WATER USE AND NUTRITIONAL WATER PRODUCTIVITY OF BUSH TEA

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

Bush tea (*Athrixia phylicoides DC.*) is an indigenous shrub of South Africa known for its medicinal properties. However, due to a lack of comprehensive information on developing suitable irrigation management strategies, the cultivation of bush tea is currently limited to small-scale operations. Considering this limitation, the first aim of this project was to investigate the effect of water regimes on growth and development, yield, and nutrient content, along with its nutritional water productivity (NWP) under field conditions. The treatment consisted of a crop water requirement (ETc) of 100%, 30% and the control (stress) in a Complete Randomised Block Design (CRBD); each treatment was replicated three times to ensure the robustness and accuracy of the findings. Results demonstrated that the highest gravimetric moisture content readings were found in the control treatment and the lowest in the 100% treatment. Conversely, the 30% ETc treatment significantly ($P<0.05$) impacted bush tea's growth, development, productivity, and NWP. However, it was observed that the 100% ETc treatment resulted in a higher biomass yield (259.1 kg/ha) compared to the 30% ETc treatment (171.2 kg/ha) and control (stress) (68.2 kg/ha). Water productivity (WP) exhibited notable differences across the various water regimes.

The result of the study highlights the significant influence of different water regimes on the growth and yield of bush tea plants. The highest yield was observed in plants subjected to 100% ETc, with a subsequent decrease in those under 30% ETc and water stress treatments. Notably, the 30% ETc treatment positively impacted soil water content, nutritional composition, and water productivity, highlighting the importance of optimizing water application in bush tea cultivation. Under control (stress) conditions, a decrease in stomatal conductance was noted, but water use efficiency increased, indicating the adaptability of bush tea to water-scarce environments. This emphasizes the plant's resilience and potential to thrive in challenging conditions. The study underscores the crucial role of varying water regimes in influencing plant physiology, nutritional composition, leaf gas exchange, and chlorophyll fluorescence. The adaptability of bush tea to stress conditions suggests its potential as a valuable resource for food and nutritional security in arid regions.

The second aim was to determine bush tea's physiological parameters, nutritional yield, nutritional water productivity, and metabolite composition under varying water regimes. The phytochemical analysis by liquid chromatography-mass spectrometry (LC-MS) coupled with visualization of the detected chemical spaces through molecular networking indicated *Athrixia phylicoides DC*. to be rich in various bioactive compound derivatives. The results showed that the 30% ETc enhanced plant growth, nutrient content, and nutritional water productivity compared to other water treatments. Nevertheless, 100% ETc yielded more (95.62 kg ha^{-1}) than 30% ETc (60.61 kg ha^{-1}) and control (12.12 kg ha^{-1}). The chlorogenic acid accumulation was higher under 30% ETc compared to 100% ETc and control. Therefore, this study is the first to determine the accumulation of various bioactive compounds in bush tea leaf extracts under varying water regimes. This confirms that bush tea is well adapted for production without limiting nutrients in areas with low water availability.

Future studies should investigate a more detailed analysis of bush tea's nutritional water productivity and use efficiency. This is especially important under diverse water regimes and in varying environmental conditions. These in-depth investigations will contribute to a comprehensive understanding of the plant's adaptive mechanisms and shed light on its potential. Such insights are crucial for advancing sustainable agriculture practices and promoting further research in the realm of natural products, ultimately contributing to the broader field of agriculture and enhancing our understanding of the plant's potential applications.

These findings contribute valuable insights for developing appropriate irrigation management strategies to overcome existing limitations in the scale of bush tea production. The research lays the groundwork for future studies and practical applications to foster the sustainable cultivation of bush tea in South Africa.

Conclusion and Recommendations

The research investigated the potential of varying water regimes to enhance growth, development, and bush tea quality while facilitating its production for medicinal purposes. As the problem of food security increases with diminishing resources and population growth, small-scale agriculture also increases to meet the increasing demand.

Research on bush tea is considered a solution to the problem of malnutrition. This approach promises to improve current food practices. In countries facing water scarcity and malnutrition, such as South Africa, bush tea cultivation is a beautiful plant with the potential to create new markets for the rural economy and contribute to the conservation of plant biodiversity and the search for plant-derived compounds for new medicines. It can also be a way to generate income for rural communities. This study provides evidence of improved bush tea yield, NWP and nutrient yield, and nutrient water efficiency in water-limited regions.

The following recommendations are based on the findings made from the investigation:

- These findings offer valuable insights for future research, suggesting exploring diverse environments and seasons, both within and outside the province, for cultivating bush tea. This approach could improve its nutritional water productivity and overall yield. It would be interesting to investigate the interaction of bush tea with other herbal medicinal teas in future studies.
- Future studies should investigate different medicinal plants' water utilisation and productivity since this study only focused on bush tea in a controlled environment and a field experiment.
- Future studies should consider exploring additional experimental variables, such as agronomic management practices like planting density, to achieve a comprehensive understanding. This will help in the scientific and systematic evaluation of their impact on the yield and quality of many bush tea varieties.
- Quantitative research of bush tea harvested under varying water regimes using comparative assessments through questionnaires in different locations to provide quality assessments.
- A molecular network on the stem and roots of bush tea is highly recommended to give more insight into the compounds available in bush tea plants under varying water treatments and in different locations and seasons.

Innovation

The study showed that bush tea is one of the herbal teas with a significant number of nutrients, which makes it a unique multi-purpose plant. Its medicinal purposes make it an important future crop for commercialisation. These research outcomes hold considerable potential for introducing novel crops into the agricultural system, contributing to enhanced livelihoods and sustainability. Furthermore, the results unveiled a positive response of various identified compounds to limited water conditions and stress, exhibiting diverse mass-to-charge characteristics. Among these compounds are chlorogenic acids, flavanols, and terpenoids, all in plants and with medicinal applications. These findings suggest that using these compounds can reduce malnutrition and mitigate food insecurity within the livelihoods of poor rural communities.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

4CL	4-coumarate-CoA ligase
A	Net CO_2 Assimilation Rate
AAS	Atomic absorption spectrometry
a.m.	Ante meridiem
A/Ci	Intercellular CO_2 concentration
AAS	Atomic Absorption Spectrometry
AES	Alternative Electron Sink
ANOVA	Analysis of Variance
ARC	Agricultural Research Council
BPI	Base peak intensity
C4H	cinnamic acid 4-hydroxylase
C_a	Ambient CO_2 Concentration
Ca	Calcium
CERU	Controlled Environment Research Unit
CGA	Chlorogenic acids
CHS	chalcone synthase
Ci	Intercellular CO_2 Concentration
Ci/Ca	Ratio of intercellular and ambient CO_2 concentrations
CNS	Central nervous system
CRBD	Complete randomised block design
Cu	Copper
CWR	Crop water requirements
DAFF	National Department of Agriculture, Forestry and Fisheries
DNA	Deoxyribonucleic Acid
E	East
ESI	Electrospray ionisation
ET_a	Crop Water Requirement
ET_o	Reference Evapotranspiration
ETR	Electron Transport Rate
FAO	Food Agriculture Organization
Fe	Iron
FNS	flavone synthase
FSU	Farmer Support Unit
Fv/Fm	Maximum Quantum Efficiency of Photosystem II Photochemistry
G	Acceleration due to gravity
GACP	Good agricultural and collection practices
GNPS	Global Natural Products Social Molecular Networking
Gs	Stomatal Conductance
HCL	Hydrochloric acid

HIV-AIDS	Human Immunodeficiency Virus - Acquired Immune Deficiency Syndrome
HNO_3	Nitric acid
HOBO	Honest observer by the onset
IBA	Indole-3-butyric acid
ICFR	Institute for Commercial Forestry Research
IGDP	Integrated Growth and Development Planning (2010)
IK	Indigenous knowledge
IPM	Integrated pest management
IRGA	Infrared Gas Analyzer
K	Potassium
K_c	Crop Factor
KCl	Potassium chloride
LAI	Leaf area index
LAN	Limestone ammonium nitrate
LC-MS/MS	Liquid Chromatography-Quadruple Time-of flight Tandem Mass Spectrometry
LCF	Leaf chamber fluorometer
LSD	Least Significant Difference
M	Mass
m/z	Mass-to-ratio
Mg	Magnesium
Mn	Manganese
MN	Molecular network/networking
N	Nitrogen
NaI	Sodium iodide
NC	Nutritional Content / Nutritional/nutrient composition
NDP	National Development Plan
NEPAD	New Partnership for Africa’s Development
NP	Natural products
NUCs	Neglected and underutilised crop species
NUFMS	Neglected and underutilised functional medicinal crops
NUS	Neglected and underutilised crop species
NWP	Nutritional Water Productivity
ΦPSII	Effective quantum efficiency of photosystem II photochemistry
P	Phosphorus
P	Precipitation
PAL	Phenylalanine ammonia-lyase
PCA	Principal component analysis
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
P_{soil}	The bulk density of that given soil
P_{water}	Water density

QC	Quality control
qN	Non-Photochemical Quenching
qP	Photochemical Quenching
QTOF	Quadrupole Time-of-Flight
RNA	Ribonucleic acid
RQ	Research question
S	South
SA	South Africa
SAHPRA	South African Health Products Regulatory Authority
SWC	Soil Water Content
T	Transpiration Rate
TQMS	Triple quadrupole mass spectrometer
Θ_{Dry}	Weight of dry soil
Θ_g	Gravimetric moisture content
Θ_v	Volumetric moisture content
Θ_{Wet}	Weight of wet soil
UHPLC-QTOF-MS/MS	Ultra-high performance liquid chromatography quadrupole time of flight mass spectrometry
UKZN	University of KwaZulu-Natal
US	United States
UV/V	Ultraviolet/visible
WHO	World Health Organization
WP	Water Productivity
WRC	Water Research Commission
WUEi	Intrinsic Water Use Efficiency
WUEins	Instantaneous Water Use Efficiency
Y_a	Actual yield
Zn	Zinc

LIST OF SYMBOLS AND UNITS

cm	Centimetres
cm^2	Square Centimetres
cmol/kg	Centimoles per Kilogram
g	Gram
g/cm^3	Grams per Cubic Centimetre
ha	Hectare
ha^{-1}	per Hectare
kg	Kilogram
kg^{-1}	per Kilogram
kg/ha; $kg.ha^{-1}$	Kilogram per Hectare
kg/m^3	Kilogram per Cubic Metre
kV	Kilovolts
kPa	Kilo Pascals
L	Litres
Lhr^{-1}	Litres per Hour
L/min	Litres per Minute
m	Metre
m^{-3}	per Cubic Metre
m^2	Square Metres
$m^3.ha^{-1}$; m^3/ha	Cubic Metre per Hectare
mg/g	Milligram per Gram
mg/L	Milligrams per Liter
min	Minutes
mL; ml	Millilitres
mL/min	Millilitres per Minute
mm	Millimetres
mmol m^{-2} s^{-1}	Millimole per Square Metre per Second
mol	Moles
ppm	Parts per million
rpm	Revolutions per minute
µL	Microlitres
µm	Micrometres
µmol e^{-1} m^{-2} s^{-1}	Micromoles of electrons per square meter per second
µmol. mol^{-1}	Micromole per Mole
µmol m^{-2}	Micromole per Square Metre
µmol m^{-2} s^{-1}	Micromole per Square Metre per Second
'	Minutes
%	Percentage
°C	Degrees Celsius

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1 INTRODUCTION AND BACKGROUND

South Africa is a semi-arid nation with a water profile quickly transitioning from water-scarce to water-stressed (Singels et al., 2010). The country's annual rainfall fluctuates around 500 mm, far below the world's average of 860 mm per annum (Mabhaudhi, 2012). Rainfall is unevenly distributed, with about 50% of the rain falling on 15% of the land (Crétat et al., 2012). It is in most of the remaining 85% of the country where rural inhabitants are concentrated (Shackleton et al., 2008). These communities are characterised by practising rainfed agriculture and generally derive low economic returns from farming activities (Beddington et al., 2011). Poverty, unemployment, and food and nutrition insecurity are concentrated within these communities (Shackleton et al., 2008). Increasing agricultural activities within rural communities has been identified as a way to improve food nutrition security, reduce household poverty, increase youth employment, and promote rural development (RSA, 2010). It is argued that current agricultural activities within these communities are too mainstream and lack the necessary innovation to foster rural economic development. Given these challenges, embracing a new paradigm that focuses on agricultural models custom-fit to changing climates and markets and filling new niche markets is necessary. One strategy could be diversifying cropping enterprises to include multipurpose medicinal plants with pharmaceutical and economic potential.

