation.

Chapter 5: Compared the nutritional value, phytochemical composition, and anti-microbial potential of wild and cultivated *T. decumbens*.

Chapter 6: Evaluated the intercropping potential of *T. decumbens* in mitigating salinity stress in salt-sensitive *Spinacia oleracea*.

Chapter 7: Investigated the phytochemical profile of saline-cultivated *T. decumbens*: *In vitro* cytotoxicity, acetylcholinesterase inhibitory activity, anti-cancer and anti-inflammatory potential.

Chapter 8: Is the general conclusion, which summarises the results and connects the chapters objectives to the aim of the study and the recommendations are made for further research.

RESEARCH OUTPUTS

The research outputs listed below acknowledge the candidate's contribution to scientific knowledge and advancement throughout the PhD duration.

Published manuscripts

1. Sogoni, A., Jimoh, M.O., Ngxabi, S., Kambizi, L., Laubscher, C.P., 2025. Evaluating the nutritional, therapeutic, and economic potential of *Tetragonia decumbens* Mill.: A promising wild leafy vegetable for bio-saline agriculture in South Africa. ***Open Agriculture*** 10. https://doi.org/10.1515/OPAG-2022-0368
2. Sogoni, A., Jimoh, M.O., Barker, A.M., Keyster, M., Kambizi, L., Laubscher, C.P., 2024. Salinity modulates morpho-physiology, biochemical and antioxidant defence system in *Tetragonia decumbens* Mill.: a neglected wild leafy vegetable in South Africa. ***Plant Physiology Reports*** 1–14. https://doi.org/10.1007/S40502-024-00811-6
3. Sogoni, A., Jimoh, M.O., Keyster, M., Kambizi, L., Laubscher, C.P., 2023. Salinity Induced Leaf Anatomical Responses and Chemical Composition in *Tetragonia decumbens* Mill.: an Underutilized Edible Halophyte in South Africa. ***Russian Journal of Plant Physiology*** 70, 1–9. https://doi.org/10.1134/S1021443723601775
4. Sogoni, A., Jimoh, M.O., Mngqawa, P., Ngxabi, S., Le Roes-Hill, M., Kambizi, L., Laubscher, C.P., 2024. Comparing the nutritional, phytochemical, and anti-microbial potential of wild and cultivated *Tetragonia decumbens* mill.: A promising leafy vegetable for bio-saline agriculture in South Africa. ***Journal of Agriculture and Food Research*** 18, 101419. https://doi.org/10.1016/J.JAFR.2024.101419
5. Sogoni, A., Jimoh, M.O., Ngxabi, S., Keyster, M., Kambizi, L., Laubscher, C.P., 2025. Intercropping the halophyte *Tetragonia decumbens* Mill. with salt-sensitive *Spinacia oleracea* L. mitigated salinity stress by enhancing the physiological, biochemical, and nutritional quality of the salt-sensitive species under saline cultivation. ***Journal of the Saudi Society of Agricultural Sciences,*** 12 (1), 2. https://doi.org/10.1007/s44447-025-00001-2

Submitted manuscript

6. Sogoni, A., Jimoh, M.O., Kerebba, N., Horn, S., Pieters, R., Kambizi, L., Laubscher, C.P., 2025. Phytochemical profiling of saline-cultivated *Tetragonia decumbens* Mill: In vitro cytotoxicity, Acetylcholinesterase inhibitory activity, anti-cancer and anti-inflammatory potential. Submitted to ***Phytomedicine Plus.***

Conference attended

7. Sogoni, A**.**, Jimoh, M.O; Kambizi, L., Laubscher, C. Comparing the nutritional value and phytochemical composition of wild and cultivated *Tetragonia decumbens* Mill.: A promising leafy vegetable for bio-saline agriculture in South Africa. *31st International Horticultural Congress: 14-20 August, Angers, France. (Oral)* ***2022.***

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APPENDIX C: PUBLISHED PAPER IN RUSSIAN JOURNAL OF PLANT PHYSIOLOGY

APPENDIX D: PUBLISHED PAPER IN JOURNAL OF AGRICULTURE AND FOOD RESEARCH

APPENDIX E: PUBLISHED PAPER IN JOURNAL OF THE SAUDI SOCIETY OF AGRICULTURAL SCIENCES

GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
ANOVA	Analysis of Variance
CAT	Catalase
CPUT	Cape Peninsula University of Technology
DPPH	2,2- diphenyl-1picrythydrazyl hydrate
FRAP	Ferric reducing antioxidant power
LC-MS	Liquid chromatography mass spectrometry
LSD	Least significant difference
MDA	Malondialdehyde
mM	Millimolar
NaCl	Sodium chloride
ORAC	Oxygen Radical Absorbance Capacity
SOD	Superoxide dismutase
ROS	Reactive oxygen species

CHAPTER ONE

INTRODUCTION, RESEARCH PROBLEM, RESEARCH AIM, OBJECTIVES, HYPOTHESES AND SIGNIFICANCE OF THE STUDY

1.1. Introduction

Globally, the agricultural industry feeds more than 7 billion people, and alarmingly by 2050, that number will exceed 8 billion (Molotoks et al., 2021). The global demand for food production has never been greater, particularly in developing countries where the population is expected to grow by 90%, implying that food insecurity will become a bigger issue (Ogunniyi et al., 2020). To meet the increased food demand, crop production should increase by 70% to 100% by 2050, according to the World Development Report (World Bank, 2007). However, poor agro-ecological conditions and the scarcity of freshwater have severely impacted the yield of essential crops such as wheat, rice and maize around the world including South Africa, making it extremely difficult to meet the required target (Corwin, 2021). Furthermore, environmental conditions such as soil salinity are anticipated to pose a major problem in many regions in the future due to climate change (Sogoni, 2020). Recent attempts to adapt to these climatic conditions include the use of wild salt-tolerant plants (halophytes) with significant economic values such as leafy vegetables, feed crops and as pharmaceutical precursors (Ventura & Sagi, 2013). This resulted in an increased interest in the crop production development of edible halophytes in combating the challenges of food and nutrient deficiency around the world (Jacobsen et al., 2013; Kashyap et al., 2021).

In Europe and Latin America, culinary halophytes such as *Cichorium spinosum*, *Cichorium intybus*, *Capparis spinosa, Crithmum maritimum* and *Portulaca oleracea* to name a few, are used in many green dishes in restaurants and are favoured as main ingredients in salad dressing due to their saltiness and crunchiness (Jang et al., 2007; Petropoulos et al., 2016). In Korea, the young stalks of the edible halophyte *Salicornia herbacea* are eaten in variety of ways, including as a seasoned vegetable, salad, and in fermented food (Sánchez-Gavilán et al., 2021). Beside their culinary applications, the medicinal properties of various halophytes have been shown to be effective in treating and preventing chronic diseases that plague modern societies, such as cancer, heart disease, and diabetes (Petropoulos et al., 2018). This led to the inclusion of these plants on supermarket shelves in Europe and Asia which resulted into the emergence of commercial cultivation due to the increasing demand of sustainable supply (Castañeda-Loaiza et al., 2020).

In South Africa, edible halophytes are still neglected and considered important mainly in the light of safeguarding biodiversity and are only valued in rural parts of the country during times of famine (Hamed & Custódio, 2019). In the Cape Floristic Region (mainly the Western Cape province) many edible halophytes species are foraged rather than cultivated and are only known by a small group of local chefs and food enthusiasts due to their nutritional offering of healthy and affordable nutrient alternatives such as vitamins and minerals (Tembo-Phiri, 2019;

Botha et al., 2020). Bvenura and Sivakumar (2017) also reported that these neglected vegetables can contribute to diets by adding both micronutrients and bioactive compounds and could help eradicate nutrient deficiencies and reduce food insecurity at the household level. Furthermore, given the increasing global climate change and severe conditions that exist around the world, the use of native halophytes for food production in South Africa could be a climate change adaptation strategy (Mndi et al., 2023).

1.2. Research problem

Soil salinity has been reported as one of the main causes of devastating crop production losses around the world including South Africa (Rehman et al., 2023). It restricts water absorption in salt-sensitive crops causing osmotic stress and nutritional imbalance which disrupt key physiological processes such as photosynthetic activity, tissue hydration, and induction of oxidative stress and membrane damage, resulting in low plant biomass and yield (Mahmood et al., 2021; Hussain et al., 2023). Thus, the alternative use of resilient crops like halophytes is crucial to fulfil the increasing food demand escalated by the increasing human population. Halophytes can tolerate high temperatures, drought, and salinity, making them the ideal nutritional crop for cultivation in regions impacted by these conditions. These species are also used in phytoremediation of saline soil as companion plants to salt-sensitive crops. However, in most African countries including South Africa, edible halophytes are still underutilised and understudied even though these species could provide an alternative source of nutrients to the growing human population. Recently, a neglected South African edible halophyte *Tetragonia decumbens* has been cultivated under saline conditions and showed promising production returns for bio- saline agriculture (Sogoni et al., 2021; Sogoni et al., 2023). Nevertheless, in-depth studies on micro-morphological, physiological, biochemical and antioxidative mechanism of this species to salinity remains underexplored for precise bio-saline agriculture. Furthermore, the lack of scientific knowledge on its nutritional profile, pharmacological activity, and its potential use as a phytoremediator in salt affected soils has contributed to its underutilisation. Thus, this study was conducted to examine the nutraceutical, phytochemical, intercropping and morpho-physiological response of *T. decumbens* to salt-stress to further support its domestication, consumption, and cultivation among South African households and in regions affected by salinity.

1.3. Research aim

This study aims to investigate the effect of salinity on crop development, salt-tolerance mechanisms, nutritional composition, phytochemical profiling, biological activities, and intercropping potential of *Tetragonia decumbens* to further support its domestication, consumption, and cultivation among South African households and in regions affected by salinity.

1.4. Objectives

The specific objectives are to:

a) Determine the morpho-physiology, biochemical, and antioxidant defence system in *T. decumbens* to better understand its salt tolerance mechanisms for bio-saline agriculture.
a) Assess the micro-morphology and chemical constituents of *T. decumbens* leaves in response to salinity to better understand the anatomical traits responsible for its salt-tolerance.
b) Compare the proximate, vitamins, anti-nutrients, minerals, and antimicrobial potential of wild and greenhouse cultivated *T. decumbens* to further support its consumption and potential commercialization.
c) Evaluate the intercropping potential of *T. decumbens* in alleviating salinity stress in salt-sensitive *Spinacia oleracea* under saline cultivation.
d) Assess the phytochemical profiling of ethanolic extracts of *T. decumbens,* their cytotoxicity, as well as their acetylcholinesterase inhibitory activity, anti-cancer and anti-inflammatory potential to promote its medicinal potential.

1.5. Hypotheses

It is hypothesized that:

a) Salinity will modulate morpho-physiology, biochemical and antioxidant defence system in *T. decumbens*
b) Salinity will inflict changes in anatomical features of *T. decumbens* leaves.
c) Greenhouse cultivated plants will be more nutritious while those collected from the wild will have high phytochemical composition with anti-microbial activity.
d) Intercropping the halophyte *T. decumbens* with salt-sensitive *Spinacia* oleracea will mitigate the negative effects of salinity and improve the physiological, biochemical, and nutritional quality of the salt-sensitive species under saline cultivation.
e) Salinity will have an impact on the phytochemical composition and the detection of novel compounds with potent biological effects in *T. decumbens*.

1.6. Significance of the study

The study has the potential of:

a) Providing scientific justification on the edibility and nutritional status of *T. decumbens* to support its consumption and domestication among coastal households.
b) Revealing the micro-physiological traits responsible for the salt-tolerance *in T.decumbens* plant parts.
c) Determining the influence of salinity on phytochemical accumulation and profiling.
d) Ascertaining for the first time the ethnopharmacological relevance of some bioactive compounds present in the plants, as sources of food, food fortificants and pharmaceutical precursors.
e) Providing sustainable solution in alleviating the negative effects of salinity in vegetable production through intercropping with halophytes.
f) Providing awareness on the cultivation of halophytes as an alternative source of nutrients in dry and salt affected regions, thus contributing to food security.

1.7. Delimitations of the study

Field cultivation trials of *T. decumbens* under saline soils are still to be investigated.

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CHAPTER TWO

EVALUATING THE NUTRITIONAL, THERAPEUTIC AND ECONOMIC POTENTIAL OF *TETRAGONIA DECUMBENS* MILL.: A PROMOSING WILD LEAFY VEGETABLE FOR BIO-SALINE AGRICULTURE IN SOUTH AFRICA

Published in Open agriculture

Evaluating the nutritional, therapeutic and economic potential of *Tetragonia decumbens* mill.: A promising wild leafy vegetable for bio-saline agriculture in South Africa

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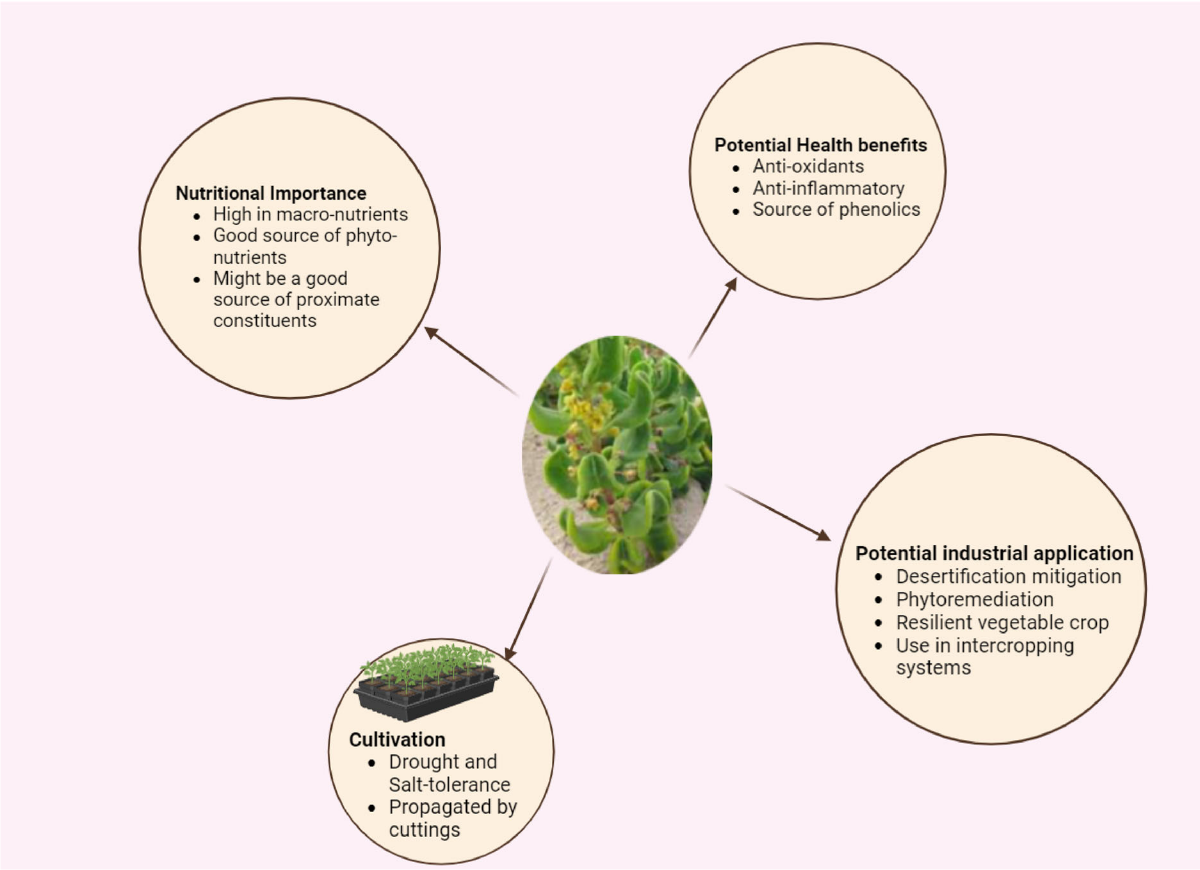
Abstract

Global agriculture feeds over 7 billion people and alarmingly, this number is expected to increase by a further 50% by 2050. To meet the additional food demand, the world development report has estimated that crop production should increase by 70% to 100% by 2050. However, Climate change, expanding soil salinization and the developing shortages of freshwater have negatively affected crop production of edible plants around the world. Current attempts to adapt to these conditions include the use of salt-tolerant plant species with potential economic value to fulfil the increasing food demand escalated by the increasing human population. The wild edible halophyte (*T. decumbens*) commonly known as dune spinach has the potential to be used as a leafy vegetable, a source of dietary salt, in phytoremediation and as a source of secondary metabolites. However, it remains underutilised in South Africa as commercial farming of this species has never been explored. This review examined the potential of domesticating the wild dune spinach as a leafy vegetable, describing its morphology and ecology, its propagation and cultivation requirements as well as its potential use on human health and in phytoremediation of saline soils. Furthermore, this analysis is expected to be useful towards further research and popularization of this underexploited halophyte.

Keywords: Dune spinach; Functional foods; Halophyte; Salt tolerance; Wild foods

Graphical Abstract



2.1. Introduction

The global agricultural industry already provides sustenance for more than 7 billion people, and it is projected that this figure will rise by an additional 50% by the year 2050 (Loconsole et al., 2019). The need for food production on a worldwide scale is now at its highest and already about 2 billion people are estimated to suffer from micronutrient deficiencies problem (Ogunniyi et al., 2020). To satisfy the growing need for food, agricultural output must rise by 70% to 100% by the year 2050 (Singh, 2022). However, increasing soil salinity and global climate change have caused major constraints for agricultural productivity of staple crops such as wheat and maize (Corwin, 2021). Furthermore, high salinity in the soil triggers osmotic balance disruption in plants, limiting water intake and transpiration and consequently reduce yield (Amerian et al., 2024). Present efforts to adjust to these conditions include the use of wild edible plants that can tolerate high temperatures and salinity, and possess economic potential such as leafy vegetable, feed crop and as pharmaceutical precursors (Ventura & Sagi, 2013; Debez et al., 2010). This sparked a global interest in the crop production development of edible halophytes in combating the challenges of food and nutrient deficiency around the world (Kashyap et al., 2021).

In Europe and Latin America, culinary halophytes such as *Cichorium spinosum*, *Cichorium intybus*, *Capparis spinosa, Crithmum maritimum* and *Portulaca oleracea* to name a few, are used in many green dishes in restaurants and are favoured as main ingredients in salad dressing due to their saltiness and crunchiness (Jang et al., 2007; Petropoulos et al., 2016). In Korea, the young stalks of the edible halophyte *Salicornia herbacea* are eaten in variety of ways, including as a seasoned vegetable, salad, and in fermented food (Sánchez-Gavilán et al., 2021). Beside their culinary applications, the medicinal properties of various halophytes have been shown to be effective in treating and preventing chronic diseases that plague modern societies, such as cancer, heart disease, and diabetes (Ksouri et al., 2012; Petropoulos et al., 2018). This led to the inclusion of these plants on supermarket shelves in Europe and Asia which resulted into the emergence of commercial cultivation due to the increasing demand of sustainable supply (Castañeda-Loaiza et al., 2020).

In South Africa, edible halophytes are still neglected and considered important mainly in the light of safeguarding biodiversity and are only valued in rural parts of the country during times of famine (Hamed & Custódio, 2019). In the Cape Floristic Region (mainly the Western Cape province) many edible halophytes species are foraged rather than cultivated and are only known by a small group of local chefs and food enthusiasts due to their nutritional offering of healthy and affordable nutrient alternatives such as vitamins and minerals (Botha et al., 2020). Afolayan and Jimoh (Afolayan & Jimoh, 2009) also reported that these neglected vegetables can contribute to diets by adding both micronutrients and bioactive compounds and could help eradicate nutrient deficiencies and reduce food insecurity at the household level. Furthermore, given the increasing global climate change and severe conditions that exist around the world, the use of native halophytes for food production in South Africa could be a climate change adaptation strategy (Mndi et al., 2023). Concurrently, it has also been stated that South Africa is approaching physical water scarcity by 2040, and its agricultural sector has been directly hampered by the recent drought (Bischoff-Mattson et al., 2020). Therefore, it is of utmost importance to cultivate crops that are adapted to harsh conditions within the framework of saline agriculture, to address the challenges of nutrient deficiency, saline soils and water scarcity (Panta et al., 2014).

Seawater and salinized lands represent potentially cultivable areas for edible salt tolerate plants. In South Africa, the majority of the fields with these qualities are located close to coastal areas and suffer from salinity (Malan et al., 2015). Therefore, saline agriculture with halophytes could enable coastal and saline soils to be productive. Hence, knowledge on the unexploited edible halophyte, *Tetragonia decumbens* is needed to support its cultivation potential on saline soils. This review examined the potential of domesticating the wild dune spinach as a leafy vegetable, describing its morphology and ecology, its propagation and cultivation requirements as well as its potential benefits to human health and in phytoremediation.

2.2. Material and methods

Research articles were downloaded from databases such as Scopus, PubMed, Web of Science and google scholar. The keywords used for searching the appropriate articles related to the title were "Dune spinach", "*Tetragonia*", "Wild edible halophytes", "Phytoremediation", Medicinal value of *Tetragonia* and "Nutritional value of halophytes" or "coastal foods". Boolean operators were used to combine search terms for optimal retrieval of relevant literature. Initial screening of search results was performed based on titles and abstracts to identify potentially relevant articles. Articles used for this research were limited to the original research articles and those written in English Language only.

2.3. Taxonomy, Morphology, and Distribution of *Tetragonia decumbens*

Tetragonia decumbens Mill. is a perennial shrub and a member of the Aizoaceae family and its common names include Dune spinach (English) and Duinespinasie (Afrikaans) (Forrester, 2004; Nkcukankcuka et al., 2021). It is a sprawling shrub with branches or runners that can grow up to 1 m long. This perennial shrub has papillose-hirsute, oblong, or lance-shaped fleshy leaves that feel hairy to the touch (Snijman, 2013). The glistening leaves have a shiny and warty appearance (Van Wyk & Gericke, 2017). This is caused by the small, shiny, water-storage cells that cover the surface of the leaf. The inflorescences are axillary with one to three flowers, which usually start appearing from August to November (spring to summer). The seeds vary in size and have a brown hard outer casing with distinct rigid wings when dry (Tembo-Phiri, 2019).

The shrub occurs in few southern African countries including South Africa, Namibia and Botswana (Figure 2.1). It is largely distributed along the coastal dunes (Figure 2.2), saline flats and inland saline areas from southern Namibia to the Eastern Cape (Klak et al., 2017).

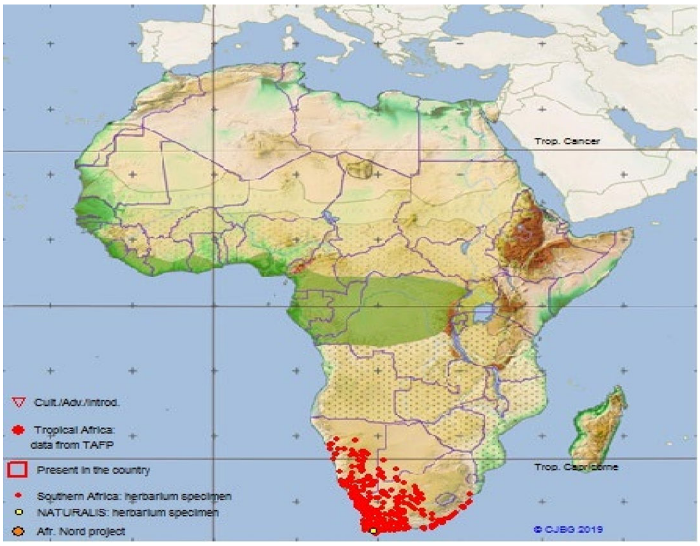


Figure 2.1: Geographical distribution map of *T. decumbens* (Adapted from: http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php?langue=an&id=48025)

Figure 2.2: *T. decumbens* growing in its natural habitat along the strand beach, Cape Town, South Africa (Photo: Avela Sogoni).

2.4. Crop production of *T. decumbens*

2.4.1. Plant propagation

Tetragonia decumbens can be easily propagated by pulling mature runners from the sand with roots attached as cuttings. These can be directly planted into a well-drain soil which must be kept moist until the plant has established itself (Rusch, 2016). However, this practice could lead to over-harvesting as the species begin to gain popularity among coastal communities. Hence an easy, suitable and cost-effective propagation protocol should be in place. A propagation study conducted by Sogoni et al. (Sogoni et al., 2022) on root initiation of nodal stem cuttings of *T. decumbens* as influenced by rooting hormone and various rooting media, found that cuttings planted on sand: peat mix (1:1) without hormone treatment had better rooting percentage suggesting that the species can be easily propagated from cuttings in greenhouse conditions. This could be a cost-saving option for potential growers of the species since no additional cost on hormone treatment will be needed. In terms of seed propagation, no studies have been conducted. However, Rusch (2016) reported the re-sprouting ability of the species when watered and suggested that this can be utilised for repeated harvest without the need to save seeds and re-sowing every year. Hence further studies are required on repeated harvests of shoots and their phytonutrients, to promote large scale production of this leafy vegetable.

2.4.2. Cultivation trials

Tetragonia decumbens has been recently piloted as a possible commercial crop at a community garden in Khayelitsha, Cape Town, South Africa (Figure 2.3). Rusch (2016) reported that rooted stem cuttings of the species can be easily grown in well-drained media such as sand or loamy soil. For optimal growth the media should be kept moist until the cuttings have established, thereafter they should be watered three times a week. This was also supported by the pot experiment of Tembo-Phiri (2019) who reported that *T. decumbens* can be grown with water level maintained at 25% pot capacity (a pot volume of 3.4 dm^3). When grown under a nutrient film hydroponic system, the species performed well in silica sand with the application of a full nutrient solution with low electrical conductivity (Nkcukankcuka et al., 2021). This leafy vegetable was also successfully cultivated by Sogoni et al. (2021) under salt stress in soilless culture (Figure 2.4). The cuttings were planted into pots containing sand: peat mix (1:1) and a total of 300 mℓ nutrient solution was prepared for each pot with and/or without NaCl addition. The plants were then watered every three days. The nutrient solution was created by adding NUTRIFEED™ (manufactured by STARKE AYRES Pty. Ltd. South Africa) to municipal water at 10 g per 5L. The nutrient solution contained the following ingredients: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Mg (22 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Zn (240 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Mo (10 mg/kg). Results revealed that the use of nutritive solution incorporated with 50 and 100 mM NaCl had a positive effect on crop development, minerals and yield, with a pronounced effect at 50 followed by 100 mM as compared to the control. These findings suggest that dune spinach is a halophyte and hold the potential to be cultivated on saline soils. Nevertheless, anatomical, physiological and biochemical responses of this species to saline conditions remains underexplored for precise bio-saline agriculture, thus further studies are recommended.

Figure 2.3: Site preparation in Khayelitsha for planting in sandy soil (Photo: Loubie Rusch) Adapted from: https://www.capetownbotanist.com/planting-wild-food-garden-at-moya-we/

Figure 2.4. A trial experiment showing dune spinach response to salt stress (Photo: Avela Sogoni).

2.5. Possible adaptation mechanisms of *T. decumbens* for bio-saline agriculture

Plants are often exposed to unfavourable conditions such as salinity stress, which in some cases dramatically alter the physiological processes within the plant, causing a reduction in growth and yield (Jimoh et al., 2018). However, some species classified as halophytes have been reported to possess various physiological, anatomical and biochemical adaptations allowing them to adapt to or cope with abiotic stress (Barreira et al., 2017). The same cannot be said for dune spinach since its adaptation behaviour to saline environments remains undocumented. Potential adaptation mechanism in dune spinach could be explored by examining studies conducted on salt-tolerance mechanisms of other halophytes which are discussed below.

2.5.1. Ion transport, accumulation, and excretion

Ion transport (Na^+ and Cl^-) from roots to leaves, where they are compartmentalized in vacuoles, has been reported as a salt mitigation strategy in dicot halophytes (Munns & Tester, 2008). Souid et al. (2016) also reported that NaCl levels in roots are maintained by ion exportation to shoots, which reduces the toxic effects of salt at the root level. Concurrently, Hassan et al. (2017) and González-Orenga et al. (2019) reported Na^+ and Cl^- accumulation in leaves of various Limonium species, lending support to this dicot model. Nonetheless, high levels of Na+ can be associated with toxic effects, resulting in a decrease in other ions such as K^+ and Ca^{2+} (Amerian et al., 2024). Thus, avoiding ion toxicity appears to be a crucial survival mechanism which is achieved by the development of specialised structures such as salt glands, and or trichomes responsible for the regulation of internal salt load for ion excretion and storage (Kuster et al., 2020). Species like *Mesembryanthemum crystallinum, Atriplex portulacoides, Chenopodium quinoa* and *Tetragonia tetragoniodes* have been reported to possess bladder cells, or specialised structures where ions are sequestered under saline conditions (Figure 2.5) (Agarie et al., 2007; Shabala, 2013; Parida et al., 2016). While in *Sporobolus ioclados, Lasiurus scindicus* and *Salsola imbricata,* anatomical modifications observed were thick cuticle and epidermis, smaller and fewer stomata, higher proportion of storage tissues, and thickened endodermis (Naz et al., 2016; Naz et al., 2018; Naz et al., 2022). Nevertheless, these anatomical modification in *T. decumbens* are not reported in literature. Thus, anatomical studies are needed to give better insight on the adaptation mechanism of this species under saline conditions.

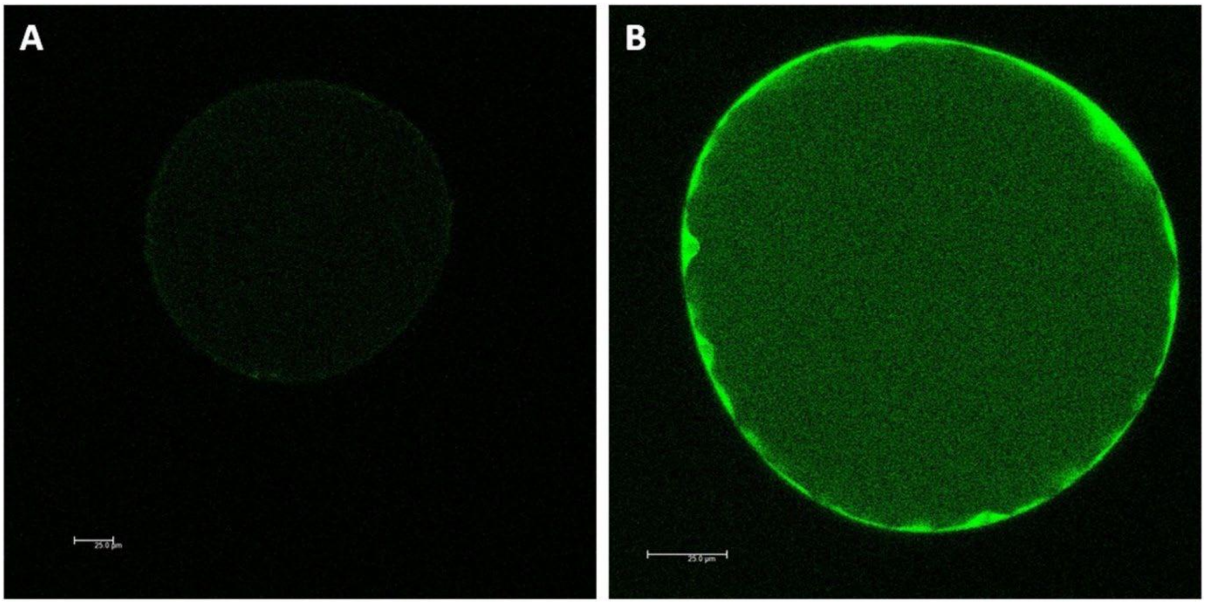


Figure 2.5: Sodium accumulation and intracellular distribution in leaf bladder cells of *T. tetragoniodes* subjected to salinity. (**A=control), (B=30 % seawater)** Adapted from Atzori et al. (Atzori et al., 2020)

2.5.2. Osmolyte production

When inorganic ions accumulate excessively in the vacuoles of plant cells, they are compensated for by compatible osmolytes/solutes in the cytoplasm (González-Orenga, Grigore, et al., 2021). These osmolytes are non-toxic organic molecules and do not interfere with cellular metabolism even at high intracellular concentrations. They play important roles in plant responses to abiotic stress, such as osmotic adjustment and protein stabilisation, as well as serving as 'reactive oxygen species' (ROS) scavengers (Ejaz et al., 2020). Osmolytes are chemically diverse, as they contain amino acids like proline, methylated proline, and quaternary ammonium compounds like glycine betaine.

Proline and glycine betaine are well known for their important roles in osmotic adjustment and anti-oxidative defence in abiotically stressed plants (Rady et al., 2018). These osmolytes have been found in a variety of halophytes and are typically lower in plants grown in non-saline conditions but show a significant increase in plants exposed to salinity and drought stress (González-Orenga, Grigore, et al., 2021). According to Alasvandyari et al. (2017), proline and glycine betaine accumulation in plant tissues is associated with higher salt tolerance in plants. As a result, determining the osmolytes in dune spinach will be critical in describing its salt-tolerance nature.

2.5.3. The activation of anti-oxidative enzymes

Different biotic and abiotic stressors, including salinity, are known to cause oxidative stress in plants by producing superoxide radicals via the Mehler reaction (González-Orenga, Grigore, et al., 2021; Rodrigues de Queiroz et al., 2023). When created in excess, these free radicals disrupt the regular metabolic mechanisms in cytoplasm, mitochondria and peroxisomes by causing oxidative damage to proteins and lipids, resulting in cell malfunctions and ultimately, death (Saleem et al., 2022). To overcome this phenomenon, plants respond by activating the production and accumulation of antioxidant enzymes. These antioxidant enzymes contribute to the elimination of ROS and the maintenance of appropriate cellular redox. For example, Superoxide Dismutase (SOD) catalyses a conversion from two O_2 radicals to H_2O_2 and O_2 (Zulfiqar & Ashraf, 2022). In alternative ways, several antioxidant enzymes can also eliminate the H_2O_2 such as catalases (CAT) and peroxidases (POX) by converting it to water (García-Caparrós et al., 2020), thus maintaining the adequate cellular redox state. High amount of SOD and CAT under saline cultivation has been reported in halophytes such as *Bupleurum tenuissimum, Chenopodium quinoa*, *Kandelia obovate* and *Mesembryanthemum crystallinum* respectively (González-Orenga, Leandro, et al., 2021; Parvez et al., 2020; Hasanuzzaman et al., 2021; Mohamed et al., 2021). Concurrently, Jeeva (Jeeva, 2020) reported that *Tetragonia tetragoniodes* a close relative of dune spinach regulated the Superoxide Dismutase (SOD) enzyme activity in mitigating cell membrane injury under saline conditions.

When excessive highly toxic ROS are not eliminated by the antioxidative enzymes, halophytes are said to activate the accumulation of non-enzymatic compounds such as phenols, flavonoids, anthocyanins, and tannins to scavage these highly toxic species (Azeem et al., 2023). This response has been reported in many halophytes, where increasing saline irrigation induced the increase of phenols and flavonoids to scavenge highly toxic free radicals (Kumari et al., 2017). Therefore, examining the activity of these anti-oxidative enzymes in dune spinach will be useful in improving the agronomical aspects of the species under saline cultivation.

2.6. Potential use of *T. decumbens*

2.6.1. Phytoremediation

Accumulation of toxic ions in agricultural lands caused by the application of chemical fertilizer and climate change has led to the loss of soil fertility and the phenomena of salinization and desertification, which makes soils unsuitable for cultivation (Hamzah et al., 2016). To overcome this catastrophic phenomenon, farmers have been predominantly using chemical amendments for the amelioration of saline and sodic soils. However, this process has become expensive in developing countries due to the competing demand from industry (Hasanuzzaman et al., 2014). Therefore, identifying cost-effective and environmentally friendly techniques are necessary (Yan et al., 2020). Numerous researchers have introduced the use of phytoremediation as a low-cost option in restoring saline soils. Phytoremediation is the use of plants to remediate contaminated soils (Jacob et al., 2018). This includes the use of salt removing species to restore saline lands. *T. decumbens* not only has the potential to remove toxic ions from the soil but it can also be used as a food crop which is a win-win scenario for potential farmers. Currently, Jeeva (2020) evaluated the performance and phytoremediation effect on the close relative of dune spinach, *Tetragonia tetragoniodes* in salt-affected soils and reported that sodic soil can be utilized for growing this crop and that it has potential for mitigation of sodic soils. Bekmirzaev et al. (2020) also found that mineral nutrition of *Tetragonia tetragoniodes* was positively affected by salt stress. This suggests that dune spinach might exhibit good potential for the amelioration of saline and sodic soils. Hence further research should be conducted on the growth performance and phytoremediation effect of dune spinach in salt-affected soils under field experiments for easy implementation by local farmers. Additionally, dune spinach might also be a good companion when intercropped with conventional cash crops on saline soils.

2.6.2. Intercropping potential of *T. decumbens*

Intercropping is primarily utilised in developing countries to improve crop productivity and is defined as the practice of growing two or more species concurrently (De La Fuente et al., 2014). In South Africa, this practice is widely utilised by small scale farmers to satisfy dietary needs and to reduce the risk of single crop failure (Bantie et al., 2014). However, intercropping with halophytes remains unexplored, even though the country is currently facing the challenges of saline soils and water shortages. The use of intercropping halophytes with conventional cash crops might be the potential solution in mitigating stress in areas where salt is of particular concern and reduce crop production (Jurado et al., 2024). Nevertheless, there are still few studies conducted on intercropping of conventional crops with halophytes around the world and South Africa is no exception. Zuccarini (2008) showed that tomatoes intercropped with purslane and garden orache had reduced Na^+ and Cl^-concentrations in tissues and had increased fruit yield under saline conditions. The fruit yield increased at approximately 44% in tomatoes that were intercropped with purslane and achieved comparable yields to those grown without saline conditions. These results were currently supported by Jurado et al. (2024), who reported that tomato plants intercropped with the halophyte *Arthrocaulon macrostachyum* had improved nutrient homeostasis and photosynthesis performance leading to enhanced fruit yield. Likewise, Simpson et al. (2018) also found that watermelon intercropped with the halophyte orache (*Atriplex hortensis* L.) substantially enhanced the yield and fruit quality of watermelon under saline conditions. While Liang and Shi (2021) discovered that cotton plants intercropped with *Suaeda salsa* and alfalfa had decreased salt accumulation in their tissues. It was also reported that these halophytes improved soil physicochemical properties and crop productivity in saline-alkali soils under mulched drip irrigation for three years under field experiment. These studies suggest that there is potential to grow conventional crops on saline lands by intercropping them with halophytes.

However, studies on field experiments will be needed for longer periods to determine which cropping system will benefit producers and increase yields while mitigating salt stress in sensitive crops. Thus, field experiments on dune spinach should be conducted to assess its viability as an intercropping candidate.

2.6.3. Desertification and soil erosion alleviation

Desertification normally known as land degradation, has resulted in significant environmental and socio-economic issues in several arid and semi-arid regions worldwide (Roy et al., 2024; Tariq et al., 2022). It causes soil erosion and nutrient degradation, significantly reducing land productivity and resulting to the decline of ecosystem functioning and services (Tariq et al., 2022). Furthermore, the rise in population growth and economic progress persistently exerts greater demands on land use, especially in emerging countries, where natural vegetation is cleared for farming and residential purposes (Yan et al., 2024; Zhao et al., 2024; Kulik & Vlasenko, 2024). The land degradation problem has been reported to affects all three elements of the critical triangle of developmental goals, namely, agricultural growth, poverty reduction, and sustainable resource management (Owusu et al., 2024). Therefore, it was pointed out by the United Nations Convention to Combat Desertification and soil erosion (Gui et al., 2024; Yan et al., 2024).

Sustainable mitigation strategies to combat desertification include the use of resilient species such as phreatophytes which uses different metabolic and physiological adaptations to prevent oxidative mutilation, nutritional imbalance, and osmotic stress under drought and saline conditions (Ali & Salem, 2024; Parnian et al., 2024). Phreatophytes generally grow in semi-arid and arid regions and satisfy their water and nutrient requirements by using extensive root systems to access groundwater, whilst stabilising the soil from erosion (Gao et al., 2022). For example, *Tamarix ramosissima* a drought-tolerant halophyte with high resistance to drought, wind erosion, and sand burial, has been widely used in desertification control in China (Zhang & Zhang, 2012). Similarly, the halophyte *Karelinia caspia* exhibits both drought and salt tolerance (Guo et al., 2022), which allows it to play a role in reducing wind erosion and desertification (Tariq et al., 2024). The species is considered as an ecosystem engineer that plays a crucial role in improving semi-arid ecosystem productivity by mitigating desertification under global climate change (Kim et al., 2024). Therefore, the use of South African halophytes such as *T. decumbens* could potentially mitigate soil erosion and desertification due to its tolerance to drought and salinity as well as its soil stabilizing ability. Hence further studies are recommended on the desertification control ability of *T. decumbens.*

2.6.4. Food security

Water scarcity and rising soil salinity are significant global issues, particularly affecting agricultural outputs of conventional crops (Singh, 2022). The alternative use of resilient crops like edible halophytes is crucial in remediating saline soils to fulfil the increasing food demand escalated by the increasing human population (Roy et al., 2024). Throughout history, wild plants have served as important sources of nutrition during periods of drought and salinity and as dietary reinforcements, particularly in communities that rely on hunting and gathering (Tariq et al., 2022). Wild vegetables, along with other wild plant foods, are often known as the 'hidden harvest' since they are gathered directly from the wild, such as agricultural fields and marshy regions, without any intentional cultivation for food purposes (Yan et al., 2024). The failure to fully utilised these green vegetables has led to conclusive proof of metabolic abnormalities, specifically classified as malnutrition, undernutrition, and stunting. Thus, the inclusion of these neglected leafy greens including *T. decumbens* in human diets is necessary in reducing hidden hunger resulting from micro-nutrient deficiencies.

2.6.5. *Tetragonia decumbens* as a promising nutritious leafy vegetable

Edible halophytes are used in many green dishes in restaurants and have gained popularity in some cultures across the world due to their saltiness and crunchiness (Agudelo et al., 2021). The species climatic adaptation to high temperatures, drought and salinity compared to conventional leafy vegetable crops makes them an ideal choice in some countries (Ebert, 2014). Recently, upscale restaurants in Europe and Asia have started including these plants into their menus as novel ingredients that promotes nutritious diet (Lima et al., 2020). In Australia and Brazil close relatives of dune spinach such as *Tetragonia tetragoniodes, Tetragonia expansa* and *Mesembryanthemum crystallinum* are commercially cultivated as a new choice of nutrient-rich leafy vegetable (Cecílio Filho et al., 2017). The leaves and soft stems of these species are consumed in a variety of ways such as seasoned vegetable and as a fermented food. Moreover, the considerable proximate and mineral composition of these species as depicted in table 1 supports their nutritional value. Carbohydrates for instance supply energy to muscle and brain and the high carbohydrate content obtained in these species indicate that they are a good source of carbohydrates. These species are also a good source of protein and contain less fibre. According to Friday and Uchenna Igwe (2021) food that contain 12% or higher protein can regulate body metabolism, cellular function and the production of amino acids (Zhao et al., 2018). Thus, reducing constipation, diabetes and cardiovascular diseases (Xiao & Guo, 2022). Also, the considerable amount of minerals recorded in these species suggests that consuming these plants could mitigate mineral deficiencies known for causing health problems.

In South Africa, *T. decumbens* as a leafy green vegetable remains underutilized as commercial farming of this species has never been explored. The leaves and soft stems of dune spinach have a salty taste and are used like green spinach, eaten raw in green salads, or cooked with other vegetables as demonstrated in figure 2.6. Dune spinach can also be fermented, pickled, used in stews and soups, and particularly tasty in a stir-fry (Rusch, 2016). When boiled it has a granular texture and tastes bland but when mixed with *Oxalis pes-caprae* (sorrel) and butter, makes a tasty dish (Van Wyk & Gericke, 2017). More recently, the species has been found to possess a substantial amount of minerals when cultivated (Table 2.1). Plant based mineral nutrients are known to be important components of human diet since they play a substantial role in the maintenance of certain physicochemical processes required to life (Salami et al., 2022). High amounts of potassium (1800-2080 mg/ 100g), calcium (900-1800 mg/ 100g) and magnesium (400-770 mg/100g) recorded in the leaves of dune spinach exceeds the daily recommended allowance for humans, suggesting that this neglected leafy vegetable can be an alternative source of nutritional minerals. Even though the edibility and promising potential of dune spinach as a fresh and processed vegetable has been documented, its nutritional composition has not been extensively explored (Nkcukankcuka et al., 2021). Therefore, nutritional profiling of the edible parts of this species is crucial to justify and support its consumption among South African households.

Figure 2.6: Oep ve Koep's Sandveld restaurant dumplings with dune spinach (A) and Dune spinach salad served with feta cheese (B), adapted from Tembo-Phiri (Tembo-Phiri, 2019).

Table 2.1: Comparison of nutritional constituents of close relatives of dune spinach (Onoiko, 2024; Walters et al., 2024; Rodríguez-Hernández & Garmendia, 2022; Kawashima & Valente Soares, 2003; Mugo et al., 2024; Sogoni et al., 2021; Cebani et al., 2024).

