The effect of different potassium concentrations and electrical conductivity on hydroponic- grown *Trachyandra divaricata*, as a green vegetable

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Declaration

I, Bakholise Bulawa, certify that the information contained in this dissertation or thesis is entirely my own original work, and it has not previously been submitted before for academic review in order to obtain a degree. In addition, it expresses my personal views rather than necessarily those of Cape Peninsula University of Technology

Abstract

The shortages of freshwater supply, inadequate groundwater, and high salinization levels in soil have become major challenges worldwide. Salinization, which refers to the presence of salts in the soil or water, is a common challenge in half of cultivated soil in South Africa and that makes it difficult for conventional crops to absorb nutrients and water. However, the salinity phenomenon has hampered the quality of arable soil and promoted the use of low-quality water (brackish or saline water) due to the lack of fresh water. Saline water has proved to harm the quality of crops and soil as it encourages soil erosion and causes a decline in soil nutrients for plants. This problem has facilitated the consideration in cultivation of coastal plants as potential alternatives as they are naturally evolved salt-tolerant species that can survive in a saline environment. *Trachyandra divaricata* is an indigenous coastal plant native to the southern African region and distributed in this hot and dry Mediterranean type climate and in high saline conditions along the coast. *Trachyandra divaricata* flower buds were historically harvested in coastal regions by indigenous people and eaten raw or boiled as a nutritious inflorescent vegetable. However, there has been insufficient scientific documentation supporting biochemical properties and nutritional content of this plant besides the knowledge collected from indigenous people. Cultivating this plant in hydroponics could be efficient as it will utilise minimal resources and nutrients but cutting plants back to encourage new growth.

This aim of this study was to investigate the nutrient profile, antioxidants potential, and flower bud development of the hydroponically grown *T. divaricata* in reaction to different potassium dosages and levels of cutting heights to discover the appropriate protocol of the plant in a hydroponic system. Four identically constructed Nutrient Film Technique (NFT) systems arranged in a complete block design were used, with each system on a separate metal mesh steel table (900 mm x 1 250 x 25000 m) having 10 replicates of each treatment. Nutrifeed™ was used as a basic nutrient in all systems, however, different potassium concentrations (0.0216, 0.0144 and 0.0072 M of K_2SO_4) were used as treatments added in into each sump, while the system with Nutrifeed only considered as a control. The different treatments comprised of plants with three different cutting height levels and non- cut plants considered as control, 5 cm (minimal), 10 cm (moderate) and 15 cm (maximum cutting). The plant height was measured using a standard ruler and the number of flower buds counted manually during and after experiment. A laboratory scale was used to measure fresh and dry weight of the plant. SPAD-502 Konic-Minolta meter was used to measure the chlorophyll content of the plants. The phytochemicals and antioxidants were analysed using dried samples of flower buds by means of assays for total flavonols, total polyphenols, 2,2'-azinobis (3-

ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Similarly to nutrient contents and proximate analysis, the dried flower buds were assessed by means of assays for carbohydrates, crude proteins, crude fat content, Ash content, moisture content, Non fiber carbohydrates (NFC), Neutral detergent fibre (NDF), energy content, macro-elements ((sodium (Na), phosphorus (P), magnesium (Mn), potassium (K and nitrogen (N)) and micro nutrients (Magnesium (Mn), Zinc (Zn), Cupper (Cu) and Iron (Fe)). The plants irrigated with 0.0072 M of K_2SO_4 without pruning showed a substantial improvement in floral bud, Neutral detergent fiber, fresh and dry weight of the shoots and roots, ash, as well as height. Contrarily, calcium and chlorophyll content were equivalent among treatments, but the treatment 0.0114 M of $K_2SO_{4,}$ and 10 cm of pruning produced the maximum production of nitrogen, sodium, potassium and Zinc. The plants that were pruned by 10 cm and irrigated with 0.0072 M of K_2SO_4 had the maximum antioxidant value (Flavonol, DPPH and Polyphenols). The results suggest that *T. divaricata* is a viable leafy vegetable since a minimal concentration of K_2SO_4 (0.0072 M) and moderate cutting back the plants optimized its productivity in relation to the biomass characteristics, growth, antioxidant potential and nutritional content.

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Dedication

This work is dedicated to:

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Structure of the thesis

The thesis is drafted differently to the alternative of a traditional format for a thesis. The article-format thesis shows examples of published, co-published and/or "ready- for-publication" articles which were prepared during candidature and apply to the format prescribed by CPUT for 100% master's studies which complies with the following principles:

- The overriding principle of the thesis is that it remains an original contribution to the discipline or field by the candidate.
- Chapters containing the journal articles form a coherent and integrated body of work, which focused on a single project or set of related questions or propositions. All journal articles form part of the sustained thesis with a coherent theme.
- The study does not include work published prior to commencement of the candidature.
- The number of articles included depending on the content and length of each article and take full account of the university's requirements for the degree as well as the one article already published, or "ready-for-publication" expected for a master's degree in this discipline.
- The thesis should be examined in the normal way and according to the normal requirements as set out by the "Guidelines for Examiners of Dissertations and Theses" (using form HDC 1.7).

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1.1 General introduction

The rapid growth of population and over-consumption of vegetables has forced the reconsideration new dimensions in the cultivation of wild leafy vegetables which are important food crops, boasting significant composition of nutrients, vitamins, and minerals for humans (Anjur *et al.,* 2022). Wild food crops are still consumed in many African countries because of their history of supplementing diets with domestic vegetables (Sultanbawa & Sivakumar, 2022; Leal Fihlo *et al.,* 2022). In South Africa wild vegetables are mainly consumed in rural areas, however some urban residents have gained knowledge of these food crops, and they harvest them in the wild or buy them from street traders (Ntuli, 2019; Zulu *et al.,* 2022). *Trachyandra divaricata* is amongst the wild vegetables that is native to South Africa however its cultivation remains underutilized due to the insufficient literature on its cultivation and nutrition value (Ngxabi *et al.,* 2021). *Trachyandra divaricata* is also wildly distributed in other parts of the world especially Australia where it was found to be toxic to livestock in the early years (Finnie *et al.,* 2011). Contrary, the ethnobotanical studies reported that the flower buds of *T. divaricata* is the edible plant part which were foraged by Traditional peoples of the Khoisan tribes in ancient times (Van Wyk, 2011; De Vynck *et al.,* 2016).

The lack of scientific studies on cultivation, exploration of medicinal properties, and nutrient potential of this species have delayed contributions to boost food insecurity. Therefor further research on nutrient supplementation and pruning during cultivation as growth stimulants could have positive outcomes in measuring vegetative traits, phytochemicals, and antioxidants potential and nutritive potential of this species.

Micro-nutrients such as potassium plays a critical role on the growth and development of the plant, particular in high crop yields (Sardans & Peñuelas, 2021). Potassium have been reported to influence abundant vital enzymes involved in photosynthesis, sugar transport, protein synthesis, nitrogen metabolism as well as the water stability and meristematic tissue growth in plant growth (Marques, 2018). The presence of potassium in plants also effects the quality of harvest yield (Gelaye *et al.,* 2022). Therefore, these qualitative traits are imperative for leafy vegetables, both in household consumption and industrial use.

Likewise, flavour in various vegetables is influenced by numerous solids, organic acids and primary sugars, also volatile compounds whose biosynthesis and concentrations are determined by potassium application (Wang *et al.,* 2009). There are reports suggesting that enhancing the potassium nutrition status of the plant can consensus to abiotic stress tolerance by decreasing the reactive oxygen species (ROS) level of the plant (Pandey & Mahiwal, 2022). Moreover, it was reported that potassium application has improved plant growth, the total chlorophyl and photosynthetic efficiency, vitamin C, total phenols and flavonoids on *Brassica juncea* cultivars under different various irrigation regimes (Rani *et al.,* 2021).

Furthermore, the application of potassium being the important factor in plant growth, pruning is also another imperative practice in producing early fruit and quality yield in plants (Mawarni & Siahaan, 2022). In addition, it has been indicated that pruning the stem is an agronomic management practice which fabricates the productivity of crops under controlled environments (Nabi *et al.,* 2022). These claims have been supported by a study on cucumber (*Cucumis sativus* L.), where the shoot pruning, and potassium supplementation improved the production and yield (Hartatik & Hudah, 2021). Similarly, the production of watermelons, sweet potatoes and tomatoes are being influenced by shoot pruning (Hasanah *et al.,* 2021). The above claims provide sufficient evidence that for the improvement of nutritional status, plant growth, quality of the yield and the antioxidants of the plant, potassium supplementation and pruning practice is vital. The objective of this study therefore was to determine the suitable growth protocol for *T. divaricata* grown in hydroponic system by evaluating the influence of pruning and potassium concentrations on vegetative development, nutrient uptake, phytochemical content, and antioxidant capacity of flower buds. The findings of this study are anticipated to support future farmers, researchers and households who may be interested in exploring the benefits of the plant.

1.1 Research Problem

The shortage of freshwater supply, inadequate groundwater, and salinization of soil have become a major challenge worldwide (Singh *et al., 2014*). Salinization, which refers to the presence of salts in the soil or water, is a common challenge in half of the arable soil in South Africa and that makes it difficult for conventional crops to absorb nutrients and water (Hassani *et al.,* 2021). However, the salinity phenomenon has hampered the quality of arable soil and promoted the use of low-quality water due to the lack of freshwater (Hussain *et al.,* 2020). Saline water was also shown to harm the quality of crops and soil as it encourages soil erosion and causes a decline in soil nutrients for plants (Li *et al.,* 2019). Thus, that has facilitated the consideration of coastal plants as potential alternatives as they are naturally evolved salt-tolerant plants that can survive in a saline environment (Sogoni *et al.,* 2021).

Water and wind dispersal mechanisms allow *T. divaricata* to spread, as a result, the plant has been reported to have the highest spreading capacity and become dominant over other species when it grows in a specific area (Brown & Brooks, 2002). *Trachyandra divaricata* and *T. ciliata* are related being members of the same genus and share similar traits regarding their edible parts. Both species have edible flowers and were used as foodstuff by ancient people who lived along the coastal regions of southern Africa (De Vynck *et al.,* 2016). *Trachyandra* species are underutilized in South Africa because they are not seen as being significant and have not been updated in recent years (Ngxabi *et al.,* 2021). However, *T. divaricata* has shown to be an important species with huge potential to be grown commercially as a fresh green vegetable (Van Wyk, 2011).

1.2 Aim

This investigation aimed to explore the flower bud development, antioxidants potential, and nutrient profile on the growth of *T divaricata* in hydroponics under different potassium dosages (0.0216, 0.0144 and 0.0072 M) and cutting height heights (5 cm, 10 cm, 15 cm) to establish an appropriate growth protocol of the plant for hydroponic cultivation.

1.3 Hypothesis

It is hypothesized that different Potassium concentrations together with cutting heights will improve morphological traits (height, chlorophyll and number of plants produced per root) and size of the flower bud of *T divaricata* and have a positive effect on the antioxidant potential and nutrient content of the plant.

1.4 Core Objective

The core objective of this study is to analyse the nutrient content, antioxidants, vegetative growth, and flower bud development of *T. divaricata* in reaction to different potassium concentrations and levels of cutting under the hydroponic cultivation.

1.4.1 Specific objectives

<u>Objective one</u>

The proposed study will determine the morphological measurements (height, fresh weight, and root weight, the number of flower buds and weight of flower buds) of *T. divaricata* in response to different potassium concentrations and pruning levels using physical measurement tools such as centimetre ruler, weight scale, and visual observation to develop an optimal hydroponic protocol for the species.

<u>Objective two</u>

To ascertain how the chlorophyll content of *T. divaricata* responds to various potassium concentrations and cutting levels using the SPAD 502Plus method to develop a suitable hydroponic protocol.

<u>Objective three</u>

To determine the nutrient content of the flower bud of *T. divaricata* using the Inductively Coupled Plasma- Optical Emission Spectrometer standard procedure to establish variation in nutrient yields in response to different potassium concentrations and cutting levels.

<u>Objective four</u>

To determine the phytochemical and antioxidant capacity of *T. divaricata* in reaction to different potassium concentrations and cutting levels that produce high amounts of antioxidant and phytochemicals in hydroponics.

<u>Objective five</u>

To determine the optimal response of *T. divaricata* from the data gathered in sub-problem 1- to develop an optimal growth protocol for producing high-quality vegetable plants under the hydroponic system.

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Chapter 2: Trachyandra divaricata: A nutritious edible halophyte and potential vegetable crop response to different potassium concentrations in a hydroponic cultivation: A review

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2.1 Abstract

Trachyandra divaricata is a coastal plant that is native to the southern African region. The plant is distributed in a hot and dry Mediterranean climate and in high saline conditions along the coast. *Trachyandra divaricata* flower buds were historically harvested in coastal regions by indigenous people and eaten raw or boiled as a nutritious inflorescent vegetable. However, there is has been insufficient scientific papers supporting the wild coastal plants besides the knowledge collected from indigenous people. There are suggestions that some halophytes are doing well in saline conditions more when they are supplemented with potassium fertilizer, as it plays a vital role in the metabolic and physiological process of the plant. Even though halophytes can survive under harsh or saline conditions some of them have a special salt-tolerant requirement. The breeding efforts have been made to extract the salt tolerant genes to inoculate them onto salt sensitive plants such as conventional crops. Those efforts have not succeeded because of the complexity of salt tolerant genes. Therefore, the domestication of some wild edible species has added a significant over the past years value. Many halophytes are used in pharmaceutical industries because of their potential of secondary metabolites, whereas the edible perspective have been abandoned because the cultivation data have been lost or not documented. Coastal edible plants have thus shown the potential to be an alternative to conventional crops in several drought-stricken African countries where saline soils remain problematic. About 70% of available water is seawater, and this alone makes conventional crops susceptible to various plant stresses and damages. This study therefore emphasised the future cultivation potential of coastal species such as *T. divaricata* to support food production and alleviate food security in South Africa.

Key words: Asphodelaceae, coastal plants, gene manipulation, domestication, edibility and food security

Abbrevations: G - Gutters**,** PVC - polyvinyl chloride, LDPE - low-density polyethylene, K2SO4 - Potassium sulphate, EC - electronic conductivity, K - potassium

2.2 Introduction

Trachyandra divaricata is an indigenous herb that belongs to Asphodelaceae family, the plant features include horizontal stem-like rhizomes with fruit sets at the tip that subsequently develop into white and purple flowers, as well as numerous (fleshy) leaves that can spread along the surface up to 1 meter long (Brown & Bettink, 2019). The species is native to South Africa, even though it was later discovered in some areas of Australia in the early years because it can easily disperse through wind and water (Hussey *et al.,* 1997). Given that *T. divaricata* leaves were found poisonous to mammals and ruminants, the plant is abandoned by animals and there is little to no investigation on its edibility and antioxidant potential (Bulawa *et al.,* 2020; Huxtable *et al.,* 2005). Poole *et al.* (2015) have stated that *T. divaricata* is palatable and Van Wyk & Gericke (2000), also maintained that *T. divaricata* is amongst the coastal edible plants which inflorescences were used as a vegetable part by Khoekhoen people in ancient times. The species is also known for its medicinal anthraquinones properties (Van Wyk *et al.,* 2022).

Several studies have been conducted to investigate the coastal edible plants that were previously used by ancient people in coastal regions and to identify the areas where these plants were previously and currently growing due to the rapidly increasing population, food insecurity, and malnutrition which resulted in limited agricultural land and shortage of fresh water to cultivate conventional crops (Piperno, 2011). For the above-mentioned reasons it is important to consider salt-tolerant plants, given that they can as well be grown using seawater hydroponically as a substitute for conventional crops (Ventura *et al.,* 2015). According to Brigand *et al.* (2018), coastal edible plants have been shown to be healthy and contain a special salt which is important for human health.

Potassium is one of the imperative macro-nutrients that are essential for plant development as it improves the size of the plant. Supplementing *T. divaricata* with potassium could improve edible parts of the plant as it plays a pivotal role in many metabolic activities of plants such as maintaining cell turgidity, triggering most of the plant enzymes involved in plant metabolism, transportation of sugars and starches uptake of nitrogen and protein synthesis. However, potassium deficiency causes major injuries to plant growth, nutrition, and leads to reduced yield (Karley & White, 2009).

Thus, investigating the influence which various potassium concentrations and cutting levels will have on *T. divaricata* vegetative growth and nutrient uptake can contribute to developing optimal growth protocols for cultivating high quality and nutritious plants, perhaps with big, improved fruit sets that can be sold on a commercial scale readily.

2.3 The valuable knowledge and contribution of coastal edible plants

South Africa is one of the countries that have diverse and rich landscape vegetation in southern Africa (Van Wyk, 2011). The region has a rich diversity of edible and medicinal plants (Ruiters-Welcome, 2017). The dispersal of ancient people has caused a lack of sufficient information that could help to assemble inclusive inventory about coastal edible plants and that might be the challenge as the comprehensive publication was lastly published in 2010 "Botanical collectors and the collection" by Germishuizen (2010).

The available documented information is scattered throughout books, articles, journals, unpublished manuscripts, museums, and herbarium, which leads to edible plants being under-utilized (Ruiters-Welcome, 2017). According to Raimondo (2015), a clear distinctive informative ethnobiological inventory of all the food plants in South Africa and wild food, plants would be beneficial as it will assist with the viable knowledge of genetic diversity of crops, also with relative wild plants and conservation of indigenous plants. Recently, an ethnobotanical inventory of edible plants in South Africa documented by Welcome & Van Wyk, (2019) suggest that Apocynaceae (137) followed by Fabaceae (135 species) are the richest edible species including in the sub- Sahara and the entire world.

The importance of gathering the indigenous knowledge of these plants as basic research in botany, horticulture, and food science, as well as science is considered as the effective strategy for these plants to be adopted and being popular (Van Wyk, 2002 & 2011). Searchinger *et al.* (2014) has predicted that by the year 2050, the world population will reach 9 billion inhabitants, hence, it is highly alarming to re-consider salt-tolerant edible plants as alternative sources of plant-based nutrients. Therefore, this kind of information fortifies food safety and widening of the alternatives of food availability and may navigate a solution to rigorous predicted climate change (Raimondo, 2015).

2.4 Importance of coastal edible plants

Halophytes have adaptation mechanisms that make them withstand or even benefit from saline conditions, as their quality and yield can be improved (Panta *et al.,* 2014). Important attributes of halophytes include, their commercial application and their potentials, such as them being the raw material for vegetables and a source of oilseed with a high nutritional value, being used as a bio-fuel precursor and as secondary metabolites in pharmaceuticals, food additives, and nutraceuticals (Shannon & Grieve, 1998; Liu *et al.,* 2005; Fan *et al.,* 2013; Flowers & Muscolo, 2015; Buhmann *et al.,* 2015). Hence, coastal edible plants appear to be the potential alternative to conventional crops as they have several benefits and the tolerance over saline conditions with levels higher than 20 mM NaCl or half the strength of seawater (Flowers & Colmer, 2008). Di Baccio *et al.* (2004) and Sgherri *et al.* (2008) have as well pointed out that moderate salinity stress in edible halophytes enhances the plant-based compounds which show important properties for human health.

2.5 *Trachyandra divaricata*

The wild vegetable *Trachyandra divaricata*, also called "Tumbling Starlily" or "Kus Waaibossies" (Afrikaans), has edible blossoms (Tshayingwe *et al.,* 2023). The plant, which is a member of the Asphodelaceae family, is primarily found in sandy areas over most of South Africa (De Vynck *et al.,* 2016). It is a robust, tufted, rhizomatous perennial that produces horizontal stalks of white to purple flowers and thick leaves that can reach a length of 1 meter (De Vynck *et al.,* 2016). The plant has ± 80 cm long height and produces several inflorescences that turn into small white flowers which are attractive to honeybees.

2.5.1 The family Asphodelaceae and species *Trachyandra* species

The family Asphodelaceae is in the order of Asperagales and consists of 17 genera and about 800 species (Dahlgren *et al.,* 1985). The family is divided into two subfamilies namely, Asphodelaceae, Str. and Aloaceae. They are arranged according to their respective vegetative and reproductive characters (Bridson *et al.,* 2009). Asphodelaceae consists of 8 genera: *Asphodeline* Reichenbach, *Asphodelus* Linné, *Bulbine* Wolf, *Bulbinella* Kunth, *Eremurus* Marschall von Bieberstein, *Jodrellia* Baijnath, *Kniphofia* Moench *and Trachyandra* Kunth. Although the genus *Trachyandra* had been previously placed under the Anthericaceae family it is now classified under Asphodelaceae (Fabaceae & Thulin. 1989).

2.5.2 Distribution *of Trachyandra divaricata*

Trachyandra spp. is wildly distributed in tropic, subtopic, arid, sub-arid, and heath areas. This is evidenced in the investigation that was done on the “the analysis of plant communities on Rotten Island (Australia)” by Poole *et al.* (2014) and other related studies. *Trachyandra divaricata* is well distributed along coastal heath and dune areas (Johnson *et al.,* 2000; Motzkin

& Foster, 2002; Rippey & Hobbs, 2003). Although the species is widely distributed in different parts of Europe it originated from the African continent (Smith & Van, 1998).

2.5.3 Climate and growth conditions

According to Heyligers (1998) *T. divaricata* is mostly adaptable to Mediterranean climates with winter rains and hot and dry summers or long and mild summers especially along the coastal region of Africa, specifically tolerating around 29-35 °C in day temperatures.

Trachyandra divaricata is thus tolerant in a diverse environment and the ever-changing climate of South Africa as it can survive winter rains and harsh temperatures as reported.

2.5.4 Water requirements

Obermeyer (1962) has indicated that *T. divaricata* has coarse roots covered by a velvet-like pubescence of interlacing root hairs, which are very active in absorbing the water and nutrients. The species have potential to be cultivated hydroponically due to its occurrence along seashores and natural wetlands which suggest that plants can easily adapt to variety of environmental conditions including disturbed areas such as next to the roadside (Todd, 2016; New *et al.,* 2021).

