VESICULAR ARBUSCULAR MYCORRHIZAL INFLUENCE ON *SUTHERLANDIA FRUTESCENS* SECONDARY METABOLITES, PERFORMANCE AND SALINE STRESS ALLEVIATION

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DECLARATION

The undersigned, Mabila Wandile, hereby attests that the Master of Science in Agriculture mini-dissertation submitted to the University of Mpumalanga has not been previously submitted for any other degree, either at this institution or elsewhere. This dissertation in design and execution is my work and does not contain other persons' graphs, pictures or data unless duly acknowledged.

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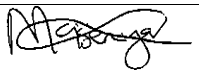
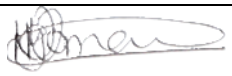
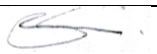
Supervisor: Dr TA Masenya	Signature: 	Date: 20/04/2026
Co-Supervisor: Dr N Khanyile	Signature: 	Date: 20/04/2026
Co-supervisor: Dr SS Thosago	Signature: 	Date: 20/04/2026

DEDICATION

I dedicate this work to my supervisor, Dr. TA Masenya, who believed in me throughout the project, providing encouragement and support. I would also like to express my gratitude to my family for allowing me to pursue this degree, as well as to the friends I made at the University of Mpumalanga, affectionately known as “Amasamariya,” who showed me love and support in every step of the way.

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ABSTRACT

The global population is continuously increasing, leading to a corresponding rise in demand for valuable medicinal plants, such as cancer bush (*Sutherlandia frutescens* L. (Br.)). Nevertheless, arable land is scanty, while the available land has high concentrations of salt ions, NaCl and $CaCl_2$. Alternative sustainable solutions to manage the one are necessary to safeguard the land for future use, while maintaining food security demands. Such solutions include the use of Vesicular Arbuscular Mycorrhiza (VAM) fungi, which haves become a hot topic and efficient solution to abiotic stress incidences. The objectives of the study were to: (1) determine whether VAM fungi will improve growth and yield of *S. frutescens* and alleviate salinity stress (2) evaluate whether biosynthesis and accumulation of phytochemicals of *S. frutescens* will be influenced using VAM fungi in reducing salinity. A shade net and microplot experiments were conducted at the University of Mpumalanga in 2024. The two independent experiments were carried out as a 4x4 factorial arrangement laid out in a randomised complete block design (RCBD) with four replications. The treatments comprised of different levels of VAM (0, 10, 20, and 30 g) and chloride salinity (0, 0.25, 0.5, and 0.75 dS m^{-1}), formulated as a 3:1 mixture of sodium chloride (NaCl) and calcium chloride ($CaCl_2$). The data collected included growth, physiological and plant biomass. The results in both experiments have shown that treatments had a significant effect ($p < 0.05$) on plant growth variables. Among the measured plant variables in experiment 1, VAM fungi had a significant impact ($p < 0.05$) on stem diameter, chlorophyll content, and the number of branches, accounting for 94.21%, 96.76%, and 98.11% of the total treatment variation (TTV). Similarly, in experiment 2, VAM fungi had a significant effect ($p < 0.05$) on chlorophyll, stem diameter, and plant fresh weight, contributing to a TTV of 91.99%, 98.12%, and 91.09%. The results revealed that, the

applied VAM fungi in Experiment 1, had relative positive effect on stem diameter, chlorophyll, and plant fresh weight compared to the control with a relative impact (RI) of 204.55 to 588.74%, 101.61 to 314.6% and 1350.96 to 5253.65%, respectively. While in experiment 2, VAM fungi increased chlorophyll content, stem diameter, and plant fresh weight with a RI of 85.59 to 278.88%, 307.34 to 890.40%, and 65.40 to 207.87%, respectively. The interactive application of salt and VAM treatment exhibited a notable impact on pH, among the measured variables in Experiment 1 only, with a TTV of 30.37%. The interactive application (0*75dS m^{-1}) resulted in the increase of soil pH by 18%. Vesicular-Arbuscular Mycorrhizal fungi, salt treatments, the interaction of salt and VAM fungi had a significant effect on the TPC in experiment 1, contributing 30.01%, 40.30%, and 29.25% of the TTV, while in Experiment 2, treatments had a substantial effect on TPC with a TTV of 98.43, 0.33, and 0.47%, respectively. Similarly, VAM fungi, salt treatments, and the interaction of salt and VAM fungi had a significant effect ($p<0.05$) on the TFC in Experiments 1 and 2, contributing to the TTV of 98.43%, 1.21%, and 0.35% and the TTV of 99.7%, 0.02% and 0.27%, respectively. The results demonstrated that, VAM boosts plant resilience to saline stress, enhances physiological traits, and affects the accumulation of secondary metabolites. The interaction of VAM and salinity treatments influenced TPC in experiment 2 only. The TPC was increased by the interaction with relative impact of 15.37% to 72.05%. Similar results were observed for TFC, where in experiment 1 and 2, the interaction increased the TFC by 0.07 to 0.57% in Experiment 1, whereas in experiment 2, TFC was reduced by 3.71% to 4.47%. Thus, the integration of VAM fungi in the cultivation of *S. frutescens* is a viable approach to augmenting plant development and resilience in saline stress conditions. It is advisable to conduct additional studies to enhance the techniques of VAM application aimed at optimising plant productivity and the biosynthesis of metabolites.

KEYWORDS

Sutherlandia frutescens., global warming, saline stress, productivity, Vesicular Arbuscular Mycorrhiza.

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CHAPTER 1

1. INTRODUCTION

1.1 Background of the study

Sutherlandia frutescens L., commonly known as cancer bush, is a remarkable leguminous plant indigenous to the sun-drenched landscapes of South Africa. Revered for its extensive pharmacological benefits and rich ethno-medicinal heritage, this plant has captured the attention of researchers and healers alike (Faleschini *et al*., 2013; Masenya *et al*., 2020). Thriving in the arid regions of Southern Africa, particularly the Western Cape, it also extends its reach to neighbouring countries like Botswana, Namibia, and Lesotho (Viljoen *et al.,* 2012).

This versatile medicinal plant that boasts a diverse array of beneficial properties, including anti-diabetic, anti-HIV, anti-stress, antibacterial, analgesic, anti-inflammatory, anticonvulsant, anti-proliferative, and antithrombotic effects. Such a rich pharmacological profile has made it a focal point of interest for its therapeutic applications (Altpeter *et al*., 2016; Masenya, 2022). Traditionally, cancer bush has been utilized to address a myriad of health concerns, ranging from common aches and pains to more severe ailments like eye diseases and digestive disorders. Additionally, it has gained recognition as a potent immune system booster for individuals living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) (Mensah *et al*., 2019; Van Wyk and Albrecht, 2008).

Despite its invaluable contributions to health and well-being, the relentless harvesting of *S. frutescens*, coupled with scant conservation efforts, threatens its ongoing survival (Masenya, 2022). As the global population continues to grow, it becomes imperative to protect this extraordinary herb and enhance its cultivation. Many medications derived from this plant have

emerged on the market, highlighting the importance of secondary plant metabolites in contemporary medicine. *Sutherlandia frutescens* is particularly celebrated for its diverse range of secondary metabolites, including flavonoids, phenolics, and tannins (Wink, 2013), which contribute to its impressive biological activities. Due to these beneficial properties, *S. frutescens* is increasingly utilized as a medicinal agent, incorporated into culinary practices, food additives for therapeutic and aromatic applications (Manuka et al., 2019). Adopting advanced agricultural techniques within the framework of climate-smart practices will be essential to meet the rising demand for its myriad benefits while ensuring its nourishment and preservation for future generations.

Medicinal plants are of paramount significance, primarily due to their secondary metabolism, which underpins their therapeutic effects. The core material behind the healing properties of these plants lies in their phytochemical properties and natural compounds that manifest as secondary metabolites (Kong *et al*., 2020). Secondary metabolism encompasses of sophisticated metabolic pathways that yield small molecule products. These compounds play crucial roles in ecological interactions, although they are not strictly necessary for the plant's immediate survival (Kong *et al*., 2020). The synthesis and accumulation of secondary metabolites are highly intricate processes that are influenced by a multitude of factors, including both internal developmental genetic circuits (such as genes and enzymes under regulation) and external environmental conditions, such as light, temperature, water availability, and salinity (Fang *et al*., 2022). These secondary metabolites are fundamental not only for the survival of the plants themselves but also for their adaptation to various abiotic and biotic stressors.

The accumulation or depletion of secondary metabolites is clearly influenced by a spectrum of factors. An alteration in a single element, such as light, temperature, soil moisture, soil fertility, or salinity, can significantly affect both the quantity and quality of secondary metabolites, even if all other factors remain constant (Manuka *et al.,* 2019). These elements are subject to daily fluctuations caused by various influences, including substandard agricultural practices, rising temperatures, and increased drought conditions (Twilley *et al*., 2020). Salinity, in particular, is one of the most pressing environmental challenges facing agriculture today, as elevated concentrations can severely limit crop production, even in plant species that can nominally tolerate salt (Cao *et al.,* 2020). Salinity causes osmotic stress (water deficit) and ion toxicity (excess Na+, Cl-), which hinders growth, prevents photosynthesis, and interferes with nutrient uptake (K+, Ca2+, N), resulting in lower yield and quality for medicinal plants (Manuka et al., 2019). With projections indicating that by 2050, more than half of the world's arable land could suffer from the adverse effects of salinity, it is imperative to address this critical agricultural issue, as it poses a severe threat to both crop health and the livelihoods of farmers globally (Khoza *et al*., 2025).

In light of these challenges, plant biologists are exploring alternative methods to cultivate crops under saline conditions, including the development of improved plant varieties. However, several obstacles remain regarding the availability, loss of genetic diversity, and accessibility of these varieties, particularly for smallholder farmers who represent a significant portion of agricultural producers (Begna, 2020). Among the strategies being employed, the use of Vesicular Arbuscular Mycorrhiza (VAM) fungi has gained traction as a promising solution. These soil fungi form

symbiotic relationships with plants, which are critical for enhancing plant growth and tolerance to abiotic stressors like salt and dehydration (Giri *et al*., 2003).

In the face of increasing climate variability and growing populations, the urgency for adaptive strategies to mitigate stressors is becoming ever more apparent. This is especially true for the cultivation of valuable medicinal plants, which are crucial to meet the rising demand for herbal remedies. Furthermore, developing effective cultivation protocols for *S. frutescens* under saline stress conditions will contribute not only to the conservation of this important species but also to the generation of high biomass enriched with conserved secondary metabolites. Such initiatives will ensure an adequate supply of quality medicinal products derived from *S. frutescens,* ultimately safeguarding this plant from potential extinction and enhancing its role as a vital resource in herbal medicine.

1.2 Problem statement

The increasing scarcity and threat of extinction of high-value medicinal plants, such *S. frutescens* warrants cultivation as a solution (Mofokeng *et al*., 2022). A significant global movement towards natural medicine has emerged, as countless traditional healing systems capitalize on naturally occurring biological resources. This increasing interest has profoundly influenced both the demand for and availability of medicinal plants (Mofokeng *et al*., 2022). With a predicted CAGR (compound annual growth rate) of around 7.2%, the worldwide phytomedicine market is expected to soar from its 2022 valuation of around $40 billion to its 2030 valuation of over $70 billion,

highlighting the medicinal potential and economic success of this industry (Soni *et al.,* 2026). As the world's population continues to grow, the demand for these invaluable medicinal plants is anticipated to surge even higher. However, providing accurate forecasts remains a challenge due to the diverse uses of these plants beyond medicinal applications. Moreover, the rising global fascination with these botanical treasures has given rise to a burgeoning "underground" trade, often shrouded in secrecy and rarely documented (Mofokeng *et al*., 2022). Cultivation is a critical conservation strategy; however, major decreases in agricultural output, terrestrial biodiversity, and ecological health, increased incidence of soil saline-alkaline stress due to global warming have hampered cultivation (Cao *et al*., 2020). It is suggested that more than 800 million hectares of land (6%) are impacted by salinity or alkalinity, and the extent of salty and alkaline soil grows each year as a result of extraordinary climatic change that has hastened land deterioration (Cao *et al.,* 2020). High saline levels not only reduce yield in medicinal plants but also reduce their curative effects (Banerjee and Roychoudhury, 2017). There is a need to find alternatives strategy to sustain the production of important medicinal plants such as *S. frutescens* with the ever-changing climatic conditions.

1.3 Rationale

Large-scale cultivation as a strategy to reduce the risk of extinction and increase *S. frutescens* availability may be hampered by a lack of improved cultivation protocols in the face of climate change. Poor harvesting techniques have put the existence of *S. frutescens* in jeopardy, as its products have gained unprecedented interest in health circles thanks to the plant's ethnomedicinal and pharmacological characteristics (Dwarka *et al*., 2020). The demand for a diverse array of indigenous medicinal plants is experiencing a significant surge, driven by the increasing human

population, evolving consumer needs, and expanding economic markets. A multitude of organizations is passionately advocating for the integration of these wild species into agricultural systems, prompted by a heightened awareness of the unsustainable harvesting practices that threaten their survival (Lambert *et al*., 1997). Supporting the cultivation of these invaluable medicinal plants is essential, not only as a strategy for fostering local economic growth and sustainability but also as a means to create jobs, rejuvenate rural economies, and generate revenue for small enterprises throughout the entire value chain (Mofokeng *et al.,* 2022). By embracing this approach, we can ensure the preservation of biodiversity while tapping into the rich potential these plants offer. The reality is that this remarkable medicinal plant has infiltrated both formal and informal markets, with its products easily accessible in pharmacies and grocery stores across South Africa. To safeguard the future of *S. frutescens* and to enhance the production of beneficial phytochemicals in its leaves, the findings of this study will play a pivotal role in promoting the plant's cultivation under saline conditions.

1.3.1 Aim

The study aimed to develop cultivation protocols for *Sutherlandia frutescens* that would enhance cultivation and secondary metabolites enrichment in the context of climate-smart agriculture.

1.3.2 Objectives

- To determine whether Vesicular Arbuscular Mycorrhiza (VAM) fungi will improve the growth and yield of *Sutherlandia frutescens* and alleviate salinity stress.

- To investigate whether biosynthesis and accumulation of secondary metabolites of *Sutherlandia frutescens* will be influenced by the use of VAM fungi in reducing salinity.

1.3.3 Hypotheses

- Vesicular Arbuscular Mycorrhiza fungi will improve the growth and yield of *Sutherlandia frutescens* and alleviate salinity stress.
- The biosynthesis and accumulation of secondary metabolites of *Sutherlandia frutescens* will be enhanced by the use of VAM fungi in reducing salinity.

1.3.4 Research questions

●Will VAM fungi improve the growth and yield of *Sutherlandia frutescens* and alleviate salinity stress?

