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The effect of shade stress and different growth media on hydroponic-grown *Trachyandra divaricata* (Jacq.) Kunth as a coastal vegetable

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DECLARATION

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

Signature Date: 14 June 2023

Abstract

The objective of the study was to ascertain how vegetative development, nutritional content, phytochemicals, and antioxidant activities of *T. divaricata* will be affected by different soilless growing media and shade conditions as well as to identify the species' ideal growing protocol. The experiment was run over the course of 12 weeks, *Trachyandra divaricata* plant divisions were collected from wild populations on the Bellville campus of the Cape Peninsula University of Technology. Divisions of the plants were done to ensure clonal propagation of identical plants. The leaves of all plants were cut to a similar length of 10 cm, measured with a ruler. It was ensured that each plant had the same number of leaves and an equal weight of 23 grams per plant weighed on an electrical scale (g). All plants were then planted into 15 cm pots and placed under controlled irrigation in the propagation house for two weeks after which they were moved to the Nutrient Film Technique (NFT) gutter hydroponic system.

Four identically NFT hydroponic systems were constructed with each system being set up on a rectangular wire mesh table (2.5 m long) which provided a flat surface. Another wire mesh table was stacked on top of each table to create a frame which were then covered with different percentages of shading as stated. Each system had its low-density polyethene (LDPE) 50 L reservoir in which the nutrient solution was prepared and supplied to the system. Four Polyvinyl Chloride (PVC) square gutters (2 m long) were placed on each table which held the 15 cm square plastic pots (ten pots per gutter). The gutters were sealed with PVC adhesive to prevent leaks and connected to a 1 × 2000 L/hr submersible pump with 2.5 m head capacity, 20 mm LDPE irrigation piping, 4 × 20 mm elbow irrigation fittings and 4 × 20 mm flow regulators. Water was pumped and circulated continuously from the reservoir tanks which contained a Nutrifeed fertilizer (65 g/kg N, 27 g/kg P, 130 g/kg K, 70 mg/kg Ca, 20 mg/kg Cu, 1500 mg/kg Fe, 10 mg/kg Mo, 22 mg/kg Mg, 240 mg/kg Mn, 75 mg/kg S, 240 mg/kg B and mg/kg Zn. Fertilizer group 1 Reg No: K2025 (Act 36/ 1947) which was bought from Starke Ayres, Cape Town. The pH and electrical conductivity (EC) were maintained at 6.5 and using a Martini Instruments PH55 pH probe. EC levels of the aqueous nutrient solutions were monitored with a calibrated hand-held digital EC meter (Hanna Instruments®™ HI 98312). The nutrient solutions were refreshed every 2 weeks to minimize the build-up of salts in the growing media.

The experiment involved two main treatments which were growth media and shading. Four different growth media namely: LECA clay (LC), Consol® silica sand (SS), peat (P) and vermiculite (V) and arranged in a completely randomized block design (CRBD). The silica sand and LECA clay were rinsed thoroughly with sterile water to clean impurities and other earthy materials. The divided parts of *T. divaricata* were then planted in pots filled with different growing media and placed in a NFT hydroponic system. White shade netting of 20% density was purchased from Stodels Garden Centre, Bellville, South Africa and used to create the shading climates. Different percentages of shading were created with 20% shade cloth by doubling

(40%), tripling (60%) and quadrupling (80%) the net into different layers. The control had no shade cover and silica sand (C=0% shade). The study's findings revealed that the use of shade nets and different soilless media had a positive effect on the vegetative growth of *T. divaricata*, with plants grown under 60% shade in peat medium producing more material than plants grown in no shade. However, leaf length increased significantly in peat soilless medium with no shade. *T. divaricata* may therefore be cultivated on vermiculite medium under 80% shade for maximum chlorophyll production because it produced the highest chlorophyll content under 80% shade. The optimum treatment for producing phytochemical composition and antioxidant activities in *T. divaricata* flower buds was moderate shade levels, as the results revealed a considerable rise in silica sand medium with 20% shade. The proximate analysis revealed that the 80% shade level yielded high fats in peat soilless medium. Furthermore, the same tendency was detected in protein yield, which was high in vermiculite under 80% shade, and the similar trend was found in ash content under 80% shade, with silica sand having the greatest value. This experiment clearly shows that *T. divaricata* can be grown beneath a shade net for optimum production of nutrients.

Overall, this work has shown that *T. divaricata* may be grown in a hydroponic system with various growth media and beneath a shade net. The vegetative development, nutritional content, antioxidant activity, and phytochemical composition in the flower buds of *T. divaricata* were all positively impacted by various soilless medium in combination with different levels of shade. The accumulation of rich mineral nutrients in flower buds is an indication of a vegetal trait that contributes nutrients. These results indicate that *T. divaricata* is a promising inflorescence vegetable with excellent nutritional value. It is recommended that the plant should be domesticated and reintroduced as inflorescent vegetable that may could serve as a dietary supplement and might significantly improve food security by providing local communities with enough access to food. Growing *T. divaricata* hydroponically will also help to availability all year round and mitigate the effects of climate change because crops grown in hydroponic systems are not influenced by seasonality.

Keywords: Shade stress; Inflorescence vegetable; soilless media; nutrient composition; phytochemicals and antioxidant activities

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DEDICATION

- To my mother, Nolungile Tshayingwe, who has always been an inspiration, supportive, believe in me, and give me hope even when things aren't going well in my academic career, as well as in memory of my late father, Booi Tshayingwe.
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1. CHAPTER ONE:

General introduction, problem statement, aims, hypothesis and objectives.

1.1. General introduction

Trachyandra divaricata, often known as "Tumbling Starlily" or "Kus Waaibossies" (Afrikaans), is an edible wild vegetable in the Asphodelaceae family. The species grows mainly in sandy areas throughout South Africa (De Vynck *et al*., 2016). It is a stout, tufted, rhizomatous perennial with fleshy evergreen leaves that are virtually flat, long, and narrow in shape and grows up to 1 m long. A horizontal stalk produces white to purple flowers that last a day; and if cut the plant readily produces more racemes (van Jaarsveld, 2001; Boatwright & Manning, 2010; De Vynck *et al*., 2016). De Vynck *et al.* (2016) further describes the green leathery fruit as a segmented ovoid capsule that turns red and later black. According to ethnobotanical sources, the flower buds of this species is edible and was devoured during times of hunger by the original Khoisan people (Van Wyk, 2011; Ngxabi *et al.,* 2021a). To date there are no recorded studies on the cultivation, nutrition, and medicinal potential of *T*. *divaricata* and its close relatives (Ngxabi *et al.,* 2021a).

Therefore, agronomical research is necessary to develop suitable cultivation protocols that will aid the domestication of *T*. *divaricata,* promote its consumption, and guarantee its long-term use in household diets as an inflorescent vegetable. Growing this wild vegetable in South Africa could be a solution for sustainable food security which are exacerbated by an increased temperatures and rainfall seasons which have become more erratic, especially in Sub-Saharan Africa (Shew *et al*., 2020). The cultivation of this prized vegetable would decrease the dependency on traditional key vegetables like potato, spinach, lettuce, and wheat, which shortages have been the root cause of malnutrition impacting two billion people globally (Mabhaudhi *et al.,* 2018).

Hydroponics is a crop production system that is utilized effectively for the cultivation of vegetables, herbs, and other crops using soilless growing substrate. The use of a fertilizer solution and the largely regulated environmental conditions, make these systems more energy efficient and productive than conventional agriculture growing. Hydroponic systems are also resource- and space-efficient growing systems and already contribute significantly to world food supplies (Manos & Xydis, 2019; Khan *et al*., 2020; Appolloni *et al*., 2021; Balasubramanya & Shaafiu, 2022). As hydroponics allows all-year round cultivation of crops, it also guarantees food security to overcome seasonal scarcity of certain food crops (Viljoen *et al.,* 2021).

Plants are frequently subjected to many pressures at the same time and as a result, they have evolved sophisticated defense mechanisms for recognizing and adapting to a wide range of environmental challenges (Holopainen & Gershenzon, 2010; Sewelam *et al*., 2014; Huang *et al*., 2022). Therefore, it is crucial for plant science studies to comprehend physiological processes, defense mechanisms and adaptive responses to abiotic and biotic stress factors. Environmental elements like light and moisture have a huge impact on how plants grow and develop (Akula & Ravishankar, 2011; He, *et al*., 2022). Light is the second most important ecological factor regulating plant growth, productivity and survival since it influences photosynthetic efficiency in plants (Li *et al.,* 2020; Sharma *et al*., 2020). It is therefore necessary to investigate the effect of varying light intensity on the vegetative growth and accumulation of dietary nutrients and phytochemicals in species such as *T. divaricata* cultivated in different growth media.

The purpose of this study therefore was to evaluate the effect of different shade levels and different growth media as growth enhancers when used to improve the vegetative characteristics, nutritional properties, phytochemicals, and antioxidant potential of *T. divaricata*, with a focus on the inflorescence due to its documented edibility. The nutrient film technique hydroponic system was used to propagate this species and to ascertain the possibility of growing the species during different seasons of the year.

1.2. Structure of the thesis

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

1.2.1 The overriding principle of the thesis is that it remains an original contribution to the discipline or field by the candidate.

1.2.2 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.

1.2.3 The study does not include work published prior to commencement of the candidature.

1.2.4 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.

1.2.5 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).

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

Chapter One: Problem statement, aims, hypothesis and objectives.
This chapter provides an overview of the research problem, aims and hypotheses of the research topic.

Chapter Two: Hydroponic cultivation of *Trachyandra divaricata* (jacq.) Kunth, panacea to support future food security: a review. This chapter provides background information on what has been researched already on the topic.

Chapter Three: Vegetative growth of *Trachyandra divaricata* (Jacq.) Kunth in response to different growing media and shade stress in hydroponics. This chapter provides the first experimental results, investigating the effect of different soilless media and shade levels in vegetative growth of *T. divaricata.*

Chapter Four: The effects of shade stress and different growing media on chlorophyll content of *Trachyandra divaricata (*Jacq.) Kunth*.* This chapter provides the results of this study show that there is potential for cultivation of *T. divaricata* on hydroponic system and LECA clay soilless medium was the best medium for chlorophyll content of this species as it performed better when combined with 80% shade compared to other treatments.

Chapter Five: Effects of different shade levels and growth media on phytochemicals, and antioxidant capacity of *Trachyandra divaricata* (Jacq.) Kunth. This chapter results recommend a combination of soilless media and moderate shade for maximum production of phytochemicals and antioxidant for *T. divaricata*.

Chapter Six: Effect of different shade levels and growth media on nutrient composition of *Trachyandra divaricata* (Jacq.) Kunth. This chapter results supports the expansion of *T. divaricata*'s commercial cultivation as a nutritious, shade-tolerant, and sustainable wild vegetable with edible flowers.

Chapter Seven: General Discussion and conclusion. This chapter deals with the general discussion which connects the previous chapters and is followed by the conclusions of the study. Recommendations are made for further work; to introduce future research topics.

Chapter eight: All the references used in this thesis.

1.3. Problem statement

Population growth, which directly contributes to the reduction of the world food basket, necessitates the production of more food to feed hundreds of thousands of people and requires the promotion or diversification of food crops to prevent hunger and malnutrition (Tripathi *et al.,* 2019). Developing countries are faced with issues of inadequate and insufficient food to feed millions of people (Sasson, 2012; Barrett *et al*., 2013). For instance, South Africa is considered as a country with high proportions of income variation in the world. The country has high levels of poverty compared to other middle-income countries, due to different underlying factors such as lack of access to agricultural land, high rate of unemployment, population growth, crime, illnesses, and death affecting the breadwinners in the family, low income, and so forth (Mathebula *et al*., 2017; Kollamparambil, 2020). To overcome these myriads of problems, there is a need to introduce some unconventional edible plants and develop cultivation strategies for the species in hydroponics to sustainably provide communities with sufficient and nutritious meals (Jimoh *et al*., 2020; Salami *et al*., 2021; Ramdwar & Siew, 2017). This will aid the propagation of more edible species such as *Trachyandra divaricata* that would be a solution to the food insecurity ravaging these developing worlds.

This study will provide commercial farmers with a successful growth protocol of this species. It will also educate people about the medicinal and nutritional benefits of the plant and its potential to grow and cook indigenous foods that are beneficial to our health since home-grown vegetables do provide a green and sustainable diet for healthy living.

1.4. Aims

The aim of this study was to cultivate *T. divaricata* in the nutrient film technique hydroponic system and to determine the effects of shade stress in different growth media on the vegetative growth, nutrient content, phytochemicals, and antioxidant potential to support future cultivation of the species.

1.5. Hypothesis

It is hypothesized that the different growth media and different levels of shading will influence the vegetative growth, nutrient content, phytochemicals, and antioxidant potential of *T. divaricata.*

1.6. Objectives

1.6.1. Main objectives

The purpose of this study was to determine the vegetative growth, nutritional composition, phytochemical properties, and antioxidants capacity of *T. divaricata* in response to different growth media and different shade percentages to establish a suitable growth protocol for *T. divaricata*.

1.6.2. Specific objectives

1.6.2.1.1 To determine the effect of different growth media and shade stress on the number and length of leaves of *T. divaricata* grown in hydroponics.

1.6.2.1.2 To evaluate the effect of different growth media and shade stress on the height of *T. divaricata* grown in hydroponics.

1.6.2.1.3 To determine the effect of different growth media and shade stress on the number of buds of *T. divaricata* grown in hydroponics.

1.6.2.1.4 To evaluate the effect of different growth media and different shade stress on the chlorophyll content *of T. divaricata* grown in hydroponics.

1.6.2.1.5 To evaluate the effect of different growth media and shade stress on the nutrient content of *T. divaricata* grown in hydroponics.

1.6.2.1.6 To quantify phytochemicals and antioxidant potential of *T. divaricata* cultivated with different growth media and shade levels in hydroponics to enhance studies of its medicinal and pharmacological potential.

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2. CHAPTER TWO:

Food security in a changing climate: A review on abiotic factors in hydroponic cultivation of *Trachyandra divaricata*, a coastal edible species from South Africa

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2.1. Abstract

Food insecurity is also considered as being closely related to poverty which may vary from one country to another. Many third world countries are faced with the lack of inadequate food quantities to feed thousands of people. South Africa is considered to have a high proportion income variation in the world, yet the level of poverty remains high compared to other middle-income countries. An edible species, *Trachyandra divaricata* (Dune onion) on the coast of South Africa has the potential to be cultivated commercially and contribute to food security. This review explores the morphology, distribution, uses and potential cultivation of Dune onions to advance research and commercialization of this neglected wild, coastal edible species.

Key words: Asphodelaceae; dune onion; photo-selective filter; shade cloth

2.2. Introduction

Despite the advantages associated with hydroponics, many wild edible coastal species such as *Trachyandra divaricata* (Dune onion) have not been studied (De Vynck *et al*., 2016). Boatwright and Manning (2010*)* described *T. divaricata* as a perennial herb to a height of 70 cm with numerous thick basal leaves up to 50 cm long. The plant is evergreen throughout the year with quill-like leaves enclosed at the base within membranous sheaths. The linear, flat leaves are dark green, succulent, smooth and hairless and often slightly rolled over along their length. Flower buds continues over a longer season compared to other *Trachyandra* species. The flower buds are edible, very much like asparagus and needs to be harvested before it branches and produces white flowers that only last a day. The plants produce more racemes if harvested at regular intervals. The green leathery fruit is a segmented ovoid capsule that turns red and later black (Heylingers, 1999; De Vynck et al., 2016).

Although *T. divaricata* has been documented as an edible plant that was traditionally used as food source by Khoi-San people living along the South African Cape coast, little is known about the cultivation of the species (De Vynck *et al*., 2016). Sharma *et al.* (2018) reported that hydroponic crop production has recently and meaningfully increased worldwide, as it tolerates or enhances a more efficient use of water and fertilizers. Hydroponics is also effective in the control of diseases control and more viable for climate change. Crop production is increased in quality and productivity, which result in attracting higher economic incomes. Due to limited data available on the cultivation of this species, further research will greatly advance the commercial cultivation in evaluating radiation levels and a suitable a growing media for T. *divaricata.*

2.3. *Trachyandra divaricata* a species of the Asphodelaceae family

Trachyandra divaricata belongs to the family Asphodelaceae in the order asparagales, which is made up of 12 genera and about 1060 species that are native to Africa, Mediterranean basin, Arabian Peninsula, west and central Europe, Madagascar, Central Asia, Australia and New Zealand (Klopper *et al*., 2013; Angiosperm Phylogeny Group 2016). Klopper *et al.* (2013) documented that the name Asphodelaceae was first scientifically published in Jussieu's *Genera Plantarum* in 1789 and this date is regarded as the starting date for the nomenclature of this family. There are also two sub-families namely Asphodeloideae and Alooideae within the asparagales family (Chase, 2000). Members of Asphodelaceae family vary broadly with some few characteristics that are common to all species. They range from small to medium-sized plants that are often succulent herbs or large trees with leaf arrangements of terminal rosettes on fibrous and woody stems. According to Smith and Van Wyk (1998), identifiable characteristics in the leaves are "dorsiventral, lanceolate-acuminate, linear, subulate, terete, often succulent and thickly conical, spirally arranged or distichous as in some species of Alooideae, amplexicaul, margins toothed, serrate or entire, sharply pointed, parallel veins often obscure.

Boatwright and Manning (2010*)* described *T. divaricata* as a perennial herb to a height of 70 cm with numerous thick basal leaves up to 50 cm long. The plant is evergreen throughout the year with quill-like leaves enclosed at the base within membranous sheaths. The linear, flat leaves are dark green, succulent, smooth and hairless and often slightly rolled over along their length. Flower buds continues over a longer season compared to other *Trachyandra* species. The flower buds are edible, very much like asparagus and needs to be harvested before it branches and produces white flowers that only last a day. The plants produce more racemes if harvested at regular intervals. The green leathery fruit is a segmented ovoid capsule that turns red and later black (Heylingers, 1999; De Vynck et al., 2016).

2.3.1. Genus Trachyandra

In 1843, Kunth described the genus *Trachyandra* for the first time when he divided *Anthericum* L. into three genera: Phalangium Mill., Bulbinella Kunth, and Trachyandra Kunth. Thereafter, most authors continued to use the name *Anthericum* to describe close relatives of *Anthericum* species until Obermeyer (1962) came up with a revision of the South African species of *Anthericum*, *Chlorophytum* and *Trachyandra* using a few distinctive characters to delimit the three genera (Perry, 1990; Ngxabi *et al.,* 2021a). *Trachyandra* is a more distinct grouping that differs from the first two genera in several ways. Among these are the adaptable anthers with caducous perianth embedded in articulated pedicels, as well as single flowers held by a single bract. *Trachyandra* is related to *Bulbine* and *Bulbinella* by these characteristics; however, *Trachyandra* differs by the presence of scabrid filaments, white to pinkish immaculate or maculate flowers, and a frequently branched inflorescence (Perry, 1990; Ngxabi *et al*., 2021a). The genus is indigenous to southern and eastern Africa, Madagascar and Yemen, with many of the species being endemic to South Africa. About 57 species are recognized globally (Table 1), out of which 48 species are predominantly found in South Africa with marked concentration in the Cape's winter rainfall region (Germishuizen & Meyer, 2003; Perry, 1990; *The Plant List,* 2010).

Table 2.1: Global distribution of Trachyandra species

S/N	Species	Authors	Countries/Provinces
1.	*Trachyandra acocksii*	Oberm.	Cape Province, South Africa
2.	*Trachyandra adamsonii*	(Compton) Oberm.	Cape Province, South Africa; Namibia
3.	*Trachyandra affinis*	Kunth	Cape Province, South Africa
4.	*Trachyandra Arenicola*	J.C.Manning & Goldblatt	Cape Province, South Africa
5.	*Trachyandra aridimontana*	J.C.Manning	Angola, Zambia, Zimbabwe, Botswana, Namibia
6.	*Trachyandra arvensis*	(Schinz) Oberm.	South Africa, Lesotho, Eswatini
7.	*Trachyandra asperata*	Kunth	Cape Province, South Africa
8.	*Trachyandra brachypoda*	(Baker) Oberm.	Cape Province, South Africa

9.	*Trachyandra bulbinifolia*	(Dinter) Oberm.	Cape Province, South Africa; Namibia
10.	*Trachyandra burkei*	(Baker) Oberm	Cape Province, Botswana, Limpopo, Free State, South Africa
11.	*Trachyandra capillata*	(Poelln.) Oberm.	Cape Province, KwaZulu-Natal, South Africa
12.	*Trachyandra chlamydophylla*	(Baker) Oberm.	Cape Province, South Africa
13.	*Trachyandra ciliata*	(L.f.) Kunth	Cape Province, South Africa; Namibia
14.	*Trachyandra dissecta*	Oberm.	Cape Province, South Africa
15.	*Trachyandra divaricata*	(Jacq.) Kunth	Cape Province, South Africa, naturalized in Australia
16.	*Trachyandra ensifolia*	(Sölch) Roessler	Namibia
17.	*Trachyandra erythrorrhiza*	(Conrath) Oberm.	Gauteng,South Africa
18.	*Trachyandra esterhuysenae*	Oberm.	Cape Province, South Africa
19.	*Trachyandra falcata*	(L.f.) Kunth	Cape Province, South Africa Namibia
20.	*Trachyandra filiformis*	(Aiton) Oberm.	Cape Province, South Africa
21.	*Trachyandra flexifolia*	(L.f.) Kunth	Cape Province, South Africa
22.	*Trachyandra gerrardii*	(Baker) Oberm.	Eswatini, South Africa
23.	*Trachyandra giffenii*	(F.M.Leight.) Oberm.	Cape Province, South Africa
24.	*Trachyandra glandulosa*	(Dinter) Oberm.	Namibia
25.	*Trachyandra gracilenta*	Oberm.	Cape Province, South Africa,
26.	*Trachyandra hantamensis*	Boatwr. & J.C.Manning	Cape Province, South Africa
27.	*Trachyandra hirsute*	(Thunb.) Kunth	Cape Province, South Africa
28.	*Trachyandra hirsutiflora*	(Adamson) Oberm.	Cape Province, South Africa
29.	*Trachyandra hispida*	(L.) Kunth	Cape Province, South Africa
30.	*Trachyandra involucrate*	(Baker) Oberm.	Cape Province, South Africa
31.	*Trachyandra jacquiniana*	(Schult. &Schult.f.) Oberm.	Cape Province, South Africa
32.	*Trachyandra kamiesbergensis*	Boatwr. & J.C.Manning	Cape Province, South Africa
33.	*Trachyandra karrooica*	Oberm.	Cape Province, South Africa Namibia,

34.	*Trachyandra lanata*	(Dinter) Oberm.	Namibia
35.	*Trachyandra laxa*	(N.E.Br.) Oberm	South Africa, Namibia, Botswana
36.	*Trachyandra malosana*	(Baker) Oberm.	Malawi, Zimbabwe
37.	*Trachyandra mandrarensis*	(H.Perrier) Marais & Reilly	Madagascar
38.	*Trachyandra margaretae*	Oberm.	Mpumalanga, KwaZulu-Natal South Africa
39.	*Trachyandra montana*	J.C.Manning & Goldblatt	Cape Province, South Africa
40.	*Trachyandra muricata*	(L.f.) Kunth	Namibia
41.	*Trachyandra oligotricha*	(Baker) Oberm.	Cape Province, South Africa
42.	*Trachyandra paniculate*	Oberm.	Cape Province, South Africa
43.	*Trachyandra patens*	Oberm.	Cape Province, South Africa
44.	*Trachyandra peculiaris*	(Dinter) Oberm.	Namibia
45.	*Trachyandra prolifera*	P.L.Perry	Cape Province, South Africa
46.	*Trachyandra pyrenicarpa*	(Welw. ex-Baker) Oberm.	Huíla Province, Angola
47.	*Trachyandra revoluta*	(L.) Kunth	Cape Province, South Africa; Namibia
48.	*Trachyandra sabulosa*	(Adamson) Oberm	Cape Province, South Africa
49.	*Trachyandra saltii*	(Baker) Oberm.	Ethiopia to Cape Province, South Africa; Yemen
50.	*Trachyandra sanguinorhiza*	Boatwr. & J.C.Manning	Cape Province, South Africa
51	*Trachyandra scabra*	(L.f.) Kunth	Cape Province, South Africa
52.	*Trachyandra smalliana*	Hilliard & B.L.Burtt	Cape Province, KZN, South Africa
53.	*Trachyandra tabularis*	(Baker) Oberm.	Cape Province, South Africa
54.	*Trachyandra thyrsoidea*	(Baker) Oberm.	Cape Province, South Africa South Africa
55.	*Trachyandra tortilis*.	(Baker) Oberm.	Cape Province, South Africa
56.	*Trachyandra triquetra*	Thulin	Somalia
57.	*Trachyandra zebrine*	(Schltr.ex-Poelln.) Oberm.	Cape Province, South Africa

2.3.2. General description of *Trachyandra divaricata*

Trachyandra divaricata, also known as Strapweed or False Onion Weed belongs to the family Asphodelaceae. The species grows with stubby branches with approximate thickness of 2.0-3.5 cm outward and upward from a compact rhizome. The basal parts of the plant bear pubescent roots having high rate of water absorption from which the leaves emerge (Heyligers, 1999). The flowers look like those of the local species *Asphodelus fistulosus* although both species are distinctly different in their leaves, shape, and stamens. A careful examination of *T. divaricata* stamens reveals yellowish anthers, flattened leaves and floppy shape (Jubase, 2015). The inflorescences have divaricated branching and their pale-lilac flowers attract honeybees (Heyligers, 1999; Jubase, 2015).