2.6 Economic importance of *Trachyandra* species as a vegetable

Coastal species are slowly gaining recognition as biological factors such as freshwater; arable soil and climate change have become a major challenge worldwide, negatively impacting the life of conventional crops (Ngxabi *et al.,* 2021). For example, saline conditions have shown harm to the quality of the crops, resulting in insufficient nutrients such as proteins, fats and calories. Thus, a need for a new alternative crop that will meet human requirements is needed (Cheeseman, 2016). *Amaranths*, *Cleome* spp and other coastal species that were healthy are being cultivated on a small scale as food crops in other countries (Van den Heever & Coertze, 1997). In East Africa plants such as *Solanum nigrum, Bidens pilosa* and *Basella alba* are mixed in one dish to offer a variety of nutrients, taste of stable food and improve palatability (Marshall, 2001; Lyimo *et al.,* 2003). It is thus economically important to enhance the cultivation of neglected species to increase food production. Table 2.1 shows an extraction for the list of 1740 edible species that is provided in a thesis titled “Food plants for Southern Africa" which showcase the edible plants of southern Africa. It specifies the edible parts of the plant in each species but is lacking the nutritional value of each species as it is not fully documented.

Table 2.1: Distribution, conservation status and uses of different parts used of *Trachyandra* species.

Family	FSA distribution	Red List	Accepted names and synonyms.	Plant parts used: Uses
Asphodelaceae	Indigenous	Least Concerned	*Trachyandra ciliata* (L.f.) Kunth	leaves: vegetable flowers: vegetable
Asphodelaceae	indigenous; endemic	Least Concerned	*Trachyandra divaricata* (Jacq.) Kunth	flowers: vegetable
Asphodelaceae	Indigenous; endemic	Least Concerned	*Trachyandra falcata* (L.f.) Kunth	flowers: vegetable
Asphodelaceae	indigenous; endemic	Least Concerned	*Trachyandra hirsuta* (Thunb.) Kunth	flowers: vegetable
Asphodelaceae	indigenous; endemic	Least Concerned	*Trachyandra hispida* (L.) Kunth	whole plant: vegetable flowers: vegetable
Asphodelaceae	Indigenous	Least Concerned	*Trachyandra revoluta* (L.) Kunth	flowers: vegetable

Adapted from: Food Plants of Southern Africa (Ruiters-Welcome 2019).

The Asphodelaceae family has many different species that are edible. Table 2.1this table reveals their botanical names and conservation status as part of encouraging the utilisation and exploitation of the family. Genius *Trachyandra* has about 6 species that are edible and least concerned because the plant seeds spread so fast through wind and water. There is little related kind of literature that has been done on available coastal edible plants in southern Africa, ever since Africa was colonized, ancient people were forced to vacate the urban areas and introduced to conventional crops (De Vynck *et al.,* 2016). It clearly shows the gap between the researchers and ancient people in terms of knowledge transfer about these plants. Therefore, Deacon, (1995) has mentioned that it will be difficult to identify the edible and toxic parts in these plants, in hotpots areas without the proper knowledge of these plants. The richness of the Cape coast particularly these underground storage organs (USO, s) marine and terrestrials-based protein has been hypothesised as the main components that have helped the ancient people during glacial phases, as they were the only inexpensive source of food available in coastal regions (Maraen, 2010). Unfortunately, in other African countries that may have been resource-poor, these food plants could not be found. In the meantime, no thorough investigations have been achieved in conjunction with the nutritional value of the wild edible species (Ruiters-Welcome, 2017 & Maraen, 2010). Singles *et al.* (2015) has discovered that Underground Storage organs that has maximum values range from (600 Kg/ha to 5,000 Kg/ha) in two different biomes in South Africa. Also, species that have a relatively large (10-100g) starch-rich and lower fibre food and are easily harvested have been discovered (Parkington, 1977; Deacon, 1993; Singels *et al.,* 2015). Most of these food plants are considered as a snack and consumed raw or boiled. In addition, these plants which are available during late winter and early spring include fruit plants such as *Carissa, Diospyros, Olea, Sarsia* as well as leaf crops such as *Trachyandra* species (Youngblood, 2004; Dominy *et al.,* 2008; De Vynck, 2011). No reports were found on the economic importance of *Trachyandra* species apart from that it is edible. *Trachyandra divaricata* develops fruits from mid-winter to early spring as it is the best time to harvest the flowers before fruit setting (Obermeyer 1962; Lewis *et al.,* 1972; Goldblatt & Manning, 1998). The flowers remain popular in selected gourmet restaurants for its salty crispy flavour and nutritious flowers in salads (Van Wyk & Gericke, 2000). The flowers are rich in nutrients and stewed as vegetables (Leipoldt, 1978; Rood, 2008; Luo *et al.,* 2019; Ngxabi *et al.,* 2021). It is documented that *T. divaricata* was consumed by the ancient Khoisan people of the Western Cape, South Africa, hence, the plant could be used to enhance the culinary values of food. Also, public sensitisation is needed to propagate its values to promote commercial cultivation as large-scale growers would require valuable information about the species for advertisement and commercial purpose.

Figure 2.6.1: shows a dish of boiled *Trachyandra divaricata* flower bud with various wild plants and bottle stored flower buds (Rusch, 2022)

2.7 Hydroponics as an option for cultivation

Hydroponics is a system that supplies the correct combination of nutrients required by a plant (Mino *et al.,* 2021). According to Searchinger *et al.,* (2014), the world population is increasing rapidly, and it is estimated that by 2050 the population will reach 9 billion inhabitants. Given this increase in the population growth rate, arable land becomes an issue as a large part of the land is used in urban expansion, while rural areas are being urbanized (Heredia *et al.,* 2014). Thus, there is a need for new strategies to produce food using limited resources efficiently to cater to the unceasing population growth. It is estimated by Martinez-Beltran & Manzur (2005) that about 70% increase in food production will be needed in the future. As a result, hydroponics is one of the effective methods of growing sustainably food throughout the year and should therefore be considered in cultivating *T. divaricata* to meet the demands of the ever-increasing population (Alshrouf, 2017).

A completely closed hydroponic system allows the environment to automatically control plant requirements such as temperature, light, plant nutrients, and water (Tablada, & Kosorić, 2022). That way an improvement in plants can be doubled compared to traditional field cultivation, specifically in terms of quality and yield. Also, unlike in the field whereby plants need to develop long roots to search nutrients and where more space in between plants is required, some plants in hydroponics can grow together to use the space efficiently, because there is no competition or search for nutrients and water, (Khan *et al.,* 2020). Given the above potentials, hydroponics has since then gained more recognition around the world and largely in developed countries, and the increasing demand for the system, hydroponics is innovative, eco-friendly, sustainable reliable, and flexible (Gwynn-Jones *et al.,* 2018; Li *et al.,* 2018). Fully integration of hydroponics in developing countries would provide various important benefits that enable the production of high-quality food and also, finding the appropriate hydroponic growth protocol of *T. divaricata* will be beneficiary as it will be available through the year and improve food security.

2.7.1 Growth substrates for hydroponic cultivation of Trachyandra

Hydroponics is also known as soilless culture because the system allows plants to grow without the presence of soil (Dan, 2007). In hydroponics, plants are either grown using a growing medium or without it as the growing medium provides support to the plant. Most of the growing mediums used in hydroponics are inert which makes the roots easily penetrate and reach a nutrient solution (Shamanshop, 2007). Although many growing substrates (coco coir, sand, vermiculite, perlite, bark, and clay stones) are commonly used in hydroponics depending on the type of plant and hydroponic system this study will focus on silica sand as it will be used. Silica sand is a white and glowed small particle from weathering rock and sieved to a length fraction of 125-250 btm, wiped clean with HNO3 to emerge as a neutral H (Osseni *et al.,* 2019). Small particles make the medium to be unable to hold moisture and normally silica sand is mixed with a lighter medium, such as vermiculite, perlite, and coco coir to enhance its water holding capacity as the silica sand has a bit of weight (Ventura & Sagi, 2013). Silica or washed river sand would be a recommendation for cultivating *T. divaricata* to simulate its natural growing medium comparable to sand dunes, however further hydroponic experimental work is required to advance a commercial success of this species.

2.7.2 The role of nutrients (potassium) in hydroponics

Potassium (K) is an essential nutrient that is required in large quantities in plant growth and development. In other words, potassium triggers various physiological and metabolic activities such as photosynthesis, protein synthesis, and resistance to diseases, pests, and so forth (Rehm & Schmitt, 2002). An adequate supply of potassium to the plants helps to maintain all the plant activities and formation of photosynthates.

Potassium also plays a pivotal role in the transportation of water through the xylem. In that order with the phloem transport systems, the role of K in xylem transport is often in conjunction with specific enzymes and plant growth hormones (Lu *et al.,* 2019). However, when there is a lack of K in a plant, some growth activities do not function to their full capacities, given that translocation of nitrates, phosphates, calcium (Ca), magnesium (Mg), and amino acids are depressed (Wang *et al.,* 2020). Therefore, a crucial component of plant nutrition and physiology is potassium (Wakeel & Ishfaq, 2022). It increases the activity of over 60 enzymes, stomatal regulation, transfer of photosynthates from the source to sink organs, detoxification of reactive oxygen species (ROS) and photosynthesis. It is referred to as a "quality element" since its sufficient supply enhances agricultural commodities' quality features and production (Kumar *et al.,* 2006; Pettigrew 2008). Additionally, it shields the plant from the damaging effects of biotic and abiotic stresses, and it enhances nitrogen uptake, which decreases environmental footprints (Wakeel & Ishfaq, 2022). Evaluating concentrations of Potassium in cultivation of *T. divaricata* would support a successful cultivation protocol for this species.

2.7.3 Electrical conductivity, and pH in plant growth and development in hydroponics

Nutrients in hydroculture are applied in liquid and solid forms. The salts dissolve in water and begin to break down into ions (Mattson & Lieth, 2019). For example, potassium changes to potash ions when dissolved. These ions carry electrical conductivity due to their negative and positive charges (Cornish, 1992). The role of electrical conductivity (EC) is to indicate the concentration of nutrients in a solution. A higher level of EC indicates higher salt concentration, while a lower level of EC indicates lower salt concentration (Cometti *et al.,* 2013). The pH is used to determine the acidity or alkalinity of the solution (Domingues *et al.*, 2012). Hence electrical conductivity and pH are critical values that need to be considered or monitored in a hydroponic system as plant growth and development depend on these values.

Plants grow faster in hydroponics because of different factors such as an automatic supply of optimum oxygen to the roots, frequently maintained EC and pH levels, and nutrient supply to the plant (Shamanshop *et al.,* 2007). Every plant species has a specific pH and EC which is suitable for its growth. For example, *T. divaricata* usually grows well in water with marginal alkaline pH (~7.5) with relatively high EC (160–180 mS/ m) and low dissolved oxygen percentage saturation levels (~40) (Malan *et al.,* 2013). However, these recommendations need to be substantiated to advance the commercial potential of the species. Different coastal plants have their specific amount nutrients that needs to be met for a proper growth, hence, the correct combination of nutrients with EC and pH being maintained correctly for the production of *T. divaricata* is essential as it will assist in finding correct procedure to grow the plant on hydroponics.

2.8 Discussion

The uncertainties of abiotic and biotic factors are highly challenging to reach the necessary goal of food accessibility to most households because their significant negative influence on the yield of crucial crops like wheat, rice, and maize around the world, including South Africa (Acquaah, 2007; Corwin, 2021). Moreover, early findings suggest that the strategy of adopting wild plants that have edible potential and tolerant ability to cope with ever climatic variations can be vital to enhance food production and add a significant value to the economies of developing countries (Debez *et al.,* 2010; Ventura & Sagi, 2013). In addition, the use of wild salt-tolerant plants (halophytes), which have considerable commercial values as green vegetables, feed crops, and medicinal precursors, is one main reason of consideration as commercial crops due to their climatic adaptations (Debez *et al.,* 2010; Ventura & Sagi, 2013). As a result, there remains an increased interest in the development of edible halophytes for use in agricultural production to address global issues with food and nutrient shortages (Jacobsen *et al.,* 2013). Therefore, with the literature provided in this review, the scientific information suggests the necessity to facilitate the use of wild edible plants to enhance their domestication for adoption as future horticulture crops in hydroponics.

Based on dietary benefits of *T. divaricata* and medicinal potential of its edible flowers (Bulawa *et al.,* 2022; Tshayingwe *et al.,* 2023), the species could be introduced into the market as an easy to grow and probably most tolerant to a variety of South African climatic regions and soils. Furthermore, embracing alternative crops that can provide essential nutrients, it may be possible to increase dietary diversity as food-based approach to improve the biological value of diets (Jimoh *et al.,* 2020). Therefore, promoting the cultivation and consumption of halophytes such as *T. divaricata* will expand dietary options and encourage additional studies to be done on other species that have previously been shown to be valuable but lack scientific evidence regarding important traits (Liu *et al.*, 2005; Fan *et al.,* 2013; Buhmann *et al.,* 2015).

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Chapter 3: Vegetative growth of *Trachyandra divaricata* in response to various cutting heights and different potassium concentrations in hydroponics

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3.1 Abstract

The constant decline in agricultural production calls for intervention to rescue food production that will create food security, and this includes strategies to use halophytic plants to substitute affected crops as they can tolerate drought and grow in saline areas. The aim of this study was to determine the effect of different potassium concentrations and pruning responses on the growth of *T. divaricata* cultivated in a hydroponic system. The experiment was carried out in four similar built nutrient film (NFT) systems, with a separate metal mesh steel table (2.5 m length) for each system. NUTRIFEED™ fertilizer served as a basic hydroponic feed in all systems while different potassium dosages (0.0216, 0.0144 and 0.0072 M) were added into each sump, and the nutrient solution without the addition of K_2SO_4 was considered as control. The plants were also cut back to different height levels (5 cm, 10 cm, and 15 cm) and the no cut plants were considered as a control. The experiment was set up in a greenhouse for 13 weeks during which different growth parameters such as leaf length, leaf number, number of flower buds, fresh and dry weight were measured. The results of this experimental trial indicated that a combination of low potassium concentration ($\leq$0.0072 M) with minimal cutting back (15 cm) had a significant effect on *T. divaricata*. However, high concentrations (0.0144 and 0.0216 M) of K_2SO_4 with low cutting back (10 and 5 cm) had negative effects on the growth and bud development. These findings will assist horticulturists, plant enthusiasts and commercial growers to develop a suitable growth protocol for the species.

Keywords: Asphodelaceae; flower buds; growth protocol; halophytes; Khoi-san people; unpruned plants

Abbreviations: G - Gutters, PVC - polyvinyl chloride, LDPE - low-density polyethylene, K2SO4 - Potassium sulphate, EC - electronic conductivity, K - potassium

3.2 Introduction

The agriculture industry in Southern African countries has been experiencing uncertainties due to biotic and abiotic factors (Ngxabi *et al*. 2021). These factors include climate variation, lack of fresh water and arable land which make it difficult to keeping up with the growing human population to maintain food and nutrition security (Ngxabi *et al.,* 2021). Due to the shortage of summer rainfall and soil deterioration, plant growth become stunted during the growing season. For instance, for the past few years, there have been significant water shortages in South Africa's Western Cape Province. Furthermore, due to the continuation of shortage, it is anticipated that this province will struggle to meet its agricultural demands in a few years (WWF, 2017). This phenomenon stimulates the need to develop more innovative techniques to enhance sustainable crop production (Ventura & Sagi, 2013).

Drought levels need to be evaluated with the development of new food production methods (Ventura *et al.,* 2011). Several writers, notably Ventura *et al.* (2011) and Klados & Tzortzakis (2014), have argued that to achieve long- term food and nutrition security, the cultivation of native, salt-tolerant, and drought-tolerant halophytic species could bring about changes, however, there are very few cultivation protocols known for most species. This has brought about an imperative need to employ different approaches in developing suitable cultivation methods for underutilized wild edible species.

There is evidence that indigenous edibles species such as *T. divaricata, T. ciliata*, and *T. falcata* were utilized as food by Khoi-San people who lived around the South African Cape coast before colonization (De Vynck *et al.,* 2016). The inflorescence of *Trachyandra* species have become popular a gourmet dish as it can be steamed or boiled or used as in a vegetable stew (De Vynck *et al.,* 2016). *Trachyandra* species (family Asphodelaceae) are distributed all over southern Africa, however, most of them are confined and unique to the south-western Cape's winter rainfall region, with just one occurring as far north as Ethiopia (Smith & Van Wyk, 1998). Most of these species occur on coastal dunes or seashores, however they can also survive under wetland conditions (Todd, 2016).

Trachyandra is a salt-tolerant food crop that grow naturally in very saline soils along the coast (Smith & Van Wyk, 1998) which provides an opportunity to be cultivated a possible commercialized salt-tolerant food crop. As a result, this would be beneficial in assuming higher agricultural productivity while also reducing the use for freshwater for irrigation during challenging water shortages. *T. divaricata* has rhizomes with coarse roots covered by a felt-like pubescence of interlacing root hairs, which are very active in absorbing the water and nutrients and thus make it a suitable specimen for hydroponic culture, however little is known about growth characteristics and suitable cultivation protocols for the species. Understanding the plant's responses to potassium and pruning, is critical for improving its performance and broadening its marketability. Therefore, this study investigated the vegetative growth of *T. divaricata* under different potassium concentrations and pruning height in hydroponic system.

3.3 Materials and Methods

3.3.1 Greenhouse Experiment

The study was carried out over a duration of 4 months at the Cape Peninsula University of Technology's greenhouse site in Bellville, Cape Town, South Africa (33^{0} 55'45.53S, 18^{0} 38' 31. 16E). The control of the inside environment of the greenhouse was ensured by the technologies used within the structure.

3.3.2 Plant Preparation

About 300 small plants were harvested at the back of Horticulture and Landscape design building on the Bellville campus of the Cape Peninsula University of Technology. Excessive plants were divided to certify the number of plant pieces (200) required for the experiment. Plant divisions were planted individually into black plastic pots (12.5 cm (height) x 12.5 cm (width) x 12.5 cm (depth) in a medium of silica sand.

The plants were placed in an environmental controlled propagation greenhouse with an automated drip irrigation system from 7 am to 7 pm daily, where they were watered for 30 seconds every hour. The plants were treated with NUTRIFEED (13:0:45) solution (100 ml per plant) once in a week as a basic nutrient. After 3 weeks, the pots were moved to the research greenhouse for acclimatization for a week. The pots were then transferred to the research greenhouse for a week for acclimatization. For the experiment, 160 healthy, uniform plants were selected from the batch, each measuring 13-20 cm in height with 10-15 leaves.

3.3.3 Experimental design

The experiment consisted of four hydroponic systems arranged in a complete block design having 10 replicates of each treatment (Table 3.3.1**)**. Each system had used a low-density polyethylene (LDPE) reservoir with a capacity of 70 litres, four polyvinyl chloride (PVC) square cutters (130 mm x 70 mm x 2 500 mm), forty 12.5 x 12.5 cm plastic pots, a 2 000 L/h submersible water pump with a 2.5 m head capacity and ten meters of 20 mm LDPE irrigation piping.

This experiment was designed with each hydroponic system being placed on top of a single rectangular steel mesh table. The four systems were marked from B1 to B4. A steel mesh table served as a flat surface for twenty white gutters while a black plastic pipe used to deliver water from the individual reservoirs. Reservoirs were kept filled with a 50 L (water and a specific amount of Potassium concentrations (0, 0.0072, 0.0144 and 0.0216 M) solution level beneath each table. Each reservoir contained a submersible water pump, which recirculated the aqueous nutrient solution to each gutter to keep the plant roots submerged with the solution. Excess waters were returned to the reservoirs. All the gutters carried pots filled with silica sand (labelled with numbers and arranged randomly). Digital timers were used to automatically control each separate system to fertigate plants with various amounts of nutrient solution (Potassium sulphate) with 0.0144 M of nutrified being used as the basic nutrients for all systems. All aqueous solutions used in this experiment was kept constant between 6.0 to 7.0 pH level and the electrical conductivity (EC) were measured weekly with a handheld digital EC meter (Hanna Instruments®™HI 98312). A calibrated handheld digital pH meter (Eurotech®™ pH 2 pen) was used to check the pH levels of every water reservoir. Potassium hydroxide was used for increasing pH and hydrochloric acid to lower pH. The EC of the aqueous nutrient solution in the reservoirs was decreased by adding reverse osmosis water, whereas the EC was increased by adding Hoagland solution to the aqueous nutrient solution in the reservoirs.

Figure 3.3.3: The experimental layout showing replicates (n = 10) in the Nutrient Filter Technique hydroponic system (Bulawa, 2021).

3.3.4 Cutting and fertigation treatments

See Table 3.3.1 below

Table 3.3.1: Treatments arranged in a randomised block design in every gutter on four hydroponic systems with different levels of cutting heights and potassium concentrations.

Treatment	Gutter one	Gutter two	Gutter three	Gutter four
1	5 cm + 0 M	10 cm + 0 M	15 cm + 0 M	No cut + 0 M
2	5 cm + 0.0072 M	10 cm + 0.0072 M	15 cm + 0.0072 M	No cut + 0.0072 M
3	5 cm + 0.0144 M	10 cm + 0.0114 M	15 cm + 0.0144 M	No cut + 0.0144 M
4	5 cm + 0.0216 M	10 cm+ 0.0216 M	15 cm+ 0.0216 M	No cut+ 0.0216 M

Note: Each gutter of each Treatment contained ten plants, one plant per pot, for a total of 40 plants in each hydroponic system and 200 plants during the entire experiment. Plant heights were cut back (cm), and Potassium nutrient concentrations (M) applied. Cutting heights of 5 cm were consider light pruning, 10 cm moderate pruning, and 15 cm heavy pruning.