● Will the biosynthesis and accumulation of secondary metabolites of *Sutherlandia frutescens* be influenced by the use of VAM fungi in reducing salinity?

1.4 Reliability, validity, and objectivity

Reliability is defined as the extent to which a measuring instrument consistently delivers the same results when the variable being measured remains stable and unchanged (Leedy and Ormrod, 2005). Various methodologies in statistical analysis provide robust assessments of data reliability (Berenson and Levine, 1996). In the present study, data reliability was rigorously established

through comprehensive statistical analysis, employing a significance level of 5%. Leedy and Ormrod (1980) articulate that validity pertains to the degree to which an instrument accurately measures what it is intended to assess. To enhance the validity of this research, the experiment was conducted in a consistent location while varying only the environmental conditions (space), thereby controlling external variables that could influence the results and ensuring that the observed outcomes were attributable to the conditions being investigated (Little and Hills, 1978) Furthermore, objectivity was achieved by grounding the findings in empirical evidence derived from statistical analyses, allowing for a thoughtful comparison and contrast with results from other studies (Little and Hills, 1978). This multifaceted approach adds depth to the integrity of the research findings.

1.5 Bias

Leedy and Ormrod (2005) articulate that bias encompasses any influence, condition, or collection of circumstances that, whether individually or in tandem, distort the integrity of data. In an effort to mitigate the risk of bias, meticulous care was taken to ensure that each experiment was executed with an adequate number of replications. This approach was essential in accounting for potential errors and enhancing the reliability of the findings (Leedy and Ormrod, 2005).

1.6 Scientific significance of the study

The global population is on a relentless upward trajectory, fueling an increasing demand for medicinal plants, among which *S. frutescens* stands out for its therapeutic potential. However, the

availability of arable land is alarmingly limited, and much of the existing land is affected by high concentrations of salt ions, such as sodium chloride (NaCl) and calcium chloride ($CaCl_2$). Saline stress poses a significant challenge, impeding agricultural productivity on a global scale. Therefore, it is imperative to explore innovative strategies that not only promote the cultivation of medicinal plants in saline environments but also preserve the integrity and richness of their secondary metabolites. The insights gained from such research will be instrumental in enhancing the production of *S. frutescens* in soils characterized by low fertility. This advancement will ensure a steady and abundant supply of therapeutic products derived from *S. frutescens*, ultimately benefiting the economic landscape of the country and providing a means to meet the rising demand for natural health solutions.

1.7 Structure of the dissertation

In Chapter 1, a thorough problem statement is articulated, accompanied by a compelling justification that outlines the necessity for this research. The chapter also defines its aims and objectives with clarity and precision. Chapter 2 provides an expansive review of the existing literature, offering critical insights and context directly relevant to the research subject at hand.

Chapters 3 and 4 are dedicated to accomplishing objectives 1 and 2, respectively, showcasing the methodologies and analyses employed to achieve these goals. In Chapter 5, the findings from all preceding chapters are meticulously consolidated, providing a rich overview of their implications and significance. This chapter also offers thoughtful recommendations for future research, paving the way for further inquiry in the field.

The concluding section masterfully encapsulates the essence of the research, weaving together the valuable insights gathered throughout the study. It is crucial to highlight that the Senate of the University of Mpumalanga has officially endorsed the application of the Harvard referencing style for all in-text citations and reference lists. This decision not only reinforces the academic rigor of the work but also ensures a consistent and uniform approach throughout the entire document, fostering clarity and precision in scholarly communication.

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CHAPTER 2

2. LITERATURE REVIEW

2.1 Introduction

Historically, people have found solace in plants for treating illnesses and injuries, today most modern drugs have their origins in these botanical sources. The demand of medicinal crops like cancer bush (*Sutherlandia frutescens* L. (Br.)) has entered both informal and formal markets and provide significant economic benefits to local communities (Shaik *et al*., 2008). In low-income regions, local therapies are often the only option for medical treatment, making traditional medicine the most accessible and cost-effective primary care solution. Despite, significant advancements in modern medicine, a striking 75-80% of the global population continues to depend on traditional medicine. This enduring reliance highlights the deep-rooted cultural significance and the holistic approaches that traditional practices offer (Shaik *et al*., 2008; Yuan *et al*., 2016).

Traditional remedies, such as *S. frutescens*, have garnered attention for their pharmacological and ethnomedicinal benefits, with some highlighted examples include the use in treating HIV/AIDS patients and helping to alleviate the negative effects of muscle atrophy (cachexia). Utilization of *S. frutescens* for the remedy of the mentioned alignments has been endorsed by numerous health ministries across Africa (Lin *et al*., 2016). The South African market in the use of indigenous medicinal plants is stipulated to be above a billion a year and this highlights the importance of medicinal plants like *S. frutescens* (Twilley *et al*., 2020).

Phytochemicals are what give medicinal plants their therapeutic qualities. Phytochemicals, or secondary metabolites found in *S. frutescens*, have therapeutic effects (Kong *et al*., 2020). Secondary metabolism, also known as specialized metabolism, is the interaction of metabolic

pathways and small-molecule products with the environment, but only through metabolic processes. The synthesis and accumulation of secondary metabolites (SMs) are influenced by a complex developmental genetic circuitry composed of enzymes and genes, as well as an external environmental circuit composed of light, temperature, water, salt, and other factors (Fang *et al*., 2022). Many medicinal products rely on secondary plant metabolites derived from this botanical species. The test plant in the current study contains a plethora of bioactive compounds. *Sutherlandia frutescens* is reported to contain a variety of secondary plant metabolites, including tannins, alkaloids, saponins, phenolics, and flavonoids (Wink, 2013). The assessment of the available secondary metabolites within this valuable medicinal plant has been conducted extensively. However, information on the extent to which the SMs are influenced by abiotic factors in *S. frutescens* is scanty. Agostini-Costa *et al*. (2012) claim that each plant species has its own set of secondary chemicals and each plant has its own adaptive mechanism, in the same extend varying reaction and changes in their metabolites when subjected to stress.

Manuka *et al*. (2019) highlight the incredible adaptability of plants, revealing that they can alter the quantity and characteristics of their secondary metabolites in response to a variety of environmental factors. These factors include light, temperature, soil moisture, nutrient levels, and even salt concentrations. Similarly, Twilley *et al*. (2020) emphasized that abiotic challenges, like drought, extreme heat, and nutrient-deficient soils, can lead to daily fluctuations in the metabolites produced by medicinal plants. This dynamic response showcases the resilience and versatility of plant life in the face of changing conditions.

The current study delves into a pressing issue: how salinity, as a form of abiotic stress, impacts secondary metabolites (SMs) in *S. frutescens*. Understanding this relationship is crucial not only for developing effective management strategies but also for highlighting the negative effects of stress on these valuable compounds. Salinity is a significant environmental challenge that threatens the yield of nearly every crop, even those known for their salt tolerance (Cao *et al.,* 2020). Yet, the degree to which salinity impacts the crucial secondary metabolites in medicinal plants like *S. frutescens* remains largely uncharted territory.

As climate variability increases salt levels due to drought and heat, the reduction in food production becomes even more alarming, placing food security at greater risk (Cao *et al*., 2020). While ongoing research aims to develop improved cultivars suited for saline environments, access to these varieties remains limited for smallholder farmers. Furthermore, at high salinity levels, these modified crops can lose their effectiveness (Manuka *et al.,* 2019). Alternative solutions, such as drainage, often fall short due to limited resources and a lack of knowledge about the appropriate cultural practices needed to effectively manage salinity (Manuka *et al.,* 2019). This highlights the urgent need for innovative strategies to combat salinity and ensure food security in the face of climate change.

Among the innovative strategies emerging to enhance plant resilience against abiotic stressors is the deployment of Vesicular Arbuscular Mycorrhiza (VAM) fungi. These incredible fungi are making waves in the agricultural world for their astonishing ability to enhance plant productivity amidst the growing challenges of climate change. By strengthening plants against various biotic and abiotic stresses, VAM fungi are becoming essential allies in modern farming (Cao *et al.,* 2020). The symbiotic partnership between plant roots and Arbuscular Mycorrhiza fungi is a brilliant

example of mutual benefit, where both organisms flourish together without causing any harm to the plant (Parihar and Rakshit, 2024). This collaboration not only boosts plant health but also holds the key to more resilient agriculture in an uncertain future. This vital association significantly enhances soil fertility, paving the way for sustainable crop production that can withstand environmental adversities. When crop plants are inoculated with VAM fungi, they exhibit accelerated growth and increased yields. This enhancement can be ascribed to the remarkable capabilities of fungi, which play a crucial role in enhancing nutrient uptake, strengthening drought resistance, and boosting resilience against salinity and various diseases (Pal and Pandey, 2014). By identifying effective solutions to adapt plants and mitigate salinity stress, we can prevent the decline of vital species, ensure the adequate production of medicinal materials, and cultivate substantial biomass with enhanced secondary metabolite content. This not only supports agricultural productivity but also contributes to ecological balance and resilience.

The extinction of *S. frutescens* is an urgent concern, mostly due to mal-harvesting and lack of conservation practices, which then necessitates improved cultivation practices (Dwarka *et al*., 2020). Raghu *et al*. (2018) discovered that just 10% of medicinal plants are farmed, implying that the practice of acquiring natural stock for its benefits is more common. The cultivation of these species, particularly those classed as endangered, represents a practical strategy to ensure an unlimited resource and may contribute to their preservation for future generations (Xego *et al*., 2016). This strategy, however, is limited by climate change challenges, such as deteriorating arable land, increasing high temperatures, drought and salinity incidence.

2.2 Description of cancer bush

Cancer bush (*Sutherlandia frutescens* L. (Br.)) is a noteworthy leguminous medicinal plant that originates from the diverse and rich ecosystems of South Africa. This remarkable species, with its striking foliage and vibrant flowers, also thrives in neighbouring regions such as Namibia, Botswana, and Lesotho. As highlighted by Faleschini *et al*. (2013), S. *frutescens* is renowned for its impressive therapeutic properties, solidifying its role as a vital component of traditional medicine practices in these areas. This medicinal plant is reported to be a perennial shrub that ranges in height from less than 0.2 to 2.5 meters. The plant can grow in both prostrate and erect positions and has a relatively short lifespan. Its stems are predominantly hairless or sparsely pubescent, with a large number of leaves concentrated at the terminals (Alamgir, 2017). The pinnate, stipulate, and short petiolate leaves have a terminal leaflet and around eight pairs of opposing leaflets. The degree of serration on each ovate-oblong, elliptic, or narrowly oblong leaflet is influenced by its source (Alamgir, 2017).

Stunning crimson blossoms are said to be found within axillary racemes with few blooms. The flowers are typically tubular and laterally compressed, featuring two small wing petals, a large standard petal that curls at the tip, and oblong, boat-shaped keel petals (Faleschini *et al*., 2013). The fruits can vary in shape from rectangular to broadly oval, depending on their origin. They possess large, bladdery, and papery pods. A wide variety of kidney-shaped seeds can be found, ranging in texture from exceedingly smooth to distinctly rugose, and in colour from light brown to dark brown (Alamgir, 2017). Figure 2.1 shows a growing cancer bush plant with its leaves.

Figure 2.2: Cancer bush (*Sutherlandia frutescens*).

Cancer bush or "kankerbos," is considered by traditional healers in South Africa to possess cancer-treating properties. According to Moteetee and Van Wyk (2007), the herb *S. frutescens* holds a profound significance among the "Khoi San" and "Nama" cultures, who initially embraced its medicinal properties. This remarkable plant predominantly thrives along the picturesque western coast of the Western Cape, where its potent extracts have been traditionally employed to combat serious ailments like stomach cancer (Aboyade *et al.,* 2017).

Belonging to the esteemed Fabaceae family, *S. frutescens* boasts a wealth of therapeutic qualities, making it an essential remedy for a range of health conditions, including influenza, diabetes, cancer, tuberculosis, chronic fatigue syndrome, rheumatoid arthritis, anxiety, clinical depression,

and more recently, HIV/AIDS (Moteetee and Van Wyk, 2007). Many symptoms associated with these disorders are often linked to disruptions in the body's stress response, which can arise from imbalances within the endocrine system.

Renowned for its resilience and adaptability, *S. frutescens* is a vital component of South Africa's rich traditional flora. Its long-standing history of use and well-documented effectiveness as a safe treatment for various health challenges have not only solidified its status locally but also contributed to its growing global popularity, albeit leading to overcultivation in some regions (Cao *et al.,* 2020).

2.3 Salinity and its effects on plants

Salinity, primarily resulting from a high concentration of sodium chloride ions within the soil substrate, stands as a significant abiotic factor that severely hinders plant growth and agricultural yield (Flowers *et al.,* 2010; Oshunsanya *et al.,* 2019). Alarmingly, Chávez-Avilés *et al*. (2013) reported that approximately thirty percent of the world's arable land and six percent of the total land area are adversely impacted by excessive salinity. This widespread salinity renders these regions increasingly inhospitable for conventional agricultural practices, posing a substantial challenge to global food security and sustainable land management. The global area of heavily salinized soils has expanded to more than 800 million hectares (Singh, 2022). Miransari and Smith (2007) stated that abiotic stress can be induced by a range of factors, in the case of salinity, drought, extreme temperatures, plant sensitivity, and specific soil and climatic conditions. Soil salinity can rise due to both natural and human causes, with climate variability being the main accelerator of this stress incidence (Oshunsanya *et al*., 2019). Furthermore, poor drainage systems, irrigation with

brackish groundwater, continuous irrigation over long periods of time, inadequate water management, and cultural approaches in irrigated agriculture can contribute to salinity. Salinity poses the greatest hazard to agricultural plants native to dry and semiarid regions such as *S. frutescens*.

Legumes such as peas, alfalfa, and barrel clover demonstrate better tolerance to higher salt levels compared to other legumes like broad beans, soybeans, green beans, and groundnuts. *Sutherlandia frutescnes* is a leguminous crop but its tolerance as a legume crop is not yet documented. Manchanda and Garg (2008) reported that rhizobia typically exhibit better salt tolerance than their host plants. This is particularly relevant as salinity poses the greatest hazard to the functioning symbiosis relative to the other partners. While some fabaceous species are resistant to soil salinity stress, these plants are frequently adversely affected by this stress at higher concentrations (Mhadhbi *et al*., 2009). In the absence of cooperative interaction linking Rhizobium and the host plant, both are susceptible to salt and osmotic stress. This vulnerability stems from the symbiotic relationship between Rhizobium and the host plant (Mhadhbi *et al*., 2009). Planting legumes in saline soils typically results in reduced yields because symbiosis is altered in some legume plants (Chávez-Avilés *et al*., 2013). Salt inhibits the ability of the legume-Rhizobium symbiosis to grow, proliferate, nodulate, and fix nitrogen. Nitrogen fixation in symbiotic relationships is influenced by a fascinating array of factors. Beyond just the interaction between plants and their Rhizobium partners, elements like the specific strain of Rhizobium, the genetic makeup of the host plant, and the unique environmental conditions all play a crucial role. The interplay of these components creates a complex web that determines how effectively these symbiotic relationships thrive (Chávez-Avilés *et al*., 2013).