2.3.3. Taxonomic description of *Trachyandra divaricata*

Kingdom: Plantae
Clade: Tracheophyte
Clade: Angiosperm
Clade: Monocot
Order: Asparagales
Family: Asphodelaceae
Subfamily: Asphodeloideae
Genus: *Trachyandra*
Species: *Trachyandra divaricata* (Jacq.) Kunth (Govaerts, 2009)

2.3.4. Salt tolerance adaptable to water and salinity stress in the distribution of *Trachyandra divaricata*

Trachyandra divaricata grows in its natural habitat on coastal dunes and on the sand flats along the western and southern coasts of South Africa (Smith & Van Wyk, 1998; De Vynck *et al.,* 2016). The species is also well-known in Western Australia and New South Wales where it is known as strap weed and has become an aggressive coastal weed. Species of *Trachyandra* occur throughout Southern Africa although most of them are endemic to winter rainfall areas of the Southwestern Cape while very few spread further northwards, with only spreading as well in Ethiopia (Smith & Van Wyk, 1998; Ngxabi *et al.*, 2021a). *Trachyandra divaricata* occurs along the coasts of the south-western corner of the African continent. It is a collective and widespread dune plant in the Cape Town region, which has a Mediterranean climate and the adjacent coasts, which are semi-arid (Heyligers, 1999; De Vynck *et al*., 2016).

Figure 2.1: ***T. divaricata*** **flourishing and fruiting in low nutrient coastal sand dunes.**

(Ivan Latte, 2007)

Figure 2.2: A geographical map showing the distribution of ***T. divaricata*** **in South Africa. Adapted from: https://repository.up.ac.za/handle/2263/8457?show=full]**

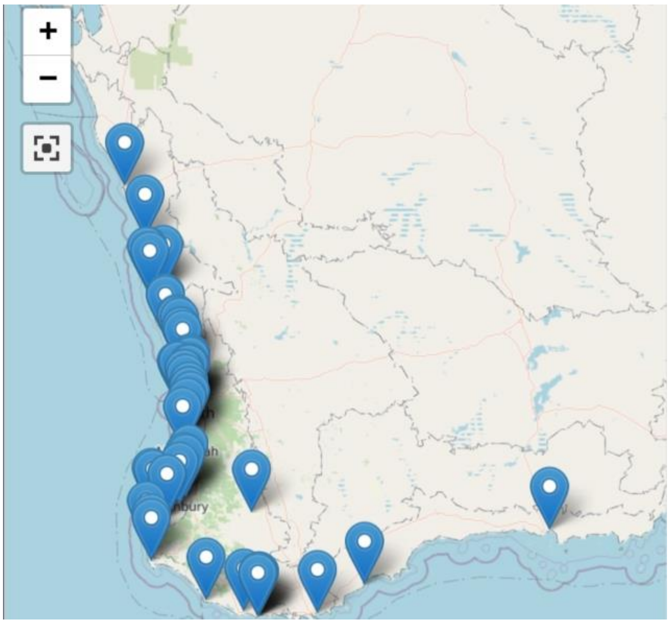

Figure 2.3: The distribution of the species in specific areas.

Figure 2.4: A closer view of the inflorescence of *T. divaricata.* (Thabo Maphisa 2007)

2.4. Ethnobotanical uses of *Trachyandra divaricata*

The flower buds can be steamed or roasted for their use in salads, as a vegetable, and they can be preserved, fermented, stir-fried or cooked as tempura. In other words, it is used cooked, in salads, stir-fries, pie fillings, stews or soups. The leaves resist well in oil or vinegar pickles and ferments (Boatwright & Manning, 2010; De Vynck *et al.,* 2016) Flowers that are edible also improve the appearance of food presentation. The species can provide physiologically active compounds such as vitamins A and C, riboflavin, niacin, calcium, phosphorous, iron, and potassium and minerals including calcium, phosphorous, iron, and potassium, all of which are good to consumers' health. discovers that edible flower buds are a good source of vitamin C, which is necessary for keeping a healthy immune system (De, 2021).

Figure 2.5: Harvested flowering buds of *T. divaricata*. (Thabo Maphisa, 2009)

2.5. Radiation and the effect of shade on plants

Boardman (1977) and Sharma *et al.* (2020) conveyed that plants grown under low light environments are unable to intensively undergo the process of photosynthesis even though this should be accomplished at the low light intensities. It was reported earlier that a shade percentage of 30-50% is ideal for vegetables while most plants will do best with a maximum of 40% - 60% shade (Chen, 2014). Though, when growing some shade loving plants such as orchids and ferns, 75% or higher shading maybe needed to get correct light levels (De, 2020). Using shade cloth can be an operative solution to reduce sunburn on plants. It also decreases the amount of radiation that reaches the plants. This is because the shade cloth can reflect the air and incoming solar radiation. Plants that are grown under the shade are found to have larger and taller leaves, and more nodes, especially if they are shade-loving plants (Randall, 2007; De, 2020).

The use of shade improves the relative humidity (RH) inside the shade structures compared to the outside the shading structure. There were many aspects of plant development that could be changed with shade cloth and as such should be observed to determine the potential impacts on crop yields, fruit set as well as fruit quality in terms of marketable or unmarketable fruit (Caillouet, 2016; Mditshwa *et al*., 2019). Shade cloth has also shown to have increased light scattering by up to 50% or more, this can affect plant growth and development (Caillouet, 2016). It was found that shade cloth on blackberries prolonged the ripening period and increased cumulative yields due to the less concentrated fruit development (Caillouet, 2016). The use of shade changed light influences in the leaf chlorophyll content. Shaded leaves had lower photosynthetic rates than non-shaded plants and have been reported to contain more total chlorophyll than sun leaves (Björkman, 1968; De, 2020). Number of leaves is one of the most crucial growth parameters which is influenced by genetic and environmental factors (Gupta *et al*., 2020). Semchenko e*t al.* (2012) reported that shade-tolerant plants grow broader, leaves become thinner to catch more sunlight for photosynthesis, and that also makes the plant to have more chlorophyll content.

Growing vegetables using 20% to 40% shade cloth allows vegetables to be deprived of heat stress and extends their productive period. It also reduces the amount of water they need and is thus a crucial contributor to water wise vegetable gardening (Brand. 1997; Sharma, *et al*., 2020). Semchenko *et al.* (2012) documented that moderate shade or plants growing under shade net, increased plant dry mass compared to the plants that are in full day sun. Furthermore, Yasoda *et al.* (2018) highlighted that 50% shade had the highest number of cauliflower leaves recorded more than one that was grown on an open space under 25% of shade also mention that 50% shade showed a greater yield than 25%. The highest number of leaves was recorded from the plants grown under shade net.

2.6. Effect of different shade cloth colour on yield and postharvest quality

Shade cloth is used as a photo-selective filter to protect plants from too much heat from the sun. It is also used to facilitate the diffusion or distribution of light to improve postharvest quality (Lara *et al.,* 2021). One of the important aspects to consider when selecting a shade cloth is the colour given that shade cloth colour influences the growth of numerous species. Selecting the right colour of shade cloth can effectively make a big difference to a specific plant (Gaurav *et al*., 2016). The selection of the right shade cloth colour is not just a matter of personal preference. White shade cloths reduce the quantity of light but not the quality of the light spectrum. Accordingly, the growth of the plant is more rapid than using green and black shade cloth. White shade cloths are often used for flowering plants. Dark colour shade cloth is known to absorb the sun’s heat while the light colours reflect the sun’s heat. Usually, green and black shade cloths behave like filters and protect the plants from receiving too much sunlight. Aluminized shade cloth may also offer additional cooling effect for the plants. Colours such as red can benefit specific plants by filtering different wavelengths of light (Chen, 2014).

2.7. Radiation and the influence of light on plants

Light intensity and quality are crucial factors for plant growth and development (Fukuda *et al.*, 2008; Ferrante & Mariani, 2018). The changes in light intensity may strongly affect the structural, physiological, and morphological aspects of a plant (Haliapas *et al*., 2008; Li, *et al*., 2020). As Walters (2005) pointed out, plants have developed photosensitive mechanisms to release light energy for photosynthesis. By so doing, the light also encourages movements of stomata, and chloroplasts, which are involved in the regulation of photosynthesis. The amount of light absorbed by the leaf surface area as well as the distribution of light influence plant growth rate, light can be measured in wavelengths, which can be short or long depending on their energy levels (Kalaitzoglou *et al*., 2019). The primary pigment in plants that is accountable for photosynthesis is chlorophyll which is reflected by green light, while red and blue spectrums are engaged (Carter, 2014). Various relevant traits for plants are influenced by photoreceptors such as size and shape of leaves, stem length, chloroplast development and even flowering period (Hudson, 2003). The photosynthetic process is influenced by the light quality, duration size of the plant canopy, and the leaf surface area. The total leaf surface area reflects the photosynthetic potential of the plant which may be influenced by factors such as nutrients, soil moisture levels, plant hormones, light distribution, and plant species types (Barritt *et al*., 1991; Liu *et al.,* 2020).

2.8. Food security in a changing climate

Havas and Salman (2011) reported that food security is a difficult concept to measure since it is simultaneously concerned with different terms, such as production, distribution and consumption of food, that cannot easily and accurately be measured or determined. Food insecurity on the other hand, offers itself more proxy indicators that can readily be used in the measurement and analysis of food (in) security. It should be stressed that food security and scarcity and hunger are not to be confused. Food security refers to the availability of food whereas scarcity and hunger are the consequences of the non-availability of food or the results of food insecurity (Napoli *et al*., 2011). The Department of Agriculture, Forestry and Fisheries (DAFF), Food and Agriculture Organization of the United Nations (FAO), as well as the Centre for Poverty, Employment and Growth (CPEG) of the Human Sciences Research Council (HSRC) recognized that food security has three dimensions namely food availability, food access and food use. Food availability implies that a country must have enough quantities of food available on a reliable basis at both national and household level. Food access suggests the ability of a nation and its households to acquire sufficient and adequate food on a sustainable basis. Food use refers to the suitable use of food through suitable diets based on knowledge of basic nutrition and care, as well as adequate water and sanitation. As Du Toit et al. (2011) and Napoli *et al*. (2011) highlighted that food security at the national level refers to the situation whereby the nation can manufacture, import, retain and sustain food needed to support its population with minimum per capita nutritional standards. At the community level, food security is defined as the situation whereby the residents in a community can acquire safe, culturally accepted, nutritionally suitable diets through a sustainable system that maximizes community self-reliance (Napoli *et al.,* 2011). At the household level, food security refers to the availability of food in one's home which one has access to. In this case, a household is regarded as food secure when the members of the family are not threatened by starvation or undernourishment within their households.

The concept of food insecurity is closely related to the poverty in country. The two concepts are interrelated, and to some extents have an influence on one another. In any food security dissertation, it is also important to briefly highlight the difficulty of poverty in the country (Naylor, 2011). Poverty refers to the condition of not having the means to afford basic human needs such as clean water, nutrition, health care, education, clothing and shelter. Schultz and De Wrachien (2002) specified that the current word food production comes from 1.5 billion hectors of land, and that is about 12% of the global land area. It has been conveyed that in the last 50 years, the cultivated land has been reduced by 13% and that global agricultural production growth is predicted to decrease by 1.5% every year until 2030, and by 0.9% from then until 2050, a decline that is equivalent to 2.3% growth per year since 1961 (FAO, 2008; Hanjra & Qureshi, 2010). As Narayanamoorthy (2007) argues, continuous decline in agricultural progress will affect world food security. To achieve realistic increases in food production, food supply will depend on the proper management of agricultural resources and investments in machinery as well as strict policies (Herrero *et al*., 2010).

Food security is perceived as a broad term, but the basic definition is that it is the continual access by all people to enough food for a vigorous healthy life or well-being (Anderson 1990; Du Toit *et al*., 2011). Food security is also viewed as being closely related to poverty in a country (Du Toit *et al*., 2011). South Africa is one of the countries with the highest disparities of income variation in the world and high levels of poverty compared to other middle -income countries (Altman *et al*., 2009; Maluleke, 2019). Different scholars have reported that rural South African households face poverty due to low income hence some households not being able to feed their members sufficiently and nutritionally (Labadarios *et al.*, 2011). In response to this problem of food insecurity in households, continuous efforts are being made by the government to obtain high productivity to increase profitability and meet the constantly increasing demand for food.

Food security is a significant challenge in many countries, nonetheless of whether you look at it from an environmental, health or urbanization angle. As people move from agricultural (rural) to urban areas, food security requirement is transferred from land cultivating to purchasing power. Those without financial means or capital in urban areas are then put at risk as they do not have access to good nutrition, and their ability to maintain food security and good health are threatened (Dubbeling *et al*., 2010; Islam, 2020). Without food security and good health, the probabilities of development are still low because development entails as well people's ability to easily and spontaneously access all those things that are needed for a sustainable well-being of humans (Lofgren & Richards, 2003). Although many things are needed for human life, not discounting self-worth and a sense of self-worth, the first step is the capability to preserve food security and good health. Drawing on the Maslow's hierarchy of needs, the author highlighted that food is one of the pre-potent physiological needs, and that without the happiness of all needs, the physiological needs, such as food, become overpowered. Similarly, an account of FAO (2010) on food security states that admission to nutritious food is a key dimension of food security. The FAO (2010) report further points out that access to nutritious food leads to better health, both physically and psychologically. Thus, better health can be provided through the good practice of eating green and sustainable food.

2.9. Wild edible plants as new staple food crops

Wild edible plants have continuously been an important food source for food-insecure families living in poverty in many developing countries (Ong & Kim, 2017). They are an important source of vegetables, fruits, tubers and nuts which are used in ensuring food supply and improving the nutritional value of food (Abbasi, 2013). Wild leafy vegetables, for example, are important sources of micronutrients in the tropics and are important in children's nutrition to ensure normal growth and intelligent development (Ong & Kim, 2017). They are also important for many communities in rural villages and even for those in urban areas, especially for the poor and the marginalized**.** Wild edible plants mainly play an important role in the life of many indigenous farming and hunter-gatherer communities. Wild edible plants are dependable alternative among marginalized groups when production of cultivated crops is condensed. (Hinnawi 2010; Ong & Kim, 2017). By leaning on this resource as an alternative to cover the gap caused by food scarcity, food shortages within households are moderated, and nourishment, to some level, is better. In poor and developing nations prone to drought and scarcity, the importance of wild edible plants as fallback opportunities or even for survival is of immeasurable worth (Mlcek & Rop, 2011; Dogan, 2016; Rome, 2018).

Through the rapid rise in the population, there is higher demand of food supply to serve millions of people as such dependence on the few major staple crops to meet the needs of the people has headed to increases in starvation and poverty (Abbasi *et al.,* 2013). Underutilized wild edible plants offer a cheaper and affordable opportunity in providing more crop modification to challenge problems associated with starvation and poverty, and provide food security, in general, to the poor in the world, and to the developing countries.

2.10. Hydroponics cultivation for water conservation

White (2004) reported that hydroponics is a technique of growing plants in nutrient solutions with or without the use of an inert medium such as gravel, vermiculite, rock wool, peat moss, saw dust, coir dust, coconut fibre, sand, etc. The term Hydroponics was derived from the two Greek words, namely ‘hydro’ meaning water and ‘ponos’ meaning labour and can literally be translated as water work. In agriculture, hydroponics consists of growing plants with their roots suspended in water containing mineral nutrients. Most hydroponic systems work automatically to control the amount of water, nutrients and photoperiod based on the requirements of different plants (White, 2004; Maucieri *et al*., 2019).

Due to rapid urbanization and industrialization, not only the cultivable land is decreasing, but also predictable agricultural practices are causing a wide range of negative impacts on the environment. To sustainably feed the world’s growing population, methods for growing sufficient food need to change. Thus, the modification of growth medium is an alternative for a safe sustainable production as it facilitates the conservation of available water resources and land, which is reducing daily due to a rapid population growth. In the present scenario, soil less cultivation might be commenced successfully and considered as alternative option for growing healthy food plants, crops or vegetables (Putra & Yuliando, 2015). Agriculture without soil includes hydro agriculture (Hydroponics), aqua agriculture (Aquaponics) and aerobic agriculture (Aeroponics) as well as substrate culture. Of these types of agriculture, hydroponics techniques have been attributed a great attention due to their efficient management of resources and food production. Many commercial and specialty crops can be grown using hydroponics including leafy vegetables, tomatoes, cucumbers, peppers, strawberries, and many more (Blidariu, & Grozea. 2011; Rajatha *et al*., 2019).

Putra and Yuliando (2015) and Tality *et al*. 2022 stated that hydroponic production of plants is becoming prevalent in the modern world with ecological inequalities such as extreme temperatures, drought, chemical toxicity, and oxidative stress threatening conventional agricultural practices. Hydroponics can be useful in places where there is limited availability of space, where there is shortage of water, where the soil is chemically excessive, and where there are high levels of pathogen infestation (Corrêa *et al.*, 2012). In South Africa, challenges like population growth, water scarcity and increasing demand for food have raised a need for sustainable and well-organized cultivation method (WWF, 2017). It is further stated that hydroponics cultivations limit the loss of water and nutrients (Siddiqi *et al*., 1998; Van So 1999). It has also been found that hydroponically grown plants grow faster and healthier because they get nutrients in particular amounts according to the grower (Ortiz *et al*., 2009; Wahome *et al*., 2011).

2.11. Different growth media used in hydroponics.

In hydroponics, soil has been replaced by artificial growth mediums since they are disease and pest free, and that they can be reused year after year (Asaduzzaman *et al.,* 2015). According to Hassain *et al.* (2014) different growth mediums lead to effective use of water and fertiliser, thus reducing the use of chemicals applied to treat plant diseases in the process. The proposed study will then focus on four growing mediums (i.e., sand, expanded clay pallet, coconut fibre and vermiculite mixed with perlite).

Expanded clay pellets (Leca clay) or grow rocks is a highly suitable growing medium for hydroponic cultures (Rebel, 2012). The pebbles vary in size from 1-18mm are filled with tiny air pockets to allow ample drainage and aeration capacity (White, 2004). Clay pellets are best for ebb and flow systems, especially in systems with circulating watering. Since expanded clay pellets do not have good water-holding capacity, salt accumulation and drying out can be the common problems in inappropriately managed systems, however the system can be flushed out. Pellets can be reused after being sterilized (Rebel, 2012). Sand on the other hand is one of the oldest known hydroponic substrates. Nevertheless, sand is not widely used today, commonly because of its low water-holding capacity and weight. Sand has an affinity to tightly pack together, reducing the amount of air available to the roots; therefore, a coarse builders' sand is best suited for hydroponic use. Alternatively, sand can be mixed with other media for greater water-holding capacity and lighter weight. The advantage of using sand is that it is of the cheapest media (Rebel, 2012). Rebel (2012) described vermiculite as a soil preservative to increase aeration and draining of the soil. Vermiculite, which is used the same way as perlite and which is frequently mixed together with it, is made from heat expanded mica and has a flaky shiny look. Since perlite and vermiculite are so lightweight, they are recommended only for starting seeds and cuttings. Perlite has a good wicking action, which makes it a good choice for wick-type hydroponic systems, plus it is relatively cheap (White, 2004). The biggest drawback to perlite is that it does not retain water very well since it dries out quickly once watered. Unlike perlite, vermiculite has a high-water retention capacity and can suffocate the plant's roots when used straight.

Coconut fibre, also called coir, is promptly becoming one of the most popular growing mediums in the world. It is the first “organic” medium that completely offers highest performance in hydroponic systems (White, 2004). Coconut fibre is a waste product of the coconut industry and is obtained from the powdered husks of coconuts. Coconut fibre is available in different grades, and the lowest grade has a very high salt content that necessitates leaching before use. The main advantages of coconut fibre lie in its oxygen and water-holding abilities. Thus, coconut fibre can maintain a larger amount of oxygen than Rockwool and yet still with a greater water-holding ability. Some researchers such as White (2004) have shown that coir might have insect-repelling abilities. High-quality coir (the grade commonly used for hydroponics), which is a constitutive of coarse fibres and has the advantage of not containing high level of nutrients that will modify the composition of a nutrient solutions.

2.12. Propagation by division

Propagation by division is a procedure of plant propagation in which a group of plants parts are cut or torn apart which each part of the divided plant contains one or more of the roots of the plant and a part of the stem of one or more stems (Megersa, 2017). Division is the simplest form of plant propagation which is suitable for most clusters and rosette forming perennials (Bagnasco, & Reidmuller, 2019). Utmost perennial plants benefited from division as they get older and begin to lose their vigour. It contains a bit more than breaking up an established clusters into a few smaller parts (Landauer *et al*., 2019). The success rate of plant propagated from division is very high compared to the other propagation methods (Rodrigues *et al*., 2020). Plants that have fibrous, rhizomatous roots, and plants that form clusters or crowns, are typically split up for propagation into new plants. The dividing line between fibrous rooted perennials, crown rhizome perennials and rhizomes are somewhat unclear (Wilsey, 2018). Rhizomes are purely underground stems and separated from the crown of roots around the base of the plant (Wilsey, 2018). These will require careful cutting and many in some case have distinct growth points. Each division will need at least one growth point if it is to strike. This sort of division is best done just as the plants are emerging from dormancy. The line between division and natural layering is overlap. Many perennials and shrubs, particularly ground covers will strike roots wherever they come in contact within the ground. If cut off at the suitable point these aerial roots will then develop as normal subterranean roots. The actual size of the divide plant differs extremely depending on the plant. Different horticultural crops can be propagated by different specialized organs propagation by division (Megersa, 2017).

2.13. Importance of nutrients, Electrical Conductivity and pH in hydroponics

Amongst factors affecting hydroponic production systems, the nutrient solution is considered as one of the most important determining aspects of crop yield and quality. A nutrient solution for hydroponic systems is an aqueous solution covering mainly inorganics ions from soluble salts of important elements for higher production of plants. In due course, some organic compounds such as iron chelates may be present in plants (Sorenson & Relf, 2009). An important element has a vibrant physiological role, and its absence prevents the complete plant life cycle (Sharma *et al.*, 2018). Thus, the 17 elements that are considered important for most of the plants are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, copper, zinc, manganese, molybdenum, boron, chlorine and nickel (Sharma *et al.,* 2018). Except the two elements, namely carbon I and oxygen (O) that are primarily obtained from the atmosphere, other important elements are supplied by the growth medium. Of these elements, such as sodium, silicon, vanadium, selenium, cobalt, aluminium and iodine among others, are considered beneficial because of the following: they can stimulate the growth, compensate the toxic effects of other elements, or may replace essential nutrients in a less specific role (Sharma *et al.,* 2018). The most basic nutrient solutions entail only nitrogen, phosphorus, potassium, calcium, magnesium and sulphur which are sometimes supplemented with micronutrients however, the nutrient arrangement determines electrical conductivity and osmotic potential of the solution (Lauguico *et al*., 2020).