3.3.5 Treatment preparation

Only silica sand (100%) was used as soilless growth substrate for cultivation in the experiment. The sand was thoroughly washed before planting to remove unwanted substances that can affect plant growth. All four hydroponic systems had the same water pumps installed to achieve fertigation regimes. The same amount of aqueous nutrient solution 450 mL for 2 minutes was used for each outlet. To measure the amount, an empty bucket was placed beneath the gutter outlet for 2 minutes. The liquid collected in the bucket was decanted and measured using a 500 mL Erlenmeyer flask. This was done to ensure uniformity. Treatment 0 M potassium and unpruned plant height were selected as the control variable, and it only received containing Nutrifeed™.

3.4 Data collection

3.4.1 Plant height

Plant height was measured prior to transplanting into the hydroponic systems and again during the experiment until post-harvest. Plant height was measured in cm using a standard ruler.

3.4.2 Plant weight

An electronic balance (Model PS 750.R2, manufactured by RADWAG®, Poland) with 0.001 g readability was used to measure the weight of the plant prior to transplanting into the hydroponic system and at the end of experiment excluding the flower buds as the plants produced them at different times as they were harvested separately.

3.4.3 Number of flower buds

Flower buds were separated from the plant as they were produced at different times, and number of flower buds were only counted manually and recorded during and at the end of the experiment.

3.4.4 Fresh weight of the flower buds

The flower buds together with its stem-like stalk were harvested separately from the plant during the production stage (8 week) and after the experiment as flower buds were not growing and maturing at the same time as mentioned on 3.3.3. The flower buds were weighed using an electronic balance and recorded.

3.4.5 Dry weight

The plants were firstly washed after being harvested with tap water until the growing substrate particles were completely removed from the roots and allowed to dry for 10 mins to be placed in a papery brown bag and dried at 30-31℃ in a pushed convention oven (Daihan Labtech LDO-15OF) until the samples became crispy dry. The dry material was weighed and recorded.

3.4.6 Statistical analyses

The collected data from the growth parameters (height, flower buds, plant, and root weight) was analysed using a two-way analysis method of variance (MINITAB) to certify the accuracy and the testing of significance levels and to be able to make correct conclusions and recommendations. Turkey's least significant difference was used to compare the significant differences between treatment means at $p \leq 0.05$. A computer software program, MINITAB was used for statistical analysis of all the calculations.

3.5 Results

3.5.1 The height of the plant

The findings of this experimental trial showed that different potassium dosages and levels of pruning had a significant ($P \leq 0.05$) influence on the plant height (Table 3.5.1). The 0.0072 M K_2SO_4 concentration had the highest values from second and fourth month as shown in the (Table 3.5.1a) compared to the other treatments including control. However, the plant leaves were pruned back in third month from the base to their respective basic leaf length level (5 cm, 10 cm and 15 cm). The plants with no cut had the highest values followed by moderate pruning (15 cm) almost all the treatments. The combination of Potassium and cutting back showed significance ($P \leq 0.05$). The maximum height mean was recorded in the unpruned treatments supplemented with 0.0072 M and 0 mL K_2SO_4 as both treatments were on the same range compared to all the treatments. The lowest values were recorded in plants treated with 0.0216 M K_2SO_4 at all the cutting back levels (Table 3.5.1).

Table 3.5.1: Plant height of *Trachyandra divaricata* in response to various potassium concentrations and various cutting back heights on monthly intervals.

K_2SO_4	Cutting	First Month	Second Month	Third Month	Fourth Month
0 M	No cut	35.02 ± 0.43a	45.25 ± 0.57a	59.29 ± 1.18a	64.14 ± 1.65a
	5 cm	8.87 ± 0.38e	18.19 ± 0.70a	14.28 ±0.63ghi	15.29 ± 2.24def
	10 cm	13.35 ± 0.45d	23.10±1.00a	19.48 ± 0.93cde	31.22 ± 0.77efg
	15 cm	18.15 ± 0.07bc	30.36 ± 0.95a	23.76 ± 0.30c	34.23 ± 1.40cd
0.0072 M	No cut	33.99 ± 0.42a	44.01± 1.35a	56.67 ± 2.13a	78.91 ± 1.85a
	5 cm	9.11 ± 0.63e	18.97 ± 1.26a	15.34 ±0.28fgh	37.00 ± 1.14de
	10 cm	13.76 ± 0.51d	29.34 ± 0.71a	20.87 ±0.72cde	44.27 ±1.68cd
	15 cm	17.63 ± 0.42c	29.97± 1.31a	23.06±0.44cd	49.43 ±1.57bc
0.0144 M	No cut	35.49± 0.90a	40.35 ± 1.51a	48.08 ± 1.67b	55.57 ± 2.28b
	5 cm	10.30 ± 0.67e	17.98 ± 0.87a	13.64 ± 0.46hi	25.50 ± 3.59fgh
	10 cm	14.17 ± 0.61d	25.52 ± 0.93a	16.84 ± 0.39ef	27.71 ± 0.85efg
	15 cm	20.42 ± 0.58b	288 ± 260a	18.40 ± 0.13cde	27.52 ± 4.74efg
0.0216 M	No cut	33.85 ± 043a	40.09 ± 0.75a	48.69 ± 1.35b	49.19 ± 2.43bc
	5 cm	9.70 ± 0.24e	17.37 ± 0.79a	11.65 ± 0.29i	13.11 ± 2.80i
	10 cm	13.78 ± 0.37d	21.50±0.92a	15.05 ± 0.59fgh	15.29 ± 2.24hi
	15 cm	18.33 ± 0.66bc	26.82 ± 0.60a	18.81 ±0.38def	21.51 ± 0 .63ghi
Two-way ANOVA Minitab					
K_2SO_4		52.2*	124071n	1091.5*	17272*
Cutting back		14431.5*	140579n	39059.7*	34194*
K_2SO_4*cutting back		26.9ns	379521n	376.3*	1582*

<u>Note</u>: The mean values ± standard Error are displayed in columns. According to Fisher's Least Squares calculations, the mean values denoted by various letters are substantially different at P ≤0.05 (*) and ns= not significant. The plants were pruned back to their basic height in the beginning of second month.

Table 3.5.2: The influence of potassium and levels of pruning on fresh and dry weight of the roots and shoots of *T. divaricata* that is grown on hydroponics.

K_2SO_4	Pruning	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root Dry Weight
0 M	NO CUT	93.48 ±19.3bc	18.27 ± 3.75ab	51.30 ± 2.39bc	27.89 ± 1.31abcd
	5 cm	60.4±9.33bcde	6.72 ±1.76d	30.98 ± 1.20 de	21.27 ± 1.72def
	10 cm	69.8 ± 12.5cd	19.19 ±4.83ab	30.86 ± 1.13 de	22.80 ± 1.78cde
	15 cm	154.3 ± 7.79a	19.38 ± 3.76a	46.06 ± 1.41cd	34.08 ± 2.25abc
0.0072 M	No cut	114.5 ± 12.6ab	16.41 ±2.34b	63.23 ± 2.68a	38.17 ± 3.81a
	5 cm	51.65±4.96d	8.65 ± 2.14 cd	30.98 ± 1.20ef	25.60 ± 0.84bcd
	10 cm	91.0 ±13.8bc	8.76 ± 2.51cd	21.22 ± 1.26e	34.86 ± 1.33def
	15 cm	89.5 ±16.0bc	15.80 ± 3.22b	38.09 ± 2.31de	26.84 ± 2.85abcd
0.0144 M	No cut	97.9 ± 17.4b	11.13± 2.25bc	51.77 ± 3.05 bc	35.78 ± 3.76ab
	5 cm	25.80 ±5.56f	6.79 ± 2.38d	59.92 ± 3.30ab	21.27 ± 1.72bcd
	10 cm	45.0±12.4e	9.28 ± 1.92cd	30.50 ± 3.59ef	17.34 ± 2.60def
	15 cm	50.0 ± 15.6de	4.95 ± 2.05e	22.93 ± 2.00fg	12.17 ± 2.83efg
0.0216 M	No cut	21.53± 4.10f	8.75 ± 1.11cd	32.88 ± 1.33ef	10.91 ± 1.67fg
	5 cm	5.91 ± 2.26g	0.42 ±0.42e	14.32 ± 0. 34g	2.62 ± 0.89g
	10 cm	3.81 ± 1.82g	2.49 ± 1.28e	19.19 ± 0.58g	3.45 ± 1.57 g
	15 cm	4.47 ± 3.67g	1.64 ± 0.57e	24.49 ± 0.95fg	1.95 ± 1.41g
			Two-way ANOVA Minitab		
K_2SO_4		18136*	3541*	11307*	13755*
Cutting back		80109*	1276*	8830*	3393*
K_2SO_4*Cutting back		23891*	1017*	9949*	2825*

Note: The mean values ± standard Error are displayed in columns. According to Fisher's Least Squares calculations, the mean values denoted by various letters are substantially differ at P ≤0.05 (*) and ns= not significant.

3.5.2 Plant fresh weight

The outcomes gathered from the present experiment showed that different potassium concentrations significantly influenced fresh weight of the plant at $\alpha = 0.05$ (table 3.5.2). Also, the pruning levels shows significant difference on the fresh weight of the plant. In contrast, the interaction of Potassium concentrations and cutting back levels showed to have a significant difference on the plant fresh weight. 15 cm plants treated with 0 ml K_2SO_4 recorded the highest mean fresh weight (154.3 g) followed by 0.0072 M + no cut, whereas a combination of 0.0216 M K_2SO_4 with all cutting back levels and unpruned recorded the least values than any other treatment (Table 3.2).

3.5.3 Plant dry weight

The results obtained from the present experimental trial indicated that different potassium concentrations had a significantly impacted ($P \leq 0.05$) on plant dry weight. Compared to pruning levels shows significant effect on the dry weight of the plant (Table 3.2). The results also indicate that there was an interaction in both factors as they show significant difference on the plant dry weight. Control variable with 15 cm cut recorded the highest mean value plant dry weight (19.38 g) while 0.0216 M in almost all the cutting back levels recorded lowest mean value expect the no cut (Table 3.2).

3.5.4 The fresh weight of the root

Table 3.5.1b shows the significant ($P \leq 0.05$) different of various potassium concentrations on the plant fresh weight. In supplemental, cutting back levels had the same significant difference on the root fresh weigh (Table 3.2). However, the results show that there was no interaction between potassium concentrations and cutting back levels on the root fresh weight. The root fresh weight obtained the highest mean value of (63.23 ± 2.68) at 0. 0072 M + No cut treatment, whereas 0.0144 M + 5 cm and 0.0216 + 10 cm recorded the lowest values (Table 3.2).

3.5.5 The dry weight of the root

The results of the current trial experiment presented on the table (3.5.2) shows the significant ($P \leq 0.05$) difference of the different potassium concentrations on the root dry weight. Also, different cutting back levels indicated significant difference on the plant dry weight. The combination of both variables proved to be significantly different on the plant dry weight. Treatment 0.0072 M + unpruned recorded the highest values, while 0.0216 M + 5 cm as well as 0.0216 M + 10 cm had the smallest mean values (Table 3.2).

Table 3.5.3: The influence of different potassium and levels of cutting back on flower bud number, plant height and the fresh and dry weight of the flower buds of hydroponically grown *Trachyandra divaricata*.

K_2SO_4	Pruning	No. of flower bud	Fresh flower Buds (g)	Dry Flower Buds (g)
0 M	No cut	5.30 ± 0.63 ab	16.14 ± 2.34 bc	13.59 ± 2.16 b
	5 cm	2.80 ± 0.29 bcd	8.01 ± 2.31 e	6.19 ± 2.11 d
	10 cm	4.60 ± 0.69 bc	9.76 ± 2.65 e	8.30 ± 2.43 c
	15 cm	3.30 ± 0.58 bcd	15.83 ± 3.23 c	13.71 ± 2.49 b
0.0072 M	No cut	7.80 ± 0.49 a	22.95 ± 3.44 a	20.06 ± 3.14 a
	5 cm	3.60 ± 0.37 bcd	5.83 ± 1.16 f	4.11 ± 0.94 de
	10 cm	4.70 ± 0.78 bc	17.75 ± 4.42 b	14.31 ± 3.83 b
	15 cm	7.60 ± 0.65 a	22.09 ± 4.01 a	19.54 ± 3.65 a
0.0144 M	No cut	4.00 ± 0.52 bcd	11.06 ± 2.22 de	7.93 ± 1.85 c
	5 cm	1.80 ± 0.47 def	5.54 ± 2.32 f	4.16 ± 1.86 de
	10 cm	2.70 ± 0.67 cde	9.28 ± 1.92 e	7.35 ± 1.66 c
	15 cm	1.00 ± 0.45 fg	4.95 ± 2.05 f	3.64 ± 1.54 e
0.0216 M	No cut	2.70 ± 0.34 cde	8.75 ± 1.11 e	6.04 ± 0.85 d
	5 cm	0.20 ± 0.13 g	0.42 ± 0.42g	0.23 ± 0.23 f
	10 cm	1.30 ± 0.54 g	2.49 ± 1.28 g	1.45 ± 0.45 f
	15 cm	1.10 ± 0.28 g	1.64 ± 0.5 g	0.86 ± 0.30 f
			Two-way ANOVA Minitab	
K_2SO_4		483.67*	4275*	3495*
Pruning		158.22*	1903*	1411*
K_2SO_4 × Pruning		95.61 *	1156*	11018*

Note: Tukey's LSD was used to rank the means along the column with a 0.05 significance threshold. Means that do not share a letter are very dissimilar. *: significant at $p \leq 0.05$.

3.5.6 Number of flower buds

The findings of this investigation showed that the number of flower buds was significantly (P≤ 0.05) influenced by different potassium concentrations, pruning levels, as well as their interaction (Table 3.3). The highest height mean values for flower buds were recorded in minimum treatment (0.0072 M). The cutting levels shown to be also significantly affected and highest values were observed on no cut and 15 cm pruning level. The minimum concentration + no cut and 15 cm recorded the highest values compared to all treatments.

3.4.7 Bud fresh weight

The findings from this investigation trial showed that different potassium dosages significantly (P≤ 0.05) influenced the bud fresh weight. Moreover, different pruning levels indicated the significant difference on the bud's fresh weight (Table 3.3). Various potassium dosages and levels of pruning had a significant difference on the fresh weight of the bud. The combination of 0.0072 M + 15 cm and unpruned had the highest mean values, and 0.0216 M + all cutting back levels had the least value than any other treatment (Table 3.3).

3.5.8 Bud dry weight

Table 4 illustrates the effects of different potassium concentrations that influenced the bud dry weight significantly (P≤ 0.05). In addition, the dry weight of the flower bud was significantly influenced by pruning levels (Table3.5.3). However, the results show relationship between various potassium dosages and levels of pruning on bud dry weight, according to the findings. The combination of 0.0072 M + no cut and 15 cm cut back had the highest mean values, whereas all the pruning levels on 0.0216 fertigation had the lowest mean values (Table 3.3).

3.6 Discussion

Recurrent droughts and extreme weather events such as floods and tropical cyclones are wreaking havoc on Southern African countries (Muzhinji & Ntuli, 2020). Cultivating intensively, a variety of plants including halophytic plants that require minimal usage of resources by means of exploiting unpopular food stuff could broaden diversity and maximize food insecurity. The present study indicated that *T. divaricata* can be grown under hydroponics due to its capacity to strive and complete its life cycle under different potassium concentrations and pruning levels. This plant has never been introduced into this type of growth protocol. This study is substantiated with previous findings by Wahome *et al.* (2011), who found that plants cultivated in hydroponics consume less resources and develop quicker. Moreover, Kausar and

Gull, (2014) reported that high concentration of potassium has influenced the growth of wheat under salt affected areas significantly.

Furthermore, the results gathered from the experiment have also showed that low concentration of potassium (0.0072 M) is effective on the height and flower bud production of *T. divaricata*, while high concentrations (0.0144 and 0.0216 M) reduce the flower bud production. Also, potassium sulphate significantly affected the dry weight and flower buds as well as both fresh and dry weight. The highest mean value for root fresh weight, root dry weight, bud fresh weight and bud dry weight were obtained at 0.0072 M potassium concentration, whereas high potassium concentrations recorded the lowest values. These results are supported by findings from Hussain *et al*. (2013) who stated that potassium is an essential macronutrient that is needed in moderate rate as the plants use it as potash (K_2SO_4) in half of the land lacks potassium element and its addition has showed to be effective on the growth and development of plants. Similarly in other plants that are salt tolerant such as wheat, barley that were previously grown in the wild and strived under salt stress environment when supplemented with potash fertilizer (Kausar & Gull, 2014).

In another study, it was reported that potash elevated some physiological growth parameters such as length and shoot ratio in wheat and barley under the stressful conditions (Hussain *et al.,* 2013). Wang *et al.* (2013) have also mentioned that the presence of potassium is critical in the sense that it controls most biochemical and physiological responses in plants. Even though potassium is instrumental for plant production and development, plants have their specific requirement in any nutrient (Ngxabi *et al.,* 2021), hence, *T. divaricata* is affected by different potassium concentrations including in the growth reduction in high concentrations. The control yielded more prominent mean values for fresh and dry weight of the plant, root weight and bud weight although the margins were close to those of 0.0072 M potassium concentration. Adams (1991) and Sayyad-Amin *et al.* (2016) concluded that chemical or excessive salinity decreases crop yield.

Pruning is done by farmers, researchers, and managers to achieve great yield and stimulate growth and development (Santos *et al.*, 2016). Plants that produce flowers and fruits are most likely to have a higher and nutritious yield during vegetative phase of their lifecycle after pruning in early stages of growth (Badrulhisham & Othman, 2016). *Trachyandra divaricata* develops many leaves that grows up to 1m (Brown & Bettink, 2019), which are not eaten so pruning leaves could be very beneficiary to the roots and flower stalk to utilise more nutrients and sunlight as they are the essential elements in the plant. Soedjarwo & Tjokrosumarto (2018), have pruned chilli sprouts on their study to encourage more fruit production, the results yielded positive result a this supports, and this substantiate the above statement. It is reported by Dasa *et al.* (2016) that plants that grow too densely struggle to distributing nutrients to the edible parts. Also, plants that are overly thick may cause the fruit to break apart during the maturity phase, hence pruning is required for optimal fruit production (Orsini, 2016). In this study, pruning had a positive influence on *T. divaricata* growth. However, plant height, weight, number of buds increased at low potassium concentrations and declined on higher concentrations because of toxicity and stress. These results revealed that unpruned treatments had the highest value in all the treatments followed by 15 cm pruning. In contrary, low level (10 & 5 cm) had the smallest values in all the treatments. Kandiah, (1971) have supported the finding of this study that pruning might imply stress on the physiology of tea plants compared to the natural growing tea plants that do not undergo pruning or trimming.

In *T. divaricata*, the inflorescence is the most significant aspect because it is the sole edible component that can be utilized as a vegetable; therefore, the importance of this study results cannot be underestimated. Low potassium concentration (± 0.0072 M) has been shown to considerably advance the weight and development of flower bud, however high concentrations inhibit flower development. Treatments with high potassium concentrations (0.0144 and 0.0216 M) developed few flower buds compared to lower concentration (0.0072 M). This occurrence might be linked to a lack of hormones necessary for floral initiation brought on by an abundance of potassium. Results from a different salinity-based investigation showed that decreased osmotic potential and reduced stomatal conductance are connected to decreased plant productivity with increasing salt. (Ngxabi *et al.*, 2021). The light concentration of K_2SO_4 with minimal pruning or unpruned were effective when compared with other combinations. The results of this study agree with previous studies done by Ventura *et al.* (2014), Zapryanova & Atanassova (2009); Stanton *et al.* (1997) who discovered that the weight of the inflorescences and blooming buds increases at low NaCl levels and declines as salinity rises. Therefore, this concludes that excess of any nutrient such as K_2SO_4 can cause flower abortion or reduction.

3.7 Conclusion

Alternative uses of less-than-ideal, as well as salt tolerant crops, will become increasingly crucial in the future to address rising food poverty and enhance agricultural production. No cut stalks of *T. divaricata* require minimal dosage of K_2SO_4 for optimal vegetative growth and bud production. In addition, cutting back is also important on plants that produce flowers and fruits, as it is done to improve plant development. Techniques such as hydroponics which uses less resource’s such water, nutrients and soil efficiently will be necessary to mitigate with crisis of food security. Hence, these findings will assist researchers, growers, and botanists with the correct protocol to grow this plant and for a wide range of other species.

3.8 Acknowledgement

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Chapter 4: The chlorophyll content of *Trachyandra divaricata* in response to various potassium concentrations and levels of pruning in a hydroponic system

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4.1 Abstract

Chlorophyll is the green pigment which is found in algae, plant and bacteria. The pigment plays a primary function of trapping sunlight which is key for the process of photosynthesis in a plant. However, in areas where salt tolerant plants such as *T. divaricata* are deprived of nitrogen and other essential nutrients, potassium and nutrient has been recommended to mitigate these challenges. Pruning leaves is a viable strategy as it allows revitalization of the edible parts of a plant and enables it to absorb nutrients effectively and get an exposure to sunlight. The goal of this study was to ascertain how pruning and various potassium concentrations affected the chlorophyll of *T. divaricata* grown in a hydroponic system. The findings of this study showed that minimal application of potassium (0.0072 M) without pruning, or 10 cm pruning level had the best chlorophyl results whereas the highest dose of potassium (0.0216 and 0.0144 M) with all the pruning levels (5,10,15 cm & unpruned) had the least chlorophyl values of *T. Divaricata*. These results will help horticulturists and industrial growers create a growth technique that is appropriate for the species and its close relatives.