Nutritional value	*T. tetragoniodes*	*T. expansa*	*M. crystallinum*	*T. decumbens*
Moisture%	60-92.7	ND	71-83	ND
Crude protein%	10-18.2	ND	13.1-15.2	ND
Crude fibre%	6-13.9	ND	18-21.6	ND
Ash%	11-13.9	ND	12-37.2	ND
Fat%	0.9-4.2	ND	1-2.1	ND
Carbohydrate%	40-50.6	ND	38-44.9	ND
Na (mg/100g)	700-3180	60-94	900-3000	840-2100
K (mg/100g)	1000-2706	420-530	430-2610	1800-2080
Ca (mg/100g)	1900-2120	35-64	1900-2115	900-1800
Mg (mg/100g)	200-459	40-55	420-640	400-770
P (mg/100g)	400-620	ND	270-330	400-540
Fe (mg/100g)	0.8-4.5	0.5-1	28-43	12-18
Zn (mg/100g)	0.7-1.29	0-0.3	6-11.7	4-6.3
Vitamin C (mg/100g)	28-50.5	58-116	4-10	ND

Note: ND= no data retrieved

2.6.6. Potential consumer acceptance of *T. decumbens*

Consumer studies on the adoption and acceptance of edible halophytes have not been widely investigated in South Africa (Sogoni, 2020). This might be attributed to the fact that the use of wild vegetables as a method to address food insecurity and mitigate climate change has been ignored and only recently has it gained attention (Borelli et al., 2020). Moreover, current research on wild leafy vegetables has focused primarily on promoting consumption through the provision of nutritional information and health advantages of including these veggies into one's diet (Bvenura & Sivakumar, 2017; Imathiu, 2021). Despite this intervention, vegetable consumption is still a significant problem in sub-Saharan Africa leading to micronutrients deficiency (Shembe et al., 2023). Hence, gaining a comprehensive understanding of customers' opinions of wild vegetables might provide valuable information on how to effectively promote their utilisation and consumption (Pieterse et al., 2023). This is because consumption and purchasing intention are critical elements in assessing the potential adoption of a food product (Shembe et al., 2023). The consumer acceptance process is significantly influenced by the appearance and taste aspects of a given food (Torrico et al., 2023). Research conducted in some regions of South Africa indicates that although the flavour of indigenous foods serves as a significant incentive for consumption, it also acts as a deterrent for others (Omotayo et al., 2021). Hence, it is important to conduct research that promotes enhancements in the taste and flavour of wild edible species to stimulate public acceptance and use. Recently, a consumer acceptance study focusing on taste, texture, consumption and purchasing intent was conducted by Tembo-Phiri (2019) on raw and cooked leaves of *T. decumbens* and *M. crystallinum*. The recipes were prepared by Ms. Loubie Rusch a local food activist and chef as shown in table 2.2. Twenty respondents consisting of 11 senior conservation ecology university students and 9 community members participated in a survey that explored the acceptance of these edible halophytes. Results revealed that cooked leaves of *T. decumbens* had a higher score of acceptance than the raw and cooked leaves of *M. crystallinum*. When assessing the consumption and purchasing intent, about 70% of the correspondence showed willingness to consume and purchase these vegetables if available in stores. These findings suggest that *T. decumbens* has potential to be adopted as leafy vegetable in South African households. However, studies with larger sample size might have a better significance in enhancing its domestication and consumption.

Table 2.2: Recipes of raw and cooked *T. decumbens* and *M. crystallinum* leaves

Raw salad meal	
1 chopped onion	**½ chilli and ¼ ring of lemon**
½ chopped green pepper	**2 cm of peeled and finely chopped ginger**
One juice lemon	**2 cups of *T. decumbens* and *M. crystallinum***
Mix the finely chopped onions, green peppers, ginger, some green chilli and lemon zest. Allow the mixture to marinate overnight in lemon juice and salt. Add chopped *T. decumbens* and *M. crystallinum* leaves and some olive oil to the mix 15 minutes before serving.	
Cooked meal	
1 chopped onion	**Olive oil for sauteing**
½ chopped green pepper and ½ chilli	**2 cm of peeled and finely chopped ginger**
¼ ring of lemon and ½ chopped tomato	**¼ teaspoon of salt**
Reduce tomato in a saucepan by half to thicken and intensify flavour. Fry finely chopped chilli, onion, green pepper, ginger and lemon rind until onion is translucent. Add reduced tomato and stir through for a minute. In a separate pan, add 1 tbsp of oil and stir fry the leaves of *T. decumbens* and *M. crystallinum* for 3 minutes or until just wilted. Stir the wilted greens into the tomato mix and adjust salt as needed.	

2.6.7. Potential of *T. decumbens* leaves as an alternative source of Sodium

According to Klein (2014) people who have switched to raw food diets and avoided salt experienced low levels of sodium in their blood as well as resultant weakness, fatigue, loss of appetite and other health issues. Most patients who suffered from severe inflammatory bowel conditions caused by high numbers of bowel movements and hyperacidity have also experienced low sodium levels (Wang et al., 2012). However, increasing the patients' sodium levels to the normal range is sometimes a long process. Traditional health practitioners have suggested and used celery, the most known source of sodium in the diet. However, in some cases, this has been inadequate for improving sodium deficits within a reasonable time frame. When this happens, some health practitioners have advised the consumption of sea salt or intravenous saline as options to help avoid further physiological dysfunction and the spectre of seizures and cardio arrest (Klein, 2014). Some edible halophytes have proven to be safe and healthful answers to sodium deficiency due to their high salt content when grown in saline conditions (Wang et al., 2012). Zhang et al. (2015) discovered that salt made from *Salicornia bigelovii* Torr. prevented the hypertensive effect that is associated with sodium deficiency. Moreover, Barroca et al. (2023) evaluated the NaCl replacement by *Sarcocornia perennis* leaf powder in white bread. The results showed that the addition of this halophyte powder as a NaCl substitute improved nutrients and minerals as well as bread quality. With such benefits, South African edible halophytes including *T. decumbens* should be popularized as an edible substitute in improving sodium deficiency.

2.6.8. Therapeutic potential of *T. decumbens*

Most edible halophytes are considered as promising nutraceutics in other countries and have been documented for the treatment and prophylaxis against various chronic diseases that afflict modern societies (Ksouri et al., 2012). *Tetragonia tetragoniodes* has been actively utilised in oriental medicine to treat stomach hypersecretion, gastric ulcers, dyspepsia and even gastric cancer. Nevertheless, its close relative *T. decumbens* endemic to south Africa has not receive any research attention, hence its potential medicinal value can be drawn from previous and current studies conducted on *T. tetragoniodes* and other closely related species

2.6.8.1. Antioxidant potential

Halophytes are known to accumulate high amounts of total phenolics and flavonoids as a defence response to saline conditions. These compounds are well known for their antioxidant potential against active oxygen species and free radicals responsible for various chronic diseases that afflict modern societies. Leaf crude extracts of *T. tetragoniodes* has been reported to show strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS radical scavenging activity. Lee et al. (2019) isolated methoxyquercetin and quercetin as the main bioactive compounds responsible for this strong antioxidant effect. Likewise, a strong ferric reducing antioxidant power was noted in crude extracts of *T. decumbens* with increasing saline treatment (Sogoni et al., 2021) Moreover, a more potent antiradical activity was also noted in *Mesembryanthemum crystallinum* a close family relative of *T. decumbens.* The methanol extract of this species was reported to possess high scavenging activity against the ABTS synthetic radicals, superoxide anion and inhibited the lipid peroxidation of linoleic acid (Hanen et al., 2009; Kang & Joo, 2023). Nevertheless, there are few studies conducted on the isolation of compounds responsible for the potent antiradical activity of the species in the genus *Tetragonia*, thus further studies are recommended.

2.6.8.2. Anti-inflammatory potential

Inflammation is induced by the response of a cell/tissue to infection, damage, or irritation. It is generally marked by fever, edoema, swelling and aches (Adeoye et al., 2024). During inflammation, mast cells are commonly mobilised to produce histamine. In this context, 6-methoxykaempferol isolated from *T. tetragoniodes* demonstrated an anti-inflammatory effect when consumed by diabetic mice by inhibiting histamine and improved blood circulation (Lee et al., 2018). Likewise, 6-methoxyflavonols, kaempferol and quercetin isolated from *T. tetragoniodes* were also tested on lipopolysaccharide (LPS) induced inducible nitric oxide synthase (iNOS) and Cyclooxygenase – 2 (COX-2) protein upregulation in RAW 264.7 cells (Lee et al., 2019). The results showed that all the isolated compounds suppressed iNOS and COX-2 respectively. These two enzymatic pathways are involved in the synthesis of nitric oxide and prostaglandin which causes inflammation. Thus, their suppression is crucial in controlling immune responses. Koa et al. (2017) also reported the suppression effect of hydrosols extracted from *T. tetragoniodes* on these two enzymatic pathways in LPS-stimulated RAW 264.7 cells. Even though these studies show potential, further research on isolated compounds are required on chronic diseases induced by inflammation.

2.7. Challenges associated with utilisation of *T. decumbens* as a leafy vegetable

2.7.1. Scarcity of indigenous knowledge transfer

Colonization, apartheid and urbanisation have led to the removal of indigenous people from cultural lands which resulted in societies completely disassociated from the land and knowledge of the useful plants it offers (Cavanagh, 2013; La Croix, 2018). As a result, many of these native plant species remain underutilised due to the loss of indigenous knowledge on their potential use. *T. decumbens* is no exception, as it is only known by a small group of local chefs and food enthusiast due to its nutritional offering of healthy and affordable nutrient-dense alternative (Botha et al., 2020).

2.7.2. Inadequate information on cultivation, post-harvest handling and storage

Very little information is available about the adoption of *T. decumbens* from the wild to cultivation, particularly regarding the environmental and agronomic processes required from propagation to post-harvest handling. The inadequate evidence-based data to support its agronomic potential is a major challenge that hinders the commercialization of this plant. This is due to researchers focusing on their areas of interest or interesting studies with few dealings with basic agronomic studies on wild vegetables which require extensive field work. According to Baldermann et al. (2016), ineffective production, storage, and processing of the by-products together with the lack of baseline knowledge on the nutritional potential negatively affect the acceptance, utilization, and value addition of many wild vegetables. Thus, further research is needed on the agronomic aspect of dune spinach to further promote its commercialization.

2.7.3. Marketing of wild vegetables in South Africa

Marketing of Halophytes in South Africa is still very low, as these species are only popular among senior citizens and are marketed through street vendors (Maseko et al., 2017). Despite their potential as nutritional and processed foods, they are rarely found in supermarkets and upmarket groceries in South Africa. This has greatly contributed to their reduced consumption among citizens. To increase their potential consumption and promotion, there should be integration between local producers and supply outlets. This will allow linkage of various market actors which will increase the supply and efficiency in the chains.

2.8. Prospects of unlocking the potential use of dune spinach

2.8.1. Holistic research approach and improved policy framework

Holistic research on halophytes in South Africa has been ignored by policymakers and researchers, although they are currently attracting interest in other parts of the world due to their adaptive nature to salinity and drought (Beletse et al., 2012). The Agricultural Research Council (ARC), Vegetables and Ornamental Plants (VOPI) and Water Research Commission (WRC), are major role players involved in the research and training of indigenous vegetables in South Africa (Maseko et al., 2017). However, there has been limited focus on halophyte research due to the current food security policy guiding research, production and marketing of agricultural produce, which pays limited or no attention to the promotion of halophytes as edible vegetables. Changing such policies and improved research funding as well as collaboration between universities and government research institutions could promote intensive research on edible halophytes. This will then unlock the resource value of dune spinach through validated findings to meet the needs of potential consumers and promote its commercialization.

2.8.2. Publication of research findings

It is through publication that the edible halophytes-based research, including its scientific and practical contributions, can be disseminated to the public in various ways, such as in journals, conferences and reports. Hence it is of utmost importance for researchers to publish their results in such platforms, so that it can be easily accessed by practitioners, potential growers and consumers with similar interests. This will then contribute to advancing knowledge on edible halophytes and their vast application on human health in South Africa.

2.8.3. Awareness and education

Appropriate awareness and education on the benefits of halophytes will ultimately boost their consumption and cultivation. This can be achieved through the inclusion of agricultural education curricula in universities that deals with the agronomic aspects of indigenous vegetables in both commercial and communal areas. This will then upgrade the knowledge base of rural dwellers and low-income groups on the importance of these neglected edible species since wild vegetables are naturally less demanding to cultivate and are economical natural resources.

2.9. Conclusion

Tetragonia decumbens, an overlooked and underutilised halophyte with edible attributes, presents a convincing argument for its incorporation into dietary regimens and agricultural practices. This is due to its exceptional easy to grow methods, accompanied by its high nutritional value, promising consumer acceptance and therapeutic potential. The promising capacity of this species to fulfil most of the recommended dietary allowance (RDA) for essential nutrients such as sodium, magnesium, potassium, iron, zinc, and vitamin C for all age groups makes it a powerful ally in combating nutrient deficiencies. Moreover, its salt accumulating capabilities makes it a good candidate for phytoremediation of saline soils through various systems such as intercropping with salt-sensitive species. Likewise, the species deep root system, drought tolerance and soil stabilization capacity, support its use in desertification control. Therefore, it can be concluded that the addition of *T. decumbens* to agricultural systems may enhance crop diversity, assure food security, improve food nutrition, and promote sustainable farming practices. Moreover, the restoration of this species may also have a significant impact in reclaiming infertile soil and improving food security. This makes it a prospective option for future food production, especially considering the changing climate.

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2.11. Author contributions

AS: Conceptualization and Writing an original draft of the manuscript; **CL**: Funding acquisition and Critical revision of the article. **MOJ** and **SN**: Critical review of the article with technical inputs. **LK:** Supervision, Critical revision of the article.

2.12. Conflict of interest

The authors wish to declare no conflict of interest.

2.13. Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

2.14. References

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CHAPTER THREE

SALINITY MODULATES MORPHO-PHYSIOLOGY, BIOCHEMICAL AND ANTIOXIDANT DEFENCE SYSTEM IN *TETRAGONIA DECUMBENS* MILL.: A NEGLECTED WILD LEAFY VEGETABLE IN SOUTH AFRICA

Salinity modulates morpho-physiology, biochemical and antioxidant defence system in *Tetragonia decumbens* mill.: a neglected wild leafy vegetable in South Africa

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Abstract

Tetragonia decumbens is an edible halophyte that grows naturally in saline environment; however, its tolerance mechanisms are poorly understood for bio-saline agriculture. So, this research was designed to look into how salinity affects vegetative growth, leaf succulence, chlorophyll content, cation accumulation, oxidative stress indicators, and antioxidative defence mechanisms involved in the salt tolerance of *T. decumbens*. Saline conditions were prepared by dissolving sodium chlorine (NaCl) in the nutritive solution. The control was maintained and only watered with nutrient solution while the tested treatments contained graded NaCl doses (250, 200, 150, 100, and 50 mM). Results revealed a substantial enhancement in shoot length, number of branches, relative water content, as well as total fresh weight in plants irrigated with 50 and 100 mM NaCl in comparison to the control, while higher saline concentrations (150-250 mM NaCl) reduced plant growth and chlorophyll content. Similarly, these high salt concentrations induced more severe oxidative stress indicated by high amounts of superoxide, cell death viability and malondialdehyde, with the most pronounced effect at the highest NaCl concentration (250 mM). Nevertheless, *T. decumbens* modulated various defence mechanisms with increasing salinity stress, these include the upregulation of superoxide dismutase, catalase, polyphenols, flavonoids, proanthocyanidins and the build-up of sodium ions in the leaves. These results show that *T. decumbens* can withstand salinity by modifying its morpho-physiological traits, antioxidant defence systems, and managing ion toxicity and oxidative stress efficiently, since all plants withstand salinity without showing signs of toxicity.

Keywords: Antioxidant defence systems; Dune spinach; Edible halophytes; Salinity tolerance; Underexploited vegetables

3.1. Introduction

Soil salinity has been reported as one of the contributing drivers of devastating crop production losses around the globe (Manuel et al., 2017; Rehman et al., 2023). Although precise measurements are unattainable, the extent of soil infected by salinity is increasing and is particularly evident in irrigated soils (Zhang et al., 2022). According to estimates, salinity affects about 10% of the world's land mass and around half of all irrigated land (Boamah et al., 2023; Medini et al., 2023). This has been attributed to the overuse of low-quality water, and extensive irrigation coupled with intensive farming and inadequate drainage systems resulting in the built-up of soluble salt ions in the soil (Hassani et al., 2020). These salt ions impede the roots' ability to absorb water, leading to osmotic stress and nutritional imbalances that impair vital physiological functions like photosynthetic activity, tissue hydration, and the initiation of oxidative stress and membrane damage, resulting in reduced plant biomass (Hussain et al., 2023; Mahmood et al., 2021). Moreover, soil salinity and the increasing global climate change will undoubtedly deteriorate food production in the coming years (Mangal et al., 2022; Zuluaga et al., 2023). Thus, alternative use of resilient crops is crucial to fulfil the increasing food demand in regions impacted by both salinity and drought. This will then provide needed calories, proteins, fats, and nutrients for inhabitants in these areas (Rasheed et al., 2020). Nevertheless, such species are still underutilised particularly in emerging countries where a 90% increase in population growth predicts that food insecurity may soon become a major concern (Ogunniyi et al., 2020). The underutilisation of these salt-tolerant species is due to limited research on their nutritional composition, growing protocols, as well little urgency shown by plant biologist and crop scientist (Salami et al., 2022). Interestingly, these underutilised potential crops are probably present among edible halophytes, which have been declared neglected in most African countries including South Africa (Mokganya & Tshisikhawe, 2019). These species can tolerate salinity and drought and some even require sodium chloride (NaCl) for optimum growth.

Halophytic species use different metabolic and physiological adaptations to prevent oxidative mutilation, nutritional imbalance, and osmotic stress (Z. Gul et al., 2022; Zhao et al., 2020). Some species increase their roots/ shoot biomass to regulate sodium (Na^+) entry to the xylem or minimize its transport to the shoots, thus minimizing ion toxicity (Parvez et al., 2020). During this process, active nutrients such as potassium, magnesium and calcium protect photosynthetic pigments that enable photosynthetic efficiency throughout the process (Ma et al., 2022). Nevertheless, ion toxicity can still occur when ion homeostasis is disrupted, which in turn affects the photosynthetic process by destroying or altering the biosynthesis of light-trapping pigments, thus reducing plant growth (Azeem et al., 2023).

Excessive build-up of Na^+ and chlorine (Cl^-) within plant cells induce oxidative stress by producing superoxide radicals via the Mehler reaction (Golldack et al., 2014; González-Orenga, Grigore, et al., 2021; Rodrigues de Queiroz et al., 2023). These free radicals disrupt the normal metabolic processes in cytoplasm, peroxisomes, and mitochondria by causing oxidative mutilation to proteins, lipids, and other essential biomolecules, resulting in acute impairment and ultimately, cell death (Flowers & Colmer, 2015; Saleem et al., 2022). To prevent the catastrophic effect of oxidative stress, plants have established defence mechanisms such as antioxidative enzymes followed by the synthesis of non-enzymatic metabolites, which scavenge the excessive reactive oxygen species (ROS). Of these several antioxidative enzymes, Superoxide Dismutase (SOD) is thought to constitute primary defence tool against oxidative stress in plants (Gill et al., 2015; Zulfiqar & Ashraf, 2022). This enzyme incites the dismutation of superoxide radicals into H_2O_2 and O_2. Subsequently, several antioxidant enzymes, such as Catalase (CAT) and Peroxidase (POX), convert H_2O_2 to water. It has been reported in the literature that halophytic plants tend to accumulate high amount of SOD under salt stress, a feature that provides an adaptation advantage over salt-sensitive species (glycophytes) (Bose et al., 2014; Huang et al., 2019; Zhao et al., 2020). Moreover, halophytes also activate the manufacturing of non-enzymatic compounds such as phenols, flavonoids, anthocyanins, and tannins to scavenge these highly toxic species when excessive highly toxic ROS are not eliminated by the antioxidative enzymes (Azeem et al., 2023). These bioactive electron-donor compounds inhibit clusters of oxidative reactions and safeguard membranes and macromolecules from damage, thus making edible halophytes good sources of nutritional antioxidants with various benefits for overall health and illness prevention.

Tetragonia decumbens Mill (Aizoaceae), also referred to as 'dune spinach' is an underutilised leafy vegetable that is mainly dispersed in coastal areas of South Africa (Klak et al., 2017). The salty leaves of this species can be eaten raw in green salads and can also be cooked like spinach and added to soups and stews (Sogoni et al., 2021; Tembo-Phiri, 2019). Earlier reports have shown that this species can withstand salinity stress (Sogoni et al., 2021). To cope with these conditions, *T. decumbens* was reported to have unchanged chlorophyll content and increased ferric reducing antioxidant power under low to moderate salinity (50–100 mM). While the highest salinity (200 mM) increased the phenolic content, nitrogen, phosphorus, and sodium. Nevertheless, in dept studies illuminating the molecular processes and physiological mechanisms responsible for the tolerance of this plant to salt levels are limited, especially on the morpho-physiological features (leaf succulency, relative water content, photosynthetic pigments), oxidative stress markers (MDA, Superoxide radicals and etc), antioxidant defence systems (superoxide dismutase, Catalase, polyphenols, flavonoids) and managing of ion toxicity. These adaptation mechanisms have been documented in numerous laboratory experiments on halophytic plants cultivated under saline conditions to better understand their salt defence mechanism (Hussain et al., 2015, 2023; Peng et al., 2019). So, this study was conceived to assess the effects of salinity on vegetative growth, leaf pigmentation, leaf succulence, cation build-up, oxidative stress indicators, and antioxidant defence systems, to better elucidate the salt tolerance traits in *T. decumbens* for bio-saline agriculture.

3.2. Materials and Methods

3.2.1. Growth Conditions and Experimental Location

The study was executed in the research greenhouse of the Cape Peninsula University of Technology. The greenhouse was configured to have daytime temperatures of 21–26 °C and nighttime temperatures of 12–17 °C, with 60% relative humidity. Under natural light conditions, the daily average photosynthetic photon flux density (PPFD) was 420 μmol/$m^{-2}s^{-1}$, with the intensity peaking at 1020 μmol/$m^{-2}s^{-1}$. The photoperiod corresponds to the prevalent conditions of early spring to summer.

3.2.2. Preparation of Plant Material and Saline conditions

T. decumbens plants were generated from cuttings following the propagation method described by Sogoni et al. (2022). Homogeneous established plants (180) were transplanted in black plastic pots (12.5 cm) filled with a combination of peat and sand (1:1) and stationed in the hardening off area to acclimatize. The plants were then watered with a full Nutrifeed™ solution produced by STARKE AYRES Pty. Ltd. South Africa. After sixteen days of plant development, plants were irrigated with reverse osmosis water for six days to eliminate any salt residue before being subjected to saline treatments, each containing ten replicates, and the study was repeated three times. Saline conditions were established on six treatments by elevating the concentration of NaCl in the nutritive solution (0, 50, 100, 150, 200, and 250 mM) as described by Sogoni et al. (2021). Each plant received 300 millilitres of nutrient solution with graded NaCl and were irrigated every two days. The control plants were only irrigated with nutritive solution without salinity. To verify that the right concentration of salinity in each pot was sustained, drain water from each pot was collected and the electrical conductivity was measured. After four months of salt treatment, plant material was harvested and used for further analysis.

3.2.3. Plant Growth Evaluation

3.2.3.1. Shoot increment and the quantity of lateral branches.

Shoot increment and the quantity of branches were employed as factors to evaluate growth development. Branch numbers were counted manually while shoot increment was determined weekly with a metre tape placed from the surface of the growth media to the shoot tip.

3.2.3.2. Plant weight

After four months of saline treatment, plants were thoroughly watered to loosen the soil and harvested cautiously to prevent damage. The harvested plants were rinsed multiple times with RO water and dried using tissue paper. Thereafter the shoots and roots were split with a sterile secateur, and the fresh samples were weighed using a RADWAG® laboratory scale. The fresh material was then dried in a LABTECH™ oven at 56 °C for 50 hours, and the dry weight was determined.

3.2.4. Leaf Hydration

3.2.4.1. Relative Water Content (RWC)

To evaluate variability in leaf RWC, three leaves were excised from three distinct plants in each of the six treatments and weighed (FW). Thereafter, were submerged in distilled water and left in the dark for 4 hours and the turgid weight (TW) was established. Lastly, the leaves were air dried in an oven at 56 °C for 24 hours to acquire the dry weight (DW). The percentage of RWC was computed using the approach provided by (Bistgani et al., 2019) with the equation shown below:

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

3.2.4.2. Leaf Succulence

Leaf succulence was calculated following Mantovani's (2011) methodology, using the below equation:

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

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). A portable AM350 leaf area meter manufactured by Bio-scientific limited, London, United Kingdom was used to measure the leaf area.

3.2.5. Photosynthetic Pigment

Chlorophyll *a* and chlorophyll *b* were estimated using spectrophotometric procedures as stated by Wang et al. (2023). Concisely, 100 mg of fine grounded fresh leaves was extracted with 5 ml of 99.5% dimethyl sulfoxide (DMSO; Sigma). The determination of the photosynthetic pigment was conducted using the absorbance of the supernatants at 649 and 665 nm, as described in the work of Lichtenthaler & Wellburn, (1983).

3.2.6. Quantification of Cations

The concentrations of Sodium, Calcium and Potassium were determined in leaves and roots following the method of Bulawa et al. (2022).

3.2.7. Determination of Oxidative Stress Markers

3.2.7.1. Cell Viability

The cell viability analysis in dune spinach leaves was assessed following the method described by Egbichi et al. (2014) with slight adjustment. Three leaf sections of 1 cm^2 were excised from three separate plants from each of the six treatments. The removed leaf sections were then stained for 30 minutes at room temperature in Eppendorf tubes filled with one millilitre of 0.25% (w/v) Evans Blue solution. After washing the leaf samples for 30 minutes in distilled water, the Evans Blue stain absorbed by dead leaf cells in the leaf tissue was extracted with one % (w/v) sodium dodecyl sulphate (SDS) after 1 hour incubation at 55 °C. The level of Evans Blue taken up by the leaf tissue was evaluated by determining the absorbance of the extract at 600 nm with a spectrophotometer.

3.2.7.2. Superoxide Radical and Malondialdehyde (MDA)

The superoxide radical content in dune spinach leaves was measured following the technique reported by Gokul et al. (2016) and the extinction coefficient of 12.8 mM cm^{-1} was used to calculate the superoxide radical levels in the leaves. To quantify the MDA content within the tested samples, the procedure explained by González-Orenga et al. (2021b) was used.

3.2.8. Activity of Antioxidant Enzymes

The method outlined by Gill et al. (2015) was followed to extract protein from the leaf samples. The protein concentration in the extracts was measured using the Bio-Rad reagent and bovine serum albumin (BSA) as the standard. Spectrophotometric experiments were used to assess the activity of the selected antioxidant enzymes in protein extracts.

The activity of superoxide dismutase was determined at 560 nm spectrophotometrically by measuring the suppression and photoreduction of nitro blue tetrazolium (NBT); the reaction solution contained riboflavin as a determinant of superoxide radicals. A unit SOD is labelled as the quantity of enzyme required to block NBT photoreduction by 50% in experimental conditions as outlined in the methodology of Brenes et al. (2020).

Catalase (CAT) activity was measured by observing a decrease in absorbance at 240 nm following the consumption of H_2O_2 added to the extracts (Baureder et al., 2014). One CAT unit was defined as the quantity of enzyme degrading one mmol of H_2O_2 at 25 °C per minute.

3.2.9. Phytochemicals and Antioxidant Assays

3.2.9.1. Crude extraction

Tetragonia decumbens leaves were harvested and oven-dried (40 °C) for 7 days. A Junkel & Kunkel type A 10 mill was used to grind the dried plant material into a fine powder. Thereafter, two grams was mixed with 80% ethanol (50 mL) for an hour for crude extraction. The supernatants were subsequently used for all analyses after being centrifuged at 4000 rpm for 5 minutes.

3.2.9.2. Total Phenol, Flavonoid and Proanthocyanidins (condensed tannins)

The total phenolic content was assessed using the Folin-Ciocalteu technique, as depicted by Ingarfield et al. (2023) and the flavonoid content was determined using the aluminium chloride spectrophotometric test reported by Jimoh et al. (2019). The Condensed tannins was assessed following the procedure reported by Jimoh et al. (2023).

3.2.9.3. DPPH

The antioxidant activity of the extract was measured using a slightly modified version of the DPPH free radical scavenging assay as reported by Jimoh et al. (2020). A 0.135 mM DPPH solution was made in a dark bottle using ethanol. The DPPH solution was reacted with 0.2 mg mL^{-1} of the extracts in equal volumes. Equal amount of 0.135 mM DPPH and the solvent were used to make the control solution. The resulting mixture was violently agitated before being incubated at room temperature for 30 minutes after which the absorbance was measured at 517 nm on a spectrophotometer. The equation below was used to calculate the scavenging activity of the plant extract.

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

3.2.10. Statistical Analysis

The experimental data was analysed using STATISTICA, version 13.5.0.17. A one-way ANOVA was conducted to determine substantial differences between treatments, followed by Fisher's (LSD) test at $p \leq 0.05$. The Shapiro-Wilk test was conducted before the analysis of variance to verify the validity of normality, while Levene's test was employed to evaluate the homogeneity of variance. The results gathered were expressed as mean values and standard error.

3.3. Results

3.3.1. Plant Growth Response

The growth responses of *T. decumbens* under NaCl concentrations varied among treatments as shown in Table 3.1. Increment in shoot length and branch number were notably influenced by NaCl, with longer shoots and more branches obtained in plants irrigated with the lowest dosage of salinity (50 mM). The same pattern was also noted in fresh weight of leaves, with plants receiving 50 mM of NaCl irrigation reporting the highest weight. Conversely, the highest dry weight of leaves was noted in plants receiving 200 mM of NaCl irrigation, but this was comparable with those irrigated with 50 mM NaCl. When assessing the roots fresh and dry weights, the control plants had the highest weights. Nevertheless, the total fresh weight was positively influenced by saline treatments with plants receiving 50 mM irrigation registering the highest weight. While there was no discernible variation in total dry weight between the treatments.

Table 3.1: Growth parameters of *T. decumbens* to salinity at the end of treatments

Treatments	SLI (cm)	NB (n)	FWL (g)	FWR (g)	DWL (g)	DWR (g)	TFW (g)	TDW (g)
Control	92.25± 2.4 ^{a}	3± 0.14 ^{c}	91.51± 6 ^{c}	28.44± 2.06 ^{a}	20.12± 0.65 ^{c}	16.7± 0.9 ^{a}	119.9± 6.1 ^{c}	36.8±1.1 a
50 mM	93.46±2.4 ^{a}	4± 0.15 ^{a}	153.7± 7 ^{a}	28.42± 1.42 ^{a}	23.8± 0.70 ^{ab}	15.9± 0.8 ^{ab}	182.13± 7.6 ^{a}	39.7±1.1 a
100 mM	83.3± 1.4 ^{b}	3.5± 0.2 ^{b}	140.13± 4.8 ^{ab}	23.39± 1.18 ^{bc}	21.6± 0.71 ^{c}	14.5± 0.45 ^{bc}	164.52±5.2 ^{ab}	36.2±0.8 a
150 mM	76.13± 2.3 ^{c}	3± 0.18 ^{c}	134.41± 7 ^{b}	23.78± 0.97 ^{bc}	22.01± 0.44 ^{bc}	14.3± 0.36 ^{bc}	158.2±6.8 ^{b}	36.3±0.6 a
200 mM	74.83± 1.7 ^{c}	2.8± 0.14 ^{c}	133.76± 5.2 ^{b}	21.96± 1.22 ^{c}	24.76± 1.6 ^{a}	13.7± 0.37 ^{c}	155.7±5.6 ^{b}	38.5±1.7 a
250 mM	75.67± 1.9 ^{c}	2.9± 0.14 ^{c}	109.28± 6.6 ^{c}	20.71± 1.81 ^{c}	21.01±0.85 ^{c}	13.35± 0.7 ^{c}	129.9±6.5 ^{c}	36.3±1.1 a
F-statistic	15.96*	8.70*	13.77*	3.16*	3.7*	2.73*	12.05*	1.59ns

Note. SLI: Shoot length increment; NB: Number of branches; FWL: Fresh weight of leaves; DWL: Dry weight of leaves; FWR: Fresh weight of roots; DWR: Dry weight of roots; TFW: Total fresh weight; TDW: Total dry weight. Means with distinct letters vary substantially from one another ($p \leq 0.05$).

3.3.2. Leaf hydration

3.3.2.1. Relative Water Content (RWC) and Leaf Succulence

Leaf RWC and leaf succulence were notably influenced by NaCl irrigation as shown in Figure 3.1. A constant RWC was noted in plants irrigated with 0 to 100 mM NaCl, then a decline was detected in plants receiving 150-250 mM of NaCl irrigation respectively. Conversely, leaf succulence increased with increasing doses of NaCl. The maximum leaf succulence was noted in plants subjected to 200 and 250 mM of NaCl irrigation and these mean values were greater than most treatments including the control.

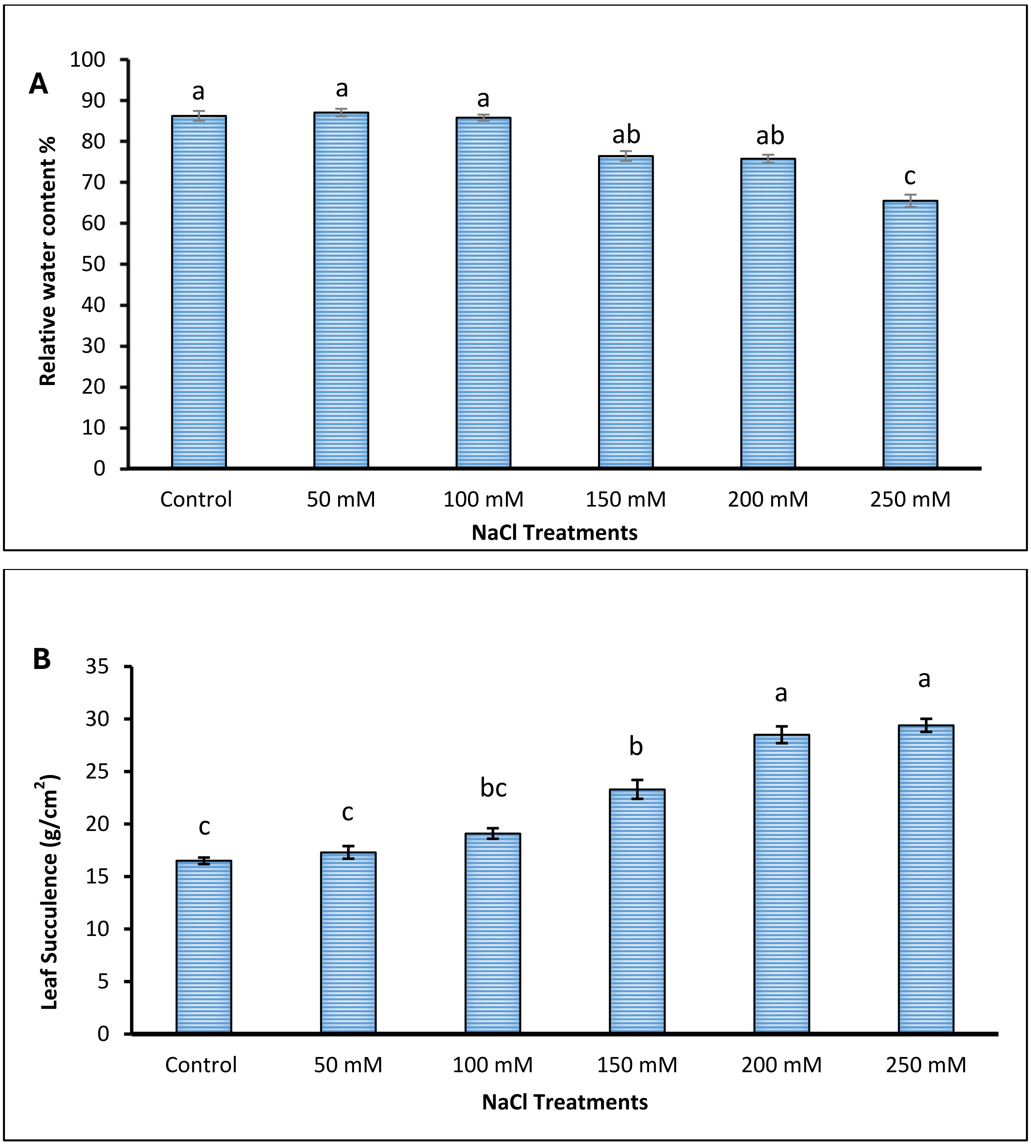

Figure 3.1: Relative water content (A) and Leaf succulency (B) of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$).

3.3.3. Photosynthetic Pigment

The effects of NaCl irrigation on chlorophyll content of dune spinach leaves are reported in Figure 3.2. Results showed that chlorophyll *a* and *b* were significantly affected and remarkably reduced with elevated NaCl concentrations of 100 up to 250 mM compared with the control. While plants treated with low NaCl concentration (50 mM) had a comparable chlorophyll content with control plants.

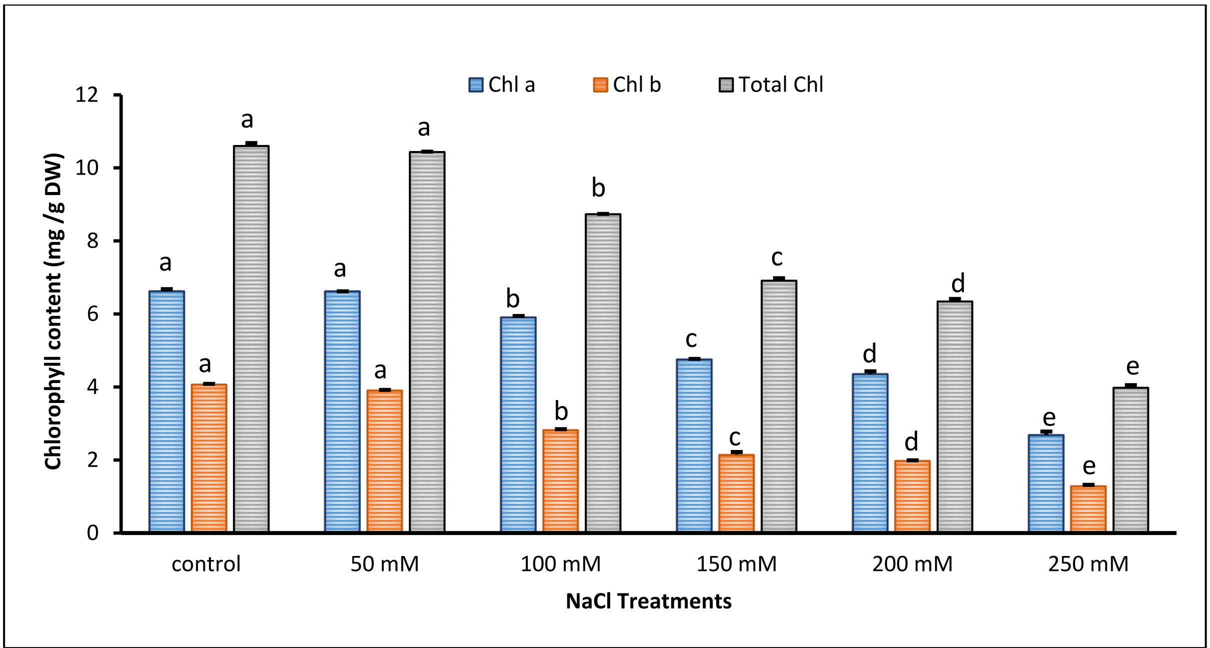

Figure 3.2: Chlorophyll content of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$).

3.3.4. Quantification of Cations

High amounts of Na^{+} were recorded with increasing NaCl irrigation in leaves and roots as indicated in Figure 3.3. The highest Na^{+} concentration was noted in plants receiving 250 mM of NaCl irrigation and the highest values were obtained in leaves than in roots. All values obtained from other treatments were notably lower than these values. On the contrary, the concentration of K^{+} and Ca^{2+} drastically decreased in plants with increasing NaCl irrigation (Figure 3.3). The control treatments yielded the maximum values of K^{+} and Ca^{2+} in both roots and leaves which were significantly higher than the values obtained in other treatments except the roots values of plants irrigated with 50 mM NaCl which were comparable to the control roots.

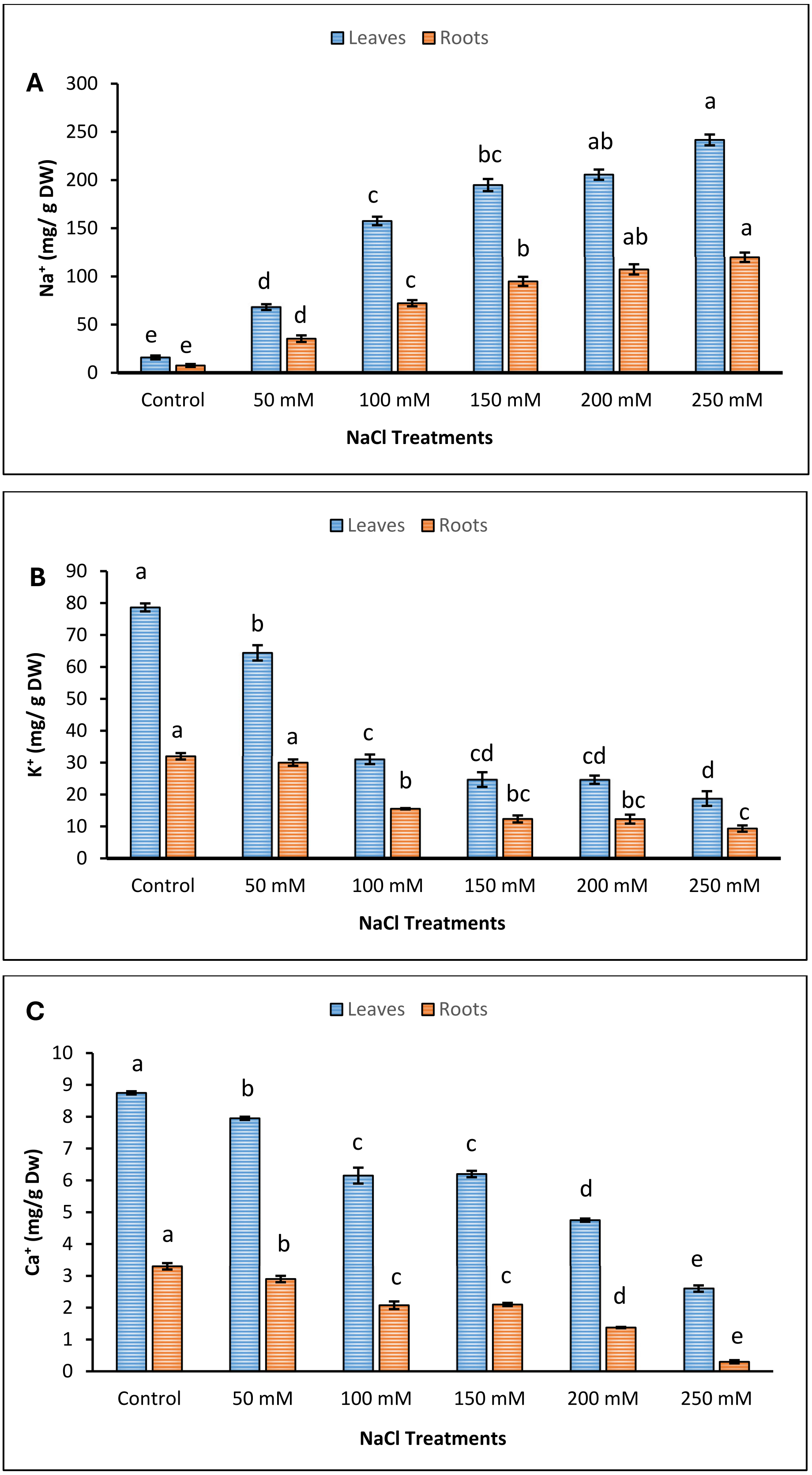

Figure 3.3: Sodium (A), Potassium (B) and Calcium (C) of dune spinach leaves and roots in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$).

3.3.5. Oxidative Stress Markers

3.3.5.1. Cell Viability, Superoxide and Malondialdehyde (MDA)

The leaf cell death and relative accumulation patterns of superoxide and MDA in response to salt stress were similar (Figure 3.4 and 3.5). Increasing NaCl treatment caused leaf cell death, with pronounced effect at highest NaCl concentration (250 mM) (Figure 3.4). This treatment had a significantly higher cell death than all other treatments. The same pattern was also noted for superoxide and MDA accumulation in the leaves of dune spinach, where 250 mM resulted in significantly higher oxidative stress than other treatments (Figure 3.5). However, it must be noted that plants watered with the lowest NaCl (50 mM) had the same leaf cell death, superoxide, and MDA content as the control plants.

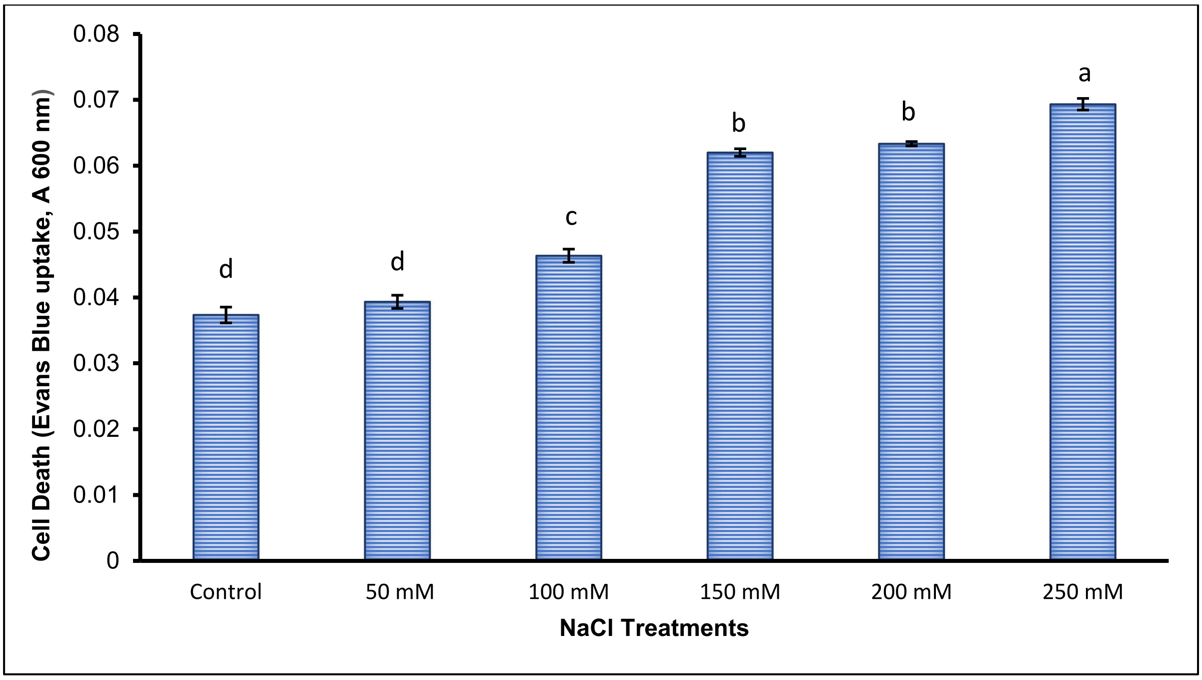

Figure 3.4: Cell viability of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$).