The total ionic concentration of an EC of the nutrient solution regulates the growth, development and production of plants (Sharma *et al.,* 2018). The total amount of ions of melted salts in the nutrient solution exerts a force called osmotic pressure, which is a colligative property of the nutrient solutions, and which is clearly dependent on the number of dissolved solutes (Sharma *et al.,* 2018). In addition, the terms 'solute potential' and 'osmotic pressure' are extensively used in nutrient solution to represent the effect of dissolved solutes on water potential while solutes reduce the free energy of water by diluting it (Cornish, 1992). Thus, both concepts (i.e., osmotic pressure and osmotic potential) can be used interchangeably if one considers important units that are used commonly, namely Atm, Bar and Mpa (Sorenson & Relf, 2009). An indirect way to estimate the osmotic pressure of the nutrient solution is the electrical conductivity (EC), an index of salt concentration that defines the total amount of salts in a solution. Therefore, EC of the nutrient solution is a good indicator of the number of available ions in the root zone of a plant (Sharma *et al*., 2018).

The pH is a parameter that measures the acidity or alkalinity of a solution. This value indicates the relationship between the concentration of free ions H+ and OH- present in a solution, and it ranges between 0 and 14 (Sharma *et al.,* 2018). An important feature of the nutrient solutions is that they must have the ions in solution and in chemical forms that can be immersed by plants. Thus, in hydroponic systems, the plant productivity is closely related to the nutrient uptake and pH regulation (Sharma *et al*., 2018). Respectively, the nutrients in a plant respond differently when exposed to changes in pH of the nutrient solution.

2.14. Conclusion

Trachyandra divaricata is an underutilized species that can address the food crisis ravaging the world. Adopting the plant from the wild will bridge the nutrient gap between the rich and poor avail food industries and commercial growers the opportunity to diversify their products. It is pertinent to develop cultivation protocols for the species to facilitate its re-introduction.

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3. CHAPTER THREE:

Vegetative growth of *Trachyandra divaricata* (Jacq.) Kunth in response to different growing media and shade stress in hydroponics

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3.1. Abstract

Trachyandra divaricata is an underutilized edible species that was traditionally used as food by Khoisan people of South Africa. The buds of this plant can be steamed, roasted, or used in salads although the plant was never commercially farmed. This necessitated the investigation of the growth response of the plant to different growth media and shade levels under hydroponic system to develop suitable techniques for its commercial cultivation. Stock of *T. divaricata* obtained at Bellville campus of the Cape Peninsula University of Technology was propagated by division methods and planted into four different growth media namely LECA clay, silica sand, peat, and vermiculite in Nutrient film technique (NFT) hydroponic system that were randomized for experiment with four different shade percentages: 20%, 40%, 60% and 80% shade levels. The results from this study showed that *T*. *divaricata* is suitable for hydroponic cultivation and that a combination of different levels of shade stress (20%, 40%, 60%, 80%) and growth media (LECA clay, peat, silica sand and vermiculite) significantly affected the vegetative growth of *T*. *divaricata*. The plants that were grown in silica sand under 20% shade level produced the highest number of leaves as they grow older during weeks 6, 8, 10 and 12 compared to other treatments. However, the peat medium under no shade treatment increased significantly, the formation of flower buds and biomass of *T. divaricata* suggesting that biomass accumulation and vegetative characteristics of the species is enhanced by longer photoperiods. Also, it is evident from the study that peat was the most suitable medium required for optimum vegetative development, flower bud formation and biomass accumulation. These findings could benefit future researchers and aid further, the commercial production of the plant.

Key words: Biomass accumulation; bud formation; Hydroponic cultivation**;** vegetative development

3.2. Introduction

Trachyandra divaricata is a perennial herb that grows up to 70 cm tall with several thick basal leaves up to 50 cm long and above (Van Jaarsveld, 2001). Its linear leaves are dark green, succulent and often slightly rolled over along their length. The raceme needs to be harvested for eating before it branches and produces white flowers that last a day; and if cut the plant readily produces more racemes (Van Jaarsveld 2001; Boatwright 2010). The leaves stay evergreen throughout the year, and the racemes of *T. divaricata* seem over a longer season compared to other *Trachyandra* species. The bright green leaves are smooth and hairless. The leaf shape is virtually flat, fleshy, long and narrow. Boatwright and Manning (2010) further described the species as quill-like with leaves enclosed at the base within membranous sheaths. The green leathery fruit is a segmented ovoid capsule that turns red and later black (Heylingers, 1999; De Vynck, *et al*., 2016).

The pace of vegetative growth is influenced by several factors in plants. These include photoperiod, temperature, water and other abiotic factors (Leakey, & Youngquist 2004; Nave *et al*., 2010; Hatfield & Prueger, 2015). In the process of vegetative growth, some plants produce storage organs such as tubers; rhizome, roots which store carbohydrates, and which are important agricultural products (Hewelt *et al*., 2000; Srivastava, 2002). Vegetative growth is the development of leaves, stems and root whereas reproductive growth is the growth of different parts such as flowers, fruits and seeds that contribute to plant reproduction (Daft & El-Giahmi. 1978; Kozlowski & Ziólko, 1988; Cervantes. 2006; Poorter *et al*., 2012). The plant divides its energy between vegetative and generative growth where the nutrients produced in the leaves are utilized and remobilized to storage organs in the plant (Mattsson *et al*., 1991; Saska & Kuzovkina, 2010). The meristematic tissues are busy making leaves during the vegetative phase, which begins at germination and lasts until tillering (Chesworth *et al*., 1998; Hewelt *et al*., 2000). This is vital because enough leaf surface area is required to capture sunlight and conduct photosynthesis (Terashima *et al*., 2006).

Many studies have revealed that the quality and intensity of light received in a specific leaf area stimulates largely the growth and development and induces physiological alterations to chloroplast ultra-structure which in turn affects the process of photosynthesis in plants (Franklin 2009; Yang *et al*., 2017; Miao *et al*., 2019; Wang *et al*., 2019). Plants alter their morphology and physiological processes in response to changes in electromagnetic spectrum absorbed from incident light resulting in adaptation to different environmental conditions

(Costa *et al*., 2010; Avgoustaki. 2019). These changes are facilitated by pigmented photoreceptors that detect variations in light composition and induce photo-morphogenetic responses due to their absorption peaks in the blue/ultraviolet and red regions of the electromagnetic spectrum that influence growth and development (Li *et al*., 2000). A practical way of regulating the intensity of incident radiation spectrum is by introducing photo selective nets as spectral filters to relieve plants from extreme environmental conditions and induce physiological changes that prompt vegetative growth in plants (Costa *et al*., 2010; Lobos *et al*., 2013). Many plants respond to different combinations of shade levels depending on the thickness or net colours used (Lobos *et al*., 2013). However, little is known about the responses and adaptation of *T. divaricata* cultivated in different growth media under different shading levels in hydroponics set up in a greenhouse. The goal of this study was to assess how different growth media and shade stress would affect the vegetative growth, morphology and the rate of development of flower buds in *T. divaricata* so that a viable propagation technique could be developed to enhance the commercial production of the species.

Figure 3.1: Showing the plant size before the experiment. (Picture by Tshayingwe, 2021)

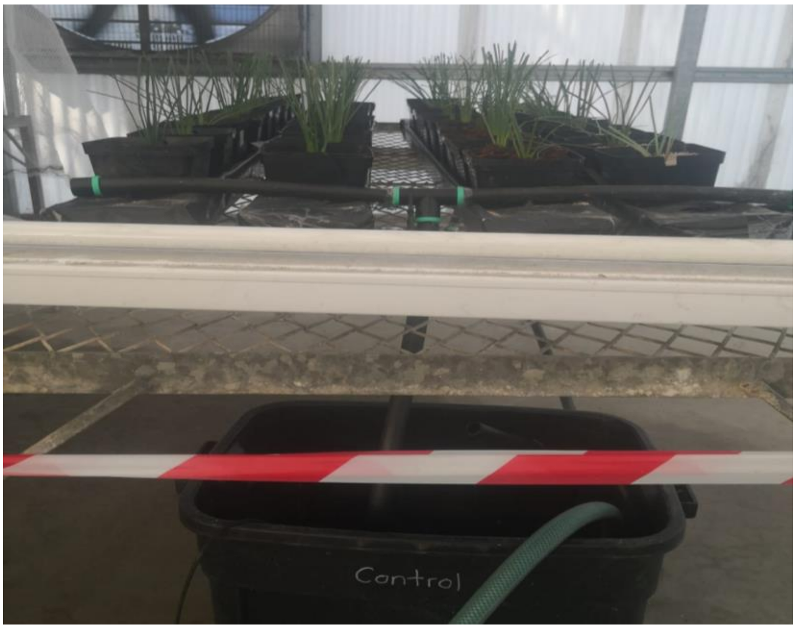

Figure 3.2: Showing the plant growth after 1 week.
(Picture by Tshayingwe, 2021)

Figure 3.3: Showing the new leaf growth in week 3.

(Picture by Tshayingwe, 2021)

Figure 3.4: Showing formation of buds in week 8.

(Picture by Tshayingwe, 2021)

3.3. Material and methods

3.3.1. Greenhouse experiment

This experiment was carried out in the Horticulture Research Glasshouse 2 of the Cape Peninsula University of Technology, Bellville campus, South Africa (33° 55’45.53S, 18° 38, 31’. 16E) over a period of twelve weeks. The environmental temperatures at the greenhouse were controlled.

3.3.2. Hydroponic system design

In this experiment, four identically manufactured nutrient film technique (NFT) hydroponic systems were employed with each system set up on a wire mesh square table (2.5 m long) that offered a flat surface. Another wire mesh square table was placed on top of each system to create a different level of shade and serve as a hanger for the shade net. Each system had its low-density polyethene (LDPE) 50 L reservoir in which the nutrient solution was prepared and supplied to the system. There were 4 Polyvinyl Chloride (PVC) square gutters (2 m long), put in place with cable ties on each table in which 15cm square plastic pots (ten pots per gutter) filled with 4 different growth media namely, LECA clay, peat, silica sand and vermiculite were arranged in the gutters. The gutters were sealed with PVC adhesive to prevent leaks and connected to a 1 × 2000 L/hr submersible pump with 2.5 m head capacity, 20 mm LDPE irrigation piping, 4 × 20 mm elbow irrigation fittings and 4 × 20 mm flow regulators (Ngxabi *et al*., 2021a).

3.3.3. Plant collection and nursery preparation

Trachyandra divaricata was obtained from Bellville campus of the Cape Peninsula University of Technology. The stock plant was propagated in 15 cm pots and placed under automatic irrigation at propagation house for two weeks and thereafter, transplanted to the hydroponic system. The division propagation method was done to ensure the species are identical to each other. The leaves were cut into the same length (10cm), number of leaves (6 leaves per plant) and equal weight of 23 g.

3.3.4. Treatment preparation

Different growth media namely: LECA clay (LC), silica sand (SS), peat (P) and vermiculite (V) used for cultivation. The silica sand and LECA clay were rinsed thoroughly with sterile water to clean impurities and other earthy materials. A shade net of 20% density percentage used for the study was purchased from Stodels Garden Centre, Bellville branch, South Africa. Different levels of shading were created from the factory made 20% density percentage net by doubling (40%), tripling (60%) and quadrupling (80%) and all these were used as light filters in the greenhouse. The nets were then used to cover the tables where pots of different growth media namely silica sand (SS), peat (P), vermiculite (V) and LECA clay (LC) were arranged in a completely randomized design (CRD) except the control, which was not covered with shade net (0% shade). The divided parts of *T. divaricata* were then planted in pots filled with different growing media under a Nutrient Film Technique hydroponic system.

Table 3.1: Experimental treatment layout for growing *T. divaricata*.

Growth media	Shading percentage
1 = LECA clay	no shade
2 = LECA clay	20% shade
3 = LECA clay	40% shade
4 = LECA clay	60% shade
5 = LECA clay	80% shade
6 = Peat	no shade
7 = Peat	20% shade
8 = Peat	40% shade
9 = Peat	60% shade
10 = Peat	80% shade
11 = Silica sand	no shade (control)
12 = Silica sand	20% shade
13 = Silica sand	40% shade
14 = Silica sand	60% shade
15 = Silica sand	80% shade
16 = Vermiculite	no shade
17 = Vermiculite	20% shade
18 = Vermiculite	40% shade
19 = Vermiculite	60% shade
20 = Vermiculite	80% shade

3.4. Results

3.4.1. Number of leaves

Even though most media had more leaves, the highest number of leaves (11.50) was found in the peat medium under 60% shade in week two, while the lowest number (8.00) was found in the peat medium with no shade in week two (Table 3.1). At week four, the highest value (13.10) of leaf number was found in LECA clay media with no shade while the lowest leaf number (9.20) was found in silica sand medium with 80% shade. At week six, the Silica sand medium under 20% shade produced the most leaves (15.50), whereas the Peat medium in no shade produced the fewest leaves (11.20). At week eight, the Silica sand medium in 20% shade produced the most leaves (19.40), whereas the Peat medium in 80% shade produced the least (13.00). At week ten, the Silica sand medium in 20% shade had the most leaves (21.50), while the Vermiculite medium in 20% shade had the fewest leaves (13.50). at week twelve, the Silica sand medium under 20% shade had the most leaves (27.90), while the fewest leaves (13.90). was recorded in the Peat medium under 20% shade (Table 3.1).

3.4.2. Leaf Length

Different growing media and different shade percentages had a significant influence on leaf length. At week 12, the longest leaf (80.80 cm) was observed in the peat medium under No shade treatment and was significantly higher ($P \leq 0.05$) than other treatments, the lowest value was recorded on Vermiculite medium under 80% shade treatment at week 12 (26.20 cm). Other treatments had an equivalent effect on the leaf length of *T. divaricata.*

3.4.3. Number of Buds

In LECA clay soilless medium, the highest number of buds were formed under 40% shade, and the lowest number was observed at 80% shade. In peat growth medium, the highest number of buds were formed under no shade, while the lowest number was recorded at 80% shade. On silica sand growth media, 60% shade was the best treatment, and the lowest was recoded at 80% shade. On vermiculite growth medium, the highest number of buds were formed under 60% shade, and the lowest number was discovered at 80% shade. The growth media Peat media No shade had the significantly different than all other treatment the largest value of (11.90) was recorded and the lowest value of (2.2) was recorded at LECA clay medium at 80% shade (Figure 3.5).

3.4.4. Plant fresh weight (g)

The LECA clay medium treatment was the least effective on the plant's fresh weight, followed by the Silica sand growth medium, vermiculite growth medium performed better than the two and peat growth medium was the best treatment and other treatments had an equivalent effect (Figure 3.6).

3.4.5. Plant dry weight (g)

Different growth media and different shade percentages had a significant influence on plant dry weight (Figure 3.7). The Peat medium with No shade treatment recorded the highest mean value of dry weight (35.17g) and was significantly higher ($P \leq 0.05$) than other treatments, the lowest value was recorded on Silica sand medium at 80% shade treatment (10.58g) and the rest of the treatments had a comparable effect on plant dry weight. (Figure 3.7).

Table 3.2: Effect of different growth media and shade stress on number of leaves of *T. divaricata.*

Growth media	Shade	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
	No shade	11.10±1.02ab	13.10±1.02a	14.90±1.12ab	16.70±1.41ab	18.60±1.66ab	24.80±2.83abc
	20% shade	9.30±0.76ab	11.00±0.58ab	12.80±0.71ab	14.10±0.82b	14.60±0.97ab	16.50±0.89bcd
LECA clay	40% shade	9.80±0.80ab	11.80±1.09ab	14.30±1.30ab	16.10±1.29ab	18.90±1.70ab	20.90±2.01abc
	60% shade	11.10±0.88ab	11.80±0.71ab	14.50±1.20ab	16.20±1.56ab	17.60±1.41ab	17.60±1.80bcd
	80% shade	11.10±0.77ab	11.20±0.61ab	13.30±0.62ab	13.30±0.62b	15.50±2.06ab	16.78.2.01bcd
	No shade	8.00±0.49b	9.80±0.42ab	11.20±0.57b	13.20±0.67b	14.70±0.86ab	19.40±1.48abc
	20% shade	9.70±0.61ab	10.80±0.71ab	11.50±0.54ab	13.20±0.73b	17.00±0.89ab	22.20±1.74abc
Peat	40% shade	9.30±0.61ab	10.30±0.52ab	12.00±0.39ab	13.30±0.63b	15.40±1.01ab	17.20±1.01bcd
	60% shade	11.50±0.40a	12.50±0.70ab	14.10±0.59ab	15.90±0.74ab	19.00±0.97ab	20.80±1.13abc
	80% shade	8.70±0.63ab	10.70±1.00ab	12.70±1.01ab	13.00±0.84b	13.90±1.16ab	14.10±1.93d
	No shade	8.90±0.66ab	11.00±0.67ab	13.80±0.79ab	17.10±0.90ab	20.00±1.50ab	26.30±2.44ab
	20% shade	8.70±0.73ab	11.40±0.76ab	15.50±0.89a	19.40±1.39a	21.50±1.15a	27.90±1.85a
Silica sand	40% shade	8.50±0.54ab	9.50±0.50b	12.10±0.57ab	13.30±0.63b	15.30±1.98ab	18.10±2.36abc
	60% shade	9.00±0.73ab	10.60±0.42ab	12.80±0.59ab	14.40±0.65ab	17.40±1.08ab	20.20±1.58abc
	80% shade	8.30±0.59ab	9.20±0.53b	11.80±0.57ab	12.30±1.74b	14.10±2.85ab	16.10±2.85cd
	No shade	8.80±0.53ab	11.60±0.56ab	12.30±0.65ab	13.00±0.62b	15.80±0.79ab	18.40±1.06abc
	20% shade	8.60±0.63ab	10.50±0.50ab	13.10±0.41ab	14.20±0.59ab	12.10±2.75b	14.50±3.37d
Vermiculite	40% shade	9.70±0.47ab	10.70±0.37ab	13.00±0.45ab	14.50±0.40ab	16.80±0.76ab	17.00±2.06bcd
	60% shade	9.90±0.55ab	11.00±0.68ab	14.00±1.23ab	16.00±1.32ab	17.70±1.68ab	19.00±2.26abc
	80% shade	9.10±0.80ab	9.70±0.82ab	11.90±0.48ab	13.30±0.68b	13.20±1.74b	14.80±1.88cd

Means that do not share a letter are significantly different at P ≤0.05.

Table 3.3: Effect of different growth media and shade stress on leaf length of *T. divaricata*.

Growth media	Shade	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
LECA clay	No shade	35.50 ± 1.74ab	45.70±2.47 ab	51.40±2.57ab	58.50±2.31 ab	63.80±2.69 abc	29.10 ± 1.04a
	20% shade	30.00 ± 1.29b	36.50±1.61 de	43.70±2.44abc	53.30±2.94 abc	61.40±2.88 abc	25.50 ± 1.38a
	40% shade	31.80 ± 1.06ab	37.50±1.25 cde	42.50±1.36abc	55.00±1.63 abc	62.40±2.03 abc	27.80 ± 0.94a
	60% shade	31.10 ± 0.46ab	38.10±0.69 bcd	44.20±0.98abc	56.20±1.55 abc	64.50±1.29 abc	28.70 ± 0.40a
	80% shade	32.90 ± 1.25ab	37.70±1.44 bcd	39.90±4.60d	46.70±5.43 abc	52.00±6.06 bc	28.90 ±1.22a
Peat	No shade	35.20±1.07ab	46.30±2.21a	52.10±2.39 a	61.90±2.76 a	80.80±3.53 a	30.10±0.75a
	20% shade	35.90±1.29ab	45.00±2.21 abc	52.50±2.15 a	62.80±2.06 a	76.20±2.85 ab	29.20±1.67a
	40% shade	33.40±1.00ab	41.70±1.24 abc	43.70±0.72 abc	63.00±2.66 a	74.00±3.61 abc	29,20±0.80a
	60% shade	35.70±0.76ab	41.80±1.20 abc	47.60±1.26 abc	63.70±2.52 a	72.60±2.71 abc	30.60±1.10a
	80% shade	32.00±0.97ab	39.10±1.51 abc	43.60±1.48 abc	57.20±3.52 abc	60.50±6.91 bc	28.50±0.76a
Silica sand	No shade	31.10±1.06ab	38.50±1.96 abc	43.60±1.81abc	49.20±2.50 abc	55.10±3.73 bc	24.00±1.63a
	20% shade	29.50±2.26b	35.80±1.53 e	40.40±1.48cd	50.40±3.50 abc	59.70±3.88 abc	25.40±1.97a
	40% shade	31.50±1.05ab	37.30±0.87cde	41.80±1.21abc	48.60±5.68 abc	55.10±6.35 bc	27.40±1.26a
	60% shade	30.10±1.06b	36.20±1.15 de	40.90±1.10bcd	51.40±2.75 abc	60.30±3.68 abc	26.70±1.64a
	80% shade	30.40±1.62ab	34.60±1.63 e	36.70±4.29d	38.00±6.45c	51.67±6.66 bc	26.30±1.66a
Vermiculite	No shade	37.00±1.97a	44.10±2.64 abc	51.30±2.69 abc	56.50±2.50 abc	66.20±3.38 abc	28.50±1.76a
	20% shade	32.60±1.13ab	37.70±1.07 bcd	43.00±1.13 abc	55.00±7.22 abc	65.40±8.32 abc	24.40±2.33a
	40% shade	33.90±0.61ab	39.00±0.89 abc	44.70±0.21 abc	63.10±2.61 a	66.40±7.70 abc	28.40±0.91a
	60% shade	32.00±1.20ab	38.10±1.36 bcd	42.20±2.05 abc	55.50±3.03 abc	62.80±3.54 abc	27.10±1.14a
	80% shade	30.30±1.43b	34.40±0.93e	39.70±1.17 d	41.80±4.91 bc	26.20±1.44 c	26.20±1.44a

Means that do not share a letter are significantly different at P ≤0.05.