Keywords: Green pigment; growth protocol; halophytes; K_2SO_4, photosynthesis, cutting and elements

Abbreviations: Chl- Chlorophyll, K_2SO_4 -Potassium sulphate, EC- Electric Conductivity, N- nitrogen

4.2 Introduction

Plants, algae and certain bacteria produce chlorophylls, which are planar and highly conjugated tetrapyrrole compounds (Perez-Galvez *et al.,* 2018). Even though chlorophyll have more than 100 forms, only two forms namely chlorophyll 'a' and 'b' are more predominant in green plants (Aramrueang *et al.,* 2019). Chlorophyll concentration correlates positively to the nitrogen status of a leaf and it is commonly used as an indication of the presence of nitrogen nutrient which is vital for growth in plants (Ercoli *et al.,* 1993; Pan et al., 2013, Meskini-vishkaee *et al.,* 2015). Chlorophylls are a regular component of human diet, present in both fresh fruits and vegetables and seaweed as well as processed foods with approved green food colourings like copper and non-copper chlorophylls (Viera *et al*., 2018).

Most of important processes such as photosynthesis, physiological and metabolic processes depend on the presence of chlorophyll for the plant to perform its full functions spontaneously (Clevers & Gitelson, 2013, Houborg *et al.,* 2015, Li *et al*., 2016). The pigment allows oxygenic photosynthetic organisms to utilise light energy to produce food and oxygen (Eberhard *et al*., 2008; Wang *et al*., 2018; Zarco-Tejada *et al.,* 2019). Nevertheless, chlorophyll is the most prevalent pigment in the biosphere, yet billions of tons of it are synthesized and destroyed yearly (Wang & Grimm, 2021).

Nitrogen, an important component of chlorophyll faces severe challenges under stressful conditions as physiological and metabolic processes that result in chlorophyll biosynthesis become hindered (Kausar *et al.,* 2014). The addition of potassium nutrient in such instances becomes more imperative as it rescues plant growth and more absorption of nitrogen minerals may be facilitated (Tzortzakis, 2010; Ashraf *et al*., 2013). The presence of potassium is critical as it controls most biochemical and physiological responses in plants, and it plays a pivotal role in enzymes activation, photosynthesis, protein synthesis, osmoregulation, energy transfer and stomatal movement (Wang *et al*., 2013).

Practices such as pruning is important as it improves the vegetative development, aesthetic value, wood quality and growth in plants, allows the revitalization of edible parts, enables efficient nutrients absorption, and positions the plant strategically to get an exposure to sunlight (Kadlec *et al.,* 2022; Pineda *et al.,* 2020). For instance, *T. divaricata* is popularly known as bush toxic leaves as its consumption lead to progressive posterior paresis in livestock due to build-up of brownish pigments in spinal and brain neurons, whose toxic precursors are not yet known (Botha & Penrith, 2008). Therefore, animals avoid grazing it and this prevents the flower bud (edible part) access to the sunlight and cause the flower bud to bend or grow unproperly (Botha & Perinth, 2008). A combination of pruning and potassium application could improve the growth of the flower bud as the former repositions the plant to access sunlight while the latter triggers the absorption of nitrogen in halophytes (Tzortzakis, 2010; Ashraf *et al.,* 2010).

The aim of this study was to evaluate the effect of pruning and potassium fertiliser on chlorophyl concentrations in *T. divaricata*. These treatments were evaluated as management alternatives that might increase the yield for *T. divaricata*. The fertilization and pruning could be used as inexpensive, simple, and eco-friendly practical recommendation for small producers to increase *T. divaricata* yield if they proved to be effective.

4.3 Materials and methods

4.3.1 Greenhouse experiment

The study was carried out over a duration of 13 weeks at the Cape Peninsula University of Technology's greenhouse site in Bellville, Cape Town, South Africa (33^0 55'45.53S, 18^0 38' 31. 16E). The control of the environment inside the greenhouse was ensured by the technologies used within the structure. The greenhouse was equipped with environmental control with temperatures set to range from 21 to 26 °C during the day and 12–18 °C at night, with relative humidity averages of 60%. The average daily photosynthetic photon flux density (PPFD) was 420µmol/m2/s and the maximum was1020 µmol/m2/s.

4.3.2 Plant Preparation

About 300 small plants were harvested at the back of Horticulture and landscape design building from Cape Peninsula University of Technology, Bellville campus. Excessive plants were divided to certify the number of plant pieces (200) required for the experiment is achieved. Plant divisions were planted individually in black pots (12.5 cm (height) x 12.5 cm (width) x 12.5 cm (depth) in a medium silica sand.

The plants were placed in an internally, environmental controlled propagation greenhouse with an automated drip irrigation system from 7 am to 7 pm, where they were watered for 30 seconds every hour. The plants were treated with NUTRIFEED (13:0:45) solution (100 ml per plant) once in a week as a basic nutrient. After 3 weeks, the pots were then moved to the research greenhouse for acclimatization for a week. Then the pots were transferred to the research greenhouse for a week for acclimatization. For the experiment, 160 healthy, uniform plants were selected from the batch, each measuring 13- 20 cm in height and having 10-15 leaves.

4.4.1 Experimental design

The experiment consisted of four hydroponic systems arranged in a complete block design having 10 replicates of each treatment (Table 3.3.1**)**. Each system had one low-density polyethylene (LDPE) reservoir with a capacity of 70 litres, four polyvinyl chloride (PVC) square cutters (130 mm x 70 mm x 2 500 mm), forty 12.5 x 12.5 cm plastic pots, a 2 000 L/h submersible water pump with a 2.5 m head capacity, ten meters of 20 mm LDPE irrigation piping, and 20 mm head connector were included in each system.

This experiment was designed with each hydroponic system being placed on top of a single rectangular steel mesh table. Each system was marked B1-B4. A steel mesh table served as a flat surface for white gutters while a black plastic pipe as a delivery pipe. Twenty plastic gutters together with twenty LDPE pipes were placed onto four steel tables held in place with cable ties for stability while gutters were sealed with PVC adhesive to avoid any leakage. Each table had four plastic gutters and surrounded with irrigation pipes, and each gutter had ten slits to insert ten plastic pots. Reservoirs were kept filled with a 50 L (water and a specific amount of Potassium concentrations (0, 0.0072, 0.0144 and 0.0216 M) solution level beneath each table. Each reservoir contained a submersible water pump, which recirculated the aqueous nutrient solution through transport pipe to each gutter so that the plant roots can submerge onto the solution. All gutters were installed with an input to direct water toward the gutters and an outlet to return excess water to the reservoir, and they were all sealed with PVC adhesive. Each input included LDPE irrigation pipping converted in a diameter from 20 mm to 15 mm to allow solution to flow in the gutter. All the gutters carried pots filled with silica sand (labelled with numbers and arranged randomly and arranged randomly). Digital timers were used to automatically control each separate system in order to fertigate plants with various amounts of nutrient solution (Potassium sulphate) with 0.0144 M of nutrified being the basic nutrients for all systems and pruned plant height. All aqueous solution used in this experiment was kept constant between 6.0 to 7.0 pH level and Electrical conductivity (EC) level of the aqueous nutrient solution weekly with a calibrated handheld digital EC meter (Hanna Instruments®™HI 98312). A calibrated handheld digital pH meter (Eurotech®™ pH 2 pen) was used to check the pH levels of every water reservoir. Potassium hydroxide was used for increasing pH and hydrochloric acid to lower pH. The EC of the aqueous nutrient solution in the reservoirs was decrease by adding reverse osmosis water, whereas the EC was increased by adding Hoagland solution to the aqueous nutrient solution in the reservoirs.

4.4.2 Treatments

Nutrifeed fertilizer (10g/5L) from Starke Ayres in Cape Town was served as the primary fertilizer and a source of foundation nutrients for all treatments since it is commonly utilized by South African vegetable growers in hydroponics. The Nutrifeed fertiliser consist of the following components: B (240 mg/kg), P (27 mg/kg), Mg (22 mg/kg), K (130 mg/kg), Mn (240 mg/kg), Zn (240 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Ca (70 mg/kg), Mo (10 mg/kg), S (75 mg/kg), and N (65 mg/kg). Potassium was added in the nutritional solution as potassium sulphate at various ratios (0.0216, 0.0144 and 0.0072 M). Only Nutrifeed was used to maintain and irrigate control variables. Table 4.4.1 illustrate the placement of each gutter on four NFT systems with various pruning levels (cm) and potassium sulphate (M) fertigation.

Table 4.4.2: The demonstration of the allocation of each gutter on four NFT systems with various pruning levels (cm) and potassium sulphate (M)

Treatments	Gutter one	Gutter two	Gutter three	Gutter four
1	5 cm + 0 M	10 cm + 0 M	15 cm + 0 M	Unpruned + 0 M
2	5 cm + 0.0072 M	10 cm + 0.0072 M	15 cm + 0.0072 M	Unpruned + 0.0072 M
3	5 cm + 0.0144 M	10 cm + 0.0144 M	15 cm + 0.0144 M	Unpruned + 0.0144 M
4	5 cm + 0.0216 M	10 cm+ 0.0216 M	15 cm+ 0.0216 M	No cut+ 0.0216 M

Note: Each gutter of each treatment contained ten plants, one plant per pot, for a total of 40 plants in each hydroponic system and 200 plants during the entire experiment. Plant heights were cut back (cm) during vegetative stage at the beginning of second month, and Potassium nutrient concentrations (mL) applied. Note: 5 cm is light pruning, 10 cm is moderate pruning, and 15 cm is heavy pruning.

4.5 Data collection

4.5.1 Chlorophyll content of the plant

A portable device (SPAD-502, China) was used to measure the chlorophyll content of the leaves of *T. divaricata* during the day so that the device can provide accurate readings at pre-planting and post-harvesting stage.

Figure 4.5.1: Collection of chlorophyl on three leaves that were marked specifically for chlorophyl data (Bulawa, 2021).

4.6 Results

4.6.1 The influence of Potassium concentrations and levels of pruning on chlorophyl content

The findings gathered from this study suggest that different potassium dosages had affected the chlorophyl content of the plant significantly (P≤ 0.05) both in pre- harvest and post-harvest stage (Figure 4). Plants were initially significantly influenced by pruning however at post-harvest stage proved otherwise as it shown non-significant (P≤0.05) influence. The combination of K_2SO_4 and pruning also did not show positive significancy. The maximum mean value was recorded in a combination of 0.0072 M K_2SO_4 + 10 cm pruning, followed by 0.0072 M with unpruned plant samples. However, the least chlorophyl values were recorded in the treatment having a combination of 0.0144 M K_2SO_4 dosage with 15 cm pruning (Figure 4.6.1).

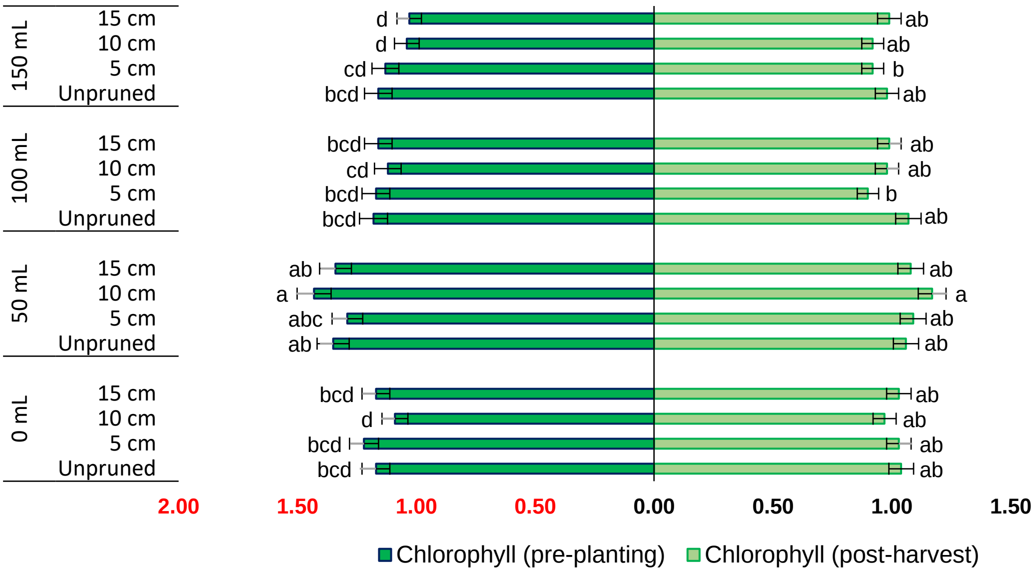

Figure 4.6.1: The influence of Potassium dosages and levels of pruning on chlorophyl content of hydroponically grown *Trachyandra divaricata.*

*Note: Tukey's LSD was used to rank the means along the column with a 0.05 significance threshold. Means that do not share a letter are very dissimilar. *: significant at $p \leq 0.05$.

4.7 Discussion

Chlorophyll is vital for life on earth because it allows oxygenic photosynthetic organisms to absorb light energy and use it for food production and release of oxygen (Eberhard *et al.,* 2008). Chlorophyll plays a crucial role in a plant as it is considered as one of the parameters that determine the wellbeing of the plant (Nkcukankcuka *et al.,* 2021). The results from this experiment have shown that 0.0072 M of K_2SO_4 had the most significant effect on chlorophyll concentration of *T. divaricata* compared to all the treatments while chlorophyl contents varied in other treatments. These findings support the assertion made by Fallovo *et al.* (2009) that applying fertilizer is one of the most practical and efficient ways to manage and enhance the production and nutritional qualities of crops intended for human consumption (Nkcukankcuka *et al.,* 2022). Also, the results align with Amujoyegbe *et al.* (2007) who reported variation in the chlorophyll content of *Zea mays* and Sorghum bicolour cultivated in soil supplemented with organic and inorganic fertilizers.

The fact that hydroponic system enables the abundant nutrient supply to increase yield in limited space make it easy to quantify significant effect of introduced factors (Treftz & Omaye 2016). However, nutrient supplementation is vital while suitable pH and electrical conductivity adjustment needs critical consideration in hydroponics as it influences the overall plant growth (Wortman, 2015; Singh & Bruce, 2016). The above statement is supported by the suggestion of Singh & Bruce (2016) that a pH range of 5.5 to 6.5 is optimal for most species for the availability of nutrients, although some species may have specific ranges. Therefore, the findings of this experiment corroborate with these reported earlier in the literature; that nutrient supplementation increases chlorophyll content.

Earlier findings by Vanhove *et al.* (2016) and Riedel *et al.* (2019), indicated that to promote the production in Cacao, proper fertilization and pruning are necessary. It was further stated that pruning allows light to reach the canopy and prevents an increase in relative humidity, it can be an effective method of preventing plant diseases in the organic cacao cultivation process (Babin, 2018). This agrees with the results of this experiment whereby a combination of the minimum concentration (0.0072 M) + 10 cm followed by 15 cm pruning level yielded the highest value of chlorophyll compared to all the treatments.

4.8 Conclusion

Chlorophyl is one of the most of important attributes that indicates the wellbeing of a plant Lack of chlorophyl result in chlorosis which affects the rate of many metabolic and physiological processes such as photosynthesis. This study has proved that minimal ($\leq$ 0.0072 M) addition of potassium sulphate on halophytic plants could improve the growth of the plant. Therefore, the results of this investigation have provided for the first time, a suitable growth protocol that have improved the chlorophyl content of *T. divaricata*. The study also suggests a reproducible cultivation method that will assist growers as plant has never been grown in hydroponics before and in a commercial scale. Future researchers could explore irrigation, photoperiods and other environmental factors that will contribute to improve the edible parts and chlorophyll content of the species.

4.9 Acknowledgment

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4.10 References

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Chapter Five: The accumulation of antioxidants under different potassium concentrations and pruning levels on *Trachyandra divaricata*

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5.1 Abstract

Plants are the most vital sources of bioactive compounds, whose synthesis are mainly influenced by biotic and abiotic factors. In this study, the antioxidant and phytochemical content of flower buds and roots of *Trachyandra divaricata* cultivated under different potassium concentrations (0.0216, 0.0144 and 0.0072 M) and levels of cutting (5 cm, 10 cm, 15 cm, and No cut) were evaluated using standard protocols. Antioxidants such as polyphenols, DPPH, FRAP, TEAC and flavanols were found to be present in the flower buds and roots of *Trachyandra divaricata*. However, the roots accumulated the highest values in all the antioxidants present in the plant compared to flower buds. Polyphenols, flavonols and FRAP all showed significant effect in the root besides DPPH and TEAC compound that was not significantly affected by potassium concentrations in all the treatments. All the variables were significant in the flower buds. However, minimum concentration with various pruning levels had the highest values in all the antioxidants. Polyphenols showed the highest value influence at moderate (0.0072 M) and all pruning levels cm, likewise flavonols which were significantly present in samples treated with 0.772 M mL+ 10 cm pruning. While FRAP, DPPH and TEAC were negatively influenced by potassium as control (0 mL) was the best compared to all other concentrations and pruning was significant at the 10 cm level. Therefore, these results suggest that potassium and pruning play a significant role in the accumulation of antioxidants in *T. divaricata.*

Keywords: Asphodelaceae; bioactive compounds; flavonols; polyphenols; *Trachyandra divaricata*.

Abbreviations: G - Gutters, PVC - polyvinyl chloride, LDPE - low-density polyethylene, K2SO4 - Potassium sulphate, EC - electronic conductivity, K - potassium

5.2 Introduction

The scientific community and epidemiological studies explicitly stated that fruits and vegetables help to prevent cancer and cardiovascular disease because of their high antioxidant content and positive effects on human health (Renaud *et al*., 1998; Temple, 2000). This has led more people to be more conscious of what they consume and accelerated the pressure on the production of more vegetable, notwithstanding, the projected 70% toxicity and salinity of arable land induced by human activities such as misuse of fertilizers, environmental degradation, and climatic, hydrological, and geomorphic factors (Stefanov *et al.,* 2016; Ma et al., 2017; Mustafa *et al*., 2019). The presence of salt in agricultural land aggravates the physiological processes of crops such as the reduction of transpiration, respiration, efficiency of stomatal conductance, and photosynthesis (Stefanov *et al*., 2016).

Hence, salt-tolerant plants are opted to be a viable source of antioxidants because when they are exposed to stressful environments, thus tend to enhance the production of secondary metabolites that are thought to be precursors of antioxidant effects in plants (Giorgi *et al.,* 2013; Jimoh & Kambizi, 2022). Therefore, these phytochemicals provide plants with protective properties against the damaging effects of stress (Ksouri *et al*., 2007). Plants also produce antioxidants that neutralize free radicals during oxidative and ionic stress, and these antioxidants are critical for human health (Jimoh *et al*., 2020; Mazouz *et al*., 2020).

In arid and semi-arid regions, crop growth and development are not favourable as most plants exhibit dehydrated symptoms that are also associated with insufficient potassium status in conventional land (Ahanger *et al*., 2017). In these conditions, potassium supplementation can mitigate the harmful effects of oxidative and ionic stress to a manageable extent to neutralize the metabolism of reactive oxygen species and enhance the synthesis of pigments (Soledad *et al*., 2015). In addition, K plays a pivotal role in sustaining plant cellular function although potassium is not a metabolic product, its deficiency has evident impacts on plant development and physiological characteristics (Tiwari et al., 1998; Sharma and Agarwal 2002; Sharma *et al*., 2006; Umar 2006; Jatav *et al*., 2014; Ahanger *et al*., 2015).

Pruning is mainly recommended as it is known to improve the plant health, arouse flowering and increases production (ALRIDIWIRSAH, 2020; Hussain *et al.*, 2014). However, it is important to be conscious about the pruning of shoots and root systems of certain species as the practice can influence biomass production, which is a vital component of antioxidants, and nutritional content. Besides the stresses caused by pruning in the plant, cutting provides a better crop yield by encouraging new growth in the shoots, flowers, and roots (Kaczorowska Dolowy *et al.,* 2019). Several reports have indicated that the level of pruning must be considered as it can influence numerous metabolisms such as pruning intensity influences growth, flower and fruit development (DuToit *et al.,* 2020). Pruning cultivated T. divaricata is an attempt to allow temperature, air circulation light so that physiological processes can happen easily.

This study investigates the influence of different potassium concentrations and levels of pruning on the antioxidant and phytochemical content of leaves of *T. divaricata* as K proved to enhance the salt-tolerant plants under various stress environments and pruning has a virtuous impact on other species when trimmed at an appropriate level. Therefore, the study will determine the suitable potassium concentration and pruning level that will have high antioxidant content, as this was never tested previously on the plant.

5.3 Materials and methods

The study was carried out over a duration of 13 weeks at the Cape Peninsula University of Technology's greenhouse site in Bellville, Cape Town, South Africa (33^0 55'45.53S, 18^0 38' 31. 16E). The control of the internal environment of the greenhouse was ensured by the technologies used within the structure.

5.3.1 Experimental design

The experiment consisted of four hydroponic systems. Each system had one low-density polyethylene (LDPE) reservoir with a capacity of 70 litres, four polyvinyl chloride (PVC) square cutters (130 mm x 70 mm x 2 500 mm), forty 12.5 x 12.5 cm plastic pots, a 2 000 L/h submersible water pump with a 2.5 m head capacity, ten meters of 20 mm LDPE irrigation piping, and 20 mm head connectors were included in each system.