Plants face a tough battle against salinity, a challenge that varies depending on their developmental stage, the types and concentrations of ions, and their interactions with the surrounding environment. When salinity levels rise, plants struggle to absorb water effectively, leading to damage in their cells and tissues. This disruption can hinder vital metabolic and physiological processes, ultimately resulting in stunted growth and reduced dry mass (Mhadhbi *et al.,* 2009).

However, plants have their own arsenal for coping with this stress. They can boost their resilience through the production of protective metabolites and by activating antioxidant enzymes. For instance, research by Ullah *et al*. (2019) highlighted that salinity stress can lead to a decrease in chlorophyll levels and an increase in reactive oxygen species (ROS), like hydrogen peroxide, within leaves. This buildup can damage their photosynthetic machinery. Given the challenges that salinity poses to crop production, it's clear that innovative solutions are essential. One promising approach gaining traction is the use of VAM fungi, which may offer an effective way to enhance plant resilience in salty conditions.

2.4 Advantages of mycorrhizal fungi under salinity stress

Mycorrhizal associations are essential allies in the journey of higher plants, significantly boosting their growth and health. These fascinating partnerships offer a host of benefits, including enhanced water and nutrient uptake, faster root development, and a remarkable increase in the roots' overall absorption capacity to adapt to salt stress. Thanks to both morphological and physiological changes in the plants, these associations play a pivotal role in transforming their ability to thrive in their environments (Phour and Sindhu, 2024). The fungi involved in these associations provide several advantages, such as an expanded surface area for nutrient absorption, a greater volume of soil exploration through their root-like structures, the development of longer-lasting absorbing roots,

more effective utilization of nutrient-poor sources, and enhanced retention and storage of soluble nutrients (Phour and Sindhu, 2024).

Mycorrhizae exist in various forms, each with unique physical structures and biological functions. For example, ectomycorrhizae are characterized by fungal cells that encircle the plant's roots. These fungi often penetrate the spaces between the epidermal cells and the initial layers of the cortex, facilitating nutrient exchange and enhancing plant growth (Bello-Bello *et al.,* 2022).

These characteristics in mycorrhiza fungi use collectively help reduce soil colloids and prevent leaching losses particularly under saline stress incidences (Maurice *et al*., 2024). They enhance the transfer and exchange of crucial nutrients such as phosphorus, nitrogen, sulfur, and micronutrients like copper and zinc from the soil to the plant. In tropical environments, it is estimated that mycorrhizal fungi can replace up to 226.796 kg of phosphorus per hectare for citrus and 190.545kg per hectare for soybeans (Frew *et al*., 2024). Moreover, mycorrhizae encourage the growth and proliferation of phosphorus-solubilizing bacteria within the mycorrhizosphere, which is essential for many smallholder farmers who cannot afford expensive mineral fertilizers necessary for enhancing crop growth (Khan *et al.,* 2022).

The intricate interplay between arbuscular mycorrhizal fungi (AMF) and salt-stressed plants offers a fascinating glimpse into the resilience of nature. This symbiotic relationship significantly enhances the selective uptake of essential nutrients, including calcium and potassium, while restricting the absorption of detrimental elements like sodium. As a result, AMF not only improve

water-use efficiency but also foster robust growth in the host plants, allowing them to thrive in challenging conditions (Hammer *et al.,* 2011; Santander *et al*., 2017).

Avio *et al*. (2017) delve into the fascinating world of AMF and their remarkable ability to interact with the metabolic processes of host plants. These incredible fungi not only encourage the accumulation of beneficial phytochemicals and antioxidant compounds but also play a pivotal role in promoting overall plant health. In saline environments, AMF work their magic by colonizing plant roots and transforming rhizosphere conditions. They enhance the absorption of organic carbon and boost essential nutrients like nitrogen, phosphorus, and potassium, while also improving the organic matter content of the soil.

Beyond this, AMF are champions of soil health, they help regulate pH levels and combat soil erosion, creating a thriving environment for plant growth (Zai *et al*., 2021). Their extensive hyphal networks extend deep into the soil, reaching far beyond the immediate rhizosphere and significantly enhancing the water and nutrient absorption capabilities of their host plants (Yang *et al*., 2016). In essence, these fungi are not just partners; they are vital allies in the quest for robust and resilient plant ecosystems.

The impact of AMF colonization goes far beyond just helping plants absorb nutrients. It plays a pivotal role in altering the levels and characteristics of polyamines and organic acids within the plants (Sheng *et al.,* 2011). Polyamines are essential for enhancing the efficiency of nutrient and water uptake, acting as vital boosters for plant health. Meanwhile, organic acids work their magic by reducing soil electrical conductivity, which in turn increases the availability of key nutrients

like nitrogen, phosphorus, and potassium. This interconnected relationship highlights the incredible influence AMF have on plant vitality and growth. This helps maintain ionic homeostasis within plant cells, ensuring optimal health and functionality (Sheng *et al*., 2011). Different species of AMF exhibit diverse capabilities in acquiring and supplying nutrients, particularly under the stress of salinity (Taylor *et al.,* 2015). Recent study by Masenya *et al.* (2023) have demonstrated that incorporating AM fungi in saline soils can significantly enhance the growth and overall effectiveness of *S. frutescens*. Similarly, research conducted by Zhao *et al.* (2022) provided compelling evidence that the application of AMF in medicinal plants not only increases the concentrations of pre-existing secondary metabolites but also aids in mitigating or controlling plant stress.

Emerging research illustrates that AMF in saline environments can lead to impressive increases in biomass output, enhancing the dry weight of both shoots and roots (Zhao *et al.* 2022). These fungi contribute to a greater number of branches, increased plant height, wider seedling diameters, expanded leaf areas, and, ultimately, improved overall plant yield (Parihar *et al*., 2020). Many plant species have shown remarkable increases in biomass under saline conditions, a testament to the indispensable role of AMF (Evelin *et al*., 2019). Mycorrhizal fungi play an amazing role in plant health by boosting the production of essential growth regulators like indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA). These vital compounds are key players in ensuring that plants grow and develop harmoniously, coordinating their growth for optimal success (Abd_Allah *et al*., 2015).

2.5 Secondary metabolites in *Sutherlandia frutescens*

Higher plants' vast secondary metabolic pathways produce a wide spectrum of chemical substances known as secondary metabolites or phytochemicals (Simsek and Whitney, 2024). Finally, these activities result in the synthesis of phytochemicals. Recent technological advances, notably in molecular biology and biochemistry, have supported the allelochemical roles of naturally occurring compounds (Chen *et al.,* 2023). While these natural compounds may not be essential for an organism's growth or reproduction, they play a fascinating role in survival. Plants, being stationary and unable to flee from their enemies, have developed remarkable strategies to defend themselves against herbivores, bacteria, viruses, and fungi. In response to these threats, they have evolved unique chemical compounds that serve as their own form of chemical defense, an incredible testament to nature's ingenuity (Pedranzani *et al.,* 2016). These allows them to improve their own survival by successfully repelling, suppressing, and eliminating predators and pathogens. Natural selection has resulted in the development of specific features. Verpoorte and Memelink (2002) delved into the fascinating world of secondary metabolites, uncovering their vital role in bolstering plant defense systems. Their research highlights how these compounds act as the plant's hidden warriors, enhancing resilience against various threats. The following section discusses some of the selected secondary metabolites responsible for the ethnomedicinal and pharmacological value of *S. frutescens*.

Sutherlandia frutescens has an uneven distribution of secondary metabolites across its organs. The leaves have the highest concentrations of bioactive amino acid derivatives including L-canavanine and pinitol, as well as flavonoids, saponins, phenolics, and triterpenoid glycosides. The stems have a few comparable chemicals, but they're not as concentrated, and the seeds and pods mostly have metabolites that help the seeds grow. Roots interact with soil through the release of defensive and

ecological compounds. All of these chemicals work together to explain the plant's great use in phytotherapy, both ancient and modern table 2.1 outlines the uneven distribution of this phytochemicals.

Table 2.1 Comparative Distribution of Secondary Metabolites in *S. frutescens*

Plant part	**Major secondary metabolites**	**Relative abundance**	**Functional significance**
Leaves	Flavonoids (sutherlandins), saponins, phenolics, alkaloids, pinitol, GABA, L-canavanine	Higher	Antioxidant, anti-inflammatory, defense
Sterrm	Cycloartane glycosides, flavonol glycosides, pinitol	Moderate	Structural protection, storage
Pods /Seeds	Saponins, phenolics, flavonoids, triterpenoids	Lower	Protection of embryo
Roots	Phenolics, terpenoids, defensive nitrogen compounds	Variable	Soil interaction and pathogen defense

Secondary metabolites exhibit a wide range of structural configurations; nevertheless, their classification is mostly based on the metabolic pathways through which they are produced (Harborne, 1999). Phenolics represent a diverse and significant class of phytochemicals, encompassing a variety of compounds such as flavonoids, tannins, coumarins, quinones, and anthocyanins. Phenolic compounds are fascinating substances celebrated for their extensive presence across the plant kingdom, playing a pivotal role in the field of pharmacognosy, the science behind medicinal drugs sourced from nature. With their diverse chemical structures and unique

properties, these compounds contribute to a range of biological activities, making them indispensable in herbal medicine and biochemistry.

From simple structures featuring a single aromatic ring to complex polymeric forms, phenolic compounds exhibit incredible versatility. They are known for their powerful effects, including anti-mutagenic, antibacterial, antiviral, and anti-inflammatory properties. Research by Scalbert *et al*. (2005) even suggests that these compounds might help inhibit ultraviolent (UV) damage and combat carcinogenic tumours, highlighting their importance in health and wellness.

Phenolics stand out as a prominent class of secondary metabolites, produced in response to various abiotic stresses in plants. According to Gumul *et al*. (2007), these compounds can be categorized into five main groups: phenolic acids, flavonoids, coumarins, lignins, and tannins. The intriguing processes that lead to their formation involve two key pathways: the shikimic acid and malonic acid pathways. Figure 2. Shows the shikimic acid pathway is chiefly responsible for synthesizing most plant phenolics, while fungi and bacteria primarily utilize the malonic acid system.

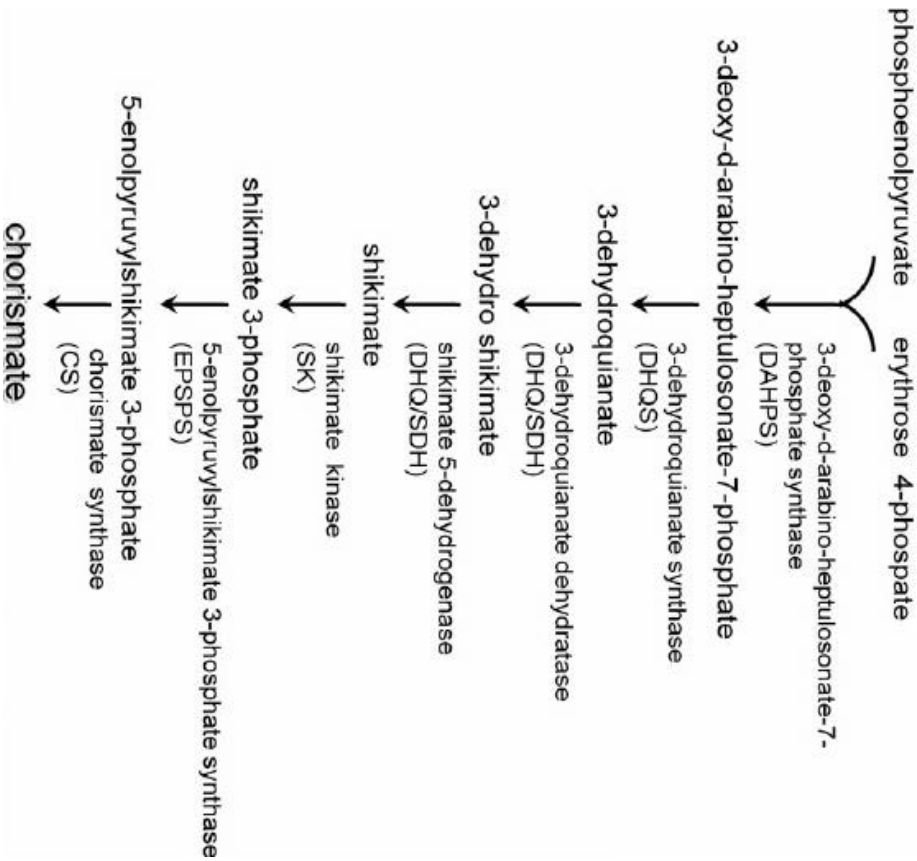

Figure 2.2 The shikimic acid pathway (Tzin and Galili, 2010)

During the conversion of shikimic acid, erythrose-4-phosphate and phosphoenolpyruvate transform into aromatic amino acids, an intricate process that underscores the complexity of nature. As noted by Foti (2007), phenols have remarkable antioxidant capabilities, notably slowing the production of free radicals. Their hydroxyl and carboxyl functional groups also enable these compounds to chelate heavy metal ions, such as iron, manganese, and copper, further showcasing their bioactivity. The below Figure 2.3 shows the molecular structure of phenols in sutherlandia frutescens.