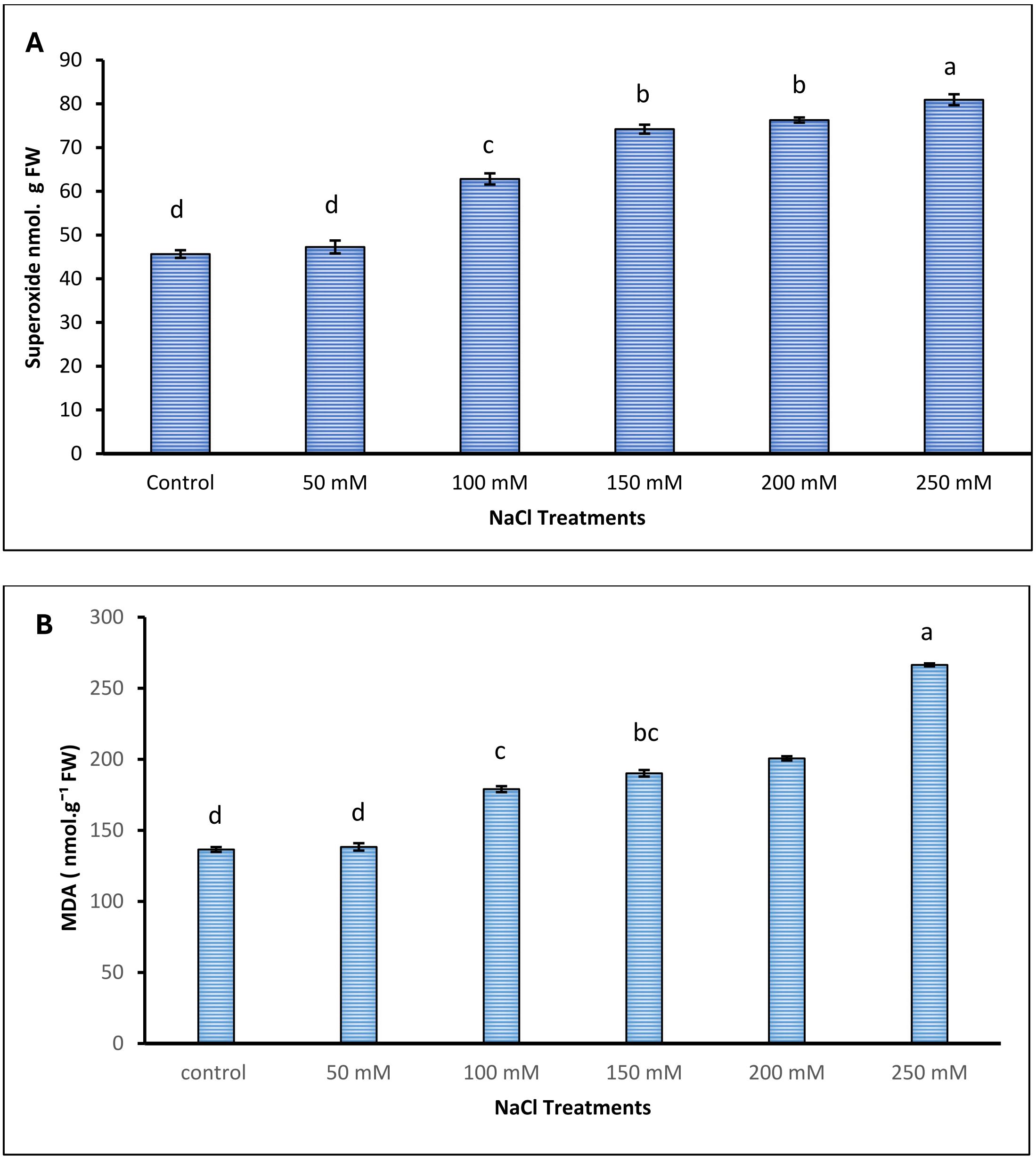

Figure 3.5: Superoxide (A) and MDA (B) of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$).

3.3.6. Activity of Antioxidant Enzymes

3.3.6.1. Superoxide Dismutase and Catalase

A substantial increase in catalase and superoxide dismutase activity with elevated NaCl concentrations was observed as illustrated in Figure 3.6. The highest activities of both enzymes were noted in plants subjected to 250 mM of NaCl irrigation.

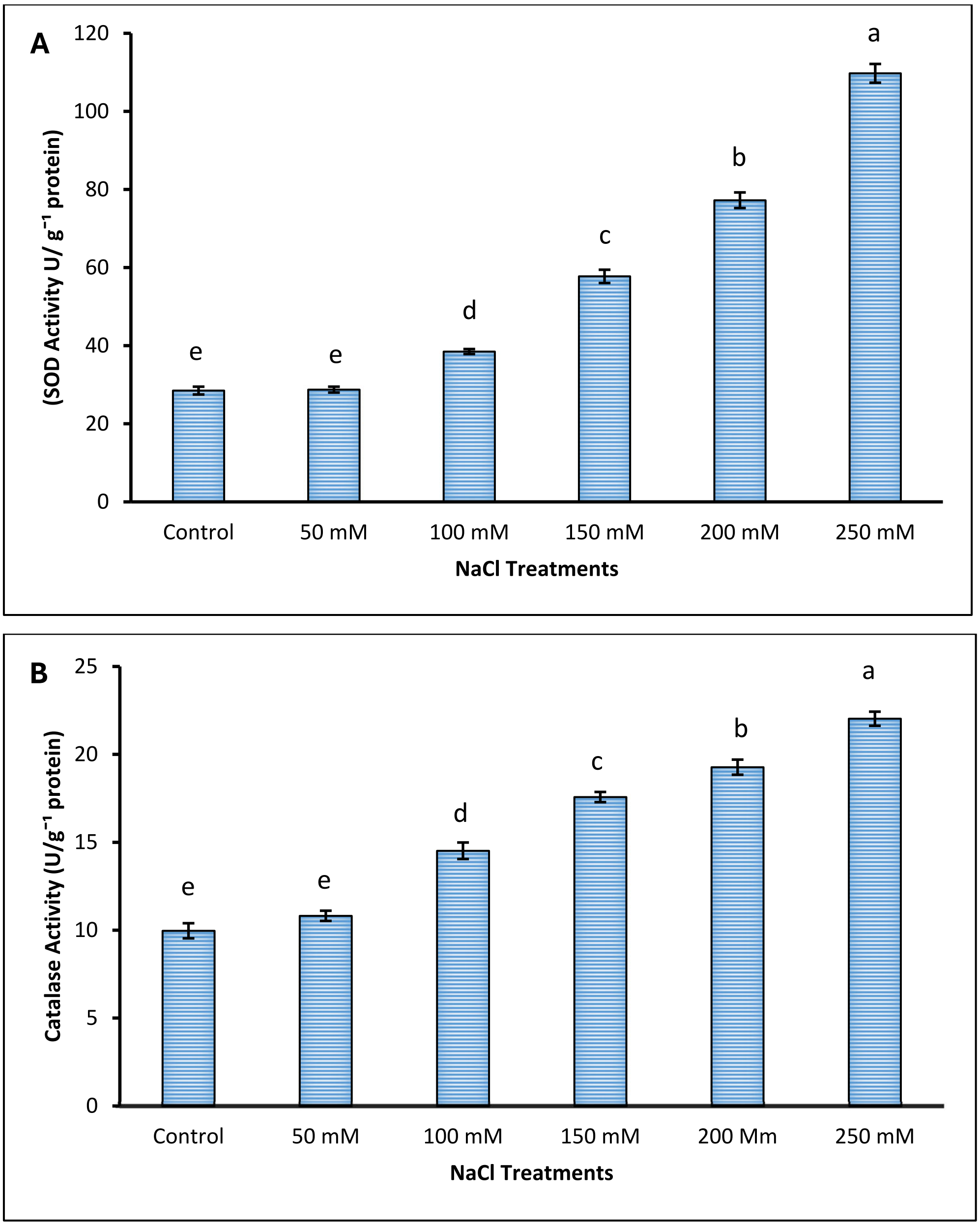

Figure 3.6: Superoxide dismutase (A) and Catalase (B) of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$).

3.3.7. Phytochemicals and Antioxidant Activity

3.3.7.1. Total Phenolic Content (TPC)

The phenolic content in dune spinach leaves varied significantly among treatments as presented in Table 2. The highest phenolic content was noted in plants irrigated with 250 mM NaCl and this was considerably higher than the values reported in control, 50, 100 and 150 mM NaCl.

3.3.7.2. Total Flavonoid Content (TFC)

Increasing NaCl concentrations optimised the flavonoids in the leaves of dune spinach. Plants irrigated with NaCl at 250 mM had substantially higher flavonoids than other treatments and the control (Table 3.2). The lowest flavonoid content was obtained in control and was equivalent with values reported for plants irrigated with 50 mM of NaCl.

3.3.7.3. Total Proanthocyanidins (TPD)

Treatments such as the control, 50, 100 and 150 mM of NaCl negatively affected the accumulation of proanthocyanidins since all these treatments had negative values, implying proanthocyanidins were not detected (Table 3.2). However, proanthocyanidin content was detected in plants subjected to 200 and 250 mM of NaCl irrigation and the recorded values were comparable.

3.3.7.4. DPPH

When assessing the DPPH scavenging activity within samples, plants irrigated with NaCl had significantly lower scavenging activity in comparison to the control and as such, the highest scavenging activity was noted in control plants.

Table 3.2: Quantification of phytochemicals and DPPH antioxidants in leaves of *T. decumbens*

Treatments	**TPC (mg GAE/g DW^{-1})**	**TFC (mg GE/g DW^{-1})**	**TPD (mg CE/g DW^{-1})**	**DPPH activity (μmol TE/g)**
Control	74.66±6.5 d	329.8±9.4 e	-13.9±2.6 c	72.5±1.3 a
50 mM	89.32±4.2 cd	339.9±7.3 e	-17.7±2.5 c	62.8±0.53 bc
100 mM	102.09±9.7 bc	447.1±6.2 d	-15.7±1.3 c	64.3±0.25 b
150 mM	114.42±2 ab	581.1±6.2 c	-3.1±0.9 b	61.2±0.13 c
200 mM	125.12±8.5 a	639.7±1.9 b	2.8±1.1 a	59±0.84 d
250 mM	128.63±6.1 a	798±9.3 a	6.46±1.2 a	61.5±0.53 c
F-statistics	9.84*	170.9*	6.69*	43.17*

Means with distinct letters vary substantially from one another ($p \leq 0.05$).

3.4. Discussion

Some plants dedicate substantial energy to multiple metabolic pathways to successfully navigate and mitigate the impact of extreme environmental challenges, such as salinity. *Tetragonia decumbens* has been reported to withstands both drought and salinity (Nkcukankcuka et al., 2021; Sogoni et al., 2021; Tembo-Phiri, 2019), however, its tolerance mechanisms and physiological responses are poorly understood. It therefore became imperative to research the impacts of salinity on growth development, leaf succulence, chlorophyll, cation concentration, oxidative stress markers, and antioxidant defence systems to elucidate its tolerance mechanisms. Furthermore, the antioxidant activity of salt-stressed *T. decumbens* and its therapeutic potential was assessed to obtain insight into the major processes conferring tolerance to salinity in this understudied plant.

3.4.1. Increasing Salinity Induced Growth Inhibition

Salinity is known to disrupts respiration, photosynthesis, and mineral uptake, resulting in decreased crop production and quality (Farooq et al., 2022; Rhaman et al., 2024). Nonetheless, halophytic species have been documented to complete their life cycles in saline environments ranging from mild to extreme salinity through osmotic adjustment (Zhao et al., 2020). In the current study, saline treatment enhanced growth development at 50 and 100 mM NaCl, while at higher salinity (200 and 250 mM NaCl), growth parameters reduced substantially in comparison to the control. However, it should be noted that even at these high salt concentrations (200 and 250 mM NaCl), plants stayed alive and showed no signs of toxicity such as chlorosis and leaf necrosis. Growth stimulation at low to moderate salinities have been reported in *T. decumbens* by Sogoni et al. (2021) and in close relative species *Tetragonia tetragoniodes* by Atzori et al. (2020) and Jeeva (2020). These findings suggest that *T. decumbens* can be regarded as a facultative halophyte due to its ability to grow in moderate saline conditions.

3.4.2. Leaf Hydration Response to Salinity

Relative water content (RWC) is an appropriate indicator of plant water level in various environmental conditions such as drought and salinity (Khatami et al., 2022). In this study, the leaf RWC remained constant from 0 to 100 mM of NaCl, then declined drastically at higher salinities (150, 200, and 250 mM). The capacity to maintain unaltered leaf RWC under mild salinity (0 to 100 mM of NaCl) shows that *T. decumbens* evolved suitable mechanisms to assure adequate water intake in saline soils. Ben Amor et al. (2020) earlier reported that the ability of dicotyledonous halophytes to tolerate salt is closely linked to their capacity to accumulate large amounts of osmolytes in their tissues. This allows them to regulate water osmotically by decreasing the osmotic potential in the cytoplasm. Osmotic adjustment is a mechanism that helps plants retain their ability to absorb water via their roots and keep their leaves firm even when the soil has limited water availability (Gul et al., 2024; Paulino et al., 2020).

Moreover, findings from this study revealed that salinity induced an increase in leaf succulency, while specific leaf area was reduced. These findings are corroborated by Atzori et al. (2020), who stated that enhanced leaf succulence (high water storage) is one of the defence strategies facultative halophytes deploy in response to low external water potential caused by salinity. In *Mesembryanthemum crystallinum, Sarcocornia quinqueflora,* and *Cakile maritima* grown under saline conditions, increased leaf succulence was reported as a mechanism to detoxify sodium ions and maintain cytoplasmic salinity below toxic levels (Ahmed et al., 2022; Yasseen & Al-Thani, 2022; Belghith et al., 2022). Based on these findings it can be inferred that *T. decumbens* plants treated with different salt treatments used this mechanism to avoid the toxic effect of sodium ions since all plants survived even at a higher salinity concentration.

3.4.3. Salinity Induced the Reduction of Photosynthetic Pigment

The total chlorophyll content is frequently employed as a salt tolerance index in plant species (Banakar et al., 2022; Sheng et al., 2024). Many plant species have been shown to have decreased photosynthetic activity and leaf pigments when exposed to salinity by means of reducing the leaf area (Khatri & Rathore, 2022). The decrease in chlorophyll content in *T. decumbens* under high salinity implies a method for protecting photosynthetic features from salt-induced damage and avoiding extreme production of reactive oxygen species (ROS). Similar patterns of chlorophyll reduction in halophytes such as *Nitraria schoberi* and *Lobularia maritima* were described by Zilaie et al. (2022) and Hsouna et al. (2020). This decrease could be attributed to disruptions in photosynthetic machinery, pigment malfunction, pigment-protein complex instability in the light-harvesting complex. Furthermore, high salt content causes ROS formation in chloroplasts, which breaks the double bonds of unsaturated fatty acids, causing chloroplast membrane damage and chlorophyll leakage from thylakoids (Azeem et al., 2023).

3.4.4. Leaf and Roots Cation Response to Salinity

When plants are exposed to salinity, ion transport and accumulation are altered as a result of nutritional imbalances induced by Na^+ and Cl^- ions competitors, which limit the uptake of nutrients such as Ca^{2+} and K^+ essential for optimal growth (Abbas et al., 2024). The reduction observed in the growth of *T. decumbens* under high salinity could be due to nutritional imbalances (Alabdallah et al., 2024). Increased Na^+ build-up in *T. decumbens* leaves was associated with a considerable decrease in K^+ and Ca^{2+} uptake, which was countered by an increase in K^+ and Ca^{2+} usage efficiency (Hualpa-Ramirez et al., 2024). As a result, when exposed to salt stress, *T. decumbens* was able to economically utilise K^+ for specified tasks, and K^+ could be substituted by Na^+ for osmotic adjustment by accumulating it in the vacuoles.

These findings suggest that *T. decumbens* employs an inclusion mechanism in which sodium ions accumulate in the leaves and are sufficiently diluted to keep cytoplasmic salinity below toxic levels. This suggests that efficient K^+ transport systems are also at action in *T. decumbens* cells to sustain mineral nutrition as well as to retain metabolic activity under saline conditions as found in several halophytes (Chen & Wang, 2024).

3.4.5. Oxidative Stress and Antioxidant System Activation

Several biochemical markers, including cell death viability, superoxide, and lipid peroxidation, are commonly employed to determine the extent of oxidative stress in plants growing under adverse conditions (Jameel et al., 2024). In the present study, cell death viability, superoxide and lipid peroxidation were all increased with increasing saline treatment, with a more pronounced influence at 250 mM of NaCl. High production of ROS that exceeds the plant controllable limit is known to damage cellular structures and membranes, which could be the reason for the substantial decline in plant growth with increasing salinity. These findings are respectively corroborated by Azeem et al. (2023) and Parvez et al. (2020) on *Moringa oleifera* and *Chenopodium quinoa* genotypes subjected to salt stress, where increasing saline treatment caused a substantial increase in oxidative stress markers leading to reduced plant growth.

However, plants have evolved defence mechanisms such as antioxidative enzymes followed by the production of non-enzymatic compounds such as phenolic compounds to combat the catastrophic effect of oxidative stress. Among these different types of antioxidative enzymes, superoxide dismutase is considered to be the primary source of defence against oxidative stress in plants (Gill et al., 2015; Zulfiqar & Ashraf, 2022). This enzyme catalyses the transformation of two O_2 radicals into H_2O_2 and O_2. Alternatively, various antioxidant enzymes, such as catalase and peroxidase (POX), convert H_2O_2 to water. Halophytic plants tend to accumulate high amount of SOD under salt stress, a feature that provides an adaptive advantage over salt-sensitive species (Huang et al., 2019; Zhao et al., 2020). In this study, high activity of SOD and CAT with increasing saline treatment was observed, confirming that *T. decumbens* uses these two enzymes as the first line of defence against ROS caused by salinity. The same observation has been reported in other halophytes such as *Bupleurum tenuissimum, Chenopodium quinoa*, *Kandelia obovate* and *Mesembryanthemum crystallinum* (González-Orenga, Leandro, et al., 2021; Hasanuzzaman et al., 2021; Mohamed et al., 2021; Parvez et al., 2020).

Halophytes are said to activate the accumulation of non-enzymatic compounds to scavage these highly toxic species when excess toxic ROS are not eliminated by the antioxidative enzymes. In this study, *T. decumbens* accumulated high quantities of polyphenols and flavonoids with increasing salinity, while proanthocyanidins were only detected in samples of *T. decumbens* irrigated with the highest salinities (200 and 250 mM NaCl). This response has been reported in many halophytes, where increasing saline irrigation induced the increase of phenols and flavonoids to scavenge highly toxic free radicals (Kumari et al., 2017). In contrast to the rise in polyphenols, flavonoids, and proanthocyanidins, antioxidant activity (DPHH) in *T. decumbens* leaves was substantially lower in saline-treated plants compared to control. These findings substantiate previous results of Sogoni et al. (2021) on *T. decumbens* where increasing NaCl treatment lowered the antioxidant activity.

3.5. Conclusion

The results of this study revealed a substantial growth enhancement in plants irrigated with lower or no NaCl dosage up to 100 mM. High concentrations of NaCl reduced growth and total chlorophyll content, but all plants remained alive without signs of toxicity. The reported high quantities of Na^+ in the leaves than in roots of *T. decumbens* indicated an ion inclusion mechanism. In this regard, the absence of dehydration and increased leaf succulence in salt-treated plants could be attributed to efficient salt ion compartmentalization. To curb the oxidative damage in plant tissues at high NaCl, *T. decumbens* regulated the production of antioxidative enzymes (CAT and SOD) and non-enzymatic compounds (polyphenols, flavonoids and proanthocyanidins) to scavenge the reactive oxygen species liable for cellular damage and reduced growth. These findings show that *T. decumbens* can withstand salinity up to 250 mM of NaCl by modulating morpho-physiological features, antioxidant defence systems, and managing ion toxicity and oxidative stress effectively. This connotes its potential use in bio-saline agriculture, particularly in semi-arid and dry regions where seawater could be diluted and used in the cultivation of this species.

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CHAPTER FOUR

SALINITY INDUCED LEAF ANATOMICAL RESPONSES AND CHEMICAL COMPOSITION IN *TETRAGONIA DECUMBENS* MILL.: AN UNDERUTILIZED EDIBLE HALOPHYTE IN SOUTH AFRICA

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Salinity induced leaf anatomical responses and chemical composition in *Tetragonia decumbens* Mill: An underutilized edible halophyte in South Africa

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Abstract

Tetragonia decumbens has recently been reported to withstand the adverse effect of salinity. However, its leaf anatomical responses are poorly understood since previous studies were focused on basic physiological and biochemical parameters. This study was designed to examine leaf micromorphological traits and internal leaf elemental compartmentalization using Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) to elucidate relative salinity tolerance mechanisms. Salt concentrations were applied to six treatments by increasing the concentrations of NaCl in a nutrient solution. The control treatment (0 mM) was irrigated solely by the nutritional solution, whereas other treatments contained graded NaCl concentrations (50, 100, 150, 200, and 250 mM). Micromorphological examination of the adaxial layer of the epidermis revealed distinctive glandular peltate trichomes among treatments. In control plants, the trichomes were flaccid and not easily detectable which resulted in low trichome density. While plants treated with salinity had uprooted trichomes which were modified to dish-like structures as salinity increases, with a more pronounce visibility at the highest salinity treatment (250 mM). On the contrary, increasing salinity reduced the stomatal density as well as stomatal opening with a more pronounced effect at 250 mM. Furthermore, the EDX revealed the presence of important elements such as potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and chlorine (Cl) which are responsible for salt tolerance in many species. Na increased with increasing saline treatment up to 100 mM then declined drastically. The lowest Na was detected in control plants which was comparable to plants irrigated with 150, 200 and 250 mM respectively. Likewise, the lowest chlorine content was detected in control plants while it increased in saline treated plants up to 150 mM and declined in plants irrigated with 200 and 250 mM respectively. When assessing K quantification, saline treatment drastically reduced K content with increasing saline irrigation. Contrarywise, the Mg content increased with saline irrigation up to 100 mM and was not detected in plants irrigated with 200 and 250 mM respectively. Interestingly, Ca was only detected in plants irrigated with 150, 200 and 250 mM respectively. These findings validate that *T. decumbens* can tolerate salinity by modulating anatomical

features such as trichomes, control of stomatal aperture and effectively managing ion toxicity with increasing salinity.

Keywords: Dune spinach; Energy dispersive x-ray; Glandular peltate trichomes; Salt glands; Salt tolerance; Scanning Electron Microscopy

4.1 Introduction

In arid and semi-arid regions of the world including South Africa, annual rainfall is limited and unequally distributed over the years (Luis de la Fuente et al., 2023; Salami et al., 2022). The use of low-quality recycled or reclaimed wastewater, and to some extent, even brackish water for irrigation is becoming more common in the production of edible crops (Atzori et al., 2020). However, this contributes to elevated soil salinity, which has been reported as a worldwide problem and poses a serious threat to world agriculture (Mangal et al., 2022; Khatri & Rathore, 2022; Zuluaga et al., 2023). The use of resistant crops such as halophytes is vital to meet the growing food demands in areas affected by both salinity and drought (Calone et al., 2022; Pérez-Romero et al., 2023; Grigore & Vicente, 2023).

The edible halophyte *Tetragonia decumbens* commonly known as dune spinach, is a leafy vegetable belonging to the Aizoaceae family which is largely distributed along the coastal areas of South Africa (Sogoni, 2020; Nkcukankcuka et al., 2021; Sogoni et al., 2022). Recently, the species has been reported to have enhanced growth and yield under saline cultivation (Sogoni et al., 2021). To cope with these conditions*, T. decumbens* modulated morpho-physiological features (unchanged chlorophyll content, K^+ homeostasis), antioxidant defence systems (increased polyphenols and ferric reducing antioxidant power) and effectively managing ion toxicity.

These adaptation strategies have also been reported in many halophytic plants cultivated under saline conditions (Hussain et al., 2015; Peng et al., 2019; Hussain et al., 2023). However, avoiding ion toxicity appears to be a crucial survival mechanism which is achieved by the development of specialised structures such as salt glands, and or trichomes responsible for the regulation of internal salt load for ion excretion and storage (Dassanayake & Larkin, 2017). Species like *Mesembryanthemum crystallinum, Atriplex portulacoides, Chenopodium quinoa* and *Salvadora persica* have been reported to possess bladder cells, or specialised structures where ions are sequestered under saline conditions (Agarie et al., 2007; Shabala, 2013; Parida et al., 2016) while in *Sporobolus ioclados, Lasiurus scindicus* and *Salsola imbricata,* anatomical modifications observed were thick cuticle and epidermis, smaller and fewer stomata, higher proportion of storage tissues, and thickened endodermis (Naz et al., 2016; Naz et al., 2018; Naz et al., 2022). Nevertheless, in *T. decumbens* such modifications are not reported in literature. Thus, anatomical studies are needed to give better insight on the adaptation of this species under saline conditions.

Leaf anatomical modifications have previously been analysed using scanning electron microscopy (Shah et al., 2018; Jimoh et al., 2019). Scanning electron microscopy (SEM) is performed at high magnifications that generate high-resolution images with high levels of precision. There have been several SEM studies examining the shape, distribution, and density of specialized structures responsible for the excretion or accumulation of ions in various halophytes (Liu et al., 2022; Zhao et al., 2022). In addition, the energy dispersive spectroscopy has also been used to detect or identify elemental composition in plant tissues to elucidate the movement of elements from the roots to the leaves under saline conditions (Chavarria et al., 2020). This study was therefore undertaken to examine leaf micromorphological traits and internal leaf elemental compartmentalization using Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) to elucidate relative salinity tolerance mechanisms in *T. decumbens*.

4.2. Materials and Methods

4.2.1. Experimental location

The cultivation experiment was conducted in the research greenhouse of the Horticultural Science Department of the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa. The greenhouse temperature was set to range between 21 and 26 °C during the day and 12-18 °C at night, with a relative humidity of 60%. Under natural light conditions, the daily average photosynthetic photon flux density (PPFD) was 420 $\mu mol/m^{-2}s^{-1}$, with the intensity peaking at 1020 $\mu mol/m^{-2}s^{-1}$. The photoperiod corresponds to the prevalent conditions of early spring to summer.

4.2.2. Plant preparation and treatments

Young plants of *T. decumbens* were obtained using softwood cuttings as described by (Sogoni et al., 2021). Uniformly rooted cuttings (180) were individually transplanted in 12.5 cm plastic pots with drainage holes underneath containing a mixture of peat and sand (1:1) and were placed in a greenhouse. During this period, the plants were irrigated with a complete nutrient solution (Nutrifeed™ manufactured by STARKE AYRES Pty. Ltd. Gauteng, South Africa) three times a week. After 10 days of growth, the established cuttings were watered with clean water for 5 days to wash off any salt residue and, thereafter, were organized into six treatments each containing 10 duplicates and the experiment was repeated three times (n= 180). Salt concentrations were set up on five treatments by adding increasing concentrations of NaCl in the nutrient solution (50, 100, 150, 200 and 250 mM). To prevent ionic shock on plants, salt treatments were gradually increased by 50 mM up to 250 mM daily. A total of 300 mL of the nutrient solution was prepared for each plant with and/or without NaCl. When the maximum salinity was reached the plants were then watered every two days. The control treatment was sustained and irrigated only by the nutritive solutions. To ensure that the appropriate salinity levels in each pot were maintained, drain water from each pot was quantitatively collected and the electrical conductivity was measured. After two months of growth under saline condition, leaf samples were harvested and prepared for micromorphological analysis.

4.2.3. Leaf examination under scanning electron microscopy (SEM) and Energy dispersive X-ray (EDX)

The examined leaves were cut in 1cm x 1cm pieces with a minora blade and placed immediately in 2.4% glutaraldehyde (GLA) for 4 hours (Jimoh et al., 2019). Leaves were then dehydrated in graded ethanol (50, 70, 90 and 100 %) for 30 minutes per rinse and dried with hexamethyldisilazane (HMDS) drying liquid. Dried specimens were mounted on aluminium stubs with double sided carbon tape and coated with a thin layer of carbon, Quorum Q150TE carbon coater (15nm thick). This was done to make the sample surface electrically conductive to avoid electron build-up on the sample surface which can cause electron charge. The samples were then loaded in a Zeiss MERLIN Field Emission Scanning Electron Microscope/Zeiss EVO MA 15 operating at an accelerated voltage of 20kV at different magnifications. A Zeiss 5-diode Back Scattered Electron (BSE) Detector (Zeiss NTS BSD) and Zeiss Smart SEM software were used to generate BSE images. The samples were chemically quantified by semi-quantitative/full quantitative Energy Dispersive X-Ray Spectrometry (EDX) using an Oxford Instruments® X-Max 20 mm2 detector and Oxford Aztec software/INCA Oxford software. Samples were analysed in area and spot mode. The area mode was taken at 250 magnifications, whereas the spot analyses were focus on the bright particles and other particles of interest including background spot analyses. The physical limitations of EDX do not allow for the analysis of elements lighter than boron therefore the elements hydrogen, helium, lithium, and beryllium cannot be analysed. The chemical composition was expressed in weight %.

The adaxial layer of the leaf epidermis was examined qualitatively and quantitatively. The quantitative features studied were the number of trichomes, trichome density, number of stomata, stomatal density, and the percentage of open and closed stomata. While the qualitative characteristics considered were trichome types, size, shape, and distribution on the leaf surface.

4.2.4. Statistical analysis

Leaf samples from each treatment were randomly selected and used for SEM and EDX examination. The data obtained was expressed as mean values and standard errors (SE) of 3 replicates and were analysed using one-way ANOVA followed by Fishers least significant test at a significance of $p \leq 0.05$. The analysis was carried out using the STATISTICA application, version 13.5.0.17.

4.3. Results

4.3.1. Micromorphological features under scanning electron microscopy.

4.3.1.1. Trichome size, shape, and distribution

Micromorphological examination of the abaxial layer of the epidermis revealed distinctive differences in response to increasing salinity. Glandular peltate trichomes were detected emerging from the epidermis of all treatments but were distinctive in size, shape, and distribution (Figure 4.1). In control plants, the trichomes were flaccid and not easily detectable which resulted in low trichome density. On the contrary, plants irrigated with salinity had more pronounced trichomes which differed in size and shape. Moreover, plants treated with the lowest salinity (50 mM) had uprooted trichomes when compared to the flat structures observed under control. These uprooted trichomes were modified to dish-like peltate structures as salinity increases, with a more pronounce effect at highest salinity treatment (250 mM NaCl).

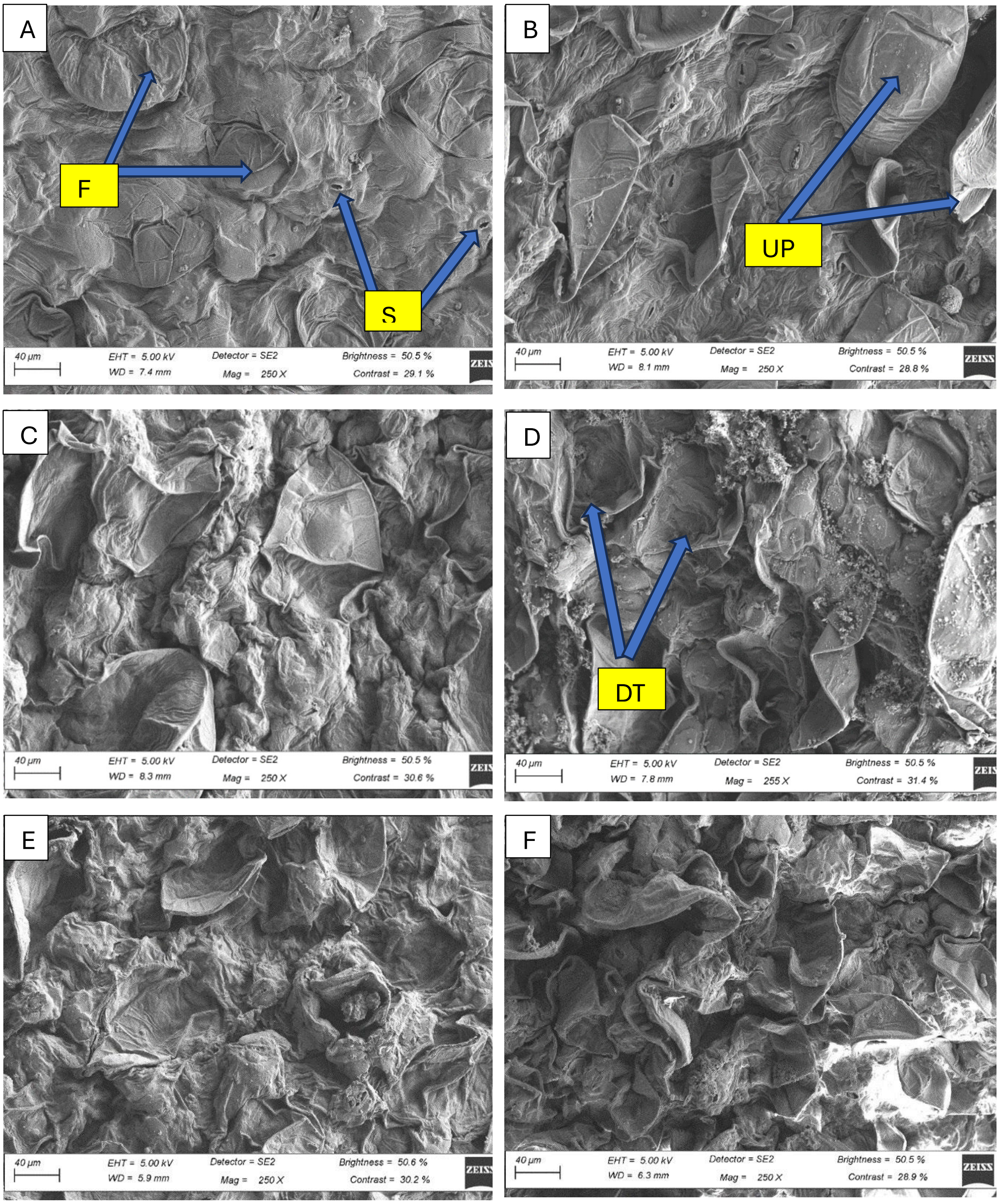


Figure 4.4: Plate A- Control, B- 50 mM NaCl, C- 100 mM NaCl, D- 150 mM NaCl, E- 200 mM NaCl and F- 250 mM NaCl shows SEM images of the abaxial leaf surface of *T. decumbens* under varying salinity treatments depicting stomatal and trichome density. Note: FT (Flaccid trichomes), ST (Stomata), UPT (Uprooted trichomes), DT (Dish-like trichomes)

4.3.1.2. Stomatal and trichome density

Saline treatment significantly influenced both the stomatal and trichome density (Table 4.1). The highest stomatal density was recorded in control plants, and this was significantly higher than all saline treatments. However, when assessing the trichome density, all plants treated with salinity had significantly higher trichome density than the control, with the highest being recorded in plants irrigated with 250 mM NaCl (Table 4.1).

Table 4.1: Effect of salinity on stomatal and trichomes density

Treatments	Stomatal density (mm^2)	Trichome density(mm^2)
Control	12.9±0.65a	4.2±0.24e
50 mM NaCl	5.7±0.24b	6.4±0.23d
100 mM NaCl	4.5±0.43bc	8.1±0.43bc
150 mM NaCl	3.2±0.24cd	7.4±0.42cd
200 mM NaCl	2.7±0.24d	9.1±0.23b
250 mM NaCl	2.8±0.41d	12.1±1a

Means that do not share a letter are significantly different at $p \leq 0.05$.

4.3.1.3Stomatal opening and closure

The microscopic examination of the stomatal opening revealed a distinctive difference among treatments (Figures 4.1 and 4.2). The control plants had the highest percentage of stomatal opening which was significantly higher than other saline treatments but was comparable to plants irrigated with 50 mM NaCl. On the contrary, increasing salinity modulated stomatal closure with more pronounced effect in plants irrigated with 200 and 250 mM NaCl.

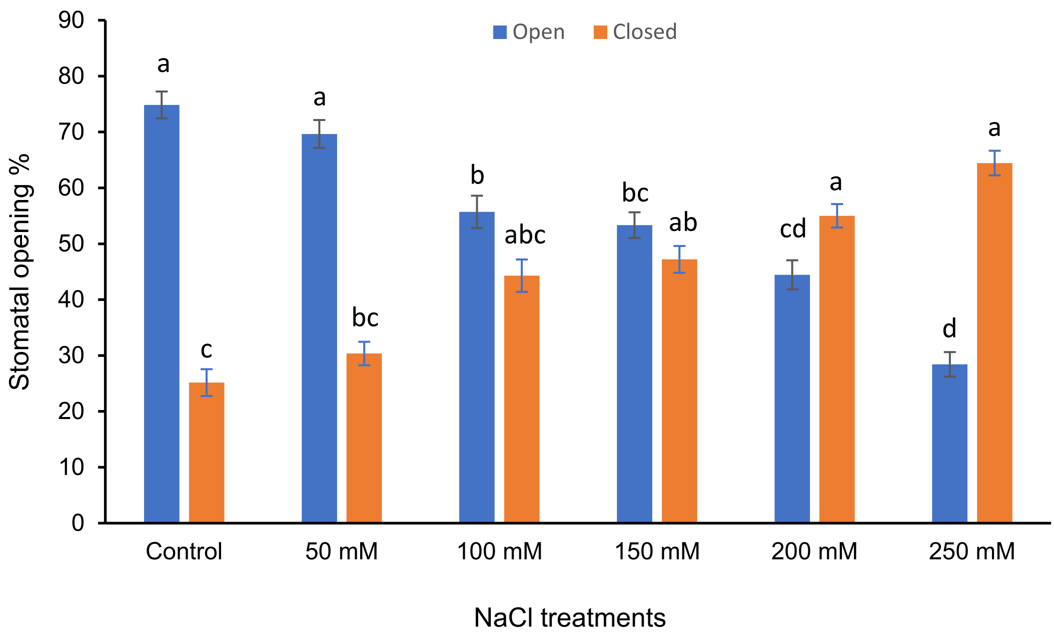


Figure 4.5: Effect of salinity on stomatal opening

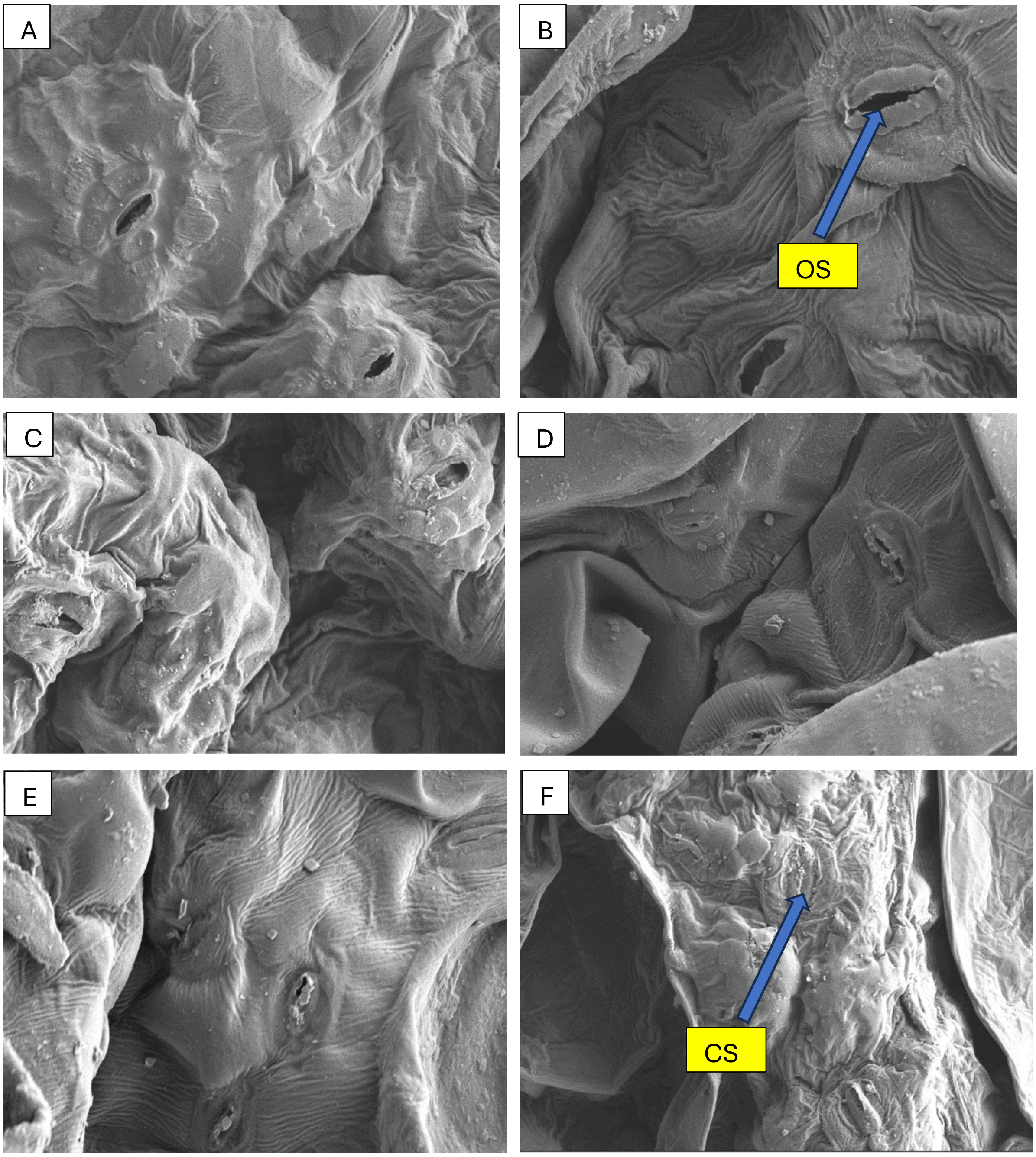


Figure 4.6: Plate A- Control, B- 50 mM NaCl, C- 100 mM NaCl, D- 150 mM NaCl, E- 200 mM NaCl and F- 250 mM NaCl depicts stomatal aperture among treatments. Note: OST (Open stomata), CST (Closed stomata)

4.3.2. Chemical elements detected by energy dispersive x-ray (EDX) on the leaf surface.

The X-ray spectra from EDX analysis revealed that saline treatment modulated the distribution of chemical elements on the leaf surface of *T. decumbens* (Table 4.2). Oxygen was found to be abundant in plants irrigated with 50 mM NaCl, while those irrigated with 150 mM had the least amount of oxygen. On the contrary, sodium increased with increasing saline treatment up to 100 mM then declined drastically. The lowest sodium was detected in control plants which was comparable to plants irrigated with 150, 200 and 250 mM respectively. Likewise, the lowest chlorine content was detected in control plants while it increased in saline treated plants up to 150 mM and declined in plants irrigated with 200 and 250 mM respectively. When assessing the potassium quantification, potassium content reduces with increasing saline irrigation. Contrarywise, the magnesium content increased with saline irrigation up to 100 mM and was not detected in plants irrigated with 200 and 250 mM respectively. Interestingly, calcium was only detected in plants irrigated with 150, 200 and 250 mM respectively.

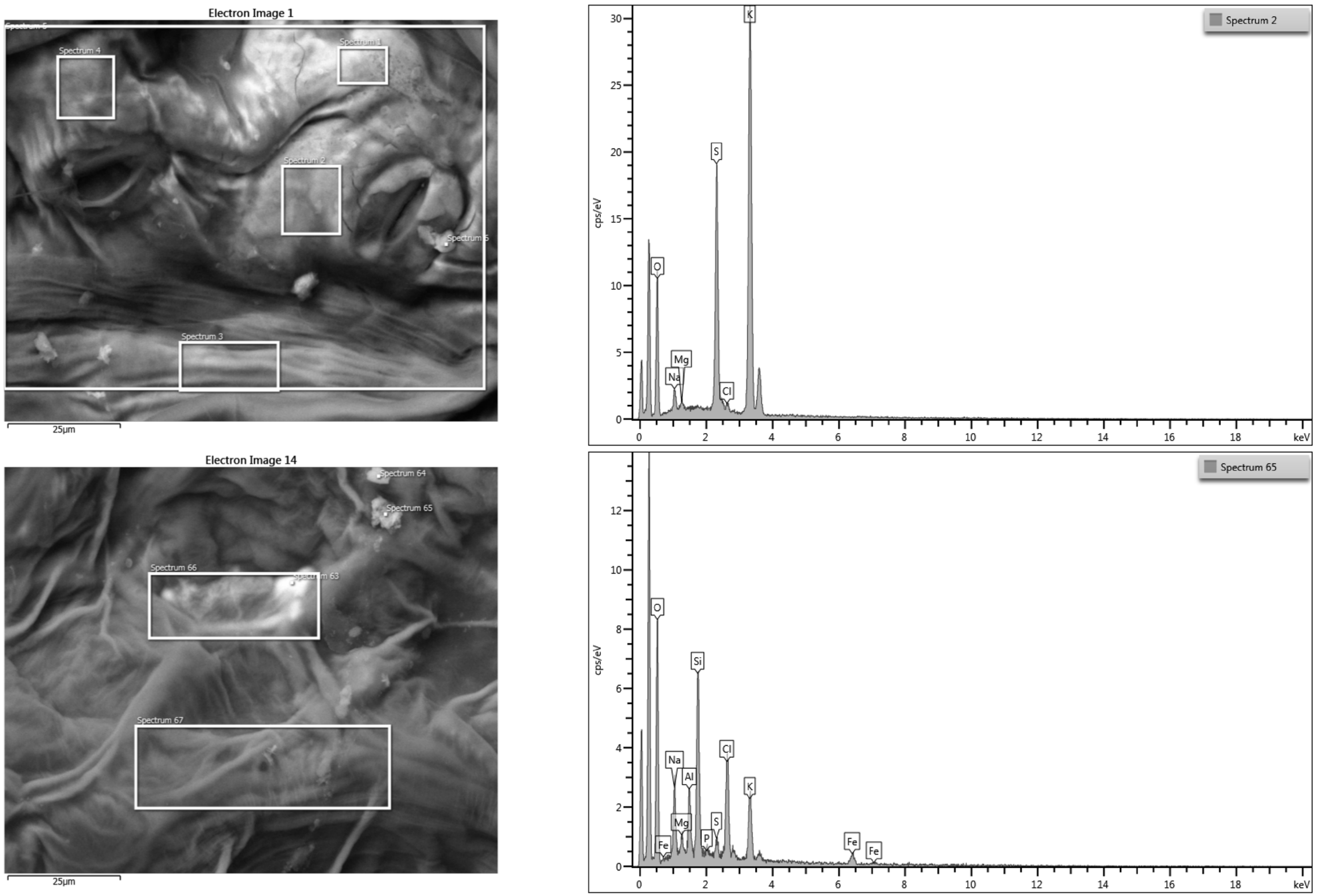
Table 4.2: Weight % of chemical elements detected on the leaf surface of *T. decumbens* using energy dispersive spectroscopy.