This experiment was designed with each hydroponic system being placed on top of a single rectangular steel mesh table. Each system was marked B1-B4. A steel mesh table served as a flat surface for white gutters while a black plastic pipe as a delivery pipe. Twenty plastic gutters together with twenty LDPE pipes were placed onto four steel tables held in place with cable ties for stability while gutters were sealed with PVC adhesive to avoid any leakage. Each table had four plastic gutters and surrounded with irrigation pipes, and each gutter had ten slits to insert ten plastic pots. Reservoirs were kept filled with a 50 L (water and a specific amount of Potassium concentrations (0.0072, 0.0144 and 0.0216 M) solution level beneath each table. Each reservoir contained a submersible water pump, which recirculated the aqueous nutrient solution through transport pipe to each gutter so that the plant roots can submerge onto the solution. All gutters were installed with an inlet to direct water toward the gutters and an outlet to return excess water to the reservoir, and they were all sealed with PVC adhesive. Each input included LDPE irrigation pipping converted in a diameter from 20 mm to 15 mm to allow solution to flow in the gutter. All the gutters carried pots filled with silica sand (labelled with numbers and arranged randomly and arranged randomly). Digital timers were used to automatically control each separate system in order to fertigate plants with various amounts of nutrient solution (Potassium sulphate) and pruned levels with 0.0144 M of nutrified being the basic nutrients for all systems. All aqueous solution used in this experiment was kept constant between 6.0 to 7.0 pH level and Electrical conductivity (EC) level of the aqueous nutrient solution weekly with a calibrated handheld digital EC meter (Hanna Instruments®™HI 98312). A calibrated handheld digital pH meter (Eurotech®™ pH 2 pen) was used to check the pH levels of every water reservoir. Potassium hydroxide was used for increasing pH and hydrochloric acid to lower pH. The EC of the aqueous nutrient solution in the reservoirs was decreased by adding reverse osmosis water, whereas the EC was increased by adding Hoagland solution to the aqueous nutrient solution in the reservoirs.

5.4 Plant Preparation

About 300 small plants were harvested at the back of Horticulture and landscape design building from Cape Peninsula University of Technology, Bellville campus. Excessive plants were divided to certify the number of plant pieces (200) required for the experiment is achieved. Plant divisions were planted individually in black pots (12.5 cm (height) x 12.5 cm (width) x 12.5 cm (depth) in a medium silica sand.

The plants were placed in an internally, environmental controlled propagation greenhouse with an automated drip irrigation system from 7 am to 7 pm, where they were watered for 30 seconds every hour. The plants were treated with NUTRIFEED (13:0:45) solution (100 ml per plant) once in a week as a basic nutrient. After 3 weeks, the pots were then moved to the research greenhouse for acclimatization for a week. Then the pots were transferred to the research greenhouse for a week for acclimatization. For the experiment, 160 healthy, uniform plants were selected from the batch, each measuring 13- 20 cm in height and having 10-15 leaves.

5.4.1 Nutrient solution

Nutrifeed fertilizer (10g/5L) from Starke Ayres in Cape Town served as the primary fertilizer and a source of foundation nutrients for all treatments since it is commonly utilized by South African vegetable growers in hydroponics. The Nutrifeed fertiliser consist of the following components: B (240 mg/kg), P (27 mg/kg), Mg (22 mg/kg) K (130 mg/kg), Mn (240 mg/kg), Zn (240 mg/kg) Cu (20 mg/kg), Fe (1500 mg/kg), Ca (70 mg/kg) Mo (10 mg/kg), S (75 mg/kg), and N (65 mg/kg). Potassium was added in the nutritional solution as treatment variable at various ratios (0.0216, 0.0144 and 0.0072 M). Nutrifeed was used to maintain and irrigate control variables. Illustration on table 4.4.1 shows the placement of each gutter on four NFT systems with various pruning levels (cm) and potassium sulphate (M) fertigation.

5.4.2 Treatment preparation

Potassium sulphate (K_2SO_4) was used to manipulate different potassium concentrations and a component (0.0144 M) of the Nutrifeed™ nutrient solutions. Potassium sulphate was added in week 4 after the system had been operating for 2 weeks with tap water and another week with the addition of Nutrifeed™. In this experiment, three potassium concentrations (0.0072, 0.0144, and 0.0216 M of K_2SO_4) were evaluated and poured into each sump, with 0 mL of K_2SO_4 serving as the control. Tap water was used to prepare the saline solutions. To prevent the build-up of potassium powder in the medium, pots, gutters, and reservoirs, all nutrient solutions containing K_2SO_4 were changed every week.

5.4.3 Pruning and fertigation treatments

Table 5.4.3 the three treatments of pruning which include 5 cm is light pruning, 10 cm is moderate pruning, and 15 cm is heavy pruning.

Table 5.4.3: The demonstration of the allocation of each gutter on four NFT systems with various pruning levels (cm) and potassium sulphate (M)

	Pruning (cm) and fertigation (mL)			
Treatment	Gutter 1	Gutter 2	Gutter 3	Gutter 4
1	5 cm + 0 M	10 cm + 0 M	15 cm + 0 M	No cut + 0 M
2	5 cm + 0.0072 M	10 cm + 0.0072 M	15 cm + 0.0072 M	No cut + 0.0072 M
3	5 cm + 0.0144 M	10 cm + 0.0144 M	15 cm + 0.0144 M	No cut + 0.0144 M
4	5 cm + 0.0216 M	10 cm+ 0.0216 M	15 cm+ 0.0216 M	No cut+ 0.0216 M

Note: Each gutter of each treatment on the system contained ten plants, one plant per pot, for a total of 40 plants in each hydroponic system and 200 plants during the entire experiment. Plant heights with 5 cm cut back is light pruning, 10 cm is moderate cut back, and 15 cm is heavy cut back at the beginning of second month, during vegetative stage and Potassium nutrient concentrations (mL) applied.

5.5 Data collection

5.5.1 Sample Preparation

Harvested flower bud materials were immediately dried in a fan-drying laboratory oven at 40 °C for 7 days. The dried material was ground into a fine powder using a Junkel and Kunkel model A 10 mill. It was then extracted by mixing 100mg of the dried powdered material with 25 mL of 80% (v/v) ethanol (EtOH) (Merck, South Africa) for 1 hour. It was centrifuged at 4000 rpm for 5 min and the supernatants were used for all analyses. Materials from harvested flower buds were immediately dried for seven days at 40 °C in a fan-drying laboratory oven. The dried material was crushed using a Junkel and Kunkel type A 10 mil to turn it into a fine powdered sample with 25 mL of 80% (v/v) ethanol (EtOH) (Merck, South Africa) and allowing the mixture to settle for one hour. The supernatants were used for every analysis after it was centrifuged at 400 rpm for 5 min.

Figure 5.5.1: The fresh parts (flower buds and roots) that were grinded into fine samples of the *Trachyandra divaricata* analysed for antioxidants and phytochemicals (Bulawa, 2021).

5.5.2 Polyphenol Assay

The total polyphenols assay ((Folin & Ciocalteu's assay) was carried out as instructed by Jimoh *et al.* (2019). A 7.5% sodium carbonate solution (Sigma, South Africa) was made by diluting Folin & Ciocalteu"s phenol reagent (2N, Sigma South Africa) ten times with distilled water. 25 µL of the crude extract was combined with 125 µL of the Folin & Ciocalteu's phenol reagent and 100 µL of sodium carbonate in a 96-well plate. The plate was incubated for 2 hours at room temperature. Then, the absorbance was determined at 765 nm using a Multiskan spectrum plate reader (Thermo Electron Corporation, USA). Gallic acid (Sigma, South Africa) standard curve with concentration ranging between 0and 500 mg/L was used to calculate the values of polyphenol samples. The results were presented in the form of mg gallic acid equivalents (GAE) for every gram of dry weight (mg GAE/g DW).

5.5.3 Estimation of Flavonol Content

The quantity of 80 mg/L in 95% ethanol (Sigma-Aldrich, Johannesburg, South Africa) and 0, 5, 10, 20, 40 concentrations of querceting were used to determine extract's flavonol content. For each sample, 225 µL of 2 % HCl and 12.5 µLl of 0.1% HCl (Merck, South Africa) in 95 % ethanol were combined with 12.5 µL of crude sample extracts. The extracts were subsequently incubated for 30 minutes at room temperature. At a temperature of 25°C, the absorbance was determined at 360 nm. The findings were presented as mg quercetin equivalent per g of dry weight (mg QE/g DW)

5.5.4 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed using the method of Benzie and Strain (1999) as described by Jimoh et al. (2020). Acetate buffer (0.3M, pH 3.6) (Merck, South Africa) was mixed with 3,2,4 and 6 tripyridyl-s-triazine (10mM in 0.1M hydrochloric acid), 3 and 6 mL of distilled water to prepare FRAP reagent. About 10 L of the crude sample extract and 300 L of the FRAP reagent were combined in a 96-well micro-plate and incubated at a room temperature for 30 minutes. Thermo Electron Corporation of the USA's Multiskan spectrum plate reader was used to measure the absorbance at 593 nm. With concentrations ranging from 0 to 1000 M. An L-ascorbic acid standard curve (Sigma-Aldrich in South Africa) was used to determine the FRAP values for plant samples that were tested. Ascorbic acid equivalents (AAE) per gram of dry weight (M AAE/g DW) were used to express the results (Sogoni *et al.,* 2021)

5.5.5 DPPH free radical scavenging activity

The DPPH radicals were measured using a 0.135 mM DPPH solution that was prepared in a dark bottle (Unuofin *et al.,* 2017). Trolox standard (6-Hydrox-2, 5, 7, 8-tetramethylchroman-2-20 carboxylic acid) solution was reacted at graded concentrations (0 and 500 M) with approximately 300 L of DPPH solution and 25 L of crude extract. The mixes were incubated for 30 minutes, and then the absorbance at 517 nm was measured. M/Trolox equivalent per gram of dry weight (M TE/g DW) was used to express the results.

5.5.6 ABTS free radical scavenging activity

The ABTS assay was performed following the method described by Jimoh et al. (2020). The stock solutions included a 7 mM ABTS and 140 mM potassium–peroxodisulphate ($K_2S_2O_8$) (Merck, Modderfontein, South Africa) solution. The working solution was then prepared by adding 88 µL of $K_2S_2O_8$ to 5 mL of ABTS solution. The two solutions were mixed well and allowed to react for 24 h at room temperature in the dark. Trolox (6-Hydrox-2, 5, 7, 8-tetramethylchroman-2-20 carboxylic acid) was used as the standard with concentrations ranging between 0 and 500 µM. Crude sample extracts (25 µL) were allowed to react with 300 µL of ABTS in the dark at room temperature for 30 minutes before the absorbance was read at 734 nm at 25 °C in a plate reader. The results were expressed as µM/Trolox equivalent per g dry weight (µM TE/g DW).

5.6 Results

5.6.1 Total polyphenols content in the roots

The data attained from this investigation have shown that different potassium dosages affected the total polyphenols content on the roots of *T. divaricata* significantly ($p \leq 0.05$). The control treatment (0 M) proved to have the highest statistical value (8.42 mg GAE/g) of polyphenols compared to other concentrations of K_2SO_4 applied. However, the sample treated with 0.0216 M of K concentration recorded the lowest value (5.01 mg GAE/g) of polyphenols. The cutting of the plants has showed a positive influence as it 10 cm prove to be suitable level. Therefore, the combination of K_2SO_4 + cutting back produced significant results (Table 5.6.2a)

Table 5.6.2a: The effects of different potassium concentrations on the antioxidants of *Trachyandra divaricata roots* under hydroponic system

Potassium Sulphate	Polyphenols (mg GAE/g)	Flavonols (mg QE/g)	FRAP (µmol AAE/g)	DPPH (µmol TE/g)	TEAC (µmol TE/g)
0 mL	8.42 ± 0.40a	4.18 ± 0.30a	42.89 ± 0.56a	27.00 ± 0.31a	20.35 ± 0.69b
0.0072 M	7.27 ± 0.13b	3.28 ± 0.09b	37.59 ± 1.09b	22.99 ± 0.90b	19.51 ± 0.21b
0.0144 M	6.32 ± 0.58c	2.79 ± 0.19b	34.29 ± 0.64c	20.69 ± 0.23c	21.09 ± 0.95ab
0.0216 M	5.02 ± 0.64d	1.96 ± 0.09c	25.39 ± 0.58d	17.19 ± 066d	23.47 ± 1.41a
			One-way ANOVA F-Statistics		
K_2SO_4	18.74*	7.78*	484.92*	152.226n	26.18n

Note: Tukey's LSD was used to rank the means along the column with a 0.05 significance threshold. Means that do not share a letter are very dissimilar. *: significant at $p \leq 0.05$.

Table 5.6.2b: The impact of potassium dosages and levels of pruning on antioxidant activity of flower buds of *Trachyandra divaricata under hydroponic conditions*

K_2SO_4	Pruning levels	Polyphenols (mg GAE/g)	Flavonols (mg QE/g)	FRAP (µmol AAE/g)	DPPH ((µmol TE/g)	TEAC (µmol TE/g)
0 M	Uprunned	3.55 ± 0.15 de	3.59 ± 0.09 def	14.08 ± 0.14 gh	5.04 ± 0.002 fg	4.45 V 0.09 gh
	5 cm	3.04 ± 0.07 ef	0.69 ± 0.03 j	15.33 ± 0.48 gh	5.37 ± 0.18 efg	2.69 ± 0.06 j
	10 cm	4.63 ± 0.14 bc	1.83 ± 0.12 hi	23.02 ± 0.80 cd	7.36 ± 0.23 cd	4.32 ± 0.19 gh
	15 cm	4.29 ± 0.25 bcd	1.99 ± 0.08 hi	20.09 ± 0.86 de	8.63 ± 0.16 bc	19.04 ± 0.49 a
0.0072 m	Unprunned	6.55 ± 0.05 a	3.33 ± 0.08 ef	19.27 ± 0.71 ef	6.44 ± 0.18 de	5.52 ± 0.06 def
	5 cm	5.84 ± 0.08 a	3.88 ± 0.21 cde	33.69 ± 1.06 a	9.34 ± 0.18 bc	5.12 ± 0.03 efgh
	10 cm	6.13 ± 0.29 a	5.22 ± 0.27 a	29.48 ± 0.85 b	11.47 ± 0.42 a	5.87 ± 0.31 b
	15 cm	6.33 ± 0.15 a	4.56 ± 0.29 abc	28.34 ± 0.63 b	9.25 ± 0.36 b	5.87 ± 0.31 bc
0.0114 M	Unpruned	6.57 ± 0.19 a	2.14 ± 0.04 hi	10.09 ±0.55 i	3.54 ± 0.09 h	5.23 ± 0.16 egh
	5 cm	2.69 ± 0.13 cd	4.29 ± 0.21 cd	16.31 ± 0.38 fg	6.37 ± 0.21 de	6.74 ± 0.07 c
	10 cm	4.26 ± 0.07 cd	4.33 ± 0.08 bc	21.14 ± 0.92 c	9.01 ± 0.32 b	6.35 ± 0.27 cd
	15 cm	5.02 ± 0.13 b	5.05 ± 0.09 ab	19.46 ± 0.23 ef	6.18 ± 0.22 def	4.17 ± 0.07 hi
0.0216	Unpruned	4.13 ± 0.13 cd	2.47 ± 0.09 gh	16.52 ± 0.71 fg	6.39 ± 0.17 de	3.27 ± 0.14 ij
	5 cm	2.93 ± 0.06 ef	3.04 ± 0.05 fg	10.99 ± 0.29 i	5.59 ± 0.47 ef	6.82 ± 0.09 c
	10 cm	3.15 ± 0.02 ef	1.53 ± 0.008hi	12.48 ± 0.55 hi	4.31 ± 0.07 gh	4.57 ± 0.06 fgh
	15 cm	5.23 ± 0.16 ef	1.82 ± 0.06 hi	12.52 ± 0.44 hi	4.10 ± 0.09 hi	3.29 ± 0.08 ij
Two- way ANOVA F-Statistics						
K_2SO_4		36.97*	47.58*	726.08*	68.19*	74.58*
Pruning		26.77*	1.20*	704.83*	57.81*	187.34*
K_2SO_4 x PRUNING		26.56*	36.38*	751.74*	97.04*	476.14*

Note: Tukey's LSD was used to rank the means along the column with a 0.05 significance threshold. Means that do not share a letter are very dissimilar. *: significant at $p \leq 0.05$.

5.6.2 Total flavonol content in the roots

The present study shows that the effect of different K_2SO_4 concentrations on the flavonol content in the roots of *T. divaricata* was statistically significant at $p \leq 0.005$. The 0.0072 M of K_2SO_4 treatment had the highest value of 4.18 mg QE/g of flavonol content in the root of *T. divaricata* (Table 5.6.2a).

5.6.3 Total FRAP content in the roots.

The influence of various potassium dosages on the FRAP content of the roots of *T.divaricata* was statistically significant at the value of $p \leq 0.005$. At $p \leq 0.005$, the control variable has shown the highest value (42.89 µmol AAE/g) of FRAP content.

5.6.4 Total DPPH content on the roots

The influence of various potassium dosages on the DPPH content of the roots of *T. divaricata* was statistically significant at the value of $p \leq 0.005$. At $p \leq 0.005$ roots of *T. divaricata* showed to have the highest value of DPPH content respectfully under 0.0072 M of 3potassium concentration (Table 5.6.2a).

5.6.5 Total TEC content on the roots

The effect of different Potassium concentrations on the TEAC content of the roots of *T. divaricata* was statistically significant at the value of $p \leq 0.005$. The control variable showed the highest value (20.35 ± 0.69a) compared to all the concentrations (Table 5.6.2a).

5.7 Results of the antioxidants on the flower buds of *T. divaricata*

5.7.1 Total polyphenols content in the flower buds

The findings gathered form obtained from this present study trial have showed that different potassium concentrations affected total polyphenols content on the flower bud of *T. divaricata* significantly at the value of $p \leq 0.001$. Pruning also had a significant influence on the plant. On the contrary, the combination of both factors (different potassium and pruning) had a significant difference. The combination of all 0.0072 M + pruning (5, 10, 15 cm) has recorded the highest concentrations of polyphenols followed by 0.0144 M + 10 cm while 0.0216 M + 15 cm cut had the least value (2.93 mg GAE/g (Table 5.6.2b).

5.7.2 Total flavonol content in the flower bud

The findings collected from this experiment have showed that different potassium dosages had a positively influenced the flavonol content on the flower bud of *T. divaricata*. Similarly, with different pruning levels have influenced the flavonol content of *T. divaricata* significantly ($P \leq 0.05$). Different Potassium concentrations with pruning levels significantly influenced ($P \leq 0.05$) *T. divaricata*. The highest height mean was recorded in treatments with 0.0072 M + 10 cm followed by 0.0144 M + 15 cm in all the potassium concentrations and pruning levels. While 0 M + 5 cm and 0.0216 M + 10 cm combination treatments recorded the lowest values in all treatments (Table 5.6.2b).

5.7.3 Total FRAP content on the flower buds

The current results of the current trial have revealed that the effect of different potassium concentrations have positively affected the FRAP content of flower buds of the *T. divaricata*. Likewise, the different pruning levels had a positive influence on the FRAP content of flower buds of the *T. divaricata.* 0 M + 10 cm was the highest concentration of FRAP recorded, while 0.0216 M + no cut cm recorded the smallest value (10.09 µmol AAE/g) (Table 5.6.2b).

5.7.4 Total DPPH content in the flower buds

The results gathered from the present study indicated that the effect of different potassium concentrations had a significant effect. Pruning height levels on the total content of DPPH of the flower buds of *T. divaricata* had a significant effect. The two-way ANOVA statistics have revealed that the combination of potassium concentration + pruning levels have a positive influence on flower buds of *T. divaricata* at 0 M + 10 followed by 0.0072 M + 5 cm, that recorded the highest content DPPH, while 0.0144 M + no cut recorded minimum value (3.54 µmol TE/g) (Table 5.6.2b).

5.7.5 Total TEAC Content on the flower buds

The influence of various potassium dosages and cutting levels on the total content of TEAC in the flower buds of *T. divaricata* have shown significance. The highest value TEAC recorded was 19.04 µmol TE/g in the treatment 0 M + 10 cm while 0 M + 15 cm had the least value (Table 5.6.2b).

5.8 Discussion

The edible plants that also produce high contents of phytochemicals are highly on demand worldwide as half of the land globally is no longer support the growth of conventional crops that are popularly known as a source of antioxidants (Renaud *et al.,* 1998; Temple, 2000)., Secondary metabolites derived from plants are known to constitute a variety of structurally distinct groups of polyphenols with potential pharmacological effects, such as anti-tumour, anti-inflammatory, antioxidant, and antipathogenic capabilities (Rajendran, 2007). Therefore, the current study investigates the influence of various potassium dosages and cutting back levels on the phytochemical content of *T. divaricata*. The study has never been carried out before, related articles and indirect comparisons of plants that are similar and other Asphodelaceae families have to be made because of scarce literature on *T. divaricata.*

The family Asphodelaceae is commonly known for their potential in antioxidants popularly in *Aloe*, *Bulbine* and *Kniphofia* species, all of which have a mixture of anthraquinones in roots such as red pigment of chrysalid which is also common in *Trachyandra* species, however, its chemistry is unknown (Dagne & Yenesew, 1994; Van Wyk *et al.,* 1995; Boatwright & Manning 2010). Plants with such antioxidants could be useful in the cosmetics, pharmacological and food industries. This study has shown the significant effect of the roots and flower buds extract in response to different potassium concentrations coupled with pruning. Similarly, the study agrees with findings from Ngxabi *et al*. (2021) who reported that NaCl had a clear effect on the antioxidants in the roots of *T. ciliata* than shoot extracts. Therefore, this suggests that *Trachyandra* species should be considered and explored broadly in order to be utilised in industries for various pharmacological purposes.

Several studies have revealed that under abiotic and biotic stress, plants tend to release reactive oxygen species (ROS) consequently of an imbalanced of ROS and production of free radicals (Mittler 2002; Vladimir- Knežević *et al.,* 2012). These ROS have the potential to damage proteins, chlorophyll, nucleic acids, impairment of enzyme activity, and promotes abnormalities that act antagonistically in several vital cellular organelles such as mitochondria and chloroplast (Mittler, 2002; Ahmad *et al*., 2010; Miyake, 2010; Demidchik, 2015). Vladimir-Knežević *et al.* (2012) concluded that the scavenging of ROS by a protective mechanism is referred to as an antioxidant effect. In this context, Ahmand *et al.* (2010) have substantiated that both enzymatic and non-enzymatic antioxidants are closely synchronized to each other to diminish the reactive oxygen species.