Athrixia phylicoides DC. (bush tea) is an indigenous shrub in South Africa, commonly used as an anti-depressant and aphrodisiac in Southern African populations (Mabogo, 1990). This shrub naturally grows in semi-arid regions with limited canopy coverage, such as grassland and forest biomes in South Africa (Limpopo, Free State, KwaZulu-Natal, Mpumalanga, and Eastern Cape Provinces) and Swaziland (Herman et al., 2000). Traditionally, the herb is greatly treasured as traditional medicine for treating diabetes, heart disease, hypertension, acne, boils, colds, cuts, headaches, infected wounds, loss of voice, and infected throat (Mudau et al., 2006; Nchabeleng et al., 2012). It is also recommended as a potent blood cleanser (Roberts, 1990) and is further substantiated by its high levels of total polyphenol content (Mudau et al., 2007a). Polyphenols are metabolic precursors of shikimate and phenylpropanoid pathways, which are regulated through sustained transcription of chalcone synthase

(CHS), flavone synthase (FNS), phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL) (Kanazawa et al., 2012). Besides polyphenols, bush tea contains quercetin, 3-o-demethyldigicitrin, 5,6,7,8,3',4'-hexamethoxyflavone (Mudau et al., 2007a), 5-hydroxy-6,7,8,3',4',5'-hexamethoxyflavan-3-ol (Mashimbye et al., 2006), tannins (Mudau et al., 2007b), and antioxidants (Daniel et al., 2007). Studies have also demonstrated the anti-depressant properties of the tea (Mashimbye et al., 2006; Mudau et al., 2007a). The commercialisation of bush tea is a potential prospect for developing high-value products for the beverage and pharmaceutical industries. However, there is a need for an alternative supply of plant material as wild plants are under extreme pressure due to increased demand from local and export markets.

Wiersum et al. (2006) reported that the intensive harvesting of bush tea, due to its increasing use, has, in many places, resulted in overexploitation and thus constitutes a serious threat to biodiversity in the region. The only option for the crop species is its large-scale cultivation to guarantee a financially viable value chain (Mudau et al., 2022). It is worth considering that water and nutrient management are critical in optimising the growth and quality attributes of bush tea and many medicinal crops. Maedza (2015) and Mudau et al. (2007a) all reported that quantifying the thresholds of macro and micro-nutrient application and water is necessary, as this has been shown to affect the growth and accumulation of total pharmaceutical content. A clear understanding of water and nutrient requirements can help farmers optimise production in different growing regions. In addition, they impact marketing and ascertain consumer affinity to accept bush tea products. Consumer perceptions toward organically produced agricultural products continue to exert enormous pressure on the farming sector due to the ecological unfriendliness of conventional inputs.

Odhav et al. (2007) stated that South Africa has many medicinal tree and shrub species. Still, the socio-economic value and water productivity, including nutritional water productivity (NWP), are under-researched. NWP quantification is still in its infancy stage, with only a handful of instances where it has been used. Examples include Chibarabada et al. (2017), who evaluated NWP for selected grain legumes [bambara groundnut (*Vigna subterranea*), cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), and dry bean (*Phaseolus vulgaris*)], and Nyathi et al. (2019), who reported on leafy vegetables [amaranth (*Amaranthus cruentus*), spider flower (*Cleome gynandra*), Swiss chard (*Beta vulgaris*), and orange-fleshed sweet potatoes (*Ipomoea batatas*)]. Previous and current research projects funded by the WRC at the University of KwaZulu-Natal have evaluated the nutritional water productivity of various traditional leafy vegetables. Specifically, Wenhold et al. (2012), a WRC Project K5/2171, assessed the 'Nutritional Water Productivity of Indigenous Food Crops,' while WRC report K5/2493//4 evaluated the "Water Use and Nutritional Water Productivity of Food Crops for Improved Nutrition and Health in Poor Rural Households." No study has evaluated the water use and NWP of a medicinal plant such as bush tea.

1.1 Project objectives

This study aimed to evaluate water use, nutritional water productivity (NWP), and the quality of bush tea in contrasting environments.

The following were the objectives of the project:

1. To determine the effect of different water levels on bush tea growth and productivity, water use, and nutritional water productivity under field and tunnel environmental conditions.
2. To determine the biochemical properties and phytochemical properties of bush tea.

1.1.1 General objective

To develop a framework for informing and guiding medicinal plant production, focusing on bush tea for poor rural communities.

1.1.2 Specific objective

Specific objective 1: To measure water use and nutritional water productivity of bush ta grown in contrasting environments.

Specific objective 2: To determine the pharmaceutical value of bush tea.

Specific objective 3: To model water use and map suitable areas for bush tea.

Specific objective 4: To formulate best management practices for maximising bush tea's water use and nutritional value.

All the contractually stated objectives were achieved and form the basis of this research report.

1.2 Scope of the report

The report is written in a series of self-contained chapters with different authors. Each Chapter addresses at least one of the project's specific objectives as set out in terms of reference. Due to the paper format used, the report does not have a general methodology section; each Chapter has its specific methodology. This may have inadvertently created cases of minor repetition, especially in the methodology section.

The report is structured to address the project objectives of the study in a logical framework.

A general overview of the report is provided below.

Chapter 1 provides an overview and conceptualisation of the overall project, a general literature review, and the study's objectives as stated in the contract.

Chapter 2 provides a global, state-of-the-art review of the nutraceutical and pharmaceutical properties of underutilised crops, which provides general views of different approaches to underutilised crops.

Chapter 3 reviews knowledge gaps on bush tea production, water use and medicinal properties.

Chapter 4 presents the findings from the investigation of responses of physiological parameters, nutritional water productivity, and phytochemical analysis of bush tea grown under a controlled environment to varying water regimes.

Chapter 5 presents the findings from evaluating the effects of different water regimes on the growth, development and productivity, nutritional water productivity (NWP), and quality of bush tea under field conditions.

Chapter 6 synthesises the various chapters, linking them to the study's main objectives as stated in Chapter 1 and the contract; it also provides the conclusions and synthesised recommendations to guide practice, application of findings, and future research.

2 NEGLECTED AND UNDERUTILISED CROPS AS PART OF FUNCTIONAL MEDICINAL CROP SPECIES: A SYSTEMATIC REVIEW

Mudau, FN, Chimonyo, VGP, Modi, AT and Mabhaudhi, T

2.1 Introduction

Rural communities within South Africa (SA) practice rainfed subsistence agriculture and generally derive low economic returns from farming activities (Beddington et al., 2011). Poverty, unemployment and food and nutrition insecurity are concentrated within these communities (Shackleton et al., 2008). Also, these communities are plagued by the coexistence of undernutrition (i.e. thinness, stunting and underweight) and over-nutrition (i.e. overweight and obesity) or diet-related non-communicable diseases (Dakora and Belane, 2019; Govender et al., 2016; Thow et al., 2018; Tsegay et al., 2014). This phenomenon is known as the double burden of malnutrition (Modjadji and Madiba, 2019). Increasing agricultural activities within these communities have often been viewed as improving food nutrition security, reducing household poverty, increasing youth employment, and promoting rural development (RSA, 2010). However, as mentioned earlier, productivity is low due to water shortages, partly changing climate and low capacity to adapt. It is argued that current agricultural activities within these communities are too mainstream and lack the necessary innovation to allow rural economic development. Given these challenges, embracing a new paradigm that focuses on the agricultural model's custom fit to changing climates and markets is necessary. Any changes should also provide the much-needed food and nutrition needs. One strategy could be diversifying cropping enterprises to include multipurpose medicinal plants with nutraceutical, pharmaceutical and economic potential.

Since recorded history, medicinal plants have been integral to human existence and the African healthcare system. Rural Africa is especially endowed with the most attainable and reasonably priced medicinal plants prescribed by herbalists and traditional healers. Africa is bestowed with huge biodiversity resources. Van Wyk (2011) reported that the “African Plant Checklist and Database Project” identified 50 136 angiosperm taxa that occur in Africa south of the Sahara (32 424 taxa in tropical Africa and 22 755 taxa in southern Africa). It is estimated to contain between 40 and 45,000 plants with development potential. Africa has more than 5000 species used in the formal and informal market as medicinal plants (Van Wyk, 2011; Tasheva and Kosturkova, 2012). Statistics from southern Africa estimate that 3,000 species or 13.8% of the flora (Van Wyk and Gericke, 2000) represent 13.5% of the flora used. Lately, alongside medicinal plants, there has been an increase in the popularity, production, and marketing of functional food crops such as amaranth, bush tea, honeybush tea, ginger, and mint. These crops also serve a dual purpose as medicinal crops; there is an emerging interest in functional medicinal food crops or plant-based dietary compounds for therapeutic, nutraceutical and pharmaceutical benefits.

In response, there has been an increase in food and nutrition, health, ethnobiological and ethnopharmacological research along the food-medicine continuum. The overwhelming evidence linking diet health and disease occurrences has reinforced these research interests. However, most studies have primarily addressed popular therapeutic, nutraceutical and pharmaceutical remedies. They have often sidelined non-conventional food crops [hereafter referred to as Neglected and underutilised functional medicinal crops (NUFMS)]. Furthermore, several attempts to promote these crops have been met with numerous constraints, including but not limited to knowledge about their true medicinal value, poorly developed value chains and conservation practices. If NUFMS contributes to rural development, employment creation, and food and nutrition security for better health and livelihoods, it is necessary to generate information to assist in mainstreaming. The research, development, and commercialisation of NUFMS can develop high-value products for the food and pharmaceutical industries. In light of this possibility and the fact that these crop species are recognised as crops for the future, productivity is needed to meet the increasing demand for local and export markets.

The study aimed to identify the range of selected functional neglected and underutilised crops in South Africa and their nutraceutical and phytochemical properties. In addition, the study outlined a possible strategy for developing the neglected and underutilised functional medicinal crops value chain, which will form the basis of the approach used in bush tea.

2.2 Materials and Methods

A mixed-method review approach, which included combining quantitative and qualitative research or outcomes with process studies, was used to compile the literature on the status of medicinal crops. Where applicable, the emphasis was placed on using literature from SA, with some comparisons to regional literature. Narrowing the search to SA allowed for assessing local knowledge relative to international knowledge on functional medicinal crops.

To fulfil the objectives of this research, the methodology of the current study was structured into four phases, namely (i) a general review of key terms and definitions to be used in the review, (ii) a mixed-methods review of the literature to establish the current status of medicinal plants and identify gaps to their mainstreaming, (ii) systematic review to quantify the amount of knowledge on (a) diversity of functional medicinal NUS (b), pharmaceutical and nutraceuticals properties, and (c) priority NUFMS and (iv) proposed production strategy for priority underutilised functional medicinal crops. Details of these stages are outlined below.

Phase 1: Definition of terms

Various definitions have been used to describe NUFMS, each with a different meaning and context, causing an incoherent body of literature on these crops. This study identifies correct and/or contextualised definitions of key terms in this review. Each definition of a term includes, as a rule, a short formal definition, some additional characteristics and references if available. The terms to be defined include "medicinal plants", "functional food crops", "functional medicinal plants", "Neglected and underutilised crops", "dietary supplements", "pharmaceutical", and "nutraceuticals". We used official guidelines, position papers, statements and reports of international societies, and original papers and review articles in scientific international journals as references.

Phase 2: Identifying priority functional medicinal crops

This part of the study was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. The main databases used were SCOPUS and Web of Science. Google Scholar was also used to identify keywords during the systematic review planning.

Phase 3: Status of underutilised functional medicinal crops in South Africa

For this phase, a mixed-method review approach, which included combining quantitative and qualitative research or outcomes with process studies, was used to compile the literature on the status of medicinal crops. Where applicable, the emphasis was placed on using literature from SA, with some comparisons to regional literature. Narrowing the search to SA allowed for assessing local knowledge relative to international knowledge on functional medicinal crops.

Phase 4: Identify a production strategy for priority underutilised functional medicinal crops

The NUFMS industry is an "uncharted" economy dominated by unsustainable informal production and market systems. Since the development of NUFMS plants in South Africa is in its infancy, a value chain approach was used to develop a guideline for commercialising NUFMS plants.

2.2.1 Search strategy

A two-step approach was used to focus the review and provide an in-depth assessment of NUFMS nutraceutical and pharmaceutical properties and priorities.

The first stage: planning the review

In this stage, research questions were identified, a protocol was developed, and eventually, the protocol was validated to see if the approach was feasible. The research questions, publication venues, initial search strings, and publication selection criteria were also defined. When all this information was defined, the protocol was revised to see if it represented a proper review protocol.

Research questions:

- The current review sought insight into NUS's nutraceutical and pharmaceutical properties. The following four research questions (RQs) were defined:
- What is the body of knowledge about the nutraceutical and pharmaceutical properties of NUFMS?
- What are the Nutraceutical and pharmaceutical characteristics of NUFMS?
- Which phytochemicals are associated with the Nutraceutical and Pharmaceutical characteristics of NUFMS?

Identifying keywords

The second step of the planning stage was an internal process to identify keywords, terms, and phrases used in the search strings. The review's objective was copied and pasted into Google Scholar, and the top 10 articles that appeared were downloaded and reviewed for keywords, terms and phrases. After identifying the articles, we verified their relevance and reliability by searching for them in indexed databases (Scopus and Web of Science).

The ten articles were:

Bhartiya, A., Aditya, J. P., & Kant, L. (2015). Nutritional and remedial potential of an underutilised food legume horse gram (Macrotyloma uniflorum): A review. Journal of Animal and Plant Sciences, 25(4), 908–920.