Elements	Control	50 mM	100 mM	150 mM	200 mM	250 mM
Oxygen	27.4±1.2bc	50.5±2.8a	34.1±0.9b	14.2±1.2d	45.8±5a	23±0.7c
Sodium	1.3±0.3c	8.9±0.9b	14.5±1.3a	2.8±0.1c	2.8±0.2c	1.5±0.09c
Magnesium	0.5±0.1b	1.3±0.1a	1.08±0.08a	0.3±0.1bc	ND	ND
Calcium	ND	ND	ND	0.7±0.01c	2.2±0.2b	4.6±0.3a
Chlorine	0.8±0.01d	10.9±1.6b	14.5±1.2a	16.14±1.1a	10.5±1.6b	4.8±0.6c
Potassium	38.9±1.8a	8.2±1b	6.5±0.6bc	5.5±0.4bc	3.8±0.9bc	1±0.09c

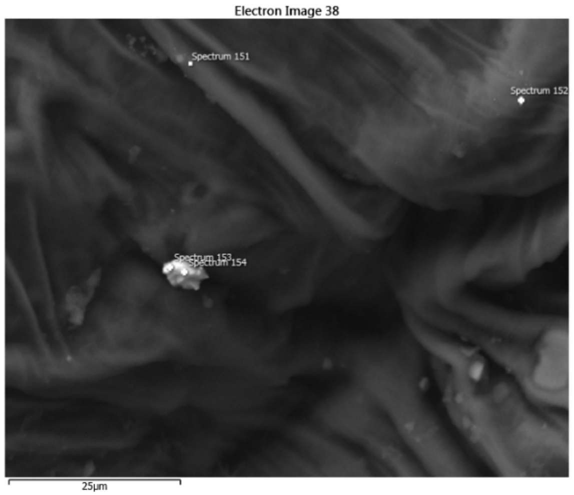
Note: ND= not detected while means that do not share a letter are significantly different at $p \leq 0.05$

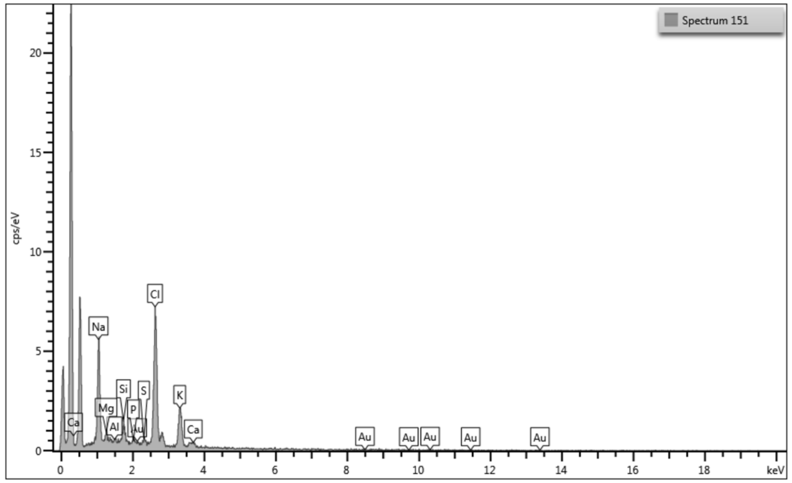
Control

50 mM

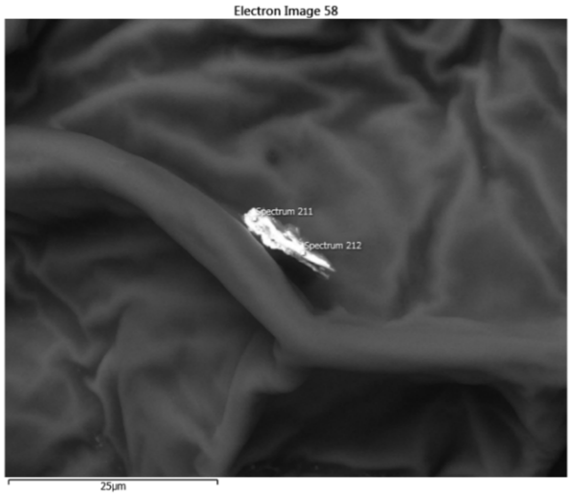


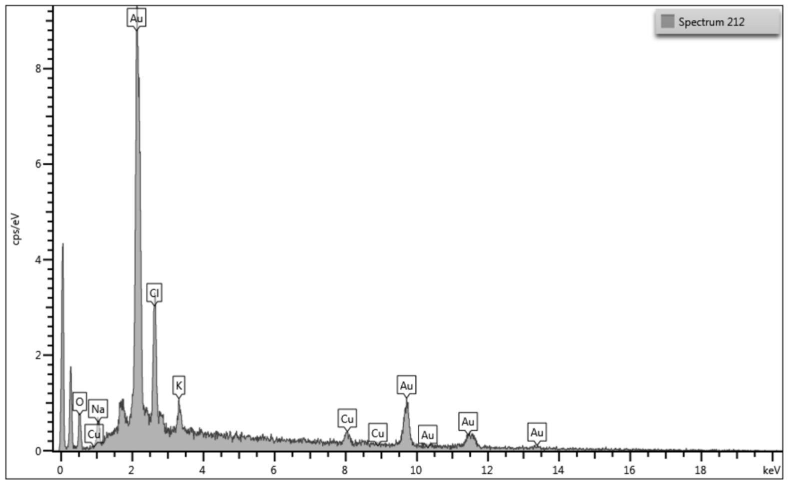
100 mM NaCl





150 mM NaCl





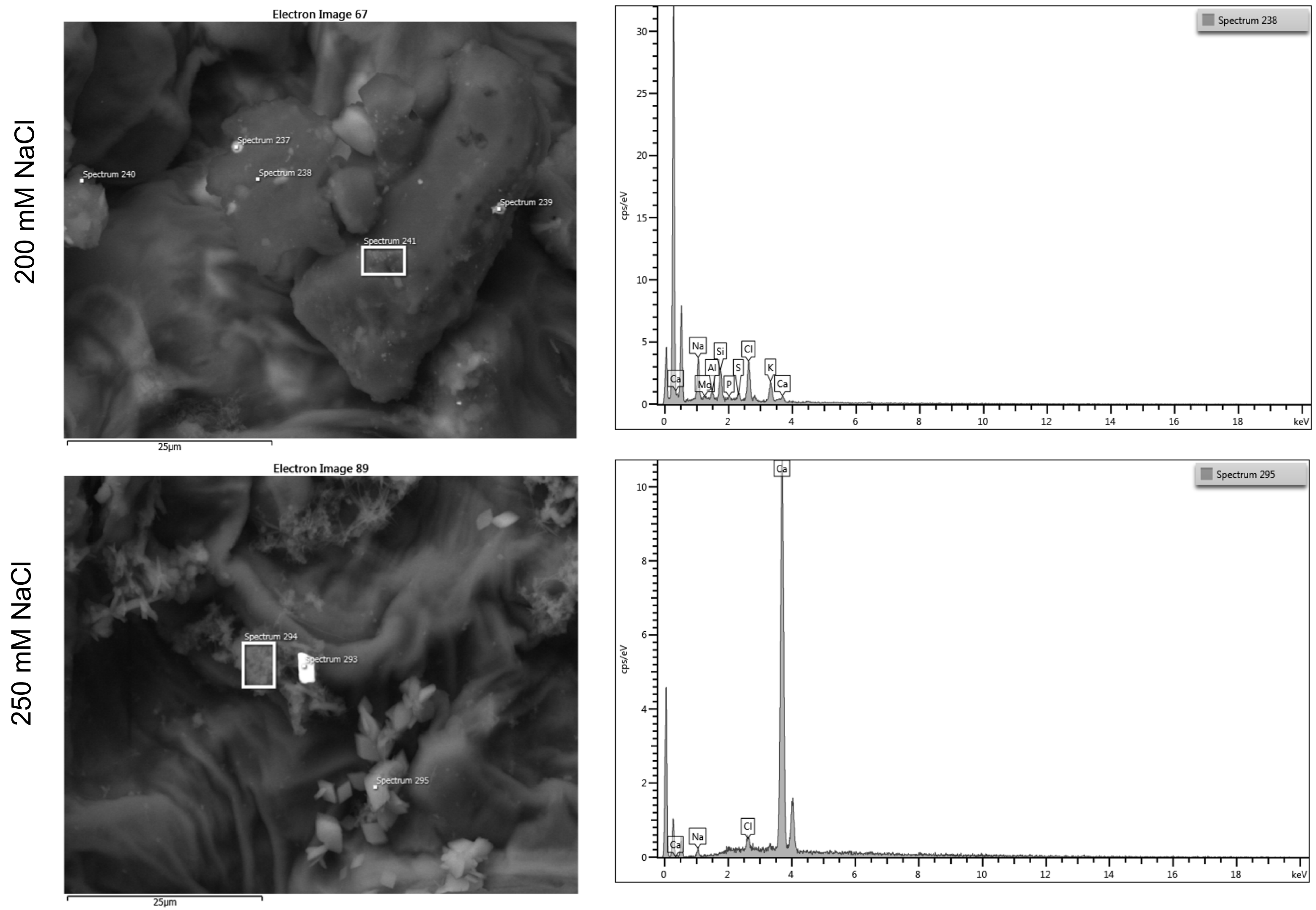


Figure 4.4: Randomly selected plates of each treatment illustrating the spectra of chemical elements detected on the leaf surface of *T. decumbens* using energy dispersive spectroscopy

4.4. Discussion

In this study, leaf micromorphological traits and internal leaf elemental compartmentalization aspects were investigated to elucidate the anatomical modulation subject to relative salinity tolerance mechanisms in *T. decumbens*. The results revealed for the first time, the presence of peltate trichomes on the abaxial layer of the epidermis. These modified structures have been reported to reduce heat load, increase tolerance to freezing, enhance water absorption, and are responsible for the storage and or excretion of toxic ions under saline conditions (Kuster et al., 2020). In the present study, peltate trichomes were more visible and enhanced in plants irrigated with increasing salinity. This suggests that *T. decumbens* utilises trichomes to store excessive Na^+ and Cl^-. These findings are supported by Tarchoune et al. (2015) on *Ocimum basilicum* and Lungoci et al. (2022) on *Nepata cataria*, where an increase in trichome density were reported under salt stress. Moreover, salt stress also induced stomatal closing as well as a decrease in stomatal density. The closing of stomata and water use efficiency under saline conditions has been reported in halophyte species such as *Chenopodium quinoa* and *C. album* (Rasouli et al., 2021). In *T. decumbens,* the stomatal closure under increasing salinity might be a strategy to optimize the balance between CO_2 assimilation and transpiration rate and potentially reduce salt transport to the leaves driven by transpirational water fluxes (Bertolino et al., 2019; Lawson & Vialet-Chabrand, 2019). Moreover, such responses enable plants to cope with adverse environmental conditions and provide superiority with regards to CO_2 uptake and water loss prevention (Kübarsepp et al., 2020).

The energy dispersive X-ray showed variation in the distribution of elemental crystals present in the leaf of *T. decumbens* as influenced by salinity. The EDX analysis revealed an increase of Na^+ and Cl^- with increasing salinity up to 100 mM, then a drastic decline was observed under 150 to 250 mM NaCl. This shows that the detected trichomes under increasing salinity have water and NaCl storage functions for osmotic adjustments in the vacuole, which enable this species to dilute excessive salt. This behaviour was also reported in *Mesembryanthemum crystallinum* bladder cells, where water and Na^+ were stored in the vacuole for osmotic adjustment and salt dilution which promotes plant growth under salt stress (Loconsole et al., 2019). Nevertheless, increasing salinity reduced the uptake of K^+ and Mg^{2+}. These two elements are responsible for a variety of physiological activities, including enzyme activation and the regulation of cation-anion balance (Pompelli et al., 2021; Rahman et al., 2021). Since K^+ is the most essential inorganic osmotic component in plant cells, adequate K^+ supply is critical for regulating turgor-driven processes such as stomatal movement and cell elongation (Tränkner et al., 2018; Bezerra-Neto et al., 2022). Even though K^+ transport was reduced under salt stress, *T. decumbens* managed to supply adequate K^+ for stomatal movement. This may be achieved by maintaining intracellular K^+ ion homeostasis by controlling the Na^+ influx into the cell, enhancing the Na^+ exclusion out of the cell, and maximizing the compartmentalization of Na^+ into the vacuole (Balasubramaniam et al., 2023).

Interestingly calcium ion was only detected in plants irrigated with the high saline concentrations (150, 200 and 250 mM NaCl). The non-detection of this ion in lower saline treatments might be due to the absence of the ion or sensitivity of the method used for energy dispersive x-ray spectrometry. Nevertheless, this element (Ca^{2+}) is required for processes that maintain the structural and functional integrity of plant cell membranes such as cell wall structure stabilization, ion transport and selectivity regulation, and control of ion-exchange behaviour and cell wall enzyme activities (Tian et al., 2015; Thor, 2019; Song et al., 2022). In the present study, the high concentration of Ca^{2+} in plants irrigated with increasing salinity is likely to be associated with cell wall structure stabilization and compartmentalization of Na^+ in the vacuole. These results are consistent with the findings of Ellouzi et al. (2011) where salinity did not affect calcium uptake in *Cakile maritima*. However, further physiological studies will be required to demonstrate this conclusively. These results support the idea that *T. decumbens*, may utilize one or more different mechanisms for coping with salinity stress, including specialised ion selectivity and or translocation of Ca^{2+} over Na^+.

4.5. Conclusion

This is the first microscopic study that describe leaf micromorphology and its chemical composition in *T. decumbens* under saline condition using SEM and EDX. For the first time the presence of peltate trichomes on the abaxial layer of the epidermis were observed as well as stomal closure with increasing salinity treatment. Moreover, high concentration of salinity decreased the weight percentage of crucial elements such as K and Mg while Na^+, Cl^- and Ca^{2+} increased. Based on these results, it is evident that this species may have utilized one or more different mechanisms for coping with salinity stress, these include the use of specialised trichomes to store or dilute excessive salt as well as the selective uptake of Ca^{2+} or translocation affinity over Na^+ may have contributed in limiting sodium uptake and accumulation in plant tissues. These findings validate that *T. decumbens* can tolerate salinity by modulating anatomical features such as trichomes, control of stomatal opening and effectively managing ion selectivity.

4.6. Author contribution statement

Conceptualisation, M.O.J. and C.P.L.; methodology, A.S., M.O.J., L.K. and C.P.L.; software, M.O.J. and A.S.; validation, M.O.J., M.K., L.K. and C.P.L.; formal analysis, A.S. and M.O.J.; investigation, A.S. and M.O.J.; resources, C.P.L., M.K. and L.K.; data curation, A.S. and M.O.J.; writing-original draft preparation, A.S..; writing, review and editing, A.S., M.O.J., M.K., L.K. and C.P.L.; supervision, A.S., M.O.J. and C.P.L.; project administration, C.P.L. and L.K.;

funding acquisition, C.P.L. and L.K. All authors have read and agreed to the final version of the manuscript.

4.7. Acknowledgement

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4.8. Compliance with ethical standards

This study was approved by the Office of the Chairperson, Research Ethics Committee, Faculty of Applied Sciences, Cape Peninsula University of Technology. Reference no: 213032120/09/2022

4.9. Conflict of interest

Authors wish to declare no conflict of interest.

4.10. Data Availability

All data are included in the manuscript.

4.11. References

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CHAPTER FIVE

COMPARING THE NUTRITIONAL, PHYTOCHEMICAL, AND ANTI-MICROBIAL POTENTIAL OF WILD AND CULTIVATED *TETRAGONIA DECUMBENS* MILL.: A PROMISING LEAFY VEGETABLE FOR BIO-SALINE AGRICULTURE IN SOUTH AFRICA

Comparing the nutritional, phytochemical, and anti-microbial potential of wild and cultivated *Tetragonia decumbens* Mill.: A promising leafy vegetable for bio-saline agriculture in South Africa

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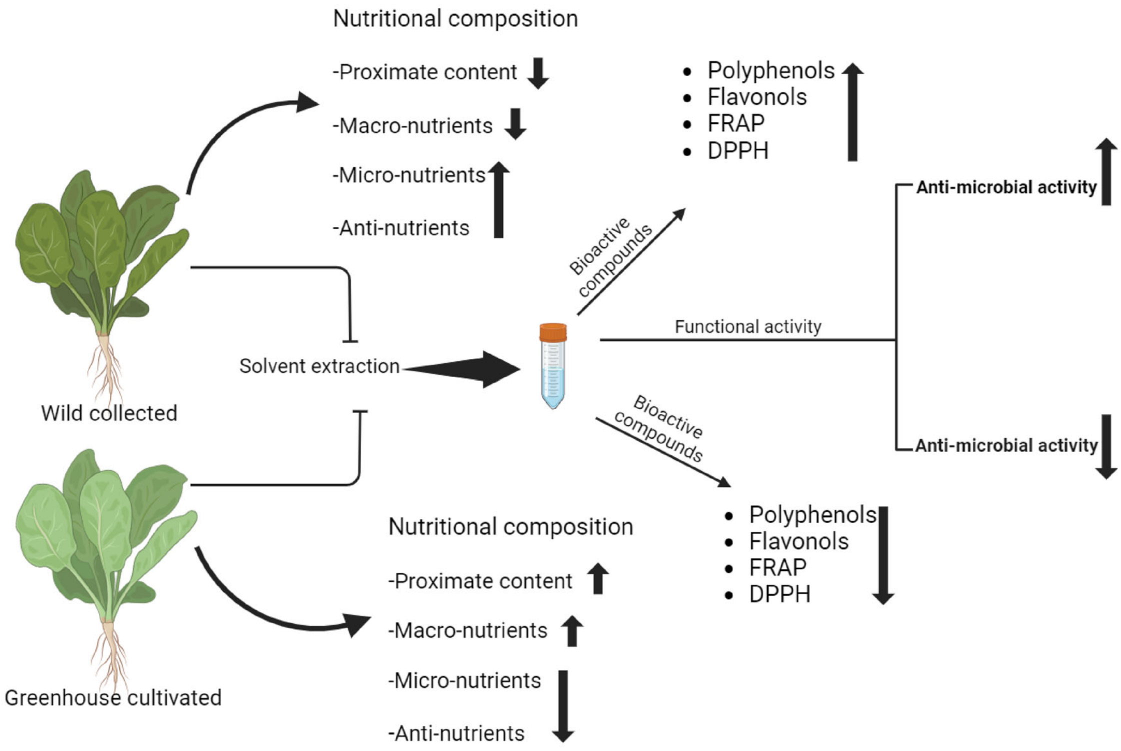
Abstract

Tetragonia decumbens is a neglected edible halophyte that grows naturally in coastal areas of South Africa. Its underutilization is due to the limited literature on its nutraceutical potential. So, this research was designed to assess the nutritional value, secondary metabolites, and anti-microbial potential of wild and greenhouse cultivated *T. decumbens* to further support its consumption and potential medicinal use. Samples of *T. decumbens* were collected from three coastal areas (Strand beach, Muizenberg beach and Blouberg beach) during the dry and wet seasons in Cape Town, and the greenhouse cultivated were subjected to varying salinity doses (0, 50, 100, 150, 200 and 250 mM). Results revealed a considerable increase in minerals (N, P and Mg) and proximate composition (Ash, moisture, and carbohydrates) in greenhouse cultivated plants subjected to 50 mM of salinity while the highest crude fat and neutral detergent fibre were recorded in wild samples. Moreover, heavy metal accumulation (Zn and Fe), phytochemicals, anti-nutrients and anti-microbial activities were more pronounced in wild plants than in cultivated samples. Wild plants collected at Blouberg beach had more heavy metals, anti-nutrients, phytochemicals, and anti-microbial activity. These findings validate for the first time, the relevance of nutritional quality of *T. decumbens* in assessing its suitability as a source of nutrients and antioxidants with possible medicinal value as shown by the inhibition of harmful bacteria and fungal strains.

Keywords: Antimicrobial metabolites; Anti-fungal activity; Edible halophytes; Neglected vegetables; nutritional value.



Graphical Abstract

5.1. Introduction

Climate change and increasing soil salinity are already having a significant effect on crop production losses around the globe (Manuel et al., 2017; Wang et al., 2023). Thus, the alternative use of resilient crops like halophytes is crucial to fulfil the increasing food demand escalated by the increasing human population (Hussain et al., 2023). Edible halophytes are crops that can tolerate high temperatures, drought, and salinity, making them the ideal choice for cultivation in regions impacted by both salinity and drought (Zaier et al., 2022). These species are known to provide needed calories, proteins, fats, and nutrients as per human daily needs (Rasheed et al., 2020). Moreover, they have recently attracted attention due to their nutritional and medicinal value. The aforementioned characteristics often arise from secondary metabolites that these species synthesise in response to oxidative damage caused by salt stress. Phenolic chemicals and some vitamins are examples of metabolites that possess well-established biological attributes such as antioxidant, anti-microbial and anti-inflammatory activity (Pothiraj et al., 2021).

Halophytes serve as a food source with functional qualities due to their nutritionally balanced composition and the presence of secondary metabolites (Duarte et al., 2022). Recently, upscale restaurants in Europe and Asia have started including these plants into their menus as a novel ingredient that also promotes a nutritious diet (Lima et al., 2020; Castañeda-Loaiza et al., 2020a). This led to the inclusion of these plants on supermarket shelves which resulted into the emergence of commercial cultivation due to the increasing demand of sustainable supply (Barkla et al., 2024).

In most African countries including South Africa, edible halophytes are still underutilised and understudied even though these species could provide an alternative source of nutrients to the growing human population (Cebani et al., 2024). Recently, *Tetragonia decumbens* a neglected South African edible halophyte has been cultivated under saline conditions and showed promising production returns for bio-saline agriculture (Sogoni et al., 2023). The salty leaves of this vegetable can be eaten raw in green salads or cooked like spinach and added to soups and stews (Sogoni et al., 2022; Nkcukankcuka et al., 2022). However, its nutritional value remains undocumented, contributing to its underutilisation among coastal households. Therefore, it became imperative to study the nutritional value, phytochemical composition, and anti-microbial potential of wild and greenhouse cultivated *T. decumbens* to encourage its domestication, consumption, and utilisation by coastal inhabitants. Results from this study are anticipated to aid as a paradigm for prospective commercial growers, researchers and coastal inhabitants who may have an interest in using this plant for enhancing food variety, food security and developing medicinal precursors.

5.2. Materials and methods

5.2.1. Wild collection and sample preparation

Fresh green leaves of *T. decumbens* were randomly harvested on three plant populations growing along the coastal areas of Strand beach (34°06'10.7"S 18°48'55.7"E), Muizenberg beach (34°06'11.0"S 18°29'05.8"E) and Blouberg beach front (33°49'31.9"S 18°28'41.5"E) during the wet (June 2023) and dry (December 2023) seasons (Mediterranean climate) in Cape Town, South Africa. The climatic conditions of the selected areas are shown in table 5.1. The harvested arial plant parts were cleaned with distilled water, oven-dried at 35 °C to complete dryness and pulverised. The pulverised material was then kept at 4 °C for further analysis.

Table 5.1: Climatic conditions of selected coastal areas (adapted from weathersa.co.za)

Wild	**Temperature (°C)**		**Precipitation (mm)**	**Humidity %**
Place	Min	Max	Average	Average
SS	13	32	27	64.5
SW	4	20	33.8	60.6
BS	12	35	25.7	67.6
BW	4	23	35.4	63.5
MS	13	32	19.5	64.7
MW	8	20	33.9	60.8

Note: SS= Strand summer, SW= Strand winter, BS= Blouberg summer, BW= Blouberg winter, MS= Muizenberg summer and MW= Muizenberg winter

5.2.2. Greenhouse cultivation and sample preparation

5.2.2.1. Growth Conditions and Experimental Location

Greenhouse cultivation of *T. decumbens* was completed in the research centre affiliated with the Department of Horticultural Sciences at the Cape Peninsula University of Technology. The design and technology of the greenhouse allowed for precise regulation of the environment within the greenhouse. The ambient temperature ranged from 20–27 °C throughout the day and 11–17 °C at night, while the relative humidity remained at a constant level of 60%. Under natural light conditions, the daily average photosynthetic photon flux density (PPFD) was 420 μmol/m^{-2}s^{-1}, with the intensity peaking at 1020 μmol/m^{-2}s^{-1}. The photoperiod corresponds to the prevalent conditions of early spring to summer.

5.2.2.2. Preparation of Plant Material and Saline conditions

Tetragonia decumbens plants were generated from cuttings following the propagation method described by Sogoni et al. (2022). Homogeneously established plants (180) were transplanted in black plastic pots (12.5 cm) filled with a combination of peat and sand (1:1) and stationed in the hardening off area to acclimatize. The plants were then watered with a full Nutrifeed ™ solution produced by STARKE AYRES Pty. Ltd., South Africa. After sixteen days of plant development, plants were irrigated with reverse osmosis water for six days to eliminate any salt residue before being subjected to saline treatments, each containing ten replicates and the study was repeated three times. Saline conditions were established on treatments by elevating the concentration of NaCl in the nutritive solution (0, 50, 100, 150, 200, and 250 mM) as described by Sogoni et al. (2021). Each plant received 300 mL of nutrient solution with graded NaCl and were irrigated every two days. The control plants were only irrigated with nutritive solution without salinity. To verify that the right concentration of salinity in each pot was sustained, drain water from each pot was collected and the electrical conductivity was measured. After four months of salt treatment, plant leaves were harvested, washed with distilled water, oven-dried at 35 °C to complete dryness and milled. The pulverised material was then kept at 4 °C for further analysis.

5.2.3. Mineral composition

The Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Varian 710–ES series, SMM Instruments, Cape Town, South Africa) was utilised to determine the mineral constituents of the samples as previously explained by Bulawa et al. (2022).

5.2.4. Proximate analysis

Proximate analyses were performed in a commercial laboratory of the Department of Agriculture and Rural Development, Kwazulu Natal Province, South Africa. The accredited in-house standard Association of Official Analytical Chemists procedures were utilised to determine moisture, ash, crude fat, crude protein, non-fibre carbohydrate and neutral detergent fibre of the samples as reported by Tshayingwe et al. (2023).

5.2.5. Anti-nutrients composition

Alkaloid and Saponin constituents of the samples were determined using the methods explained by Jimoh et al. (2020). While Oxalate was evaluated using a modified titration approach previously described by Adetunji et al. (2023).

5.2.6. Phytochemicals and Antioxidant Assays

5.2.6.1. Preparation of the crude extract

Approximately, 100 mg of the grinded samples was mixed with 70% ethanol (10 mL) for 24 hours and centrifuged at 400 rpm for 5 minutes. The supernatants were subsequently used for phytochemical and antioxidant activity analyses.

5.2.6.2. Determination of phytochemicals and antioxidant activity

The Folin-Ciocalteu technique explained by Ingarfield et al. (2019) was utilised to determine the total phenolic content of the samples. The results were expressed as mg gallic acid equivalent per g dry weight (mg GAE. g^{-1} DW). While the flavonoid content was determined using the aluminium chloride spectrophotometric test reported by Cebani et al. (2024) and expressed as mg quercetin equivalent per g dry weight (mg QE. g^{-1} DW). The radical scavenging activity was determined using the DPPH free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay following the methods described by Ngxabi et al. (2021). The results were expressed as μM Trolox equivalent per g dry weight (μM TE. g^{-1} DW) for DPPH and μM ascorbic acid equivalents (AAE) per g dry weight (μM AAE. g^{-1} DW) for FRAP.

5.2.7. Antimicrobial Activity

5.2.7.1. Test strains/micro-organisms

The antimicrobial efficacy of the crude extracts was assess using the following strains: Gram negative bacteria (*Acinetobacter baumannii* ATCC 19606T, *Burkholderia cepaciae* ATCC 25416T and *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 700603), Gram positive bacteria (*Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 10876 and *Staphylococcus aureus* subsp. *aureus* ATCC 33591) and Fungi (*Candida albicans* ATCC 90028D-5, *Candida krusei* ATCC 14243 and *Candida tropicalis* ATCC 750).

5.2.7.2. Disc diffusion assay

Approximately 2 g of the dried leaf powder was harmonized with 20 mL of 70 % ethanol for 24 hours and centrifuged at 400 rpm for 5 minutes. The supernatants of the samples were evaporated to dryness and later reconstituted in the same solvent to obtain a concentration of 50 $mg.mL^{-1}$. A disc diffusion assay was utilised to evaluate the antimicrobial activity of the samples as described by Akter et al. (2019). For each test strain, a commercial antibiotic was used as a positive control. Gentamicin (20 $mg.mL^{-1}$ stock) and ampicillin (100 $mg.mL^{-1}$ stock) were used for the bacterial test strains, while fluconazole (50 $mg.mL^{-1}$ stock) was used for the yeast/fungi test strains. The solvent without the extract was included as the negative control. The inoculated plates were incubated at 37 °C overnight (30 °C for the *C. krusei* and *C. tropicalis* strains). The zones of inhibition were assessed using a 150 mm Digital Calliper after incubation. The antimicrobial activity was categorised as follows: strong (> 13 mm), moderate (6–12 mm), weak (≥ 5 mm), and no activity (< 5 mm).

5.2.8. Statistical analysis

The results of this study were analysed using STATISTICA, version 13.5.0.17. A multivariate ANOVA was conducted to determine substantial differences among treatments, followed by Tukey's (LSD) test at $p \leq 0.05$. The Shapiro-Wilk test was conducted before the analysis of variance to verify the validity of normality, while Levene's test was employed to evaluate the homogeneity of variance. The data was shown as mean values ± standard error.

5.3. Results

5.3.1. The mineral composition of wild and greenhouse cultivated *T. decumbens*

The study revealed significant differences in mineral constituents of both the wild and greenhouse cultivated *T. decumbens* as shown in Table 5.2 and 5.3. Plants cultivated in the greenhouse had a substantial increase in macro-nutrients such as N (3300 $mg.100g^{-1}$), P (700 $mg.100g^{-1}$), K (7800 $mg.100g^{-1}$), Mg (1100 $mg.100g^{-1}$) and Na (24100 $mg.100g^{-1}$) compared to values obtained from the wild samples. Most of these minerals were more pronounced in plants subjected to 50 mM salinity except K and Na, where the highest yields were recorded in the control plants for K, and in 250 mM for Na. Conversely, Ca was more pronounced in plants collected from the wild than in greenhouse cultivated plants. The highest Ca (2100 $mg.100g^{-1}$) was noted in plants collected at Strand beach during the summer period.

Accumulation of micro-nutrients such as Zn, Mn, Cu and Fe varied among the wild and greenhouse cultivated plants (Tables 3) consderably. These heavy metals were more pronounced in wild samples than those cultivated in the greenhouse. As a result, the highest yields of Zn (8.2 $mg.100g^{-1}$), Cu (1.5 $mg.100g^{-1}$) and Fe (71.5 $mg.100g^{-1}$) were all recorded from plant samples collected at Blouberg beach during the summer period.

Table 5.2. Macronutrient composition of wild and greenhouse cultivated *T. decumbens* expressed in mg.100 g^{-1} DW.

		N	P	K	Mg	Na	Ca
Cultivated	Control	2400±1.5e	600±0.2b	7800±1.1a	900±0.23b	1500±0.4j	900±0.8f
	50 mM	3300±0.6a	700±0.1a	5200±0.86b	1100±0.5a	5600±0.9h	600±1.3g
	100 mM	2400±0.1e	500±0.6c	3100±1.5c	400±1.8e	15700±1.3d	200±0.01i
	150 mM	2400±1.1e	500±0.9c	2400±0.5e	400±0.42e	19400±0.4c	300±0.05h
	200 mM	2600±0.4d	600±0.2b	2400±0.9e	300±0.98f	20500±1.2b	200±0.11i
	250 mM	2600±1.3d	400±0.8d	1800±1.1g	300±0.5f	24100±1.5a	300±0.8h
Wild	SS	1800±0.07g	100±0.02h	2200±00.5f	900±0.02b	6100±0.9g	2100±0.2a
	SW	3100±0.4b	260±0.06e	2500±0.1d	600±0.01c	5000±0.4i	1600±0.5c
	BS	2800±0.7c	170±0.01g	1300±0.1k	600±0.04c	7300±0.5e	1700±0.2b
	BW	2000±0.1f	200±0.01f	1400±0.2j	500±0.06d	6600±0.1f	1500±0.1d
	MS	1600±0.1h	100±0.8h	1600±0.5h	600±0.07c	7400±0.5e	1000±0.2e
	MW	2600±0.2d	190±0.03f	1500±0.9i	700±0.0b	4900±0.2i	1000±0.2e

Means with the different letters along the columns are significantly different at ($P \leq 0.05$), (n=3). Note: SS= Strand summer, SW= Strand winter, BS= Blouberg summer, BW= Blouberg winter, MS= Muizenberg summer and MW= Muizenberg winter

Table 5.3. Micronutrient composition of wild and greenhouse cultivated *T. decumbens* expressed in mg.100 g^{-1} DW.

		Zn	Mn	Cu	Fe
Cultivated	Control	4.75±0.5e	4.85±0.05d	0.1±0.02g	19.2±0.03f
	50 mM	6.05±0.1c	5±0.02cd	0.2±0.01f	24.6±0.04d
	100 mM	2.75±0.5h	5.6±0.03bc	0.2±0.01f	16.1±0.01h
	150 mM	4.7±0.02ef	7.5±0.2a	0.05±0.01h	24±0.03d
	200 mM	6.05±0.1c	7.3±0.03a	Nd	18.3±0.8g
	250 mM	4.5±0.05f	6±0.4b	Nd	21.4±0.1e
Wild	SS	6.65±0.06b	2.5±0.02e	0.5±0.1b	26.5±0.05c
	SW	4.6±0.02ef	0.85±0.03g	0.4±0.01c	12.7±0.9i
	BS	8.2±0.04a	2.25±0.05e	1.45±0.5a	71.5±0.2a
	BW	6.75±0.07b	1.6±0.06f	0.5±0.01b	28.2±0.6b
	MS	5.75±0.01d	0.7±0.07g	0.35±0.05d	16.2±0.01h
	MW	3.1±0.01g	0.65±0.09g	0.3±0.01e	11.1±0.034j

Means with the different letters along the columns are significantly different at ($P \leq 0.05$), (n=3). Note: nd= not detected, SS= Strand summer, SW= Strand winter, BS= Blouberg summer, BW= Blouberg winter, MS= Muizenberg summer and MW= Muizenberg winter

5.3.2. The proximate composition of wild and greenhouse cultivated *T. decumbens*

The proximate composition of tested samples varied significantly as depicted in Table 5.4. The ash content was at its highest (50.7%) in greenhouse cultivated plants subjected to 50 mM of salinity. On the contrary, crude fat content was more pronounced in plants collected at strand beach (4.4%) during the summer season. However, this was comparable to greenhouse cultivated plants subjected to 50 mM of salinity. Similarly, the highest protein content (20.8%) was obtained in wild samples collected at Strand beach during the winter season, which was comparable to greenhouse cultivated plants subjected to 50 mM of salinity. Conversely, the moisture contents of all tested samples were more noticeable in greenhouse cultivated plants. The highest moisture value (9.8%) was obtained in control plants, which was equivalent to plants treated with 50 mM (9%). The non-fibre carbohydrate was at its highest in plants subjected to 50 mM of salinity (30.6%) in cultivated samples which was comparable to control plants (28.4%) and wild plants collected at Strand beach during winter season (28.6%). The wild samples had higher neutral detergent fibre substantially than the cultivated plants. The highest neutral detergent fibre content (48.2%) was recorded in plants harvested at Muizenberg beach during the winter season.

Table 5.4. Proximate composition of wild and cultivated *T. decumbens*.

		Ash%	Crude fat%	Protein%	Moisture%	NFC%	NDF%
Cultivated	Control	25.8±0.6g	3.6±0.6bc	15.4±0.4d	9.8±0.3a	28.4±0.1ab	39.8±0.7cd
	50 mM	50.7±0.2a	4.3±0.5ab	19.4±0.2ab	9±0.3ab	30.6±0.9a	29.8±0.9d
	100 mM	40.6±0.3c	3.7±0.1b	15.1±0.3d	8.7±0.1b	26.2±0.6bc	26.6±0.2e
	150 mM	45.8±0.1b	3.8±0.5bc	15.1±0.1d	8.3±0.2b	22.2±0.7e	28.2±1.2de
	200 mM	45.1±0.6b	3.4±0.3cd	16.5±0.9c	8.3±0.7b	26.5±0.7bc	24.9±0.3f
	250 mM	40.2±1c	3.1±0.2d	16.4±0.4c	8.1±0.9b	23.7±0.5de	22.4±0.4g
Wild	SS	30.5±1f	4.4±0.4a	11.5±0.05f	4.2±0.1cd	27.1±0.5b	40.3±0.07c
	SW	28.3±0.04f	2.4±0.2f	20.8±0.9a	4.9±0.1c	28.6±0.04ab	40.5±0.03c
	BS	36.7±0.02d	2.6±0.2e	17.8±0.4b	4.5±0.2cd	22.7±0.5e	40.5±0.3c
	BW	45.3±0.8b	3±0.7de	13±0.2e	3.6±0.2d	7.7±0.9g	43.7±0.1b
	MS	36.1±0.7de	3±0.6de	10±0.9g	4.7±0.7c	18.6±1.3f	42.1±0.2bc
	MW	25.2±0.3g	2.2±0.2f	16±0.01c	4.9±0.2c	24.1±1.5d	48.2±0.9a

Means with the different letters along the columns are significantly different at ($P \leq 0.05$), (n=3). Note: NFC= non fibre carbohydrate, NDF= neutral detergent fibre, SS= Strand summer, SW= Strand winter, BS= Blouberg summer, BW= Blouberg winter, MS= Muizenberg summer, MW= Muizenberg winter

5.3.3. Anti-nutrient composition of wild and greenhouse cultivated *T. decumbens.*

Table 5.5 shows the variability in anti-nutrient contents of wild and greenhouse cultivated plant samples. Plants cultivated under greenhouse conditions had far less alkaloids compared to those harvested in the wild except samples collected at Muizenberg during winter. The highest alkaloid content (1.4%) was recorded in plant samples harvested at Blouberg beach during the summer compared to cultivated and other wild collections. As for saponin content, greenhouse cultivated plants subjected to 250 mM had substantially more saponin (8.7%) than other saline treatment including the wild harvested samples. The same trend was also observed for oxalate, where plants subjected to higher saline treatments (200 and 250 mM) had more oxalate content at 3.3% for both treatments.

Table 5.5. Anti-nutrient composition of wild and greenhouse cultivated *T. decumbens*

		Alkaloids%	Saponin%	Oxalate%
Cultivated	Control	0.2±0.01h	6.4±0.02g	2.4±0.01h
	50 mM	0.2±0.02h	7±0.01e	2.4±0.01g
	100 mM	0.49±0.03g	7.2±0.04d	2.7±0.02e
	150 mM	0.55±0.01f	7.2±0.02d	2.8±0.05d
	200 mM	0.62±0.01e	7.6±0.04c	3.3±0.01a
	250 mM	0.62±0.03e	8.7±0.05a	3.3±0.02a
Wild	SS	1.2±0.02b	7.7±0.3b	2.9±0.03c
	SW	0.7±0.06d	6.6±0.1f	2.7±0.05f
	BS	1.4±0.02a	6.1±0.1h	3.1±0.04b
	BW	0.89±0.05c	5.6±0.1i	2.7±0.01f
	MS	0.89±0.03c	7.7±0.1b	2.7±0.03f
	MW	0.2±0.02h	6.1±0.2h	3.1±0.05b

Means with the different letters along the columns are significantly different at ($P \leq 0.05$), (n=3). Note: SS= Strand summer, SW= Strand winter, BS= Blouberg summer, BW= Blouberg winter, MS= Muizenberg summer and MW= Muizenberg winter

5.3.4. Phytochemical composition and antioxidant activity of wild and cultivated *T. decumbens*

The wild environment had a substantial impact on the build-up of phytochemical and antioxidant contents in *T. decumbens* (Table 5.6). Polyphenols were more pronounced in quantity in plants harvested from the wild, with Blouberg samples collected during summer (9.6 mg GAE. g^{-1}) followed by samples collected at Strand during summer (7.8 mg GAE. g^{-1}). A similar trend was also noted for flavonols where the highest content (1.9 mg QAE. g^{-1}) was recorded in winter Blouberg samples. Moreover, the highest antioxidant activity was again noted in wild samples compared to the cultivated ones. Similarly, the highest FRAP (101.9 µmol AAE. g^{-1}) and DPPH (56.6 µmol TE. g^{-1}) contents were recorded in Blouberg samples during summer.

Table 5.6. Phytochemical and antioxidant activity of wild and greenhouse cultivated *T. decumbens*

		Polyphenols (mg GAE. g^{-1})	Flavonols (mg QE. g^{-1})	FRAP (µmol AAE. g^{-1})	DPPH (µmol TE. g^{-1})
Cultivated	Control	5.2±0.02g	0.9±0.07e	27.9±1.4g	4.2±0.1h
	50 mM	5.3±0.01g	1.1±0.05d	32.1±0.7f	18.2±0.2e
	100 mM	6.9±0.06d	1.5±0.02b	47.2±1.2cd	17.4±0.2ef
	150 mM	7.0±0.01d	1.3±0.01c	39.5±0.2e	21.4±0.5cd
	200 mM	7.0±0.07d	1.3±0.05c	39.1±0.1e	22.5±0.5cd
	250 mM	7.5±0.05c	1.4±0.2bc	51.3±1.1b	28.3±0.4b
Wild	SS	7.8±0.05b	1.0±0.05de	48.8±1.4bcd	28.5±0.3b
	SW	5.7±0.02f	1.1±0.02d	31.8±1.3f	16±0.2f
	BS	9.6±0.1a	1.5±0.05b	101.9±2.7a	56.6±1a
	BW	7.7±0.1b	1.9±0.07a	49.1±1.1bc	23.4±1.1c
	MS	6.3±0.5e	1.5±0.05b	45.2±0.3d	20.9±1.2d
	MW	4.7±0.1h	0.8±0.01f	17.6±0.9h	8±0.7g

Means with the different letters along the columns are significantly different at ($P \leq 0.05$), (n=3). Note: SS= Strand summer, SW= Strand winter, BS= Blouberg summer, BW= Blouberg winter, MS= Muizenberg summer and MW= Muizenberg winter.

5.3.5. Anti-microbial activity of wild collected and greenhouse cultivated *T. decumbens*

Crude extracts of wild and greenhouse cultivated *T. decumbens* had a significant effect on some bacterial and fungal strains as seen in Table 5.7. Strains where the crude extract did not show zone of inhibition were excluded from the table. Crude extract of samples harvested at Blouberg beach during summer had more anti-microbial activity which was shown by the highest zone of inhibition (13.1 mm) against *A. baumannii,* and this was higher than all tested treatments. The same trend was also observed for *S. aureus* and *C. Krusei*, where the highest zones of inhibition (11 mm for *S. aureus* and 15.2 mm for *C. Krusei*) were recorded in crude extract of plant samples collected at Blouberg beach during the summer period. Surprisingly, the highest zone of inhibition (16.2 mm) against *C. tropicalis* was again noted in crude extract of samples collected at Blouberg beach during summer but this was comparable to the zone of inhibition (16 mm) recorded in greenhouse cultivated plants subjected to 250 mM of salinity.

Table 5.7. Antimicrobial activity of wild and greenhouse cultivated *T. decumbens*

		Zone of inhibition (mm)			
		A. baumannii	*S. aureus*	*C. krusei*	*C. tropicalis*
Cultivated	Control	4.2±0.04g	3.0±0.07i	2.7±0.4h	5.7±0.1e
	50 mM	4.2±0.03g	3.8±0.08gh	3.4±0.3g	6.1±0.04e
	100 mM	5.4±0.1f	4.0±0.1g	6.5±0.2d	6.2±0.06e
	150 mM	7.2±0.01d	4.2±0.03f	6.9±0.01d	9.1±0.1d
	200 mM	7.8±0.02c	5.0±0.04e	8.8±0.1c	10.8±0.2c
	250 mM	8.0±0.01c	5.2±0.03d	9.0±0.01c	16.0±0.4a
Wild	SS	10.1±0.05b	8.2±0.04b	12.3±0.01b	14±0.2b
	SW	4.2±0.05g	2.1±0.01j	5.0±0.02f	5.8±0.2e
	BS	13.1±0.01a	11.0±0.01a	15.2±0.01a	16.2±0.02a
	BW	6.8±0.02e	3.7±0.1h	6.0±0.03e	9.1±0.2
	MS	6.9±0.03e	7.1±0.05c	8.6±0.05c	11.5±0.3c
	MW	2.2±0.01h	1.9±0.04k	3.1±0.03gh	4.2±0.04f

Means with the different letters along the columns are significantly different at ($P \leq 0.05$), (n=3). Note: SS= Strand summer, SW= Strand winter, BS= Blouberg summer, BW= Blouberg winter, MS= Muizenberg summer and MW= Muizenberg winter

5.4. Discussion

Climate change and increasing soil salinity are already having a significant effect on crop production losses around the globe (Manuel et al., 2017; Wang et al., 2023; Guo et al., 2018; Peng et al., 2019). Thus, the alternative use of resilient crops like halophytes is crucial to fulfil the increasing food demand escalated by the increasing human population. Recently, a neglected edible halophyte (*Tetragonia decumbens*) has been cultivated under saline conditions and showed promising production returns for bio-saline agriculture (Sogoni et al., 2023). However, its nutritional value remains undocumented, contributing to its underutilisation among coastal households. Therefore, it became imperative to study the nutritional value, phytochemical composition, and anti-microbial potential of wild and greenhouse cultivated *T. decumbens* to support its domestication, consumption, and utilisation among coastal households.