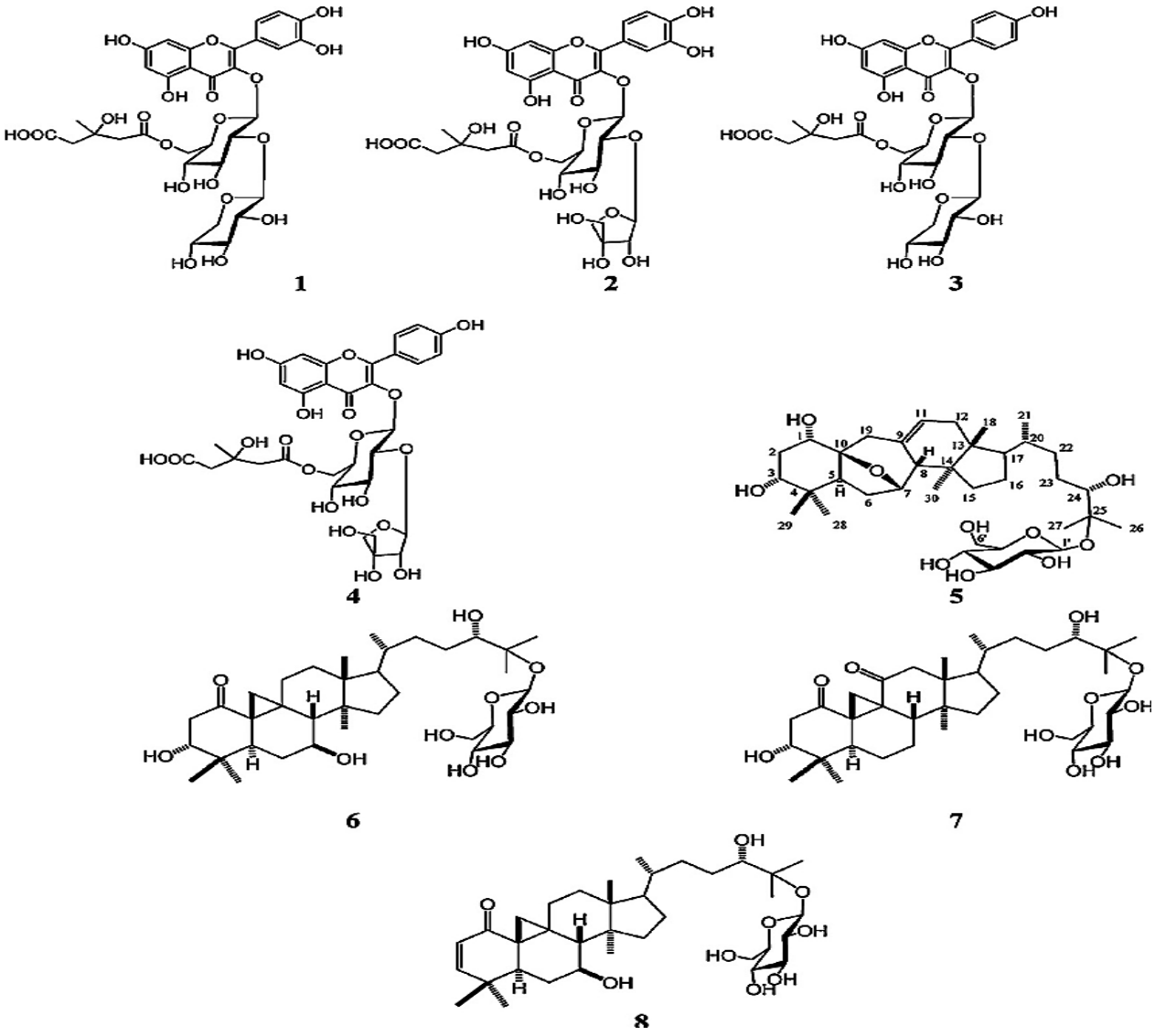

Figure 2 3: Molecular structure of Phenols in *Sutherlandia frutescens* (Fu, 2012)

Flavonoids have the unique property of having unbound hydroxyl groups that are connected to aromatic rings. According to Harborne and Williams (2000), the components in question protect against coronary heart disease while also demonstrating antibacterial, antitumor, and anti-inflammatory activities. Mncwangi and Viljoen (2012) highlighted the critical role of measuring amino acid content in Sutherlandia extracts for effective quality control. They discovered that the unique flavonoids and terpenoids found in Sutherlandia are the key compounds driving its

beneficial effects (Van Wyk and Albrecht, 2008). A fascinating array of research has emerged, employing advanced metabolomic technologies to delve into the phytochemical profiles of various Sutherlandia populations. In their innovative study, Mncwangi *et al.* (2009) utilized high-performance thin-layer chromatography (HPTLC), near-infrared spectroscopy (NIRS), and Fourier transform infrared (FT-IR) spectrometry to explore the remarkable chemotypic variations among wild Sutherlandia populations. This cutting-edge approach reveals the rich complexity and potential of this intriguing plant.

Alkaloids are fascinating compounds and rank as the third most abundant group of secondary metabolites in nature. What sets them apart? It's all about their unique structure, which features heterocyclic nitrogen atoms (Figure 2.3). Alkaloids can be categorized into three types: genuine alkaloids, protoalkaloids, and pseudoalkaloids, each with its own distinctive characteristics.

These intriguing substances, containing one or more nitrogen atoms, are often closely associated with specific plant genera and species (Bourgaud *et al.,* 2001). According to Shamsa *et al*. (2008), alkaloids play a pivotal role in influencing physiological systems in mammals, showcasing their importance beyond the plant kingdom.

True alkaloids are particularly interesting; they are defined by their fundamental structure, which includes the presence of heterocyclic nitrogen (Foti, 2007). They derive from various amino acids such as ornithine, lysine, phenylalanine, tryptophan, tyrosine, histidine, and aspartic acid. Well-known examples from this group include nicotine, morphine, and atropine, all of which have significant effects on both plants and animals.

Protoalkaloids, on the other hand, share a connection with amino acids but lack nitrogen in their heterocyclic structure. Meanwhile, saponins, another intriguing class of compounds, belong to the terpenoids and are characterized by their amphipathic nature. These molecules consist of a saccharide linked to either a steroid or a triterpene, showcasing yet another fascinating interaction of chemical structures (Foti, 2007). In the world of alkaloids and saponins, we find a rich tapestry of chemicals that not only define the identity of various plants but also hold profound implications for physiology and pharmacology. Figure 2. 3 shows the Molecular structure of alkaloids in Sutherlandia frutescens.

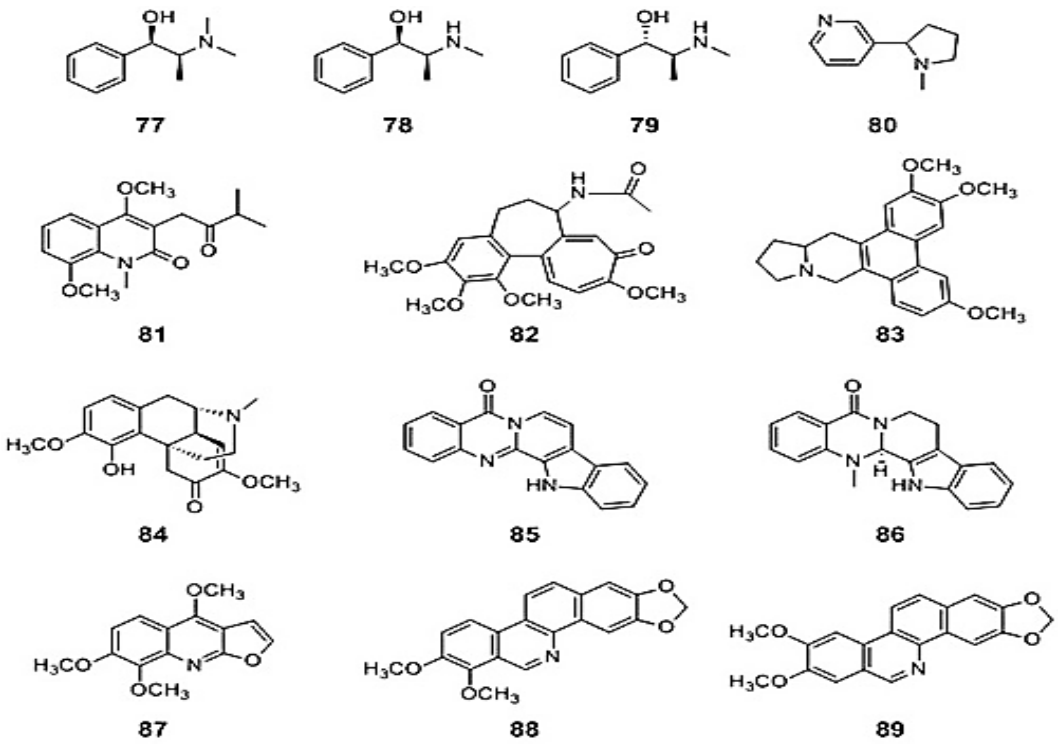

Figure 2.4: Molecular structure of alkaloids in *Sutherlandia frutescens* (Fu, 2012).

Furthermore, the material contains an alkaloid called "mescaline," which is produced from phenylethylamine. Pseudoalkaloids, including theobromine, caffeine, and solanidine, are basic chemicals that are not derived from amino acids (Irchhaiya *et al.,* 2014). The nitrogen atom in these alkaloids is produced through a process known as transamination. Abiotic stresses stimulate alkaloidal production. In addition to alkaloids, there are two major types of secondary metabolites that contain nitrogen: cyanogenic glycosides and glucosinolates. Typically, cyanogenic glycosides and glucosinolates are nontoxic (Gumul *et al*., 2007). However, crushing the plant produces volatile poisons. The secondary metabolites containing nitrogen exhibit different and diverse pharmacological actions.

Terpenes, also known as isoprenes, are a highly diverse group of chemicals found in various types of metabolites. This group contains approximately 30,000 species of both plant and animal origin. Terpenoids are fascinating compounds that boast an incredible variety of chemical structures, making them stand out among phytochemicals (Yazaki, 2006). These multifunctional molecules play a multitude of roles in nature, from serving as powerful antibiotics to acting like hormones in both plants and animals. They are essential lipids found within plant and animal cells, and they possess the unique ability to attract or repel insects. Additionally, terpenoids play a crucial role in mediating the electron transport systems necessary for processes like photosynthesis and respiration (Foster *et al*., 2006). With such a diverse array of applications, terpenoids are truly vital players in the natural world! Terpenes, which are found in a variety of bioactive natural plant products, are formed from the phytol side chain of chlorophylls and the diterpenoid skeleton. These compounds are a key family of chemicals that serve an important part in the advancement of

medicine and human health. The evidence above clearly indicates that this indigenous plant possesses critical secondary metabolites yet, there is limited information regarding their response to abiotic stressors.

2.6 Effect of salinity on secondary metabolites in plants

Sutherlandia, an adaptogen, is known for its chemical diversity, which is reflected in a variety of metabolites such as amino acids, vitamins, flavonoids, and terpenoids. The metabolites have the capacity to undergo significant changes when exposed to various environmental factors. When plants are exposed to abiotic stressors such excessive salinity, a number of explanations have been put forth to explain the observed rise or decreased in secondary metabolites. Plants' amino acid and polyamine levels indicate their physiological condition. According to Rai (2002), polyamines are essential for regulating plant growth and development (Hunter and Burritt, 2012), as well as helping plants adapt to stress incidences (Kuznetsov and Shevyakova, 2007).

Wang et al., (2018) emphasized that a diverse array of natural products, particularly plant secondary metabolites (SMs), plays a pivotal role in the defense mechanisms of plants against various environmental stressors and pathogenic invasions. These plant SMs are not only crucial for the vitality of the plant but also serve as valuable food additives and key components in medicinal formulations, culinary applications, and aromatic products due to their remarkable biological activities (Yang *et al*., 2018).

The synthesis and accumulation of SMs are influenced by multiple factors, including genetic makeup, developmental stages, morphological variations, and environmental conditions. For example, literature suggests that the accumulation of SMs is significantly affected by

environmental parameters such as light intensity, temperature fluctuations, soil moisture levels, nutrient availability, and salinity. As indicated by Yang *et al.* (2018), even a slight alteration in any one of these factors can lead to variations in SM content, highlighting the intricate balance and sensitivity of plant biochemistry, especially when other environmental conditions remain unchanged.

Salt stress stands out as one of the most daunting challenges for plants, posing a major threat to the successful cultivation of vital species like *S. frutescens.* The process of salinization triggers a cascade of intricate interactions among a plant's morphological, physiological, and metabolic systems. This phenomenon also brings about oxidative stress through the heightened production of reactive oxygen species (ROS), leading to significant shifts in plant metabolism. In response to this stress, plants ramp up their production of secondary metabolites (SMs) to effectively scavenge or neutralize ROS, showcasing their remarkable resilience (Menezes-Benavente *et al.,* 2004).

Reactive oxygen species (ROS) cause oxidative damage, which is brought on by salt-induced osmotic stress. According to Ahmad *et al*. (2010), ROS can harm proteins by altering amino acids at sites, fragmenting and aggregating cross-linked reaction products, and making proteins more vulnerable to proteolysis. To counteract ROS-mediated oxidative damage, plants have evolved a sophisticated antioxidant defense system that includes antioxidant enzymes like catalase (CAT), reduced glutathione, ascorbate, tocopherol, carotenoids, and flavonoids, as well as low-molecular mass antioxidants like SOD, peroxidase (POD), ascorbate peroxidase (APX), and catalase (de Azevedo-Neto *et al*., 2006).

2.7 Salinity Stress on Plants: Mechanism of Action and Mycorrhiza's Role Reduces this stress.

Extensive research demonstrates that the symbiotic relationship between plants and arbuscular mycorrhizal fungus (AMF) improves plant resilience to salinity stress. This symbiosis typically leads to better nutritional absorption, accumulation of osmoregulatory substances, improved photosynthetic efficacy, and heightened water-use efficiency. The responses indicate that AMF-mediated salinity tolerance encompasses a blend of dietary, metabolic, physiological, and molecular processes. The extent of these advantageous impacts, however, is frequently species-specific (Muhilan and Bagavathi Ammal, 2025). These processes enhance the fitness and growth performance of host plants in saline circumstances through mycorrhizal colonization.

Salinity stress impacts plant growth mainly via osmotic stress and ionic toxicity. Excessive salt accumulation in the soil reduces the osmotic potential of the soil solution, hindering water absorption by plant roots. Thus, plants undergo physiological drought despite the availability of water in the soil. Under these circumstances, plant growth and development are frequently hindered due to diminished cell proliferation and compromised metabolic functions. In extreme instances, plants may enter a withering phase when water absorption is inadequate to maintain physiological processes. Besides osmotic stress, salinity causes nutritional imbalance and ion toxicity. Elevated levels of sodium ions (Na^+) disrupt the absorption of vital nutrients like potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). This disturbance impacts enzyme activity, protein synthesis, and overall plant metabolism. Consequently, numerous plants demonstrate diminished growth, reduced photosynthetic efficiency, and decreased biomass output in saline

environments.

Numerous studies have shown that plants associated with arbuscular mycorrhizal fungi have enhanced growth relative to non-mycorrhizal plants in saline conditions. Cantrell and Linderman (2001), Giri *et al*. (2003), and Sannazzaro *et al*. (2007) documented that AMF-inoculated plants demonstrated superior growth and stress resilience compared to non-inoculated plants. Hajiboland *et al*. (2010) similarly found that mycorrhizal plants outperformed non-mycorrhizal plants in both normal and elevated salinity environments, with salt markedly diminishing dry matter output in two tomato varieties. A primary advantage of AMF colonization is enhanced nutrient absorption. Mycorrhizal relationships are recognized for their ability to augment phosphorus absorption in host plants (Smith and Read, 1997).