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Donno, D., Cerutti, A. K., Prgomet, I., Mellano, M. G., & Beccaro, G. L. (2015). Foodomics for mulberry fruit (Morus spp.): Analytical fingerprint as an anti-oxidant and health properties determination tool. Food Research International, 69, 179–188. https://doi.org/10.1016/j.foodres.2014.12.020

Kumari, A., & Chaudhary, H. K. (2020). Nutraceutical crop buckwheat: a concealed wealth in the lap of Himalayas. Critical Reviews in Biotechnology, 40(4), 539–554. https://doi.org/10.1080/07388551.2020.1747387

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The second stage: a systematic review

We reviewed the ten articles, and 34 terms (Table 2.1) were identified for use as keywords.

Table 2.1: Results of the systematic review of neglected functional medicinal crop species. [1]Crop type -Legume (L), Herb (H), Cereal (C), Cucurbit (Cu), Root and tuber (RT), Tree (T), Shrub (S), Leafy vegetable (LV), Pseudocereal (P). [2]Growth behaviour - Herb (H), Creeper (Cr), Climber (Cl), Tree (T), Shrub (S). [3]Plant part – Root (R), Shoot (Sh), Seed (Sd), Stem (St), Flower (Fr), Pod (Pd), Leaves (Lv), Bark (B), Corm (Cm), Tuber (Tu).

Common name	**Scientific name**	**Citation Frequency**	**Country of use**	**Crop type**[1]	**Growth behaviour**[2]	**Parts used**[3]	**Pharmaceutical and Nutraceutical properties**
Honeybush	*Cyclopia spp*	5	South Africa	L	S	Lv	Reduce digestive problems, gives relief for arthritis and to treat diabetes, relaxation and stress relief, colic, hypertension and hypotension, chest ailments, diarrhoea, immune-boosting, blood circulation and blood cleanser, kidney ailments, diabetes, eczema (internally), stomach ailments, constipation, appetite stimulant, breastfeed (stimulate milk in the mother), provide nutrition for the baby and animals when mother's milk dries up, colds and flu, cosmetics, Contains flavonoids and polyphenols. Has anti-tumour, anti-inflammatory, anti-obesity, anti-oxidant, cardioprotective and anti-diabetic properties.
Tigernut	*Cyperus esculentus*	2	Benin	L	H	Sd	To treat colon cancer, heart disease, diabetics, obesity, gastrointestinal, Aphrodisiac, Carminative, diuretic, emmenagogue, flatulence, indigestion, diarrhoea, dysentery, excessive thirst, vein expansion, and it is gluten-free. Used as a source of crude fibre, calcium and iron
Ground Bean	*Macrotyloma geocarpum*	2	Benin and Togo	L	H	Sd	Source of crude fat, arginine, amino acids, protein, calcium, potassium, Phosphorus Iron, Zinc, Lysine,

[1] Crop type -Legume (L), Herb (H), Cereal (C), Cucurbit (Cu), Root and tuber (RT), Tree (T), Shrub (S), Leafy vegetable (LV), Pseudocereal (P)
[2] Growth behavior - Herb (H), Creeper (Cr), Climber (Cl), Tree (T), Shrub (S),
[3] Plant part – Root (R), Shoot (Sh), Seed (Sd), Stem (St), Flower (Fr), Pod (Pd), Leaves (Lv), Bark (B), Corm (Cm), Tuber (Tu)

							Methionine, thiamine, Riboflavin, niacin, Phenylalanine, Histidine, Tryptophane
Wing bean	*Psophocarpus tetragonolobus*	1	India	L	Cl	Sd	Rich in proteins, oils, vitamins and carbohydrates. It has antioxidant, anti-inflammatory, anti-nociceptive, anti-bacterial, anti-fungal, anti-proliferative and cytotoxic activity
Sword bean	*Canavalia gladiata*	1	India	L	Cl	Sd, Pd	treatment of vomiting, abdominal dropsy, kidney-related lumbago, asthma, obesity, stomach-ache, dysentery, coughs, headache, intercostal neuralgia, epilepsy, schizophrenia, inflammatory diseases and swellings. It is rich in carbohydrates, proteins, oils and minerals (K, Mg, Ca, P and S)
Sunn hemp	*Crotolaria juncea*	1	India	L	S	F, Sh	The plant is used to purify the blood and is used to treat impetigo and psoriasis
Lablab	*Dolichos lablab*	1	India	L	Cl	Sd	
Pigeon pea	*Cajanus cajan*	3	Canada, Benin, India	L	S	Lv, St, Sd	It is a source of proteins, fibre, calcium, potassium, magnesium, phosphate
Winged bean	*Psophocarpus tetragonolobus*	1	Malaysia	L	Cl	Sd	Source of peptides and treatment of ailments caused by microbes
Bambara groundnut	*Vigna subterranea*	2	Benin, Southern Africa	L	H	Sd	Source of moisture, protein, carbohydrate, energy, crude fibre, calcium, potassium, magnesium, sodium, phosphate, iron, zinc, copper, ascorbic acid, B carotene, Lysine, methionine, thiamine, riboflavin, phenylalanine, histidine, niacin, tryptophan, valine, threonine, leucine, isoleucine, fat, ash, phenolics and flavonoids.
Velvet bean	*Mucuna pruriens* (L.) DC. var utilis	5	India	L	Cl	Sd	Treatment of cancer and microbial diseases.
Grass pea	*Lathyrus sativus* L	1		L	Cl	Sd	Its pharmacological effects include anti-oxidant, nervous, anti-diabetic, analgesic, anti-pyretic and

							cardioprotective effects. The current review discussed the chemical constituents and pharmacological effects of Lathyrus sativus. The oil from the seeds is a powerful and dangerous cathartic (stimulating bowel evacuation). It contains starch, cane sugar, leguminvicilin, legumelin, fixed oil, gum resin, oleoresin, alkaloids, carbohydrates, flavonoids, terpenes, phenols, tannins, vitamin C, riboflavin, carotenoids, beta-carotene, proteins and amino acid.
Clusterbean#	*Cyamopsis tetragonoloba*	1	India	L	Cl	Sd	Source of amino acids, ascorbic acid, and Lysine used to treat heart diseases and colon cancer.
Broad bean	*Vicia faba*	1	Spain	L	Cl	Sd	protein, carbohydrates, B group vitamins and minerals, volute vitamins, folic acid, niacin, and vitamin C, dietary fibre and macro and micronutrient
African yam bean	*Sphenostylis stenocarpa*	1	Benin	L		Sd	Source of B carotene and phenolics. Treatment of cancer
Black gram	*Vigna mungo*	2	India	L	H	Sd	The seedpods are diuretic and lithotripic, and the inside of the green pods is rubbed on warts to remove them. Source for lipids, proteins, carbohydrates, amino acids, energy, fibre, ash, lysine, phenolics and leucine. Used for the treatment of cancer and heart diseases.
Drumstick	*Moringa oleifera*	7	Nigeria, India, Mauritius, Spain, South Africa	T	T	Sd, Pd, Lv, R	Almost all tree parts are eaten or used as ingredients in traditional herbal medicines. This especially applies to the leaves and pods commonly eaten in parts of India and Africa. To date, Moringa oleifera may lead to modest blood sugar and cholesterol reductions. It may also have antioxidant and anti-inflammatory effects and protect against arsenic toxicity. In addition, it has anti-microbial, anti-oxidant, anti-cancer, cardiovascular, hepatoprotective, anti-ulcer, diuretic, anti-urolithiatic,

							and anti-helminthic. Its multiple pharmaceutical effects are capitalized as therapeutic remedies for various diseases in the traditional medicinal system. Moringa leaves are an excellent source of calcium, potassium Iron, magnesium, phosphorus, zinc, vitamin A, vitamin B1 (thiamine), B2 (riboflavin), B3 (niacin), B-6 folate and ascorbic acid (vitamin C), oils, fatty acids, micro-macro minerals elements and various phenolics,
Baobab/ African Baobab	*Adansonia digitata*	3	Benin, Ghana	T	T	Fr, Sd, R, B, St, Lv, F, Sh	To treat diabetes, cancer, diuretics, inflammation, hypolipidemic, fever and flavonoids. It is a source of Arginine, minerals, calcium, sodium, potassium, magnesium, manganese, iron, zinc, phenolics, Vitamin A, B, C; proteins and carbohydrates
Breadfruit	*Artocarpus altilis*	1	Benin	T	T		The sources are fats, carbohydrates, proteins, crude fibre, calcium, sodium, phosphate, magnesium, zinc, manganese, potassium, copper, molybdenum, and vitamins. It assists in treating indigestion, diarrhoea, malaria, vomiting, and fever. It can be used externally for wound cleaning.
African fan palm	*Borassus aethiopum*	1	Benin	T	T	Fr, R	The roots may be used to treat stomach parasites, bronchitis, sore throats, and asthma. The leaves are said to be an aphrodisiac, and the sap is reported to have many uses. The African fan plant is a good source of protein, fat, ash, fibre, amino acids, aspartic acid, threonine, serine, glutamic acid, proline glycine, methionine, isoleucine, leucine, minerals, sulphur, potassium, magnesium, calcium, sodium, zinc, iron, manganese, and copper.
	Parkia biglobosa	1	Benin	T	T		Used to treat different ailments. Virtually all parts of the plants are used traditionally to treat skin-related diseases, diabetes, diarrhoea, hypertension, cough

							and bronchitis. Source of protein, crude fibre, ash, carbohydrates, carotenoids, vitamin C, and minerals.
Blackberry	*Rubus fruticosus*	1	Benin	T	T		Useful in treating cancer, diarrhoea, dysentery, whooping cough, anaemia, toothache, mouth ulcer, sore throat, haemorrhoids, and minor bleeding. Blackberry is a good source of nutrients, such as manganese, copper, dietary fibre, carbohydrates, zinc, magnesium, minerals, and vitamins.
Mulberry	*Morus alba L.*	2	India	T	T	Fr, Lv	Used for treating dizziness, insomnia, premature ageing, skin irritation and DM2. Contains vitamin C, zinc, calcium, iron, potassium, phosphorus, and magnesium
Physic nut	*Jatropha curcas L.*	1	India	T	T	Fr, Sd, R, B, St, Lv, F, Sh	Source of Ascorbic acid, phenolics, anthocyanins and flavonoids. Treatment of cancer, inflammation, and diabetes.
Jackfruit	*Artocarpus heterophyllus*	1	India	T	S		Presence of many secondary metabolites, including diterpenoids, sesquiterpenoids, alkaloids, flavonoids, phenols, lignans, coumarins and cyclic peptides. Pharmacological activities, such as anti-inflammatory, anti-oxidant, anti-microbial, anti-viral, anti-cancer, anti-diabetic, anti-coagulant, hepatoprotective, analgesic and abortifacient effects
Velvet tamarind,	*Dialium guineense*	1	Benin	T	H		Sources of proteins, carbohydrates and amino acids.
Marvel of Peru or four o'clock flower#	*Mirabilis jalapa*	1	India	S		Lv, Sd, Sh, St	Treating ulcers, diarrhoea, boils, stomach-ache, boils, skin diseases and asthma. It is an anti-syphilitic, anti-bacterial and a vermifuge
Chocolate weed	*Melochia corchorifolia*	1	India	S	H	Sd, Lv, St	diuretic, purgative, and for vulnerary, aphrodisiac, reduce inflammation. For candida, chagas disease,

							colic, constipation, contusions, diarrhoea, dysentery, earache, oedema, eczema, freckles, herpes, hives, itch, intestinal parasites, liver problems, pain, skin problems, skin infections, syphilis, vaginal discharge, urinary insufficiency, wounds, worms
Cannabis	*Cannabis sativa*	2	Canada	S	S	Sd, L	Cannabis is commonly used for long-term or severe pain, nausea caused by chemotherapy and painful muscle spasms. Cannabis is a good source of essential fatty acids, amino acids, dietary fibre, enzymes, vitamins, proteins, carbohydrates, water, fat, trace amounts of calcium, potassium, sodium, minerals, flavonoids, carotenoids, terpenes and phytocannabinoid acids.
Hibiscus or Roselle	*Hibiscus sabdariffa*	3	Egypt, India, Japan	S	H	Lv, F, Sd	Reduces inflammation treatment of pain, spasms, asthma, insomnia, depression, and loss of appetite
Cape Periwinkle; graveyard plant	*Catharanthus roseus* (L.)	1	Israel	S	H	Lv, Sh	Source of K, Mg, Mn, Fe, CHO, Zn, P and vitamin C. Used for the treatment of diabetes, indigestion, and blood pressure. It also has biotic functions.
Cassava	*Manihot esculenta*	1	Philippines	S	S	R, Tu, Lv, Sh	Treatment of high blood pressure, liver diseases, and fevers, as well as mild laxatives and anti-bacterial treatments. Rich in phytochemicals like polyphenols, especially anthocyanins, polysaccharides and organic acids. Source of manganese, copper, molybdenum and ascorbic acid.
Donkey berry	*Grewia flavescens Juss*	3	Niger, India	S	S	B Lv Fr	It can be used for wound cleaning, menstrual, and stomach problems. It is a good source of potassium, calcium, manganese, iron, copper, zinc, crude fibre, ash, and carbohydrates.
Carob	*Ceratonia siliqua*	1	South Africa	S	S	St, Lv, F, Pd, R	Treatment of Aphrodisiac, diarrhoea, cancer, heart disease, inflammation, microbial. Provide flavonoids, tannins, and steroids.