The results from this experiment indicate that all cutting back levels (5, 10, 15 cm) + 0.0072 M of potassium concentration showed a positive significance with almost similar values on the antioxidants such as Polyphenol of the flower bud of *T. divaricata* compared to all treatments the plant samples were subjected to. This is in correlation with the results of Rezazadeh *et al.* (2012) who concluded that moderate salinity induced high concentration of phenolics in the halophytic plant, *Cynara scolymus L*. However, high potassium concentration proved otherwise as the results showed no significance on the polyphenols. Similar findings were made by Ben-Abdallah *et al.* (2019) and Ksouri *et al*. (2007), who discovered that *Cakile maritima*, a halophytic plant, could not accumulate phenolic chemicals at high salinity levels. According to other reports, the reduced absorption of potassium and phosphorus, which are essential nutrients for the formation of secondary metabolites like polyphenols, is the reason why polyphenol production declines at high salt concentrations (Waring & Pitman, 1985; Shabala & Pottosin, 2014). This further corroborates the fact that the salinity and some nutrients such as potassium and phosphorus have a similar functional influence on the plants. Dolowy *et al*. (2019) have also suggested that pruning methods cause stress on the plants and Mittler (2002), Ahmad *et al.* (2010), Miyake, (2010) & Demidchik, (2015) have stated that plants release phytochemicals in stressful conditions to neutralise ROS to survive.

The flower buds of *T. divaricata* showed a significant effect on the concentration of 0.0072 M + 10 cm pruning in flavonols compared to all the treatments. This result substantiates the statement of Rezazadeh *et al.* (2012) that moderate treatment influences the accumulation of flavonols, similarly to polyphenols on the flower buds. whereas high concentrations such as 0.0216 M + no cut showed non-significance in DPPH and FRAP polyphenols present in the flower bud of the plant (Polyphenols, DPPH and FRAP). This is also in correspondence with claims made by (Waring & Pitman, 1985; Rezazadeh *et al.,* 2012) that minimal to moderate salt concentrations facilitate the favourable build-up of flavonols.

According to this study, both FRAP and DPPH assays have yielded more or less similar results but are unique from other antioxidants as potassium concentrations have not significantly influenced the accumulation of antioxidants in flower buds of *T. divaricata*. However, pruning (10 cm) had significant effect on the plant while moderate to higher potassium concentrations proved not to be significant. Cakmak, (2005) claimed that inappropriate K levels may also trigger stress reactions, and because of stressors, reactive oxygen species may accumulate. In addition, a build-up of ROS should be avoided since it can cause DNA damage, lipid peroxidation, protein oxidation, and other oxidative reactions that can kill cells (Gill & Tuteja, 2010).

Root samples were amongst the plant parts that were used to identify phytochemicals that are present in *T. divaricata* as Boatwright & Manning, (2010) claimed that a layer of red or orange tissue in outer cortex of the roots of most *Trachyandra* species is alleged to have phytochemicals. Earlier, Dagne & Yenesew, (1994), Van Wyk *et al.* (1995) indicated that a red pigment chryslandicin has been extracted from the roots of Bulbine Wolf and Kniphofia Moench species of the Asphodelaceae family and the pigment is known to be present in the roots of this family. In this study, different potassium concentrations were applied to the plants to evaluate the effect they may have on the plant. The results have shown that the sample treated with 50 mL of potassium concentration accumulated the highest values and has a significant effect on polyphenols, flavonols, FRAP, and DPPH in all the treatments and TEAC had been significantly influenced in all the potassium concentration treatments. This result was supported by findings from Ngxabi *et al*. (2021) who found that moderate NaCl (0.0114 M) had a positive significant effect on the DPPH, TEAC, FRAP and flavanols content of *T. ciliata*, a close relative of *T. divaricata*. In addition to having an impact on plant production, treatment of aromatic and medicinal crops with potassium may also increase the manufacture of other biologically active compounds, such as phenolics, which have a protective effect against induced stress reactions (Khattak *et al.,* 2007).

5.9 Conclusion

The results of this study have suggested that the highest values of bioactive compounds such as (phenolics, DPPH, TEAC, FRAP and flavanols) being obtained at the presence of potassium sulphate together with cutting back level proved that this combination treatment is subsequently suitable. Therefore, indigenous underutilised plants need to be taken into consideration as the plants such as *T. divaricata* have shown these important biologically compounds that plays vital roles in human health.

5.10 Acknowledgement

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Chapter 6: The influence of various potassium dosages and cutting back on the nutritional content of *Trachyandra divaricata*

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6.1 Abstract

The need for exploitation of underutilised plants needs to be reviewed to maximize the food security to those who cannot afford. The intensive exploration of these food plants will help to reveal their proximate, micro and macronutrients. In this study. The proximate, micro and macronutrients contents of *T. divaricata* cultivated under different potassium (0.0072, 0.0144 and 0.0216 M) and cutting back levels (5 cm, 10 cm, 15 cm) were evaluated using standard protocols. All the proximate components (Ash, crude protein, energy, NFC, crude fat, NDC, Moisture) were significantly influenced by the treatments (K_2SO_4 + cutting back). The results of almost all the macro-nutrients such as Ca, Na, P, K, N and K/Ca+Mg were significantly influenced by various K_2SO_4 concentration and cutting back levels except Mg, which is not significant. Moreover, micro-nutrients such as Zn, Fe, Mn -had the positive results, besides Cu. Therefore, the overall results of this study indicate that potassium sulphate together with cutting back the plants are suitable growing protocol for *T. divaricata.*

Keywords: Proximate, Micro and Macro nutrients, food security, edible plants, gene manipulation

Abbbrevations: G - Gutters, PVC - polyvinyl chloride, LDPE - low-density polyethylene, K2SO4 - Potassium sulphate, EC - electronic conductivity, K - potassium

6.2 Introduction

Food availability in South Africa has become a severe challenge because of limited resources and employment opportunities for job creation that leads to poverty (Ngxabi *et al.,* 2021). The world has about 1.5 billion hectors, and that is about 12% of the arable land that is responsible for the production of food, which is very low to support the ever-increasing population (Herrero *et al.*, 2012). It has been further predicted that in 50 years to come, the land will reduce by 13% due to urbanisation and the worldwide agriculture production will continue to decrease by 1.5 yearly until 2030 (Herrero *et al.,* 2010; Berchoux *et al*., 2023). This is due to too heavily dependence on a few existing staple food crops such as rice, wheat and maize (de Fátima Lisboa Fernandes *et al*., 2012, Stein, 2010) instead of exploring and introducing other food products that have potential of providing essential nutrients and diversity in diet (Jimoh *et al*., 2018).

Unavailability of arable land, fresh water and the adverse effects of climate change are the main constraints that have greatly influenced the agricultural production negatively in many parts of Sub- Saharan Africa (Kotir, 2011). Hence, the integration of new innovative agriculture techniques that are more sustainable, water wise and innovative cultivation practices is important as they ensure sufficient food is available all times through proper management of agricultural resources and investments in automation (Yamaguchi & Blumwald, 2005).

The suggestion has enforced a huge pressure on growers to swiftly find efficient systems to produce good quality food quantities considering other edible species (Kyriacou & Rouphael, 2018). Similarly, it is important that nutritional requirements are met for food supply. Leafy vegetables are commonly known to be supplemented with Nitrogen (N) fertilizers to maximise the yield and boost the production commercially. Similarly, Potassium fertilizer, especially Potash is seen as one of the most important nutrients that is popularly known to boost flower and fruit development when applied correctly (Borgognone *et al.,* 2013).

Utilization of plants that are salt and drought tolerant such as *Trachyandra* species can contribute to promote food security as they were anciently used (Ngxabi *et al.,* 2021). As halophytes have a special adaptation mechanism that make them withstand or even benefit from saline conditions, thus, their quality and yield can be improved (Panta *et al.,* 2014). These important attributes of halophytes namely, their commercial application and their potentials, such as them being the raw material for vegetables and a source of oilseed with a high nutritional value, being used as a bio-fuel precursor and as secondary metabolites in pharmaceuticals, food additives, and nutraceuticals have made good candidates (Shannon & Grieve, 1998; Flowers & Muscolo, 2015; Liu *et al.,* 2005); Fan *et al.,* 2013; Buhmann *et al.,* 2015). Thus, a need for new alternative crops that will meet human requirements is imperative (Cheeseman, 2016). *Trachyandra divaricata* is considered as an edible plant because of its flowers buds that are eaten as a snack or salad especially by the indigenous Khoisan people of South Africa as reported by van Wyk *et al.* (2016) and Ngxabi *et al.,* (2021). This experiment investigates the potential of *T. divaricata* as a green vegetable, its potential as functional food, and as an important edible crop of high economic value.

6.3 Materials and methods

The study was carried out over a duration of 13 weeks at the Cape Peninsula University of Technology's greenhouse site in Bellville, Cape Town, South Africa (33^0 55'45.53S, 18^0 38' 31. 16E). The control of the inside environment of the greenhouse was ensured by the technologies used within the structure.

6.3.1 Plant Preparation

About 300 small plants were harvested at the back of Horticulture and Landscape design building on the Bellville campus of the Cape Peninsula University of Technology. Excessive plants were divided to certify the number of plant pieces (200) required for the experiment. Plant divisions were planted individually into black plastic pots (12.5 cm (height) x 12.5 cm (width) x 12.5 cm (depth) in a medium of silica sand.

The plants were placed in an environmental controlled propagation greenhouse with an automated drip irrigation system from 7 am to 7 pm daily, where they were watered for 30 seconds every hour. The plants were treated with NUTRIFEED (13:0:45) solution (100 ml per plant) once in a week as a basic nutrient. After 3 weeks, the pots were moved to the research greenhouse for acclimatization for a week. The pots were then transferred to the research greenhouse for a week for acclimatization. For the experiment, 160 healthy, uniform plants were selected from the batch, each measuring 13-20 cm in height with 10-15 leaves.

6.3.2 Experimental design

The experiment consisted of four hydroponic systems. Each system had used a low-density polyethylene (LDPE) reservoir with a capacity of 70 litres, four polyvinyl chloride (PVC) square cutters (130 mm x 70 mm x 2 500 mm), forty 12.5 x 12.5 cm plastic pots, a 2 000 L/h submersible water pump with a 2.5 m head capacity and ten meters of 20 mm LDPE irrigation piping.

This experiment was designed with each hydroponic system being placed on top of a single rectangular steel mesh table. The four systems were marked from B1 to B4. A steel mesh table served as a flat surface for twenty white gutters while a black plastic pipe used to deliver water from the individual reservoirs. Reservoirs were kept filled with a 50 L (water and a specific amount of Potassium concentrations (0, 0.0072, 0.0144 and 0.0216 M) solution level beneath each table. Each reservoir contained a submersible water pump, which recirculated the aqueous nutrient solution to each gutter to keep the plant roots submerged with the solution. Excess waters were returned to the reservoirs. All the gutters carried pots filled with silica sand (labelled with numbers and arranged randomly). Digital timers were used to automatically control each separate system to fertigate plants with various amounts of nutrient solution (Potassium sulphate) with 0.0144 M of nutrified being used as the basic nutrients for all systems. All aqueous solutions used in this experiment was kept constant between 6.0 to 7.0 pH level and the electrical conductivity (EC) were measured weekly with a handheld digital EC meter (Hanna Instruments®™HI 98312). A calibrated handheld digital pH meter (Eurotech®™ pH 2 pen) was used to check the pH levels of every water reservoir. Potassium hydroxide was used for increasing pH and hydrochloric acid to lower pH. The EC of the aqueous nutrient solution in the reservoirs was decreased by adding reverse osmosis water, whereas the EC was increased by adding Hoagland solution to the aqueous nutrient solution in the reservoirs.

6.3.3 Pruning and fertigation treatments

Table 6.3.1: The demonstration of the allocation of each gutter on four NFT systems with various pruning levels (cm) and potassium sulphate (M)

Pruning (cm) and fertigation (mL)				
Table	Gutter 1	Gutter 2	Gutter 3	Gutter 4
1	5 cm + 0 M	10 cm + 0 mL	15 cm + 0 mL	No cut + 0 mL
2	5 cm + 0.0072 M	10 cm + 0.0072 M	15 cm + 0.0072 M	No cut + 0.0072 M
3	5 cm + 0.0144 M	10 cm + 0.0144 M	15 cm + 0.0144 M	No cut + 0.0144 M
4	5 cm + 0.0216 M	10 cm+ 0.0216 M	15 cm+ 0.0216 M	No cut+ 0.0216 M

Note: Each gutter of each treatment contained ten plants, one plant per pot, for a total of 40 plants in each hydroponic system and 200 plants during the entire experiment. Plant heights were cut back (cm), and Potassium nutrient concentrations (mL) applied. Cutting back, 5 cm is light pruning, 10 cm is moderate pruning, and 15 cm is heavy pruning.

6.4 Data collection

6.4.1 Flower bud collection

The flower buds were collected manually by a pruner shear at the height ranges from 8-12 cm during and at post- harvest as the flower bud did not mature or develop at the same time and some plants produced multiple flower buds. Each plant had its specific marked brown paper bag (16 x 24 cm) where the flower buds stored. After some data recorded such as flower buds' weight, the buds produced in each system per gutter were combined nutritional content was analysed.

6.4.2 Nutritional analysis

The nutrient uptake was only analysed on the flower buds of the plant. The plant flower buds of each gutter per system were mixed in the same brown paper bag (16 x 24 cm) according to their respective concentrations and pruning levels during the experiment and at post-harvest stage. After the experiment the marked brown paper bags containing flower buds were taken to the air forced oven (Dainhan Labtech LDO-150F). The plant edible parts were grinded using a capacity standard coffee grinder (Mellerware - Aromatic Coffee bn Mill & Grinder) and packed, labelled, and sent out to the Department of Agriculture and Rural Development analytical laboratory, Province of KwaZulu Natal in powdery form for proximate analysis.

6.4.3 Mineral analysis

The mineral composition of each set of replicates in the experiment was determined using the Inductively in the analytical laboratory of the Department of Agriculture and Rural Development, KwaZulu Natal Province was used to carry out the elemental analysis as described by Sogoni *et al.* (2021). The Coupled Plasma- Optical Emission Spectrometer was used to determine mineral composition of each set of replicates in the experiment at analytical laboratory of the Department of Agriculture and Rural Development, KwaZulu Natal Province, was utilized to carry-out the elemental analysis as outlined by Sogoni *et al.* (2021).

6.5. Proximate Analysis

6.5.1 Moisture content

The procedures outlined by Jimoh *et al*. (2020) with slight modification were used to determine the moisture content. Empty porcelain containers were dried in an oven at 105 °C for an hour, cooled, and weighed W1. Approximately 1 g of the pulverized samples of *T. divaricata* were placed in a receptacle and oven-dried to a constant weight at 105 °C. Prior to being reweighted (W3), the jar and its contents were cooled in a desiccated. The equation below was used to get the moisture content percentage.

% Moisture content = $\frac{W2 - W3}{W2 - W1}$

6.5.2 Crude Fibre content

This was evaluated with slight adjustment in accordance with Miechowska and Dmoski (2006) description. A digestive tablet was used to boil 2 g of powdered materials in 100 mL of 12.5% concentrated H_2SO_4 for 30 minutes, then filtered under pressure. After digestive residue being repeatedly washed with boiling water until the clear mixture was achieved, then was rinsed with 100 ml of 12.5 NaOH solution. The resulting residue was cooled in a desiccated, dried at 100 °C and weighed (F1). The residues ere then chilled in a desiccated environment for 5 hours in a muffle furnace at 550 °C before being re-weighed (F2). The following estimation was made for the crude fiber percentage:

% Crude fibre = $\frac{\mathrm{F1} - \mathrm{F2}}{\text{Original weight of the pulverised sample}} \times 100$

6.5.3 Crude fat content

The Association of Official Analytical chemists (AOAC), (2016) criteria were followed while calculating the crude fat. On an orbital shaker for 24 hours, approximately 1 g of the powdered sample was extracted in 100 mL of diethyl ether. After filtering the mixture, the filtrate collected in clean beakers that had previously been weighed (W1). The ether extract was then mixed with 100 mL of diethyl ether to equilibrate it. The mixture was then agitated for a further 24 hours on an orbital shaker, and the filtrate was collected in a beaker (W1). After being dried out in a steam bath and oven-dried at 55 C, the ether filtration was weighed in the beaker (W2). Thus, the crude fat content was determined as follows.

% Crude fat content = $\times 100$

6.5.4 Ash content

The technique created by the Association of Official Analytical Chemists (AOAC, 2016) was used to calculate the percentage of ash content in plant samples. After being marked with sample codes with a heat-resistant marker, porcelain crucibles were over-dried at 105 °C for an hour. After cooling in a desiccated (W1), the crucible was weighed. Thereafter, 1 g of ground samples were heated to 550 °C for 5 hours after being placed in a muffle furnace set to 250 °C for 2 hours. The samples were weighed (W3) after cooling in a desiccant. The samples of an ash content were determined as follows.

% Ash content = $\frac{\mathrm{W2-W3}}{\mathrm{W2-W1}} \times 100$

6.5.5 Crude protein

This was discovered by heating 2 g of pulverized materials and 20 mL of concentrated H_2SO_4 to a clear mixture while using as a catalyst (Adegbajubet *et al*., 2019). Following filtration and distillation, the digested extracts were dissolved in 250mL. Thereafter a second distillation in 500 mL round-bottom flask using the aliquot containing 50 mL of 45% NaOH, 150 mL of the distillate was placed into a flask containing 100 mL of 0.1 M HCl. A yellow colour signalled the endpoint of the titration process, and equation below was used to compute the nitrogen content percentage.

CP=$\frac{[(\text{ml std} \times \text{N of acid}) - (\text{ml bank} \times \text{ N of base})] - (\text{ml std base} \times N\ of\ base) \times 1.4007}{\text{Pulverised sample original weight}}$

Where N= stands for the crude percentage and normality were acquired by multiplying the nitrogen value by a constant factor of 6.25 (Idris *et al*., 2019)

6.5.6 Neutral detergent fibre (NDF)

The NDF composition of the samples was determined using the equation below, as described by (Idris *et al*., 2019).

% NDF = $\frac{(W1+W2)-W1}{\text{sample weight}} \times 100$

6.5.7 Non-fibre carbohydrate (NFC)

The sample's non- fiber carbohydrates samples were compute using the formula presented below.

% NFC = 100 - (% Ash + % Crude fat + Crude protein + % NDF)

6.5.8 Energy content

The combination of the various values for crude lipids (excluding fibre), crude protein and total carbohydrates using factors (37 KJ, 17 KJ and 17 KJ) by means of FAO (2003) conversion factor, the energy content of each sample of *T.divaricata* from various treatments was calculated. Energy content (KJ/100 mg) = $(\text{crude protein} \times 17) + (\text{Crude fat} \times 37) + (\text{CHO} \times 17)$

Where CHO indicate the total carbohydrate as determined by the equation below; (Tylutki et al., 2008).

CHO = NFC + NDF

6.6 Results

6.6.1 Macronutrients

The level of accumulation of macro elements such as potassium (K), phosphorus (P), nitrogen (N), calcium (C), magnesium (Mg) in the elevated flower bud samples is presented in table 6.7.1. The increasing of mineral yields per 100 g of plant sample is Mg < K < Na < P < Ca. In all evaluated treatments, including the control, different potassium application, cutting levels and their interaction did not significantly influence the Ca yield of the flower buds, however, Mg was positively influenced by the treatments (Table 6.7.1). Samples from the treatment 0 M + 10 cm cutting had the highest Mg yield, which was statistically comparable to the control + 5 and 15 cm pruning levels, respectively. Contrarily, the Na composition of the samples varied in all treatments, and the treatments with highest value was 0.0144 M + 10 cm pruning. similarly, treatments 0.0144 M + 10 cm pruning had the highest potassium composition, however, this was not statistically different from control,0 ml + 5 cm, 10 cm, and 15 cm pruning, respectively. Furthermore, treatment 0.0144 M with the following levels of pruning (no cut, 5 cm and 10 cm) have obtained the highest values of nitrogen compositions compared to all other treatments, including the control and the values were noticeably higher. In addition, treatments 0. 0144 M with no cut, 5 and 10 cm showed the highest values of N. These values were noticeably greater than those of the control and all other combination treatments. In contrast, the treatment with the highest yield of K was 0.0072 M with no cut, which was much higher than all other treatments, including the control. (Table 6.5.1).

6.6.2 Micro-elements

Table 6.5.3 shows the extent of the accumulation of heavy metals like magnesium (Mn), iron (Fe), cupper (Cu) and Zinc (Zn) in the raised flower bud samples. The accumulation of heavy metals in *T. divaricata* was significantly influenced by potassium application and pruning levels in this study compared to control treatment. The treatment 0.0144 M with 10 cm of pruning had the highest yield of Zinc, which was significantly higher than any other treatment, including the control treatment. Similarly, the highest Mn yield was obtained in treatment 0.0072 M with no cut, which was significantly higher than all other treatments, including control treatment. This was different for Cu, where the highest yield (0.53 mg/100 g) was obtained from treatment with 0.214 M and no cut, respectively. The same pattern was observed for iron, with the highest yield (13.3 mg/100 g) obtained from treatment 0.0144 M with no pruning treatment (Table 6.5.2).

Figure 6.6.2: The flower quality of buds were collected in the hydroponic system under 0.0072 M where each plant produced more than two flower buds (Bulawa, 2021).