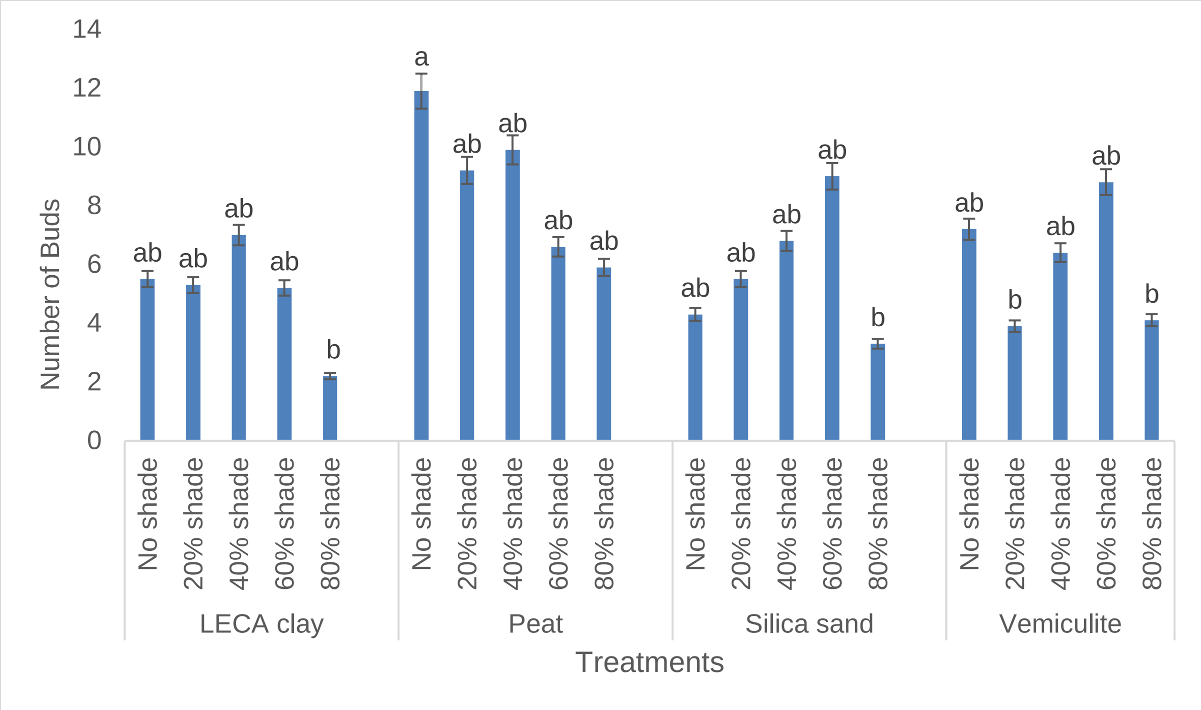

Figure 3.5: The effect of different growth media and shade stress on formation of buds of *T. divaricata.*

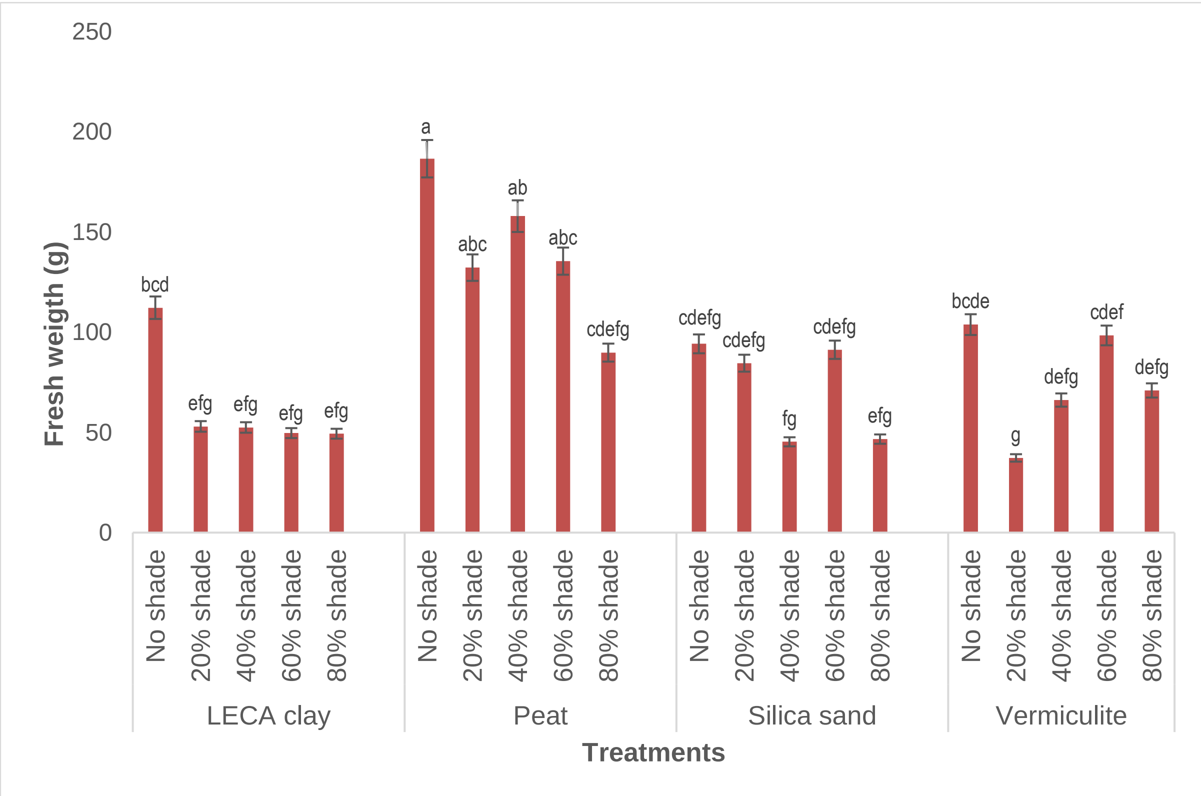

Figure 3.6: The effect of different growth media and different shade percentages on fresh weight of *T. divaricata.*

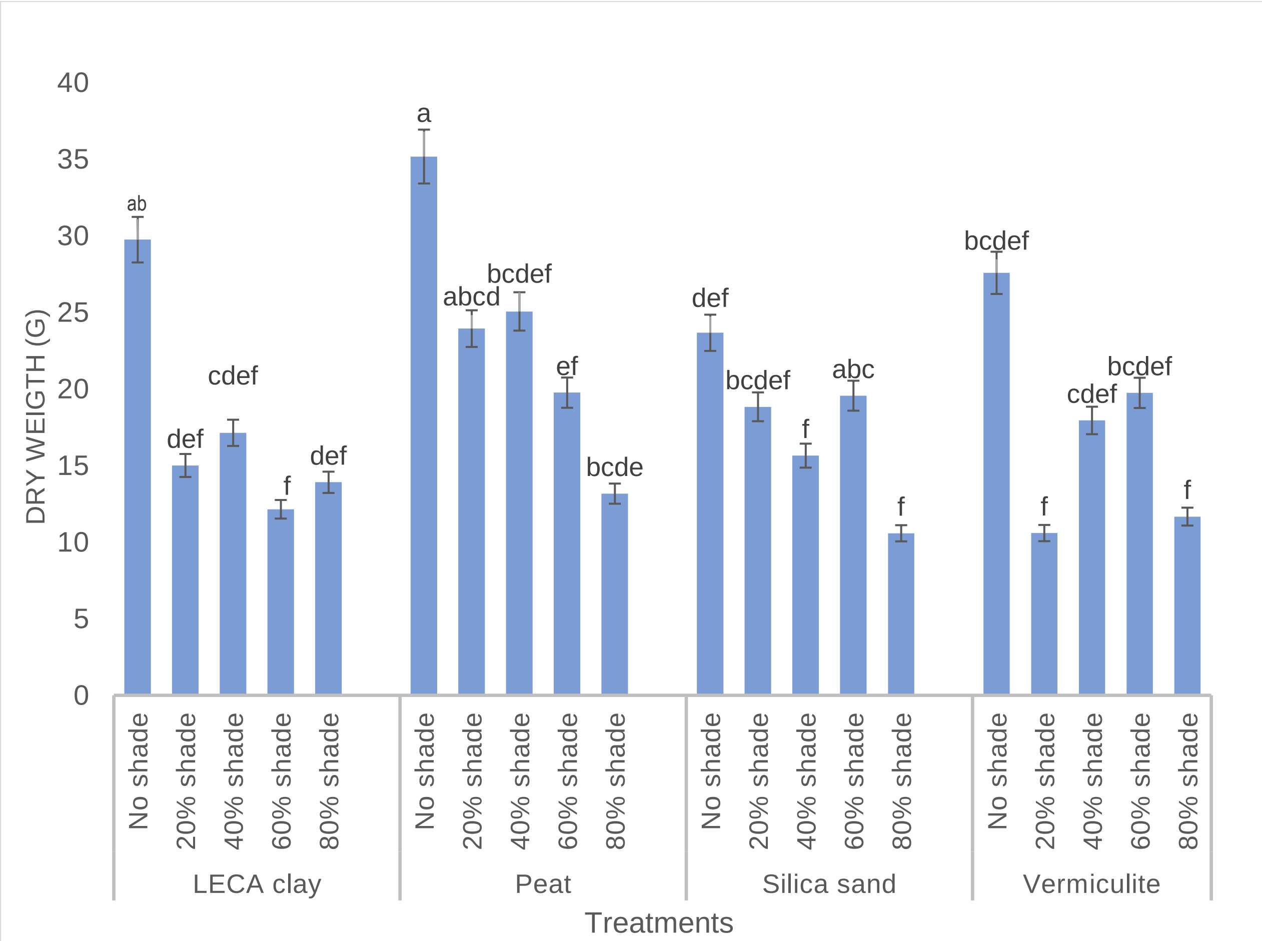

Figure 3.7: The effect of different growth media and different shade percentages on dry weight of *T. divaricata.*

3.5. Discussions

In plants, growth and development are induced by reduced light intensity incident on certain regions of the leaf (Nave *et al*., 2010; Yasin *et al*., 2019). The quality of light for which a plant is exposed to causes some physiological changes that alter the photosynthetic capacity of a plant (Franklin 2009; Yang *et al*., 2017; Ahmed *et al*.,2020). Presence of shade intercepts the intensity of electromagnetic spectrum received from incident light by pigmented photoreceptors resulting in modification of some morphological characteristics perceived to down-regulate or up-scale vegetative growth in plants (Li *et al*., 2000; Costa *et al*., 2010; Wang *et al*., 2019; Yasin *et al*., 2019).

In this study, a combination of different levels of shade stress (20%, 40%, 60%, 80%) and growth media (LECA clay, peat, silica sand and vermiculite) significantly affected the vegetative growth of *T. divaricata*. The plants that were grown in silica sand under 20% shade level produced the highest number of leaves as they grow older during weeks 6, 8, 10 and 12 compared to other treatments whereas the plants cultivated in peat medium under 60% shade and LECA clay under no shade had the highest number of leaves at week two and week four respectively suggesting that LECA clay and peat support seedling establishment by means of water conservation by LECA clay and moisture retention in peat due to its high humus content as respectively reported earlier by (Fekri & Kasmaei, 2013) and (Markoska *et al*., 2018). The consistency recorded in the highest number of leaves in plants cultivated in silica sand corroborates earlier report that *Trachyandra* species thrives well naturally in sand dunes (Ngxabi *et al*., 2021a; Ngxabi *et al*., 2021b). However, findings from this study contradict earlier research by Bande *et al*. (2013) where 50% shade was reported to have had a greater impact on leaf formation.

The organic components of growth media influence the vegetative and flowering characteristics of a plant in number of ways (Dubey *et al*., 2013; Olle *et al*., 2012). Over the years, peat has been used for nursery production of plants due to its high cation exchange capacity, high porosity and high water-retention capacity (Karimi *et al*., 2013; Zaller, 2007). The peat medium under no shade treatment increased significantly, the formation of flower buds and biomass of *T. divaricata* suggesting that the species is light-loving, thus, its vegetative characteristics will be enhanced by long photoperiods. The highest yield of flower buds and biomass of *T. divaricata* in peat medium may have been facilitated by high humus content and high water and nutrient retention capacity of peat medium which enables it to retain water efficiently and supply the plant with growth nutrients required for vegetative development effectively than other growing media (Zaller, 2007; Kitir *et al*., 2018; & Markoska *et al*., 2018). These results agree with the findings of Khandaker *et al*. (2019) on *Petunia sp.* bud formation where the highest number of buds was recorded in plants cultivated using Peat growth medium. These findings emphasize further, the significance of water and nutrient retention capacity of a growth medium for the enhancement of vegetative development and plant biomass accumulation variously reported by Olle *et al*. (2012), Shrestha *et al*. (2018) and Qin & Leskovar, (2020).

3.6. Conclusion

The current study demonstrates that *T. divaricata* is a fast-growing species that thrives well in longer photoperiods although moderate shade levels may be used successfully in hydroponics for its cultivation on a commercial scale. It is evident from the study that peat was the most suitable medium required for optimum vegetative development, flower bud formation and biomass accumulation. These findings could benefit future researchers and aid further, the commercial production of the plant.

3.7. Acknowledgements

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4. CHAPTER FOUR:

The effect of shade stress and different growing media on the chlorophyll content of *Trachyandra divaricata* (Jacq.) Kunth

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4.1. Abstract

In most hydroponic systems, the amount of water, nutrients, and photoperiod are controlled automatically based on the demands of individual plants. Hydroponics is employed as a cultivated strategy in this study since photoperiod can be manipulated easily and plants can be cultivated all year-round while requiring less water. The effect of shade levels and different growing media on the chlorophyll content of *Trachyandra divaricata* was investigated in this study. Shade treatment was set up on hydroponic system by mounting four different shade levels (20%, 40%, 60% and 80% shade nets) on different growth media (LECA clay, Peat, Silica sand and Vermiculite) set up in hydroponic system. Plants cultivated with silica sand with no shade were used as control. The results of this study show that there is potential for cultivation of *T. divaricata* on hydroponic system and LECA clay soilless medium was the best medium for chlorophyll content of this species as it performed better when combined with 80% shade compared to other treatments. These findings suggest that *T. divaricata* is shade tolerant and could survive in a well-drained and aerated medium as the plant attained the climax of chlorophyl content in LECA clay treated with maximum shading.

Key words: Chlorophyll concentration; photoperiod; shade tolerance; *Trachyandra divaricata*

4.2. Introduction

Soil is the ordinary growth medium for cultivation of many crops (Sharma *et al.,* 2018). However, problems such as soil borne diseases, undesirable microbial activities and nematodes, changing acidity levels, salinity, poor drainage, poor nutrient levels and undesirable soil characteristics are among major factors affecting soil productivity (Ananda & Ahundeniya, 2012; Shanmugam *et al*., 2020; Asif *et al*., 2021). To achieve optimal yield in agricultural production, soil has been substituted by many organic and inorganic growing media, since these substrates are pest and disease-free inert material capable of holding sufficient nutrients and moisture needed by plants for optimal growth and can be reused year after year (Asaduzzaman *et al*., 2015; Kommu & Pilli 2019; Udemezue & Kanu, 2019). The use of a soilless culture system derived from artificial substrates could result in well-organized and effective use of water and fertilizers and minimize the use of chemicals for pest and disease control (Hassan & Ali, 2014; Jamwal & Sharma, 2019).

Hydroponic culture involves indoor cultivation of crops which are protected from the potential negative effects of elements supplied in moderate proportion organized under vertical growing techniques which are designed to maximize space limits (Ropokis *et al*., 2019). Hydroponic cultivation provides various advanced production benefits, including a reduced environmental effects and minimal usage of water due to the closed-loop nature of hydroponic system (Chen et al., 2020; Faber *et al*., 2020; Rufí-Salís, *et al*., 2020; Ullah *et al*., 2019). With soilless culture, the agricultural activity can be scaled to any facility size while improving the health and nutritional value of a plant (Ropokis *et al*., 2019) although the physico-chemical properties of the growth media could have direct and indirect effects on plant growth and development (Ghehsareh *et al*., 2012; Gruda, 2019). Due to the increasing use of soilless media, many diseases brought on by soil-borne plant pathogens in the production of vegetables and ornamentals plants have been eradicated (Garibaldi *et al*., 2014; De Corato. 2020; Phani *et al*., 2021).

The vegetative growth of a plant can be improved by manipulating its light trapping capacity when subjected to shade stress (Yao *et al*., 2017) thought to limit significantly, chlorophyl biosynthesis and net photosynthesis which are the most important foundation of energy for plant growth (Yao *et al*., 2017; Kommu & Pilli, 2019; Hussain *et al*., 2019; Hayes & Ferruzzi, 2020). In shaded leaves, there are more chloroplasts in the thinner layer of mesophyll cells, allowing them to harvest more sunlight at lower levels of radiation (Kume, 2017; Kirchhoff, 2019; Mounir *et al*., 2021). This suggests that among other environmental factors, light plays a crucial role in chlorophyll production, although, temperature, metal ions and other physico-chemical factors can promote or reduce chlorophyll production (Nakajima *et al*., 2012; Frugé *et al*., 2019).

This chapter focus on the effect of different shade levels (20% shade net, 40% shade net, 60% shade net and 80% shade net) on the chlorophyll content of *T. divaricata* propagated in four different soilless growing media (LECA clay, Peat, silica sand and vermiculite) set up under Nutrient film technique (NFT) hydroponic system with a view to develop suitable cultivation protocol for the species.

4.3. Materials and methods

4.3.1. Greenhouse experiment location

This experiment was carried out over a period of twelve weeks in the Horticulture Research Greenhouse 2 of the Cape Peninsula University of Technology, Bellville campus, South Africa (33□ 55’45.53S, 18□ 38, 31’. 16E). The nature of the structure and the technology installed ensured full control of the environment within the greenhouse.

4.3.2. Plant collection and propagation

Trachyandra divaricata plant divisions were collected from wild populations on the Bellville campus of the Cape Peninsula University of Technology. Divisions of the plants were done to ensure clonal propagation of identical plants. The leaves of all plants were cut to a similar length of 10 cm with 6 leaves and an equal weight of 23 grams per plant. All plants were then planted into 15 cm pots and placed under controlled irrigation in the propagation house for two weeks after which they were moved to the Nutrient Film Technique (NFT) gutter hydroponic system.

4.3.3. Hydroponic system design

Four identically NFT hydroponic systems were constructed with each system being set up on a rectangular wire mesh table (2.5 m long) which provided a flat surface. Another wire mesh table was stacked on top of each table to create a frame which were then covered with different percentages of shading as stated. Each system had its low-density polyethene (LDPE) 50 L reservoir in which the nutrient solution was prepared and supplied to the system. Four Polyvinyl Chloride (PVC) square gutters (2 m long) were placed on each table which held the 15 cm square plastic pots (ten pots per gutter). The gutters were sealed with PVC adhesive to prevent leaks and connected to a 1 × 2000 L/hr submersible pump with 2.5 m head capacity, 20 mm LDPE irrigation piping, 4 × 20 mm elbow irrigation fittings and 4 × 20 mm flow regulators (Ngxabi *et al.*, 2021a). Water was pumped and circulated continuously from the reservoir tanks which contained a Nutrifeed fertilizer (65 g/kg N, 27 g/kg P, 130 g/kg K, 70 mg/kg Ca, 20 mg/kg Cu, 1500 mg/kg Fe, 10 mg/kg Mo, 22 mg/kg Mg, 240 mg/kg Mn, 75 mg/kg S, 240 mg/kg B and mg/kg Zn. Fertilizer group 1 Reg No: K2025 (Act 36/ 1947) which was bought from Starke Ayres, Cape Town. The pH and electrical conductivity (EC) were maintained at 6.5 and using a Martini Instruments PH55 pH probe and for adjusting the pH hydrochloric acid (HCl) was used to lower the pH, sodium hydroxide (NaOH) was used for raising the pH. EC levels of the aqueous nutrient solutions were monitored with a calibrated hand-held digital EC meter

(Hanna Instruments®™ HI 98312). The nutrient solutions were refreshed every 2 weeks to minimize the build-up of salts in the growing media.

4.3.4. Experimental treatments

The experiment work involved two main treatments which were growth media and shading. Four different growth media namely: LECA clay (LC), Consol® silica sand (SS), peat (P) and vermiculite (V) and arranged in a completely randomized block design (CRBD) (Faber et al., 2020). The silica sand and LECA clay were rinsed thoroughly with sterile water to clean impurities and other earthy materials. The divided parts of *T. divaricata* were then planted in pots filled with different growing media and placed in a NFT hydroponic system. Black shade netting of 20% density was purchased from Stodels Garden Centre, Bellville, South Africa and used to create the shading climates. Different percentages of shading were created with 20% shade cloth by doubling (40%), tripling (60%) and quadrupling (80%) the net into different layers. The control had no shade cover (C=0% shade). Each treatment was numbered, as presented in the Table 5.1 below.

Table 4.1: Experimental treatment layout for growing *T. divaricata.*

Growth media	Shading percentage
1 = LECA clay	no shade
2 = LECA clay	20% shade
3 = LECA clay	40% shade
4 = LECA clay	60% shade
5 = LECA clay	80% shade
6 = Peat	no shade
7 = Peat	20% shade
8 = Peat	40% shade
9 = Peat	60% shade
10 = Peat	80% shade
11 = Silica sand	no shade (control)
12 = Silica sand	20% shade
13 = Silica sand	40% shade
14 = Silica sand	60% shade
15 = Silica sand	80% shade
16 = Vermiculite	no shade
17 = Vermiculite	20% shade
18 = Vermiculite	40% shade
19 = Vermiculite	60% shade
20 = Vermiculite	80% shade

4.3.5. Chlorophyll content of the leaves

The data for chlorophyll was taken from week two and after every two weeks till week 12 directly from actively growing fully developed leaves of *T. divaricata*. The chlorophyll content was measured from the three largest matured leaves from each plant. The chlorophyll measures of each plant were averaged to one value and recorded. The data was collected between 11 am and 16 pm and the chlorophyll meter was first calibrated to adjust the chlorophyll meter to the greenhouse light intensity. All the data collected from the vegetative growth parameters of this study were statistically analysed using 2-way analysis of variance (ANOVA) using Minitab software. Tukey's least significant difference was used to compare the significant differences between treatment means at $p \leq 0.05$. The calculations carried out to obtain statistical data were accomplished through the computer program (Minitab) and were tabulated. The data results are represented by graphs and tabulations.

4.3.6. Data collection and statistical analysis

The data for chlorophyll was taken from week two and after every two weeks till week 12 directly from actively growing fully developed leaves of *T. divaricata*. The chlorophyll content was measured from the three largest matured leaves from each plant. The chlorophyll measures of each plant were averaged to one value and recorded. The data was collected between 11 am and 16 pm and the chlorophyll meter was first calibrated to adjust the chlorophyll meter to the greenhouse light intensity. All the data collected from the vegetative growth parameters of this study were statistically analysed using 2-way analysis of variance (ANOVA) using Minitab software. Tukey's least significant difference was used to compare the significant differences between treatment means at $p \leq 0.05$. The calculations carried out to obtain statistical data were accomplished through the computer program (Minitab) and were tabulated. The data results are represented by graphs and tabulations.

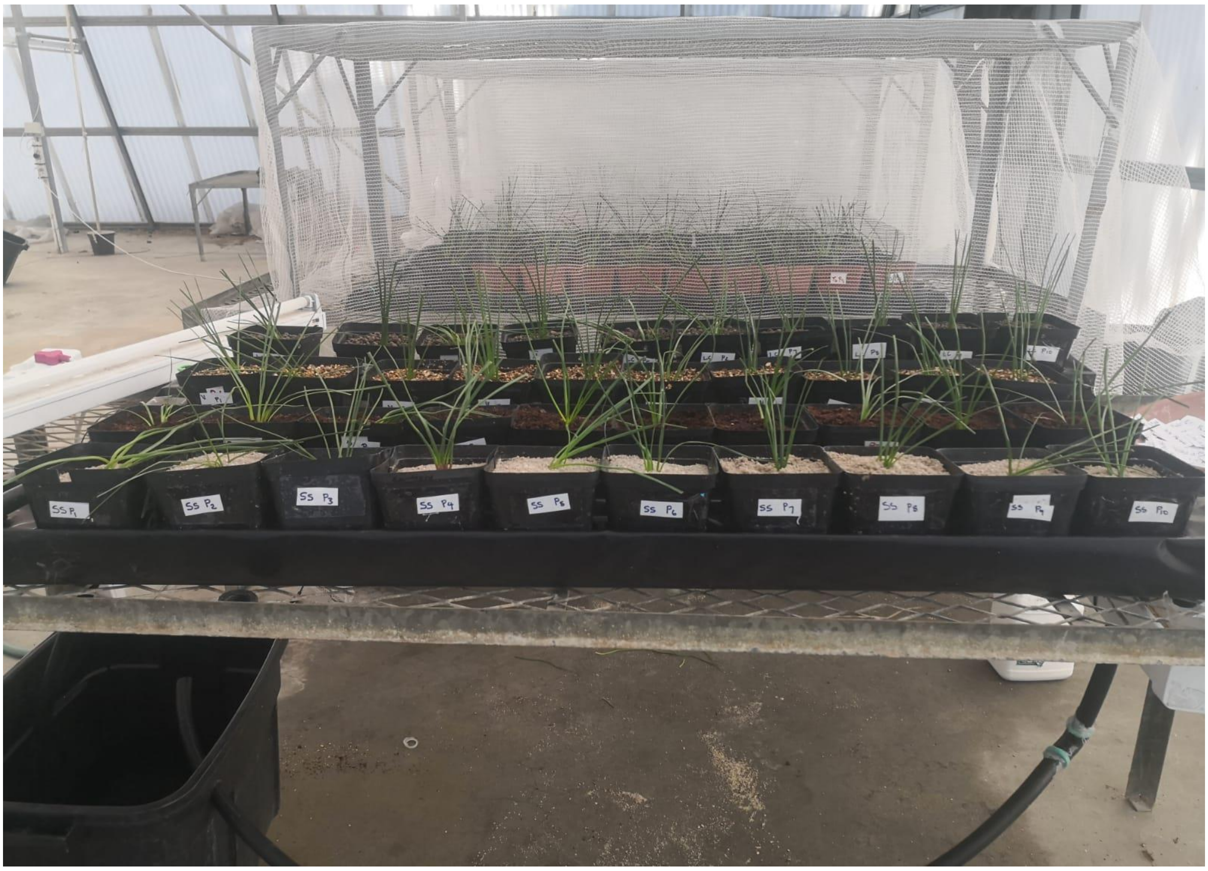

Figure 4.1: Experimental layout with 4 different growing media and treatments with and without shading. (Picture: Tshayingwe, 2021).