The large hyphal network of arbuscular mycorrhizal fungi expands the soil volume accessible to plant roots, enabling plants to obtain nutrients that would otherwise be inaccessible. The augmented nutrient absorption substantially enhances plant development in saline environments. Additionally, AMF colonization has been demonstrated to mitigate oxidative stress in plants subjected to salt. Salinity stress frequently results in the excessive generation of reactive oxygen species (ROS), which induce oxidative damage to biological constituents including lipids, proteins, and nucleic acids. AMF inoculation has been shown to diminish the accumulation of oxidative stress markers, including malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), while concurrently elevating the levels of protective metabolites such as soluble sugars, reducing sugars, soluble proteins, and organic acids, including oxalic, fumaric, acetic, malic, and citric acids (Jabborova *et al*., 2022). The control of these metabolites is intricately connected to metabolic

pathways related to energy production, specifically glycolysis and the tricarboxylic acid (TCA) cycle. By activating these pathways, AMF augment the plant's energy status and raise its capacity to withstand saline environments. This metabolic adaptation facilitates the preservation of cellular homeostasis and promotes ongoing growth and development, even in the face of stress.

The mitigation of salt stress by arbuscular mycorrhizal fungi is linked to their colonization process within plant roots. During colonization, fungal hyphae adhere to the root surface and develop specialized structures termed appressoria. The hyphae subsequently infiltrate the root cortex, where they form extensively branching structures termed arbuscules and storage formations referred to as vesicles (Rengasamy, 2006). These structures enable effective nutrient transfer between the fungus and the host plant. Moreover, AMF colonization enhances plant hydration dynamics and osmotic regulation. The accumulation of osmolytes, augmented water-use efficiency, and elevated antioxidant production promote plant tolerance to salinity stress. Antioxidants generated in mycorrhizal plants mitigate reactive oxygen species, consequently diminishing oxidative damage induced by salt stress (Aktar et al., 2025; Chaithra et al., 2025).

An other significant process pertains to the augmentation of the effective root system via the fungal hyphal network. The hyphae proliferate extensively in the rhizosphere, investigating soil areas beyond the root depletion zone where nutrients and water are scarce. During root colonization, arbuscular mycorrhizal fungi (AMF) can markedly enhance a plant's ability to assimilate minerals, nutrients, and water from the soil. Mycorrhizal colonization can, in certain instances, include up to 90% of the total root length (Chen *et al*., 2020). Under saline circumstances that frequently hinder root activity, arbuscular mycorrhizal fungi (AMF) are essential for sustaining nutrient

absorption. By enabling selective mineral uptake via their hyphal networks, AMF enhance the accumulation of vital minerals necessary for plant growth and development.

The improved nutrient absorption is especially crucial in salt-affected soils, where nutrient availability and transport are significantly restricted (Verma, 2022). The alleviation of salt stress by arbuscular mycorrhizal fungi (AMF) encompasses various interrelated mechanisms, including as higher nutrient uptake, improved osmotic management, elevated antioxidant activity, metabolic modifications, and greater root exploration via fungal hyphae. These systems jointly enhance plant growth and stress resilience in saline environments.

2.8 Research gap

The availability of knowledge regarding the process of domesticating *S. frutescens* in a saline environment and the effects of salt on its secondary metabolites is scanty. Due to the increase in global temperature, drought and rainfall fluctuations which all results in salts accumulation as a result of climate change and climatic variability, it is imperative for researchers to undertake investigations on the influence of mycorrhiza-symbiosis with higher order medicinal plants and their adaptation to stress, as well as the influence on the secondary metabolites. The research seeks to bridge the knowledge gap regarding saline soils and how commercial mycorrhiza products can be used to adapt plants to stress incidents, and their impact on the secondary metabolites of *S. frutescens*.

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CHAPTER 3

VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI INFLUENCE ON *SUTHERLANDIA FRUTESCENS* PERFORMANCE AND SALINE STRESS ALLEVIATION

3.1 Introduction

Natural saline soils cover almost 1 billion hectares of the total 14 billion hectares of land accessible on the planet, with reports suggesting that more than half of the world's arable land might be salinized by 2050 (Masenya *et al*., 2023). Plant biologists are investigating alternative methods to cultivate crops under saline conditions, such as the use of improved varieties, which would be affordable for all farmers. Among these strategies, the use of Vesicular Arbuscular Mycorrhiza (VAM) fungi has remained highly effective among sustainable climate-smart agriculture techniques for reducing salinity stress and increasing crop yield (Giri *et al.*, 2007).

Vesicular Arbuscular Mycorrhizal (VAM) fungi are soil fungi that form symbiotic associations. These are vital in plant growth, allowing the agricultural plants to develop tolerance to abiotic stress factors such as salt and dehydration (Giri *et al*., 2007). The development of cultivation protocols for *S. frutescens* under soil-saline stress conditions would contribute to increased production of this crop with the aim of conserving the plant and preventing its extinction. Examining these symbiotic connections provides insights into how biological modifications might mitigate salt-induced stress and boost biochemical parameters, including micronutrient accumulation, thereby improving plant performance (Kumar *et al*., 2019). Therefore, the objective of the study was to determine whether VAM fungi will improve the growth of *S. frutescens* and

alleviate salinity stress. The null hypothesis was that VAM fungi would improve the growth of *S. frutescens* and alleviate salinity stress.

3.2 Materials and methods

3.2.1 Description of study area

The shade nets and micro-plots experiments were conducted at the University of Mpumalanga's farm, nestled in the scenic Mbombela Campus of South Africa (25°26′11″S 30°58′54″E). This vibrant region experiences an average annual rainfall of less than 500 mm, making it a fascinating backdrop for the study. Relative humidity at the site typically ranges from 70–90% in the mornings to 40–60% in the afternoons, depending on seasonal and daily variations (SA Weather Service, 2020). Nelspruit experiences an average minimum temperature of 13°C and a high temperature of 27°C. The site had a H range of 4.53 to 5.54 and a light texture (sandy loam to loamy) (Shilenge, 2020).

3.2.2 Experimental design and cultural practices

In 2024, two independent experimental trials were conducted: one in a micro-plot setting (Experiment 1) and the other in a shade net environment (Experiment 2). Both research trials utilized a 4x4 factorial design arranged in a randomized complete block design (RCBD), with four replications. The treatments included four different levels of arbuscular mycorrhizal fungi (VAM) applied at 0, 10, 20, and 30 grams, as well as chloride salinity levels of 0, 0.25, 0.5, and 0.75 dS m^{-1}. These salinity levels were generated by mixing sodium chloride (NaCl) and calcium chloride ($CaCl_2$) in a 3:1 ratio (Mashela, 2017). The fungus employed was RRHIZ-UP, and the isolates

identified in the product were *Funneliformis coronatum*, *Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *Rhizophagus irregularis* species.

Cancer bush seeds were directly sown into plastic pots measuring 30 cm in diameter. The pots were filled with a mixture of steam-pasteurized loam and sandy soil in a 3:1 volumetric ratio. The plants were irrigated with non-chlorinated water, using 250 ml every other day. After seven days, each plant was administered 2.5 grams of NPK fertilizer at a ratio of 2:3:2. After 14 days of planting, chlorine-free water was replaced with the treatment. Insect pest scouting was performed from the tume of germination, every other day, and necessary preventative measures were taken if pests were found. Hand weeding was conducted with hand hoes whenever weeds appeared in the containers. A micro-plot experiment was conducted by placing 30 cm square plastic pots (80) into 15 cm deep holes spaced 0.5 m apart both inter and intra spacing. The shade nets were implemented using a 40% alunet shade cloth that was strung 2 metres above the ground. A shade-net experiment was conducted by arranging 30 cm plastic pots (80) at intervals of 0.5 m inter × 0.5 cm intra spacing, with a sample unit of 80 pots per experiment (Laka, 2025).

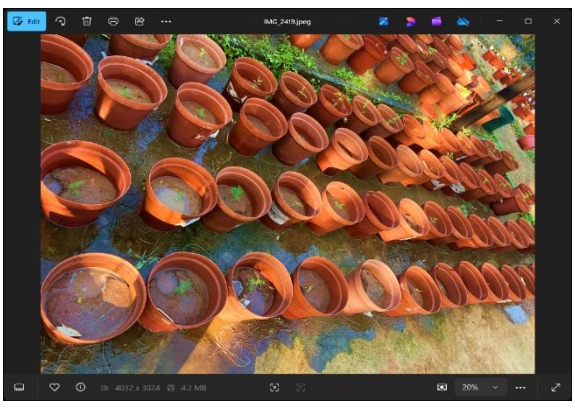

Figure 3.1 shade-net experiment 2

Figure 3.2 Micro-plot experiment 1

3.3 Data collection

The plant parameters were measured on day 90 after initiating the treatments. The monitored plant parameters included plant height (cm), stem diameter (mm), number of branches, root length (cm), fresh weight (g), dry matter content and chlorophyll content (CCI). The height of the plants was measured from their crowns to the tips of their flag leaves, the stem diameter was measured using the Vernier calliper, the number of branches were manually counted, and chlorophyll content was measured using the chlorophyll meter. The root length was measured using a measuring tape and fresh weight was determined using a weighing scale. Subsequently, the roots and shoots samples were dried at a temperature of 55°C in an oven for 72 hours to determine the dry matter content. Following the removal of roots from the shoots, the roots were cleaned to eliminate any residual soil particles, dried using a laboratory towel to remove any extra water, and subsequently weighed to ascertain their fresh weight before estimating their dry weight. The soil in each container was well blended, and subsequently, a 250 mL sample was extracted for the purpose of analysing the pH and electrical conductivity (EC). After undergoing air-drying and screening through a 2-mm mesh, a soil subsample weighing 10 grams was introduced into a glass beaker containing 25 mL of deionised water. The resulting mixture was vigorously spun for 5 minutes using a glass rod, followed by incubation for 50 minutes (Rayment and Higginson, 1992). Following the incubation, the contents were agitated once more and allowed to rest for 10 minutes. Subsequently, pH and EC measurements were obtained from the supernatant using a HANNA Smart Bench-Top pH/EC/DO Metre.

3.4 Data analysis

The collected data was subjected to Statistix 10.0. To determine if the data were normally distributed, and the Shapiro-Wilk test was used (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965). A two-way ANOVA was utilised to assess the influence of the treatments. In cases where the treatment was significant ($p< 0.05$), mean comparisons were accomplished using Turkey HSD test, and the degrees of freedom and their mean sum of squares (MSS) were partitioned to obtain the total treatment variation (TTV). The results were discussed at the probability level of 5% or less only, unless otherwise stated (Mashela *et al*., 2015).

3.5 Results

3.5.1 Plant growth variables

The results in both experiments have shown that the treatments had a significant effect ($p< 0.05$) on plant growth variables. Among the measured plant variables in Experiment 1, VAM fungi had a significant impact ($p< 0.05$) on stem diameter, chlorophyll content, and the number of branches, accounting for 94.21%, 96.76%, and 98.11% of the total treatment variation (TTV), respectively, (Table 3.1). The results revealed that the applied VAM fungi in Experiment 1 had relative positive effect on stem diameter with a relative impact (RI) of 204.55% to 588.74%, also similar observations were noted on chlorophyll content and plant fresh weight with a RI of 101.61% to 314.60%, and 1350.96% to 5253.65%, respectively (Table 3.3). Similarly in Experiment 2, VAM fungi had a significant effect ($p< 0.05$) on chlorophyll content, stem diameter, and plant fresh weight, contributing to a total treatment variance (TTV) of 91.99%, 98.12%, and 91.09%, respectively (Table 3.2). Vesicular Arbuscular Mycorrhizal fungi increased chlorophyll content,

stem diameter, and plant fresh weight in Experiment 2 with a RI of 85.59% to 278.88%, 307.34% to 890.40%, and 65.40% to 207.87%, respectively (Table 3.4).

Table 3.1: Source of variation affecting stem diameter (SD), chlorophyll content (CL) and plant fresh weight (PFW) of *Sutherlandia frutescens* at 90 days after application of treatments in Experiment 1

		SD		CL		PFW	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	3	2.388	1.70	0.1544	0.48	16,65	0.62
VAM	3	132.307	94.21***	30.0313	96.76***	2645,85	98.11***
Salt	3	1.893	1.35	0.1199	0.39^{ns}	13,97	0.52^{ns}
VAM*Salt	9	2.195	1.56	0.3214	1.04^{ns}	4,93	0.71^{ns}
Error	61	1.662	1.18	0.4109	1.32	15,48	0.57
Total	79	140.445	100	31.0379	100	2696.88	100

***Highly significant at p≤0.001, **significant at p≤0.05, ns not significant, TTV (%) Total treatment Variation

Table 3.2: Source of variation affecting chlorophyll (CL), plant fresh weight (PFW), and stem diameter (SD) of *Sutherlandia frutescens* at 90 days after application of treatments in Experiment 2

		CL		PFW		SD	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	3	0.7528	1.98	8.37	0.37	3,581	2.79
VAM	3	35.0611	91.99***	2208.85	98.12***	117,018	91.09***
Salt	3	0.4324	1.13^{ns}	3.85	0.17^{ns}	2.51	1.95^{ns}
VAM*Salt	9	1.1542	3.03^{ns}	7.63	0.34^{ns}	2.84	2.21^{ns}
Error	61	0.7126	1.87	22.56	1	2.53	1.96
Total	79	38.1131	100	2251.26	100	128.47	100

***Highly significant at $p \leq 0.001$, **significant at $p \leq 0.05$, ns not significant, TTV (%) Total treatment Variation

Table 3. 3: The effect of VAM on stem diameter (SD), chlorophyll content (CL), plant fresh weight (PFW) of *Sutherlandia frutescence* at 90 days after initiation of treatment application in Experiment 1

VAM	SD	RI (%)	CL	RI (%)	PFW	RI (%)
0	1.2735^{d}±0.2943	0	0.9338^{d}±0.1463	0	0.520^{d}±0.8980	0
10	3.8784^{c}±0.2934	204.55	1.8826^{c}±0.1459	101.61	7.545^{c}±0.8953	1350.96
20	5.6989^{b}±0.3213	347.50	2.8749^{b}±0.0.1598	207.87	17.221^{b}±0.9804	3211.73
30	7.4976^{a}±0.3001	588.74	3.8715^{a}±0.1492	314.60	27.839^{a}±0.9158	5253.65

[y]Column means ± standard error followed by the same superscripts were not different (p≤ 0. 05) according to Tukey HSD significant different test. [z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 3.4:Relative impact effect of VAM affecting chlorophyll content (CL), plant fresh weight (PFW) and stem diameter (SD) at 90 days after initiation of treatment application in Experiment 2

VAM (g)	CL	RI (%)	PFW	RI (%)	SD	RI (%)
0	1.1582^{D}±0.1927	0	2.833^{D}±1.0842	0	2.7798^{D}±0.3627	0
10	2.1495^{C}±0.1921	85.59	11.540^{C}±1.0810	307.34	4.5979^{C}±0.3616	65.40
20	2.9696^{B}±0.2104	156.40	19.969^{B}±1.1838	604.87	6.6182^{B}±0.3960	138.08
30	4.3882A±0.2199	278.88	28.058^{A}±1.1058	890.40	6.6182^{B}±0.3960	207.87

[y]Column means ± standard error, followed by the same superscripts, were not different ($p \leq 0.05$) according to Tukey HSD significant difference test.