Cancer bush#	*Sutherlandia frutescens*	3	South Africa	S	S	St, Lv, F, Pd, R	Used to treat chickenpox, diabetes, cancer, menopausal symptoms, influenza, rheumatoid arthritis, peptic ulcers, anxiety, clinical depression, HIV infection and external wounds. Caner bush is amino acids, proline, and alanine.
Ethiopian eggplant	*Solanum aethiopicum*	1	Ghana	S	S	Fr	Treatment of cancer and diabetics.
African eggplant	*Solanum macrocarpon*	2	Ghana	S	S		Source of fats, carbohydrates, proteins, crude fibre, Ca, Na, P, Mg, Mn, K, Fe, Zn, Cu, Mo, vitamin A, Vitamin C. Treatment of diabetes, dysentery haemorrhoids, bowel movement and Blood pressure.
Bitter eggplant	*Solatium insanum, L*	1	Sri Lanka	S	S	Sd, Lv, R	Source of fats, CHO, proteins, C-fibre, Ca, Na, P, Mg, Mn, K, Fe, Zn, Cu, Mo and vitamin C.
Miracle fruit#	*Synsepalum dulcificum*	1	Africa	S	H	Fr	Source of carbohydrates, vitamin A, vitamin C and phenolics.
Cactus pear#	*Opuntia robusta*	2	South Africa	S	S	Fr	Used for type 2 diabetes, high cholesterol, obesity, alcohol hangover, colitis, diarrhoea, and benign prostatic hypertrophy (BPH). It is a good source of minerals, amino acids, vitamins C, E, K, and beta-carotene, flavonoids, and antioxidants.
Pea eggplant	*Solanum torvum*	2	India	S	S	R, Fr	Source of vitamin C, Leucine, flavonoids and anthocyanin. Treatment of diabetes and gastrointestinal conditions.
Quinoa	*Chenopodium quinoa*	1	China	P	H	Sd	It is rich in fibre, minerals, antioxidants, and all nine essential amino acids. Quinoa is a good source of protein, lipids, ash, crude fibre, carbohydrate, and energy.
Buck wheat	*Fagopyrum esculentum*	3		P	H	Sd, F	Treatment of bacterial diseases

Amaranth	*Amaranthus spinosus*	1	India	P	H	Lv, Sh	Amaranth is used to treat diarrhoea, ulcers, and a swollen mouth and throat. The leafy vegetable is rich in fibre, protein, calories, protein, carbohydrates, fat, manganese, magnesium, phosphorus, iron, selenium, copper
Amaranth	*Amaranthus tricolor*	1	India	P	H	Lv, Sh	Treatment of eczema. It is a source of flavonoids, steroids, lipids, carbohydrates, crude fibre, amino acids, minerals, protein, ash, B carotene and phenolics
Elephant foot yam	*Amorphophalus campanulatus*	1	India	RT	H	Tu	Source of crude fat, crude protein, crude fibre, Calcium, Manganese, iron, zinc, copper, Vitamin A, B-carotene, foliate, lysine and methionine. Used as a diuretic.
Up yam	*Dioscorea bulbiferaa*	1	India	RT	H	Tu	Source of calcium, manganese, phosphate, iron, zinc, copper, Vitamin A, B-carotene and foliate. Used for the treatment of eczema and inflammation.
Lesser yam	*Dioscorea esculanta*	2	India, Philippines	RT	H	Tu, St	Lesser yam is used to treat piles, dysentery, syphilis, ulcers, leprosy, diabetes, asthma, cough, and cancer. Nutritional composition includes protein, crude fibre, ash, and fat.
Taro	*Colocasia esculenta*	5	India, Benin, Philippines, Tanzania	RT	H	Cm, Lv, Sh, St	Treatment of diarrhoea, helps control blood sugar, reduces the risk of heart disease, weight loss, and has anti-cancer properties. Good source of protein, carbohydrates, fat, fibre, vitamins, potassium, folate, calcium, magnesium, phosphorus, and iron
Greater yam	*Dioscorea alata*	2	India, Philippines	RT	H	Tu	Treatment of ulcers, boosts brain health, reduces inflammation, and improves blood sugar control. Source of carbohydrate, vitamin B6, copper, manganese, potassium.
Yam	*Dioscorea dumetorum*	4	Benin, India	RT	Cl	Tu	Rich in Vitamin C as well as starch. It contains calcium, phosphorous, thiamine, riboflavin, niacin, oxalic acid, calcium oxalate, sapotoxin and flavones, apigenin and luteolin. Has Anti-microbial, Anti-hepatotoxic, Anti-

							cancer, Anti-Lipidperoxidative, Anti-bacterial and Anti-fungal, Anti-diabetic, Anti-melanogenic, Anthelmintic, Anti-microbial, Anti-hepatotoxic, Anti-cancer, Anti-Lipidperoxidative, anti-bacterial and Anti-fungal, Anti-diabetic, Anti-melanogenic, Anthelmintic properties
Sweet potato	*Ipomea batatas*	1	Benin	RT	Cr		Sweet potato may be used to promote gut health, treat cancer and vision, and support the immune system and brain function. Great source of fibre, minerals and vitamins.
Giant taro	*Alocasia macrorrhiza*	1	Philippines	RT	H	St, Lv	It provides moisture, crude fat, protein, ash, crude fibre, minerals, calcium, sodium, potassium, magnesium, manganese, phosphate, iron, zinc, copper, and phenolics. It treats cancer, heart disease, diabetes, dysentery, inflammation, gonorrhoea, haemorrhages, hypertension, and helminths. It is a source of tannins and flavonoids.
Ethiopian potato	*Plectranthus edulis*	1	Ethiopia	RT	H	Tu	Used to treat urinary disorders, including bladder infection (cystitis), prostatic hyperplasia, prostate cancer, lung diseases, and other cancers. It contains fat, fibre, sodium, potassium, carbohydrates, vitamins, minerals, and other essential nutrients.
Wild ginger	*Siphonochilus aethiopicus*	1	South Africa	RT	H		Used to treat intestinal ailments and relieve stomach aches and cramps. Reduces stress, pain, and anxiety. Contains fat, sodium, carbohydrates, sugars, protein, and calories.
Tannia	*Xanthosoma sagittifolium*	3	India, Ghana, Philippines	RT	H	Cm, St, Lv, Tu, R	Source of moisture, fats, crude protein, protein, crude fibre, minerals, sodium, potassium, manganese, phosphates, zinc, iron, copper, lysine, methionine, histidine, and Isoleucine.

White seed melon	*Cucumeropsis manni*	1	Benin	Cu	Cr	Sd, Fr	Juice from the fruit is mixed with other ingredients to treat the cord-relic of newborn babies until it drops off. Source of carbohydrates, proteins, essential amino acids, fatty acids, minerals, and vitamins
Watermelon	*Citrullus lanatus*	1	Benin	Cu	Cr	Sd, Fr	Used to treat urinary tract infections, alcohol poisoning, hypertension, diabetes, gonorrhoea, and diarrhoea. It is a good source of copper, vitamin B5, lycopene, and vitamin C.
Bottle gourd	*Lagenaria siceraria*	1	China	Cu	Cr		It is a source of crude fats, moisture, proteins, carbohydrates, energy, amino acids, minerals and Vitamin A.
Bitter gourd	*Momordica charanti-a*	2	India, South Africa	Cu	Cr	Fr	It provides proteins, potassium, iron, and fibre. It is used in the treatment of cancer and as an aphrodisiac
Wax gourd	*Benincasa hispida*	1	India	Cu	Cr		Source of proteins, fibre, amino acids, ascorbic acid and B carotene
Bitter melon	*Momordica charanti-a*	1	Korea	Cu	Cr	Fr	It is used to fight cancer, diabetes, and many infectious diseases and treat eye-related diseases. Contains calories, fat, sodium, carbohydrates, fibre, sugar, and protein.
Pumpkin	*Cua pepo var. styriaca*	1	Iran	Cu	Cr	Sd, R, F	Source of vitamin A, B carotene, Fat, Protein, Crude fibre, Minerals, Calcium, sodium, potassium, magnesium, phosphates, iron, and copper. Assists with bowel movement and treatment of helminths, liver complaints and intestinal diseases.
Millet	Millet	1	India	C	H		Control of blood sugar and improve digestive health. It is a good source of vitamins, phosphorus, potassium, antioxidants, niacin, calcium, and iron.
Napier grass	*Pennisetum purpureum* (Schumach)	1	Africa	C	H		Treatment of cancer and viral diseases. Reduces inflammation and

Pearl millet	*Pennisetum glaucum*	2	Benin, Kenya	C	H	Sd	Treat iron deficiency anaemia, reduces blood sugar levels, and aid in weight loss and microbial actions. Good source of energy, moisture, protein, fat, mineral, fibre, carbohydrate, calcium, phosphorus and iron.
Barley	*Hordeum vulgare*	1	Himalaya	C	H	Sd	Sources of Na, P, Mg, Mn, K, Fe, Zn, Cu, Mo, Ca, and oleic, palmitic and stearic acids.
Proso millet	*Panicum miliaceum*	1	Himalaya	C	H	Sd	Source of moisture, fat, protein, ash, carbohydrate, amino acid and Vitamin A
Fonio millet	*Digitaria exilis*	1	Benin	C	H		The amino acid methionine is important for the body's cartilage production. It helps strengthen nails and hair. Fonio millet is a good source of thiamine niacin, riboflavin carbohydrates, protein, fat, fibre, and iron.
Foxtail millet	*Setaria italic*	1	Himalaya	C	H	Sd	It is good for cardiac health, regulates blood sugar levels, lowers blood cholesterol, and improves digestion and immunity.
Finger millet	*Eleusine coracana* (L.)	4	India, USA, Canada	C	H	Sd, Lv	Source of protein, carbohydrates, crude fibre, energy, thiamine, riboflavin, niacin, phenylalanine, threonine, valine, leucine and isoleucine
Sorghum	Sorghum bicolor	2	Benin	C	H		Source of moisture, fat, protein, ash, carbohydrate, amino acid and Vitamin A, Source of phenolics
Maize	*Zea mays* L.	3	Philippines, Iran, Vietnam	C	H	Sh, Sd	Source of moisture, fat, protein, ash, carbohydrate, amino
Scarlet pimpernel	*Chenopodium album*	1	Egypt	H	H		Source of phenolics: Treat iron deficiency anaemia, reduce blood sugar levels, and microbial actions.
Bush tea	*Athrixia phylicoides DC.*	5	South Africa	H	S	Lv	cleansing or purifying the blood, treating boils, headaches, infected wounds, and cuts, and the solution may also be used as a foam bath. Treatment of various ailments such as boils, acne, colds, loss of voice, and throat infection as a gargle. Significantly

							high polyphenols, tannins, antioxidants, quercetin, flavonoids, alkaloids, polysaccharides, amino acids, lipids, vitamins, and inorganic elements
Sweet clover	Sweet clover	1	Egypt	H	H		Used to treat microbial infections and diabetes. It is a source of carbohydrates, sodium, potassium, magnesium, manganese, phosphates, iron, zinc, Mo, Vitamin A
Lemongrass	*Cymbopogon flexuosus*	3	India, Saudi Arabia,	H	H	Fr	Used to treat stomach and intestinal spasms, aches, high blood pressure, convulsions, pain, vomiting, cough, achy joints, and fever. Rich in minerals, various essential nutrients and vitamins.
Rapeseed/ Sarson	*Brassica napus* L.			H	H		Source of moisture, fat, CHO, protein, fibre, Fe, Zn, B carotene, lysine, phenolics and flavonoids.
Water hyssop	*Bacopa monnieri*	1	India	H	H	Lv	It contains powerful antioxidants, may reduce inflammation, boost brain function, reduce ADHD symptoms, and prevent anxiety and stress. Source of carbohydrates, fat, proteins, and minerals.
Safflower	*Carthamus tinctorius*	3	China, Pakistan	H	H	Sd, F	Used to traditionally treat painful joints, trauma, dysmenorrhea, amenorrhea, postpartum, and abdominal pain. The nutritional value includes copper, tryptophan, fat, vitamin B1, and phosphorus.
Fennel flower	*Nigella sativa L*	1	Iran	H	H	R, Sh	Treatment of diabetes and gastro-intestinal bowel movement. It is used as an aphrodisiac and diuretic and assists with indigestion
Ladies' fingers or Okra	*Abelmoschus esculentus*	1	Iran	H	H	Sd, R, St, Fr	An infusion of the root is used to treat syphilis, and the juice of the roots is used to treat cuts, wounds, and boils. It is a good source of calories, fats, sodium, potassium, carbohydrates, protein, dietary fibre, proteins, vitamins, and iron.
Plantain	*Plantago major*	1	Sweden	H	H	Lv	Helps with memory. Sources of carbohydrates. fibre, Ca, K, P, Fe, Cu, Vitamin A