4.4. Results

4.4.1. Plant chlorophyll content

The chlorophyl content of *T. divaricata* varies considerably as the plant ages with respect to the growth media. At weeks 2, 4, 6, 8 and 10, the highest mean values of chlorophyl content were recorded in LECA clay treated with different shade stress (Table 4.1). The 80% shade stress yielded the highest chlorophyl values at weeks 2, 4 and 6 whereas during weeks 8 and 10, the 60% and 20% shade stress respectively produced the highest chlorophyl values than other treatments. At week 12, vermiculite medium treated with 80% shade stress produced the highest chlorophyl value although the medium also recorded the lowest chlorophyl from weeks 2 to 12 under different shade levels except for week 8 when silica sand yielded the least chlorophyl value under 40% shade stress (Table 4.1).

Table 4.2: Effect of different growth media and different shade levels on chlorophyll content of *T. divaricata*.

Media	Shade	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
LECA clay	No shade	1.020 ± 0.25 cde	1.026 ± 0.41 def	0.99 ± 0.02 d	1.09 ± 0.23 bcd	1.19 ± 0.02 ab	1.15 ± 0.03 ab
	20% shade	0.961 ± 0.34 fg	0.99 ± 0.37 efg	1.32 ± 0.05 ab	1.00 ± 0.02 cde	1.27 ± 0.05 a	1.02 ± 0.02 ab
	40% shade	0.980 ± 0.02 def	1.02 ± 0.02 def	1.09 ± 0.03 cd	1.12 ± 0.26 abc	1.06 ± 0.03 abc	1.12 ± 0.03 ab
	60% shade	1.20 ± 0.27 ab	1.35 ± 0.03 ab	1.41 ± 0.04 ab	1.35 ± 0.04 a	1.12 ± 0.02 ab	1.03 ± 0.04 ab
	80% shade	1.32 ± 0.49 a	1.34 ± 0.05 a	1.43 ± 0.04 a	0.96 ± 0.10 de	0.96 ± 0.12 abc	1.20 ± 0.14 ab
Peat	No shade	1.14±0.02 bcd	0.95±0.02 g	1.00 ± 0.02 d	1.06 ± 0.03 bcd	1.031 ±0.19 abc	0.99±0.04 b
	20% shade	1.11±0.03 bcd	1.20 ± 0.07 bcd	1.10 ± 0.04 cd	1.09 ± 0.03 bcd	1.14±0.06 ab	1.02±0.04 ab
	40% shade	0.97±0.02 efg	1.04±0.02 def	1.11 ± 0.03 cd	1.09 ± 0.02 bcd	1.08±0.04 abc	1.16±0.08 ab
	60% shade	1.15±0.02 abc	1.28±0.05 abc	1.40 ± 0.06 ab	1.15 ± 0.01 abc	1.16±0.03 ab	1.11±0.02 ab
	80% shade	1.17±0.02 abc	1.26±0.04 abc	1.19 ± 0.06 d	1.11 ± 0.04 bcd	1.19±0.04 ab	1.15±0.13 ab
Silica sand	No shade	0.96±0.03 fg	1.00±0.03 efg	1.03 ± 0.03 d	1.21 ± 0.03 abc	1.16±0.03 ab	0.98±0.03 b
	20% shade	1.13±0.02 bcd	0.99±0.02 efg	1.04 ± 0.02 d	1.08 ± 0.03 bcd	1.08±0.03 abc	1.01±0.02 b
	40% shade	1.02±0.03 cde	1.04±0.02 def	1.02 ± 0.03 d	0.90 ± 0.04 e	1.00±0.12 bc	0.88±0.03 b
	60% shade	1.17±0.03 abc	1.16±0.05 bcd	1.35 ± 0.04 ab	1.25 ± 0.03 ab	1.97±0.22 ab	1.13±0.02 ab
	80% shade	1.23±0.05 ab	1.24±0.03 abc	1.33±0.04 ab	1.15±0.13 abc	1.22±0.14 ab	1.29±0.03 ab
Vermiculite	No shade	1.00±0.04 cde	1.14±0.03 cde	1.11±0.03 cd	1.12±0.02 bcd	1.04±0.02 abc	1.16±0.03 ab
	20% shade	0.99±0.02 efg	1.27±0.05 abc	1.10±0.04 cd	1.13±0.03 abc	0.81±0.21 c	0.84±0.18 b
	40% shade	0.89±0.04 g	0.96±0.02 fg	1.01±0.01 d	0.98±0.03 cde	1.08±0.03 abc	1.07±0.03 ab
	60% shade	1.09±0.04 bcd	1.27±0.05 abc	1.24±0.04 bc	1.14±0.03 abc	1.08±0.02 abc	1.04±0.02 ab
	80% shade	1.22±0.07 ab	1.26±0.05 abc	1.09±0.02 cd	1.14±0.02 abc	1.24±0.04 ab	1.42±0.02 a

4.5. Discussions

Chlorophyll is a crucial photosynthetic pigment to the plant growth as it determines largely, the photosynthetic capacity which is the most important foundation of energy for plant growth (Papageorgiou, & Giese, 1971; Ying *et al.,* 2018). With the aid of chlorophyl, plants can harvest energy from the sun, separate charges, and transfer electrons within reaction centers in photosynthetic antenna systems (Tanaka & Tanaka, 2006). This is possible after chlorophyl is biosynthesized in a well-coordinated process facilitated by a series of cooperative enzymes (Beale, 1999; Tanaka &Tanaka, 2006; Mounir *et al*., 2021).

However, the use of shade nets is a way of manipulating incident light rays to reduce photosynthetic activities and therefore net carbon acquisition, biomass accumulation, vegetative growth, and development in plants (Khan *et al.,* 2000; Silva *et al.,* 2016). A combination of factors such as morphological adaptations, photosynthetic light trapping, utilization, and compensation determines shade tolerance of plants and their survival under low light conditions (Walters *et al.,* 1993; Gommers *et al.,* 2013). This affects allocation of photosynthate to vertical or lateral growth depending on the level of shade tolerance of plants (Wang *et al.*, 1994; Chen *et al.,* 1996; Gommers *et al.,* 2013; Khan *et al.,* 2000).

Nevertheless, exposure of plants to shade may optimize or slow down their growth as reported by Brand (1997), Liu *et al*. (2018) and Wan *et al.* (2020) depending on their morphological adaptation to shade. Due to the slower relative growth rate and biomass build-up, several research claim that shade can limit plant growth by decreasing the photosynthetic capacity of leaves (Yang *et al.,* 2018). Other studies show that there is a positive relationship between shade and plant growth (Liu *et al*., 2018). However, the result from this study disagrees with findings Downey *et al*. (2004) who suggested that chlorophyl production is reduced when light is absent and agrees with of Brand (1997) and Wan *et al*. (2020) that shade improves chlorophyl biosynthesis and gives plants a healthier green leaf colour.

4.6. Conclusion

The findings from the study revealed that *T. divaricata* is a shade tolerant plant and different soilless media can be used successful in the propagation of this species. It is obvious from the study that LECA clay combined with shade was the best treatment required for optimum production of chlorophyll content in *T. divaricata* however there is a potential in cultivating *T. divaricata* successfully in using 80% shading combined with vermiculite growing medium as found in week 12 of the study to produce optimum chlorophyl levels in *T. divaricata*. This suggests that the possibility for commercial production of the plant as an edible crop is promising to produce more chlorophyl with larger leaves and possibly improved flowering. Further studies on the production of flowering under shady conditions of the species are recommended to elucidate further advances in growing the important species for commercial purposes.

4.7. Acknowledgement

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4.8. References

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5. CHAPTER FIVE:

Effects of different shade levels and growth media on phytochemicals, and antioxidant capacity of *Trachyandra divaricata* (Jacq.) Kunth

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5.1. Abstract

Phytochemicals are bioactive nutritional plant molecules present in fruits, vegetables, grains, and other plant foods that reduce the risk of significant chronic diseases. These benefits might go beyond basic dietary needs. However, throughout the past few decades, the use of chemically produced medicines has not significantly improved the overall survival rate. This study evaluated the effect of different growth soilless media and shade levels on the phytochemicals, and antioxidant capacity of *Trachyandra divaricata.* Findings from the study showed that silica sand medium with 20% shade was the best treatment for polyphenols and DPPH. The LECA clay medium with no shade was the most productive treatment for TEAC (umol TE/g) antioxidant generation in *T. divaricata*. Vermiculite medium with 60% shade showed significantly increased flavonols (mg QE/g) while FRAP content (umol AAE/g) performed better in LECA clay medium under 60% shade. A combination of soilless media and moderate shade is recommended for maximum production of phytochemicals and antioxidant for *T. divaricata*.

Key words: Asphodelaceae; flavonols; phenols; shade stress; soilless growth media

5.2. Introduction

The process of secondary metabolism in plants is crucial to the biosynthesis of natural compounds otherwise known as phytochemicals (Pourcel & Grotewold, 2009; Tijjani *et al*., 2018). This process is chiefly directed by the evolution of new genes or natural selection of certain characteristics that trigger the production of these phytochemicals which may in turn modulate the expression of certain genes (Pichersky & Gang, 2000; Saito, 2013). These novel compounds, most of which chemical structures are yet to be elucidated, are synthesized, and accumulated in tissues of some selected group of plants and may be used as markers for taxonomic delimitation (Pichersky & Gang, 2000; Payne *et al*., 2012; Spínola & Castilho, 2016).

Phytochemicals are amazing compounds with low molecular mass whose biosynthesis may be dictated by family, variety, biotic and abiotic stressors within the environment (King & Young, 1999; Sharma & Sharma, 1999; Grotewold, 2005). The specificity and diversity of these natural chemicals from plants may be instrumental to the specific and diverse evolutionary and ecological roles they play within the plant and its environment such as allelopathy and protection against herbivory (Friedman, & Rasooly, 2013; Wetzel & Whitehead, 2020; Jimoh *et al.,* 2021). The biological activity of the natural compounds such as storage lipids, fragrances, essential oils, flavonoids, polyphenols, and pharmaceutics is mostly driven by the evolutionary process as related plant families usually make use of equivalent chemical structures in building up resistance against diseases to develop their defence mechanism (García-Cañas *et al*., 2010; Wagner *et al*., 2016; Jimoh *et al*., 2019; Hsu & Tain, 2020). For instance, production of toxic chemicals from some plants may suppress the growth of other plants within the surrounding or prevent them from being fed upon by herbivores or potential pathogens (Jelassi *et al*., 2016; Richards *et al*., 2016).

Phytochemicals can also improve the shelve life and sensory attributes of food, aid pollination, make plants survive harsh environmental conditions and cure chronic diseases (Ferlay *et al*., 2015; Kotilainen *et al*., 2018; Siegel., *et al.,* 2018; Ketnawa *et al*., 2021; Sharifi-Rad *et al.,* 2021). Among important abiotic factors that stimulate the production of secondary metabolites in plants are shade and growth media. The presence of either or both factors may modify the plant's environment in a manner that the diversity, concentration, and chemical profiles of phytochemicals present in the plant are manipulated (Estell *et al.,* 2016; Katerova *et al.,* 2017; Eljounaidi, & Lichman, 2020; Faber *et al*., 2020;).

Trachyandra divaricata is an underutilized perennial herb with edible flower buds. The raceme produces white flowers that last a day; and if cut the plant readily produces more racemes which need to be harvested for eating before they branch (Boatwright & Manning, 2010*)*. Despite the ethnobotanical claim that the plant was consumed by the indigenous Khoisan people of South Africa, there is dearth of information on its ability to tolerate shade, and a variability in the yield of secondary metabolites when cultivated under different growth media remains unknown. This research, therefore aimed at quantifying phytochemicals and antioxidant activities of *T. divaricata* cultivated on different growth media and under different shade levels in hydroponic system to serve as a baseline study for the propagation of its potential pharmacological benefits.

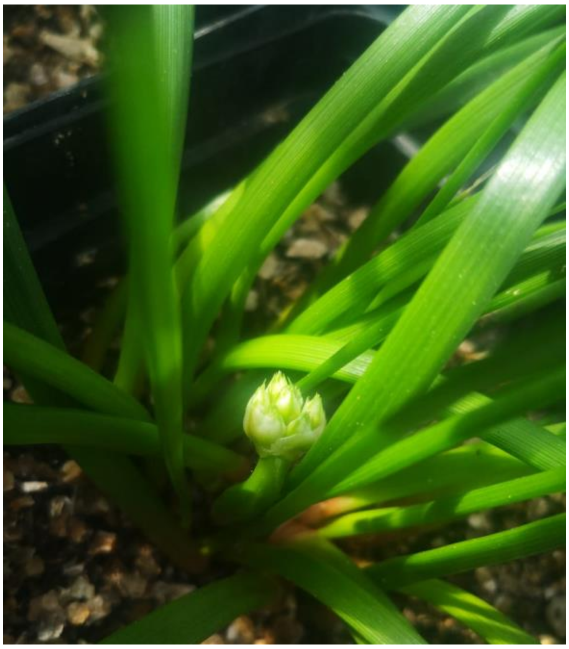

Figure 5.2: Flower bud formation during cultivation of *T. divaricata.*

Picture: Tshayingwe, 2021

Figure 5.1: The flower of *T. divaricata* resembles that of edible asparagus.

Picture: Tshayingwe, 2021

Figure 5.4: Prolific sprouting of *T. divaricata* in hydroponic cultivation.

(Picture: Tshayingwe, 2021)

Figure 5.3: Flower buds of *T. divaricata* harvested and washed in preparations for cooking.

(Picture: Tshayingwe, 2021)

5.3. Materials and methods

5.3.1. Greenhouse experiment location

This experiment was carried out over a period of twelve weeks in the Horticulture Research Greenhouse 2 of the Cape Peninsula University of Technology, Bellville campus, South Africa (33° 55'45.53S, 18° 38, 31'. 16E). The nature of the structure and the technology installed ensured full control of the environment within the greenhouse.

5.3.2. Plant collection and propagation

Trachyandra divaricata plant divisions were collected from wild populations on the Bellville campus of the Cape Peninsula University of Technology. Divisions of the plants were done to ensure clonal propagation of identical plants. The leaves of all plants were cut to a similar length of 10 centimetre with 6 leaves and an equal weight of 23 grams per plant. All plants were then planted into 15 cm pots and placed under controlled irrigation in the propagation house for two weeks after which they were moved to the Nutrient Film Technique (NFT) gutter hydroponic system.

5.3.3. Hydroponic system design

Four identically NFT hydroponic systems were constructed with each system being set up on a rectangular wire mesh table (2.5 m long) which provided a flat surface. Another wire mesh table was stacked on top of each table to create a frame which were then covered with different percentages of shading as stated. Each system had its low-density polyethene (LDPE) 50 L reservoir in which the nutrient solution was prepared and supplied to the system. Four Polyvinyl Chloride (PVC) square gutters (2 m long) were placed on each table which held the 15 cm square plastic pots (ten pots per gutter). The gutters were sealed with PVC adhesive to prevent leaks and connected to a 1 × 2000 L/hr submersible pump with 2.5 m head capacity, 20 mm LDPE irrigation piping, 4 × 20 mm elbow irrigation fittings and 4 × 20 mm flow regulators (Ngxabi *et al*., 2021a). Water was pumped and circulated continuously from the reservoir tanks which contained a Nutrifeed fertilizer (65 g/kg N, 27 g/kg P, 130 g/kg K, 70 mg/kg Ca, 20 mg/kg Cu, 1500 mg/kg Fe, 10 mg/kg Mo, 22 mg/kg Mg, 240 mg/kg Mn, 75 mg/kg S, 240 mg/kg B and mg/kg Zn. Fertilizer group 1 Reg No: K2025 (Act 36/ 1947) which was bought from Starke Ayres, Cape Town. The pH and electrical conductivity (EC) were maintained at 6.5 and using a Martini Instruments PH55 pH probe and for adjusting the pH hydrochloric acid (HCl) was used to lower the pH, sodium hydroxide (NaOH) was used for raising the pH. EC levels of the aqueous nutrient solutions were monitored with a calibrated hand-held digital EC meter

(Hanna Instruments®™ HI 98312). The nutrient solutions were refreshed every 2 weeks to minimize the build-up of salts in the growing media.

5.3.4. Experimental treatments

The experiment work involved two main treatments which were growth media and shading. Four different growth media namely: LECA clay (LC), Consol® silica sand (SS), peat (P) and vermiculite (V) and arranged in a completely randomized block design (CRBD) (Faber et al., 2020). The silica sand and LECA clay were rinsed thoroughly with sterile water to clean impurities and other earthy materials. The divided parts of *T. divaricata* were then planted in pots filled with different growing media and placed in a NFT hydroponic system. Black shade netting of 20% density was purchased from Stodels Garden Centre, Bellville, South Africa and used to create the shading climates. Different percentages of shading were created with 20% shade cloth by doubling (40%), tripling (60%) and quadrupling (80%) the net into different layers. The control had no shade cover (C=0% shade). Each treatment was numbered, as presented in the Table 5.1 below.

Table 5.1: Experimental treatment layout.

Growth media	Shading percentage
1 = LECA clay	no shade
2 = LECA clay	20% shade
3 = LECA clay	40% shade
4 = LECA clay	60% shade
5 = LECA clay	80% shade
6 = Peat	no shade
7 = Peat	20% shade
8 = Peat	40% shade
9 = Peat	60% shade
10 = Peat	80% shade
11 = Silica sand	no shade (control)
12 = Silica sand	20% shade
13 = Silica sand	40% shade
14 = Silica sand	60% shade
15 = Silica sand	80% shade
16 = Vermiculite	no shade
17 = Vermiculite	20% shade
18 = Vermiculite	40% shade
19 = Vermiculite	60% shade
20 = Vermiculite	80% shade

5.3.5. Data collection and phytochemical and antioxidant analysis

5.3.5.1. Sample preparation

Mature flower buds harvested from fully grown *T. divaricata* post-flowering (Figures 5.1a-d) were first air-dried in a room maintained at 32 °C for 2 weeks at the Botanical Lab of the Cape Peninsula University of Technology, Art and Design building. After that, they were further dried in a fan-drying laboratory oven at 40 °C for 7 days to make it crispy dry. The type A 10 miller from Junkel and Kunkel was used to grind the dried buds into a fine powder. By combining 100 mg of the dried, powdered material with 25 mL of ethanol (EtOH) (Merck, South Africa) at a 70/30 ratio for one hour, flower bud material was extracted. The supernatants were used for all studies after it was centrifuged at 4000 rpm for 5 min. All chemicals used for the experiments were of standard analytical grade obtained from either Merck or Sigma South Africa.

5.3.5.2. Total polyphenol assay

The total polyphenol assay of the extracts was performed using the Folin–Ciocalteu method as reported by Ngxabi *et al.* (2021a). About 25 µL of the sample was mixed with 125 µL Folin–Ciocalteu reagent (Merck, Johannesburg, South Africa) that was diluted 10 times with distilled water. Thereafter, 7.5% sodium carbonate solution was prepared and added in a 96-well microplate with extracts. The plate was incubated for 2 h at room temperature and the absorbance was then measured at 765 nm in a Multiskan spectrum plate reader (Thermo Electron Corporation, Waltham, MA, USA). The standard curve was prepared using 0, 20, 50, 100, 250, and 500 mg/L gallic acid (Sigma, South Africa) in 10% EtOH, from which the polyphenolic content was extrapolated, and the results were expressed as mg gallic acid equivalent per g dry weight (mg GAE/g DW).

5.3.5.3. Estimation of Flavonol Content

The flavonol content of the extracts was determined using quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, Johannesburg, South Africa) as standard. About 12.5 µL of the crude sample extracts were mixed with 12.5 µL 0.1% HCl (Merck, South Africa) in 95% ethanol, 225 µL 2% HCl for each sample. The extracts were then incubated for 30 min at room temperature. The absorbance was read at 360 nm at a temperature of 25 °C [37]. The results were expressed as mg quercetin equivalent per g dry weight (mg QE/g DW).

5.3.5.4. Antioxidant capacity of DPPH Radicals

The DPPH radical was generated from a solution of 0.135 mM DPPH prepared in a dark bottle Ohikhena *et al*. (2018). About 300 µL of DPPH solution was reacted with graded concentrations (0 and 500 µM) of Trolox standard (6-Hydrox-2,5,7,8-tetramethylchroman-2-20 carboxylic acid) solution and 25 µL of crude extract. The mixtures were incubated for 30 min, after which absorbance was taken at 517nM. The results were expressed as µM/Trolox equivalent per g dry weight (µM TE/g DW).

5.3.5.5. Determination of TEAC (µmol TE/g)

The TEAC assay was carried out by generating $ABTS^+$ from potassium persulphate mixed with ABTS dissolved in a buffer (Özgen *et al.,* 2009). The assay was based on the ability of the tested plant samples of *T. divaricata* to inhibit the ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radicals ($ABTS^+$) relative to a standard antioxidant Trolox, as described by Re *et al*. (1999) and Zulueta *et al*., (2009). To ensure stability, the $ABTS^+$ was diluted with 20 mM sodium acetate buffer (pH 4.5) at a wavelength of 734 nm and pH range 0.700 ± 0.02. About 3 mL $ABTS^+$ was reacted with 20 µL of the samples, and absorbance of the solution was read at 734 nm after 10 min. The experiment was run in triplicate for each sample. The results are estimated as µmol TE/g dry weight of the plant sample, extrapolated from a trolox calibration curve generated within a concentration range of 0-250 µM.

5.3.5.6. Determination of Ferric Reducing Antioxidant Power (FRAP)

The method described by Jimoh *et al.* (2020) was used to perform the FRAP assay. FRAP reagent was prepared by mixing 30 mL Acetate buffer (0.3 M, pH 3.6) with 3 mL 2,4,6-tripyridyl-s-triazine (10 mM in 0.1M Hydrochloric acid), 3 mL Iron (III) chloride hexahydrate (FeCl3·6H2O) (Sigma, South Africa), 6 mL of distilled water and incubated for 30 min at 37 °C. Then, 10 µL of the crude sample extract was mixed with 300 µL of the FRAP reagent in a 96-well plate. The absorbance was then measured at 593 nm in a Multiskan spectrum plate reader. An L-Ascorbic acid (Sigma-Aldrich, South Africa) was used as a standard to calculate the FRAP sample values, with the concentration curve varying from 0 to 100 µM. The results were expressed as µM ascorbic acid equivalents (AAE) per g dry weight (µM AAE/g DW).

5.3.6. Statistical analysis

Data for phytochemical and antioxidant analysis was analysed using two-way analysis of variance (ANOVA) and Fisher's Least Significant Difference (Tukey's L.S.D) test used to evaluate between treatments using Statistica (StatSoft, Inc., Tulsa, OK, US). Different treatments were considered as independent variables and the significance threshold was set at P ≤ 0.05 (Steel & Torrie, 1980).

5.4. Results

5.4.1. Total phenol

Different growth media and different shade percentages had a significant effect on polyphenols in *T. divaricata* investigated in this study (Figure 5.1). The highest mean value of (10.44) polyphenols was recorded in *T. divaricata* cultivated in silica sand medium under 20% shade, followed by LECA clay (20% and 60% shade levels) and peat medium (60% shade level), all of which have equivalent phenolic content whereas the lowest value was recorded in samples grown in LECA clay under 80% shade treatment although most of the remaining treatments had an equivalent effect on polyphenols (Figure 5.6).