[z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

Salt had a significant impact ($p < 0.05$) on plant height and number of branches in both experiments, contributing a TTV of 81.69% and 79.44% in Experiment 1, and a TTV of 48.93 and 60.02%, in Experiment 2 (Table 3.5). The results in Experiment 1 showed that relative to the control, plant height and branch number increased by 82.69 to 271.83% and 75.31 to 201.89%, respectively. Also in Experiment 2, salt seemed to not harm the plant, improving number of branches and plant height by 18.58 to 57.87% and 9.03 to 60.29%, respectively (Table 3,6).

Table 3.5: Source of variation affecting plant height (PH) and number of branches (NB) of *Sutherlandia frutescence* at 90 days after application of treatments in Experiments 1 and 2

		Experiment 1				Experiment 2			
		PH		NB		NB		PH	
Source	Df	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	3	612,97	12.48	100.639	11.35	185.346	27.47	2297.59	41.01
VAM	3	93,73	1.91^{ns}	25.514	2.88^{ns}	5.097	0.76^{ns}	154.55	2.76^{ns}
Salt	3	4011,72	81.69***	704.631	79.44***	405.004	60.02***	2741.34	48.93***
VAM*Salt	9	56,66	1.15^{ns}	31.946	3.60^{ns}	49.455	7.33^{ns}	141.29	2.52^{ns}
Error	61	135,56	2.76	24.290	2.74	29.830	4.42	268.02	4.78
Total	79	4910,64	100	887.02	100	674.732	100	5602.79	100

***Highly significant at p≤0.001, **significant at p≤0.05, ns not significant, TTV (%) Total Treatment Variation.

Table 3.6: The relative effect of salt on the number of branches (NB**) and plant height (PH) of *Sutherlandia frutescens* at 90 days after initiation in Experiment 1 and 2**

	Experiment 1				Experiment 2			
Salt (dS m^{-1})	PH	RI%	NB	RI%	NB	RI%	PH	RI%
0	12.339^{D}±2.33	0	6.804^{C}±0.99	0	18.013^{C}±1.09	0	41.472^{B}±3.28	0
10	22.542^{C}±2.64	82.69	11.928^{B}±1.12	75.31	21.360^{BC}±1.24	18.58	45.215^{B}±3.71	9.03
20	34.825^{B}±2.85	182.24	17.540^{A}±1.21	157.79	25.648^{AB}±1.34	42.39	61.355^{A}±3.02	47.94
30	45.880^{A}±3.03	271.83	20.540A±1.28	201.89	28.438^{A}±1.43	57.87	66.474^{A}±4.27	60.29

yColumn means ± standard error, followed by the same superscripts, were not different ($p \leq 0.05$) according to Tukey HSD significant difference test. [z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

3.5.2 Soil Variables

The application of salt treatment had a significant influence ($p \leq 0.05$) on electrical conductivity (EC) in both experiment 1 and 2. Salt treatments had an effect with a total treatment variance (TTV) of 33.51 and 11.55% for EC, respectively (Table 3.7). In experiment 1, the salts decreased EC by 12.41 to 40.33% as compared to the control, while in experiment 2, the EC diminished by -3.65 to 59.95% (Table 3.8). The interactive application of salt and VAM treatment exhibited a notable impact on pH only, among the measured variables in experiment 1, with a TTV of 30.37% (Table 3.9). The interactive application (0*75dS m^{-1}) resulted in the increase of soil pH by 18% increase.

Table 3.7: Source of variation affecting soil pH and EC at 90 days after initiation in Experiments 1 and 2

		Experiment 1				Experiment 2	
		EC		pH		EC	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	3	17645.8	38.93	0.97335	45.72	167108	7.53
VAM	3	533.1	1.17^{ns}	0.16673	7.83^{ns}	1621369	73.02^{ns}
Salt	3	15187.7	33.51**	0.07243	3.40^{ns}	256479	11.55**
VAM*Salt	9	7740.5	17.08^{ns}	0.64650	30.37**	93341	4.20^{ns}
Error	61	4220	9.31	0.26986	12.68	82296	3.71
Total	79	45327.1	100	2.1289	100	2220593	100

***Highly significant at p≤0.001, **significant at p≤0.05, ns not significant, TTV (%) Total treatment Variation

Table 3.8: The effect of salt on soil EC at 90 days after initiation in Experiments 1 and 2

	Experiment 1		Experiment 2	
Salt (dS m^{-1})	EC	RI (%)	EC	RI (%)
0	145.42^{A}±13.012	0	431.06^{A}±57.463	0
10	95.28^{AB}±14.708	-34.48	391.69^{AB}±64.952	-9.13
20	86.77^{B}±15.919	-40.33	172.64^{B}±70.300	-59.95
30	127.37^{AB}±16.922	-12.41	415.33^{AB}±74.727	-3.65

yColumn means ± standard error, followed by the same superscripts, were not different ($p \leq 0.05$) according to Tukey HSD significant difference test. Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 3.9: The interactive effect of salt and VAM on soil pH after 90 days of initiation of treatment application in Experiments 1

VAM		Salt(dS m^{-1})	pH	RI (%)
0	*	0	5.8250^{B}±0.1849	0
0	*	0.25	6.4273^{AB}±0.2333	10.34
0	*	0.50	6.4211^{AB}±0.3020	10.23
0	*	0.75	6.9151^{A}±0.2151	18.71
10	*	0	6.3078^{AB}±0.1985	8.29
10	*	0.25	6.2500^{AB}±0.2597	7.30
10	*	0.50	6.4056^{AB}±0.2150	9.97
10	*	0.75	5.9728^{AB}±0.2634	2.54
20	*	0	6.3763^{AB}±0.2332	9.46
20	*	0.25	6.0490^{AB}±0.2333	3.85
20	*	0.50	6.0000^{AB}±0.2597	3.0
20	*	0.75	6.2879^{AB}±0.3019	7.95
30	*	0	6.6031^{AB}±0.2130	13.36
30	*	0.25	6.4789^{AB}±0.2130	11.23
30	*	0.50	5.9507^{AB}±0.2385	2.16
30	*	0.75	6.2516^{AB}±0.3020	7.32

yColumn means ± standard error, followed by the same superscripts, were not different ($p \leq 0.05$) according to Tukey HSD significant difference test. zRelative impact (RI %) = [(treatment/control) – 1] × 100

3.6 Discussion

3.6.1 Plant variables

The results in both experiments have shown that treatments had a significant effect ($p < 0.05$) on plant growth variables. “This observation was also reported by Başak *et al*. (2011), who found that when Mycorrhiza treatment was performed by using ROOTS-novozymes endo-mycorrhiza fungus (VAM) on two tomato varieties grown under salinity, the treatments had a significant effect on plant length, fresh root weight and dry root weight for ‘Aspendos’ species seedlings were found to be significant at the statistical level. Although the interaction of salts and VAM fungi was significant, the results demonstrated that VAM seemed to improve plant growth. The results revealed that the applied VAM fungi improved stem diameter, chlorophyll content, and the number of branches in Experiments 1 and 2, with a relative impact that was high, indicating the effect of the treatment. The increased number of branches improves the plant's ability to capture sunlight and effectively use nutrients, adding to greater biomass. As reported by Başak *et al*. (2011), several studies have demonstrated that plants inoculated with VAM improve various plant variables. Başak *et al*. (2011) reported observations in pepper, onion and vegetables showing that AMF inoculation resulted in higher growth as compared to the control treatment. Biological growth of natural arbuscular mycorrhiza fungus increased the quality of seedlings in seedbeds. Experimental evidence shows that VAM colonization significantly increases chlorophyll content, biomass, and root-to-shoot ratios in saline environments (Başak *et al*., 2011). These enhancements show VAM's ability to regulate physiological activities and boost growth under challenging conditions (Masenya *et al*., 2023).

The incorporation of AM fungi into the cultivation of *S. frutescens* offers a viable approach to alleviate saline stress, consequently promoting plant development and resistance. Experimental findings demonstrate that AMF markedly enhances root development and total biomass across different salinity levels, suggesting its capacity to enhance crop resilience in salt-affected soils, a pressing issue exacerbated by climate change and soil degradation (Masenya *et al*., 2025). The data suggest that integrating AMF into agricultural techniques may facilitate the sustainable production of this valued plant, enhancing both conservation and yield. Future studies should concentrate on refining AMF inoculation methods and investigating their interactions with pest management measures to maximize their advantages in saline conditions and ensure the plant's long-term sustainability.

Salt had a considerable impact ($p< 0.05$) on plant height and number of branches in both Experiments 1 and 2. Muhammad and Hussain (2010) reported corresponding results to the current study when evaluating the effect of NaCl salinity on the germination and seedling growth of medicinal plants, where salts resulted in significant differences for the measured variables. The results in Experiments 1 and 2, showed that, salt does not harm the plant, but rather improves the number of branches and plant height. Qados (2011) reports analogous results to the current study when evaluating the effect of salt stress on plant growth and metabolism of bean plant (*Vicia faba* (L.). Qados (2011) reported how salinity increased both fresh and dry weights of the shoot in the two measurement periods. The observations are not common in most studies. As a result, Atta *et al*. (2023) suggested that, such observations are due to the fact that in high-salinity soils, plants develop a range of physiological and biochemical adaptations. In addition to ion homeostasis and

compartmentalization, the principal responses include the biosynthesis of compatible solutes, osmo-protectants, antioxidant compounds, polyamines, and nitrous oxide, as well as the regulation of phyto-hormones. *Sutherlandia frutescens* demonstrates adaptive responses, including osmolyte accumulation and the activation of antioxidant defences, to alleviate the adverse effects of salinity. Comprehending these physiological responses is essential for augmenting the robustness of this medicinal plant.

Nevertheless, the positive view in the current study is that plants exposed to saline conditions face substantial challenges such as osmotic stress, ion toxicity, and nutritional deficits. These stresses jointly impede physiological and biochemical processes required for development and production. Salinity hinders water absorption by lowering soil water potential, causing dehydration and stomatal closure. This limits photosynthesis and reduces biomass accumulation (Munns and Tester, 2008). Excess sodium (Na^+) and chloride (Cl^-) ions can disrupt cellular ion balance, damage membranes, and delay metabolic processes (Roy *et al*., 2020).

3.6.2 Soil variables

The experimental results indicated that, salt treatment significantly influenced both electrical conductivity (EC) and pH levels, highlighting notable distinctions between Experiment 1 and Experiment 2. The observed reduction in EC across both trials suggests that salt treatments effectively alter ion mobility and concentration, key factors impacting conductivity. The more pronounced decrease in EC in Experiment 1 as compared to Experiment 2 may be attributed to varying ambient conditions, salt compositions, or initial ion concentrations (Masenya *et al*., 2025).

Electrical conductivity serves as a vital parameter in soil science, functioning as an indirect measure of soil salinity and nutrient availability. By assessing EC, the quantity of soluble salts in the soil, which correlates with the ionic composition that influences plant growth and soil health. In saline soils, elevated EC levels as seen in Experiment 2 can have adverse effects on crop yields, which is essential for maintaining agricultural sustainability and formulating informed policies. Numerous studies underscore the toxicity risks posed by the accumulation of sodium (Na^+) and chloride (Cl^-) ions, which can hinder nutrient uptake, leading to issues such as stunted growth and reduced agricultural productivity (Yan and Marschner, 2013).

Research by Corwin and Lesch (2003) emphasized that, soil EC is one of the most reliable and comprehensible metrics for illustrating field variability in precision agriculture. Birrell *et al*. (1996) discussed the potential of using geographic measurements of EC to anticipate agricultural yields based on soil water changes. Furthermore, soil salinity can significantly influence plant EC, which in turn affects root development (Doran *et al*., 1996). Additionally, it wasnoted that low EC values, as seen in Experiment 1, can negatively impact soil health by indicating nutrient deficiencies. As pointed out by Rhodes and Corwin (1990), maintaining a moderate EC value neither too high nor too low is crucial for optimal soil conditions. The findings from this study suggest that salt treatments lead to elevated EC values, reinforcing the idea that these treatments have a substantial impact on soil properties.

3.7 Conclusion

The results showed no significant difference between the interaction of salt and VAM fungi. The hypothesis that stated VAM fungi would improve growth of *S. frutescens* under salinity conditions is rejected. However, VAM showed a positive influence on the growth of plants, in the absence of salinity, suggesting it may be utilised to improve growth in agricultural systems. Additional research, including the optimization of VAM application in various environmental conditions is required to fully realize its potential in sustainable agriculture, particularly for medicinal plant production in saline-affected areas. In the next research chapter, the researcher investigated how the secondary metabolites of *S. frutescens* were influenced by VAM fungi under salinity conditions.

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CHAPTER 4

THE BIOSYNTHESIS AND ACCUMULATION OF SECONDARY METABOLITES OF *SUTHERLADIA FRUTESCENS* INFLUENCED BY VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI UNDER SALINITY CONDITIONS

Abstract

4.1 Introduction

The production of medicinal plants has attracted increasing interest due to the many secondary metabolites they possess, which are crucial in pharmacology and therapy. Environmental conditions, including soil salinity, significantly influence plant development and metabolite production (ABD-Allah *et al*. 2015). These conditions often trigger stress responses that affect the pathways involved in metabolic activities. ABD-Allah *et al*. (2015) alluded that, the capacity of medicinal plants to acclimatize to environmental stresses, including soil salinity and nutrient deficiency, is essential for their survival and development. *Sutherlandia frutescens*, a plant renowned for its diverse array of secondary metabolites with potential medicinal applications, thrives in many settings characterized by persistent stress.

This adaptive potential is closely linked to its ecological interactions, particularly with soil microorganisms, like arbuscular mycorrhizal (AM) fungi, which facilitate nutrient absorption and improve the plant's stress tolerance. Through the establishment of symbiotic relationships, these fungi enhance the plant's capacity to absorb essential minerals and water, thereby facilitating the plant's growth and the production of bioactive compounds vital for its therapeutic properties. Research has shown that advantageous AM fungi can establish a mutualistic symbiosis with medicinal plants, enhancing the plants' development, resistance to abiotic stresses, and accumulation of useful secondary metabolites (Pratyusha *et al.*, 2022). This connection is crucial

as it facilitates *S. frutescens'* adaptation to its environment, allowing it to thrive in challenging settings. During periods of abiotic stress, *S. frutescens* plays a crucial role in maintaining soil health and the stability of plant communities, underscoring its ecological significance. Research indicates that VAM fungi play a vital role in these processes. They accelerate the synthesis of secondary metabolites and aid the plant in managing salt stress, both of which are essential for agricultural sustainability and conservation initiatives (ABD-Allah *et al.,* 2015). Investigating the relationship between AMF colonization and decreased salinity in *S. frutescens*, a plant renowned for its therapeutic properties, may yield significant insights for optimizing secondary metabolite profiles for medicinal purposes. A comprehensive study of these dynamics is essential for the sustainable utilization and development of medicinal plants rich in therapeutic compounds (Zhang *et al*., 2021).