Plant based mineral nutrients are known to be important components of human diet since they play a substantial role in the maintenance of certain physicochemical processes required to life (Salami et al., 2022). Hence scouting for new resilient crops with high mineral composition is crucial in the mist of global changes in climate and food insecurity. In this study, high mineral content obtained in wild and cultivated samples of this leafy vegetable attest to earlier reports that neglected wild leafy vegetables are a good source of minerals. Furthermore, the variability in mineral composition of wild and cultivated *T. decumbens* showed that increasing salinity and place of collection modulated the accumulation of minerals in plant samples. In greenhouse saline cultivation, this could be attributed to excessive Na^+ absorption by the plant roots which inhibits the absorption, transport, and buildup of other nutrients. While the variability in plant samples collected from the wild could be linked to abiotic factors such as microclimate (temperature and rainfall), soil texture and availability of nutrients in each site of collection (Zhang et al., 2022). Nevertheless, almost all the minerals assessed in both the wild and in cultivation were above the recommended daily allowance (RDA). Magnesium (Mg) for instance has a 55 mg RDA and this is far below the values obtained in both the wild and cultivated samples. The Mg yield ranged from 300-1100 mg/100g with the highest value attained in greenhouse cultivated plants subjected to 50 mM of salinity. The recorded Mg values in this study are substantially greater than those reported in commercially cultivated leafy vegetables such spinach (Ferreira et al., 2018; Kim et al., 2021) and lettuce (Breś et al., 2022). This suggests that this neglected leafy vegetable can be an alternative source of nutritional Mg. Moreover, magnesium performs essential roles in several biological processes in humans, including structural support in proteins, nucleic acids, polyribosomes, and facilitating neurotransmitter release, promoting cell adhesion, maintaining calcium-potassium balance, and serving as a cofactor for enzyme activities (Fiorentini et al., 2021). Hence, consistently consuming this plant will ensure the serum maintains an ideal level of magnesium content.

When assessing the accumulation of potassium in leaf samples, most greenhouse cultivated plants meet the RDA of 2000 mg except plants subjected to 250 mM of salinity. Increasing salinity has been reported to reduce the potassium levels due to the antagonistic effect of sodium in plant roots (Abbasi et al., 2015). These findings substantiate those of Atzori et al. (2020) where *Tetragonia tetragonioides* cultivated under saline conditions had reduced potassium with increasing salinity. On the contrary, most wild samples did not meet the RDA except plant samples collected at Strand beach during summer and winter seasons. This could be due to the physiochemical composition of the soil of which potassium was sufficiently available. *Tetragonia decumbens* has shown to be a valuable source of potassium when cultivated, which is a crucial element for a holistic diet. Potassium is physiologically important as intracellular and extracellular cations that are necessary for regulating blood pressure, muscle contraction, and transmission of nerve signals (Wrzecí et al., 2022).

When examining the phosphorus composition of the tested samples, only the greenhouse cultivated plants subjected to 50 mM of salinity meet the RDA of 700 mg. These findings are contrary to those obtained by Castañeda-Loaiza et al. (2020b) on commercially cultivated halophytes suchs as *Suaeda maritima, Mesembryanthemum nodiflorum,* and *Sarcocornia fruticosa*, where leaf phosphorus was below the daily recommended allowance. Thus, suggest that this neglected vegetable can be a source of dietary phosphorus. Calcium and phosphorus are important component of bone and teeth minerals in human skeleton (Raskh, 2020). The calcium content of the tested samples was more pronounced in wild samples than those in greenhouse cultivation. As a result, all samples collected from the wild met the RDA of 1000 mg, while all cultivated treatments fell short. This is due to the alteration of ion transport and accumulation of nutrients caused by Na^{+} and Cl^{-} ions competitors, which limits the uptake of calcium (Orlovsky et al., 2016). These findings substantiate those of Šamec et al. (2021) on *Brassica* leafy vegetables where increasing salinity reduced the calcium content in leaves and roots.

High accumulation of sodium in edible parts of halophytes under bio-saline agriculture has been reported by Accogli et al. (2023). This was the scenario in this study where both the wild and cultivated *T. decumbens* had sodium that is well above the RDA of 200-500 mg. Sodium is the predominant cation in extracellular fluid and is crucial for the regulation of acid-base equilibrium and serves as a precursor for the transmission of nerve signals (Jomova et al., 2022). However, high consumption of Na mineral may cause some health complications such as cardiovascular disease, hypertension, and stroke (Barroca et al., 2023). It is therefore crucial to boil the leaves for 5 minutes to reduce the sodium composition before consumption as stated by Caparrotta et al. (2019).

Micronutrients, particularly iron, zinc, copper, and manganese, are essential for the well-being of human diets since they contribute to the prevention of malnutrition (Freeland-Graves et al., 2020). It is estimated that over 3.7 million children worldwide suffer from malnutrition due to deficits in certain micronutrients each year (Webb et al., 2021). Even though these trace elements are needed in small quantities, their daily intake of no less than 20 mg is necessary. In this study, the micronutrients of the tested samples fell below the RDA except iron. The reduction in micro-nutrients under increasing saline conditions have been reported on numerous edible halophytes such as *Salicornia bigelovii, Salicornia ramosissima* and *Tetragonia tetragonioides* respectively (Lima et al., 2020; Atzori et al., 2020). Nevertheless, the iron content of some samples in both the wild and in cultivation meet the RDA of 20 mg. Iron is often found to be lacking in school children, making it the most commonly deficient micronutrient (Mantadakis et al., 2020). This deficiency has been linked to anaemia, weariness, and blood-related disorders (Habib et al., 2023). Most individuals get iron by consuming vegetables, specifically spinach (Duarte et al., 2022). Compared with spinach, it is evident that the leaves of *T. decumbens* contain much higher quantities of iron, with values that are 5-10 times greater than those found in spinach. Moreover, the lower values in heavy metals within the tested samples suggest that this neglected vegetable is safe for consumption.

Proximate analysis is used to determine the nutritional relevance of a particular food source (Kendler et al., 2023; Biel et al., 2023). In the study, moisture content, ash, crude protein, crude fat, non-fibre carbohydrate and neutral detergent fibre of the wild and cultivated samples were determined. The ash content within samples ranged from 25.2-50.7%, which was higher than the values obtained in other wild edible halophytes such as *Arthrocnemum indicum* (12%), *Halocnemum strobilaceum* (15%) and *Suaeda fruticose* (6%) (Zaier et al., 2022). The elevated ash content in the leaves of this vegetable suggests that it has a significant quantity of nutritional fibres, which serve as a habitat for digesting microorganisms in the gastrointestinal system. Generally, wild leafy vegetables have been reported to have low fat content that usually range from 1-3% (Srivastava et al., 2023). This was this case in this study, where the fat content of the examined samples ranged from 2.2-4.4%. High levels of dietary fat may result in elevated cholesterol levels, which are a significant contributing factor to cardiovascular problems. Thus, the intake of this vegetable would be effective in controlling weight loss and problems induced by excessive fat content.

The protein composition of wild and cultivated *T. decumbens* leaves ranged from 10-20.8%. These values were comparable to the results obtained by Jimoh et al. (2020) on *Amaranthus caudatus (20.2%),* but higher than most commercially cultivated edible halophytes such as *Salicornia ramosissima* (12.6%), *Sarcocornia fruticose* (14.4%)*, Crithmum maritimum* (13.9%) and *Mesembryanthemum crystallinum* (12.7%) (Oliveira-Alves et al., 2023). While the non-fibre carbohydrates within the samples ranged from 7.7-30.6%, this was higher than most consumed wild vegetables in southern Africa as reported by Bvenura and Sivakumar (2017). Moreover, the moisture content of the tested samples was substantially higher in cultivated plants than those collected in the wild. Nevertheless, all samples had lower moisture content which ranged from 3.6-9.8%. Thus, connoting that this vegetable could possess lesser microbial contamination and chemical deterioration, which are generally linked with high moisture content (Mndi et al., 2023).

Halophytes have been reported to possess high amounts of anti-nutrients in edible parts which impair the uptake of nutrients or diminish their bioavailability (Barkla et al., 2024). In most edible halophytes, oxalate, alkaloids and saponins have been reported as major anti-nutrients (Kumar et al., 2019). In this study the alkaloid content of tested samples were very low in both the wild and cultivated plants, while oxalate and saponin were substantially higher ranging from 2-8.7%. Various strategies to reduce anti-nutrients in halophytes have been documented, these include soaking, blanching, and cooking (Caparrotta et al., 2019; Barkla et al., 2024). In *Tetragonia expansa,* blanching the leaves lowered the total oxalate and saponin content by 25 % when immersed into hot water for two minutes (Kawashima & Valente Soares, 2003; Barkla et al., 2024). This method has proven to be the best anti-nutrient reduction in most vegetables (Badawy et al., 2018). However, blanching causes leaf wilting and about 35% reduction in vitamin C and is not suitable when crips leaves are needed for salad dressing. This method can be utilised for *T. decumbens* to reduce the composition of anti-nutrients on edible leaves.

Halophytes are also recognised for their production of various secondary metabolites such as phenolics under extreme saline conditions for defence against free radicals (Pereira et al., 2024). These phenolics compounds provide added-value products in food due to their health benefits (Calleja-Gómez et al., 2024). In the present study, secondary metabolites such as polyphenols were more pronounced in quantity in plants harvested from the wild, with Blouberg samples collected during the summer season having the highest content. A similar trend was also noted for flavonols where the highest content was again recorded in wild samples. The variability in metabolites between the wild and cultivated samples may be linked to the differences in climatic conditions, salinity concentration within the soil and anthropogenic disturbances. These results, substantiate the findings of Mangoale and Afolayan (2020) on

Alepidea amatymbica and Xie et al. (2021) on *Corydalis Saxicola*, where wild collected samples had more phytochemicals than the cultivated plants.

Phytochemicals in halophytes such as phenolics are well known to have numerous biological activities such as antioxidant, anti-microbial and anti-inflammatory activities (Pothiraj et al., 2021). Thus, in this study, the crude extracts of both the wild and cultivated samples of this edible halophyte were tested for their anti-microbial activities against gram-negative and gram-positive bacterial strains as well as fungal strains. Plant samples harvested from the wild had more anti-microbial activity than the cultivated ones. Crude extract of plants collected at Blouberg beach during the summer period had the highest zone of inhibition against the gram-positive *Acinetobacter baumannii* ATCC 19606T (13.1 mm) and gram-negative *Staphylococcus aureus* subsp. *aureus* ATCC 33591 (11 mm). These results correlate to the findings of Pandey and Gupta (2014) on *Chenopodium album* and Mouderas et al. (2019) on *Traganum nudatum*, where the methanolic extract inhibited the growth of *A. baumannii and S. aureus* respectively. Thus, suggesting that the crude extract of *T. decumbens* is susceptible to bacterial strains and might be suitable for anti-bacterial applications. The same trend was also observed on fungal strains such as *Candida Krusei and Candida tropicalis,* where the highest zones of inhibition (15.2 mm for *C. Krusei* and 16.2 for *C. tropicalis*) were recorded in crude extract of plant samples collected at Blouberg beach during the summer period. These results are in agreement with those reported by Chekroun-Bechlaghem et al. (2021) on *Arthrocnemum macrostachyum* and *Salicornia europaea* where the extracts inhibited the growth of *Candida Krusei and Candida tropicalis* respectively. The inhibition activity of *T. decumbens* leaf extracts against bacterial and fungal strains can be linked to the high level of phenolics and flavonols quantified in this halophyte, which are known for their anti-microbial properties (Calleja-Gómez et al., 2024).

5.5. Conclusion

The current study revealed that greenhouse cultivated plants subjected to low saline treatment had enhanced minerals and proximate properties as compared to wild collected samples. Given the significant amount of minerals, proximate composition and low anti-nutrients found in this study, it is evident that consuming *T. decumbens* may meet the daily needs for minerals and proximate nutrients. Moreover, the efficacy of antimicrobial metabolites of the crude extract of wild collection of *T. decumbens*, suggest that this halophyte might be suitable for both anti-bacterial and anti-fungal applications. Therefore, for optimal yield of minerals and proximate constituents, it is recommended to cultivate this species under 50 mM of salinity while for medicinal purposes, wild collected samples are recommended.

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CHAPTER SIX

INTERCROPPING THE HALOPHYTE *TETRAGONIA DECUMBENS* MILL. WITH SALT-SENSITIVE *SPINACIA OLERACEA* L. MITIGATED SALINITY STRESS BY ENHANCING THE PHYSIOLOGICAL, BIOCHEMICAL, AND NUTRITIONAL QUALITY OF THE SALT-SENSITIVE SPECIES UNDER SALINE CULTIVATION

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Intercropping the halophyte *Tetragonia decumbens* Mill. with salt-sensitive *Spinacia oleracea* L. mitigated salinity stress by enhancing the physiological, biochemical, and nutritional quality of the salt-sensitive species under saline cultivation.

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Abstract

Increasing soil salinity is already having a significant effect on production losses of commercial vegetables around the globe. Thus, the implementation of innovative techniques is crucial to cultivate these vegetables amidst these unfavourable conditions. Halophytes are potential plants for resilient agricultural systems, such as intercropping with glycophytes, to enhance their productivity in saline soils. Therefore, the purpose of this study was to examine the intercropping potential of the halophyte *Tetragonia decumbens* in alleviating the damaging effects of salinity stress on spinach (*Spinacia oleracea*). Spinach seedlings were grown alone and in consociation with the halophyte under various salt stresses (50, 100, 150 and 200 mM NaCl). Results showed that increasing salinity reduced crop growth, relative water content, chlorophyll, and nutritional quality of spinach in monocultured system. Similarly, high salinity treatment induced severe oxidative stress depicted by high amounts of superoxide, malondialdehyde and the upregulation of superoxide dismutase, catalase, peroxidase, polyphenols, and flavonoids. Interestingly, intercropped spinach irrigated with 50 and 100 mM revealed a substantial enhancement in crop performance, reduction in oxidative stress and had improved nutritional quality depicted by high amounts of minerals, proximate constituents, and vitamins. These results support the introduction of *T. decumbens* in vegetable farming systems and highlights its positive impact on improving the overall crop performance of salt sensitive vegetables under saline condition.

Keywords: Food security; Intercropping with halophytes; phyto-desalination; Saline agriculture

6.1. Introduction

Global climate change and increasing soil salinity are already having a significant effect on production losses of commercial vegetables around the globe and will undoubtedly deteriorate food production in the coming years (Giordano et al., 2021; Shah et al., 2024). Salinity has been linked to the excessive utilisation of low-quality water, widespread irrigation, intensive agriculture, and insufficient drainage systems, leading to the accumulation of soluble salt ions in the soil (Hassani et al., 2020). These salt ions hinder the capacity of the roots to absorb water, causing osmotic stress and nutritional deficiencies that disrupt essential physiological processes such as photosynthesis, tissue hydration, and the onset of oxidative stress and membrane damage, ultimately leading to diminished plant biomass (Hussain et al., 2023a; Mahmood et al., 2021). Thus, the application of innovative techniques is crucial to cultivate these vegetables in the mist of these unfavourable conditions. Phyto-desalination, an inexpensive plant-based bioremediation method, can drastically improve salt-affected soils through planting of appropriate salt extracting halophyte species (Trang et al., 2023; Zhang et al., 2023).

Halophytes can be utilised as a cost-effective and eco-friendly method of phytoremediation to remove excess salt from salt-affected soils due to their reliance on osmotic adjustment, which involves the absorption of salt ions from the soil solutions of saline soils (Atzori et al., 2022). According to Jurado et al. (2024), intercropping with halophytes has been shown to significantly decrease sodium adsorption ratio, chloride content, and electrical conductivity at the soil level, resulting in a reduction in soil salinity. Similarly, Liang and Shi (2021) found that intercropping cotton with the halophyte *Suaeda salsa* L. enhanced the total aboveground biomass and seed cotton yield under saline conditions through halophyte root extraction of sodium ions in the soil. While Hu et al. (2020) noted an increase of vitamin C in cauliflower intercropped with salt tolerance grass under saline conditions. These findings suggest that intercropping halophytes with salt sensitive horticultural crops under saline conditions might alleviate the detrimental effects of salinity on crop production.

Tetragonia decumbens Mill. is an underutilised edible halophyte of South Africa (Nkcukankcuka et al., 2022). Recently, the species has been shown to extract and accumulate Na^+ and Cl^- in the leaf vacuole when cultivated under increasing salinity (Sogoni et al., 2023). Moreover, enhanced bladder cells such as peltate trichomes were noted in the leaves under scanning electron microscopy, suggesting that *T. decumbens* is a salt accumulator. Nevertheless, there are no reported studies on the phyto-desalination potential of this halophyte in an intercropping system under saline conditions. Thus, it became imperative to examine the intercropping potential of *T. decumbens* in alleviating the detrimental effects of salinity on plant growth, physiological, biochemical, and nutritional value of *Spinacia oleracea* “Beet Fordhook Giant” a widely consumed vegetable. Therefore, findings from this study are hoped to support the inclusion of this halophyte in vegetable farming systems and its adoption as a candidate species for phytoremediation in marginal arid and semi-arid regions affected by salinity.

6.2. Materials and methods

6.2.1. Greenhouse cultivation

6.2.1.1. Growth conditions

This study was conducted in the research greenhouse of the Cape peninsula University of Technology during October and November. The greenhouse was configured to have daytime temperatures of 21–26 °C and nighttime temperatures of 12–17 °C, with 60% relative humidity. Under natural light conditions, the daily average photosynthetic photon flux density (PPFD) was 420 $\mu mol/m^{-2}s^{-1}$, with the intensity peaking at 1020 $\mu mol/m^{-2}s^{-1}$. The photoperiod corresponds to the prevalent conditions of early spring to summer.

6.2.1.2. Preparation of plant materials and saline treatments

Seeds of salt sensitive *S. oleracea* were bought from a commercial garden store (Stodels™) and germinated on seedling trays following the procedure outlined by Yavuz et al. (2022) while the salt tolerant halophyte *Tetragonia decumbens* was propagated from stem cuttings using the technique described by Sogoni et al. (2022). Homogeneously established *S. oleracea* seedlings were transplanted individually (monoculture) and others were intercropped with *T. decumbens* in black plastic pots filled with a combination of peat and sand (1:1) and stationed in the hardening off area to acclimatize. The plants were then watered daily with a full Nutrifeed™ solution produced by STARKE AYRES Pty. Ltd., South Africa. After sixteen days of plant development, plants were irrigated with distilled water for six days to eliminate any salt residue before being subjected to saline treatments. Saline conditions were established on monoculture (*S. oleracea*) and on intercropping system (*S. oleracea* + *T. decumbens*) by gradually elevating the concentration of NaCl in the nutritive solution to formulate four treatments (50, 100, 150, and 200 mM) as described by Sogoni et al. (2021). These concentrations were chosen based on the salt tolerance limit of the chosen halophyte. The control (0 mM) plants were only irrigated with nutritive solution without salinity. Each pot received 300 mL of nutrient solution with or without the graded NaCl and were irrigated every two days. Drain water from each pot was collected and the electrical conductivity was measured to ensure that the right concentration of salinity in each pot was sustained. After two months of saline treatments, plants were harvested and used for further analysis.

6.2.2. Biomass yield assessment

After 60 days of plant growth under saline conditions, plants were thoroughly watered with distilled water to loosen up the soil and were harvested cautiously to prevent damage. They were then rinsed multiple times with distilled water and dried with tissue paper. Thereafter, the shoots and roots were separated with secateur, and the fresh samples were weighed using a laboratory scale, followed by oven-drying at 35 °C to complete dryness, and the dry samples were also weighed.

6.2.3. Physiological attributes

Photosynthetic leaf pigments: Chlorophyll a and b were estimated by spectrophotometric procedures by measuring the absorbance of the supernatants at 649 and 665 nm as explained by Wang et al. (2023). The variability in the leaf relative water content was evaluated following the procedure previously described by Bistgani et al., (2019).

6.2.4. Oxidative markers

Cell viability of the leaves was determined using the procedure described by Egbichi et al. (2014). The superoxide radical content was measured following the technique reported by Gokul et al. (2016) where the extinction coefficient of 12.8 mM cm^{-1} was used to calculate the superoxide radical levels. The malondialdehyde content of the tested samples was quantify using the procedure described by González-Orenga et al. (2021).

6.2.5. Anti-oxidative enzymes

The method outlined by Gill et al. (2015) was utilised to extract protein from the tested leaf samples. The protein concentration in the extracts was measured using the Bio-Rad reagent and bovine serum albumin (BSA) as the standard. Spectrophotometric techniques were used to assess the activity of the selected antioxidant enzymes in protein extracts.

The activity of superoxide dismutase (SOD) was determined spectrophotometrically by measuring the suppression and photoreduction of nitro blue tetrazolium (NBT) at 560 nm. The NBT reaction solution uses riboflavin as a determinant of superoxide radicals. A unit SOD was labelled as the quantity of enzyme required to block NBT photoreduction by 50% in experimental conditions as outlined in the methodology of Brenes et al. (2020). Catalase (CAT) activity was measured by observing a decrease in absorbance at 240 nm following the consumption of H_2O_2 added to the extracts (Din Muhammad et al., 2024). One CAT unit was defined as the quantity of enzyme degrading one mmol of H_2O_2 at 25 °C per minute. While the enzyme activity of peroxidase (POD) was determined using the techniques and principles described by González-Orenga et al. (2021).

6.2.6. Phytochemicals and antioxidant activity

The Folin-Ciocalteu technique described by Ingarfield et al. (2023) was utilised to evaluate the total phenolic content of the tested samples. Flavonol content was determined using the spectrophotometric aluminium chloride test reported by Cebani et al. (2024). The radical scavenging activity was determined using the DPPH free radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays following the methods described by Ngxabi et al. (2021).

6.2.7. Nutritional assessment

The mineral constituents (Macro and Micro-nutrients) of the tested samples were determined with the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Varian 710–ES series, SMM Instruments, Cape Town, South Africa) as previously described by Bulawa et al. (2023). The proximate composition (moisture, ash, crude fat, crude protein, non-fibre carbohydrate and neutral detergent fibre) was determined using the accredited in-house standard Association of Official Analytical Chemists (AOAC) procedures reported by Tshayingwe et al. (2023). The composition of Vitamin A-retinol, Vitamin C-Ascorbic acid, and Vitamin E-α-tocopherol of the leaf samples were also evaluated using the AOAC methods described by Nemzer et al. (2021).

6.2.8. Anti-nutrients

The saponin content of the leaf samples was determined using the procedure explained by Jimoh et al. (2020), while oxalate was evaluated by a modified titration technique previously described by Adetunji et al. (2023).

6.2.9. Statistical analysis

The experimental data were analysed with Minitab statistical software and a multivariate analysis of variance was used to distinguish significant differences among treatments followed by Tukey's (LSD) test at $p \leq 0.05$. Prior to the analysis of variance, the validity of normality was evaluated by Shapiro-Wilk test, while Levene's test was used to examine the homogeneity of variance. The data were then expressed as mean ± standard error.

6.3. Results

6.3.1. Biomass yield assessment

Saline treatment had a considerable impact on the biomass yield of spinach grown in different cropping systems (Table 6.1). A significantly higher yield in shoot fresh weight was noted in plants subjected to 50 mM of salinity under intercropping system, however this was statistically similar to the yield obtained in control plants. A similar trend was also noted for shoot dry weight, where the maximum yield was again obtained in plants treated with 50 mM under intercropping system while the lowest shoot dry weight was obtained in plants subjected to

200 mM under monocultured system. On the contrary, both the root fresh weight and root dry weight were significantly higher in control plants as compared to both cropping systems. When assessing the total fresh weight, plants subjected to 50 mM under intercropping system had the highest total fresh weight, but these values did not differ significantly to the control. The lowest values in total fresh weights were all obtained in plants subjected to increasing salinity under monocultured system. This trend was also noted for total dry weight, where significantly higher values were recorded in most plants under intercropping system. While the least values were noted in plants cultivated under mono cropping system.

Table 6.1. Biomass yield assessment of spinach grown in saline conditions under monoculture and intercropping system.

Salinity	Shoot wet weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Total fresh weight (g)	Total dry weight (g)
Control	213±1.6ab	12.8±04bcd	36.9±1.6a	9.6±1.1a	249.9±1.2ab	22.4±0.8b
M-50 mM	158.9±1.2cde	12.5±1.2bcd	16.3±0.8bcd	4.2±0.9cd	175.3±1.3cde	16.7±1c
M-100 mM	139.02±1.3def	9.8±0.5d	14.2±0.4cd	5.2±0.2bc	153.2±0.9def	15.1±0.4c
M-150 mM	115.3±1.6ef	10.3±0.8d	14.4±0.8bcd	5.1±0.3bc	129.8±1ef	15.4±0.9c
M-200 mM	93.74±1.9f	8.8±1.4d	10.2±0.3d	1.8±0.8d	104±1.1f	10.7±1.1d
I-50 mM	249±1.3a	21.7±1.3a	22.9±0.7b	7.9±0.6ab	272±0.8a	29.6±0.7a
I-100 mM	189.2±0.9bc	16.2±0.6bc	21.8±0.6bc	4.4±0.1cd	211±0.3bc	20.6±0.9b
I-150 mM	175.1±1.7bcd	16.8±1.7b	17.9±0.5bcd	3.3±0.cd	193±0.8bcd	20.1±0.5b
I-200 mM	141.8±1cdef	12.3±0.6cd	19.6±0.9bc	3±0.7cd	161.4±1cde	15.4±0.3c

Means with different letters along the columns are significantly different at $P \leq 0.05$ (Tukey's Test). Data are means of thirty replicates per treatment (n=30). Note: M= Monoculture, I= Intercropping

6.3.2. Physiological traits

Salinity induced physiological changes in spinach leaves grown in different cropping systems (Table 6.2). The highest photosynthetic pigments (Chl a and b) were noted in the control, but these values were statistically similar to those recorded in plants subjected to 50 mM of salinity under intercropping system. Increasing salinity caused a significant reduction of photosynthetic pigments in plants subjected to mono cropping system as compared to those in intercropping system. As such, the least values were obtained in plants subjected to 150 and 200 mM under mono cropping system. When assessing the relative water content within treatments, the control plants had the highest relative water content, and this was significantly higher than all other treatments. Nevertheless, plants subjected to increasing salinity (50 and 100 mM) under intercropping system had significantly higher relative water content than all plants subjected to mono cropping system.

Table 6.2. Photosynthetic pigments and relative water content (RWC) of spinach leaves grown in saline conditions under monoculture and intercropping system.

Salinity	Chl a (mg/ g FW)	Chl b (mg/ g FW)	Chl a+b (mg/ g FW)	Relative water content %
Control	6.8±0.8a	4.1±0.2a	10.9±0.3a	90±03a
M-50 mM	4.8±0.5c	3.4±0.6b	8.2±0.8c	75.7±1.1d
M-100 mM	4±0.3e	2.1±0.5d	6.1±0.6e	68.7±1.4e
M-150 mM	3.4±0.2g	1.8±0.8e	5.3±0.3f	66.8±1f
M-200 mM	2.7±0.4h	0.9±0.5g	3.7±0.3g	65.2±1.2g
I-50 mM	6.7±0.8a	4.1±0.2a	10.8±0.8a	88.6±1.1b
I-100 mM	5.2±0.3b	3.1±0.2c	8.4±0.4b	81.4±1.2c
I-150 mM	4.4±0.2d	2.1±0.3d	6.5±0.1d	75±0.01d
I-200 mM	3.7±0.6f	1.5±0.1f	5.3±0.5f	67.9±0.5ef

Means with different letters along the columns are significantly different at $P \leq 0.05$ (Tukey's Test). Data are means of six replicates per treatment (n=6). Note: M= Monoculture, I= Intercropping

6.3.3. Oxidative stress markers

Salinity and cropping system had an influence on the accumulation of superoxide radicals and Malondialdehyde (MDA) within the leaves of spinach as displayed in figure 6.1. A significant increase in superoxide was noted in plants subjected to increasing salinity under mono cropping system. The highest content of superoxide was obtained in plants subjected to 200 mM of salinity under mono cropping system and was significantly higher than treatments. While the least value was obtained in control treatment followed by plants subjected to 50 and 100 mM under intercropping system. A similar trend was also noted for MDA, where the highest content was obtained in plants subjected to 200 mM under mono cropping system. The least value of MDA was again noted in control plants, but did not differ significantly to plants subjected to 50 and 100 mM under intercropping system respectively.

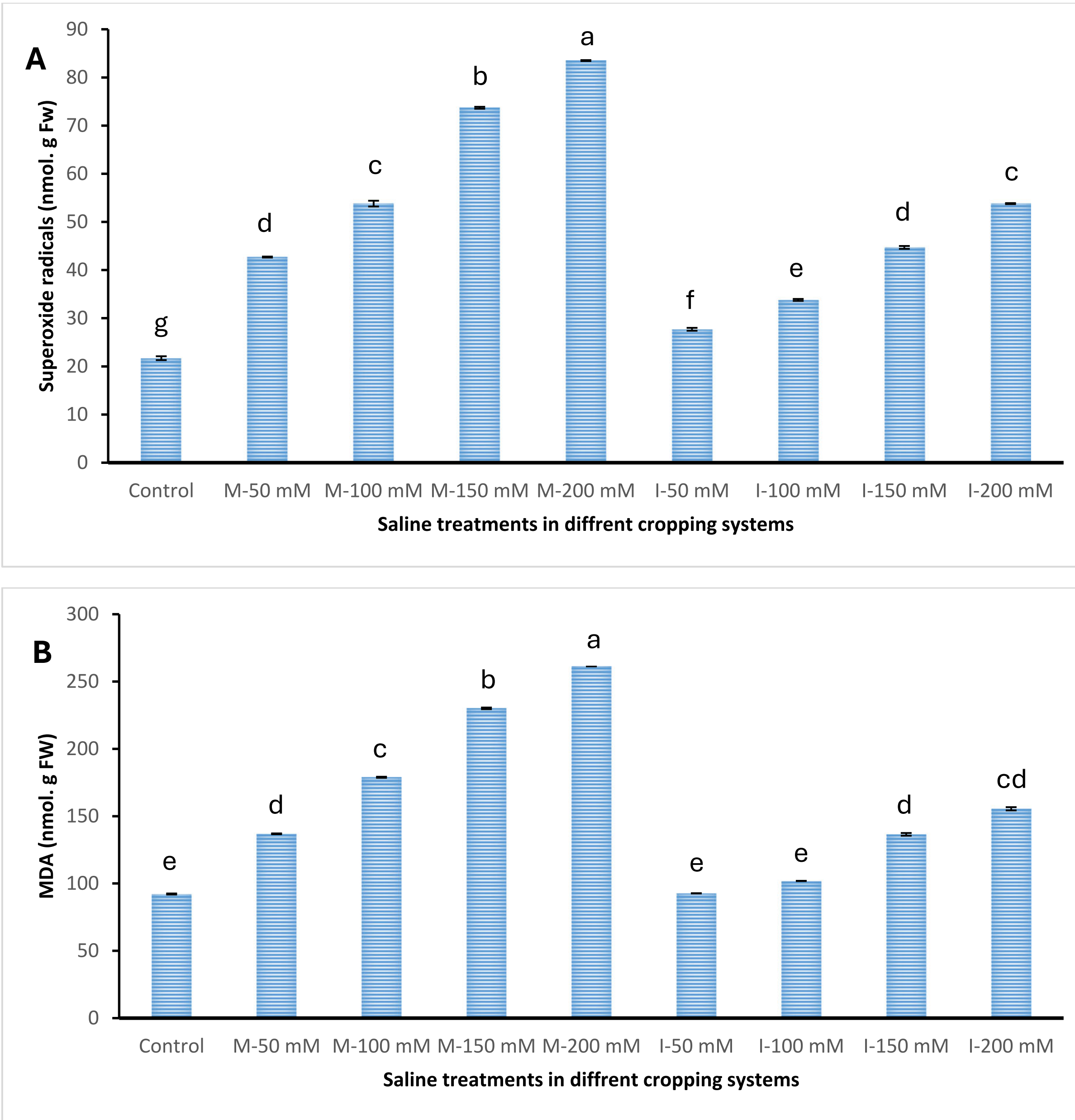

Figure 6.1: Oxidative stress markers (A= superoxide and B=MDA) in spinach leaves grown in saline conditions under monoculture (M) and intercropping (I) system. Means with the different letters are significantly different at $P \leq 0.05$ (Tukey's Test). Data are means of six replicates per treatment (n=6).

6.3.4. Antioxidant defence system

Increasing salinity stimulated the activation of antioxidative defence system in spinach under different cropping systems (Table 6.3). The antioxidative enzymes (SOD, CAT, and POD) were all enhanced with increasing salinity in plants subjected to mono cropping system. High amounts of SOD, CAT and POD were all obtained in plants irrigated with 200 mM of salinity under mono cropping system and these values were significantly higher than all treatments. While the least amounts were recorded in control, they were statistically similar to the values recorded in plants treated with 50 mM under intercropping system. A similar pattern was also observed when assessing the phytochemicals and antioxidant activity of the leaf samples, where the highest polyphenols, flavonols, FRAP and DPPH were all noted in plants irrigated with 150 and 200 mM of salinity under mono cropping system. Again, the least values were obtained in control but did not significantly to the plants subjected to 50 mM under intercropping system.

Table 6.3. Antioxidant defence system of spinach leaves grown in saline conditions under monoculture and intercropping system.

Salinity	**Superoxide dismutase activity (U/ g^{-1} protein)**	**Catalase activity (U/ g^{-1} protein)**	**Peroxidase activity (U/ g^{-1} protein)**	**Polyphenols (mg GAE/g)**	**Flavonols (mg QE/g)**	**Ferric Reducing Antioxidant Power (µmol AAE/g)**	**DPPH (µmol TE/g)**
Control	27.6±1.4e	9.7±0.4f	13.4±0.4f	5.2±0.4c	0.9±0.04e	27.9±0.9f	3.6±0.5d
M-50 mM	38±0.9cd	14.2±0.1d	16.5±0.1d	6.9±0.1a	1.1±0.04d	36.2±0.5d	18.4±0.3ab
M-100 mM	41.3±0.6c	17.3±0.3c	18.2±0.6c	7±0.6a	1.2±0.03c	39.7±0.2c	14.2±0.3bc
M-150 mM	59.3±0.8b	19.2±0.6b	20.4±0.2b	7.2±0.2a	1.4±0.01b	45.9±1b	17.4±0.1ab
M-200 mM	76±0.7a	21±0.9a	24.7±0.5a	7.3±0.1a	1.5±0.06a	50.6±0.9a	22.9±0.9a
I-50 mM	28.6±0.6e	10.1±0.5ef	13.8±0.3f	5.4±0.3c	0.9±0.02e	26.8±0.5f	4.4±0.1d
I-100 mM	31±0.4e	11.4±0.2e	15.2±0.2e	5.4±0.2c	1±0.01d	31.3±0.8e	5.4±0.6d
I-150 mM	37±0.8d	15.3±0.3d	16.7±0.3d	6.2±0.3b	1.1±0.04d	39.7±0.2c	11.5±0.5c
I-200 mM	57±0.5b	18.9±0.1bc	18.4±0.1c	6.4±0.1b	1.5±0.6a	45.8±1.2b	17.1±0.8bc

Means with the different letters along the columns are significantly different at $P \leq 0.05$ (Tukey's Test). Data are means of six replicates per treatment (n=6). Note: M= Monoculture, I= Intercropping

6.3.5. Nutritional assessment

6.3.5.1. Minerals and vitamins

Saline treatment had a significant effect on minerals and vitamin content of spinach leaves under different cropping systems as shown in Table 6.4.1 and 6.4.2 respectively. A significantly higher amount of nitrogen was obtained in control plants. Interestingly, higher phosphorus was noted in plants grown under intercropping system with a pronounce accumulation in plants subjected to 200 mM. When examining the accumulation of potassium and magnesium, the control plants had the highest amount, but these were statistically similar to the values obtained in plants treated with 50 mM under intercropping system. Nevertheless, increasing saline irrigation enhanced the accumulation of sodium in mono cropping system, with the highest value attained in plants subjected to 200 mM. The lowest sodium content was attained in control followed by plants subjected to intercropping system. Conversely, the calcium content was significantly higher in control plants followed by intercropped plants subjected to 50 mM.

Saline treatment also had a significant effect on the accumulation of micro-nutrients and vitamins in the leaves of spinach under different cropping systems. Zinc, Manganese, copper and iron were all reduced with increasing salinity under mono cropping system, while they were gradually enhanced under intercropping system. The highest yield of Zn, Mn and Fe were all noted in plants subjected to 100 mM under intercropping and these values were significantly different from all other treatments including the control. Nevertheless, Copper, vitamin C and vitamin A were more pronounced in control plants, however the vitamin C content was statistically similar to the plants subjected to 50 mM under intercropping system.

Table 6.4.1. Macronutrient composition of spinach leaves grown in saline conditions under monoculture and intercropping system.

Salinity	**N (mg/100g)**	**P (mg/100g)**	**K (mg/100g)**	**Mg (mg/100g)**	**Na (mg/100g)**	**Ca (mg/100g)**
Control	4208±15a	615±11e	6845±11a	775±2a	4629±14i	527±8a
M-50 mM	3756±6b	525±12f	6558±16b	725±5b	5649±9g	375±13d
M-100 mM	3754±9b	489±6g	5459±9c	705±18c	6970±11c	295±6f
M-150 mM	3593±11c	449±9h	4859±11d	639±14d	7958±14b	265±5g
M-200 mM	3505±8e	405±12i	4125±9e	511±9e	8210±12a	240±10h
I-50 mM	3116±13g	665±18d	6640±19ab	765±15a	4865±15h	459±8b
I-100 mM	3114±16g	837±15b	6558±11b	725±5b	6385±11f	405±10c
I-150 mM	3528±8d	785±11c	5459±9c	725±15b	6490±13e	345±6e
I-200 mM	3399±13f	880±19a	5070±15d	705±18c	6650±19d	235±7h

Means with different letters along the columns are significantly different at P≤ 0.05 (Tukey's Test). Data are means of six replicates per treatment (n=6).

Table 6.4.2. Micronutrients and vitamin composition of spinach leaves grown in saline conditions under monoculture and intercropping system.

Salinity	**Zn (mg/100g)**	**Mn (mg/100g)**	**Cu (mg/100g)**	**Fe (mg/100g)**	**Vitamin C (mg/100g)**	**Vitamin E (µg/100g)**
Control	9.5±0.5b	11.3±0.5g	0.3±0.0a	44.1±0.9g	51.8±0.1a	15.2±0.5a
M-50 mM	6.2±0.1e	11.8±0.2f	0.1±0.0c	38.7±0.4i	40±0.1c	8.8±0.9d
M-100 mM	6.7±0.5d	12.8±0.3e	0.0±0.0e	42.2±0.8h	35.4±0.5d	5.5±0.2g
M-150 mM	6.1±0.2e	12.9±0.2e	0.0±0.0e	70.7±0.7d	30±0.01e	4.2±0.6h
M-200 mM	5.5±0.1f	12±0.3f	0.0±0.0e	72.9±0.7c	20.5±0.5f	2.6±0.1i
I-50 mM	9.4±0.5b	16.9±0.4d	0.2±0.0b	45.9±0.9f	52.1±0.1a	15±0.04b
I-100 mM	10.5±0.6a	23.2±0.8a	0.1±0.0d	173.6±0.9a	50.1±1b	10.2±0.5c
I-150 mM	9.5±0.5b	20.2±0.1b	0.1±0.0d	120.7±1.2b	40±0.8c	8.3±0.1e
I-200 mM	7.2±0.1c	18.85±0.8c	0.0±0.0e	60.6±0.6e	35.4±0.9d	6.2±0.05f

Means with different letters along the columns are significantly different at P ≤ 0.05 (Tukey's Test). Data are means of six replicates per treatment (n=6). Note:

M= Monoculture, I= Intercropping

6.3.5.2. Proximate composition

The proximate composition varied significantly in the tested samples in both cropping systems under saline treatment (Table 6.5). The ash content was enhanced by increasing salinity with the highest yield recorded in plants subjected to 200 mM under mono cropping system. While the least value was noted in control plants. Conversely, crude fat was significantly higher in plants subjected to 50, 100 and 150 mM under intercropping system. The lowest values of fat were obtained in control and in mono cropping system respectively. Nevertheless, the protein content within the tested samples was found in abundant in control and this was significantly higher than all treatments. When assessing the moisture content of the samples, plants subjected to 50 and 100 mM under intercropping system had significantly higher moisture content than other treatments. Moreover, the non-fibre carbohydrate was significantly enhanced in control plants, while the neutral detergent fibre and Acid detergent fibre were substantially higher in plants subject to 200 mM under mono cropping system.

Table 6.5. Proximate composition of spinach grown in saline conditions under monoculture and intercropping system.

Salinity	Ash%	Crude fat%	Protein%	Moisture%	Non fibre carbohydrate%	Neutral detergent fibre%	Acid detergent fibre%
Control	34.2±1.5i	1.6±0.1e	26.3±1.4a	6.3±0.3b	32±0.1a	32±1.1d	21.4±0.6cd
M-50 mM	37.3±1.1g	1.9±0.1d	19.4±0.9f	6±0.3d	29.4±1.2c	31.3±1.1f	18.3±0.3e
M-100 mM	45.6±0.8c	1.6±0.1e	19.4±1.3f	5.4±0.1e	28.1±1.6d	31±0.9g	20.7±0.3cd
M-150 mM	46.2±1.1b	1.1±0.0f	22±1.1c	4.9±0.2f	16.9±0.7h	34.8±1.3b	24.5±1.1b
M-200 mM	54.9±1.1a	1.1±0.0f	21.2±1.9e	4.2±0.7g	1.3±0.7i	42±0.7a	34.7±0.2a
I-50 mM	36.9±1.1h	2.8±0.5a	23.4±1.6b	7.1±0.9a	30±1.1b	30±0.4h	20.1±0.9de
I-100 mM	38±0.9f	2.3±0.2b	23.4±1.3b	7±0.2a	19±1.3g	34.1±0.2c	22.8±0.2bc
I-150 mM	44.5±1.5d	2±0.5c	22.4±1.1c	6.3±0.2b	22.5±1.2f	31.2±1.1f	21.8±0.9cd
I-200 mM	44.1±1.2e	1.6±0.1e	21.9±1.9d	6.2±0.1c	23±0.1e	31.6±0.3e	20.7±1.2cd

Means with the different letters along the columns are significantly different at $P \leq 0.05$ (Tukey's Test). Data are means of six replicates per treatment (n=6). Note: M= Monoculture, I= Intercropping

6.3.5.3. Anti-nutrients

Salinity and cropping system influenced the buildup of anti-nutrients in the leaves of spinach as shown in figure 6.2. A trend was noted, where increasing salinity significantly enhanced the accumulation of anti-nutrients with a pronounced effect in plants grown under mono cropping system. As a results, the highest yield of oxalate and saponin were all obtained in plants subjected to 200 mM of salinity under mono cropping system these values were significantly higher than all treatment including the control. While the least values were recorded in control and in plants subjected to 50, 100 and 150 mM under intercropping system.

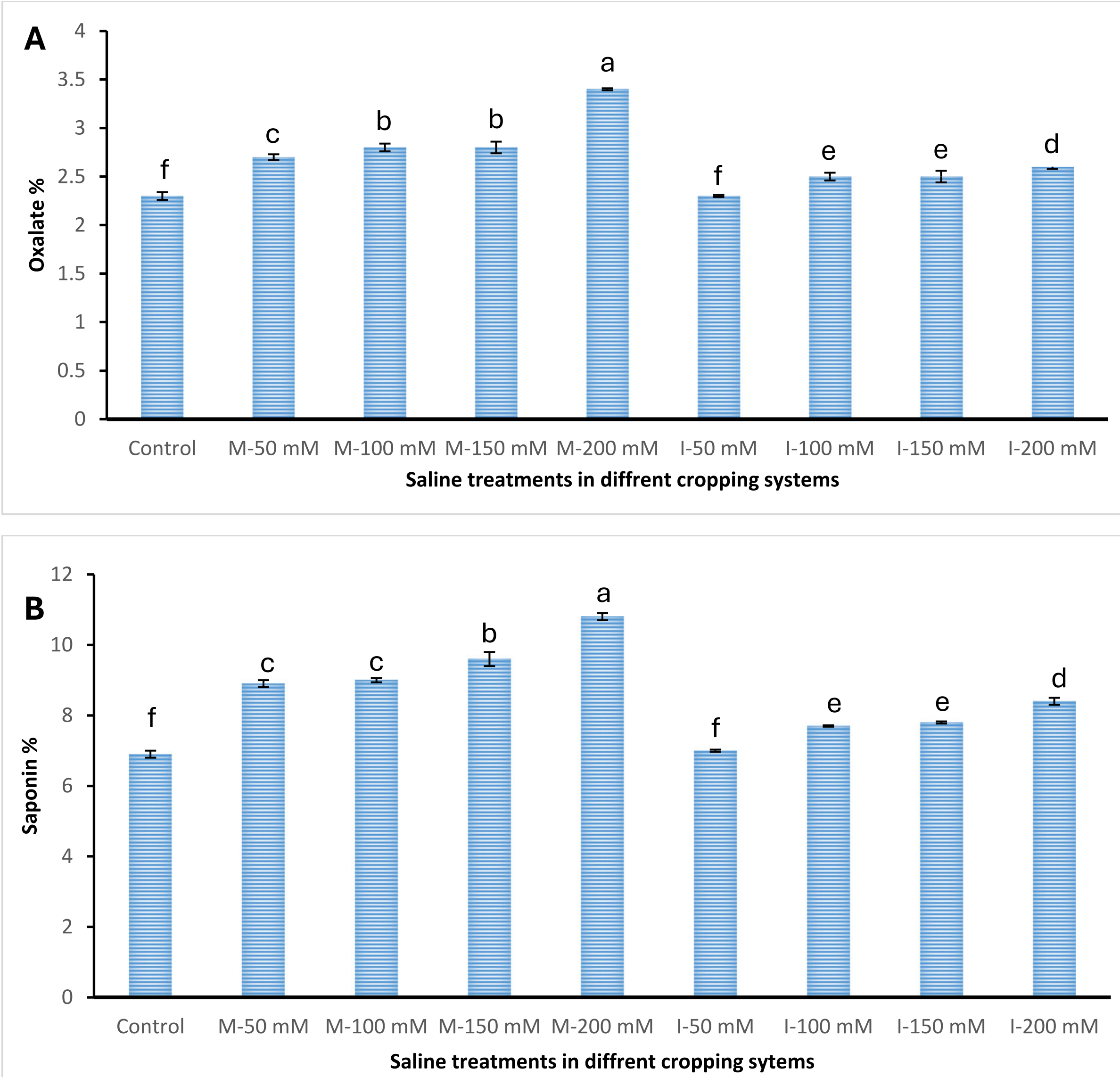

Figure 6.2: Anti-nutrient composition (A=Oxalate and B=Saponin) of spinach leaves grown in saline conditions under monoculture (M) and intercropping (I) system. Means with the different letters are significantly different at $P \leq 0.05$ (Tukey's Test). Data are means of six replicates per treatment (n=6).