Table: 6.6.2: Major elements obtained from flower buds of *Trachyandra divaricata* grown in hydroponics under different potassium dosages and levels of pruning.

K_2SO_4	Pruning	Ca (mg/100 g DW)	Mg (mg/100 g DW)	Na (mg/100 g DW)	P (mg/100 g DW)	N (mg/100 g DW)	K (mg/100 g DW)	K/Ca+Mg (mg/100 g DW)
`230 M	No cut	610 ± 0.01ab	340 ± 0.01ab	220 ± 0.00c	740 ± 0.01ab	3790 ± 0.07b	5700 ± 0.01hi	2530 ± 0.05h
	5 cm	480 ± 0.01abc	340 ± 0.01ab	190 ± 0.00cde	700 ± 0.01abc	3630 ± 0.07b	5460 ± 0.02ij	2580 ± 0.09h
	10 cm	650 ± 0.01ab	370 ± 0.02a	180 ± 0.01def	700 ± 0.01abc	3800 ± 0.00b	5130 ± 0.01jk	2030 ± 0.017i
	15 cm	640 ± 0.01ab	350 ± 0.00a	210 ± 0.01cd	660 ± 0.02cde	3690 ± 0.05b	5130 ±0.09jk	2160 ± 0.00i
0.0072 M	Unpruned	260 ± 0.01cd	170 ± 0.01e	120 ± 0.01h	670 ± 0.01cd	3550 ± 0.08b	9460 ± 0.78a	9490 ± 0.02a
	5 cm	440 ± 0.01ab	280 ± 0.28bc	130 ± 0.00gh	710 ± 0.01abc	3690 ± 0.05b	6490 ± 0.03ef	3750 ± 0.05g
	10 cm	250 ± 0.01cd	210 ± 0.01cde	120 ± 0.00gh	750 ± 0.01a	3770 ± 0.06b	7090 ± 0.04cd	5680 ± 0.14b
	15 cm	390 ± 0.0bc	250 ± 0.01cd	130 ± 0.01gh	740 ± 0.01ab	3700 ± 0.058b	7530 ± 0.07b	7530 ± 0.07d
0.0144 M	No cut	280 ± 0.01cd	240 ± 0.01cde	270 ± 0.01b	670± 0.24cd	4300 ± 0.06a	6460 ± 0.03ef	4930 ± 0.04d
	5 cm	330 ± 0.01bc	250 ± 0.01cd	140 ± 0.01fgh	660 ± 0.15cde	4300 ± 0.06b	5820 ± 0.0h	4180 ± 0.04ef
	10 cm	310 ± 0.01bc	260 ± 0.00cd	340 ± 0.01a	760 ± 0.01a	4470 ± 0.09a	7160 ± 0.07bc	4950 ± 0.01d
	15 cm	270 ± 0.01cd	230 ± 0.00cde	180 ± 0.01cde	620 ± 0.01def	4340 ± 0.02a	6730 ± 0.03 def	5350 ± 0.02c
0.0216 M	No cut	700 ± 10.2a	220 ± 0.01cde	270 ± 0.01b	590 ± 0.01ef	2970± 0.03cd	6360 ± 0.04fg	3910 ± 0.06fg
	5 cm	230 ± 0.01cd	210 ± 0.01cde	130 ± 0.01gh	680 ± 0.02bcd	3530 ± 0.145b	3530 ± 0.15k	4150 ± 0.02ef
	10 cm	240± 0.01cd	270 ± 0.03bc	300 ± 0.01b	560 ± 0.02cde	3170 ± 0.07c	6770± 0.13de	4400 ± 0.05e
	15 cm	500 ± 0.88ab	190 ± 0.01de	160 ± 0.01efg	550 ± 0.02f	2800 ± 0.06d	6070 ± 0.027fg	4760 ± 0.04d
				Two-way ANOVA F-Statistic				
K_2SO_4		1.06*	75.85*	137.70*	43.30*	181.01*	706.71*	2898*
Pruning		0.96ns	5.40*	100.60*	19.48*	21.50*	228.54*	519*
K_2SO_4*pruning		0.95ns	4.72*	45.47*	11.32*	9.99*	92.39*	532*

Note: Tukey's LSD was used to rank the means along the column with a 0.05 significance threshold. Means that do not share a letter are very dissimilar. *: significant at $p \leq 0.05$.

Table 6.6.3: The present micro-nutrients on the *T.divaricata* influenced by different potassium dosages and levels of pruning grown in hydroponics

K_2SO_4	Pruning	Zn (mg/100 g DW)	Mn (mg/100 g DW)	Cu (mg/100 g DW)	Fe (mg/100 g DW)
0 mL	Unpruned	10.8 ± 1.15bcd	4.23± 1.20def	0.5. ± 0.00a	12.3± 0.88abc
	5 cm	8.9 ± 0.58ef	2.9± 0.88jk	0.373±0.15bc	12.23± 0.67bc
	10 cm	8.4. ± 1.15fg	3.36± 0.33ij	0.464 ± 0.31ab	8.9 ± 0.58fg
	15 cm	10.2 ± 1.15bcd	3.8± 1.00gh	0.5 ± 0.00a	8.72± 0.64fg
0.0072 M	Unpruned	10.9± 1.53bc	6.7± 0.58a	0.2 ± 0.00d	9.23± 2.03ef
	5 cm	8.6 ± 0.88f	4.4± 1.15de	0 ± 0.00f	7.1± 0.58h
	10 cm	7.3 ± 0.58h	4.9± 0.58bc	0.1± 0.00e	5.6± 1.53i
	15 cm	11± 0.58b	5.2 ±1.20b	0.1± 0.00e	8.2± 1.13g
0.0144 M	Unpruned	10.8 ± 1.53bcd	3.8± 0.58gh	0.4± 0.00bc	13± 0.58ab
	5 cm	10.1± 0.58bcd	4.2± 0.58efg	0.4± 0.00bc	8.1± 1.15g
	10 cm	12.6± 0.88a	4.03± 0.35efgh	0.4± 0.00bc	12± 4.51c
	15 cm	9.8 ± 0.58de	2.9± 0.58k	0.2± 0.00d	13.3± 2.65a
0.0216 M	Unpruned	10.16± 1.67bcd	3.9± 0.58fgh	0.537± 0.34a	10.7± 3.71d
	5 cm	9.83± 6.94cde	4.633± 0.88cd	0.357± 0.32c	8.8± 0.56fg
	10 cm	8.86± 0.33ef	3.7± 0.58hi	0.370± 0. 21bc	9.43± 0.89ef
	15 cm	7.5± 2.52gh	2.8± 0.00k	0.467± 0.41ab	10.13± 0.89de
Two-way ANOVA F-Statistic					
K_2SO_4		2017.8*	2364.29 *	96.497*	10710.7 *
Pruning		1456.4*	597. 59 *	10.313*	4358.4 *
K_2SO_4*pruning		5253.8*	1365.23*	14.407*	7284.4 *

Note: Tukey's LSD was used to rank the means along the column with a 0.05 significance threshold. Means that do not share a letter are very dissimilar. *: significant at $p \leq 0.05$.

6.6.3 The influence of potassium and pruning on proximate composition of flower buds

This experimental trial revealed significant effects in proximate contents in flower buds of hydroponically grown *T. divaricata* under different potassium concentrations and levels of pruning regarding ash, non-fibre-carbohydrate, crude protein, moisture, energy values n neutral detergent fibre, and crude fat (Table 6.5.3). The highest ash content of the flower buds was recorded in treatments 0.0072 M + 15 cm pruning respectively. The values obtained were positively significant than all other treatments including the control. Th. On the contrary the highest crude fat content was recorded from treatment 0.144 M + 15 cm pruning. However, this did not differ from the control, 0 M + 5 cm, 0.0072 M +10 cm, 0.0144 M with no pruning and 0.0216 M with no pruning respectively. Moreover, the highest yield of crude protein was recorded in treatment 0.0144 M with 5 cm pruning, and this was significantly higher than all treatments including the control. Similarly, the highest NDF (37.8 %) was significantly higher than all treatments including the control (Table 6.5.4).

The content of NFC was high in treatment 0.0216 M + no cut, and this was similar with treatment 0.0216 M in all pruning levels. The treatments with the highest moisture percentage were 0.0072 M + 15 cm and 0.0.144 M + 5 cm pruning, respectively. The energy content of *T. divaricata* flower buds, measured in (KJ/ 100g), differed between treatments. According to an analysis of the results, plants that were pruned into height of 15 cm without the potassium application had the highest energy density (1507.3 mg/ 100g), which was equivalent to many other treatments, including the control treatment.

Table 6.6.4: The influence of various potassium concentrations and levels of cutting on proximate composition of flower buds of hydroponically grown *Trachyandra divaricata*

K_2SO_4	Pruning levels	% Ash	% Crude fat	% Crude protein	% NDF	% NFC	% Moisture	Energy value (KJ/100 g)
0 mL	Unpruned	15.7 ± 0.02bcd	2.2± 0.05abc	24.2 ± 0.04cd	33.2± 0.2cd	24.6± 0.2def	10.08± 0.04g	1476.4± 0.3ab
	5 cm	14.9 ± 0.20cd	2.3± 0.08abc	23.6 ± 0.04cde	31.8± 0.4ef	27.2± 0.4bcd	10.3± 0.1efg	1491.1± 2.2ab
	10 cm	14.5 ± 0.20d	2.0 ± 0.05bc	24.1 ± 0.01cd	34.6± 0.1bc	24.7± 01de	10.5± 0.07defg	1493.5± 6a
	15 cm	14.0 ± 2.30d	2.0 ± 0.02bc	23.46 ± 0.1de	35.3± 0.2b	25.1± 2.5de	10.23±0.08fg	1503.2± 3.2a
50 mL	Unpruned	23.6 ± 0.30a	1.9 ± 0.02bc	22.3 ± 0.2ef	37.8± 0.2a	14.2± 0.4g	10.9± 0.03cde	1335.7 ±0.5d
	5 cm	16.0 ± 2.10bcd	2.0 ± 0.01bc	23.7 ± 0.4cde	32.3± 0.3de	25.8± 2.1cde	10.8± 0.1cdef	1469± 0.3abc
	10 cm	19.1 ± 0.05bc	2.1± 0.06abc	23.7 ± 0.1cde	31.6± 0.3ef	23.3± 0.2def	12.19± 0.1a	1417.4± 0.2bc
	15 cm	20.0 ± 0.04ab	1.8 ± 0.01bc	23.19 ± 0.2def	35.05± 0.08b	19.85± 0.3f	11.8± 0.08ab	1395± 0.4cd
100 mL	Unpruned	16.5 ± 0.30bcd	2.3 ± 0.08ab	25.1 ± 0.3bc	31.94± 0.08def	24± 0.6def	11.4± 0.2bc	1464.9± 4abc
	5 cm	14.8 ± 0.10cd	1.9 ± 0.02bc	23.5 ± 0.3de	30.± 0.1gh	26.5± 0.2cde	11.82± 0.01ab	1486.8± 1.8ab
	10 cm	16.8 ± 0.01bcd	2.3 ± 0.1ab	26.0 ± 0.1ab	32.6± 0.3de	22.1± 0.4ef	11.46± 0.1bc	1459.9± 2.1abc
	15 cm	17.1 ± 0.07bcd	2.6 ± 0.29a	26.6 ± 0.3a	31± 0.01fg	25.7± 0.02cde	11.± 0.06cd	1461.2± 2.1abc
150 mL	Unpruned	15.7 ± 0.20bcd	2.1 ± 0.08abc	22.5 ± 0.3ef	26.9± 0.1j	59.5± 0.5ab	10.9± 0.03cde	1475.5± 2.1ab
	5 cm	13.3 ± 0.10d	1.7 ± 0.06c	24.3 ± 0.2cd	30.1± 0.04gh	60.5± 0.2a	10.2± 0.1fg	1507.3± 3.1a
	10 cm	15.6 ± 0.20cd	1.9 ± 0.01bc	23.2 ± 0.2de	28.9± 0.2hi	59± 0.1ab	9.9± 0.2g	1473.2± 3.6ab
	15 cm	16.2 ± 0.30bcd	1.9 ± 0.1bc	21.7 ± 0.5f	28.2± 0.3ij	60.1± 0.4a	10.1± 0.1fg	1462.2± 3.3abc
Two-way ANOVA F-Statistic								
K_2SO_4		28.05*	10.56*	56.51*	394*	85.82*	103.53*	29.46*
Pruning		9.59*	1.32ns	26.97*	24.2*	10.59*	3.04*	8.56*
K_2SO_4*pruning		3.02*	3.80*	6.31*	58.38*	8.85*	14.15*	3.18*

Note: Turkey"s LSD was used to rank the means along the column with a 0.05 significance threshold. Means that do not share a letter are very dissimilar. *: significant at $p \leq 0.05$. * NDF= Neutral Detergent fibre; NFC= Non-Fibre Carbohydrates, ns= not significant

6.7 Discussion

Indigenous coastal edible crops were once popular in the early days before the colonization, as plants such as Amaranthus were consumed in the pre- Colombian America, Aztec, Africa (Li *et al.,* 2015). Coastal plants provide various proximate, macro and micronutrients. Schroeder & Backhed, (2016) have reported that excessive amount of Ash content gives an indication that the plant is rich in dietary fibres that protects digestive organisms in the alimentary tract. In the present study *Trachyandra divaricata* has accumulated the highest value (23.6 %) of Ash and this value was the highest compared to other treatments (Table 6.3). These results are similar with study done by Jimoh *et al.* (2020) on *A. caudatus* who recorded more than 5% of ash content, which is the minimum required value. Mlakar *et al.* (2009); Nascimento *et al.* (2014) and Soria- Garcia *et al.* (2018) reported on their studies lower results compared to those collected by the present study, as they have accumulated 7.1%, 7% and 6.43% on other salt tolerant plants. In contrary, Akin- Idowu *et al.* (2018); Nascimento *et al.* (2014) results produced otherwise results earlier as they had less than 5% of Ash content.

Trachyandra divaricata contains the lowest values of crude fat, as the significant treatment 0.0114 M + 15 cm obtained the lowest values, and this is evident that K_2SO_4 + cutting back had less effect on the protein. These results agree with those of Bressani (2018), who recorded the lowest values of crude fat from *A. cactus* under different soil at different growth stages. Bressani (2018), further explained that most plants with high Ash are likely not to contain high amount of crude fat. Hence, it can be concluded that ash content act conversely with fat. However, crude fat is commonly known for its major role in human diet, organoleptic properties, and absorption of fat-soluble vitamins (Jimo *et al*., 2020). Jimoh *et al.* (2020) also mentioned that food plants with high fat (unsaturated) content could be associated with chronic and cardiovascular diseases such as obesity, heart complications. Therefore, *T. divaricata* with low fat could be used as a dietary supplement formulated for weight loss purposes.

The minimal preferred standard number by the United States Department of Agriculture for protein value of *Amaranthus* is 13.6% (Jimoh *et al*., 2018). The minimum preferred protein value recorded in *Amaranthus* species is doubled by the crude protein value of 26.6% recorded in *T. divaricata* flower bud. Comparing the protein values of other various studies of *A. caudatus*, Mekonnen *et al. (*2018) obtains 14.96%, Nascimento *et al.* (2014) recorded 13.4% and Tapia- Blacido *et al.* (2007) recorded 15.1%. Thus, findings from this study implies that *T. divaricata* is rich in crude protein. These results are further supported by a study done by Alli *et al*. (2021) where 50% of K_2SO_4 had the best results on the quality of potatoes resulting in specific quality parameters such as starch, sugars and protein content. These results are followed by the 100% of K_2SO_4. In addition, starch synthesis (carbohydrates) in potatoes is associated with the potassium availability. This is in correlation with our study despite the plant, as 0.0144 M of K_2SO_4 had proved to alleviate crude protein as well NFC in 0.0144 M of K_2SO_4. However, cutting back could be detrimental as it is unusual practise in plants and associated with stress. However, in this study it was different case as it has significantly influenced *T. divaricata* on the accumulation of proximate (crude protein, NFC, and energy content). Shabala & Pottosin (2014) agrees with statement above, with the emphasis that potassium assists plants in adapting to biotic and abiotic stressors.

The combination of high potassium concentration (0.0216 M mL) together with unpruned recorded the highest value of non-fibre carbohydrates (NFC) content (60.5± 0.2) and that was the only treatment that has an outstanding value compared to other concentrations. The results of this study are way higher than those of Jimoh *et al.* (2020) who recorded 17.51 % at the post-harvest of *A. caudatus*. However, the values are still slightly low compared to the results of Amaranthus which proves to be rich in this carbohydrate as it recorded 61.4 % and 52.18% in the study done by Alvarez-Jubete *et al.* (2010) & Akubugwo *et al.* (2007) respectively. Jimoh *et al.* (2020), has suggested that the low values of carbohydrate might be traded off for protein production in *A. caudatus*. Therefore, the claims made by Jimoh *et al.* (2020) are also supported by (Elsheikha & Elzidany, 1997) that protein is in inverse manner with carbohydrates. Moreover, the highest values of Ash content might be the reason of low content of protein. With *T. divaricata* managed to have high values in almost all the compounds make these different potassium sulphate concentrations + various pruning levels to be the suitable growing protocol. The results of energy have recorded the lowest value on 0.0072 M + unpruned, while 0.0216 M + 5 cm recorded the highest value, and this suggests that employing 0.0216 M + 5 cm has the best energy levels on *T. divaricata*. Thus, the results gathered in this study agree with Jimoh *et al.* (2020) who reported that protein, carbohydrates, fats, and fibre are the small building blocks of energy so when these compositions have the high values, energy will ultimately have the high value.

Minerals are inorganic substances that perform vital metabolic processes that cannot be generated by living organisms (Alemayehu *et al.,* 2021). The structural functions of soft tissues and the skeleton, as well as regulatory processes including blood coagulation, oxygen transport, neuromuscular transmission, and enzyme activity, are all significantly influenced by minerals (NCR, 1989).

The calcium shows to be significantly influenced by the K_2SO_4, while cutting back was not significant. Considering the fact that calcium (Ca) is essential for neuron function, muscular contraction, and cell signalling. By preserving healthy bones and teeth, it also supports the skeletal system. When communicating with other cells, calcium is used by body cells to activate certain enzymes, move ions across cellular membranes, and send and receive neurotransmitters (Sadler, 2011). The result of this study is far higher compared to the results obtained by Alemayehu *et al.* (2021) on the different Oat (*Avena sativa*) varieties which is also an underutilised cereal grain in Ethiopia, their highest values ranged from 44 to 102.7 mg/100 g. Wasonga *et al*. (2020), claimed that the excessive application of potassium rate lead to higher rates of the following nutrients, Ca, P, Mg, Zn, Fe and S both in leaves and roots regardless of the irrigation dose.

Magnesium serves as a co-factor for enzymatic activities, releases neurotransmitters, promotes cell adhesion, and maintains calcium-potassium equilibrium and structural roles in nucleic acids, proteins and polyribosomes are vital roles played by Mg (Rude 1998; Stein, 2010). Potassium together with cutting back the plants had no influence on the magnesium composition of *T. divaricata* as the highest value of Mg was obtained at 0 mL + no cut. However, the fact that *T. divaricata* has accumulated the highest value (370 mg/100 g) that is more than (55 mg/ 100 g) of cooked *Amaranthus* food recommended by USDA (2018), suggests that *T. divaricata* is a rich source of magnesium. The study of Adegbaju (2019), had the same results as *C. argentea* has not significant at 53.9 mg/ 100 g on different growth levels and the (450 mg/ 100 g) to meet the recommended daily consumption magnesium for survival. However, the recommended value of Amaranthus is also far below to all the values gathered from all the treatments in the present study. Mekonne *et al.* (2018); Nascimento *et al.* (2014) on the proximate analysis of *A. caudatus* had slightly similar results of Mg compared to those of this study. Thus, this implies that plants that have high level of Mg content such as *T. divaricata* they could be used to supplement.

Howeler (2014), also found that over-supplied with K cassava had decreased Ca, Mg, and S levels. The combination of these elements (K/CA + mg) have yielded positive results of potassium supplemented together with cutting back *T. divaricata. The value of* 9490 mg /100 g considering the daily recommended content of both Ca+ Mg being mentioned above, this will provide the human body with the necessary benefits of these nutrients. The significance of each of these elements have already been discussed above.

Sodium is a micronutrient that is required for human body, about 1500 mg recommended daily allowance (RDA) for adult (McCarron *et al.*, 2013). However, He & MacGregor (2009), have urged that the RDA of 200 -500 mg of sodium for healthy living. K_2SO_4 together with cutting back have proven to influence sodium content of *T. divaricata* as the highest rate was recorded at 0.0114 M + 10 cm. A sodium hormone regulator termed aldosterone, which functions differently depending on sodium level, is housed in the renal tubule of the kidney. Despite the kidney's ability to retain salt, some significant sodium losses still happen through sweating and defaecation, which may not always necessitate supplementation with sodium (Aburto *et al*., 2013; Food and Nutrition Board, 1989; Stein, 2010). Phosphorus is another micronutrient that is required about 700 mg/ day quantity (Chang *et al*., 2014).

Phosphorus is one of the main essential micro-nutrients that is significant for cell growth and maintenance, also with kidney functioning and repair (Adegbaju *et al.* 2019). Considering the fact that it also plays a crucial role on the formation of Adenosine triphosphate (ATP), DNA and RNA. In addition, phosphorus promotes calcium absorption and preserves the acid-base balance (Gharibzahedi & Jafari, 2017).

In this study, the highest value of phosphorus has superbases 760 mg/ 100 g at 0.0114 M + 10 cm and this quantify the plant to be considered as phosphorus supplement as the potassium and cutting back had also significant influence on the plant obtaining the highest value. Adegbaaju *et al*. (2019) have endorse *C. argentea* as a good phosphorus supplement by obtaining 845 mg/ 100 g. This is similar to the study done by Makobo *et al*. (2010) who recorded the higher amount of P of *A. cruentus* at the early growth stages. Thus 118.3 mg of *T. divaricata* could be able to deliver the daily requirement of 1000 mg/ day for adults as suggested by (NHMRC, 2006).