The presence of decreased salinity amplifies this interaction, hence enhancing metabolic pathways that ultimately lead to the accumulation of bioactive compounds. Symbiotic processes are becoming recognized for their role in plant stress physiology and enhancement of plant productivity, as noted by ABD-Allah *et al*. (2015). Mycorrhizal fungi augment nutrient absorption and stress tolerance, thus indirectly affecting the production and storage of essential secondary metabolites, including total flavonoids and phenols. This improvement is associated with enhanced physiological responses, such as osmotic adjustment and antioxidant processes, which aid the plants' adaptation to lower salinity conditions. Hence, the objective of the study was to investigate whether biosynthesis and accumulation of secondary metabolites of *S. frutescens* will be influenced by the use of VAM fungi in reducing salinity.

4.2 Methods and materials

4.2.1 Study area

The study was conducted from May to July 2024, encompassing the unique conditions of microplots and shade nets, as outlined in Chapter 3.

4.2.2 Experimental design and cultural practices

The experimental design and cultural practices are similar to those previously described (Chapter 3).

4.2.3 Data collection

After a 90-day growing period, *S. frutescens* plants were harvested and processed for examination to evaluate their phenolic and flavonoid content. The collected plants were transported to the University of Mpumalanga laboratory, where the shoots and roots were desiccated, pulverized, and subjected to a 500 μm sieve to yield a fine powder appropriate for extraction. Methanol served as the solvent for the extracting both phenolic compounds and flavonoids from the powdered materials. The total phenolics were quantified utilizing a modified Folin–Ciocalteu colorimetric technique for this investigation. In this procedure, 0.50 mL of the methanol extract (1 mg/mL) was combined with Folin–Ciocalteu reagent and sodium carbonate, incubated for 2 hours, and the absorbance was assessed at 760 nm with a UV–vis spectrophotometer. A calibration curve for gallic acid was established as the standard for quantifying phenolic content, given as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g), with values averaged over three replicates. The aluminium chloride ($AlCl_3$) colorimetric method, including modifications, was

employed for flavonoid measurement. Four hundred (400) microliters of extracts were combined with sodium nitrite, $AlCl_3$, and sodium hydroxide, and subsequently diluted to a final amount of one milliliter with distilled water. Following a 15-minu te incubation in darkness, the absorbance was measured at 510 nm. A calibration curve based on quercetin was employed to quantify flavonoid concentration as milligrams of quercetin equivalent per gram of dry weight (mg QE/g), with standard deviation values derived from triplicate measurements (Singh *et al*., 2022). This comprehensive methodology facilitated an accurate evaluation of the phytochemical properties of *S. frutescens* under the specified investigation conditions.

To determine the flavonoid content, an $AlCl_3$ reagent and a quercetin standard were utilized (Zhishen *et al.,* 1999). The concentration of quercetin in the leaves was quantified as milligrams of quercetin equivalents per gram of dry leaf weight (mg QE/g DW). The method for quantifying tannins by measuring the intensity of a color formed during a chemical reaction, which is directly proportional to the tannin content in the sample, as described by Sun *et al*. (1998), was also employed, and the findings were reported. Each sample underwent three analyses, and results were expressed as milligrams of catechin per gram (mg catechin/g).

4.2.4 Data analysis

This research utilized the Shapiro-Wilk test to assess the normality of the data distribution (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965). Data analysis was conducted using Statistix 10.0, following the normality assessment. Mean separation was done using Tukey HSD at the probability of 5%. The absorbance of quercetin at 760 nm was measured using a standard

quercetin concentration. The results focused exclusively on parameters that were significant at the probability threshold of $p \leq 0.05$ unless stated otherwise.

4.3 Results

The applied treatments, as shown in Table 4.1 below, had a significant impact ($p \leq 0.05$) on total phenolic content (TPC) and total flavonoid content (TFC) in both experiments 1 and 2. Vesicular-Arbuscular Mycorrhizal (VAM) fungi, salt treatments, the interaction of salt and VAM fungi had a significant effect on the TPC in experiment 1, contributing 30.01%, 40.30%, and 29.25% of the total treatment variation (TTV). In contrast, in experiment 2, treatments had a substantial effect on TPC with a TTV of 98.43, 0.33, and 0.47%, respectively (Tables 4.1). Similarly, VAM fungi, salt treatments, and the interaction of salt and VAM fungi had a significant effect on the TFC in experiments 1 and 2, contributing to the TTV of 98.43%, 1.21%, and 0.35% and in experiment 2, with the TTV of 99.7%, 0.02% and 0.27%, respectively (Table 4.1).

Vesicular-Arbuscular Mycorrhizal fungi increased the TPC relative to the control, with a relative impact (RI) of 5.79%, to 9.22% in Experiment 1, and from 26.15%, to 80.50% in Experiment 2 (Table 4.2). The salt treatments reduced both TPC and TFC in both experiments. The application of salt resulted in a reduced TPC in Experiments 1 and 2, by -9.09% to -12.29%, and from -1.62% to -2.95%, in Experiment 2 (Table 4.3). The TFC was reduced by a RI of -0.87% to -4.55 in Experiment 1, while in Experiment 2 the reduction was -.0.66%. However, salt did increase TFC by 0.14% in Experiment 2 when applied at the lowest concentration of 0.25 dS m^{-1}.

The interaction of VAM and salinity treatments influenced TPC in Experiment 2 (Table 4.4) only. The TPC was increased by the interaction with relative impact of 15.37% to 72.05%. Similar results were observed for TFC, where in Experiment 1 and 2 (Table 4.4), the interaction increased the TFC by 0.07 to 0.57% in Experiment 1, whereas in Experiment 2, TFC was reduced by 3.71% to 4.47%.

Table 4.1: Source of variation affecting Total Phenolic Content (TPC) and Total Flavonoids Content (TFC) of *Sutherlandia frutescens* after 90 days of initiation in Experiments 1 & 2

		TPC in EXP 1		TPC in EXP 2		TFC in EXP 1		TFC in EXP 2	
Source	DF	MS	TTV	MS	TTV	MS	TTV	MS	TTV
Rep	2	0.0437	0.05	0.131	0.11	0.002	0.04	0.003	0.14
VAM	3	24.2438	30.01***	114.939	98.99***	559.150	98.43**	214.147	99.7**
Salt	3	32.5530	40.30***	0.387	0.33***	6.868	1.21**	0.048	0.02**
VAM*Salt	9	23.6285	29.25***	0.542	0.47***	2.022	0.35**	0.573	0.27**
Error	30	0.3036	0.38	0.103	0.09	0.002	0.04	0.002	0.09
Total	47	80.7726	100	116.102	100	568.044	100	214.773	100

***Highly significant at p≤0.001, **significant at p≤0.05, ns not significant, TTV (%) Total Treatment Variation.

Table 4.2: Effect of VAM on Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of *Sutherlandia frutescens* at 90 days after initiation of treatment application in Experiments 1 & 2

VAM	TPC in EXP 1	RI (%)	TPC in EXP 2	RI(%)	TFC in EXP 1	RI(%)	TFC in EXP 2	RI(%)
0	25.249^{D}±0.1699	0	9.852^{D}±0.0989	0	50.225^{A}±0.0131	0	28.841^{A}±0.01149	0
10	26.710^{C}±0.1847	5.79	12.428^{C}±0.1075	26.15	46.028^{B}±0.0130	-8.36	22.434^{B}±0.0162	-22.21
20	28.784^{A}±0.1591	14	15.383^{B}±0.0926	56.14	38.914^{C}±0.0133	-22.52	19.630^{C}±0.0139	-31.94
30	27.576^{B}±0.1866	9.22	17.783^{A}±0.1087	80.50	32.632^{D}±0.0164	-35.03	18.659^{D}±0.0163	-35.30

[y]Column means ± standard error, followed by the same superscripts were not different (Pp 0 < 05) according to Tukey's HSD significant difference test.

[z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 4.3: Effect of Salt on Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of *Sutherlandia frutescens* at 90 days after initiation of treatment application in Experiments 1 & 2

Salt(dS m^{-1})	TPC in EXP 1	RI	TPC in EXP 2	RI	TFC in EXP 1	RI	TFC in EXP 2	RI
0	29.438^{A}±0.1387	0	14.147^{A}±0.0808	0	42.815^{A}±0.0107	0	22.420^{A}±0.0122	0
0.25	26.763^{B}±0.1695	-9.09	13.918^{AB}±0.0987	-1.62	42.444^{B}±0.0130	-0.87	22.451^{A}±0.0148	0.14
0.50	26.298^{BC}±0.1733	-10.67	13.807^{AB}±0.1009	-2.40	41.673^{C}±0.0133	-2.67	22.421^{A}±0.0152	0
0.75	25.820^{C}±0.2128	-12.29	13.729^{B}±0.1239	-2.95	40.867^{D}±0.0164	-4.55	22.273^{B}±0.0186	-0.66

[y]Column means ± standard error, followed by the same superscripts were not different (Pp 0 < 05) according to Tukey's HSD significant difference test.

[z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

Table 4.4: Effect of VAM*Salt on Total Phenolic Content (TPC) in Experiment 1, Total Phenolic Content (TPC) and Total Flavonoids Content (TFC) in Experiment 2 of *Sutherlandia frutescens* at 90 days after initiation of treatment application

VAM *Salt (dS m^{-1})	TPC in EXP 2	RI	TFC in EXP 1	RI	TFC in EXP 2	RI
0*0	10.572^{E}±0.1648	0	50.099^{A}±0.0218	0	27.975^{C}±0.0248	0
0*0.25	9.861^{EF}±0.2301	-6.73	50.133^{AB}±0.0304	0.07	29.014^{B}±0.0346	3.71
0*0.50	9.681^{EF}±0.2301	-8.43	50.283^{BC}±0.0304	0.37	29.149^{AB}±0.0346	4.20
0*0.75	9.295^{F}±0.1685	-12.08	50.383^{C}±0.0222	0.57	29.227^{A}±0.0253	4.47
10*0	12.741^{D}±0.1649	20.52	47.863^{D}±0.0218	-4.46	22.857^{D}±0.0248	-18.29
10*0.25	12.197^{D}±0.2300	15.37	46.747^{E}±0.0304	-6.69	22.428^{E}±0.0346	-19.83
10*0.50	12.377^{D}±0.2300	17.07	45.322^{F}±0.0304	-9.54	22.323^{E}±0.0346	-20.20
10*0.75	12.396^{D}±0.2301	17.25	44.178^{G}±0.0304	-11.82	22.129^{F}±0.0346	-20.90
20*0	15.084^{C}±0.1615	42.68	40.144^{H}±0.0213	-19.87	19.831^{G}±0.0243	-29.11
20*0.25	15.249^{C}±0.1615	44.24	39.862^{I}±0.0213	-20.43	19.644^{H}±0.0243	-29.78
20*0.50	15.790^{BC}±0.1853	49.36	38.557^{J}±0.0245	-23.04	19.563^{HI}±0.0279	-30.07
20*0.75	16.027^{BC}±0.2300	51.60	37.092^{K}±0.0304	-25.96	19.483^{L}±0.0346	-30.35
30*0	18.189^{A}±0.1615	72.05	33.152^{L}±0.0213	-33.83	19.016^{J}±0.0243	-32.03
30*0.25	17.919^{A}±0.1615	69.49	33.034^{M}±0.0213	-34.06	18.719^{K}±0.0243	-33.09
30*0.50	17.825^{A}±0.1642	68.61	32.530^{N}±0.0217	-35.07	18.648^{K}±0.0247	-33.34
30*0.75	17.198^{AB}±0.1641	62.67	31.814^{O}±0.0435	-36.50	18.252^{L}±0.0496	-34.76

[y]Column means ± standard error, followed by the same superscripts were not different (p< 05) according to Tukey's HSD significant difference test. [z]Relative impact (RI %) = [(treatment/control) – 1] × 100.

4.4 Discussion

The fascinating world of medicinal plants, such as *S. frutescens*, reveald the crucial role that secondary metabolites play in their therapeutic potential. Compounds like phenols and flavonoids act as the plant's armor, responding dynamically to abiotic stressors like salt. This adaptive defense mechanism not only highlights the resilience of these plants but also underscores their importance in traditional medicine (Ghassemi-Golezani *et al.,* 2020).

In our experiments, the results demonstrated that various treatments, including VAM fungi and salt exposure, significantly influenced the total phenolic content (TPC) across both trials as depicted in Table 4.1 showing an increase in the TPC. This aligns with the findings of Alizadeh *et al.* (2021), who explored how Arbuscular mycorrhizal fungi can help mitigate salinity stress while simultaneously enhancing phenolic compounds in Moldavian balm. Their research showcased substantial variations in both total phenols and flavonoids, further emphasizing the intricate relationship between these treatments and the plants' biochemical responses.

Vesicular-Arbuscular Mycorrhizal fungi increased the TPC relative to the control in Experiments 1 and 2 (Table 4.3). Various studies over the past decade have highlighted the role of VAM in mediating physiological changes that lead to increased levels of phenolic and flavonoid compounds. These studies demonstrate significant improvements in the profiles of secondary metabolites when plants are exposed to saline conditions (Ahmad *et al*., 2021). Arbuscular mycorrhizal fungi (AMF) symbiosis increases growth and stimulates the biosynthesis of bioactive

compounds, thereby influencing polyphenolic content in medicinal plants under salt stress (Alizadeh *et al*., 2021)

Interestingly, even though there was a notable increase in TPC, the presence of VAM fungi actually led to a decrease in TFC in both Experiments 1 and 2. This observation highlights the complex role that secondary phenolic metabolites play in plant defense mechanisms, which are not just crucial for plant health but also hold significant benefits for human well-being.

Surprisingly, there are only a few studies delving into how different mycorrhizal fungi influence the secondary metabolites of their host plants. For instance, Eftekhari *et al*. (2012) reported contrasting results, finding an increase in TFC when analyzing total phenolics and TFC in mycorrhizal grape varieties (*Vitis vinifera* L.) and examining how postharvest drying impacted quercetin yield.