6.4. Discussion

6.4.1. Intercropping system improved biomass yield

Salinity is a crucial environmental factor that hinders plants from achieving their full genetic potential (Balasubramaniam et al., 2023; Hussain et al., 2023b; Lu et al., 2021; Singh, 2022; Zarbakhsh and Shahsavar, 2023). In addition, it hinders respiration, photosynthesis, and mineral absorption, leading to a decline in both crop yield and quality (Din Muhammad et al., 2024; Rasool et al., 2024). In the present study, the intercropping system using the halophyte *T. decumbens* improved the yield of spinach under low (50 mM) and moderate (150 mM) salinity levels compared to the mono cropping system (spinach only under saline conditions).

This could be attributed to the salt accumulation and extraction ability of *T. decumbens* as reported by Sogoni et al. (2023) who showed through scanning electron microscopy that this halophyte utilises salt bladder cells such as peltate trichomes to store excessive Na^+ and Cl^- in the epidermal layer of the leaf. These results are supported by the findings of Liang and Shi (2021) on cotton plants where intercropping with the halophyte *Suaeda salsa* enhanced the total aboveground biomass and seed cotton yield under saline conditions through the root extraction of sodium ions in the soil. Similarly, Simpson et al. (2018) found that intercropping watermelon with the halophyte *Atriplex hortensis* substantially enhanced the yield and fruit quality of watermelon. Nevertheless, growth reduction in *Lactuca sativa* intercropped with the halophyte *Salsola soda* was also reported by Atzori et al. (2022), suggesting that not all halophytes can mitigate the detrimental effects of salinity when intercropped, some may outcompete the salt sensitive species for nutrients and light resulting in reduced growth. Hence specific halophyte selection as companion plants for horticultural crops still requires further studies under saline cultivation.

6.4.2. Cropping system induced changes in photosynthetic pigments and water relative content in response to salinity

The measurement of total chlorophyll content is often used as an indicator of salt tolerance in several plant species (Banakar et al., 2022), while relative water content is used as a suitable indicator of plant water level. Several plant species have shown reduced photosynthetic activity and relative water content when exposed to saline conditions mostly via a reduction in leaf area and the disruption of photosynthetic machinery (Khatri and Rathore, 2022). In this study, the reduction in chlorophyll and relative water content in spinach treated with graded salinity under mono cropping system could be attributed to the disruption of photosynthetic machinery caused by the excessive build-up of sodium ions in the chloroplast. This affects the carbon metabolism and photophosphorylation resulting in reduced photosynthesis (Lawson and Vialet-Chabrand, 2019). Similar reports of chlorophyll reduction in monocultured spinach and cowpea under increasing salinity were reported by Muchate et al. (2018) and Nanhapo et al. (2017) respectively. Nevertheless, spinach subjected to 50 mM under intercropping system had chlorophyll and relative water contents that were comparable to the control plants. This suggests that the uptake of sodium through osmotic adjustment and accumulation of compatible solutes in the chosen halophyte, played an important role in the absorption of water and nutrients by the roots of the intercropped spinach. Thus, maintaining a suitable chlorophyll and relative leaf water content is required for plant growth.

6.4.3. Generation of oxidative stress and antioxidant defence system

Various biotic and abiotic stimuli induce oxidative stress in plants by generating superoxide radicals via the Mehler reaction (Golldack et al., 2014; Rodrigues de Queiroz et al., 2023).

Excessive production of these free radicals disrupts normal metabolic processes in the cytoplasm, mitochondria, and peroxisomes by causing oxidative harm to proteins and lipids. This leads to cellular dysfunction and, ultimately, cell death (Saleem et al., 2022). Various biochemical indicators, such as cell death, cell viability, superoxide, and lipid peroxidation, are often utilised to assess the level of oxidative stress in plants that are exposed to unfavourable environmental circumstances. The current study found that superoxide levels and lipid peroxidation increased in monocultured spinach, while there was a substantial decrease in intercropped plants treated with 50 and 100 mM of salinity. High production of free radicals with increasing salinity has been reported on numerous salt sensitive species (Manuel et al., 2017; Zulfiqar and Ashraf, 2022). To curb this effect, plants activate the production of antioxidative enzymes and non-enzymatic compounds as a line of defence (Alfosea-Simón et al., 2020; Arif et al., 2020; Naz et al., 2016; Rahman et al., 2021). This was the case in this study where high amounts of antioxidative enzymes (SOD, CAT, and POD) and non-enzymatic compounds (Polyphenols and flavonols) were all produced in monocultured spinach to scavenge these highly toxic free radicals. Nonetheless, intercropped spinach had lower antioxidants suggesting the establishment of controlled oxidative stress within plant cells. These findings support earlier reports on the alleviation of salinity tolerance in salt sensitive species through halophyte intercropping (Jurado-Mañogil et al., 2023).

6.4.4. Impact of intercropping system on nutritional constituents

6.4.4.1. Minerals and vitamins

Salinity has been reported as a significant environmental element that impacts the ability of commercial vegetables to acquire nutrients. This is due to the alteration of ion transport and accumulation as a result of nutritional imbalances induced by Na^+ and Cl^- ions competitors, which limit the uptake of essential nutrients required for optimal growth (Saddique et al., 2022; Vajjiravel et al., 2024). This was the case in this study, where an increase in sodium build-up in monocultured spinach leaves caused a significant decline in vital nutrients such as P, K, Ca, Mg and vitamin C and E. The observed outcome is a direct result of the antagonistic absorption of sodium and macro-cations by plants resulting in nutritional imbalances. This corroborates the findings of Neocleous et al. (2014), where increasing salinity caused a decline in essential minerals in green and red baby lettuce. Similarly, Ors and Suarez (2017) and Kim et al. (2021) also noted reduced minerals with increasing salinity treatment in spinach. Nonetheless, the intercropped spinach subjected to 50 and 100 mM of salinity had a balanced Na/K, Na/Ca and Na/Mg ratio which is a determining factor of counteracting the negative effects of salinity (Petretto et al., 2019). This could be attributed to the enhanced phytodesalinating capacity of *T. decumbens* as illustrated in supplementary data. Similar findings were also obtained by Jurado et al. (2024) and Karakas et al. (2021) on tomato and strawberry plants intercropped with halophytes under saline cultivation, where sodium ions were reduced drastically in leaves, thus maintaining a balance in nutrient composition required for growth. In addition, the intercropped spinach subjected to 50 and 100 mM also had an enhanced vitamin C and E. These findings correspond to those of Hu et al. (2020) on cauliflower intercropped with salt tolerance grass, where an increase in vitamin content was noted under saline conditions.

Moreover, intercropped spinach showed an increase in Zn, Mn, and Fe. This augmentation might have caused a positive impact on the response of spinach plants when subjected to salinity. Research has shown that supplementing plants with Zn and Mn enhances their performance in saline conditions by promoting plant growth through photosynthesis protection and reducing oxidative stress (Hasan et al., 2024; Khan et al., 2024; Ye et al., 2020). In this study, intercropped spinach exposed to 50 and 100 mM had the highest levels of Zn and Mn in their leaves, along with the highest levels of K^+, $Ca^{2+,}$ and Mg. This may be connected to the higher crop productivity noted in these treatments which supports the idea that halophytes have a positive impact on improving plant mineral nutrition in *S. oleracea*.

6.4.4.2. Proximate and anti-nutrient composition

Nutritional value of commercial vegetables across the globe has been substantially affected by saline conditions, with a particularly noticeable effect in arid regions (Manuel et al., 2017). This has sparked worldwide interest in discovering strategies to sustain plant nutrition in the mist of salinity and to improve food security in impacted nations (Munir et al., 2022). In the present study, increasing salinity had an influence on the proximate composition of spinach subjected to different cropping systems. The highest ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were all recorded in monocultured spinach. As salinity increases, plants absorb large quantities of sodium ions and subsequently translocate more into shoots leading to higher ash content (Nabati et al., 2014). Moreover, increasing salinity enhances the polysaccharides in cell walls and decrease soluble carbohydrates, thereby leading to increased insoluble fibres such as ADF and NDF (Hedayati-Firoozabadi et al., 2020). Interestingly, intercropped spinach had lower contents of ash, ADF and NDF respectively while the non-fibre carbohydrate content was increased in intercropped spinach subjected to 50 mM of salinity. This suggests that the intercropping system reduced the production of polysaccharides in the cell wall, thus increasing the yield of soluble carbohydrates in spinach.

Also, intercropped spinach had more protein and moisture content than the monocultured plants. These findings agree with the findings of Hedayati-Firoozabadi et al. (2020) on *Sorghum bicolor* intercropped with *Bassia indica* under saline conditions, where an increase in protein content was observed in intercropped samples, while reduced protein was reported in monocultured samples. The reduction in moisture content of monocultured plants can be linked to low amounts of minerals absorbed under saline stress (Kim et al., 2021). However, reduced protein could be attributed to the reduction in proteolysis synthesis as well as limited supply of amino acids and denaturation of enzymes involved in protein and amino acid synthesis (Athar et al., 2022).

Vegetables cultivated under saline conditions tend to possess high amounts of anti-nutrients such as oxalate and saponin which impair the uptake of nutrients or diminish their bioavailability (Barkla et al., 2024). Various strategies such as soaking, steaming, and cooking have been documented to reduce anti-nutrients in plants (Caparrotta et al., 2019). In this study, increasing salinity enhanced the build-up of anti-nutrients in monocultured spinach, while they decreased in intercropped system. Hence, intercropping system can be harnessed to reduce the anti-nutrient composition of spinach when grown under saline treatment instead of boiling and steaming, which has been reported to reduce the vitamin constituents of the edible leaves (Badawy et al., 2018).

6.5. Conclusion

Salinity is one of the most critical concerns for agriculture in arid and semi-arid regions. This study shows that intercropping with the halophyte *Tetragonia decumbens* in control environment enhances growth, physiological, biochemical, and nutritional value of spinach plants irrigated with 50 and 100 mM of salinity. Nevertheless, high concentration of salinity prompted a mild oxidative stress in intercropped plants, which was reflected by a slight decline in plant growth. Nonetheless, this research supports the introduction of *T. decumbens* in vegetable farming systems and highlights its positive impact on improving the overall crop performance of spinach under saline condition. Furthermore, field studies in saline soils are recommended to broaden the mechanism of action and to ascertain the phytodesalinating potential of *T. decumbens*.

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6.7. Conflict of interest

The authors wish to declare no conflict of interest.

6.8. Funding

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6.9. Supplementary data

Relative phytodesalination rate

The relative phytodesalination rate (RPR) of *Tetragonia decumbens* was assessed on six replicates per treatment, in accordance with Rabhi et al. (2015), and measured as the capacity of shoots to accumulate sodium ions per unit of biomass over time, as follows:

RPR (mg Na^+ g^{-1} DW day^{-1}) = RGR * (Na_f^+ - Na_i^+) / (DW_f - DW_i)

RGR denotes the relative growth rate, Na_f^+ represents the sodium concentration in leaves at the termination of the experiment, Na_i^+ indicates the sodium concentration in leaves at the commencement of the experiment, DW_f refers to the dry weight of leaves at the end of the experiment, and DW_i signifies the dry weight of leaves at the commencement of the experiment.

Results

Phytodesalination capacity of *T. decumbens*

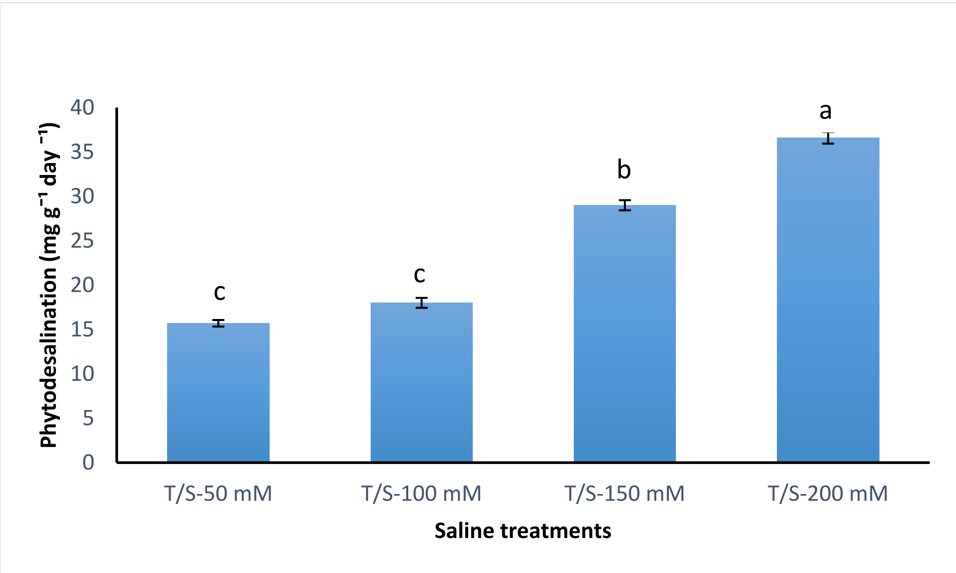

Figure 6.3: Relative phytodesalination capacity of *T. decumbens* intercropped with spinach (T/S) expressed in mg g^{-1} day^{-1}. Data are means of six replicates per treatment (n=6) and different letters indicate significant differences at $P \leq 0.05$ (Tukey's Test).

6.10. Authors contribution statement

Avela Sogoni: Investigation, Data collection, documentation and analysis, Methodology, Writing an original draft of the manuscript. **Charles Petrus Laubscher**: Conceptualization, Supervision, Funding acquisition, Critical revision of the article. **Muhali Olaide Jimoh and**

Sihle Ngxabi: Referencing and statistical software, Critical review of the article with technical inputs, Final approval of the article and manuscript preparation. **Learnmore Kambizi and Marshall Keyster**: Validation of experimental design, Supervision, Critical revision of the article.

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CHAPTER SEVEN

PHYTOCHEMICAL PROFILING OF SALINE-CULTIVATED *TETRAGONIA DECUMBENS* MILL: IN VITRO CYTOTOXICITY, ACETYLCHOLINESTERASE INHIBITORY ACTIVITY, ANTI-CANCER AND ANTI-INFLAMMATORY POTENTIAL

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Phytochemical profiling of saline cultivated *Tetragonia decumbens* Mill: In vitro cytotoxicity, acetylcholinesterase inhibitory activity, anti-cancer and anti-inflammatory potential

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Abstract

Cancer, alzheimer's disease and chronic inflammation present considerable health issues for individuals worldwide. Current treatment with synthetic drugs has been associated with adverse side effects. Thus, the search for plant-based options that are affordable, reliable, and safe is highly imperative. This study explored the phytochemical profiling of ethanolic extracts of *Tetragonia decumbens*, their cytotoxicity, as well as their acetylcholinesterase inhibitory activity, anti-inflammatory and anti-cancer potential. Bioactive compounds from leaf extracts collected from saline cultivated samples (0, 50, 100, 150, 200 and 250 mM NaCl) were identified with ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Cytotoxicity of the cell lines were assessed with MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). Cell line of RAW 264.7 mouse macrophage was used to assess the anti-inflammatory activity of the extracts by monitoring the reduction of nitrites, while acetylcholinesterase inhibitory activity of the extract on Human SH-SY5Y neuroblastoma cells was evaluated using Ellman colorimetric method. For the first time, the UPLC-MS analysis identified 98 compounds in *T. decumbens,* most of which were flavonoids, flavonols, amino acids, hydroxybenzoic acid derivatives, organic and fatty acids. Potent anti-inflammatory activity was noted in the dose of 100 µg/mL in crude extract of 100 mM NaCl treatment, and this was significantly higher than cells treated with Aminoguanidine (positive control). There was also a significant inhibitory activity of acetylcholinesterase in cells treated with crude extracts of 0 mM NaCl, followed by 50 and 100 mM NaCl respectively, when compared with untreated cells. Additionally, the extract of 150 mM NaCl treatment showed cytotoxic effect against cancer cells at high concentration (1mg/mL) but were toxic to non-cancer cells. These findings suggest that the identified compounds in *T. decumbens* extracts have potential anti-inflammatory and acetylcholinesterase inhibitory activities. Consequently, these compounds can be further explored as potential therapeutic agents for the dual treatment of inflammation and alzheimer's disease.

Keywords: Alzheimer's Disease; Edible halophyte; Functional food; Inflammation; Saline cultivation

7.1. Introduction

Inflammation is primarily a defensive response to pathogens and tissue injury (Borquaye et al., 2020). During the initial stages of this process, macrophages serve as the primary line of defence, generating various pro-inflammatory mediators, including nitric oxide (NO), cytokines, and prostaglandins, in response to stimuli such as microbial lipopolysaccharide (LPS) (Silva et al., 2021). Under normal conditions, the release of these molecules is of paramount importance, manifesting severely, rapidly and only for a short period of time upon injury, until the resolution of the harmful stimuli (Jo & Choi, 2024). However, the aberrant synthesis of these pro-inflammatory mediators over a lengthy period may evolve to chronic inflammation-related disorders (Nkemzi et al., 2024). The use of non-steroidal anti-inflammatory drugs in preventing the production of these pro-inflammatory mediators, including NO has shown potential in treating chronic inflammation (Gonfa et al., 2023). Nevertheless, adverse consequences linked to the extended use of these anti-inflammatory drugs have created a demand for novel treatments with minimal side effects. Natural compounds obtained from medicinal plants have been reported to effectively treat inflammatory related illnesses with minimal side effects (Kim et al., 2024).

The degeneration of neurological function due to the reduction in levels of the neurotransmitter acetylcholine in the brains of the elderly, results in loss of cognitive ability (Nguyen et al., 2020; Chauhan et al., 2024). This condition is referred to as Alzheimer's disease (AD), the most common form of dementia. Primary intervention in treating AD necessitates the regulation of acetylcholine within the synaptic region, thereby restoring deficient cholinergic neurotransmission (Huang et al., 2022). Synthetic medicines such as tacrine, donepezil and rivastigmine, used in the regulation of acetylcholine are known to cause gastrointestinal disturbances. However, plant-based extracts with strong acetylcholinesterase (AChE) inhibitory activity have been reported to enhance acetylcholine levels in the synaptic area of the brain (Prasathkumar et al., 2022). As a result, the pursuit of novel AChE inhibitors, especially those from natural sources, with enhanced potency persists.

Besides chronic inflammation and Alzheimer's disease, cancer ranks among the most prevalent causes of mortality globally, with an accelerating death rate particularly evident in developing nations (Soerjomataram et al., 2023). Thus, attracting focus for an efficacious anticancer treatment. Chemotherapy is one of the ways to treat cancer and the advances in anticancer drugs have improved patient care (Anand et al., 2023). However, conventional synthetic drugs also cause undesirable side effects on normal cells or tissues, such as bone marrow function suppression, nausea, vomiting, and alopecia (Guchhait et al., 2022). Hence natural antioxidants and many phytochemicals have been recently recommended as anti-cancer adjuvant therapies because of their anti-proliferative and pro-apoptotic properties (Chimento et al., 2023). These include plant compounds such as podophyllotoxin, vincristine, etoposide, irinotecan, vinblastine, topotecan and paclitaxel, which have significantly contributed to the development of efficient anticancer pharmaceuticals (Jimoh et al., 2024). Moreover, several other bioactive compounds of plant origin, such as gimatecan and topotecan, are currently undergoing clinical research for cancer treatment due to their targeted efficacy (Asma et al., 2022). Nonetheless, there are still several plant species that might possess anticancer potential which have not yet been fully investigated.

Edible halophytes have recently gained popularity as a source food with functional properties due to their nutritionally balanced composition and the presence of secondary metabolites (Ngxabi et al., 2025). These species have been shown to be effective in the treatment of chronic diseases emerging from cognitive impairment, metabolic disorders, cardiovascular complications, cancer and diabetes (Custodio et al., 2022). Recently, *Tetragonia decumbens*, a neglected South African edible halophyte has been cultivated under saline conditions and demonstrated encouraging production returns for bio-saline agriculture with enhanced nutritional value and anti-microbial activity (Sogoni et al., 2024a). Nonetheless, a comprehensive profiling of its phytochemicals and their biological activities remains unexploited. Thus, this study aimed at elucidating the chemical profile of metabolites aggregated in the crude extracts of *T. decumbens* as well as their cytotoxicity, acetylcholinesterase inhibitory activity, anti-cancer and anti-inflammatory potential to further supports its propagation, consumption and commercialization.

7.2. Material and methods

7.2.1. Greenhouse cultivation

7.2.1.1. Preparation of plant material, growth conditions and Saline treatments

Tetragonia decumbens plants were generated from cuttings following the propagation method described by Sogoni et al. (2022). Thereafter the plants were subjected to saline conditions recently reported by (Sogoni et al. (2024). After four months of saline treatment, the plant material was harvested, desiccated, and pulverised for subsequent analysis.

7.2.2. Plant extraction technique

Approximately 10 g of pulverised *T. decumbens* leaves from each salt treatment was deposited into a spherical flask containing 200 mL of 70% ethanol and shaken violently with an orbital shaker at 120 rpm for 48 hours. The mixture was subsequently filtered and concentrated following the method of Okaiyeto et al. (2023). Thereafter, the concentrated samples were preserved at 4 °C in the refrigerator and utilised for all analyses.

7.2.3. Phytochemical analysis using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS)

The LC-MS analysis utilised a QA Waters Synapt G2 quadrupole time-of-flight mass spectrometer. It was equipped with a Waters ultra-pressure liquid chromatography (UPLC-MS) system utilising Waters ms^E technology and photodiode array detection. The phenolic method and instrument specifications were described by and adapted from Jimoh et al. (2024), utilising the negative ion mode with slight adjustments. In the positive ion mode, solvents A and B each comprised 0.1% formic acid, while the mobile phase consisted of water and acetonitrile. After 0.5 min of 100 % solvent A, the gradient transitioned to 100% B for over 0.5 min to 12.5 min. Thereafter, 13 min into the runtime, it then changed to 100% A for the following 2 min in a total run time of 15 min. The flow rate was 0.4 mL/min, the seal wash was 5 min, and the column temperature was maintained at 55 °C. Ionizing electrospray 275 °C desolvation temperature, 15 V cone voltage, and ESI Pos. Leucine encephalin was injected as a lock mass in the background and sodium formate was applied for calibration to acquire exact mass readings. The MassLynx software platform offered with Waters Mass spectrometers was used for manually processing each chromatogram.

7.2.3.1. Identification of compounds with UPLC-MS

The identified metabolites were assigned preliminary names based on precise mass matches obtained from automated searches in databases such as Metlin, massBank, NIST, and other repositories like PubChem, as well as mass fragmentation patterns of compounds and the number of carbon atoms for isotope relative abundance. All compounds were identified based on accurate mass matching, provided their accurate mass error (AME) exceeded 5 ppm (Kerebba et al., 2022). To ascertain a specific compound through retention time, mass fragmentation, and ionisation modes, several standards of phenolic compounds were introduced under uniform LC/MS settings (positive and negative ion modes). Given the capability to obtain all standards and detect numerous compounds by UPLC-MS, the MS and MS^2 fragment ions of analogous compounds were utilised for annotation. Compound structures were clarified using MS-MS analysis of the sample's compounds, which were fragmented to correspond with product ion mass spectra. Upon obtaining isotope abundances, the quantity of carbon atoms in the peak was calculated as a concluding step. Erroneous annotations were reduced by employing the anticipated number of carbon atoms in the ostensibly identified molecule.

7.2.4. Cytotoxicity

7.2.4.1. Cell culturing

The H4IIE-luc (cancerous rat hepatoma) and Vero (non-cancerous African monkey kidney) cell lines were cultured following the procedure described by Idris et al. (2024).

7.2.4.2. MTT assay

Following the manufacturer's instructions, the MTT (3-[4.5-dimethylthiazol-2-yl]−25 diphenyl tetrazolium bromide) cell proliferation assay kit from Thermo Fisher Scientific (Johannesburg, South Africa) was used to evaluate the cytotoxicity of the ethanolic extract of *T. decumbens* leaves (Palshetkar et al., 2020). Specifically, H4IIE-luc and Vero cells were seeded in the inner 60 wells of a 96-well microplate at a density of 10,000 cells/mL media (Dulbecco's Modified Eagle's Medium, Sigma: D2902; St. Louis, MO, USA) and incubated for 48 hours at 37 °C in humidified air with 5% CO_2. Following incubation, the medium was removed and replaced with the one that included *T. decumbens* extract at concentrations ranging from 0.03125 to 1 mg/L and incubated for another 24 hours. Thereafter, a colorimetric experiment was conducted using the yellow dye 3-(4,5-dimethyltiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT, Montigny-le-Bretonneux, France) to assess the vitality based on metabolic activity of cells. In this experiment, mitochondrial reductase enzymes in live cells transform MTT into formazan (blue). Each well received a final concentration of 500 µg/mL MTT, which was then incubated for 30 minutes. After dissolving the blue formazan crystals created by decreased MTT with dimethylsulfoxide, the formazan's absorbance was measured spectrophotometrically at 560 nm. The fraction of viable to dead cells was computed by comparison with a control (solvent), and the amount of blue formazan generated is proportionate to the number of viable cells.

7.2.5. Anti-inflammatory activity

With a few minor adjustments, the approach of Nkemzi et al. (2024) was used to conduct the anti-inflammatory analysis. In 96-well plates, RAW 264.7 cells were seeded at a density of 1×10^5 cells per well in RPMI1640 culture medium supplemented with 10% foetal bovine serum (RPMI complete medium). The cells were then allowed to adhere for the entire night. On the next day, the utilised growth material was disposed and 50 µL sample aliquots were added, diluted in RPMI complete medium, to reach final concentrations of 50, 100, and 200 µg/mL. Thereafter, 50 µL of medium containing lipopolysaccharide (final concentration of 500 µg/mL) was added to the indicated wells to assess the anti-inflammatory activity. Cells were grown for a further 24 hours with the positive control, aminoguanidine (AG), at a concentration of 100 µM. To quantify the generation of nitric oxide (NO), 50 µL of the wasted culture media was moved to a fresh 96-well plate. N-(1-Naphtyl)-ethylenediamine dihydrochloride (NED) solution and sulfanilamide solution were prepared in accordance with the manufacturer's instructions.

The wasted culture medium was mixed with 50 µL of sulfanilamide solution and allowed to sit at room temperature for 10 minutes in the dark. Each well then received fifty (50) µL of NED solution, which was then allowed to sit at room temperature for five to ten minutes in the dark. A BioTek® PowerWave XS spectrophotometer was used to measure absorbance at 540 nm. The amount of NO in each sample was determined using a nitrite standard curve that was created from sodium nitrite diluted in a culture medium. As mentioned above, the MTT assay was used to measure cell viability to confirm that toxicity was not a contributing factor.

7.2.6. Acetylcholinesterase (AChE) activity

With a few minor adjustments, the spectrophotometric approach described by Ellman et al. (1961) was used to measure the AChE activity. Human SH-SY5Y neuroblastoma cells were lysed in 50 µL saline phosphate-buffered containing a final concentration of 0.5% Triton X-100 (Härtl et al., 2011) and centrifuged at 4°C for 10 min at 12 000 g. The supernatant (10 µL) was pipetted into a 96-well microtiter plate containing Ellman buffer and tetraisopropylpyrophosphoramide at a concentration of 100 µM. Thereafter, 50 µL of crude extract was added together with acetylthiocholine and dithionitrobenzoic acid at a concentration of 1 mM and 500 µM respectively. The supernatant without the crude extract served as the untreated cells. A Victor multilabel plate reader (Perkin Elmer, Waltham, MA, USA) was used to measuring the absorbance at 405 nm over 20-minutes. The standard curve prepared with each assay was used to calculate the enzyme activity. Remaining lysate material was assessed for protein amount by means of a Bradford assay to indicate activity as mg/protein.

7.2.7. Statistical analysis

All data were statistically analysed with Minitab 17 statistical software. A one-way analysis of variance was used to assess the significance of treatment differences. Parametric statistics' presumptions were validated. The Kolmogorov-Smirnov test was used to validate normality, while Levine's test was used to confirm homogeneity of variance. Using Tukey's (LSD) test, all significance assertions were predicated on a probability of $p < 0.05$.

7.3. Results

7.3.1. Profiling of phytochemicals in crude extracts of *T. decumbens*

7.3.1.1. Identification of flavonoids

Identification of flavan-3-ols: Fourteen monomeric flavan-3-ols were detected and identified, peaks 45, 46, 50, 51, 54, 56, 59, 62, 64, 70, 75, 76 and 77). Stereoisomers: catechin and epicatechin and (Table 1) were the basis of identification. The order of hydrophobicity for flavan-3ol monomers in the reversed-phase liquid chromatography i.e. (-/+)-epicatechin > (-

/+)-catechin > (-/+)-epigallocatechin > (-/+)- gallocatechin also enabled identification of the monomers and the oligoners. The order of elution from the column is in the opposite direction. Thus (-/+)-gallocatechin elutes earlier and epicatechin comes out last. Therefore, (-/+)-gallocatechin (peak 11), and (-/+)-epicatechin (peak 17) could be identified. Generally, catechin and epicatechin exhibit fragments m/z 151, 135 (due to retro Diels Alder (RDA) fragmentation) upon collision induced dissociation (CID) of their precursor ions $[M -H]^-$; m/z 289 (mzCloud). In addition, fragment ions m/z 245 ($[epicatechin -H-44]^-$, loss of CO_2), m/z 205 ($[epicatechin -H-84]^-$, loss of flavonoid A ring) and m/z 179 ($[M-H-110]^-$, loss of flavonoid B ring).

7.3.1.2. Identification of flavonols

Compound 73 with MS1 ion m/z 615.1926 was tentatively identified as quercetin 3-O-β-D-(6^{11}-galloyl) galactopyranoside, Quercetin galactoside showed ion at m/z 463 then MS2 fragments m/z 300 signifying loss of sugar moiety (-162). The fragment m/z 300 $[M -H-glc]^-$. Additional acylation of quercetin galactoside with a galloyl group could result in quercetin-3-O-β-D-(6^{11}-galloyl) glucopyranoside. Peaks 80, 81 and 84 were identified as those of isorhamnetin 3- O -coumaryl rutinoside-7- O -rhamnoside, isorhamnetin 3- O -coumaryl derivative (for 81 and 84). The precursor ion m/z 843.2032 was due to loss of 72Da. The fragment ion with an m/z of 72 in negative ion mode is a characteristic ring fragment of a (1→6)-linked “anhydro” galactose-H_2O, which lost a (1→3)-branch. The fragment ion for isorhamnetin 3-O -coumaryl rutinoside-7- O -rhamnoside implied 435 [M-H-isorhmatin-coumaryl]-, 675 [M-H-239]-, 239$[M-H-675]^-$, 299[Isorhamnetin -CH_3]- or 299$[M-H- Isorhamnetin -rutinoside-rhamnoside]^-$ the ion m/z 675 was due to loss of two rhamnoside. Compound 85 was identified as isorhamnetin -O-glucurunoside with m/z 494.3253 with MS^2 fragment m/z 316 $[Isorhamnetin - H]^-$, 298 $[Isorhamnetin – H-CH3]^-$, while 82 was aglycone isorhamnetin 298 $[Isorhamnetin + H-CH3]^+$, $275[M+H-CH3-CO]^+$.

7.3.1.3. Identification of Amino acids

Compounds 1, 12, 13, 14, 15, 16, 18, 27 and 55 were identified as amino acids. Amino acids were identified following abundance of the protonated ions fragments and derivatives which could indicate loss of water (-18 Da) yielding their residue mass or loss of (H_2O + CO) to give their immonium ions (-46 Da). Peak 16 was identified as tyrosine, based on a molecular ion peak $[M+H]^+$ at m/z 182.0847 (calcd 182.0812) and a fragment ion at m/z 165[M+H- NH3]+. 136, 153. Peak 27 was identified in both positive and negative ion modes as tryptophan, based on a protonated molecular ion peak $[M+H]^+$ at m/z 205.0995 (calcd for 205.0972). Additionally, its MS/MS spectrum displayed a fragment ion $[M+H- NH3]^+$ at m/z 188. Notably, this is a preferential loss of ammonia (NH3) from the protonated tryptophan. It is generally previously as a fragmentation pattern, generating the diagnostic 2-carboxyspiro[cyclopropane-indolium] fragment ion. Its derivative was identified in compound 55 and 16. N-alpha-Acetyl-L-ornithine was detected in peak 1 with $[Ornithine + H]^+$ at m/z 133 due to loss of 42Da of acetyl group. The ion at 147 was due to loss of CO (28Da). Compound 14 and 15 were identified as trans-4-hydroxy-L-proline and cis-4-hydroxy-L-proline respectively with characteristic fragment ion of m/z 86 due to consecutive loss of water and carbon monoxide (H_2O + CO) $[M+H- H_2O - CO]^+$ in accordance with earlier reports (Piraudi et al., 2003). Compounds 12 and 13 were identified as hydroxy-D-proline and hydroxy-D-proline isomer respectively using accurate mass.

7.3.1.4. Identification of Alkaloids

Four alkaloids were detected and identified (4, 7, 21 57), The protonated ion of trigonelline exhibited m/z 138.0585 $[M + H]^+$) and caffeine, m/z 195.0910 $[M + H]^+$) in the positive ion mode. And gave major product ions at m/z 120 $[M + H- H_2O]^+$ and 138 $[M +H –O-C –NCH3]^+$; (methyl isocyanate, 57 Da), respectively. Compound 21 was identified as phenylalanine consistent with previous studies (Harnly et al., 2007). Compound 57 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 $[M-H- coumaric\ acid -CO2]^-$.

7.3.1.5. Identification of stilbenoids

Piceatannol (m/z 243.0592, peak 17) was also identified by previous report and accurate mass. The MS^2 spectrum produced ions at m/z 194 $[M - H -18]^-$, arising from the loss of a water molecule, and at m/z 201 $[M - H - 42]^-$ due to loss of C_2H_2O.

7.3.1.6. Identification of hydroxycinnamic acid derivatives

Eight hydroxybenzoic acids and their derivatives were identified (peaks 26, 65, 66, 67, 72, 74, 78 and 79). Most of them could absorb UV at about 325 nm (those with caffeoyl moiety) compounds or 309 nm (with coumaroyl). Compound 26 was identified as coumaroyl disulphate using its accurate mass and fragment ions m/z 119, 204. Due to the presence of coumaric acid, its derivatives could display m/z 163 ($[coumaric\ acid-H]^-$) and MS2 fragment m/z 119 ($[coumaric\ acid –CO2]^-$) (Hokkanen et al., 2009). Thus peak 67 was identified as coumaroyl malate. In line with earlier report, Peak 78 was tentatively identified as sinapaldehyde according to earlier reports (Moqbel et al., 2018) and m/z 162 from the glucose conjugation to form sinapaldehyde-o-glucoside.

7.3.1.7. Identification of hydroxybenzoic acid derivatives

About nine hydroxybenzoic acids and their derivatives could be identified (compounds; 28, 29, 34, 35, 36, 37, 49, 67 and 71). Their uv absorption maxima ranges between 270-282nm). In the negative ion mode, the quasi-molecular ion $[M-H]^-$ of compound with m/z 293.1149 belonged to that of beta-hydroxy ketones; 6-gingerol. According to the information of the high-resolution accurate mass, fragment ions at m/z 193.0856 were identified in the cleavage between C6 and C7 bonds of such a compound (Jiang et al., 2005). Gingerol pyrolysis results in production of zingiberone with m/z 193.0363 and main fragment 175($[M-H-H_2O]$-). Gingerols in positive ion mode can show protonated molecular ions ($[M+H]^+$), H_2O subtracted protonated molecular ion ($[M-H_2O + H]^+$). Compounds 29 and 35 are, named as 8-gingerol and 6-gingerol respectively. Fragment ions were 275 ($[M-H_2O-H]^-$), and 305 ($[M-H_2O-H]^-$), or 277 ($[M-H_2O-CH_3-H]^-$ for methyl-6-gingerol are common. The MS^1 ions was m/z 293 and 321 respectively producing common fragment ion m/z 113 $[C_7H_{13}O]^-$in MS^2 formed as a result of inductive cleavage of the side chain. The ion fragment at m/z 137 was due to cleavage of alkyl chain with its acetoxy group. The formation of fragment ions at m/z 321, [M-H-AcOH]-, suggests di-acetylated derivative of 6-gingerdiol thus compound 67 was identified as diacetoxy-6-gingerdiol (Jolad et al., 2004). Compound 36 with some similarity in fragmentation pattern showed an increase of pentose sugar (28 Da) thus identified as 6-gingerol pentose. Methyl gingerol showed precursor ion at m/z 307.0823 after addition of 15 Da on 293 and fragment 249 $[gingerol-H-CO_2]^-$. Compound 87 was identified as gallic acid monohydrate with m/z 187.0990 and fragments 169[Galic acid-H]-, and $[gallic\ acid-H_2O-CO_2]^-$. A digallate could be identified at 321.1002 $[M- H]^-$.

7.3.1.8. Identification of Tannins

Compounds 89 and 90 were identified as 3 -O-methylellagic acid and 3,3′-di-O-methyl ellagic acid respectively owing to absorptions at 405 nm and typical fragments in MS^2 spectra (m/z 316, 298, 227, 275, 195) (Kramberger et al., 2020). The fragments were identified as: 316 $[M+H-CH_3]^+$, 298 $[M-H-OCH_3]^-$, 282 $[M-CO-H_2O]$, 242 $[M-H-_2CO_2]$- and 227 $[M-2CO_2-OH +2H]^+$. The fragments indicate the loss of the methyl [M-H-15]-, methoxy $[M-H-31]^-$, carbonyl (acylium ion) $[M-H-28]^-$, and carbon dioxide [M-44] moieties from the compound.

7.3.1.9. Identification of coumarin

Coumarins and furanocoumarins were detected at peaks 2, 3, 38, 42, and 44. Most of the peaks exhibited UV λmax at 274 nm, characteristic of coumarin. Peak 38 was tentatively identified as xanthotoxin (hydroxyl psoralen) with an MS^2 of characteristic ion m/z 192 $[M+H-CO]^+$ and 175 $[M+H-CO_2]^+$. The $[M+H]^+$ ion was shown a m/z 232. The fragment at m/z 188 was formed from the RDA cleavage of the furano ring, losing the C_2H_2O- fragment ion. Peak 42 was identified to be merazin as identified in the previous report.

7.3.1.10. Identification of organic and fatty acids

The spectra of fatty acid compounds displayed abundant ions due to the loss of CH_2, CO_2, and H_2O groups. Some derivatives of lineolic acid methyl jasmonate with MS1 ion at m/z 225.1602 MS^2 fragment m/z 193 perhaps due to loss of CH_3O (-32 Da). Ten fatty acids were characterised (compounds 83, 86, 89, 90, 91, 92, 94, 95, 96, and 97). Polyunsaturated and hydroxylated fatty acids were identified compounds. Hydroxylated fatty acids showed extra loss of water molecules. Hydroxy decenoic acid fragment showed at 187 in positive ion mode and at 185 Da in negative ion mode. The polyunsaturated fatty acids included dihydroxydecanoic acid at peaks of 83, 86, 95 and 96, dihydroxy-octadecenoic acid (m/z 312.1224) and trihydroxyoctadecadienoic acid (m/z 327.2162), all found in the water and ethanol extracts. Trihydroxyoctadecanoic 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. Additionally, dihydroxy-octadecadienoic acid were identified at peak 96. Besides, LC-MS revealed a monohydroxy-fatty acid peaks, hydroxy octadecadienoic acid with m/z 293.2092. (peak 94), saturated fatty acid, hydroxy palmitic acid was also identified at peaks 97, in agreement with previous work (Ibrahim et al., 2023).

7.3.1.11. Identification of Nucleoside/Nucleotide

These included adenosine, cytidine, guanosine and uridine Nucleosides such as guanosine involve neutral losses of CO, NH_3, CH_2CH_2, $NHCH_2$, NHCO, and NH_2CN and glycosidic C–N bond cleavage (Liu et al., 2008). Fragment ions m/z 282, m/z 284 correspond to the deprotonated/protonated molecular ions of guanosine, and ions m/z 150, m/z 152 are deprotonated and protonated of guanine. The ion m/z 150 or m/z 152 was due to glycosidic C–N bond cleavage after losing 132 Da of sugar moiety along with its respective nucleoside. 2'-Deoxyadenosine monohydrate (peak 47, m/z 337.0935 $[M - H]^-$) too involves this kind of rupture as only option (Flosadóttir et al., 2011). Pyrimidine nucleosides included uridine-5'-monophosphate (compound 23) and uridine-5'-diphosphate compound 61) identified with precursor ion m/z 323.1161 and 403.1593 respectively. Its methyl derivative: 5,2'-O-dimethyluridine showed ion m/z 273.1465. Pyrimidine nucleosides can use two channels of fragmentation; glycosidic C–N bond cleavage and base fragmentation leading to the formation of NCO- and $[M\text{-}H\text{–}HNCO]^-$ (Flosadóttir et al., 2011). The MS^2 fragment ions consisted of 97 $[PO_4]^{2-}$, 184$[M - C_2H_4O_2]^-$ (0,3X sugar cross ring cleavage), 175$[M - C_3H_4N_2]^-$ (base fragmentation). Phosphoribose-derived signals included m/z 213 for ribonucleotide phosphate RbP. Database (mzCloud) and (Flosadóttir et al., 2011).