Toothache plant	*Acmella oleracea*	1	Benin	H	H		Used to treat toothache, throat and gum infections, stomach, diuretic, dry mouth, and gastric ulcers. It is a good source of fat, carbohydrates, nitric oxide, and hydroxytoluene.
Creeping woodsorrel	*Oxalis corniculata*	1	India	H	H	Lv	Source of proteins, moisture, carbohydrates, minerals, Ca, Mg, Na, Mn, P, Fe, vitamin A, Vitamin C, B carotene, oleic and palmitic acid. Treatment of heart disease, cancer, inflammation, blood pressure, hypertension, ulcers, tannins and flavonoids.
Chinese water chestnut	*Eleocharis dulcis*	1	India	H	H	Tu	Source of fats and lipids, crude proteins, energy, crude fibre, calcium, manganese, sodium, potassium, iron, zinc, copper and vitamins A and C. It is used for the treatment of gastrointestinal problems, including dysentery. It is used as a diuretic and as a treatment for gonorrhoea and fever.
Lamb's quarters	*Chenopodium album*	3	India, Egypt	LV	H	Lv, Sh	Source of flavonoids. Treatment of inflammation, cardiovascular diseases, diarrhoea and helminths.
False sesame	*Ceratotheca sesamoides*	1	Benin	LV	H	Lv	Source of proteins, carbohydrates and amino acids.
Black sesame	*Sesamum radiatum*	1	Benin	LV	H	Sd, Lv	Sources of carbohydrates. fibre, Ca, K, P, Fe, Cu, Vitamin A, Vitamin C, ascorbic acid and B carotene
	Crassocephalum rubens	1	Benin	LV	H	Lv	Used to treat indigestion, upset stomach, headaches, epilepsy, fresh wounds, to stop nose bleeding, swollen lips and sleeping sickness. It is a good source of crude protein, lipids, ash, fibre, carbohydrates, and energy.
Spiderflower	*Cleome gynandra*	2	Benin	LV	H	Lv	Source of phenolics. Treatment of cancer, viral diseases, diabetes, hypertension, inflammatory conditions, ulcers and wound cleaning
Jute mallow	*Corchorus olitorius*	2	Benin, Ghana	LV	H	Lv, Sd	A good remedy for aches, pains, dysentery, enteritis, fever, pectoral pains, ascites, piles, and tumours. It is

							also a rich source of potassium, iron copper, manganese and zinc
Thickhead, redflower ragleaf	*Crassocephalum crepidioides*	1	Benin	LV	H	Lv	Source of proteins, iron and potassium
Wild mustard	*Brassica juncea*	1	India	LV	H		Source of moisture, fats, proteins, carbohydrates, fibre, minerals, Ca, Na, K, Mg, Mn, P, linoleic, oleic, Palmitic and stearic acid.
Kales	*Brassica oleracea*	3	Spain, Netherlands	LV	H	Lv	Source of proteins, lipids Cu, Fe, Zn, Na, Zn, Mg, Mn and vitamin C. Used for treating diabetes, inflammation, hypertension, Malaria, liver complaints, helminths, hepatic insufficiency and has biotic functions.
Common dandelion	*Taraxacum officinale F. H. Wigg*	1	Iran	LV	H	R, Sh	Source of moisture, fat, protein, carbohydrates, crude fibre, energy, calcium, potassium, iron, ascorbic acid, phosphorus, copper. Used in treating inflammation, blood pressure, lactogenic cancer, viral diseases and haemorrhoids. It is used as an aphrodisiac.
Purslane	*Portulaca oleracea*	1	NA	LV	H	Sd	It can be used as a febrifuge, anti-septic, or vermifuge. Has a good content of sodium, potassium, carbohydrates, protein, and vitamin C.
Hyacinthus[#]	*Hyacinthaceae*	2	India	LV	Cr	R, Fr, Sh	Used to treat rheumatism, cardiac, urinary infection, dermatological problems, stomach, haemorrhoid, and prostate disease. Hyacinthus is a good source of crude lipids, ash, fibre, proteins, minerals, potassium, and sodium

From the review of the ten articles, it was observed that, in many instances, research articles on NUS often insinuate nutraceutical and pharmaceutical benefits without mentioning the actual attribute. However, research articles expanded on the phytochemical characteristics that allow nutraceutical and pharmaceutical benefits later in the main text. Therefore, it was important to include *nutraceutical, pharmacological, phytochemical, pharmaceutical, and medicinal*. Overall, the main objective was to articulate the nutraceutical and pharmaceutical attributes of NUFMS. Within the ten articles, we identified the following attributes: *antifungal, anti-bacterial, anti-viral, anti-mutagenic, antihepatotoxic, anti-inflammatory, antihistaminic, anti-immuno-modulatory, anti-hypolipidemic, anti-diabetic, anti-convulsant, anti-carcinogenic, anti-hypolipidemic, anti-acetylcholinesterase, anti-neuropathic, anti-hypertensive, anti-analgesic, anti-helminths, anti-malaria, lactogenic, aphrodisiaque, diuretic, hepatoprotective, hypotensive, carminative*. We also observed that these terms have variations, e.g. anti-fungal can be written as “anti-fungal” or “anti-fungal”. These variants were also included in the search string. In the selected ten articles, it was also observed that several terms had been used to refer to neglected and underutilised crops. Using some of the identified terms and incorporating those obtained from expert knowledge, we came up with the following: *indigenous, neglected, traditional, orphan, native, underutilis(z)ed, future, and medicinal crops terms* were also used as keywords.

To address RQ 1 and 2, searches were conducted using the above-stated keywords and search combinations outlined in Table 2.1.

2.2.2 Data mining, analysis and presentation

The second stage was conducting the review to identify priority NUFMS. When conducting the literature search, the cluster of key terms was systematically used in each database, and these were later combined to come up with a list of articles. The lists from the different databases were concocted, and duplicates were removed (Supplementary Table 1 and Figure 2.1).

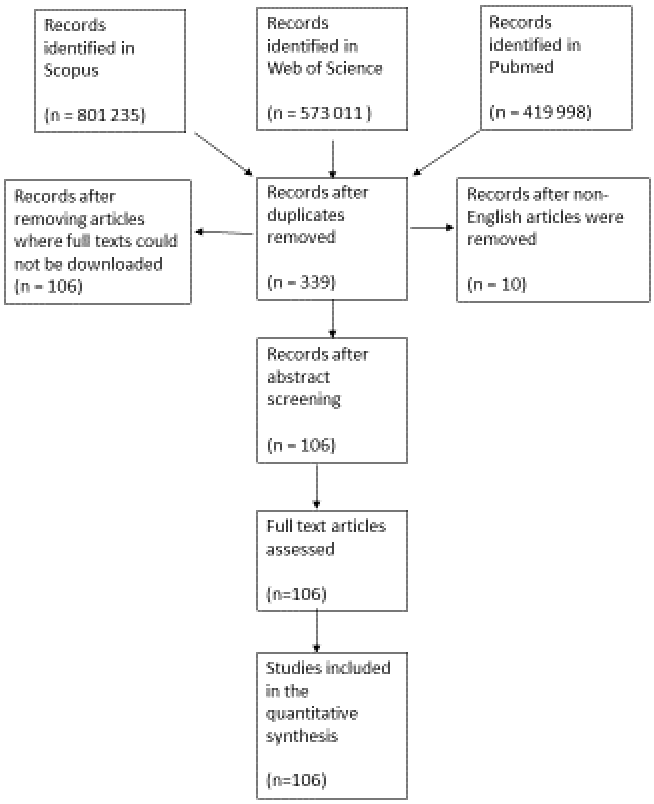

Figure 2.1: PRISMA diagram of the selected articles for the literature synthesis

The list of articles was exported to Excel, where data on authors, year of publication, type of publication, crop species and type, nutraceutical and pharmacological characteristics, phytochemical properties and any other information to help answer the research questions were extracted and stored. Articles excluded from the final list were those discussing crop species not found in Africa, whose focus was very broad and not on nutraceutical and pharmacological properties of neglected and underutilised crop species and were not available in English. After all, the relevant articles were left, and necessary data was extracted, and the data was subjected to bibliometric analysis. Bibliometric analysis is a quantitative method used to assess published articles and has become helpful in evaluating peer-reviewed studies in a specific field of research (Lozano et al., 2019; Small, 1973). The evolutionary trends were inferred from statistically assessing the occurrence and co-occurrence of key terms used to map trends in NUFMS using VOSviewer software. The titles and abstracts of articles in the final database (with 105 articles in Table 2.2) were used in the VOSviewer to investigate how concepts and topics have evolved over the years.

Table 2.2: Identified key experts and their role in the commercialisation process of medicinal plants

Expertise	**Role**
Agronomist	To improve techniques for the cultivation of medicinal plants
Conservation campaigners	To persuade the public of the need to conserve medicinal plants
Ecologists	To understand the ecosystem in which medicinal plants grow
Ethnobotanists	To identify the use of plants as medicines in traditional science
Health policy-makers	To include conservation and utilisation of medicinal plants in their policy and planning
Horticulturists	To cultivate medicinal plants
Legal experts	To develop effective legal mechanisms that ensure that the collection of medicinal plants is at sustainable levels
Manufacturers	To develop processed products and by-products such as pills, lotions, ointments
Nurseries	To produce and supply propagules and seedlings of medicinal plants
Pharmacognosists	To study the application of medicinal plants
Plant breeders	To breed improved strains of medicinal plants for cultivation
Plant genetic resources specialist	To assess and map the genetic variation in medicinal plants and maintain seed banks of medicinal plants
Plant pathologist	To protect the cultivated medicinal plants from pests and diseases without using dangerous chemicals
Seed biologists	To understand the germination and storage requirements of the seeds of different medicinal plants

Taxonomist	To identify medicinal plants accurately
Traditional health practitioners	To provide information on the use and availability of medicinal pants

2.3 Results and discussion

2.3.1 Conceptual definitions of key terms

According to Blum (2016), “correct definitions and use of terms are not just a matter of formality but are essential for emerging research.” Consensus in the use of terms and definitions determines the success or failure of research, and the lack of it undermines attempts to reproduce the specific outputs, hence outcomes (Blum, 2016). Currently, there is an array of terms used to define the medicinal properties of crops, each with a different meaning and context, and this has tended to work against NUFMS. In this section, we define and explain key terms that have been used to describe.

Medicinal plants

A medicinal plant contains active compounds or therapeutic properties in one or more of its organs, which can be pharmacologically beneficial to the human body (Rasool, 2012; Namdeo, 2018). Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Medicinal plants are widely used in non-industrialised societies, mainly because they are cheaper than modern medicines. Currently, the science of medicinal plants has been recognised as ‘alternative medicine’ where synergetic effects and preventative properties have been studied (Rasool, 2012).

Functional food crops

Functional foods are defined as “a food category in which the products are either modified or fortified with substances that have a preventive or therapeutic effect beyond their original nutritional value” (Jonas and Beckmann, 1998). Functional foods can also be defined as “foods that are similar in appearance to a conventional food and possess physiological benefits or reduce the risk of chronic disease beyond basic nutritional functions" (Sawahla, 2014). Functional foods should be consumed at sufficient levels to positively impact the body (Vattem and Maitin, 2015).

Neglected and underutilised crops

Neglected and underutilised crop species (NUS) have been used to refer to crop species that were primarily grown in their native communities but are currently losing their popularity (Padulosi et al., 2002). These crops have significant potential as food and industrial crops but are marginalised, if not entirely sidelined, by researchers, breeders, policymakers, producers and traders (Mabhaudhi et al., 2017). Consequently, they have poorly developed and understood value chains. They are non-commodity crops and belong to a large, biodiverse group of domesticated, semi-domesticated or wild species and, in most instances, are locally adapted (Padulosi et al., 2013). They are cultivated in traditional systems, mostly at the subsistence level, while using informal seed systems (Dansi et al., 2012). Due to the need to increase agricultural productivity, NUS have been receiving attention because of their potential to promote food security.

Dietary supplement

Dietary supplements are another major nutraceutical class that includes concentrated food-derived nutrients (Phillips and Rimmer, 2013). Dietary supplements are not intended to replace food but are designed to add added nutrients or perceived health benefits to daily food consumption.

Pharmaceuticals

Pharmaceuticals are therapeutic and biologically active substances used to treat diseases (Taylor, 2015). They are structures that makeup drugs that can be complex or simple aromatic molecules (Taylor, 2015). Pharmaceuticals can be packaged as capsules, tablets, liquid or gel, and the quantity taken should be within a prescribed limit; otherwise, effectiveness will be diminished.

Nutraceuticals

Nutraceuticals refer to the science dealing with bioactive plant-based compounds that are isolated, packaged to be distributed in medicinal form and consumed to alter or maintain normal body functions (Das et al., 2012; Verma et al., 2017). The term also includes whole plants providing nutritional supplementation, such as honeybush tea, which provides antioxidants. The term ‘nutraceutical' was derived from 'nutrition' and 'pharmaceutical'. Nutraceuticals can be used as part of the diet to prevent or treat diseases. Some countries have not adopted the term 'nutraceutical'; hence, other terminologies are used to describe them in that regard. For example, in the United States of America, they are labelled as 'dietary supplements' while in India, they are referred to as 'foods for special dietary use'. In South Africa, the term 'nutraceuticals' is not recognised in its drug-control law; hence, it is referred to as 'complementary medicine' and is regulated by the South African Health Products Regulatory Authority (SAHPRA).