Adding to the complexity, Yildirim *et al*. (2016) noted that plants exhibit varied responses to different abiotic stress conditions, which might explain the discrepancies between TPC and TFC levels. Similarly, Abdelhalim *et al.* (2022) reported compelling findings: all sorghum cultivars treated with arbuscular mycorrhizal fungi (AMF) recorded significantly higher concentrations of total phenolics, flavonoids, carotenoids, and tannins compared to the control group ($P < 0.05$). This suggests an intriguing interplay between mycorrhizal associations and plant metabolite production, inviting further exploration into this fascinating subject.

The salt treatments reduced both TPC and TFC in both experiments. The highest phenolic content was found in VAM-treated plants exposed to moderate salinity (30*0.25 dS m^{-1}.), demonstrating a synergistic relationship between mycorrhizal colonization and moderate stress levels. This finding is consistent with previous research, which has shown that VAM treatment in saline conditions increased the accumulation of polyphenolic compounds (Evelin *et al*., 2009). Prolonged exposure to high salt levels can hinder nutrient absorption and restrict growth, reducing the plant's biosynthetic potential.

The present study found significant differences in the contents of phenols, flavonoids, and tannins in *S. frutescens* after various VAM and salt treatments. The correlation between saline treatments and VAM fungus with TFC and TPC was statistically significant, lending credence to the findings of Olsson *et al*. (2014), who found that mycorrhizal connections improve plant growth and nutrient uptake in salt stress. Mycorrhizal fungi may improve nutrient uptake or increase the plant's tolerance to osmotic stress, as suggested by the moderate TTV of 0.35% for TFC in the context of VAM and saline treatment (Alizadeh *et al*., 2021; Khalid *et al.,* 2022).

Vesicular-Arbuscular Mycorrhizal (VAM) fungi symbiosis improves plant salt tolerance by increasing water and nutrient uptake, particularly phosphorus and nitrogen, which are required for secondary metabolite synthesis (Smith and Read, 2008). These effects are likely due to VAM-induced modulation of antioxidant pathways and enhanced biosynthetic gene expression (Ruiz-Lozano *et al.,* 2012). From an ecological and pharmacological standpoint, secondary metabolite enhancement via VAM-salinity interactions can improve *S. frutescens'* therapeutic qualities. This is particularly significant because the plant is widely known in traditional South African medicine for its adaptogenic and immunomodulatory properties (Wyk & Gericke, 2000). The findings

provide a platform for implementing VAM biotechnology into *S. frutescens* farming, particularly in salinized marginal soils.

Yildirim *et al*. (2016) stressed the importance of studying how biotic and abiotic stresses interact to influence plant physiology and chemistry. Important findings about how salinity affects the biochemical makeup of *S. frutescens* have been uncovered by the research. It is clear that additional research into adaptation techniques to improve plant resistance is necessary in light of the fact that TPC and TFC significantly decrease under salt stress. To improve agricultural techniques for resilient crops in salty soils, future research should investigate how mycorrhizal fungi affect phenolic production in areas prone to salinity.

4.5 Conclusion

The findings of this research are quite intriguing, as they demonstrated that, the use of VAM fungi in saline conditions significantly boosts the accumulation and biosynthesis of Total Phenolic Compounds (TPC), even the TFC was increased, although at low rates. This supports our hypothesis that VAM fungi can enhance the production of vital secondary metabolites in *S. frutescens* faced with salinity stress. However, there's much more to explore. Further investigation is needed to fully understand how VAM fungi impact the secondary metabolites of medicinal plants in saline environments, especially given the challenges posed by our changing climate. In the next

chapter, the researcher will wrap up the key discoveries, discuss their implications, identify existing knowledge gaps, and offer recommendations for future studies.

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CHAPTER 5

5.1 Summary

In conclusion, the integration of vesicular arbuscular mycorrhizal (VAM) fungi in the cultivation of *S. frutescens* is a viable approach to augmenting plant development and resilience in saline stress conditions. The significant enhancements noted in stem diameter, chlorophyll production, and branching ability underscore the vital function of VAM in promoting nutrient uptake, optimising water relations, and bolstering antioxidant defences, which together alleviate the detrimental impacts of salinity. Experimental research substantiates that VAM enhances both structural and metabolic growth while also improving the plant's overall health and resilience against abiotic and biotic challenges. With the exception of the increase in deterioration caused by climate change, which escalates difficulties in agriculture and ecosystem sustainability, the practical implementation of VAM may be crucial in promoting sustainable practices, especially in the cultivation of medicinal plants such as *S. frutescens*. Additional study is crucial to enhance VAM application methods and investigate its synergistic potential with pest control tactics to optimise benefits in saline environments, ultimately resulting in enhanced yields and conservation outcomes in agricultural systems.

5.2 Scientific contribution

Farmlands in South Africa are largely deteriorating due to malpractices, increased drought and high temperatures, which result in an increased incidence of salinity. The findings of this study will expand knowledge in the cultivation of *S. frutescens* with the aim of preventing its potential

extinction. Furthermore, the generated knowledge will improve potential for the domestication of *S. frutescens* on a large scale and in smallholder farming sectors under saline soil conditions, ensuring abundant availability and supply of *S. frutescens* medicinal products, and also improving the economy of the country.

5.3 Recommendations

This investigation highlights the considerable influence of VAM fungi and salt treatments on the growth, secondary metabolite production, and stress alleviation of *S. frutescens*. The results demonstrate that the application of VAM significantly improved plant performance, as shown by the notable increase in stem diameter, chlorophyll content, and plant fresh weight. The noted enhancements suggest that VAM aids in more effective nutrient absorption, boosts photosynthetic performance, and supports general plant health. Considering these advantages, incorporating VAM into cultivation methods can serve as a valuable approach to enhance plant health and productivity, particularly in environmental conditions.

Furthermore, VAM significantly contributed to mitigating the detrimental effects of saline stress, as evidenced by its capacity to lessen decreases in electrical conductivity and to counteract negative influences on plant growth. The interconnectedness of VAM and plant roots likely leads to improved soil structure, increased water retention, and better plant adaptation to high salinity environments. Consequently, VAM ought to be extensively utilised as a biological instrument to improve plant resilience in soils affected by salinity. The integration of VAM with organic soil amendments has the potential to enhance plant resilience to salt stress, promoting improved survival and growth in challenging conditions.

The study indicated that VAM had a significant impact on total phenolic content (TPC) while leading to a decrease in total flavonoid content (TFC) regarding secondary metabolite production. The rise in phenolic compounds suggests that VAM may potentially boost the production of bioactive compounds linked to the medicinal qualities of *S. frutescens*. Nonetheless, the reduction in flavonoid levels suggests the potential for targeted modulation of metabolites, necessitating additional research to enhance metabolite production for use in pharmaceuticals. Comprehending these interactions can enhance VAM application strategies to optimise the therapeutic potential of the plant.

The application of salt treatment notably influenced electrical conductivity and pH levels, yet its detrimental effect on the accumulation of secondary metabolites underscores the importance of meticulous salinity management in plant cultivation. High levels of salinity hinder the production of metabolites, potentially impacting the medicinal properties of *S. frutescens*. Consequently, it is essential to investigate strategies like controlled irrigation and soil amendments to mitigate the negative impacts of salt stress. Moreover, additional investigation is necessary to explore the relationship between salt and VAM, aiming to enhance cultivation techniques that support plant health and maximise the yield of secondary metabolites.

The findings indicate that the incorporation of VAM into the cultivation of *S. frutescens* promotes plant growth, alleviates saline stress, and affects the production of secondary metabolites. Future investigations should focus on pinpointing the most effective VAM strains to enhance phenolic compound accumulation, while maintaining plant resilience across diverse environmental conditions. By implementing these recommendations, individuals in the field can enhance the

sustainability and medicinal value of *S. frutescens*, establishing it as a viable option for cultivation across various landscapes.

5.4 Conclusion

This investigation demonstrates that VAM fungi enhance the growth, stress resilience, and secondary metabolite synthesis of *S. frutescens*. The application of VAM resulted in notable improvements in stem diameter, chlorophyll content, and plant fresh weight, indicating its positive impact on plant health. Furthermore, VAM elevated TPC, potentially augmenting the medicinal attributes of the plant; however, it decreased total flavonoid content (TFC), indicating the need for additional investigation. The influence of salt treatment on electrical conductivity and pH reveals a significant relationship, while its adverse effects on metabolite production highlight the necessity for meticulous management strategies. Overall, VAM serves as an effective instrument for enhancing plant performance in stressful environments, and additional investigation can refine its advantages.

Appendices

Appendix 1: Analysis of variance for stem diameter (SD) experiment 1

Source	DF	SS	MS	F	P
Rep	3	7.164	2.388		
VAM	3	396.920	132.307	79.60	0.0000
Salt	3	5.680	1.893	1.14	0.3405
VAM*Salt	9	19.754	2.195	1.32	0.2453
Error	61	101.392	1.662		
Total	79				

Appendix 2: Analysis of variance for chlorophyll (CL) in experiment 1

Source	DF	SS	MS	F	P
Rep	3	0.4633	0.1544		
VAM	3	90.0938	30.0313	73.08	0.0000
Salt	3	0.3596	0.1199	0.29	0.8313
VAM*Salt	9	2.8928	0.3214	0.78	0.6334
Error	61	25.0670	0.4109		
Total	79				

Appendix 3: Analysis of variance for plant fresh weight (PFW) in experiment 1

Source	DF	SS	MS	F	P
Rep	3	49.95	16.65		
VAM	3	7937.56	2645.85	170.94	0.0000
Salt	3	41.90	13.97	0.90	0.4453
VAM*Salt	9	44.36	4.93	0.32	0.9659
Error	61	944.16	15.48		
Total	79				

Appendix 4: Analysis of variance for chlorophyll (CL) in experiment 2

Source	DF	SS	MS	F	P
Rep	3	2.258	0.7528		
VAM	3	105.183	35.0611	49.20	0.0000
Salt	3	1.297	0.4324	0.61	0.6131
VAM*Salt	9	10.388	1.1542	1.62	0.1297
Error	61	43.466	0.7126		
Total	79				

Appendix 5: Analysis of variance for plant fresh wright (PFW) in experiment 2

Source	DF	SS	MS	F	P
Rep	3	25.10	8.37		
VAM	3	6626.56	2208.85	97.89	0.0000
Salt	3	11.56	3.85	0.17	0.9157
VAM*Salt	9	68.68	7.63	0.34	0.9586
Error	61	1376.41	22.56		
Total	79				

Appendix 6: Analysis of variance for stem diameter (SD) in experiment 2

Source	DF	SS	MS	F	P
Rep	3	10.742	3.581		
VAM	3	351.054	117.018	46.34	0.0000
Salt	3	7.531	2.510	0.99	0.4017
VAM*Salt	9	25.526	2.836	1.12	0.3606
Error	61	154.023	2.525		
Total	79				

Appendix 7: Analysis of variance for plant height (PH) in experiment 1

Source	DF	SS	MS	F	P
Rep	3	1838.9	612.97		
VAM	3	281.2	93.73	0.69	0.5608
Salt	3	12035.2	4011.72	29.59	0.0000
VAM*Salt	9	509.9	56.66	0.42	0.9207
Error	61	8269.0	135.56		
Total	79				

Appendix 8: Analysis of variance for the number of branches (NB) experiment 1

Source	DF	SS	MS	F	P
Rep	3	301.92	100.639		
VAM	3	76.54	25.514	1.05	0.3769
Salt	3	2113.89	704.631	29.01	0.0000
VAM*Salt	9	287.51	31.946	1.32	0.2480
Error	61	1481.68	24.290		
Total	79				

Appendix 9: Analysis of variance for the number of branches (NB) in experiment 2

Source	DF	SS	MS	F	P
Rep	3	556.04	185.346		
VAM	3	15.29	5.097	0.17	0.9157
Salt	3	1215.01	405.004	13.58	0.0000
VAM*Salt	9	445.10	49.455	1.66	0.1192
Error	61	1819.61	29.830		
Total	79				

Appendix 10: Analysis of variance for plant height (PH) in experiment 2

Source	DF	SS	MS	F	P
Rep	3	6892.8	2297.59		
VAM	3	463.6	154.55	0.58	0.6326
Salt	3	8224.0	2741.34	10.23	0.0000
VAM*Salt	9	1271.6	141.29	0.53	0.8492
Error	61	16349.3	268.02		
Total	79				

Appendix 11: Analysis of variance for EC Experiment 1

Source	DF	SS	MS	F	P
Rep	3	52937	17645.8		
VAM	3	1599	533.1	0.13	0.9442
Salt	3	45563	15187.7	3.60	0.0184
VAM*Salt	9	69665	7740.5	1.83	0.0800
Error	61	257420	4220.0		
Total	79				

Appendix 12: Analysis of variance for pH Experiment 1

DF	SS	MS	F	P
3	2.9200	0.97335		
3	0.5002	0.16673	0.62	0.6061
3	0.2173	0.07243	0.27	0.8479
9	5.8185	0.64650	2.40	0.0214
61	16.4615	0.26986		
79				

Appendix 13: Analysis of variance for EC Experiment 2

Source	DF	SS	MS	F	P
Rep	3	501324	167108		
VAM	3	487106	162639	1.97	0.1274
Salt	3	7694436	256479	3.12	0.0325
VAM*Salt	9	84006	93341	1.13	0.3533
Error	61	5020068	85596		
total	79				

Appendix 14: Analysis of variance for Phenol (TPC) in experiment 1

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	2	0.087	0.0437		
VAM	3	72.731	24.2438	79.86	0.0000
Salt	3	97.659	32.5530	107.24	0.0000
VAM*Salt	9	212.657	23.6285	77.84	0.0000
Error	30	9.107	0.3036		
Total	47				

Appendix 15: Analysis of variance for Phenol (TPC) in experiment 2

Source	DF	SS	MS	F	P
Rep	2	0.261	0.131		
VAM	3	344.816	114.939	1116.35	0.0000
Salt	3	1.160	0.387	3.76	0.0211
VAM*Salt	9	4.882	0.542	5.27	0.0003
Error	30	3.089	0.103		
Total	47				

Appendix 16: Analysis of variance for flavonoid (TFC) in experiment 1

Source	DF	SS	MS	F	P
Rep	2	4.943E-03	0.002		
VAM	3	1677.45	559.150	311654.45	0.0000
Salt	3	20.6029	6.868	3827.82	0.0000
VAM*Salt	9	18.2016	2.022	1127.23	0.0000
Error	30	0.05382	0.002		
Total	47				

Appendix 17: Analysis of variance for Flavonoid (TFC) in experiment 2

Source	DF	SS	MS	F	P
Rep	2	0.007	0.003		
VAM	3	642.442	214.147	91917.90	0.0000
Salt	3	0.143	0.048	20.40	0.0000
VAM*Salt	9	5.159	0.573	246.06	0.0000
Error	30	0.070	0.002		
Total	47				