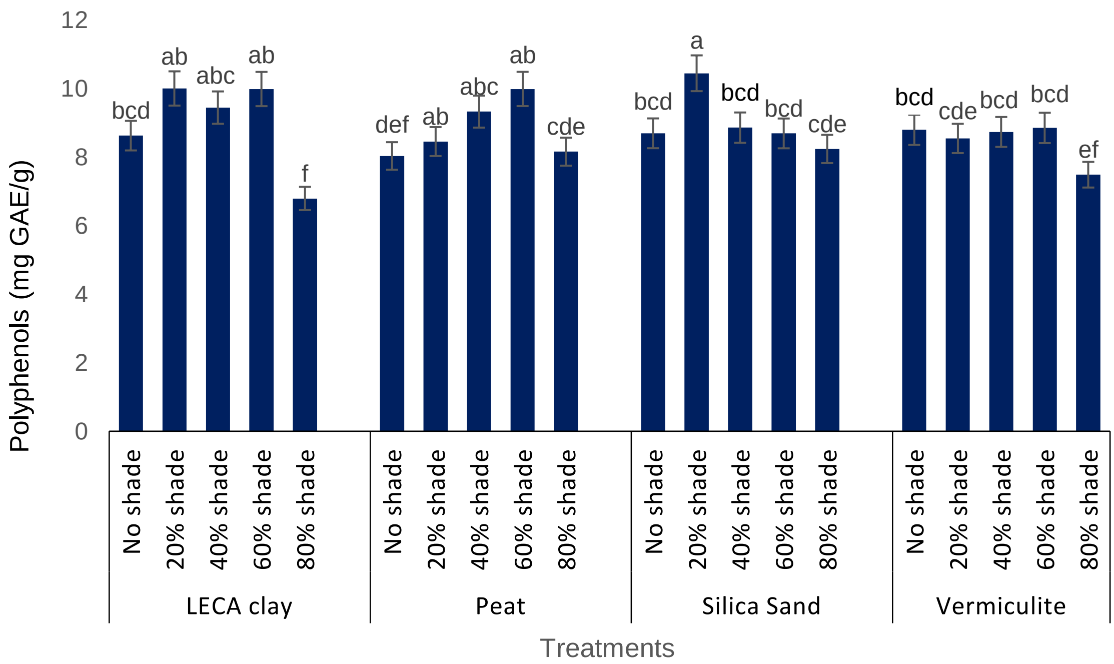

Figure 5.5: The effect of different growth media and shade levels on total polyphenol in the flower buds of *T. divaricata.*

5.4.2. Total flavonols

The highest flavonol content was recorded in samples of *T. divaricata* grown in vermiculite medium under 20% and 60% shade levels while LECA clay treated with 80% shade was least effective (Figure 5.7). The total flavonol values varied considerably in all growth media used in this study. LECA clay had the highest flavonols in the control treatment (no shade) whereas 40% shade yielded the highest flavonols in the peat medium. In silica sand, 80% shade affected flavonol production significantly than other treatments (Figure 5.7).

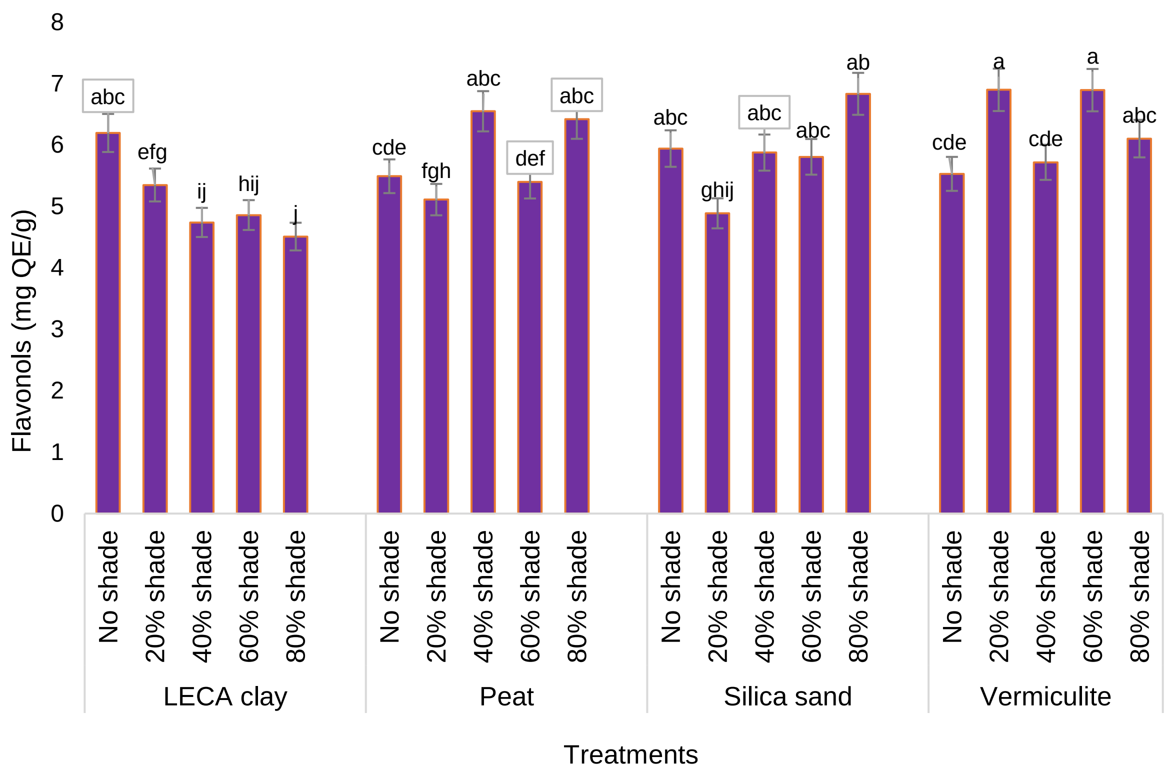

Figure 5.6: The effect of different growth media and shade levels% on total flavonols in the flower buds of *T. divaricata.*

5.4.3. Total DPPH antioxidant content

The total DPPH antioxidant content was highest in samples of *T. divaricata* cultivated under 60% shade treated LECA clay soilless medium while the lowest DPPH content was recorded in samples treated with 80% shade levels in both LECA clay and vermiculite media (Figure 5.8). In peat growth medium, the highest value of DPPH was produced under 60% shade while the lowest number was recorded at 80% shade. For silica sand growth medium, 20% shade was the best treatment for DPPH antioxidant formation, whereas the lowest was recoded under 80% shade levels. The vermiculite growth medium produced the highest DPPH under no shade, and the lowest number was recorded at 80% shade. The silica sand under 20% shade had the highest DPPH antioxidant which is significantly different from other plant samples from the same treatment whereas the vermiculite medium under 80% shade produced the lowest DPPH antioxidant value which is equivalent to LECA clay medium with 80% shade treatment (Figure 5.8).

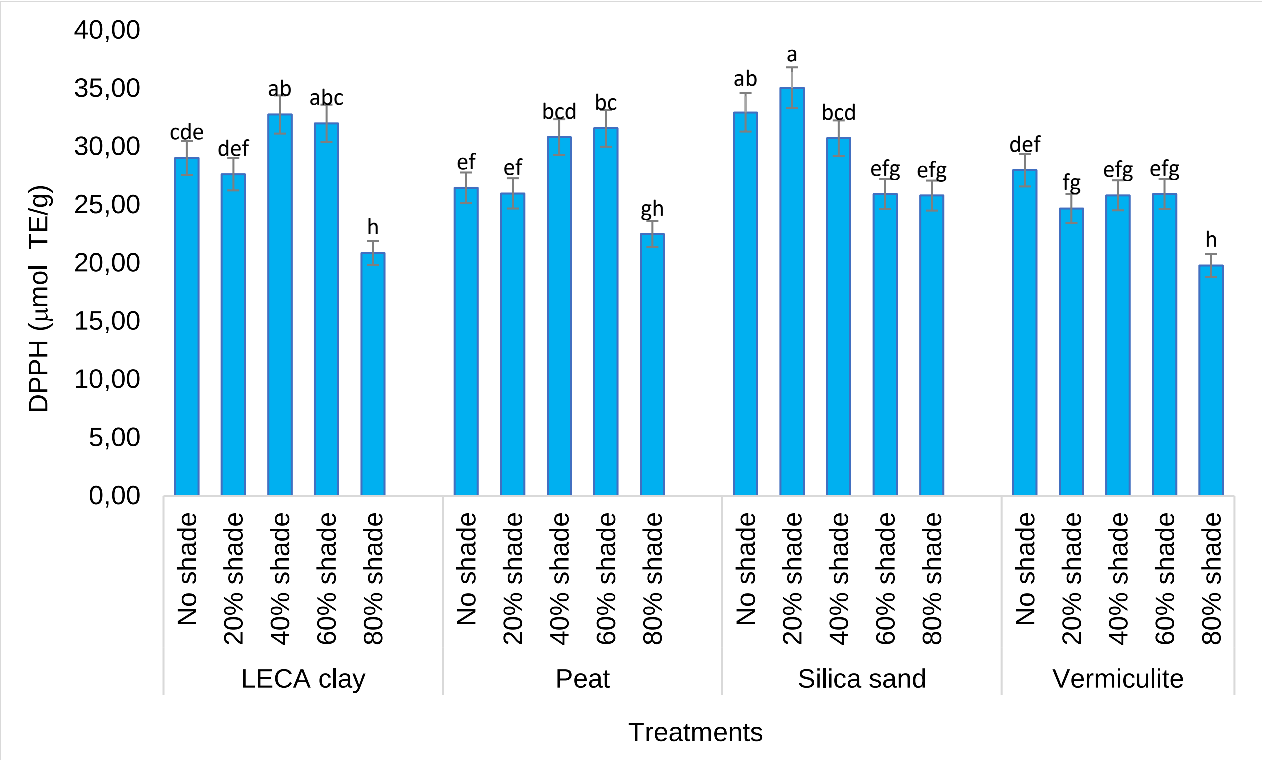

Figure 5.7: The effect of different growth media and different shade levels on total DPPH content in the flower buds of *T. divaricata.*

5.4.4. Total TEAC antioxidant content

Different growth media and different shade percentages had a significant effect on the estimated TEAC values (µmol TE/g). The LECA clay medium with no shade treatment yielded the highest mean value of TEAC while the lowest was recorded in the 80% shade treated samples of the same medium. Samples of *T. divaricata* grown in vermiculite performed better than other treatments especially under no shade while variability was observed in the effects of other treatments on TEAC values of experimented samples of *T. divaricata* (Figure 5.9).

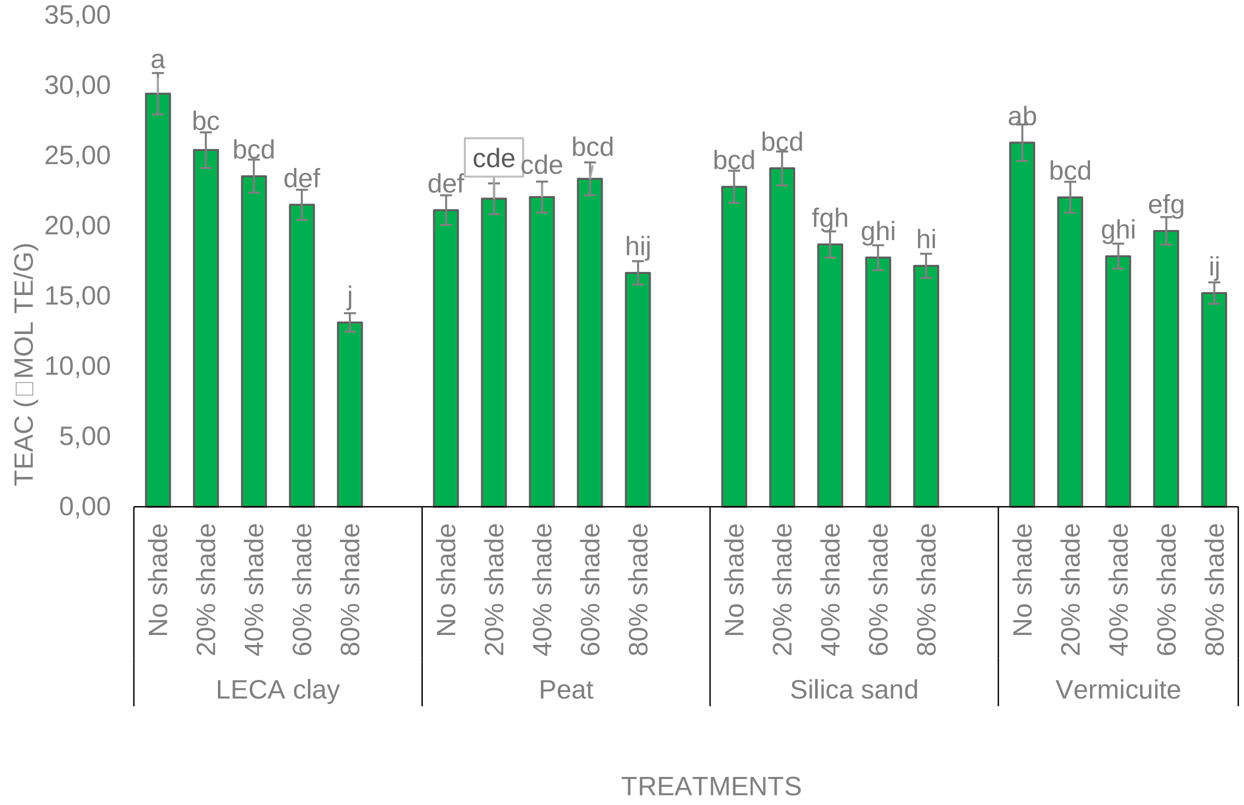

Figure 5.8: The effect of different growth media and different shade levels on total DPPH content in the flower buds of *T. divaricata.*

5.4.5. The FRAP antioxidant assay.

The FRAP antioxidants in *T. divaricata* showed significant variability in the tested samples (Figure 5.10). The 60% and 80% shade treated samples of *T. divaricata* in LECA clay respectively had the overall highest and lowest FRAP values. In peat growth medium, the highest value of FRAP content was obtained under 60% shade while the lowest was recorded at 80% shade. With silica sand, 20% shade was the best treatment, whereas both 40% and 80% shaded samples had an equivalent and the lowest FRAP content. The vermiculite medium had the highest and lowest FRAP contents in the 60% and 80% shaded plants respectively (Figure 5.10).

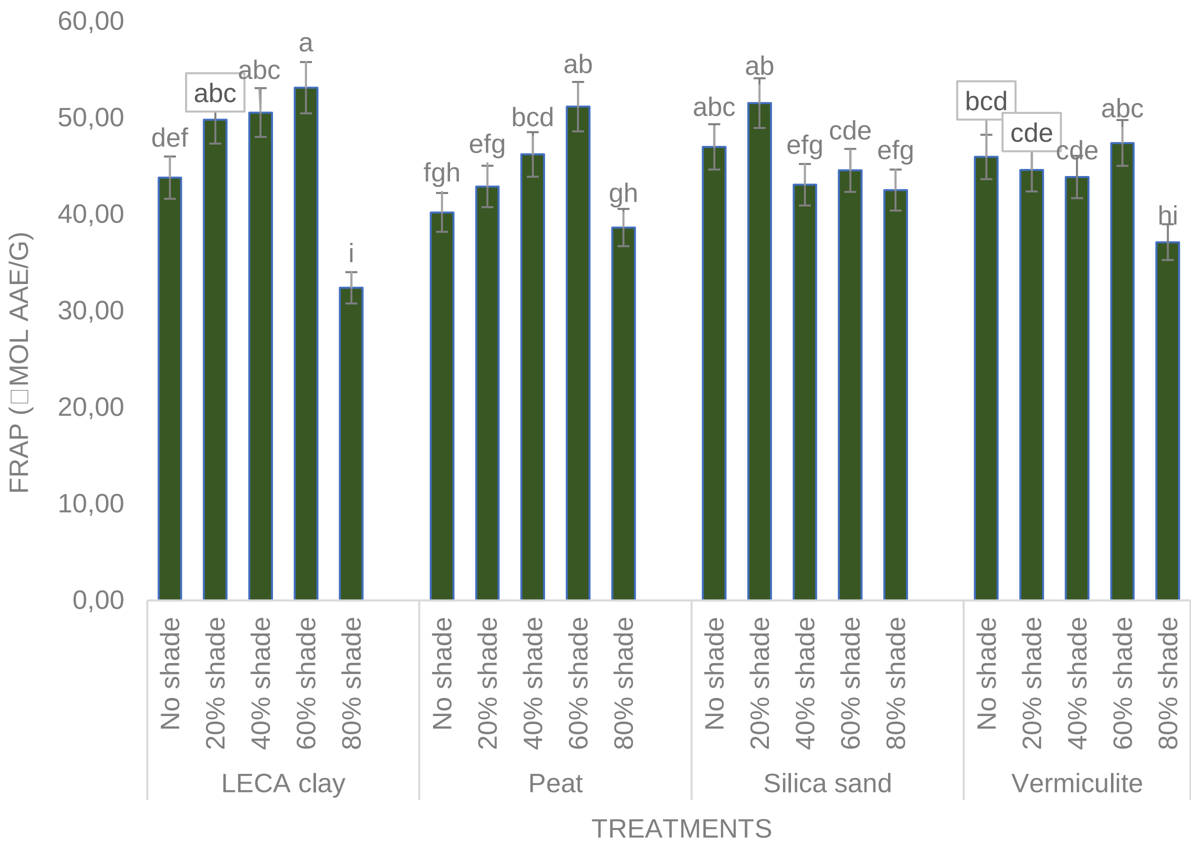

Figure 5.9: The effect of different growth media and different shade levels on total FRAP content in the flower buds of *T. divaricata.*

5.5. Discussions

Antioxidants are substances that prevent oxidation, a chemical process that can result in free radicals and cascade events that could harm an organism's cells. (Halliwell, 1996; Shahidi, & Zhong, 2005; Santos-Sánchez *et al*., 2019). Fruits, vegetables, cereals, and other plant-based diets contain phytochemicals which are biologically active nutritious plant compounds that lower the risk of serious chronic diseases. (Liu, 2004; Chun *et al*., 2005; Payne *et al*., 2012; Siegel., *et al* 2018; Choudhari *et al*., 2020; Teng *et al*., 2021). These advantages may extend beyond dietary requirements to food preservation, pollinators attraction, signalling cascading, regulation of pathways, modulation of intracellular events in the stem cells, and participation as growth factors (Dadashpour *et al*., 2017; Jamieson *et al*., 2017; Redondo-Blanco *et al*., 2020; Jideani *et al*., 2021).

The accumulation of phytochemicals and antioxidants varied significantly in *T. divaricata* cultivated in different growth media at various shade levels. The highest value of DPPH was found in silica sand medium under 20% shade compared to other treatments while the lowest value was found on vermiculite medium at 80% shade which is equivalent to LECA clay medium with 80% shade treatment. Also, the silica sand medium with 20% shade treatment recorded the highest mean value of polyphenols than other treatments whereas the lowest value was recorded in the samples harvested from LECA clay medium at 80% shade treatment and most of the treatments had an equivalent effect on polyphenols. The similar trend observed in the polyphenols and DPPH antioxidant in the tested samples of *T. divaricata* reiterates the strong correlation between polyphenols and antioxidant activities in plants (Wong *et al.,* 2006; Zhao *et al.,* 2008; Jimoh *et al*., 2019).

The peat, silica sand, and LECA clay growth media, all outperformed each other in terms of their ability to accumulate flavonols in the tested plant samples. The maximum value of flavonols was obtained in the vermiculite growth medium with 20% shade, while the lowest value was found in the vermiculite growth medium with 80% shade, which is equivalent to 80% shade in LECA clay. These findings support earlier reports that biosynthesis of flavonols is light dependent (Hollman & Arts, 2000). Flavonols have enormous therapeutic functions mainly in the prevention and treatment of chronic diseases such as cancer, Alzheimer's disease, type-2 diabetes, cardiovascular complications, blood platelet aggregation, and osteoporosis (Survay *et al.,* 2011). Bioavailability of flavonols and other phytochemicals in flower buds of *T. divaricata* as reported in this study validates the ethnobotanical use of the flower buds of the plant and its close relatives for food and herbal therapy by the indigenous Khoisan residents of South Africa (Boatwright & Manning, 2010; Ngxabi *et al*., 2021b).

Different growth media and different shade percentages had a significant effect ($P \leq 0.05$) on TEAC antioxidant content. The LECA clay medium with no shade treatment recorded the highest mean value of TEAC while the lowest was observed under 80% shade treatment of the same medium. Similar trend was found in the FRAP content of *T. divaricata* grown in LECA clay except that the highest FRAP value was recorded under 60% shade treatment. These results agree with findings of Rubio *et al*. (2016) that both TEAC and FRAP assays are based on the same single electron transfer mechanism except for the fact that FRAP is performed under pH 3.6 (acidic) while TEAC is performed at pH 7.0 (neutral) conditions (Huang *et al*., 2005).

Furthermore, findings from the study suggest that the spectral quality of incident light rays can be modified using different levels of shade nets as physiological modulators of light intensity to improve the environmental conditions of the greenhouse (Ahemd, *et al.,* 2016; Caruso, *et al*., 2020), thereby manipulating the yield, quality, and concentration of phytochemicals in plants (Buthelezi *et al*., 2016; Tmušić *et al.,* 2021). Results from this study corroborate these findings as shaded plants especially at 20% shade levels improved the level of phytochemicals in *T. divaricata*. However, the results from disagree with the finding of Caruso *et al*. (2020) who observed that the shading net reduces the antioxidant capacity of plants.

5.6. Conclusion

The result from the study indicates that there is a potential of cultivating *T. divaricata* for consumption and different soilless media can be used successfully in the propagation of this species. It is obvious from the study that minimum shade level (20%) combined with silica sand was the best treatment required for optimum production of phytochemicals and antioxidants in *T. divaricata*. This suggests why the plant thrives well in coastal sand dunes owing to its capacity to produce more antioxidants and phytochemicals to improve its adaptation to the environment. Further studies on the chemical diversity of the species are recommended to elucidate further, the therapeutic potential of *T. divaricata*.

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6. CHAPTER SIX:

Effect of different shade levels and growth media on nutrient composition of *Trachyandra divaricata* (Jacq.) Kunth

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6.1. Abstract

Wild edible plants have consistently served as a significant source of food for households in many developing countries. These crops are among underutilized vegetables that can be utilized to alleviate nutrient shortage. Costal edible plants increase the nutritional values of food consumed by numerous rural and urban dwellers, especially the downtrodden and poor. *Trachyandra divaricata* is amongst these costal species which has edible inflorescence that was once consumed by the native Khoisan people of South Africa, however, the species is neglected despite its potential to be used as vegetable. In the present study, the effect of different shade levels and different growth media on the nutrient composition of buds of hydroponically cultivated *T. divaricata* was evaluated. The results of this study indicated that there is a potential for commercial cultivation of *T. divaricata* from Nutrient Film Technique hydroponic system. The results of the study reveal that Peat medium with 20% shade was the best treatment for Acid-Detergent Fibre (ADF) content, whereas the lowest value was found at LECA clay medium in 80% shade. It was also observed that 60% shade had a significant impact on nitrogen content, with the highest value found in vermiculite medium. Furthermore, vermiculite medium with 80% shade was the best treatment for high protein yield. However, successful propagation of this species can help close the nutritional gap.

Key words: Dune onion; edible flowers; food security; inflorescence vegetables; macronutrients; micronutrients

6.2. Introduction

Wild edible plants play a crucial role in ensuring food security and balanced diets for many people in developing countries (Duguma, 2020). These plants may serve as a source of domesticated species and offer beneficial genetic features that can be used in breeding and selection to increase the yield and nutritional qualities of new crops (Thakur, *et al*., 2020). Although cultivated plants are the main source of food and income for rural dwellers, they cannot supply all the food needed each year. Tender stems and leaves are the main parts of wild vegetables that can be eaten. Collecting and consuming tender stems and leaves of edible wild vegetables has become a way of life for many rural populations around the world to meet their nutritional demands (Ju. *et al*., 2013). Different parts of wild vegetables can be prepared by boiling, stir-frying, adding to soups, or stewing with meat (Cheng *et al.,* 2022).

From environmental, health, or urbanization standpoint, food security is a big concern in many countries (Kidane, & Kejela. 2021). The need for food security shifts from land cultivation to purchasing power as people move from rural to urban regions (Temesgen. & Retta. 2015; Koffi *et al*., 2020). Consequently, those in metropolitan areas who lack resources or capital are at danger because they lack access to a healthy diet and face threats to their capacity to sustain food security and good health (Dubbeling *et al*., 2010). Without food security and sound health, there are still little opportunities for development, as this is what allows people to harness all the resources readily and naturally, they require for their well-being (Khan *et al*., 2020). Human communities experience acute food shortages during natural or man-made calamities, making them significantly dependent on wild food plants for survival (Afolayan, & Jimoh. 2009; Cheng *et al*., 2022). Naturally, many overlook these vegetables, commonly referred to as famine foods and only consider them during times of nutritional crisis (McBurney *et al*. 2004; Afolayan, & Jimoh. 2009; Cheng *et al*., 2022). However, the lack of knowledge about the nutritional value of wild food plants and their potential for food security has been blamed for the marginalization (McBurney *et al*., 2004; Afolayan, & Jimoh. 2009; Cheng *et al*., 2022). Although several things, such as a sense of self-worth and social status are necessary for human life, the ability to sustain food security and good health tops human priority scale (McBurney *et al*., 2004; Cheng *et al*., 2022).