2.3.2 Characteristics of searched literature

2.3.2.1 Diversity of functional medicinal NUCs

The NUFMS in this study were of nutritional or medicinal significance and were being used to treat specific ailments within communities. The study listed 226 plants belonging to 105 medicinal species sampled worldwide (Table 2.1), but found in South Africa. The medicinal plants were pooled and were of use in 29 countries. The regions represented by the sampled articles included Western Africa (32%), Northern Africa (4%), Eastern Africa (2%), Southern Africa (6%), Asia (47%), North America (3%), South America (1%), Europe (4%) and Middle East (1%). The plants included legumes (16), pseudocereals (4), cereals (9), cucurbit (7), herbs (9), leafy vegetables (10), roots and tubers (10), shrubs (14) and trees (9). The plants presented a variety of growth behaviours, and the parts also utilised varied.

From the literature review results, South African research has focused on seven of the 105 neglected and underutilised functional medicinal crops. These include honeybush (*Cyclopia*), drumstick (*Moringa oleifera*), carob (*Ceratonia silique*), cancer bush

(*Sutherlandia frutescens*), wild ginger (*Siphonochilus aethiopicus*), bitter gourd (*Momordica charantia* L) and cactus pear (*Opuntia robusta*). Among these, the honeybush was the most frequently cited plant, appearing in five of the 105 articles of the sampled pool. Collectively, the plants had medicinal uses as anti-oxidants, anti-inflammatory and anti-cancer properties. Other ailments and conditions treated included arthritis, diabetes, helminthiasis, liver problems and fever. Nutritionally, the plants were utilised as appetite stimulants and a source of minerals. The plants grew as legumes (honeybush), shrubs (carob, cancer bush, cactus pear), root and tuber (wild ginger), tree (drumstick) and cucurbit (bitter gourd) (Table 2.1).

2.3.2.2 Main research themes coming out of the literature

In evaluating the sourced literature on NUMFS, results (Figures 2.2 and 2.3) showed that research into nutraceutical and pharmaceutical properties started trending globally in 2012, mostly in China and Southeast Asia.

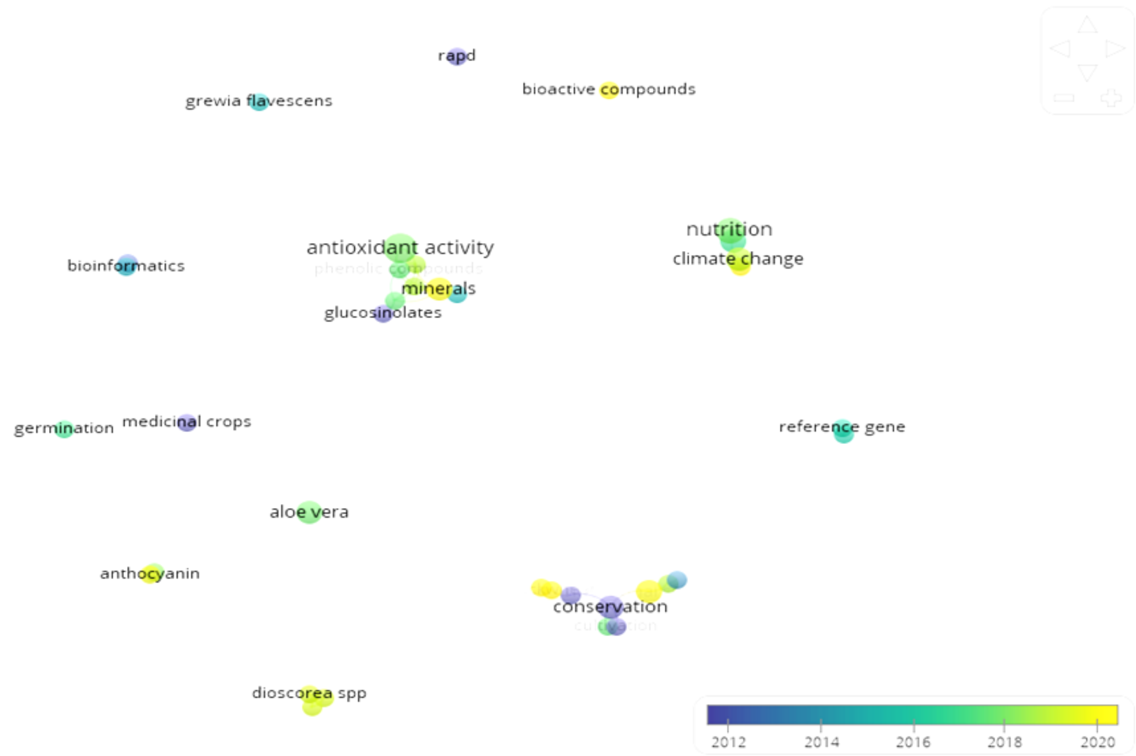

Figure 2.2: Direction and revolution of topical concepts on neglected and underutilised functional medicinal crops (NUFMS) derived using data from titles and abstracts in VosViewer

During this period, the emphasis was placed on conservation issues of many of these underutilised crops. From 2014 to 2015, we witnessed a shift from conservation issues to outlining the potential benefits of these crops (Figure 2.3).

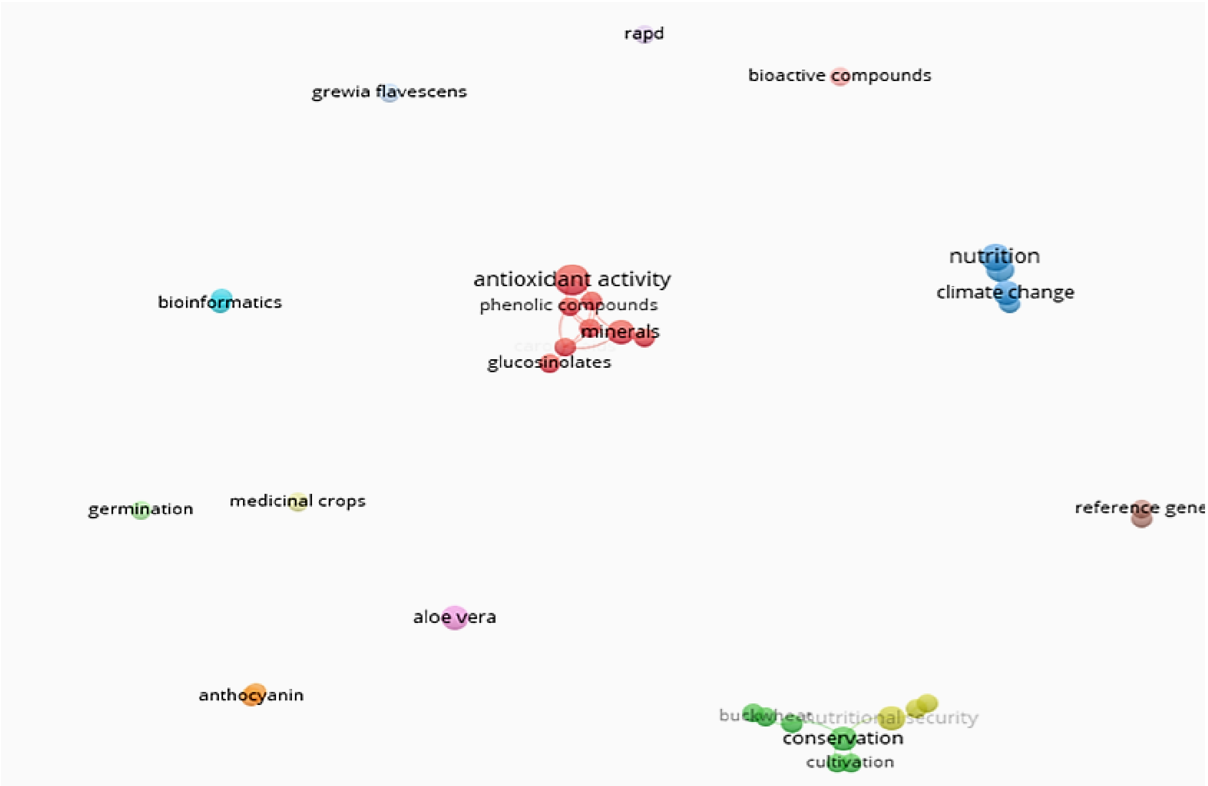

Figure 2.3: Topical concepts on neglected and underutilised functional medicinal crops (NUFMS) derived using data from titles and abstracts in VosViewer

More recently, studies have focused on understanding the mechanisms that confer the purported benefits. A closer look at the keywords shows three main thematic areas coming out of the source literature (Figure 2.2 and 2.3).

The first thematic area, denoted by the red cluster, comprised terms that highlighted the biochemical properties of NUFMS (Supplementary Table 1). The thematic area represented the largest cluster; the keyword antioxidant activity had the highest citation (Figure 2.3). The compounds in this cluster associated with the anti-oxidant properties of NUFMS include phenolic compounds, minerals, carotenoids, glucosinolates and proteins. Glucosinolates and proteins are located on the cluster's periphery, indicating their role as indirect anti-oxidants. Glucosinolates stimulate natural anti-oxidant systems in the body, while proteins work by inhibiting lipid oxidation. Purslane and amaranth were the only NUFMS frequently cited in the pool of research sampled for the network mapping.

The second thematic area donated by the green cluster showed that research is still focusing on the sustainable use and production of NUFMS. The keyword 'conservation' was mostly cited in articles that fell into this cluster, followed by tissue culture (Figure 2.3 and Supplementary Table 1). Tissue culture has been used to clone desirable NUFMS genomes, including buckwheat. Buckwheat is one of the most genetically diverse NUFMS, with 10,000 concessions currently stored in various germ banks globally (Chauhan et al., 2010). In addition, its popularity has increased due to the diversity of properties, which has enabled it to be used for nutritional security, medicinal purposes, industrial use, and as a cash crop. Such research efforts are being made to document the NUFMS in different world regions, one of the crucial and basic steps in their developmental journey. For instance, tissue culture technology has opened extensive research areas for micro-propagation, secondary metabolite production and biodiversity conservation for medicinal and endangered crop species (Tasheva and Kosturkova, 2012). Other biotechnological tools, such as cryopreservation and genetic transformation, are important for selecting, multiplying, and conserving the critical genotypes of medicinal plants. Cryopreservation is a long-term conservation method in liquid nitrogen and allows for the conservation of endangered medicinal plants (Wang et al., 2021). Genetic transformation may be a powerful tool for enhancing the productivity of novel secondary metabolites (Narayani and Srivastava, 2017). Genetic improvement of NUFMS is also currently underway (Jayaramachandran et al., 2020). Efforts are being made to genetically engineer Sesame (*Sesamum indicum* L.) to improve its productivity through mutagenesis (Jayaramachandran et al., 2020). Sesame production has been scarred by low productivity, and the genetically modified mutant produced a larger seed weight and higher seed yield. DNA technology has also been used to ascertain, confirm and trace plant origins, origins and phylogenetic associations. Barcoding using DNA has been used to ascertain the genealogy of some legumes, such as lablab [*Lablab purpureus*

(L.) Sweet], marama bean [*Tylosema esculentum* (Burch.) Schreiber] and cowpea [*Vigna unguiculata* (L.) Walp] (Popoola et al., 2019).

The blue cluster, which represents the third thematic area, is associated with the role of NUFMS in food and nutritional security. The keywords in this cluster included nutrition, food security, climate change and intercropping (Figure 2.3 and Supplementary Table 1). The research in this cluster indicates a response to the impacts of climate change on food production systems to ensure food and nutritional security. Intercropping, therefore, has been suggested as an intervention. According to Mabhaudhi et al. (2017), a key strategy to adapt to a changing climate is developing and promoting underutilised crop species (in our case, NUFMS). Exploiting the large reservoir of minor and underutilised crop plants would provide a more diversified agricultural system and food sources necessary to address food and nutrition security concerns in the face of climate change. Furthermore, there is vast potential for these crops to increase agricultural diversification and minimise environmental degradation; this has a direct impact on climate adaptation and mitigation (Mabhaudhi et al., 2017; Beddington et al., 2011).

The rest of the clustering was species-dependent (e.g. Dioscorea and bioactive compounds; safflower and gene sequencing). The studies falling into these clusters assessed (quantitatively and qualitatively) the properties of NUFMS using biotechniques (Figure 2.3 and Supplementary Table 1).

2.3.2.3 Identified crop types and their distribution

Legumes

The most cited leguminous plants included honeybush tea (*Cyclopia spp*), velvet bean (*Mucuna pruriens*) and pigeon pea (*Cajanus cajan*). They are mostly utilised in India and Africa, growing as climbers or shrubs. Seeds are the most used parts in 60% of the legumes listed in this study. The legumes were used as dual-purpose plants to provide nutritional elements and medicinal use. Legumes such as Tigernut (*Cyperus esculentus*), Bambara groundnut (*Vigna subterranean*), grass pea (*Lathyrus sativus L*), broad bean (*Vicia faba*) have been cited in the treatment of cardiovascular diseases, cancer, and diabetes in addition to being a source of nutritional compounds.

Additional properties were reported in honeybush, where an increase in appetite, reduction in digestive problems, and relief for arthritis have been noticed in patients taking the tea. The elements provided by legumes included crude fibre, crude fat, various amino acids, and mineral elements such as iron, calcium, magnesium, and phosphorus. Only ground beans (*Macrotyloma geocarpum*) and pigeon peas (*Cajanus cajan*) were singularly used to provide nutrients (Table 2.1).