Table 7.1: Phytochemical compounds identified from *T. decumbens*

No	t_R (min)	$UV_{\lambda max}$ (nm)	*m/z* $(M-H)^-$/ $(M+H)^+$	MS/MS	Tentative identification	Class	Sample
1	1.58	298	175.1221*	147, 133, 156	N-alpha-Acetyl-L-ornithine	Amino acid	D0
2	1.58	299	195.0510	165, 147	Trihydroxycoumarin	Coumarin	D0
3	1.60		229.0500	195, 165, 146	Trihydroxycoumarin derivative	Coumarin derivative	D1, D2, D3, D4, D5
4	1.60	273	195.0910*	127, 166	Caffeine	Alkaloid	D0
5	1.60		217.0695*Na	195, 212, 148, 127	Caffeine	Alkaloid	D1, D2, D3
6	1.72	267	189.0041	133	Catechol sulphate	Phenylsulfate	D0, D1, D2, D3
7	1.77	264	138.0585*	127	Trigonelline	Alkaloid	D0, D1, D3
8	2.21		133.0131	71	Malic acid	Organic acid	D2, D3, D4
9	2.61		191.0206	111, 173	Citric acid	Organic acid	D1, D2, D3, D4, D5
10	2.97	263	136.0666*		Adenine	Nucleoside	D0
11	3.65	285	191.0190	173, 111, 129	Isocitric acid	Organic acid	D1, D2, D3, D4, D5
12	3.66		130.0527*		Hydroxy-D-proline	Amino acid	D0, D1, D2, D3
13	3.95	284	130.0536*		Hydroxy-D-proline isomer	Amino acid	D0, D1, D2, D3
14	4.62	258	132.1053*	86	trans-4-Hydroxy-L-proline	Amino acid	D0, D1, D2, D3
15	5.05		132.1053*	86	Cis-4-Hydroxy-L-proline	Amino acid	D0, D1, D2, D3
16	5.20	267	182.0847*	165, 136, 153	Tyrosine	Amino acid	D0, D1, D2, D3
17	5.35	265	243.0592	194, 201	(E)-Piceatannol	Stilbenoid	D4, D5
18	5.43	264	196.1015*	150, 165	N'-Formylkynurenine/	Amino acid derivative	D0, D1, D2, D3
	5.45	274	194.0805	161, 128	N'-Formylkynurenine	Amino acid derivative	D0
19	5.58		161.0443	147, 134	Cinnamic acid methyl ester	Benzoic acid ester	D2, D3, D4, D5
20	6.39		282.0841	150, 204	Guanosine	Nucleoside	D1, D2, D3
	6.40	252	284.1026*	152, 149	Guanosine	Nucleoside	D0, D1, D2, D3
21	7.10	257	164.0706	147, 122	Phenylalanine	Amino acid	D1, D2, D3, D4, D5
	7.17	257	166.0881*	120	Phenylalanine	Amino acid	D0, D1, D2, D3

22	9.10		273.1465	175, 184	5,2'-O-dimethyluridine	Nucleotide	D3
23	9.13	273	323.1161	273, 184, 213, 97, 175	Uridine-5'-monophosphate	Nucleotide	D0, D1, D2, D4, D5
24	9.14		275.1622*	234	5,2'-O-dimethyluridine isomer	Nucleotide	D0, D1, D2, D3
25	9.52		273.1451	161, 201	5,2'-O-dimethyluridine isomer	Nucleotide	D3
26	9.75	275	323.1158	204, 119	Coumaric--O-disulfate	Hydroxycinnamic acid derivative	D3
27	9.85	279	203.0809	175	L-Tryptophane	Amino acid	D1, D2, D3, D4, D5
	9.92	279	205.0995*	188	L-Tryptophane	Amino acid	D0, D1, D2
28	10.12	279	321.1013/ 323.1281		digallate	Hydroxybenzoic acid derivative	D0
29	10.20		323.1312		8-Gingerol	Hydroxybenzoic acid derivative	D1, D2, D3, D4, D5
30	10.53	280	252.0886^{-2H}	201, 234, 232	N-oleoylsphingenine	Ceramide	D1, D2, D3
	10.53	279	254.1043*		N-oleoylsphingenine	Ceramide	D0, D1, D2
31	10.59	274	325.1312 340.1311	252, 232	DL-Corydine	Alkaloid	D0, D1, D2, D3
32	10.76	274	193.0363	193	β-D-Glucopyranuronic acid	Saccharide	D0, D1, D3, D4, D5
33	11.13	279	175.0363	115, 113, 136	2-Isopropylmalic acid	Organic acid	D1, D2, D3, D4, D5
34	11.35		193.0363	175, 138	Zingiberone	Hydroxybenzoic acid derivative	D1, D2, D3, D4
35	11.66	272	293.1149	193	6-Gingerol	Hydroxybenzoic acid derivative	D1, D2, D3, D4, D5
	11.69		295.1290*	166, 270, 283	6-Gingerol	Hydroxybenzoic acid derivative	D0, D1, D2, D3
36	11.76	272	425.0770	327, 293	6-Gingerol-o- pentose	Hydroxybenzoic acid derivative	D1, D2, D3, D4, D5
37	11.87	270	307.0823	293, 249	Methyl-6-gingerol	Hydroxybenzoic acid derivative	D1, D2, D3, D4, D5
38	11.97		217.1557*	200, 147, 177, 111, 88	Xanthotoxin	Coumarin	D1, D2, D3, D4, D5

	12.26	270	215.0833	192	Xanthotoxin	Coumarin	D1, D2, D3, D4, D5
39	12.27	270	217.0993*	144, 130	Bergapten	Coumarin	D0, D1, D2, D3
40	12.55	263sh	349.0585	97, 148	N-Coumaroyl-L-tryptophan	Alkaloid	D0, D1
41	12.81	272sh	236.0555		Culmorin	Sesquiterpenoid	D1, D2, D3, D4, D5
	12.81	272	238.0726*	105	Culmorin	Sesquiterpenoid	D0, D1
42	12.99	280	259.0713	215	Meranzin	Coumarin	D1, D2, D3, D4, D5
	12.98	281	261.0875*	188, 217, 145	Meranzin	Coumarin	D0, D1, D2
43	13.25	286sh	561.2178[M-2HM-	323, 214, 157	Medioresinol-C- gluconide	Lignan	D0, D1, D2, D3, D4, D5
44	13.28		189.1278*	163, 145	Hydroxycoumarin derivative	Coumarin	D1, D2
45	13.41	281	323.1151^{+H2O}	97	(-/+) Gallocatechin	Flavan-3-ol	D1, D2, D3, D4, D5
46	13.64	281	307.1218^{-2H}	192, 203, 279	(-/-) Gallocatechin	Flavan-3-ol	D1, D2, D3, D4, D5
47	13.97	284sh	337.0935	250, 132, 97, 88	2'-Deoxyadenosine monohydrate	Nucleoside	D0, D1
48	14.05	278sh	250.0714	132, 97	2'-Deoxyadenosine monohydrate	Nucleoside	D1, D2, D3, D4, D5
	14.05		252.0878*	134, 234, 204	2'-Deoxyadenosine monohydrate	Nucleoside	D0, D1, D2, D3
49	14.21		321.1002	241, 97	Digallate	Hydroxybenzoic Acid derivative	D1, D2, D3, D4, D5
50	14.45	280	307.1194	280, 275, 262	(-/+) (epi)Gallocatechin	Flavan-3-ol	D1, D2, D3, D4, D5
	14.46		282.0981*	236, 193	(-/+) (epi)Gallocatechin metabolite	Flavan-3-ol	D0
51	15.08	270	305.1060	234, 273	(-/-) (epi)Gallocatechin	Flavan-3-ol	D0, D1, D2, D3
52	15.21	267	185.0385	97	2,5-Dimethylbenzenesulfonic acid	Benzene derivatives	D0, D3, D4, D5

53	15.37		371.0952$^{[2M-H]-}$	185, 264	2,5-Dimethylbenzenesulfonic acid dimer	Benzene derivatives	D2, D3, D4, D5
54	15.80	281	305.1073	97, 203	(-/+) (Epi)gallocatechin	Flavan-3-ol	D0, D1, D2, D4, D5
55	16.01		252.0893*	206	3-Hydroxy-DL-kynurenine	Amino acid derivative	D1, D2
	16.02		206.0818$^{[M-H-H2O]}$	164	3-Hydroxy-DL-kynurenine	Amino acid derivative	D1, D2, D3, D4, D5
56	16.16		305.1041	97, 241	(-/-) (Epi)gallocatechin	Flavan-3-ol	D0, D3, D4, D5
57	16.30	309	280.0804	163, 262, 244	N-cis-Coumaroyl-tyramine	Alkaloid	D0, D1, D2, D4, D5
	16.33	309	282.0966*	264, 136, 165, 207	N-cis-Coumaroyl-tyramine	Alkaloid	D0, D1, D2
58	16.89		291.0898	206, 97, 279	(-/+) Catechin	Flava-3-ol	D0, D1, D2, D4, D5
59	16.96	311	266.1017$^{*[M+2H+Na]2+}$ (288.0789)	248, 166, 208, 120	(-/+) Catechin	Flavan-3-ol	D0, D1, D2
60	17.32		259.1665*	197, 84	Not identified	-	D0, D1, D2, D3
	17.34	318	257.1502	215, 197			D1, D2, D4, D5
61	17.62		403.1593	223, 97, 321	Uridine-5'-diphosphate	Nucleotide	D2, D3
62	17.77	283	275.1046	245, 193	Unidentified flavanol metabolite	Flavan-3-ol	D4, D5
63	17.79		289.0802	245, 275, 193	(-/-) Catechin	Flavan-3-ol	D2
64	18.33		413.1462	269, 349, 183, 125, 59	Catechin benzoyl ester	Flavan-3-ol	D1, D3
65	18.42	278, 312	355.1996*	149	Ferulic acid glucoside	Hydroxycinnamic acid derivative	D0, D1, D3
66	18.79	279, 312	197.1191*	162, 179	Dihydroferulic acid	Hydroxycinnamic acid derivative	D0, D1, D2, D3
67	18.95	264, 312	279.1244	258, 269, 144	Coumaroyl malate	Hydroxycinnamic acid derivative	D1, D3
68	19.26	325sh	379.0658	262	Diacetoxy-6-gingerdiol	Hydroxybenzoic acid derivative	D1, D2, D4, D5

69	19.35	279	262.0728^{-2H}	146, 218	Abscisic acid	Carotene derivative (terpenoids)	D1, D2, D3, D4, D5
	19.36	278	264.0886*	131	Abscisic acid	Carotene derivative (terpenoids)	D0, D1, D2, D3
70	20.20	275, 317	291.1267^{-2H}	207, 165, 147	(-/+) Epicatechin	Flavan-3-ol	D1, D2, D3, D4, D5
71	20.56	281, 312	187.0990	169, 125	Gallic acid monohydrate	Hydroxybenzoic acid derivative	D0, D1, D3, D4, D5
72	20.87	311	429.1724	335, 89, 205, 119, 249, 317	1,3-O-Feruloyl-caffeoylglycerol	Hydroxycinnamic acid derivative	D1, D2, D3, D4, D5
73	21.04	276, 323	615.1926	463, 464, 300, 445, 405, 289	Quercetin 3-O-β-D-(611-galloyl) galactopyranoside	Flavonol	D2, D4, D5
74	21.28	272, 324	837.3243	691, 335, 417, 477, 245, 805, 191, 162	Caffeoyl shikimic acid –O-sulphate dimer	Hydroxycinnamic acid derivative	D0, D5
	21.30	273, 319	839.3311*	693, 337, 211	Caffeoyl shikimic acid –O-sulphate dimer	Hydroxycinnamic acid derivative	D0
75	21.67	278, 332	637.1368	289, 305, 573, 403, 203	(Epi) gallocatechin- 3-O-(6^{11}-O-gallate) glucoside	Flavan-3-ol	D3
76	21.96		289.1105	245	(-/-) Epicatechin	Flavan-3-ol	D1, D2, D3, D4, D5
77	22.25	281, 316	307.1208	97	(-/-) (Epi)gallocatechin	Flavan-3-ol	D1, D2, D3, D4, D5
78	22.74	324	209.0821*	191, 161, 193	Sinapaldehyde	Hydroxycinnamic acid derivative	D0, D1, D2
79	22.76	324sh	393.0822$^{[M-Na-2H]-}$	243, 185	Sinapaldehyde-4-O-beta-D-glucopyranoside	Hydroxycinnamic acid derivative	D1, D2, D3, D4, D5
80	23.06	281, 329	915.1870$^{[M-2H]-}$	435, 675, 239, 299	Isorhamnetin 3- O -coumaryl rutinoside-7- O -rhamnoside	Flavonol	D4, D5
81	23.26	281, 329	843.2032	299, 161, 343	Isorhamnetin 3- O -coumaryl hexoside derivative	Flavonol	D2, D5
82	24.34		316.2860*	221, 298, 275, 195	Isorhamnetin	Flavonol	D2, D3

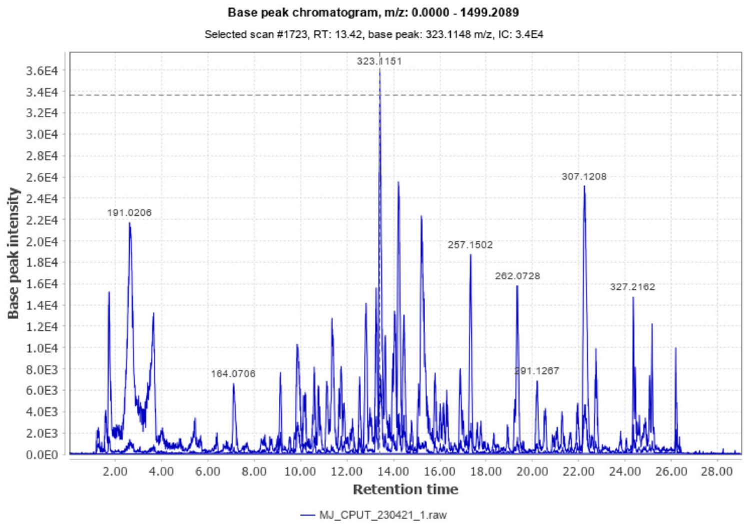
83	23.80		312.1224	263, 269	Dihydroxy-octadecenoic acid	Fatty acid	D2, D5
84	24.05	282, 326	813.1809	675, 163	Isorhamnetin 3- O -coumaryl hexoside derivative	Flavonol	D2, D3, D4, D5
85	24.28	284, 313	494.3253	316, 298	Isorhamnetin -O-glucurunoside	Flavonol	D4
86	24.36	284	327.2162	215	Trihydroxy-octadecadienoic acid	Fatty acid	D1, D2, D3, D4, D5
87	24.36	284	316.2854*	316, 298, 293, 221, 275, 195	3 -O-methylellagic acid	Tannin	D0, D1
88	24.45	318, 405	329.2289	227, 282	3,3'-di-O-methylellagic acid	Tannin	D1, D2, D3, D4, D5
89	24.57		454.2946*	326, 187, 275	Trihydroxy-octadecadienoic acid derivative	Fatty acid	D2
90	24.59		520.3604*	326, 187, 332	Trihydroxy-octadecadienoic acid derivative	Fatty acid	D5
91	24.79		225.1602*	193, 100, 211, 158	Methyl jasmonate	Lipid	D0, D1, D2, D3, D4
92	24.87		265.1463	145	Decarbonylated hydroxy octadecadienoic acid	Fatty acid	D0, D1, D2, D3, D4, D5
93	24.90	275	313.1062*	225, 306, 275 220	5,7,4′-Trimethoxyflavone	Flavone	D1, D2, D3, D4, D5
94	25.07		293.2092	265, 145	Hydroxy octadecadienoic acid	Fatty acid	D0, D1, D2, D3, D4, D5
95	25.10		326.3033	225, 277	Trihydroxy-octadecadienoic acid isomer	Fatty acid	D2, D4
96	25.17		311.2208	293, 265	Dihydroxyoctadecadienoic acid	Fatty acid	D0, D5 D1, D2, D3, D4, D5
97	26.20		271.2258	265	Hydroxy palmitic acid	Fatty acid	D0, D1, D2, D3, D4, D5
98	26.38		247.1668*	227, 124	Zederone	Sesquiterpene	D1, D2, D3, D4, D5

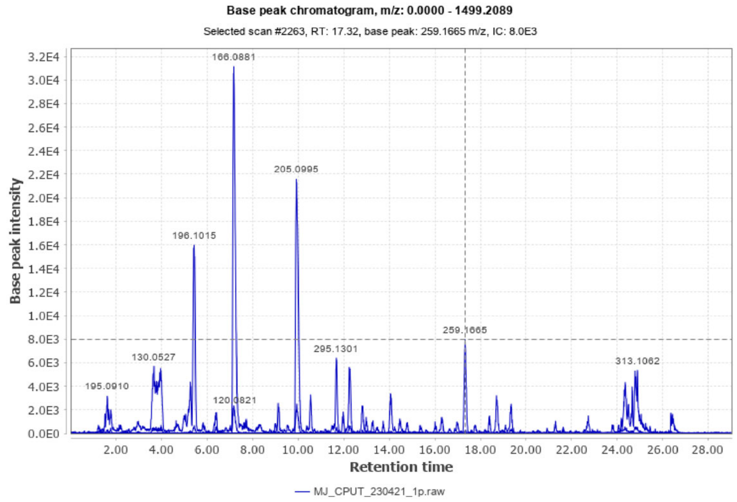
Results are presented as D0= Control, D1= 50 mM NaCl, D2= 100 mM NaCl, D3= 150 mM NaCl, D4= 200 mM NaCl and D5= 250 mM NaCl.

Negative

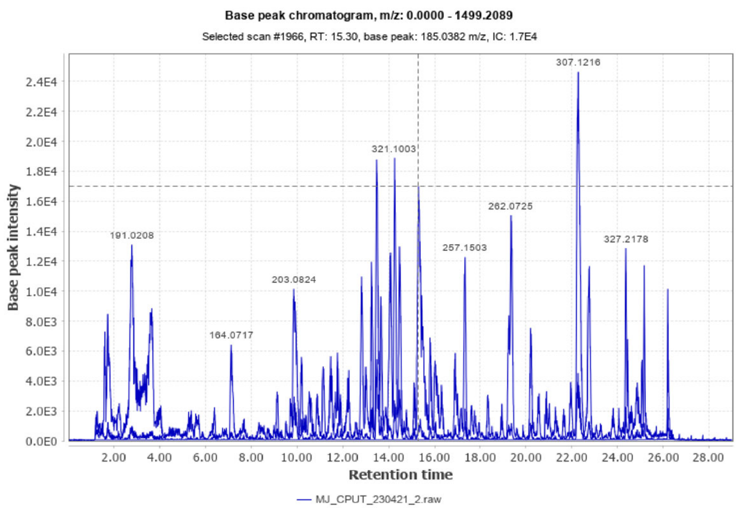
Positive

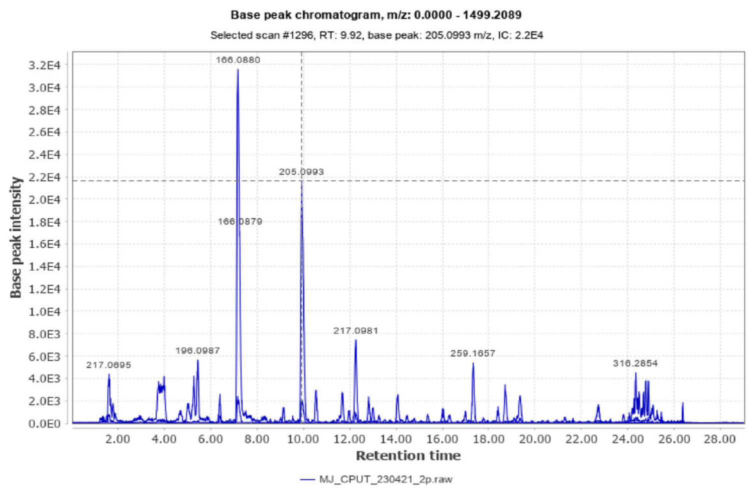
Sample 1: D0- Control (0 mM NaCl)



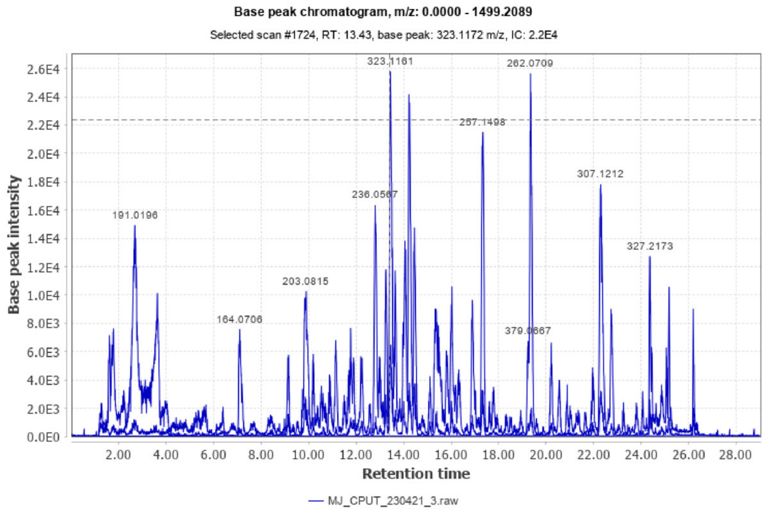


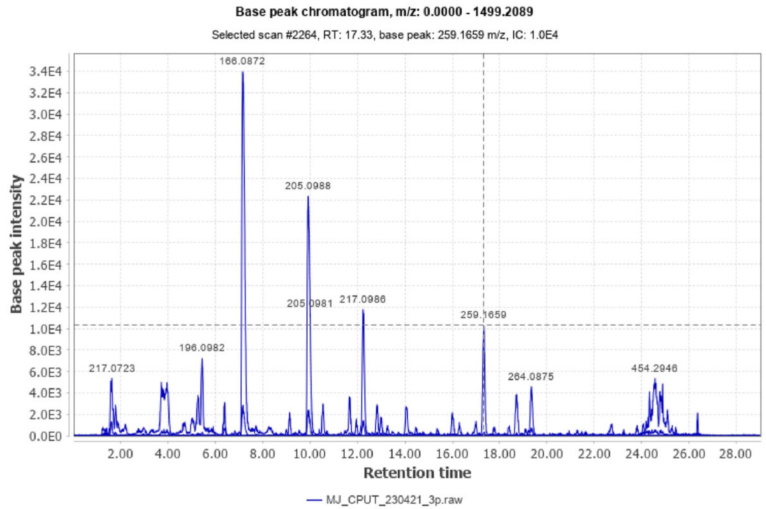
Sample 2: D1- 50 mM NaCl



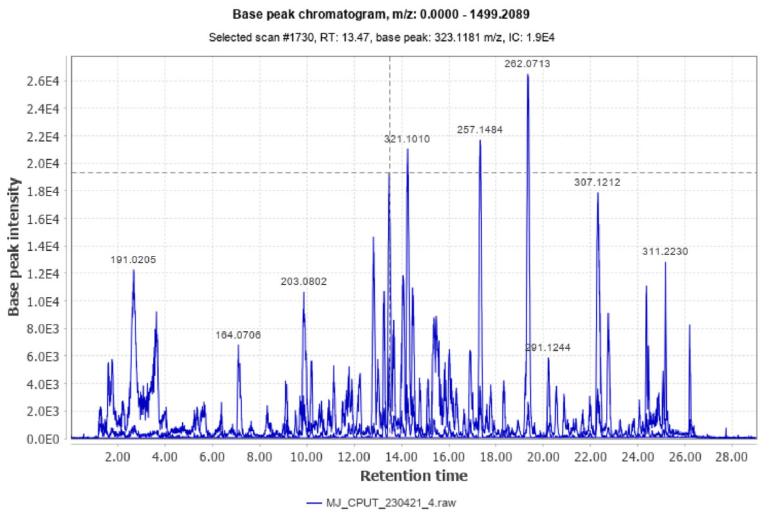


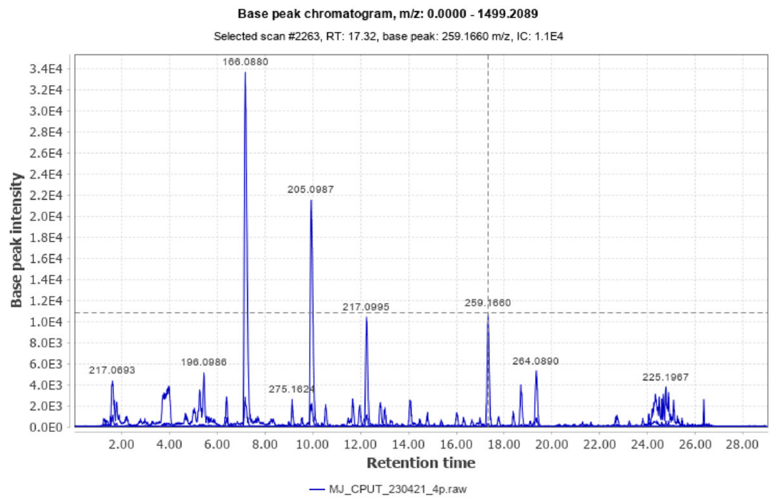
Sample 3: D2- 100 mM NaCl





Sample 4: D3- 150 mM NaCl





Sample 5: D4- 200 mM NaCl

Sample 6: D5- 250 mM NaCl

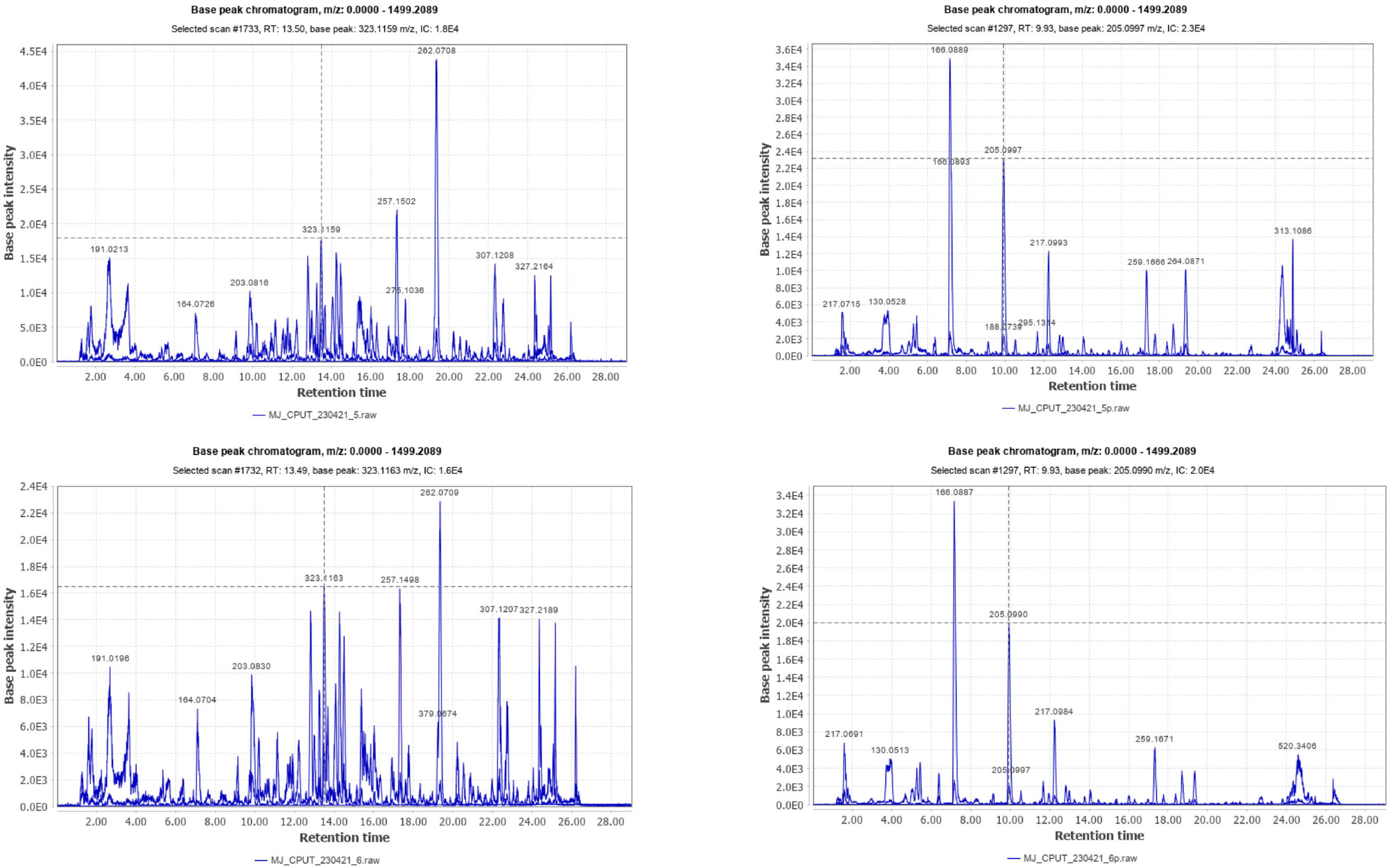
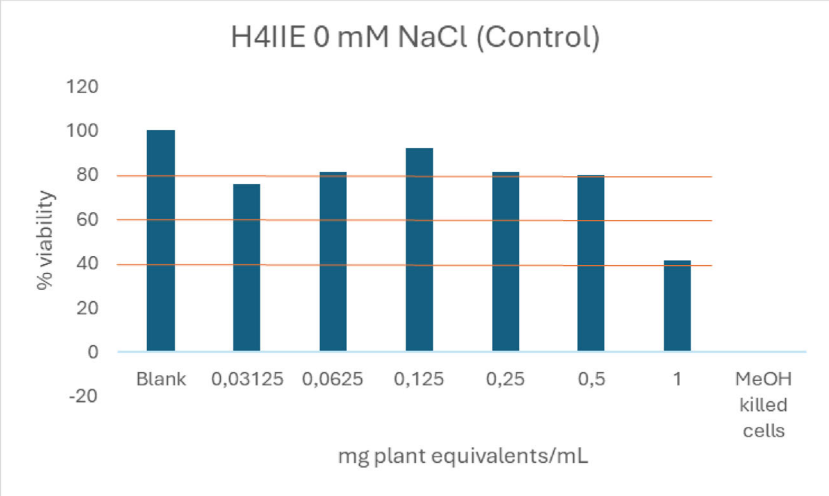
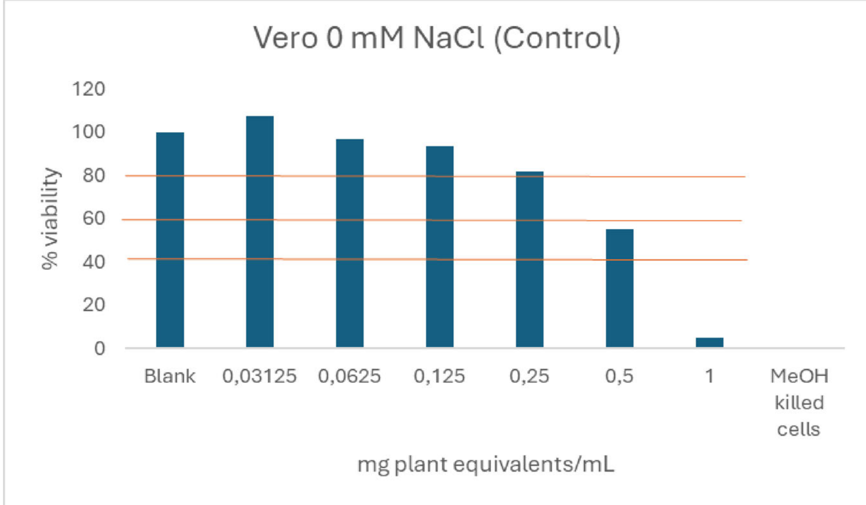


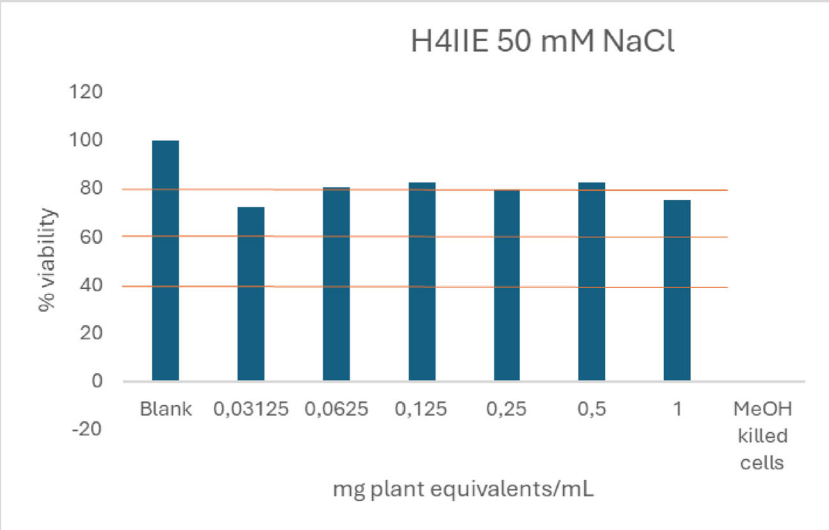
Figure 7.7: UHPLC-ESI-MS base peak chromatograms of ethanol extracts *of T. decumbens* analysed in positive and negative ion modes.

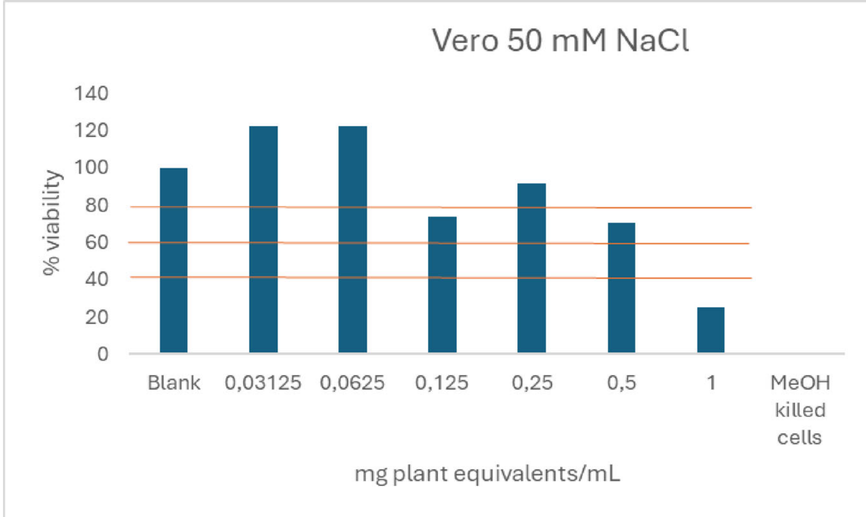
7.3.2. Cytotoxicity of *T. decumbens* crude extract against cancerous H4IIE-*luc* and non-cancerous Vero cells

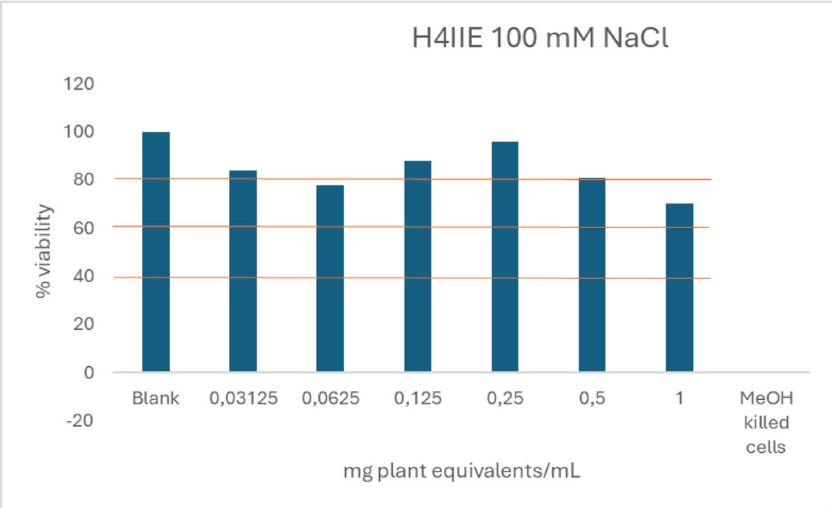
The cytotoxicity effect of ethanolic extracts of *T. decumbens* cultivated under varying salinity concentrations was examined on H4IIE-luc (cancerous Rat hepatoma) and Vero (non-cancerous African monkey kidney) cells, in order to determine what concentrations of the extract are safe for use in cancer treatment. The viability of cells was measured according to ISO 10993 template, where viability of >80% = non-cytotoxic, 60-80 = weak cytotoxic, 40-60 = moderate cytotoxic and viability of <40% = strong cytotoxic. Cells were then treated with extracts at various concentrations (0.03125 mg/mL; 0.0625 mg/mL; 0.125 mg/mL; 0.25 mg/mL; 0.5 mg/mL and 1 mg/mL) for 24 h. The results showed that the crude extracts of saline treatments (0-250 mM NaCl) at the concentrations of 0.03125 mg/mL; 0.0625 mg/mL; 0.125 mg/mL; 0.25 mg/mL and 0.5 mg/mL had a weaker cytotoxicity against H4IIE-luc (cancerous Rat hepatoma) cells, while the highest concentration (1 mg/mL) from 150 mM treatment showed a stronger cytotoxicity. Nonetheless, this concentration (1 mg/mL) also showed a stronger cytotoxicity against Vero (non-cancerous African monkey kidney) cells. These results demonstrates that the crude extracts of *T. decumbens* possess a weaker anti-cancer potential in most concentrations except (1mg/mL). However, this concentration is toxic to non-cancer cells, thus it not safe for use.

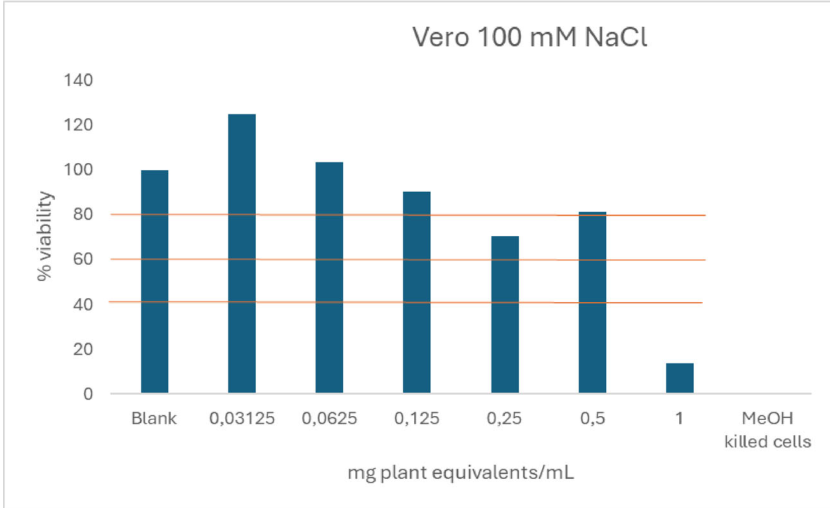

H4IIE 0 mM NaCl (Control)
% viability
120
100
80
60
40
20
0
-20
Blank
0,03125
0,0625
0,125
0,25
0,5
1
MeOH killed cells
mg plant equivalents/mL

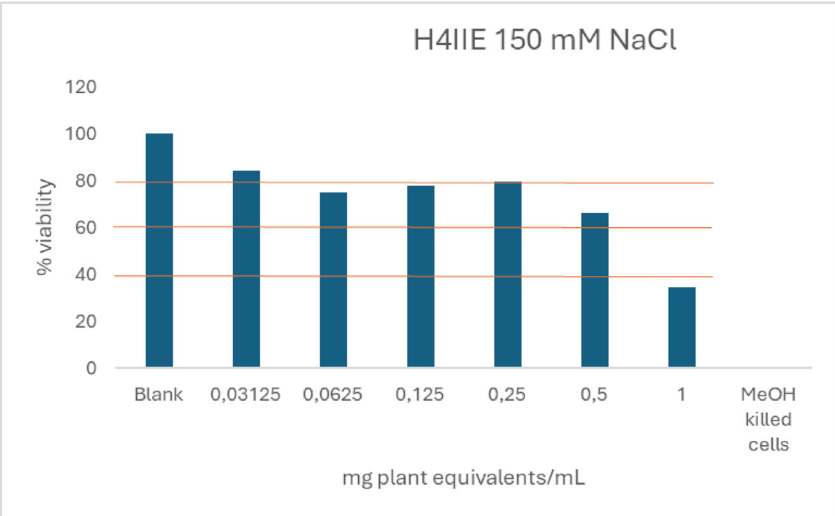

Vero 0 mM NaCl (Control)
% viability
120
100
80
60
40
20
0
Blank
0,03125
0,0625
0,125
0,25
0,5
1
MeOH killed cells
mg plant equivalents/mL

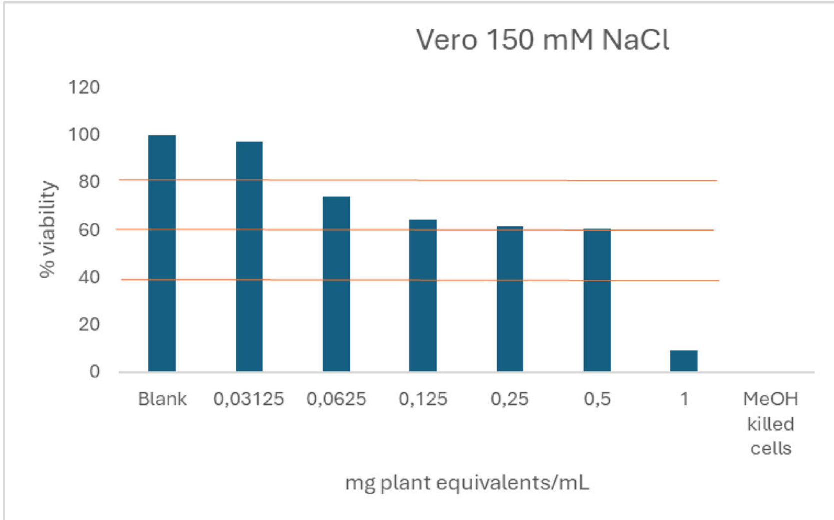

H4IIE 50 mM NaCl
% viability
120
100
80
60
40
20
0
-20
Blank
0,03125
0,0625
0,125
0,25
0,5
1
MeOH killed cells
mg plant equivalents/mL


Vero 50 mM NaCl
% viability
140
120
100
80
60
40
20
0
Blank
0,03125
0,0625
0,125
0,25
0,5
1
MeOH killed cells
mg plant equivalents/mL


H4IIE 100 mM NaCl
% viability
120
100
80
60
40
20
0
-20
Blank
0,03125
0,0625
0,125
0,25
0,5
1
MeOH killed cells
mg plant equivalents/mL


Vero 100 mM NaCl
% viability
140
120
100
80
60
40
20
0
Blank
0,03125
0,0625
0,125
0,25
0,5
1
MeOH killed cells
mg plant equivalents/mL


H4IIE 150 mM NaCl
% viability
120
100
80
60
40
20
0
Blank
0,03125
0,0625
0,125
0,25
0,5
1
MeOH killed cells
mg plant equivalents/mL


Vero 150 mM NaCl
% viability
120
100
80
60
40
20
0
Blank
0,03125
0,0625
0,125
0,25
0,5
1
MeOH killed cells
mg plant equivalents/mL

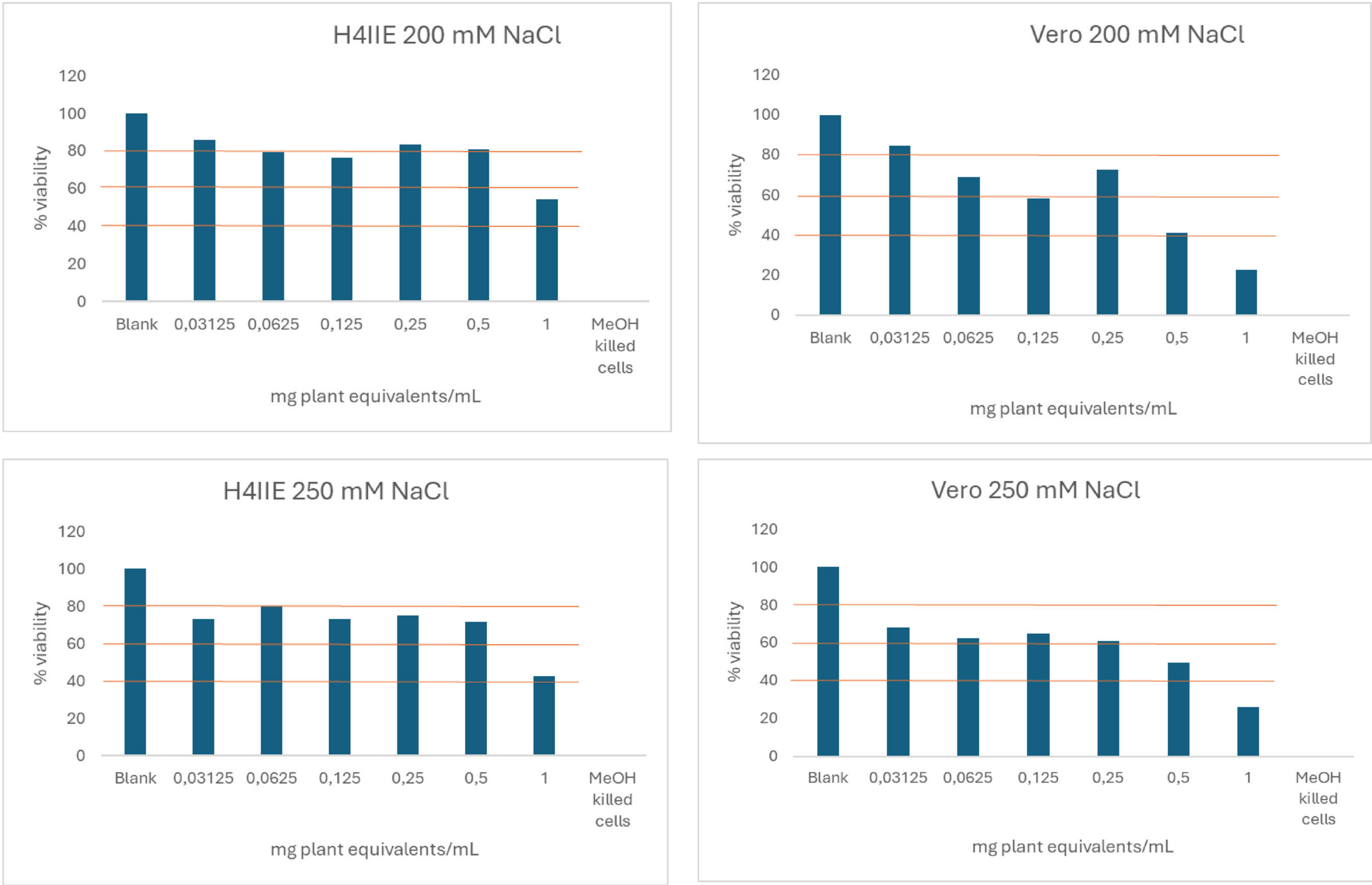
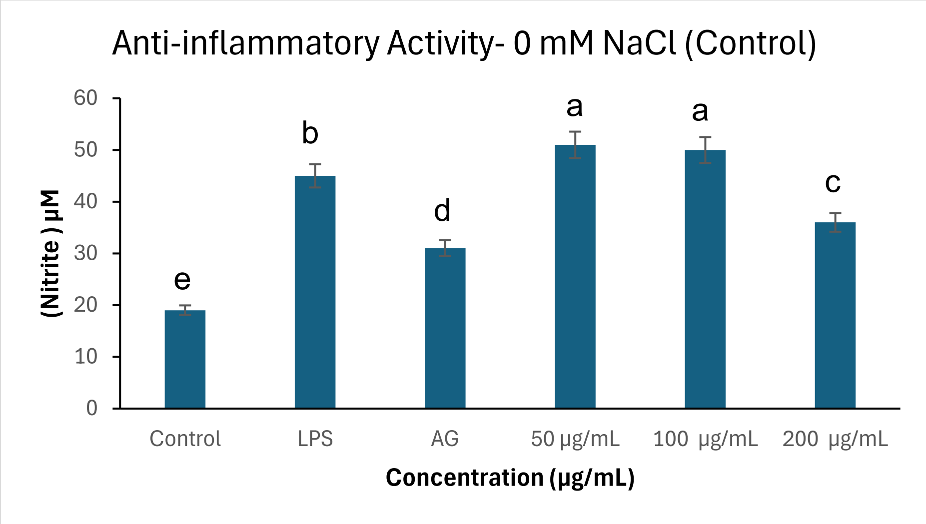
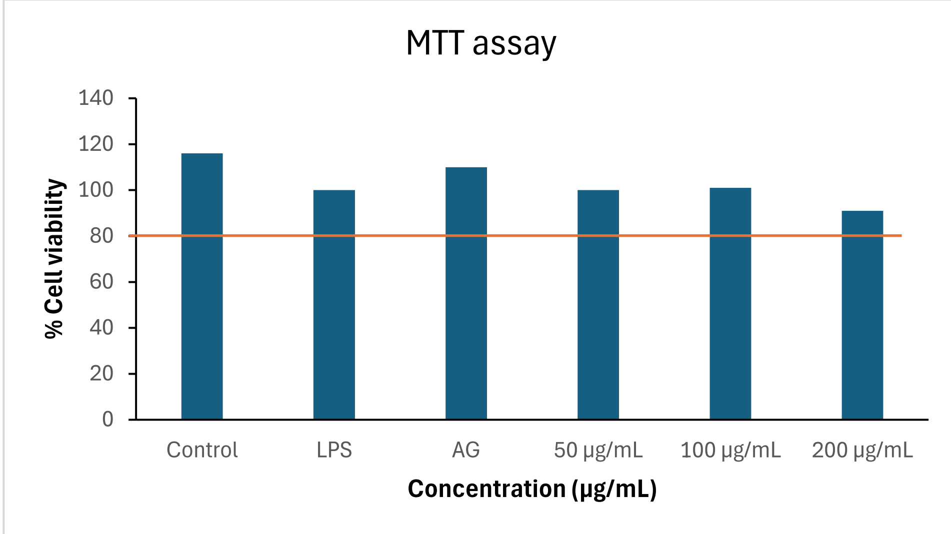


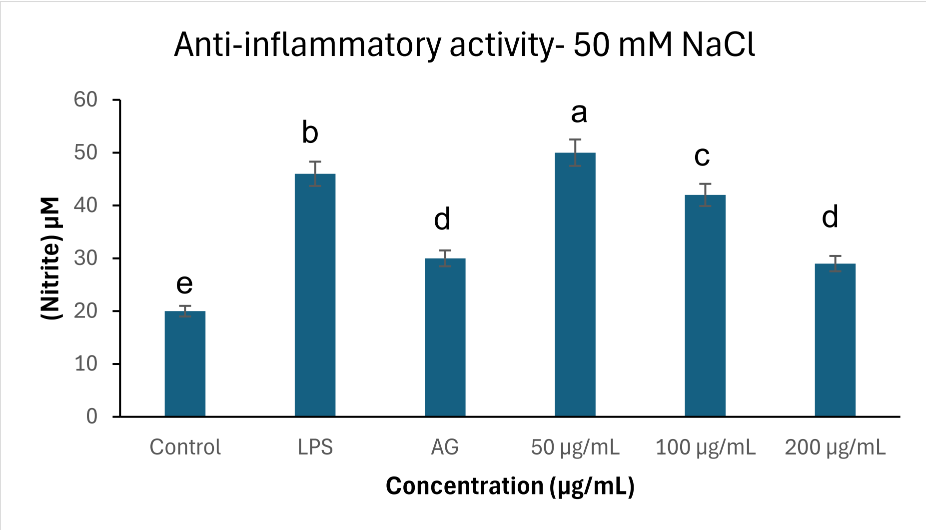
Figure 7.2: Cytotoxicity of *T. decumbens* crude extract against cancerous H4IIE-luc and non-cancerous Vero cells

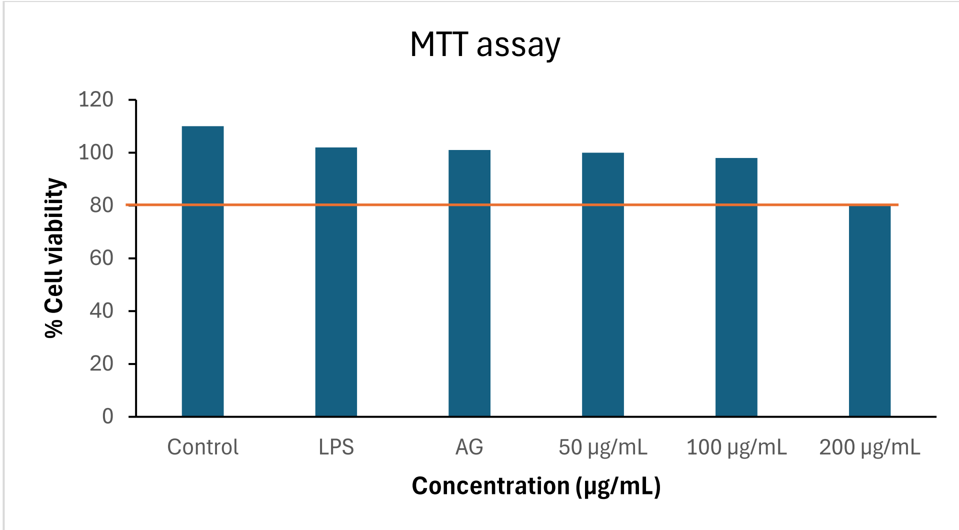
7.3.3. Anti-inflammatory activity

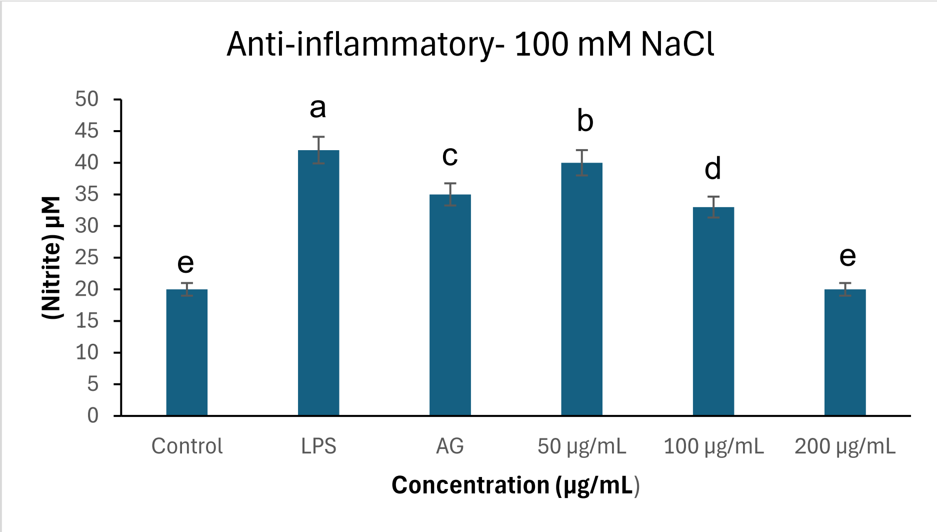
In Fig. 7.3, the anti-inflammatory efficacy is demonstrated by the reduction in nitrite concentration in response to lipopolysaccharide (LPS) activation of RAW macrophages with no effect on cell survival, as shown in Aminoguanidine (AG) treated cells which significantly ($p < 0.05$) lowered the nitrites to less than 40% with increase cell viability of 100% and above. A significant reduction in nitrite concentration was observed in comparison to the LPS activated cells, with the dose of 100 μg/mL in 100 mM treatment showing a significantly lower concentration of nitrite compared to other treatment concentrations of the extract that are non-toxic to cells. Moreover, this concentration had a significantly higher level of anti-inflammatory activity than cells treated with AG (positive control), thus showcasing a reasonable anti-inflammatory activity.