Potassium is a vital mineral needed by the body for controlling blood pressure, nerve transmission, muscle contraction, regulating waste disposal, and maintaining optimum fluid balance (Gharibzahedi &Jafari, 2017). WHO (2012) have suggested that at least 3510 mg of potassium should be consumed daily by adults. Thus, potassium sulphate has managed to elevate the P composition of *T. divaricata* as it acquired 9460 mg/ 100 *g.* The results of this of study have obtained high values compared to the average potassium of different varieties of oats (248.3 mg/ 100 g) Alemayehu *et al.* (2021) and Ozcan *et al.* (2017) had previously recorded lower values. The potassium results of this study are even higher than the staple food as Mckevith (2004) reported their values as follows, Wheat (150 mg/100 g), rice (250 mg/100g), maize (220 mg/100 g) and barley (270 mg/100 g). Therefore, reported figures in this study potassium supplemented *T. divaricata* is a potential source of potassium.

Trachyandra divaricata has accumulated numerous micronutrients such as Fe, Zn, Mn, and Cu. These types of nutrients also play significant in human body but are frequently required in small amounts unlike macro- nutrients. The different potassium concentrations and cutting back the plants have been significant on the accumulation of all micronutrients. However, cutting back the plants in most cases causes stress to the plants but the potassium seems to have mitigate the stress as it is one of the potassium functions because the all the micro-nutrients are significant.

Iron (Fe) is one the most insufficient micronutrient on the school children and adults as a result many are using medicinal supplements. Iron is usually associated with anaemia, fatigue, and other blood related diseases (Haimi & Lerner, 2014). The amount of 9 and 15 mg/day of an iron is recommended for children and adults (Jahnen-Dechent & Ketteler, 2012). However, Hurrell & Egoli (2010), have emphasized that the minimum Fe requirement for adults is 1.8 mg/ day. *Trachyandra divaricata* have accumulated 13.3 mg/ 100 g, which quantify the plant to be consumed daily as it will meet the RDA of iron. Therefore, the result of this study agrees with Wasonga *et al*. (2021) who had proved that potassium had a positive effect on Cassava plant as the highest values were obtained on the maximum potassium concentration (32 Mm). The Zinc (Zn) is needed by human body for healthy growth and maintenance. It is an essential element of more than 300 enzymes involved in the production and breakdown of lipids, proteins, nucleic acids, carbohydrates, and carbohydrates (WHO, 2005). It is present in many biological systems and processes and is essential for the thyroid, blood coagulation, immune system, and wound healing processes. Potassium together with cutting back *T. divaricata* as the treatments has played a positive impact on the accumulation of Zn as it has obtained highest value at 0.0072 M + 10 cm. This is comparable with study done by Wasongo *et al.* (2021), who recorded highest amounts in 32 Mm (30.2) on the leaves of cassava. Thus, *T. divaricata* have accumulated the highest value of Zn compared to the results of an oats (1.6 mg to 2.1 mg / 100 g) provided by Alemayehu *et al*. (2021). Mn (0.0072 M + no cut). Manganese (Mn) serves as a co-factor for several enzymes and is essential for the efficient operation of the neurological system throughout the body, including the brain (Jahnen-Dechent & Kettele, 2012). While, the efficient functioning of organs depends on copper, which also functions as a co-factor and a component of various enzymes, Cu also boosts the immune system's ability to combat infections (DiNicolantonio *et al.,* 2018). Copper (Cu) compound have been significantly influenced by the potassium and cutting back of *T. divaricata* as the highest value is 6.7 mg/ 100 g. Therefore, *T. divaricata* could not provide sufficient Cu at all different potassium sulphate and cutting as John et al. (2009) suggested that it does not meet the RDA for Cu as the RDA is specified (0.7 or 1.1 mg/ 100 g per day) for children including adults. The present results suggest that *T. divaricata* supplemented with K_2SO_4 has low values of Cu because the highest value is 0.54 mg/100 g. This suggest that only plants like *Celosia argentea* could manage to provide with sufficient Cu, Adegbaju *et al.* (2019), recorded the highest value of 7.65 mg/ 100 g in his study. However, *T. divaricata* had the higher values than those obtained by Alemayehu *et al*. (2021), different oats varieties that range from 0.2 to 0.4.2 mg/100 g. This agrees with the suggestion that micronutrients are required in small quantities in human body unlike macronutrients.

6.8 Conclusion

This study revealed that potassium sulphate has managed to work conjunction with cutting back because almost all the minerals, micro and macronutrients are significantly influenced. Macro nutrients such as N, P, and K are found to be high in this plant which means the utilization of this underutilised plants could be beneficiary as they have potential to combat malnutrition. The study also reveals that potassium sulphate could mitigate physiological stress induced by cutting back. Therefore, *T. divaricata* contains essential minerals in adequate proportion required in a balanced diet and may be considered as conventional crop to foster food security.

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6.10 References

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CHAPTER 7: THE GENERAL DISCUSSION AND CONCLUSION

7.1 General discussion

The shortages of nutrient supply, inadequate groundwater, and salinization of soil have become a major challenge worldwide (Singh *et al., 2014*). Salinization, which refers to the presence of salts in the soil or water, is a common challenge that has impacted half of the land in southern Africa (Dimsey, 2006). The above-mentioned challenges have affected food production severely in such that conventional crops struggle to strive under such circumstances. Therefore, this is an alarm for food producers to adopt new strategies that could be imperative for food production or domestication of ancient wild food plants. The potassium supplementation strategy reported to mitigate the plant growth and development in salt affected areas and other stress conditions (Ngxabi *et al.,* 2021). This becomes interesting as Kausar & Gull (2014) discovered that wheat and barley grown in the wild were able to survive under salt stress because of potassium supplementation. Potassium has been reported to be lacking in most arable land and therefore, the addition of this micronutrient is essential as the plant uses it in stressful conditions to enhance their growth and development (Hussain *et al.,* 2013). However, a combination of potassium supplementation and pruning has never been investigated before, but few studies have attested that pruning is beneficial to dense plants as it encourages new growth and allocates nutrients to edible parts (Badrulhisham & Othman, 2016). This will encourage the extension of the extinct knowledge and exploitation of underutilised edible species such as *T. divaricata* as part of solving food insecurity crisis and embracing diversity.

The effect of different potassium concentrations and pruning levels on the growth of hydroponically grown *T. divaricata* was investigated in chapter 3. The morphological growth parameters such as height, weight, flower bud yield, total dry and wet weight of shoots and roots were significantly affected by different potassium concentrations and pruning levels. Plants that were fertigated with 0.0072 M K_2SO_4 had the highest values in terms of morphological growth parameters. Similar results have been reported on the length and shoot ratio of wheat and barley supplemented with potassium under saline condition (Hussain *et al.,* 2013). Likewise, Wanshnong *et al*. (2022) reported the same findings on *Papaya* var. Red Lady, where the application of potassium increased the plant height, plant girth and the total number of leaves. In addition, pruning of some flowering and fruit plants are mostly likely to have high and nutritious yield when the leaves are pruned (Badrulhisham & Othman, 2016). However, in this study no cut and 15 cm cutting had the highest values in almost all the parameter while 5 & 10 cm had smallest values. This may be because *T. divaricata* has its specific pruning level.

The minimum concentration of K_2SO_4 had the highest formation of flower buds, fresh and dry weight. These results may be due to the claim made by Sardans and Peñuelas (2021) that potassium plays a crucial role in plant development and making a point of interest in improving yield. In addition, the highest cutting level (15 cm) and no cut recorded the highest formation of flower bud and fresh/dry weight. Orsini (2016) has claimed that pruning is vital in plants that produce flowers and fruits as it encourages the new growth and more stems. Similar results were reported by Pongki and Tjokrosumarto (2017), on the growth of chilies as pruning encouraged maximum fruit production.

The effect of different potassium concentrations and cutting levels on chlorophyll content of *T. divaricata* was evaluated in chapter 4. The minimum K_2SO_4 concentration (0.0072 M) had the significant results on the chlorophyll content compared to other concentrations. Also, 15 cm and no cut recorded the positive results of chlorophyll yield in *T. divaricata.* These findings suggest that 0.0072 M and 15 cm or no cut could be the optimal protocol for chlorophyl yield of *T. divaricata*. This study indicates that the minimum addition of potassium and 15 cm cut back to no cut could have a uniform effect on the chlorophyll content, as the chlorophyll reflects the state of plant health. These results align with the claim made by Tränkner, & Tavakol, (2018) that the additional applicant of potassium enhance the photosynthetic CO2 fixation as the transportation and consumption of photo assimilates in plants regulate chlorophyll content during oxidative stress conditions. Siddiqui *et al*. (2018) also reported similar findings on tomato seedlings exposed to drought stress which was mediated by the exogenous application of potassium that further regulated endogenous potassium, which in turn enhanced the chlorophyll content and photosynthesis in tomato seedlings.

The lack of fresh water and arable land has promoted the reconsideration of cultivation of wild plants with antioxidant potential same as vegetables which are popularly known for their antioxidant capacity by consumer and food industry (Pinto *et al.,* 2022). Halophytes are opted to be a viable source of antioxidants because when they are exposed to stressful environments, they tend to increase the production of secondary metabolites that are alleged to be precursors of antioxidant effects in plants (Giorgi *et al.,* 2013; Jimoh & Kambizi, 2022). Therefore, these phytochemicals carry defensive traits on the plant from the adverse effect of stress (Ksouri *et al.,* 2007). Plants also produce antioxidants that neutralize free radicals during oxidative and ionic stress, and these antioxidants are critical for human health (Jimoh et al., 2020 & Mazouz *et al.,* 2020). The constant improvement of these phytochemicals and antioxidant in plants is essential as they are important for human health. This can be accomplished by modifying fertilizer and growing conditions. This chapter focused on the phytochemical and antioxidant activity of flower buds under different potassium concentrations and pruning levels on *T. divaricata* (Jacq.) Kunth and findings from the current study demonstrate that potassium concentrations along with cutting levels had favourable effects on phytochemicals and antioxidants. Under various cutting levels in this study*, T. divaricata* accumulated the maximum values of polyphenols, flavonols, FRAP, and DPPH on 0.0072 M (K_2SO_4). These outcomes are supported by the findings of Mahmoud *et al*. (2019), who applied a moderate potassium concentration to Solanum tuberosum tubers to promote phytochemical accumulation. A similar pattern was also noted by Zikalala *et al*. (2017) when they examined how fertilization with nitrogen, phosphorus, and potassium affected the nutritional quality of baby spinach (*Spinacia oleracea L*.), finding that a moderate amount of K increased the concentrations of total phenols, total antioxidant activity, total flavonoids, and vitamin C. On the contrary, TEAC got the highest value in control and 15 cm cutting level, so this was different. This may be related to the preferred K_2SO_4 concentration by the species. Cakmak, (2005) supported the claims by stating that inappropriate K levels may also trigger stress reactions, and because of stressors, reactive oxygen species may accumulate and infringe some phytochemicals. In this case, pruning may be played a crucial role as it previously stated that it helps with encouraging a new growth and nutrient allocation on edible organs. In this case, pruning can play a crucial role since it has already been mentioned as helpful in encouraging new growth and nutrient allocation on edible organs.

Chapter 6 covers the effect of different potassium concentrations and pruning levels on the nutrient content of *T. divaricata*. All the proximate compositions that were found to be present in *T. divaricata* were significantly affected by different potassium concentrations and pruning levels. The results of this study revealed that the ash content of the flower bud of *T. divaricata* has shown a positive result of 13.3% to 23.6% composition in all treatments, and normally wild plants does not exceed 5%. The ash content of food is a measure of its nutritional value and is thought to be a reflection of the mineral contents preserved in food materials (Akbari *et al.,* 2022). Ntuli (2019), reported similar findings on two water spinach species (*Ipomea plebeian* R.Br. and *Ipomea wightii* (Wall.) Choisy), where the ash content reported was 24%. Therefore, the outcome of the present study is comparable with ash content of the processed foods (Muñoz-Arrieta *et al.,* 2021). This high ash value indicates that the plant is high in dietary fibres, which provide shelter for digestive organisms in the gastrointestinal tract (Schroeder & Bäckhed, 2016).

The fat content in flower buds of *T. divaricata* ranged from 1.7–2.3%, and this was like the findings Ntuli, (2019) on two water spinach species. With 0.144 M of K_2SO_4 + 15 cm cut back treatment having highest values (2.6g/100g) compared to all. This is in coloration with the claim made by Bressani (2018) who explained that most plants with high ash are likely not to contain high amount of crude fat. However, the fat in leafy vegetables provides energy, essential fatty acids, and vitamins and which adds to palatability through absorption and retaining flavour.

The moderate treatment (0.144 M) with 5 cm cutting had the highest protein composition and values ranged from 21.7 to 26.6% in tested samples, and these were lower than those reported in *A. caudatus* (30.2%) and S. nigrum (38.98%), respectively by Jimoh *et al*. (2020) and Ogundola *et al*. (2018). Nevertheless, the protein content of the flower buds *of T. divaricata* was comparable with that of *Moringa oleifera* leaves (27.3%), as reported by Sultana (2020). However, the highest carbohydrates contents (60.5%) found in *T. divaricata* at 0.0114 M + 5 cm treatment has exceeded those presented by Bvenura *et al.* (2017) for wild edible vegetables in Southern Africa. This indicates that *T. divaricata* is a rich source of carbohydrates and has a high calories content that can assist in providing the body with calories. The presented moisture content of flower buds was between 9.9 to 12.1% of all tested treatments. These lower moisture values imply that the flower buds of this plant may have a long storage lifespan, benefiting the producers and sellers and its potential use for prolonging the shelf life of foods or as preservatives may be researched. The absorption and concentrations of additional nutrients that are available to plants have been demonstrated a growth and developmental change because of fertilizer application (Jimoh *et al.,* 2020). This might be caused by interactions in the transport of other minerals or ion absorption within plant cells (Davarpanah *et al.,* 2016; Norozi *et al.,* 2019). Therefore, it is essential to use the proper fertilizer doses for plant growth and development. These nutrients support life by supplying the body the essential nutrients it needs for its psychophysical well-being (Bulawa *et al.,* 2022). The harvests from treatment 0 M (K_2SO_4) + 10 cm pruning had the maximum Mg yield, which was statistically comparable to the control, 0 M + 5 and 15 cm pruning levels, respectively.

The highest mineral contents discovered in this study confirm earlier claims that certain wild vegetable species are a source of necessary minerals (Jimoh *et al.,* 2018). Variations in the study's findings further demonstrated that K, Calcium (Ca), and Magnesium (Mg) ions had an antagonistic effect since Ca and Mg levels declined when K was added. Even though these minerals were marginally decreased in samples that had 0.0072 M of potassium treatment and various amounts of cutting levels, they improved the growth and development of flower buds. These findings support prior research by Norozi *et al.* (2019), who found that potassium fertilizer application diminished calcium and magnesium leaf concentrations while increasing nitrogen, phosphorus, potassium, and sodium concentrations. These findings demonstrate that the calcium content of *T. divaricata* was below the 1000 mg RDA for adults that was suggested. Although magnesium level in all investigated treatments was significantly higher than the RDA value of 55 mg/100 g, as reported by the (USDA, 2008). In addition to its structural roles in proteins, nucleic acids, and polyribosomes, magnesium also plays important roles in the release of neurotransmitters, cell adhesion, calcium-potassium homeostasis stabilization, and as an enzyme cofactor (Stein, 2010). So, regular consumption of this plant will guarantee ideal serum magnesium concentration.

Several micronutrients, including iron, zinc, aluminium, copper, and manganese, are crucial for human nutrition (Ntuli, 2019). Less than 20 mg of these trace minerals must be consumed daily, and they make up less than 0.01% of body weight (Ogundola *et al.,* 2018). According to the USDA (2008), all trace elements examined in *T. divaricata* flower buds, with the exluding of copper, are within the required daily intake. *Trachyandra divaricata* recorded the highest values of 13.3 mg/100g iron at 0.0114 M + 15 cm treament, which was lower than the values reported for other wild vegetables consumed in South Africa, such as *Lecaniodiscus cupaniodes* (27.8 mg/100 g), *Sterculia tragacantha* (803.7 mg/100 g), and Ipomea plebeian (1225 mg/100 g) (Ntuli, 2019). Similar to manganese, *T. divaricata* flower buds were found to have between 2.8 to 6.7 mg/100 g of manganese, which was less than the value found in Moring oleifera (252 mg/100 g) (Gopalakrishna *et al.,* 2016). In addition to its role as an antioxidant, manganese also plays a role in bone development, blood sugar regulation, immune system function, and cellular reproduction (Ghanbarzadeh *et al.,* 2022). Fe is necessary for the creation of haemoglobin, reducing anaemia rates, and healthy central nervous system function (Kaur *et al.,* 2017). Based on these results, it may be considered that *T. divaricata* flower buds are suitable for consumption because they accumulate fewer heavy metals than other wild vegetables.

7.2 General Conclusion

This current study supports the hypothesis that have been made on chapter one as different potassium concentrations and cutting levels have influenced the growth, chlorophyll content, proximate composition, nutrient content, and antioxidants content of *T. divaricata.* The minimum concentration (0.0072 M) with pruned or unpruned levels showed to be an outstanding amongst the others. The growth parameters of *T.divaricata* together with chlorophyl have shown to be positively influenced by the treatments. With various essential nutrients such as Zinc, Potassium, nitrogen, Iron calcium, manganese, phosphorus, potassium, sodium, and proximity such ash, protein carbohydrates and fat being affected by the treatments showed to be vital for *T. divaricata*. The plant's high fibre content confirms its digestive effectiveness in humans, and its high protein content confirms its worth as an immune booster, an important nutraceutical, and a potential functional food. In addition, moisture has been significantly affected with low values and this showed that this plant has high lifespan. Therefore, the findings suggest that the edible, antioxidants and phytochemicals of this plant have been influenced by the treatments and quantifies it to be considered for public consumption and make it available in commercial market for the purposes of embracing diverse culinary.

7.3 Recommendations

The study suggested that hydroponic cultivation of *Trachyandra divaricata* with a minimal K_2SO_4 concentration (0.0072 M) and moderate pruning (15 cm) optimizes productivity, resulting in improved floral bud development, nutritional content, and antioxidant properties. This approach offers a sustainable solution for addressing salinization challenges and obtaining a valuable leafy vegetable. Overall, the dissertation aimed to contribute to sustainable agriculture and food security by exploring the potential of *Trachyandra divaricata* as a valuable crop and providing insights into the cultivation practices that can optimize its growth and nutritional value. However, further research can be done on the physiological, medicinal and biological activities of *T. divaricata* to widen and popularise its potential pharmacological uses.

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Appendix 1

plants

Article

Potassium Application Enhanced Plant Growth, Mineral Composition, Proximate and Phytochemical Content in *Trachyandra divaricata* Kunth (Sandkool)

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Abstract: Wild leafy vegetables are commonly included in the diet of people in rural homesteads. Among various wild edible vegetables in South Africa, *Trachyandra divaricata* (Sandkool) is one of the most abundant but underutilized due to the dearth of literature on its cultivation and nutritional value. In the present study, the effect of potassium application and pruning on growth dynamics, mineral composition, and proximate and phytochemical content in *T. divaricata* were evaluated. Treatments consisted of three potassium concentrations (0.0072, 0.0144, and 0.0216 M) supplemented in the form of potassium sulphate (K_2SO_4) with four pruning levels (unpruned, 5, 10, and 15 cm) applied in each treatment. The potassium doses were added to the nutrient solution, while the control treatment was sustained and irrigated with nutrient solution only. The results revealed a significant increase in flower bud yield, height, total dry and wet weight of shoots and roots, as well as ash and neutral detergent fibre in plants irrigated with 0.0072 M of K_2SO_4 without pruning. Conversely, chlorophyll content and Ca were comparable among treatments, while the highest yield of Na, P, N, and Zn was recorded in treatment 100 mL of K_2SO_4 with 10 cm pruning. Likewise, the highest antioxidant value (Polyphenols, Flavonol and DPPH) was obtained from plants irrigated with 0.0072 M of K_2SO_4 with 10 cm pruning. Based on these findings, *T. divaricata* is a promising leafy vegetable as a minimum dose (0.0072 M) of K with moderate pruning optimised its productivity in terms of growth, biomass parameters, nutritional content, and antioxidant potential. Due to its rich nutritional value, the plant should be domesticated and studied further for its potential nutraceutical benefits.

Keywords: Asphodelaceae; functional food; inflorescent vegetables; nutraceuticals; potassium sulphate; underutilized vegetables

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1. Introduction

Wild leafy vegetables are important food crops because they contain adequate amounts of nutrients, vitamins, and minerals for humans [1]. African communities have a long history of supplementing their diets with traditional vegetables [2,3]. In South Africa, most rural residents rely on foraged leaves as their primary source of leafy vegetables [4]. Although wild leafy vegetables are commonly consumed in rural areas, they are also consumed by urban residents who either buy them from traders or collect them in the wild [5,6].

Among various wild edible vegetables in South Africa, *Trachyandra divaricata* (Sandkool) is one of the most abundant but underutilized herbs due to the dearth of literature on its cultivation and nutritional value [7]. Although it was found in the early years in some parts of Australia, *T. divaricata* is native to South Africa. The plant is an invasive species within Asphodelaceae, and it has rhizomes which produce numerous fleshy leaves which spread along the surface up to 1 m long and a horizontal stem-like structure that produces white and purple flowers which turn into fruit sets at the tip, at the later stage [8]. Ethnobotanical reports claim that the inflorescence of this plant is palatable as it was consumed