Cucurbit

Cucurbits were popular in Asia (Korea, India, Iran, and China) and Africa (South Africa and Benin). Most plants in this category are creepers and have a dual function of providing nutrients and treating diseases, except for *Cucumeropsis manni*, bottle gourd (*Lagenaria siceraria*), and pumpkins (*Cucurbita pepo*), which provide nutritional compounds only. Their seeds and fruits provide nutrients such as vitamins, carbohydrates, fibre and minerals. The ailments treated by the listed cucurbits include bowel movements, helminthes, cancer, and liver disease, and cancer (Table 2.1).

Leafy vegetables

Leafy vegetables are mostly utilised in Africa (Benin, Ghana), Europe (Spain and the Netherlands), and Asia (India and Iran). Lamb's quarters (*Chenopodium album*) and kale (*Brassica oleracea*) were the most frequently cited plants among other leafy vegetables. The leaves were primarily consumed, while in the case of common dandelion (*Taraxacum officinale*), jute mallow (*Corchorus olitorius*), black sesame (*Sesamum radiatum*), lamb's quarters' seeds, roots and shoots were utilised (Table 2.1). They have been listed as sources of B-carotene, phenolics, carbohydrates, fatty acids and minerals. The legumes were collectively used to treat diabetes, inflammation, hypertension, malaria, liver problems, helminths, cancer, haemorrhoids and indigestion. The Spider flower (*Cleome gynandra*) is particularly used as an aphrodisiac.

Cereals

This group is mostly associated with grasses, and their seeds are consumed primarily to provide carbohydrates. In addition, the seeds have been explored for containing lysine, minerals such as sodium, potassium, magnesium, manganese, phosphates, iron, zinc, vitamins A and B, phenolics and flavonoids (Table 2.1). The listed cereals were rarely used for medicinal purposes except for finger millet (*Eleusine coracana*), which treat some microbial infections and diabetes. In maize (*Zea mays*) and finger millet, shoots and leaves are consumed and their seeds. Cereals were reported in Africa (Benin and Kenya), Asia (India, Philippines, Iran, Vietnam) and America (Canada and USA). Maize and finger millet were the most frequently cited plants in the group.

Pseudocereals

Six articles mentioned the use of pseudocereals as NUFMS in India (2 articles), China (1 article) and unspecified geographical locations (3 articles). Buckwheat (*Fagopyrum esculentum*) and amaranth (*Amaranthus tricolor*) were used medicinally to treat eczema, while *Amaranthus spinosus* (L.) was a diuretic. Nutritionally, pseudocereals were sources of flavonoids, steroids, lipids, carbohydrates, crude fibre, amino acids, minerals, protein, ash, β-carotene and phenolics. Parts utilised included seeds, leaves, flowers and shoots.

Roots and tubers

The geographical distribution of roots and tubers cited in articles selected for the study was confined to Asia (India and the Philippines) and Africa (Ethiopia, Benin, South Africa, and Tanzania). The most popular plant in the group was Taro (*Colocasia esculenta*), mentioned in five articles (Table 2.1). Tubers were mostly consumed, while stems, leaves, shoots, and corm were used in crops such as taro. Medicinal functions included use in the treatment of diarrhoea (*Dioscorea bulbifera*), ulcers (*Dioscorea esculenta*), kidney stones and appendicitis (*Colocasia esculenta*). The yam (*Dioscorea dumetorum*) has been used to treat or relieve many ailments associated with cancer, heart disease, diabetes, dysentery, inflammation, gonorrhoea, haemorrhages, and hypertension helminths. Nutritional contributions of roots and tubers included carbohydrates, crude fat, protein, crude fibre, minerals and phenolics (Table 2.1).

Shrubs

Another popular class of NUFMS was made up of plants classified as shrubs, being cited in 25 articles. The plants were identified in Africa (South Africa, Ghana, Sri Lanka, Niger) and Asia (India, China, Philippines). Dual-purpose species used for both medicinal and nutritive purposes included donkey berry (*Grewia flavescens Juss*), Ethiopian eggplant (*Solanum aethiopicum*) and miracle fruit (*Synsepalum dulcificum*) (Table 2.1). Shrubs used for the sole purpose of treating ailments included Cannabis (*Cannabis sativa*) for inflammation; cancer bush (*Sutherlandia frutescens*) for cancer and diabetes; pea eggplant (*Solanum torvum*) for curbing bacterial diseases, and Cassava (*Manihot esculenta*) was used externally to clean wounds. Shrubs used to provide nutrition only included Hibiscus or Roselle (*Hibiscus sabdariffa*), which is a source of manganese, copper, molybdenum and ascorbic acid; African eggplant (*Solanum macrocarpon*) for provision of fats, carbohydrates, proteins, crude fibre, minerals and vitamin c; bitter eggplant (*Solatium insanum,* L) for provision of carbohydrates, vitamin A, vitamin C and phenolics (Table 2.1).

Trees

Tree species considered were from many regions, particularly West Africa (7), Southern Africa (2), Asia (4) and Southern Europe (1) (Table 2.1). The group is home to drumstick (Moringa oleifera), cited most frequently (7 articles) among all the plant species considered in this study. A wide variety of plant parts were used, and of note was the baobab (*Adansonia digitate*), which had eight parts that were utilised for medicinal and nutritional purposes. These parts included fruits, seeds, roots, bark, stem, leaves, flowers and shoots (Table 2.1). Trees were used to treat diseases and conditions such as cancer, blood pressure, ulcers, inflammation, diabetes, diarrhoea, malaria and fever. Nutritional contribution by this group of plants included the provision of fats, carbohydrates, proteins, crude fibre, minerals and vitamins.

Herbs

Plants classified as herbs were mentioned in articles mainly from Asia. Fruits, seeds, leaves, flowers and roots were used to utilise their medicinal and nutritional properties. Most herbs listed worked exclusively as either medicinal or nutritional. Medicinal herbs included lemongrass (*Cymbopogon flexuosus*) and bush tea (*Athrixia phylicoides* DC.) for diabetes, improving bowel movements, enhancing sexual potency and as a diuretic substance and water hyssop (*Bacopa monnieri*) to enhance memory. Herbs used for nutritional purposes were fennel flower (*Nigella sativa* L.) and ladies' fingers or okra (*Abelmoschus esculentus*). Dual-purpose herbs included safflower (*Carthamus tinctorius*), used as a source of flavonoids and for the treatment of diarrhoea, cardiovascular diseases, helminths and diarrhoea and plantain (*Plantago major*), used as a source of phenolics and for the treatment of cancer, viral diseases, diabetes, hypertension, inflammatory conditions, ulcers and wound cleaning (Table 2.1).

2.3.3 Chemical constituents of functional medicinal crops

Plants have been used for thousands of years to flavour and conserve food, treat health disorders, and prevent diseases. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various nutraceutical and pharmaceutical purposes. These active compounds also enhance their survival. These constituents have been identified as alkaloids (Varsha et al., 2013), glycosides (Firn, 2010), flavonoids (Varsha et al., 2013), phenolics (Puupponen-Pimiä et al., 2001), saponins (Vashist and Sharma, 2013), tannins (Varsha et al., 2013), and essential oils (Canales-Martinez et al., 2008), steroids (Madziga et al., 2010). In this section, we provide an overview of these constituents.

Alkaloids are found in one of the largest plants containing highly active nitrogenous molecules (Shakkarpude et al., 2020). Alkaloids have anti-cancer and immune-stimulant properties. Examples of alkaloids found in plants include caffeine, theobromine and theophylline, classified as purine alkaloids and act as stimulants that increase heart function, vasodilation, and metabolism (Anaya et al., 2006). Alkaloids are being used to synthesise drugs, including vincristine, a common drug for leukaemia, made from Madagascar periwinkle (*Catharanthus roseus*) (Barrales-Cureño et al., 2019; Shakkarpude et al., 2020).

Bitters: The plants in this group have a characteristic bitter taste that stimulates the digestive system, including the salivary glands (Shakkarpude et al., 2020). As a result, bitters are considered effective as appetite stimulants and ensure a well-functioning digestive system (Sahu et al., 2013). Examples of bitters are Aloes, wormwood and hops.

Cardiac Glycosides: The group comprises steroids that work directly on the cardiac muscle. They have been used traditionally as poison and placed on arrows' tips for hunting (Morsy, 2017). With advances in medical technology, drugs have been synthesised with the correct levels to treat heart diseases. In addition to cardiac muscle-stimulating properties, cardiac glycosides are diuretics that assist in draining fluids out of the body (Shakkarpude et al., 2020). Examples of cardiac glycosides include digitoxin, digoxin and convallotaxin. Cardiac glycosides are found in the families Apocynaceae and Asclepiadaceae (Shakkarpude et al., 2020).

Cyanogenic Glycosides: Some plants, such as the wild cherry and the elderberry, produce hydrogen cyanide as a defence mechanism that relaxes the muscle, and in high amounts, it can be highly poisonous to humans (Yulvianti and Zidorn, 2021). Some examples of cyanogenic glycosides include amygdalin, which can be isolated from bitter almonds (*Prunus dulcis* (Mill.). In wild cherry and elderberry, the compounds are used to pacify coughs (Shakkarpude et al., 2020).

Flavonoids: Flavonoids are broadly found in nature and comprise secondary polyphenol metabolites with a wide range of medicinal properties (Wang et al., 2018). More than 9000 types of flavonoids have been isolated from plants so far, and these are known to have 'anti-viral, anti-inflammatory, cardioprotective, anti-cancer, anti-ageing properties’ (Wang et al., 2018; Shakkarpude et al., 2020). Flavonoids can be found in various plants, including vegetables such as quercetin and kaempferol (Ngameni et al., 2013). Chalcones, flavones, and isoflavones can be found in the *Dorstenia angusticornis* (L.) and cashew plants (*A. occidentale* L.). Lemons also contain flavonoids and assist in strengthening the capillaries. They are responsible for colouring some plants characterised by red, blue, and purple pigments or yellow and white pigmentation on fruits and flowers (Ngameni et al., 2013; Shakkarpude et al., 2020).

Minerals: Minerals are acquired by plants from the soil and hence are found in many plants. Minerals are responsible for ensuring general health, including disease prevention. Intake of sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine in the diet reduces the risk of cardiovascular disease (Sanchez-Castillo et al., 1998). Trace elements are significant in ensuring human metabolic processes as components of proteins such as haemoprotein and haemoglobin (Imelouane et al., 2011). Calcium is responsible for healthy teeth and bones, while sodium and potassium act as regulators. The horsetail plant is known to assist in repairing connective tissue.

Phenols: Phenolic compounds have chemopreventative properties, which enable them to be anti-carcinogenic (Huang et al., 2009). Examples of phenolic compounds found in medicinal plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans and quinones. Apart from anti-cancer properties, phenolic compounds have anti-microbial properties that prevent infection (Shakkarpude et al., 2020). More than 8000 phenolic compounds have been isolated from medicinal plants, including wintergreen, mint and willow (Tungmunnithum et al., 2018).

Polysaccharides: Polyssachaccharides are macromolecules that exist as structural molecules in plants. They exist in plants as monosaccharides in the form of starch and cellulose. Cellulose and hemicellulose are insoluble in water and bulky, and hence, they assist in bowel movements. Other bioactivities of polysaccharides in medicinal plants include anti-tumour activity, anti-oxidant activity, anti-coagulant activity, anti-diabetic activity, radioprotection effect, anti-viral activity, hypolipidemic immunomodulatory (Wang et al., 2008; Xie et al., 2016).

Proanthocyanins are compounds closely related to tannins and flavonoids, which give plants their colouring. The compounds have anti-inflammatory, anti-oxidant and anti-cancer properties; hence, they are important in heart, eyes and feet health (Shakkarpude et al., 2020). Plants that contain large quantities of proanthocyanins include blackberries and hawthorn berries. The bioavailability of proanthocyanidins is primarily influenced by polymerization, which is rendered insignificant in the gastric tract (Golovinskaia and Wang 2021).

Saponins: Saponins are secondary metabolites, surface-active glycosides with a characteristic foaming ability (Shakkarpude et al., 2020). Steroidal and triterpenoid are the two types of naturally occurring saponins. Their benefits include lowering cholesterol levels, treating diarrhoea and having anti-microbial properties (Desai et al., 2009). Plants containing high levels of saponins include agave, wild yam, and several lily family members.

Tannins: Tannins are polyphenols of a high molecular weight converted to quinones when oxidised (Pereira et al., 2015). Their bitter taste is part of the protective mechanisms in plants to protect them from herbivores. They are, however, utilised in processing leather during the tanning stage. They have a binding effect on proteins, thereby creating a protective layer that prevents microbes' action (Pereira et al., 2015). They are highly concentrated in the bark, sap, fruits and leaves of plants such as oak bark and black catechu.

Vitamins: Vitamins are compounds with many uses and are required in small amounts to maintain a constant body environment. Antioxidant vitamins, particularly Vitamins A, C, and E, scavenge for free radicals in the body. Fruits and vegetables are sources of vitamins, while plants such as rose hips and sea buckthorn are rich in vitamins B, C and E. Ascorbate or vitamin C is also a cofactor for ascorbate peroxidase (Smith et al., 2007) and phylloquinone or vitamin K is involved in the electron transport chain.