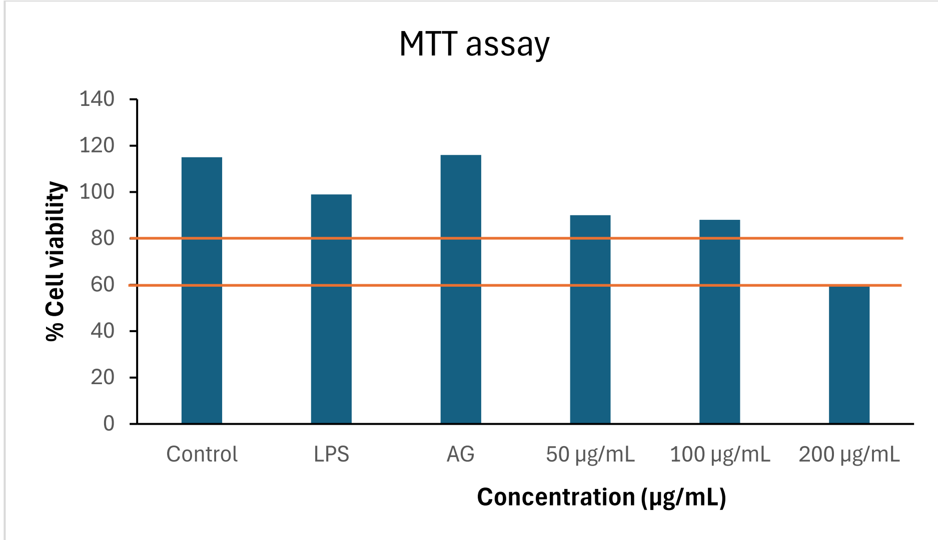

Anti-inflammatory Activity- 0 mM NaCl (Control)
(Nitrite) µM
60
50
40
30
20
10
0
e
b
d
a
a
c
Control
LPS
AG
50 µg/mL
100 µg/mL
200 µg/mL
Concentration (µg/mL)

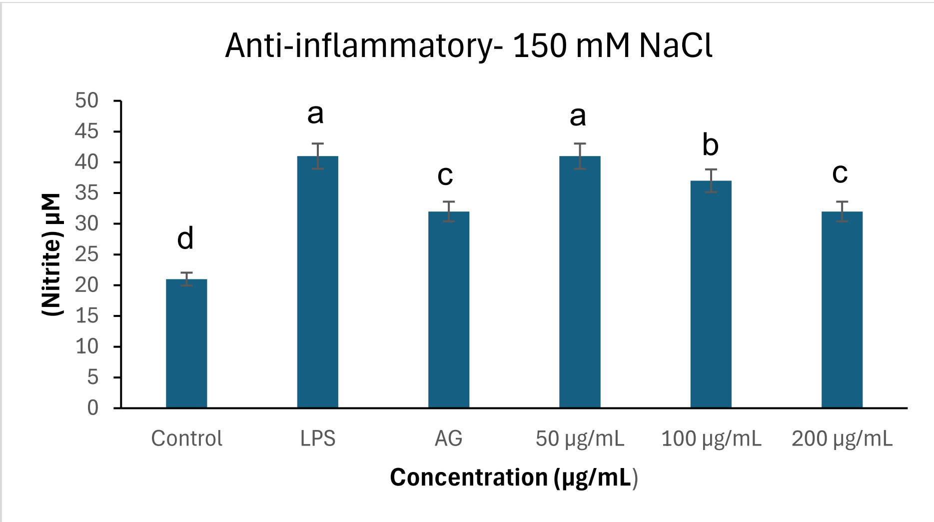

MTT assay
% Cell viability
140
120
100
80
60
40
20
0
Control
LPS
AG
50 µg/mL
100 µg/mL
200 µg/mL
Concentration (µg/mL)

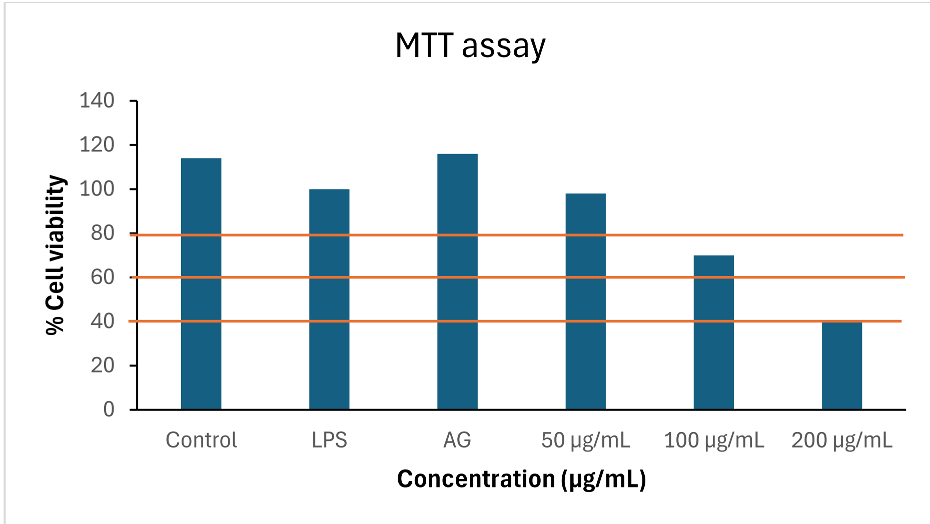

Anti-inflammatory activity- 50 mM NaCl
(Nitrite) µM
60
50
40
30
20
10
0
e
b
d
a
c
d
Control
LPS
AG
50 µg/mL
100 µg/mL
200 µg/mL
Concentration (µg/mL)


MTT assay
% Cell viability
120
100
80
60
40
20
0
Control
LPS
AG
50 µg/mL
100 µg/mL
200 µg/mL
Concentration (µg/mL)


Anti-inflammatory- 100 mM NaCl
(Nitrite) µM
50
45
40
35
30
25
20
15
10
5
0
e
a
c
b
d
e
Control
LPS
AG
50 µg/mL
100 µg/mL
200 µg/mL
Concentration (µg/mL)


MTT assay
% Cell viability
140
120
100
80
60
40
20
0
Control
LPS
AG
50 µg/mL
100 µg/mL
200 µg/mL
Concentration (µg/mL)


Anti-inflammatory- 150 mM NaCl
(Nitrite) µM
50
45
40
35
30
25
20
15
10
5
0
d
a
c
a
b
c
Control
LPS
AG
50 µg/mL
100 µg/mL
200 µg/mL
Concentration (µg/mL)


MTT assay
% Cell viability
140
120
100
80
60
40
20
0
Control
LPS
AG
50 µg/mL
100 µg/mL
200 µg/mL
Concentration (µg/mL)

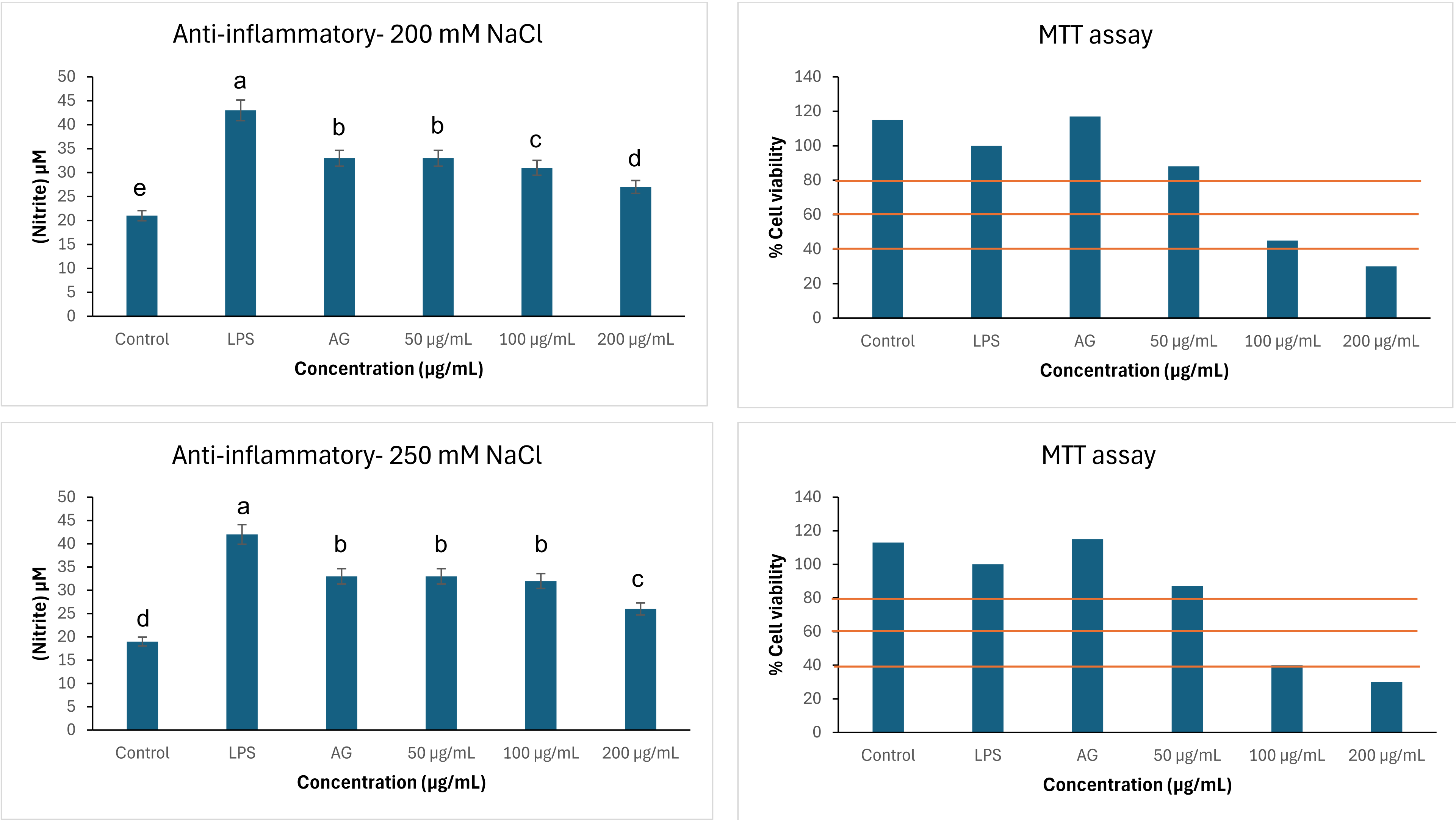


Figure 7.3: Nitric oxide production in LPS-activated macrophages treated with samples and Cell viability (%) of LPS-activated macrophages after 24 h of exposure to treatments. The bar graph represents the quadruplicate values of the experiment.

7.3.4. Acetylcholinesterase (AChE) activity

The results illustrated in Figure 7.4, show that salinity stress significantly influenced the acetylcholinesterase inhibitory activity of *T. decumbens* extracts on Human SH-SY5Y neuroblastoma cells. Extract with significantly higher acetylcholinesterase inhibition activity was the control (0 mM NaCl), followed by 50 and 100 mM NaCl respectively, compared to the untreated cells.

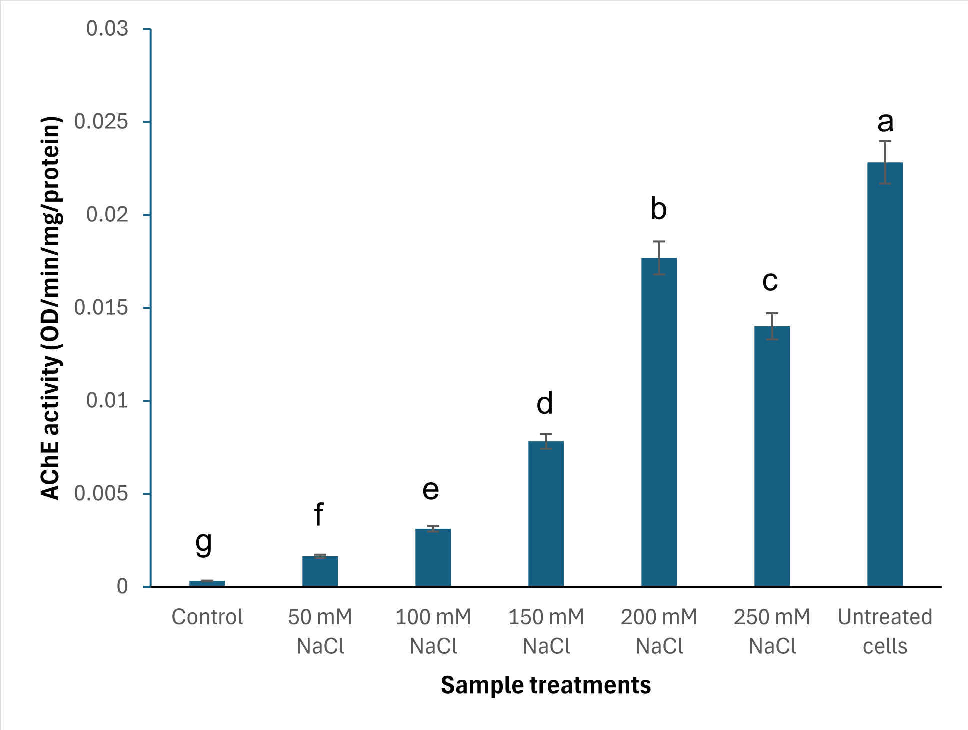


Figure 7.4: Acetylcholinesterase (AChE) inhibition potential of *T. decumbens* extracts cultivated under various saline concentrations.

7.4. Discussion

Edible halophytes have recently gained popularity as a source of food with functional qualities due to their nutritionally balanced composition and the presence of secondary metabolites (Sogoni et al., 2025). These species have been reported to produce novel bioactive compounds under salinity stress and are used in the management of various ailments in humans due to their distinct biological effectiveness and therapeutic potential (El-Saadony et al., 2023; Mohammed et al., 2023). In this study, UHPLC-MS analysis identified 98 compounds in *T. decumbens* for the first time, most of which were flavonols, stilbenoid, hydroxybenzoic acid derivatives, alkaloids, coumarin and hydroxycinnamic acids among others. These compounds are known to exert various biological activities ranging from cardio-protection, neuroprotection, anti-diabetic properties, antimicrobial, anti-cancer and anti-inflammation among others.

Cancer is one of the leading causes of death worldwide and the rising death rate is more rapidly in Africa, Asia and Central America (Soerjomataram et al., 2023). Thus, drawing attention for an affordable and effective anticancer treatment. Natural antioxidants and many phytochemicals have been recently suggested as anti-cancer adjuvant therapies because of their anti-proliferative and pro-apoptotic properties (Chimento et al., 2023).

Anticancer properties of plant based phenolic compounds have been reported extensively in the literature (Bhattarai et al., 2021; Maheshwari & Sharma, 2023). For instance, hydroxycinnamic acids has been reported to increase the cytotoxicity and apostosis of DU145 of prostate cancer cells (Jimoh et al., 2024). Sesquiterpene also showed good inhibition of cancer cells (HeLa and MCF-7) (Shoaib et al., 2017), while stilbenes have been reported to block the metabolic activation of pro-carcinogens by inhibiting specific isoforms of cytochrome P450 (CYP) enzymes and thus preventing the initiation of carcinogenesis in cultured human tumour cells (Akinwumi et al., 2018). Interestingly, prominent anti-cancer compounds such as stilbenoid ((E)-Piceatannol) (Piotrowska et al., 2012), hydroxybenzoic acid derivatives (6-Gingerol and 6-Gingerol-o- pentose) (Salari et al., 2023), tannin (3,3'-di-O-methylellagic acid) (Harper, 2023) and coumarins (Bergapten and Xanthotoxin) (Mirzaei et al., 2017) were identified in *T. decumbens.* Nonetheless, the ethanolic extracts possessed mild anti-cancer potential against H4IIE-luc (cancerous Rat hepatoma) in most concentrations. These results suggest that the presence of these anti-cancer metabolites might have been low in the tested extracts, and this may be due to the extraction solvent used. Thus, encouraging their isolation and the use of different extraction solvents for further research.

Inflammation is a biological defence mechanism that enables cells to protect themselves against pathogens and toxins, as well as defective immune regulation (Borquaye et al., 2020). However, unending inflammatory actions result in tissue dysfunction and damages leading to numerous ailment’s (Nkemzi et al., 2024). Current treatment for inflammation has been accomplished by the use of non-steroidal anti-inflammatory drugs (Gonfa et al., 2023). Nevertheless, adverse consequences linked to the extended use of these anti-inflammatory drugs have created a demand for novel treatments with minimal side effects. Previous studies have uncovered natural anti-inflammatory compounds such as phenolics and flavonoids to be beneficial in the treatment of chronic inflammatory diseases associated with overproduction of nitric oxide (Oguntibeju, 2018).

In this study, the anti-inflammatory activity was determined and indicated by the reduction in nitrite concentration in response to lipopolysaccharide activation of RAW macrophages with no effect on cell viability. A potent anti-inflammatory activity was observed in the dose of 100 μg/mL in crude extract of 100 mM NaCl treatment, and this was significantly higher than cells treated with aminoguanidine (positive control) with no effect on cell viability. The potent anti-inflammatory activity observed in *T. decumbens* could be due to the presence of identified phenolic derived compounds such as Phenylsulfate (catechol sulphate), Sesquiterpene (Zederone), hydroxybenzoic acid (6-Gingerol-o- pentose) and alkaloid (Trigonelline), which are reported to have anti-inflammatory activity (Pintatum et al., 2020; Promdam & Panichayupakaranant, 2022; Khalili et al., 2018; Ha et al., 2017). These results concur with findings of (Ayertey et al., 2021), who reported a commendable anti-inflammatory activity of the ethanolic leaf extract of *Morinda lucida* and was linked to the presence of phenolic derived compounds such as alkaloid and flavonoids known for their anti-inflammatory potential. Similarly, Nethengwe et al. (2024) also reported a significant reduction in nitrite concentration in a dose-dependent manner of *Garcinia livingstonei* aqueous extract in lipopolysaccharide (LPS)-induced cells with no signs of toxicity. Therefore, based on these results, *T. decumbens* could be considered as a potential source of anti-inflammatory treatment.

Alzheimer's disease (AD) is the predominant form of dementia, a progressive age-associated condition marked by the deterioration of brain function (Chauhan et al., 2024). This is attributable to the diminished amounts of the neurotransmitter acetylcholine in the brains of the elderly as the disease advances, leading to a decline in cognitive function (Nguyen et al., 2020; Taqui et al., 2022). The use of plant-based extracts as Acetylcholinesterase inhibitors (AChEIs) in AD has been reported to enhance acetylcholine levels in the synaptic area, thus reinstating impaired cholinergic neurotransmission (Prasathkumar et al., 2022; Huang et al., 2022). In this study, ethanolic extracts of *T. decumbens* significantly inhibited the AChE activity in Human SH-SY5Y neuroblastoma cells with pronounced effect in 0 and 50 mM NaCl samples as compared to untreated cells. This inhibition could be linked to the presence of 3-Hydroxy-DL-kynurenine, an amino acid known to regulated oxidative stress and neurotoxicity in the brain (Liang et al., 2022; Sharma et al., 2022). These findings are in agreement with the study conducted by Asaduzzaman et al. (2014), where *Aegle marmelos* extracts inhibited rat brain AChE. Moreover, the effectiveness of plant-based extracts in inhibiting AChE was also reported by Uddin et al. (2021) on several medicinal plants and was attributed to presence of anticholinesterase compounds. The presence of anticholinesterase compounds in the leaf extracts of *T. decumbens* suggest that this species could be used as a potential acetylcholinesterase inhibitor for the treatment of Alzheimer’s disease.

7.5. Conclusion

The present study was the first to explore the *in vitro* anti-cancer, anti-inflammatory and acetylcholinesterase inhibitory potential of ethanolic leaf extract of saline cultivated *T. decumbens*. Identification of compounds in leaf extracts revealed the presence of flavonoids, flavonols, stilbenoid, hydroxybenzoic acids, alkaloids and Hydroxycinnamic acids among others. Moreover, the crude extracts demonstrated a potent inhibitory effect on inflammation and acetylcholinesterase, while they had mild effect on cancer cells. The biological effects exhibited by the plant extract is due to the identified phytochemical compounds as previously documented, which indicates its potential as a promising source for isolating lead compounds that could be useful in developing new anti-inflammatory and acetylcholinesterase inhibitor drugs. Therefore, these findings could be beneficial for future research avenues on the application of this species in the formulation of nutraceuticals and pharmaceuticals.

7.6. Acknowledgement

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CHAPTER EIGHT

GENERAL CONCLUSION AND RECOMMENDATIONS

8. General conclusion and recommendation

8.1. Summary and conclusions

Soil salinity has been reported as one of the main causes of devastating crop production losses around the world including South Africa. The use of resilient crops like halophytes is crucial to fulfil the increasing food demand escalated by the increasing human population. Additionally, these species are also used in phytoremediation of saline soil as companion plants to salt-sensitive crops. However, in most African countries including South Africa, edible halophytes are still underutilised and understudied even though these species could provide an alternative source of nutrients to the growing human population. Recently, a neglected South African edible halophyte *Tetragonia decumbens* has been cultivated under saline conditions and showed promising production returns for bio- saline agriculture. Nevertheless, in-depth studies on the micro-morphological, physiological, biochemical and antioxidative mechanism of this species to salinity remains underexplored for precise bio-saline agriculture. Furthermore, the lack of scientific knowledge on its nutritional profile, pharmacological activity, and its potential use as a phytoremediator in salt affected soils has contributed to its underutilisation. Thus, this study examined the nutraceutical, phytochemical, intercropping and morpho-physiological response of *T. decumbens* to salt-stress to further support its domestication, consumption, and cultivation among South African households and in regions affected by salinity.

In Chapter 2 a review paper evaluating the potential of domesticating the wild dune spinach (*T. decumbens*) as a leafy vegetable, describing its morphology and ecology, its propagation and cultivation requirements as well as its potential use on human health and in phytoremediation of saline soils was conducted. The literature demonstrated that *T. decumbens* is an overlooked and underutilised halophyte with edible attributes and presents a convincing argument for its incorporation into dietary regimens and agricultural practices. This was due to its exceptional easy to grow methods, accompanied by its high nutritional value, promising consumer acceptance and therapeutic potential. The promising capacity of this species to fulfil most of the recommended dietary allowance of essential nutrients such as sodium, magnesium, potassium, iron, zinc, and vitamin C for all age groups makes it a powerful ally in combating nutritional deficiencies. Moreover, its salt accumulating capabilities makes it a good candidate for phytoremediation of saline soils through various systems such as intercropping with salt-sensitive species. Therefore, it was concluded that the addition of *T. decumbens* to agricultural systems may enhance crop diversity, assure food security, improve food nutrition, and promote sustainable farming practices. Thus, further studies on its nutraceutical, phytochemical, intercropping and morpho-physiological response to salt-stress was recommended to further support its domestication, consumption, and cultivation among South African households and in regions affected by salinity.

In Chapter 3 & 4 the investigation into the effect of salinity on micro-morphological, physiological, biochemical and antioxidative mechanisms in *T. decumbens* was conducted to elucidate its salt-tolerance mechanisms. Results revealed a substantial enhancement in shoot length, number of branches, relative water content, as well as total fresh weight in plants irrigated with 50 and 100 mM NaCl in comparison to the control, while higher saline concentrations (150-250 mM NaCl) reduced plant growth, chlorophyll content and stomatal density. Similarly, these high salt concentrations induced more severe oxidative stress indicated by high amounts of superoxide, cell death viability and malondialdehyde, with the most pronounced effect at the highest NaCl concentration (250 mM). Nevertheless, *T. decumbens* modulated various defence mechanisms with increasing salinity stress, these include the upregulation of superoxide dismutase, catalase, polyphenols, flavonoids, proanthocyanidins and the build-up of sodium ions in the leaves. Moreover, micromorphological examination of the adaxial layer of the epidermis revealed distinctive uprooted glandular peltate trichomes with increasing salinity, thus suggesting this species may have utilized these specialised structures to store or dilute excessive sodium ions. These results showed that *T. decumbens* can withstand salinity by modifying its anatomical features, morpho-physiological traits, antioxidant defence systems, and managing ion toxicity and oxidative stress efficiently, since all plants withstand salinity without showing signs of toxicity.

In Chapter 5 the investigation into the nutritional composition, secondary metabolites, and anti-microbial potential of wild and greenhouse cultivated *T. decumbens* was conducted to further support its consumption and potential medicinal use. Samples of *T. decumbens* were collected from three coastal areas (Strand beach, Muizenberg beach and Blouberg beach) during the dry (summer) and wet (winter) seasons in Cape Town, and the greenhouse cultivated were subjected to varying salinity doses (0, 50, 100, 150, 200 and 250 mM). Results revealed a considerable increase in minerals (N, P and Mg) and proximate composition (Ash, moisture, and carbohydrates) in greenhouse cultivated plants subjected to 50 mM of salinity while the highest crude fat and neutral detergent fibre were recorded in wild samples. Moreover, heavy metal accumulation (Zn and Fe), phytochemicals, anti-nutrients and anti-microbial activities were more pronounced in wild plants than in cultivated samples. Wild plants collected at Blouberg beach had more heavy metals, anti-nutrients, phytochemicals, and anti-microbial activity. These findings validated for the first time, the relevance of nutritional quality of *T. decumbens* in assessing its suitability as a source of nutrients. It was evident that consuming *T. decumbens* may meet the daily needs for minerals and proximate nutrients. Moreover, the efficacy of antimicrobial metabolites of the crude extract of wild collected *T.*

decumbens, suggest that this halophyte might be suitable for both anti-bacterial and anti-fungal applications. Therefore, for optimal yield of minerals and proximate constituents, it was recommended to cultivate this species under 50 mM of salinity while for medicinal purposes, wild collected samples were recommended.

In Chapter 6 the intercropping potential of the halophyte *Tetragonia decumbens* in alleviating the damaging effects of salinity stress on spinach (*Spinacia oleracea*) was examined. Spinach seedlings were grown alone and in consociation with the halophyte under various salt stresses (50, 100, 150 and 200 mM NaCl). Results showed that increasing salinity reduced crop growth, relative water content, chlorophyll, and nutritional quality of spinach in monocultured system. Similarly, high salinity treatment induced severe oxidative stress depicted by high amounts of superoxide, malondialdehyde and the upregulation of superoxide dismutase, catalase, peroxidase, polyphenols, and flavonoids. Interestingly, intercropped spinach irrigated with 50 and 100 mM of NaCl revealed a substantial enhancement in crop performance, reduction in oxidative stress and had improved nutritional quality depicted by high amounts of minerals, proximate constituents, and vitamins. These results support the introduction of *T. decumbens* in vegetable farming systems and highlighted its positive impact on improving the overall crop performance of salt sensitive vegetables under saline condition. Furthermore, field studies on saline soils are recommended to broaden the mechanism of action and to ascertain the phytodesalinating potential of *T. decumbens*.

In Chapter 7 the chemical profile of metabolites aggregated in the crude extracts of *T. decumbens* as well as their cytotoxicity, acetylcholinesterase inhibitory activity, anti-cancer and anti-inflammatory potential was evaluated to further supports its consumption and commercialization. For the first time, the ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis identified 98 compounds in saline cultivated samples (0, 50, 100, 150, 200 and 250 mM NaCl) of *T. decumbens,* most of which were stilbenoids ((E)-Piceatannol), hydroxybenzoic acid derivatives (6-Gingerol and 6-Gingerol-o- pentose), tannin (3,3'-di-O-methylellagic acid), coumarins (Bergapten and Xanthotoxin), Phenylsulfate (catechol sulphate), Sesquiterpene (Zederone), and alkaloid (Trigonelline) among others. Potent anti-inflammatory activity in the form of nitrate reduction in lipopolysaccharide (LPS)-induced cells was noted in the dose of 100 µg/mL in crude extract of 100 mM NaCl treatment, and this was significantly higher than cells treated with Aminoguanidine (positive control). There was also a significant inhibitory activity of acetylcholinesterase in Human SH-SY5Y neuroblastoma cells treated with crude extracts of 0 mM NaCl, followed by 50 and 100 mM NaCl respectively, when compared with untreated cells. Additionally, the extract of 150 mM NaCl treatment showed cytotoxic effect against cancer cells at high concentration (1mg/mL) but were toxic to non-cancer cells. These findings suggest that the identified bioactive compounds in *T. decumbens* extracts could have potential anti-inflammatory and acetylcholinesterase inhibitory activities. Consequently, these compounds can be further explored as potential therapeutic agents for the dual treatment of inflammation and alzheimer's disease. Therefore, it was recommended that isolating these lead compounds could be beneficial for future research avenues on the application of this species in the formulation of nutraceuticals and pharmaceuticals.

8.2. Recommendations

This consolidated work has shown the potential value of *T. decumbens* as a nutritious vegetable with numerous pharmacological values. Moreover, its phytoremediation potential of saline soils supports its introduction in vegetable farming systems (intercropping with salt sensitive species) in saline affected areas. Nonetheless, more studies are recommended on identifying key genes and proteins responsible for the salt tolerance mechanism in this species. Moreover, the synthesis, characterization and assessment of potential pharmacological effect of bio-metallic nanoparticles produced from aqueous extract of this species is recommended in future medical studies or trials. Furthermore, it is also important to isolate pure compounds in this species and elucidate their chemical structures using nuclear magnetic resonance (NMR) spectroscopy to underpin their biological activities.

LIST OF APPENDICES

APPENDIX A: PUBLISHED PAPER IN JOURNAL OF OPEN AGRICULTURE

DE GRUYTER

Review Article

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

Evaluating the nutritional, therapeutic, and economic potential of *Tetragonia decumbens* Mill.: A promising wild leafy vegetable for bio-saline agriculture in South Africa

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Abstract: Global agriculture feeds over seven billion people and alarmingly, this number is expected to increase by a further 50% by 2050. To meet the additional food demand, the world development report has estimated that crop production should increase by 70–100% by 2050. However, climate change, expanding soil salinization, and the developing shortages of freshwater have negatively affected crop production of edible plants around the world. Current attempts to adapt to these conditions include the use of salt-tolerant plant species with potential economic value to fulfil the increasing food demand escalated by the increasing human population. The wild edible halophyte *Tetragonia decumbens* commonly known as dune spinach has the potential to be used as a leafy vegetable, a source of dietary salt, in phytoremediation and as a source of secondary metabolites. However, it remains underutilized in South Africa as commercial farming of this species has never been explored. This review examined the potential of domesticating the wild dune spinach as a leafy vegetable, describing its morphology and ecology, its propagation and cultivation requirements as well as its potential use on human health and in phytoremediation of saline soils. Furthermore, this analysis is expected to be useful towards further research and popularization of this underexploited halophyte.

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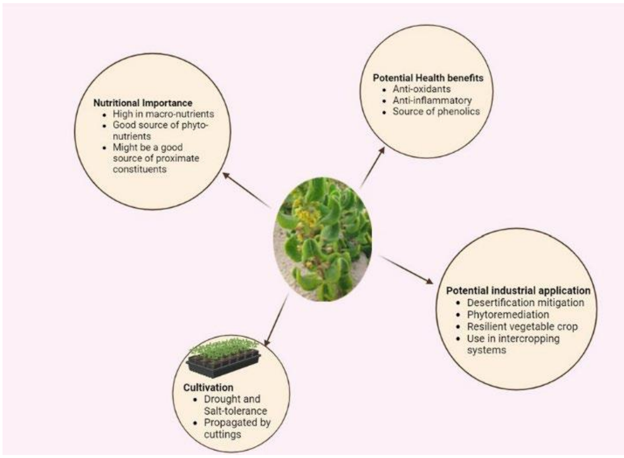

Source: Avela Sogoni.

Keywords: dune spinach, functional foods, halophyte, salt tolerance, wild foods

1 Introduction

The global agricultural industry already provides sustenance for more than seven billion people, and it is projected that this figure will rise by an additional 50% by the year 2050 [1]. The need for food production on a worldwide scale is now at its highest and already about 2 billion people are estimated to suffer from micronutrient deficiency problem [2]. To satisfy the growing need for food, agricultural output must rise by 70–100% by the year 2050 [3]. However, increasing soil salinity and global climate change have caused major constraints for agricultural productivity of staple crops such as wheat and maize [4]. Furthermore, high salinity in the soil triggers osmotic balance disruption in plants, limiting water intake an

APPENDIX B: PUBLISHED PAPER IN PLANT PHYSIOLOGY REPORTS

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ORIGINAL ARTICLE

Salinity modulates morpho-physiology, biochemical and antioxidant defence system in *Tetragonia decumbens* Mill.: a neglected wild leafy vegetable in South Africa

Avela Sogoni[1] · Muhali Olaide Jimoh[1,2] · Adelé Mariska Barker[3] · Marshall Keyster[3] · Learnmore Kambizi[1] · Charles Petrus Laubscher[1]

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Abstract

Tetragonia decumbens is an edible halophyte that grows naturally in saline environment; however, its tolerance mechanisms are poorly understood for bio-saline agriculture. So, this research was designed to look into how salinity affects vegetative growth, leaf succulence, chlorophyll content, cation accumulation, oxidative stress indicators, and antioxidative defence mechanisms involved in the salt tolerance of *T. decumbens*. Saline conditions were prepared by dissolving sodium chlorine (NaCl) in the nutritive solution. The control was maintained and only watered with nutrient solution while the tested treat- ments contained graded NaCl doses (250, 200, 150, 100, and 50 mM). Results revealed a substantial enhancement in shoot length, number of branches, relative water content, as well as total fresh weight in plants irrigated with 50 and 100 mM NaCl in comparison to the control, while higher saline concentrations (150–250 mM NaCl) reduced plant growth and chlo- rophyll content. Similarly, these high salt concentrations induced more severe oxidative stress indicated by high amounts of superoxide, cell death viability and malondialdehyde, with the most pronounced effect at the highest NaCl concentration (250 mM). Nevertheless, *T. decumbens* modulated various defence mechanisms with increasing salinity stress, these include the upregulation of superoxide dismutase, catalase, polyphenols, flavonoids, proanthocyanidins and the build-up of sodium ions in the leaves. These results show that *T. decumbens* can withstand salinity by modifying its morpho-physiological traits, antioxidant defence systems, and managing ion toxicity and oxidative stress efficiently, since all plants withstand salinity without showing signs of toxicity.

Keywords Antioxidant defence systems · Dune spinach · Edible halophytes · Salinity tolerance · Underexploited vegetables

APPENDIX C: PUBLISHED PAPER IN RUSSIAN JOURNAL OF PLANT PHYSIOLOGY

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RESEARCH PAPERS

Salinity Induced Leaf Anatomical Responses and Chemical Composition in *Tetragonia decumbens* Mill.: an Underutilized Edible Halophyte in South Africa

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Abstract—*Tetragonia decumbens* Mill. has recently been reported to withstand the adverse effect of salinity. However, its leaf anatomical responses are poorly understood since previous studies were focused on basic physiological and biochemical parameters. This study was designed to examine leaf micromorphological traits and internal leaf elemental compartmentalization using Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) to elucidate relative salinity tolerance mechanisms. Salt concentrations were applied to six treatments by increasing the concentrations of NaCl in a nutrient solution. The control treatment (0 mM) was irrigated solely by the nutritional solution, whereas other treatments contained graded NaCl concentrations (50, 100, 150, 200, and 250 mM). Micromorphological examination of the adaxial layer of the epidermis revealed distinctive glandular peltate trichomes among treatments. In control plants, the tri- chomes were flaccid and not easily detectable which resulted in low trichome density. While plants treated with salinity had uprooted trichomes which were modified to dish-like structures as salinity increases, with a more pronounced visibility at the highest salinity treatment (250 mM). On the contrary, increasing salinity reduced the stomatal density as well as stomatal opening with a more pronounced effect at 250 mM. Furthermore, the EDX revealed the presence of important elements such as potassium (K), calcium (Ca), magne- sium (Mg), sodium (Na) and chlorine (Cl) which are responsible for salt tolerance in many species. Na increased with increasing saline treatment up to 100 mM then declined drastically. The lowest Na was detected in control plants which were comparable to plants irrigated with 150, 200 and 250 mM respectively. Likewise, the lowest chlorine content was detected in control plants while it increased in saline treated plants up to 150 mM and declined in plants irrigated with 200 and 250 mM respectively. When assessing K quanti- fication, saline treatment drastically reduced K content with increasing saline irrigation. Contrariwise, the Mg content increased with saline irrigation up to 100 mM and was not detected in plants irrigated with 200 and 250 mM respectively. Interestingly, Ca was only detected in plants irrigated with 150, 200 and 250 mM respectively. These findings validate that *T. decumbens* can tolerate salinity by modulating anatomical features such as trichomes, control of stomatal aperture and effectively managing ion toxicity with increasing salinity.

Keywords: *Tetragonia decumbens*, dune spinach, energy dispersive X-ray, glandular peltate trichomes, salt glands, salt tolerance

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APPENDIX D: PUBLISHED PAPER IN JOURNAL OF AGRICULTURE AND FOOD RESEARCH

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Journal of Agriculture and Food Research

Comparing the nutritional, phytochemical, and anti-microbial potential of wild and cultivated *Tetragonia decumbens* mill.: A promising leafy vegetable for bio-saline agriculture in South Africa

Avela Sogoni [a], Muhali Olaide Jimoh [a,b], Pamella Mngqawa [c], Sihle Ngxabi [a], Marilize Le Roes-Hill [c], Learnmore Kambizi [a], Charles Petrus Laubscher [a,*]

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A R T I C L E I N F O

Keywords:
Antimicrobial metabolites
Anti-fungal activity
Edible halophytes
Neglected vegetables
Nutritional value

A B S T R A C T

Tetragonia decumbens is a neglected edible halophyte that grows naturally in coastal areas of South Africa. Its underutilisation is due to the limited literature on its nutraceutical potential. So, this research was designed to assess the nutritional value, secondary metabolites, and anti-microbial potential of wild and greenhouse culti- vated *T. decumbens* to further support its consumption and potential medicinal use. Samples of *T. decumbens* were collected from three coastal areas (Strand beach, Muizenberg beach and Blouberg beach) during the dry and wet seasons in Cape Town, and the greenhouse cultivated were subjected to varying salinity doses (0, 50, 100, 150, 200 and 250 mM). Results revealed a considerable increase in minerals (N, P and Mg) and proximate composition (Ash, moisture, and carbohydrates) in greenhouse cultivated plants subjected to 50 mM of salinity while the highest crude fat and neutral detergent fibre were recorded in wild samples. Moreover, heavy metal accumu- lation (Zn and Fe), phytochemicals, anti-nutrients and anti-microbial activities were more pronounced in wild plants than in cultivated samples. Wild plants collected at Blouberg beach had more heavy metals, anti-nutrients, phytochemicals, and anti-microbial activity. These findings validate for the first time, the relevance of nutritional quality of *T. decumbens* in assessing its suitability as a source of nutrients and antioxidants with possible me- dicinal value as shown by the inhibition of harmful bacteria and fungal strains.

1. Introduction

Climate change and increasing soil salinity are already having a significant effect on crop production losses around the globe [1,2]. Thus, the alternative use of resilient crops like halophytes is crucial to fulfil the increasing food demand escalated by the increasing human population [3]. Edible halophytes are crops that can tolerate high temperatures, drought, and salinity, making them the ideal choice for cultivation in regions impacted by both salinity and drought [4]. These species are known to provide needed calories, proteins, fats, and nutrients as per human daily needs [5]. Moreover, they have recently attracted attention due to their nutritional and medicinal value. The aforementioned characteristics often arise from secondary metabolites that these species synthesise in response to oxidative damage caused by salt stress. Phenolic chemicals and some vitamins are examples of metabolites that possess well-established biological attributes such as antioxidant, anti-microbial and anti-inflammatory activity [6].

Halophytes serve as a food source with functional qualities due to their nutritionally balanced composition and the presence of secondary metabolites [7]. Recently, upscale restaurants in Europe and Asia have started including these plants into their menus as a novel ingredient that also promotes a nutritious diet [8,9]. This led to the inclusion of these plants on supermarket shelves which resulted into the emergence of commercial cultivation due to the increasing demand of sustainable supply [10].

In most African countries including South Africa, edible halophytes are still underutilized and understudied even though these species could provide an alternative source of nutrients to the growing huma

APPENDIX E: PUBLISHED PAPER IN JOURNAL OF THE SAUDI SOCIETY OF AGRICULTURAL SCIENCES

Journal of the Saudi Society of Agricultural Sciences (2025) 24:2 https://doi.org/10.1007/s44447-025-00001-2

ORIGINAL PAPER

Intercropping the halophyte *Tetragonia decumbens* Mill. with salt-sensitive *Spinacia oleracea* L. mitigated salinity stress by enhancing the physiological, biochemical, and nutritional quality of the salt-sensitive species under saline cultivation

Avela Sogoni[1] · Muhali Olaide Jimoh[1,2] · Sihle Ngxabi[1] · Marshall Keyster[3] · Learnmore Kambizi[1] · Charles Petrus Laubscher[1]

Received: 13 February 2025 / Accepted: 15 February 2025

Abstract

Increasing soil salinity is already having a significant effect on production losses of commercial vegetables around the globe. Thus, the implementation of innovative techniques is crucial to cultivate these vegetables amidst these unfavourable condi- tions. Halophytes are potential plants for resilient agricultural systems, such as intercropping with glycophytes, to enhance their productivity in saline soils. Therefore, the purpose of this study was to examine the intercropping potential of the halo- phyte *Tetragonia decumbens* in alleviating the damaging effects of salinity stress on spinach (*Spinacia oleracea*). Spinach seedlings were grown alone and in consociation with the halophyte under various salt stresses (50, 100, 150 and 200 mM NaCl). Results showed that increasing salinity reduced crop growth, relative water content, chlorophyll, and nutritional qual- ity of spinach in monocultured system. Similarly, high salinity treatment induced severe oxidative stress depicted by high amounts of superoxide, malondialdehyde and the upregulation of superoxide dismutase, catalase, peroxidase, polyphenols, and flavonoids. Interestingly, intercropped spinach irrigated with 50 and 100 mM revealed a substantial enhancement in crop performance, reduction in oxidative stress and had improved nutritional quality depicted by high amounts of minerals, proxi- mate constituents, and vitamins. These results support the introduction of *T. decumbens* in vegetable farming systems and highlights its positive impact on improving the overall crop performance of salt sensitive vegetables under saline condition.

Keywords Food security · Intercropping with halophytes · Phyto-desalination · Saline agriculture