The human diet is made up of a variety of elements, a complicated mixture of various ingredients, including nutrients, which provide the raw materials needed to power various metabolic processes in every body cell (Desai *et al*., 2014; Chen *et al*., 2018; Raza *et al*., 2021). In addition to acting as the building blocks and fuel for cellular structures, nutrients and their metabolites also act as powerful signalling molecules, inducers and repressors of gene expression, as well as direct moderators of protein function (Bender. 2003, Gibson. 2005; Liu, *et al*., 2009) Many of them actively control the actions of transcription factors and the changes in the genome's epigenetic markers to regulate gene expression (Liu *et al*., 2009 Maseko *et al*., 2019). Acquired metabolic disorders are linked to nutrition transition, which involves switching from traditional food to modern diets that are high in calorie density and poor in nutritional diversity (Choi & Friso, 2010; Chen *et al*., 2018). Since macronutrients' constituent parts are used by all living things as both energy substrates and as the building blocks of cellular structures, their advantages are obvious. Together with minerals, these important metabolites make up a group of compounds known as essential nutrients (Chen *et al*., 2018; Hardy, & Tollefsbol, 2011; Mennen *et al*., 2005; Mousa. *et al*., 2019).

To provide a secure and resilient food supply in the face of ongoing population increase and climate change, it is imperative to consider inflorescent food plants such as *T. divaricata* that can enhance the nutritional quality of human diet. Despite this potential, *T. divaricata* has been examined less and has little to no literature on its nutritional properties. Nevertheless, it is known that the Khoisan people of the South African cape coast used this plant as food because it is edible (De Vynck *et al*., 2016). Successful propagation of this species as a marketable vegetable and its re-introduction in daily diets will create options for nutrient diversity, hence promoting food security.

6.3. Materials and methods

6.3.1. Greenhouse experiment location

This experiment was carried out over a period of twelve weeks in the Horticulture Research Greenhouse 2 of the Cape Peninsula University of Technology, Bellville campus, South Africa (33□ 55'45.53S, 18□ 38, 31'. 16E). The nature of the structure and the technology installed ensured full control of the environment within the greenhouse.

6.3.2. Plant collection and propagation

Trachyandra divaricata plant divisions were collected from wild populations on the Bellville campus of the Cape Peninsula University of Technology. Divisions of the plants were done to ensure clonal propagation of identical plants. The leaves of all plants were cut to a similar length of 10 centimetre with 6 leaves and an equal weight of 23 grams per plant. All plants were then planted into 15 cm pots and placed under controlled irrigation in the propagation house for two weeks after which they were moved to the Nutrient Film Technique (NFT) gutter hydroponic system.

6.3.3. Hydroponic system design

Four identically NFT hydroponic systems were constructed with each system being set up on a rectangular wire mesh table (2.5 m long) which provided a flat surface. Another wire mesh table was stacked on top of each table to create a frame which were then covered with different percentages of shading as stated. Each system had its low-density polyethene (LDPE) 50 L reservoir in which the nutrient solution was prepared and supplied to the system. Four Polyvinyl Chloride (PVC) square gutters (2 m long) were placed on each table which held the 15 cm square plastic pots (ten pots per gutter). The gutters were sealed with PVC adhesive to prevent leaks and connected to a 1 × 2000 L/hr submersible pump with 2.5 m head capacity, 20 mm LDPE irrigation piping, 4 × 20 mm elbow irrigation fittings and 4 × 20 mm flow regulators (Ngxabi *et al*., 2021a). Water was pumped and circulated continuously from the reservoir tanks which contained a Nutrifeed fertilizer (65 g/kg N, 27 g/kg P, 130 g/kg K, 70 mg/kg Ca, 20 mg/kg Cu, 1500 mg/kg Fe, 10 mg/kg Mo, 22 mg/kg Mg, 240 mg/kg Mn, 75 mg/kg S, 240 mg/kg B and mg/kg Zn. Fertilizer group 1 Reg No: K2025 (Act 36/ 1947) which was bought from Starke Ayres, Cape Town. The pH and electrical conductivity (EC) were maintained at 6.5 and using a Martini Instruments PH55 pH probe and for adjusting the pH hydrochloric acid (HCl) was used to lower the pH, sodium hydroxide (NaOH) was used for raising the pH. EC levels of the aqueous nutrient solutions were monitored with a calibrated hand-held digital EC meter (Hanna Instruments®™ HI 98312). The nutrient solutions were refreshed every 2 weeks to minimize the build-up of salts in the growing media.

6.3.4. Experimental treatments

The experiment work involved two main treatments which were growth media and shading. Four different growth media namely: LECA clay (LC), Consol® silica sand (SS), peat (P) and vermiculite (V) and arranged in a completely randomized block design (CRBD) (Faber et al., 2020). The silica sand and LECA clay were rinsed thoroughly with sterile water to clean impurities and other earthy materials. The divided parts of *T. divaricata* were then planted in pots filled with different growing media and placed in a NFT hydroponic system. Black shade netting of 20% density was purchased from Stodels Garden Centre, Bellville, South Africa and used to create the shading climates. Different percentages of shading were created with 20% shade cloth by doubling (40%), tripling (60%) and quadrupling (80%) the net into different layers. The control had no shade cover (C=0% shade). Each treatment was numbered, as presented in the Table .61 below.

Table 6.1: Experimental treatment layout of the treatment trials.

Growth media	Shading percentage
1 = LECA clay	no shade
2 = LECA clay	20% shade
3 = LECA clay	40% shade
4 = LECA clay	60% shade
5 = LECA clay	80% shade
6 = Peat	no shade
7 = Peat	20% shade
8 = Peat	40% shade
9 = Peat	60% shade
10 = Peat	80% shade
11 = Silica sand	no shade (control)
12 = Silica sand	20% shade
13 = Silica sand	40% shade
14 = Silica sand	60% shade
15 = Silica sand	80% shade
16 = Vermiculite	no shade
17 = Vermiculite	20% shade
18 = Vermiculite	40% shade
19 = Vermiculite	60% shade
20 = Vermiculite	80% shade

6.3.5. The nutrient analysis

6.3.6. Sample preparation

Harvested flower buds and plant materials were first dried in a room at 32 °C for 3 weeks at the Botanical Lab of the Cape Peninsula University of Technology, Department of Horticultural Science, Art and Design building. After that, they were dried in a fan-drying laboratory oven at 40 °C for 7 days. The type A 10 mill from Junkel and Kunkel was used to grind the dried plants into a fine powder. The pulverized flower buds were used for both proximate and elemental analyses. The elemental analysis was performed using the Inductively Coupled Plasma-Optical Emission Spectrometer (Varian Vista-MPX, Victoria 3170, Australia) in the analytical laboratory of the Department of Agriculture and Rural Development, KwaZulu Natal Province. The Ca, Fe, Mg, P, K, Na, Mn, and Zn contents were evaluated while Nitrogen % was calculated by dividing protein value by 6.25.

6.3.6.1. Ash content

A procedure developed by the Association of Official Analytical Chemist (AOAC, 2016), was used for the percentage ash content of plant samples was determined. Ceramic crucibles were oven-dried at 105 °C for one hour after being marked with sample codes using a heat-resistant marker. The crucibles were weighed after cooling in a desiccator (W_1). Later, 1 g of ground samples from various formed soils were placed in the porcelain crucibles that had already been pre-weighed and reweighed (W_2). The crucibles and their contents were placed in a muffle furnace set at 250 °C for 1 hour, followed by 550°C for 5 hours to totally ash the samples. Samples were weighed after being allowed to cool in a desiccator (W_3). The percentage ash content of the samples was valued as

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

6.3.6.2. Determination of moisture content

The protocols outlined by (AOAC, 2016) were followed, albeit with a few minor modifications, to determine the moisture content. Empty porcelain containers were dried in an oven at 105 degrees for an hour, cooled in a desiccator, and weighed at W1. Ground samples of *T. divaricata* shoot and plant sample harvested from the different soilless media and shade levels each weighing (1.000 ± 0.001) g W2 was placed in a vessel and oven-dried to a constant weight at 105 ˚C. The vessel and its content were moved into a desiccator to cool off and reweighed (W3). The percentage of moisture content was assessed as given in equation:

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

6.3.6.3. Neutral detergent fibre (NDF)

The samples' NDF compositions were identified in accordance with (Idris *et al.,* 2019) using the equation below.

$$\% \text{ NDF content} = \frac{W1 + W2 - W1}{Weight\ of\ sample} \times 100$$

6.3.6.4. The crude fat contents.

The crude fat content was estimated following the AOAC (2016) guidelines. About 1 g of the pulverized sample was extracted in 100 mL of diethyl ether on an orbital shaker for 24 hours.

The mixture was then filtered, and the filtrate was collected in clean beakers that had previously been weighed. The ether extract was mixed with 100 mL of diethyl ether and then shaken on an orbital shaker for a further 24 hours. The filtrate was likewise collected in a beaker (W1). The ether filtrate was concentrated in a steam bath, dried in an oven at 55 °C, and reweighed in the beaker (W2). The crude fat content was therefore evaluated as

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

6.3.6.5. Statistical analysis

Data for phytochemical and antioxidant analysis were analysed using two-way analysis.

of variance (ANOVA) and Fisher's Least Significant Difference (Tukey's L.S.D) test

used to evaluate between treatments using Statistica (StatSoft, Inc., Tulsa, OK, US).

Different treatments were considered as independent variables and the significance threshold was set at P ≤ 0.05 (Steel & amp; Torrie, 1980).

6.4. Results

6.4.1. The effect of different growth media and shade levels on proximate composition of the flower buds of *T. divaricata.*

The highest value of Acid-Detergent Fibre (ADF) (41.17) was recorded at 20% shade in peat medium while the lowest value of (23.57) was found at LECA clay medium in 80% shade. At 80% shade in Silica sand and LECA clay media the highest Ash content was recorded followed by 40% Silica sand whereas the lowest value was recorded at Silica sand medium with No shade treatment and the rest of the treatments had equivalent effect on Ash content. Furthermore, the highest value of fat content was found in Peat soilless medium with 80% shade while the lowest was recorded at 20% shade in Peat medium. The highest amount of (11.53) moisture content was recorded in peat medium with 60% shade followed by vermiculite at 40% shade with the highest value of (11.37) while the lowest was found at 80% shade in vermiculite medium and all other treatments had the equivalent effects. The 20% shade combined with soilless growth media was the best treatments for neutral detergent fibre (NDF), the highest value was obtained in peat medium with 20% shade, however NDF, didn't perform any better at 80% shade treatment and the LECA clay medium had the lowest value at 80% shade. The highest value of protein content was recorded in Vermiculite medium with 80% shade whereas the least value was obtained in Silica sand medium at 40% shade while the rest of the treatments had comparable effect on protein composition of *T. divaricata.*

6.4.2. The effect of different growth media and shade levels on macronutrients in the flower buds of *T. divaricata.*

The macronutrients in *T. divaricata* are presented in Table 6.3. The main elements examined were calcium (Ca), K/Ca+Mg, magnesium (Mg), sodium (Na), phosphorous (P), potassium (k) and nitrogen. The highest Ca value was recorded at 80% shade in LECA clay and silica sand media, while the lowest was obtained in vermiculite with no shade. The best growth medium for K/Ca+Mg, under 20% shade was LECA clay where the highest value was recorded followed by peat whereas the lowest value of K/Ca+Mg, was found in vermiculite medium. At 40% shade, the peat medium was the best for K/Ca+Mg, with the highest value of (3019.50±0.50k) while the lowest value was found at LECA clay. The silica sand at 60% shade was the best medium whereas the lowest value of K/Ca+Mg, at 60% was recorded in LECA clay medium. The peat medium had the highest K/Ca+Mg value, which was observed at 80% shade, whereas LECA clay media had the lowest value. However, from no shade the highest value of K/Ca+Mg were obtained in Peat soilless medium while other media were comparable to the control which is silica sand. The best treatment for potassium content in *T. divaricata* it was peat medium with 20% shade, it was further observed that potassium was higher in peat under 40% shade whereas the lowest was recorded in LECA clay with 60% shade.

The highest value of N (3719.50) was obtained in plants grown with vermiculite medium under 60% shade followed by (3689.00) at 80% shade in vermiculite soilless medium while the lowest value of (2720.00) was found under 40% shade in silica sand medium. Moreover, the highest Mg was recorded at 80% shade in LECA clay whereas all other treatments had the equivalent effect, and the least value was obtained at 20% shade in LECA clay medium. In comparison to other treatments, phosphorus increased significantly in 60% shade when four different soilless media (LECA clay, Peat, Silica sand, and Vermiculite) were used and it also performed better at 80%, while plants from 40% and 20% shade had a comparable effect. Additionally, it was discovered that phosphorus production depleted under no shade. The highest Na content in tested buds of *T. divaricata* was recorded at 40% shade in silica sand and, LECA clay with 80% shade, however, peat medium had the lowest Na under 80% shade.

6.4.3. The effect of different growth media and shade levels on micronutrients in the flower buds of *T. divaricata*

The micronutrients such as Copper (Cu), Iron (Fe), Manganese (Mn) and Zinc (Zn) present in the flower buds of *T. divaricata* are presented in Table 6.4. The best treatment for cupper was 80% shade, and all other treatments had equivalent effects when compared to the control, however there was no effect found in peat soilless growth medium under 60%, 40%, and with no shade. The plant sample with the highest Fe value was grown in 80% shade in LECA clay, while the plant sample with the lowest Fe value was found in 60% shade in peat medium. At 80% shade Manganese had significantly increased in LECA clay medium whereas other treatments had a comparable effect. Zinc levels were higher in Silica sand and LECA clay media with 80% shade, but lowest in LECA clay with no shade treatment.

Table 6.2: The effect of different growth media and shade levels on proximate analysis in the flower buds of *T. divaricata.*

Shade	Media	ADF %	Ash %	Fat %	Moisture %	NDF %	Protein %
No shade	LECA clay	33.78±1.16 bcd	12.45±0.15 bcd	2.00±0.05 abc	9.87±0.51 abc	47.93±0.68 ab	19.14±0.23 bcd
	Peat	32.80±0.29 bcd	12.59±0.66 bcd	1.91±0.03 bcd	9.76±0.31 abc	44.3±0.77 bc	17.63±0.38 cd
	Silica sand	31.12±0.87 def	11.01±0.32 d	1.82±0.05 bef	10.62±0.39 ab	40.85±1.23 bcd	19.87±0.51abc
	Vermiculite	32.19±0.32 cde	11.92±0.27 cd	2.01±0.02 abc	10.26± 0.70abc	40.55±1.30 bcd	18.04±0.48 cd
20% Shade	LECA clay	35.91±0.31 b	13.76±0.36 bcd	1.73±0.70 efg	10.29±0.21 abc	44.45±3.09 bc	21.86±0.50 ab
	Peat	41.17±0,28 a	14.97±0.33 b	1.56±0.65 g	10.32±0.46 abc	53.87±0.57 a	17.71±0.26 cd
	Silica sand	29.09±0.25 ef	11.91±0.46 cd	2.26±0.65 ab	10.29±0.21abc	40.85±0.53 bcd	19.23±0.24 abc
	Vermiculite	35.09±1.54 bc	14.01±0.36 bc	2.05±0.55 abc	9.70±0.15 abc	43.98±3.62 bc	17.83±0.58 cd
40%Shade	LECA clay	24.84±0.18 ij	14.34±0.50 bc	2.04±0.15 abc	11.27±0.41 a	34.21±0.65 ef	18.02±0.48 cd
	Peat	30.48±0.41 def	14.39±0.37 bc	1.90±0.20 bcd	11.12±0.48 ab	39.24±1.04 cde	18.97±0.11 bcd
	Silica sand	31.67±0.41 cde	18.89±0.34 a	1.82±0.45 def	10.02±0.66 abc	39.53±1.27 cde	16.88±0.13 d
	Vermiculite	28.23±0.42 efg	13.14±0.28 bcd	2.14±0.40 abc	11.37±0.38 a	35.80±1.55 def	17.67±1.80 cd
60% Shade	LECA clay	30.08±0.78 def	12.23±0.34 bcd	1.83±0.01 def	11.30±0.52 a	42.83±0.47 bcd	17.90±0.35 cd
	Peat	27.81±0.22 fgh	14.38±0.54 bc	1.84±0.02 cde	11.53±0.23 a	40.93±0.67 bcd	20.94±0.38 abc
	Silica sand	31.15±0.49 def	14.21±0.76 bc	1.68±0.03 fg	9.40±0.75 abc	43.78±0.53 bc	21.91±0.61 ab
	Vermiculite	31.94±1.04 cde	14.14±0.69 bc	1.79±0.05 def	8.81±0.17 bc	40.93±0.57 bcd	21.80±1.43 ab
80% Shade	LECA clay	23.57±1.07 j	19.19±0.54 a	2.09±0.01 abc	8.05±0.18 cd	32.91±0.51 f	18.26±0.63 bcd
	Peat	24.84±0.54 ij	13.06±0.50 bcd	2.25±0.02 a	9.76±0.40 abc	32.91±0.66 f	20.65±0.42 abc
	Silica sand	26.31±0.01 ghi	20.86±0.87 a	1.81±0.16 def	9.88±0.25 bcd	35.81±0.40 ef	19.04±0.08 bcd
	Vermiculite	26.02±0.52 hij	13.76±0.48 bcd	1.93±0.11 bcd	6.25±0.65 d	34.28±0.72 ef	22.82±0.26 a

Table 6.3 The effect of different growth media and shade levels on macronutrients content in the flower buds of *T. divaricata*.

Shade	Media	Calcium (ca) mg/100g	K/Ca+Mg mg/100g	Potassium (K) mg/100g	Nitrogen (N) mg/100g	Magnesium (Mg) mg/100g	Phosphorus (P) mg/100g	Sodium (Na) mg/100g
No shade	LECA clay	409.50±0.50 i	3145.00±5.00 e	5865±5.00 h	3019.00±1.00 k	324.50±5.50 fg	511.00±1.00 ef	139.00±1.00 jk
	Peat	409.50±0.50 i	3419.50±0.50 cd	5926.50±3.50 g	2880.50±0.50 n	289.00±1.00h	529.00±1.00e	157.50±2.50gh
	Silica sand	347.50±2.50 m	3079.00±1.00 h	4979.00±1.00 m	3257.50±2.50 g	286.00±3.50 h	444.50±5.50 g	169.00±1.00 e
	Vermiculite	327.50±2.50 o	3039.00±1.00 j	5570.00±10.00 j	2957.00±2.50 l	371.00±1.00 cde	489.00±1.00 f	137.50±2.50 k
20% Shade	LECA clay	389.50±0.50 k	4039.00±1.00 a	6329.00±1.00 d	3579.00±1.00 d	249.00±1.00 i	427.50±2.50 g	147.50±2.50 ij
	Peat	469.50±0.50 f	3619.00±0.50 b	7167.50±2.50 a	2867.50±2.50 o	329.00±1.00 fg	689.00±1.00 bc	149.00±1.00 hi
	Silica sand	347.50±2.50 m	3147.50±2.50 e	5390.50±0.50 k	3119.00±1.00 h	319.00±1.00 gh	511.50±1.50 ef	144.00±4.00ijk
	Vermiculite	449.00±1.00 h	2839.00±1.00 m	6319.50±0.50 d	2939.00±1.00 m	419.00±1.00 b	528.50±1.50 e	149.00±1.00 hi
40%Shade	LECA clay	689.00±1.00 d	1979.00±1.00 p	4961.00±1.00 m	2960.50±0.50 l	359.00±1.00 def	611.00±1.00 d	179.00±1.00 d
	Peat	519.00±1.00 e	3019.50±0.50 k	6648.00±2.00 b	3047.50±2.50 j	369.50±0.50 cde	678.50±1.50 c	158.00±2.00gh
	Silica sand	829.50±0.50 c	2219.00±1.00 o	6169.00±1.00 f	2720.00±1.00 q	357.50±2.50 def	419.00±1.00 g	230.50±0.50a
	Vermiculite	409.00±1.00 i	2519.50±0.50 n	5239.50±20.50 l	3119.00±1.00 h	399.00±1.00 bc	747.50±2.50 a	147.50±2.50 ij
60% Shade	LECA clay	359.50±0.50 l	2979.00±1.00 l	4865.00±5.00 n	2811.00±1.00 p	289.00±1.00 h	611.00±1.00 d	138.50±1.50 jk
	Peat	458.50±1.50 g	3111.00±1.00 f	6189.00±1.00 ef	3412.00±2.50 e	337.50±2.50 efg	747.50±2.50 a	159±1.00 fg
	Silica sand	409.00±1.00 i	3428.00±2.00 c	6389.00±1.00 c	3599.00±1.00 c	328.00±1.50 fg	744.00±26.00 a	168.00±2.00 ef
	Vermiculite	360.50±0.50 l	3411.00±1.00 d	6223.50±6.50 e	3719.50±0.50 a	347.00±3.00def	716.00±4.00 ab	169.50±0.50de
80% Shade	LECA clay	1101.00±1.00 a	1536.50±3.50 r	5692.50±7.50 i	3018.50±1.50 k	478.50±1.50 a	529.00±1.00 e	219.50±0.50 b
	Peat	339.00±1.00 n	3100.50±0.50 g	5228.00±12.00 l	3365.00±5.00 f	319.00±1.00 gh	678.50±1.50 c	79.00±1.00 m
	Silica sand	1059.00±1.00 b	1911.00±1.00 q	6392.50±7.50 c	3060±0.50 p	374.00±26.00 cd	516.00±4.00 ef	199.00±1.00 c
	Vermiculite	400.00±1.00 j	3069.00±1.00 i	5559.00±1.00 j	3689.00±1.00 b	318.50±1.50 gh	727.50±2.50 a	99.50±0.50 l

Table 6.4: The effect of different growth media and shade levels on micronutrients analysis in the flower buds of *T. divaricata.*

Shade	Media	Copper (Cu) mg/100g	Iron (Fe) mg/100g	Manganese (Mn) mg/100g	Zinc (Zn) mg/100g
No shade	LECA clay	0.40±0.01 abc	7.50±0.50 h	3.69±0.01 g	5.81±0.01 h
	Peat	0.00± 0.00 d	6.25±0.05 ij	3.47±0.03 g	5.84±0.06 h
	Silica sand	0.40±0.01 abc	9.25±0.05 fg	2.19±0.01 h	6.58±0.02 g
	Vermiculite	0.69±0.01 ab	9.45±0.05 ef	3.79±0.02 g	5.58±0.02 i
20% Shade	LECA clay	0.53±0.43 abc	5.75±0.05 ij	3.55±0.30 g	5.36±0.04 j
	Peat	0.11±0.01 cd	5.85±0.05 ij	5.85±0.05 cd	7.58±0.02 e
	Silica sand	0.29±0.01 bcd	7.55±0.05 h	2.79±0.02 h	6.41±0.01 g
	Vermiculite	0.69±0.01 ab	15.75±0.05 d	5.48±0.02 d	6.47±0.03 g
40%Shade	LECA clay	0.49±0.02 abc	22.11±0.01 c	5.84±0.05 cd	7.59±0.01 e
	Peat	0.00±0.00 d	6.55±0.05 i	2.80±0.20 h	6.38±0.03 g
	Silica sand	0.39±0.01 abc	23.89±0.01 b	5.29±0.02 de	8.47±0.03 c
	Vermiculite	0.79±0.01 ab	6.20±0.01 ij	3.58±0.02 g	6.58±0.02 g
60% Shade	LECA clay	0.29±0.01 bcd	6.29±0.01 ij	3.45±0.35 g	8.28±0.02 c
	Peat	0.00±0.00 d	6.29±0.01 ij	6.47±0.03 bc	7.58±0.02 e
	Silica sand	0.49±0.01 abc	8.50±0.50 g	3.93±0.08 g	6.57±0.03 g
	Vermiculite	0.80 ±0.01 ab	5.49±0.01 j	4.68±0.02 ef	7.95±0.05 d
80% Shade	LECA clay	0.89±0.01 a	40.39±0.01 a	9.48±0.03 a	9.19±0.01 b
	Peat	0.58±0.03 abc	5.68±0.03 ij	3.68±0.02 g	5.93±0.07 h
	Silica sand	0.58±0.02 abc	22.11±0.01 c	6.58±0.03 b	10.50±0.10 a
	Vermiculite	0.81±0.02 ab	10.29±0.02 e	4.65±0.05 f	7.11±0.01 f

6.5. Discussions

The suitability of organic substrates for plant growth is significantly influenced by nutrient availability (Khalaj, *et al*., 2019), seasonality and shade stress to which the plant was subjected (Dodd *et al*., 2005), which may alter their elemental composition as well as other variables affecting dynamics and nutrient forms, such as the presence of dissolved organic compounds (Khalaj, *at al*., 2019). This agrees with findings of current study results as the nutritional composition amongst all treatments has proven to be highly influenced different by different growth media and different shade levels on *T. divaricata.* Increased shade levels resulted in an increase in copper Cu composition in plants propagated in vermiculite medium (Table 6.4). The same pattern was observed in Zn content, where the relationship between shade and concentration was linearly proportional from 40% shade in LECA medium. Additionally, there was no significant difference in Fe content of *T. divaricata* flower buds in plant samples harvested from peat medium. Furthermore, Mn yield was high in LECA clay under 80% shade, contradicting previous findings. Erickson *et al*., (2021) claims that manganese increases in soil with high water holding capacity and low pH. Wolcott *et al.* (2022) suggested that PH levels in LECA clay is 7 and it has poor water holding capacity. These findings correlate with Erickson *et al*. (2021) study findings where organic matter decreased with manganese value as a result manganese had a poor performance in peat medium under 40% shade and peat medium contains organic material (Kitir *et al*., 2018).

Micronutrients are crucial for the body's ability to produce energy, make haemoglobin, maintain bone health, have a healthy immune system, and resist oxidative damage (Wishart, 2017). As the human body is subjected to physical and psychological stresses regularly, disrupting its internal balance, the body responds by producing enzymes, hormones, and other compounds needed for energy production, cell maintenance and repair, immune function and recovery from illness, blood formation, and maintenance of vital organs which also play important roles in several homeostatic processes and all these processes are highly influenced by nutritional sufficiency. Thus, micronutrients are essential for enabling appropriate reactions to stimuli that can threaten the homeostasis of the organism. Since the human body typically cannot generate micronutrients, an appropriate daily consumption at amounts advised by various governing agencies is required (Graham, & Welch, 1996; Gammoh, & Rink 2017; Wishart, 2017 & Khan *et al*., 2021). *Trachyandra divaricata* is rich in micronutrients required by the body, such as iron, magnesium, and zinc, as shown in Table 6.3, and because these micronutrients are suggested to be consumed daily, successfully cultivating this species can help to close the nutritional gap.

Zinc level in *T. divaricata* flower buds was 10.50 mg/100g which was less than 60.70 mg recorded for *Colocasia esculenta L.* inflorescence however it met the daily requirements of 6.8mg to 9.4mg as stated by Okechukwu, *et al*. (2020). The results from this study showed that *T. divaricata* buds are good source of Zn. Increased intake of zinc especially among children, would reduce the likelihood of Zn insufficiency. Due to evidence linking Zn shortage to increased morbidity, alopecia, diarrhoea, mental problems, and decreased growth, Zn deficiency in developing countries is becoming a major concern (Prasad. 2013; Okechukwu, *et al*., 2020; Raza *et al*., 2021). Consuming flowers of *T. divaricata* will guarantee the minimum daily requirement for Zn in household diets.

The iron content was high under 80% shade in LECA clay (40.39 mg/100g) in contrast to a previous report (Okechukwu, *et al*., 2020) that claimed that vegetables are typically a poor source of iron. This value was higher than the values found in cocoyam inflorescence (0.4 mg) and sweet potato leaves (16 mg) (Okechukwu, *et al*., 2020). Iron is a necessary component of red blood cells, which transport oxygen. It helps the muscles maintain oxygen reservoirs and makes the body more resistant to infections (Szklarz, *et al*., 2022). Anemia, fatigue, headache, insomnia, and heart palpitations are all symptoms of iron deficiency (Peyssonnaux. *et al*., 2008; Bhadra. & Deb., 2020). The current study found that flower buds *T. divaricata* can be used as Fe supplement in situations where Fe is greatly needed.

Findings from this study have suggested that accumulation of minerals and proximate composition of a plant can be manipulated by adjusting the intensity of incident light using shade nets of varying thickness. The concurrent impact of soilless growth media also manifested in the variation recorded in the proximate and mineral compositions of the tested samples. The results further demonstrated that *T. divaricata* is a nutrient-dense wild edible vegetable that can contribute to the nutrient supply in addition to common leafy vegetables. However, these results contradict earlier reports by Whitehead and Isaac (2012) who claimed that shade reduces nitrogen content of plants whereas the highest nitrogen content of *T. divaricata* was recorded in vermiculite medium at 60% and 80% shade levels. It has also been observed that shade and growing medium had a significant influence on the nitrogen composition of *T. divaricata* as the highest mean value of nitrogen (3719.50) were recorded under 60% and 80% under vermiculate soilless medium. This corroborates the earlier study on wheat, where nitrogen concentration was significantly increased by shade while recorded lower under no shade treatment (Whitehead & Isaac, 2012).

Phosphorus contents increased significantly in all peat mediums except for those under 40% and 80% shade, phosphorus had increased in vermiculite medium these findings agree with the findings of (Gungor, & Yildirim., 2013), who discovered that peat medium increases the phosphorus content in *Capsicum annuum L*. The daily recommended intake of phosphorus is 700 mg (Jimoh, *et al*., 2020), *T. divaricata* met this requirement as shown in table 6.3.

Potassium is a vital electrolyte and mineral that is necessary for many bodily processes. For instance, potassium is necessary for controlling your heart rate and blood pressure as well as for healthy nerve conduction, protein synthesis, glycogen levels and muscular contraction (Wang *et al*., 2020). At least, 2000 mg of it is desirable for an adult (Jimoh, *et al*., 2020), the potassium content of the analysed plant samples was recorded in large quantities in *T. divaricata* as indicated in Table 6.3.

Dairy products in most industrialized countries give 50–80% of the daily recommended amount of calcium, while plant-based diets only provide around 25% of that amount. Calcium is regarded as a vital structural component (Hussain *et al*. 2011), from the current study LECA clay and silica sand soilless media in 80% shade were the most effective for calcium concentration; LECA clay had the greatest value (1101.00), whereas vermiculite soilless medium with no shade treatment had the lowest value (327.50).

In contrast to the previous study's findings by Valenzuela-Cobos *et al.* (2019), which claimed that peat decreases the concentration of acid detergent fibre (ADF) and neutral detergent fibre (NDF), the results from the current study show that peat medium with 20% shade produced the best ADF content results of the tested plant samples, however there was no significant difference in ADF concentration under no shade treatment when compared to the control (table 6.2). The magnesium composition of the current study' s analysed sample is higher than that of *A. sativum* reported by Hussain *et al*. 2011, This suggests that *T. divaricata* is a high-magnesium sauce. For adults, a daily magnesium intake of between 300 and 400 mg is advised (Uwitonze & Razzaque, 2018), therefore *T. divaricata* met these requirements. Including magnesium-rich foods in your plant-based diet is an effective way to lower blood pressure and lower your risk of developing heart disease (McCarty, 2005; Boone, 2013). Additionally, the USAD (2018), report claims that the typical magnesium concentration in 100 g of cooked broccoli is 12 mg, while amaranth meal has a magnesium value of 55 mg. This is much lower than the values found in all samples examined, where Mg levels ranged from 249.00 to 478.50 mg/100g, indicating that growth media and shade net favourably modify this mineral in *T. divaricata.* Hirzel *et al*. (2020) reported that using shade cloth increases sodium content on apple trees. In the current study, sodium increased significantly under 40% shade while the lowest value of sodium was found under 80% shade on vermiculite medium, implying that for sodium production, 40% or partial shade is recommended.

Protein is one of the most important factors in the development of the human anatomy (Maton, 1997). It is one of the most fundamental building blocks of our bodies. Proteins make up most of our muscles, organs, and immune system (Schönfeldt, & Hall, 2012; Chasapis, *et al*., 2020). The protein content of the tested sample was high when compared to an earlier report on spinach, which had a protein value of 8.67. (Irfan *et al*., 2021). Protein consumption leads to less inflammation, which lowers the risk of chronic illness and lowers cholesterol. (Iddir, *et al*., 2020). One of the main causes of nutritional disease is a lack of protein in the diet. A good source of proteins is a plant-based food that gets more than 12.00% of its calories from proteins (Abbasi.*et al*., 2015). Because wild food sources have the highest crude protein content, several food formulations choose to employ them as high protein sources. The current study discovered that the protein content in *T. divaricata* ranged from 16.88%, proving that this species is a high protein source.

Due to its versatility, broccoli is one of the most well-known vegetables. It is filled with essential nutrients, including fibre that makes you feel full. In addition to having several critical elements that promote weight loss, broccoli also has phytochemicals that can break down fats (Nagraj, *et al*., 2020; Irfan *et al*.,2021). Furthermore, compared to the current study, the protein content of broccoli reported by (Irfan *et al*., 2021) is lower because it is less than 10%. The fat content recorded in the current study was low when compared to the early report on wild edible vegetables listed by Abbasi.*et al*. (2015), however the fat value recorded in *Dioscorea deltoidea* of 0.690% means this value is lower when compared to the current study Table 6.2.

The ash content of the tested flowers of *T. divaricata* increased significantly as the shade levels increased, with the highest value of ash found under 80% shade (Table 6.2). This finding contradicts a previous report by Mataa *et al*. (2018) on *Hibiscus sabdariffa*, in which the shade effect decreased the ash content. The ash percentage of the flower buds varied between treatments, ranging from 11% to 20.86%, which corresponds to the composition seen in processed meals (Tie *et al.,* 2021). These findings are consistent with the findings of (Fernandes *et al*., 2017) on Cauliflower, Pumpkin, and Broccoli, where the ash level ranged from 12 to 15%. The nutritional value of food is determined by its ash content, which is assumed to reflect the mineral content preserved in food items (Radha, 2021). A high ash value indicates that the plant is high in dietary fibres, which provide protection for digestive organisms in the gastrointestinal tract (Jimoh, *et al*., 2020). Moludi, *et al.* (2020) reported that some dietary fibres act as prebiotics, which can encourage the development of beneficial bacteria in the gut and support the balance of the gut microbiota. When compared to the wild vegetables listed from the prior study by Aneja. (2007); Mataa *et al*. (2018), the moisture content from the current study is lower.

6.6. Conclusion

This study demonstrated that shade had a good impact on the mineral elements, and proximate contents in the flower buds of *T. divaricata.* The flower buds may be used as a natural vegetable source of nourishment based on the findings of these experiments. Additionally, the current study supports the expansion of *T. divaricata*'s commercial cultivation as a nutritious, shade-tolerant, and sustainable wild vegetable with edible fluorescence. To better support its consumption as a wild vegetable, more research on the impact of various media on key metabolites responsible for high nutrient profile of the species and its potential pharmacological uses is recommended.

6.7. Acknowledgment

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7. Chapter Seven: General discussions, conclusion, and recommendations

7.1. General discussions

Population growth necessitates the production of more food to feed hundreds of thousands of people, as well as the promotion or diversification of food crops to prevent hunger and malnutrition (Tripathi *et al*., 2019). Developing countries are dealing with issues of insufficient and inadequate food to feed millions of people (Sasson, 2012; Barrett *et al*., 2013) which has necessitated the adoption of new strategies for food production to encourage diet diversification. One of these approaches is the modulation of light intensity to influence growth and biosynthesis of minerals and phytochemicals in plants. This becomes imperative as there are few studies conducted on the interactive effects of light intensity and media on plant growth, nutrition, and biosynthesis of secondary metabolites in edible inflorescence species (Ncise *et al*., 2020; Appolloni *et al*., 2022). Light has long been recognized as the most important factor influencing growth and mineral composition in plants (Appolloni *et al*., 2022). Furthermore, this will assist to understand plants response to light intensity and growth media to enhance their nutritional value and supplement the world's food and nutritional security although there is extensive literature on the reduction of growth caused by high light intensity in many plant species (Pinto *et al*., 2022). In addition, this will support the growth of more edible species like *Trachyandra divaricata*, which would provide a remedy for the widespread food insecurity in these developing countries. However, there is a scarcity of literature on this species and on pharmacological activities of this plant.

Results of the current study (chapter 3) revealed that indices of new growth namely leaf number, leaf length, fresh and dry weight, and number of flower buds measured in *T. divaricata* were significantly affected by the different growing media and light intensity moderated by shade levels. Plants cultivated in peat under normal greenhouse lighting (no shade) had the highest leaf number, flower buds, fresh and dry weight than those grown under shade levels and varying growth media. Similar findings have been observed on *Mitragyna speciosa* Korth by Zhang *et al*. (2022) where height, average leaf size, and total leaf dry mass were increased in response to unshaded greenhouse conditions. In addition, peat has been reported to play a crucial role in water and nutrient retention in hydroponically grown plants (Bian *et al*., 2015). Thus, an increase in plant growth parameters were observed in this medium (peat) as compared to sand, LECA clay and vermiculite. This may be attributed to the high-water holding capacity of the peat medium

(Agus *et al*., 2020), enhanced by the high shade levels, as reported by Depauw *et al.* (2020) who reported that increased shade reduces plant height.

Plant cultivated in peat medium with no shade had the highest formation of flower buds when compared to other treatments. The highest yield of flower buds and biomass of *T. divaricata* in peat medium may have been facilitated by high humus content and high water and nutrient retention capacity of peat medium which enables it to retain water efficiently and supply the plant with growth nutrients required for both flower induction and vegetative development effectively than other growing media (Zaller, 2007; Kitir *et al*., 2018; & Markoska *et al*., 2018). In comparison with the control, silica sand under no shade, vermiculite and peat media had the highest content of plant fresh weight. Furthermore, plant dry weight was high in peat medium with no shade while the lowest value was observed in silica sand with 80% shade. These findings corroborate previous reports that peat plays a crucial role in water and nutrient retention in hydroponically grown plants (Bian *et al*., 2015), thereby justifying an increase in growth parameters observed in plants cultivated with peat as compared to sand, LECA clay and vermiculite media.

The effect of different growth media and shade levels on the chlorophyll content of *T. divaricata* was studied in Chapter 4. Shade cloth had a positive effect on the production of chlorophyll in *T. divaricata*, according to the findings. It was then determined that this species could be grown in vermiculite medium under 80% shade for optimal chlorophyll yield. These findings indicate that *T. divaricata* can optimize captured light and to maximize its photosynthetic efficiency and gain carbon under shaded conditions. This was also reported respectively by Zhang *et al*. (2022) and Tran, (2018) on chlorophyl concentration in seedlings of *Mitragyna speciosa* and *Fraxinus latifolia* under varying light exposure.

Vegetables with high antioxidant capacity add value to food products and are well received by consumers and the food industry (Pinto *et al*., 2022). Consumption of plant products high in phenolic content can protect human tissue oxidation by scavenging free radicals and inhibiting lipid peroxidation, thereby improving nutritional quality, and avoiding potential problems caused by excessive consumption of synthetic additives (Akbari *et al*., 2022). As a result, it is critical to enhance the phytochemical components of fruits and vegetables during cultivation. One of the most important environmental variables in regulating vegetable growth, development, and phytochemical accumulation is light condition (light quality, light intensity, and photoperiod), which is especially important for vegetables grown in controlled environments (Corrêa *et al*., 2012; Bian *et al*., 2015). In the present study, the flower buds of plants grown under 20% shade had higher polyphenols than other treatments while the media had no significant effect on the accumulation of polyphenols. This supports the findings of Ncise *et al*. (2020) on *Tulbaghia violacea* flower stalk, where polyphenolic content was observed to observed to have been increased by low light intensity. On the contrary, the flavonols compositions were variable among light intensities. This contradicts the results of Gao *et al*. (2021) on Broccoli microgreens where high light intensity increased the flavonol content. Nevertheless, the antioxidant capacity was increased in plants grown in LECA clay and peat under 40 and 60% shade except TEAC. These results shows that light intensity and growth media may affect not only plant yield but also the synthesis of other biologically active substances, such as phenolics, which have protective role against induced stress responses (Bulgari *et al*., 2019). Moreover, phytochemical content and antioxidant activities of harvested of flower buds of *T. divaricata* subjected to different growth media and different shade levels, showed the best results under 20% shade in silica sand medium, however, FRAP results was better under 60% shade in LECA clay medium. This could be related to light conditions as it influences the phytochemical accumulation and antioxidant activities (Machado *et al*., 2018), same trend was observed in DPPH concentration in vermiculite growth medium. Based on the results from the current study, the flower buds of *T. divaricata* may be used as a good natural source of dietary antioxidants.

The effect of different shade levels and growth media on the nutrient diversity in *T. divaricata* was evaluated in Chapter 6. The results show that using a shade net had a positive effect on the flower buds of this species in proximate analysis, as the results of 80% shade level yielded high fats in peat soilless medium. Furthermore, the same trend was observed in protein yield, which was high under 80% shade in vermiculite, possibly due to the availability of microbiota in these two soilless media. The same trend was found in ash content under 80% shade; however, the highest value was recorded in silica sand. The fat content obtained from the flower buds of *T. divaricata* (1.56 to 2.25%) fall within range of these well-known edible flowers such as *Erythrina caribaea, Aloe vera, Allium schoenoprasum, Brassica oleracea var. italica, Tropaeolum majus* and *Erythrina americana* which have been reported to have low levels of unsaturated fats (Fernandes *et al*., 2017; Sotelo *et al*., 2007; Navarro-Gonzalez *et al*., 2014). The moisture content of tested flowers of *T. divaricata* ranged from 6.25 to 11.53% in tested treatments including the control. These values are too low when compared to other edible flowers which average between 70 to 80% in moisture (Sotelo *et al*., 2007; Navarro-Gonzalez *et al*., 2014; Fernandes *et al*., 2017). This suggest that the flower buds of this plant might have long storage lifespan which will favour the growers and sellers. Also, the plant may be explored further for its potential use as plant-based preservative used in enhancing the shelve life of food.

The result of this experiment clearly indicates that *T. divaricata* could be cultivated under shade net for optimum production of nutrients. Moreover, the micronutrients present in the flower bud of *T. divaricata* were far below the recommended dietary allowance (RDA) except Iron in few treatments while most macro-elements in the tested samples exceeded the RDA. These trace elements were lower than values reported on other edible inflorescence species such as Banana (*Musa spp.)* (Lau *et al*., 2020), *Asparagus officinalis* L. (Guo *et al*., 2020) and *Cynara cardunculus* L. (Gostin *et al*., 2019). Based on these findings, it can be assumed that the flower buds of *T. divaricata* are safe to consume because they accumulated fewer heavy metals than other inflorescence vegetables.

7.2. Conclusion and recommendations

The study's findings suggest that *T. divaricata* can be easily propagated in big quantities for commercial uses and that growing more crop plants doesn't require expensive seed or machinery as the propagation method used from this study to propagate this species was the plant division method. On the other hand, shade stress had a considerable positive impact on plant growth, chlorophyll content, nutrient content, and antioxidant potential, which supported the growth of healthy plants. As a result, 80% shade yielded the highest chlorophyll content in vermiculite medium. According to the findings, peat was the best medium for optimal vegetative development, floral bud production, and biomass accumulation.

These findings suggest that *T. divaricata* has the potential to be grown in hydroponics using various growing media under shade cloth as a possible inflorescence's vegetable. This suggests that commercial growers should consider this cultivation method for other inflorescences vegetables for improved production, although the mineral content, proximate composition, and phytochemical content were comparable among tested treatments including the control. However, the high fibre content of the flower buds validates its digestive effectiveness in humans, and its high protein content validates its value as an immune booster, important nutraceutical, and as a potential functional food. The lower moisture content indicates that the plant may have a long storage lifespan, and a potential in improving the shelve life of food. Plants grown in peat under normal greenhouse conditions had an improved growth parameters while variation was observed in proximate compositions as well as phytochemical composition in response to light intensity and growth substrate.

The study showed that the mineral components and proximate contents in *T. divaricata* flower buds were positively impacted by 20% shade. The results of this study suggest that the flower buds could be utilised as a natural vegetable source of nutrition. In comparison to other treatments, the highest value of DPPH was discovered in silica sand medium under 20% shade, while the lowest value was observed on vermiculite medium at 80% shade, which is equal to LECA clay medium with 80% shade treatment. Furthermore, the silica sand medium with 20% shade treatment had the highest mean value of polyphenols compared to other treatments, but the samples collected from LECA clay medium with 80% shade treatment had the lowest value, and most of the treatments had an equivalent effect on polyphenols. These findings imply that 20% shade is optimal for phytochemical and antioxidant accumulation in *T. divaricata*. To better support its consumption as a wild vegetable, more research on the impact of various media on key metabolites responsible for high nutrient profile of the species and its potential pharmacological uses is recommended.

Based on these findings, *T. divaricata* should be domesticated due to its rich nutritional value. This species entry into the market will contribute to the country's economy, job creation, and food security by increase in vegetable crops and ensures sustainability in underserved areas. As a result, further research is needed on other cultivation methods that are likely to improve flower buds' formation, as it is the most important part of this species, as well as on the effect of shade on flower buds' formation and nutritional content to support the potential of its commercial viability as an inflorescence vegetable.

7.3. References

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8. CHAPTER EIGHT:

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Light Intensity and Growth Media Influence Growth, Nutrition, and Phytochemical Content in *Trachyandra divaricata* Kunth

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Abstract: *Trachyandra divaricata* (Sandkool) is one of the most abundant wild edible inflorescence vegetables in South Africa. The dearth of literature on its edibility, nutrient composition, and conservation has contributed to its underutilisation. This study investigated mineral and proximate content, phytochemical compositions, and growth response of *T. divaricata* to light intensity and soilless media. Treatments comprised four media (LECA clay, silica sand, peat, and vermiculite) which were subjected to different shade levels (no shade, 20, 40, 60, and 80%) created from a factory-made 20% density net by doubling (40%), tripling (60%), and quadrupling (80%). All treatments were irrigated with a standard nutrient solution. The results showed that the treatments impacted the yield of *T. divaricata* significantly in terms of biomass and flower buds, especially in plants cultivated in peat under normal greenhouse lighting (no shade). Conversely, plants developed significantly more specific leaf size and total chlorophyll content under shade levels (20, 40, 60, and 80%) in different growth media, even though the values were comparable among treatments. The highest Ca, Mg, Cu, Fe, and Mn levels were consistently recorded in flowers of *T. divaricata* grown in LECA clay under 80% shade level, while other minerals varied in tested treatments. The peat medium under 20% shade optimised the neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of the flowers, whereas both fat and protein contents were greatly enhanced by peat and vermiculite, respectively, under the 80% shade. Consistently, the lowest phytochemical contents were recorded in LECA clay subjected to 80% shade, whereas the highest polyphenols and DPPH antioxidants were produced by silica sand medium treated with 20% shade. Both TEAC and FRAP antioxidants were improved significantly in LECA clay under no shade and the 60% shade level. However, both 20% and 60% shade levels enhanced the flavonol content significantly. On the basis of these findings, *T. divaricata* is a promising inflorescent vegetable that may be considered for domestication and further research due to its potential pharmacological and nutraceutical values.

Keywords: dune onion; edible flowers; functional food; inflorescent vegetables; nutraceutical; shade levels; underutilised vegetables

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1. Introduction

Plants are subjected to a variety of abiotic stresses such as variable light intensity, fluctuating temperatures, water scarcity, salinity, heavy metals, and mechanical injuries and herbivory [1,2]. In most cases, plants are subjected to multiple stresses at the same time, and thus they have evolved sophisticated defence mechanisms to recognise and adapt to a wide range of stresses [3]. Hence, understanding their physiological processes and defence mechanisms is of critical importance for plant science research. Light and moisture are two environmental factors that have a significant impact on plant growth and development [4]. Light is the second most important ecological factor influencing plant growth, production, and survival, as it plays a significant role in determining photosynthetic efficiency in plants [5]. Moisture, which is determined by soil structure, is one of the