Volatile oils: Volatile oils are highly complex oils extracted from plants and are used to produce essential oils. They contain more than 100 compounds (Shakkarpude et al., 2020). Essential oils have anti-microbial, anti-carcinogenic, anti-diabetic and anti-oxidant properties (Reddy, 2019). *Apiaceae, Lamiaceae, Myrtaceae, Poaceae*, and *Rutaceae* families are significant sources of essential oils.

Antioxidants are molecules that inhibit the production of free radicals in the body through oxidation. Compounds that act as antioxidants include flavonoids, phenolics, sterols, alkaloids, carotenoids and glucosinolates (Saranya et al., 2017). Medicinal plants such as turmeric, cinnamon, *Allium sativum, Zingiber officinale, Crocus sativus, Dodonaea viscosa, Barleria noctiflora, Anacardium occidentale, Datura fastuosa* and *Caesalpinia bonducella* are among many other plants that have anti-oxidant properties.

2.3.4 Pharmacological properties

Studies to determine medicinal plants' chemical profile and composition reveal the complexity and variety of compounds contributing to plants' various uses in treating numerous ailments, including life-threatening diseases such as HIV-AIDS, cancer, cardiovascular diseases, and diabetes. Sexually transmitted infections are among the most common reasons people use herbal medicines and visit traditional healers in South Africa. NUFMS plays an important role in providing nutrition and treating chronic diseases, especially in many minority communities and developing regions. Neglected and underutilised functional medicinal crop species exhibit various pharmaceutical properties that include but are not limited to anti-inflammatory, anti-spasmodic, anti-oxidative, anti-bacterial, anti-fungal, anti-cancer, anti-allergic, hypoglycemic, analgesic, immunomodulatory, anti-stress, anti-ulcerogenic, anti-hypertensive, CNS depressant, hepatoprotective, chemopreventive, radioprotective, anti-tumour, and antipyretic (Figure 2.4).

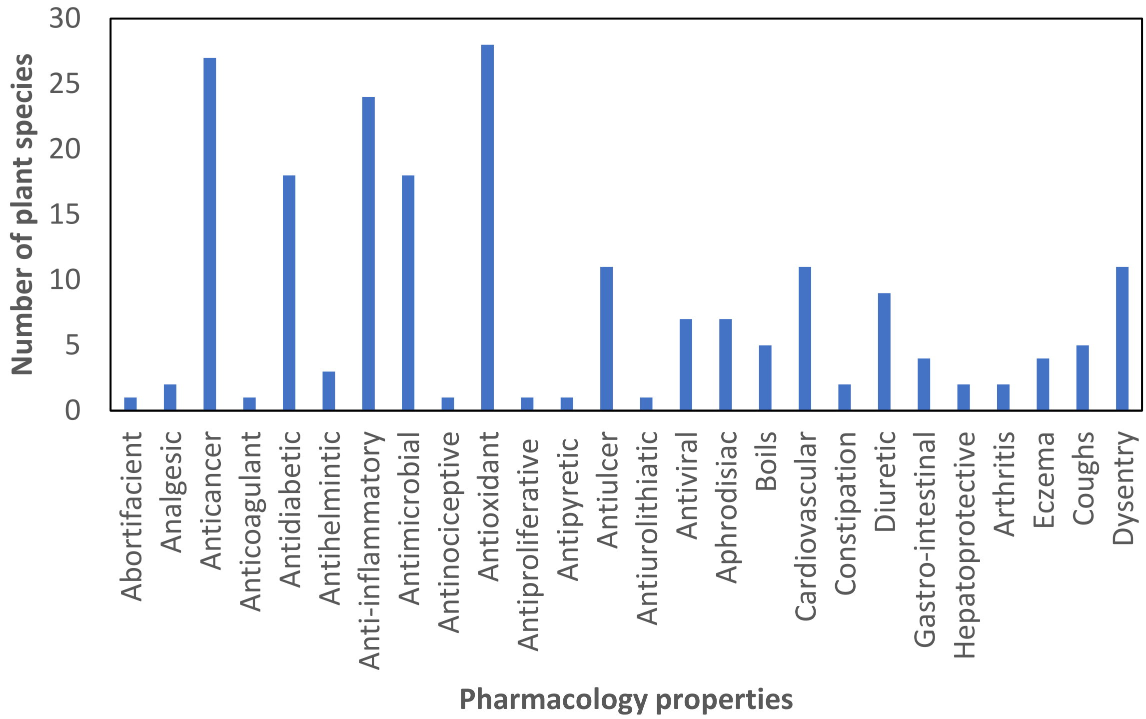

Figure 2.4: Total frequency of articles citing neglected and underutilised crops found in South Africa as compared to the rest of the world

The majority of the plant species identified (91.3%) had multiple pharmacological uses, with 45 species (42.4%) used in the treatment of five or more ailments, 18 species (16.5%) treating between five and three ailments, 15 species (11.5%) treating three or fewer ailments (Table 2.1). Gastrointestinal disorders, sexually transmitted infections, colds, cough and sore throat and gynaecological problems were treated with the most medicinal plant species (Figure 2.4). Gastrointestinal disorders, particularly cholera, diarrhoea, and dysentery, are a major concern in South Africa and the whole region (Ribeiro et al., 2010). Sexually transmitted infections are a major public health concern in developing countries, with their transmission rate regarded as one of the highest in the world (Van Vuuren and Naidoo, 2010).

2.4 Production strategy for neglected and underutilised functional medicinal crops

The NUFMS industry is considered an "uncharted" economy dominated by unsustainable informal production and market systems. In response to high population growth rates, rapid urbanisation and the important cultural value placed on traditional medicines and health remedies, the demand for these plants is expected to grow (Mander, 2004). Currently, the demand for NUFMS plants outstrips supplies. This is evidenced by the high level of erosion and extinction from the wild and high prices in the market. Most crop species harvested from the wild have been considered endangered medicinal plant species.

On the other hand, low-yielding potential has meant low productivity due to low genetic and breeding focus and undeveloped agronomic practices. The depletion of wild stocks and low productivity have been attributed to the unsustainable access and availability of NUFMS. Concurrently, it has now been recognised that their conservation and production should be coupled with poverty alleviation initiatives for rural communities. There is a growing need to increase research on NUMFS since they are i) an important plant genetic resource, ii) a resource for the synthesis of natural medicines and nutrients, and iii) support the health and nutrition of poor South Africans. The motivation to conserve and cultivate these plants should involve a socio-economic and technical approach in which attention is given to the synergistic interlinkages between economic, technical, cultural and institutional dimensions of domestication. Key activities will thus be used to formulate a strategic business plan. These will include conservation and propagation, research and development, marketing and distribution and policy.

Since the development of NUFMS plants in South Africa is in its infancy, understanding their current status using a value chain approach will be considered in developing a guideline for the commercialisation of NUFMS plants.

2.4.1 Understanding the status of functional medicinal crops

There is a need to improve information, the supply, marketing and production of functional medicinal plant material (Akinola et al., 2020). This is because wild plants are under extreme pressure due to increased demand from local and export markets, and the productivity of many of these crop species remains far too low for meaningful economic returns (Sivakumar et al., 2004; Tasheva and Kosturkova, 2012; Mabhaudhi et al., 2017; Akinola et al., 2020). In addition, climate change and variability have resulted in reduced availability and potency of these crop species owing to unfavourable growing conditions (Mabhaudhi et al., 2017). Therefore, the status of functional medicinal crops in SA is reviewed to identify existing gaps, opportunities, and challenges for developing future research capacity. It is hoped that a framework to transform these crops into a commercially viable enterprise can be developed through understanding their status. Also, within the context of SA, we can identify priority medicinal crops. This can be achieved through the research value chain, i.e. breeding/crop improvement – production – agro-processing – marketing (Figure 2.5).

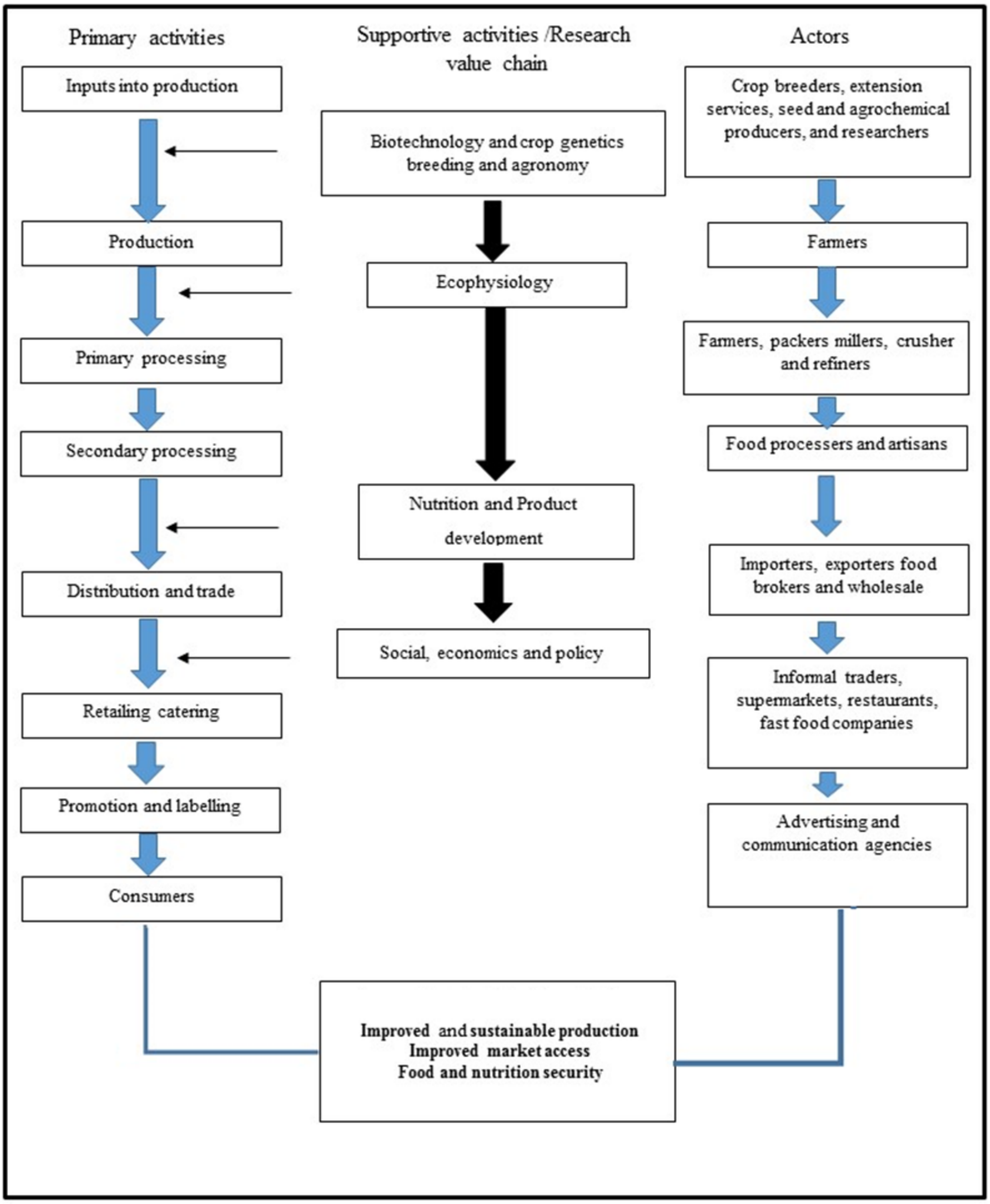

Figure 2.5: Value chain of neglected and underutilised crops indicating primary and support activities and actors involved during the primary activities (Source: Mabhaudhi et al., 2017).

A value chain refers to the "full range of activities which are required to bring a product or service from its commencement, through the different phases of product delivery to final consumers, and final disposal after use" (Padulosi et al., 2002). Such an approach was considered ideal for the development of medicinal plants. It would allow for a holistic strategy that gave equal focus on all aspects required to transform medicinal plants successfully (Gómez and Ricketts, 2013; Manyise and Dentoni, 2021). In the context of rural agricultural development, value-chain approaches have since become a popular strategy to encourage greater participation in national and international markets (Sharma and Chen, 2020). Such a strategy has also been proposed to promote lesser products such as NUFMS (Padulosi et al., 2002); however, little has been done to develop sustainable value chains. Value chains of NUFMS have been immature, with few activities and actors. There is a need to identify actors and their key roles in successfully translating NUFMS into commodity crops. Figure 2.5 proposes a simple food value chain. It outlines the "who", "when", and "how" considerations to guide upscaling NUFMS value chains. Support activities play a pivotal role in identifying and enhancing the "value" that must be added, thus incentivising a broader spectrum of actors. This set of activities has been called "research value chain" (Figure 2.5).

The value chain approach is also accepted for developing and promoting emerging technologies across different sectors (Sharma and Chen, 2020). The lack of an explicit focus on the supportive role of research and development may partly explain the slow progress in developing NUFMS value chains. Research and development are particularly true since much information remains in indigenous knowledge systems. A clear and targeted research and development agenda could unlock the potential of NUFMS. For starters, there is a need to consider key activities for sustainable production and use of these crop species (Figure 2.6).

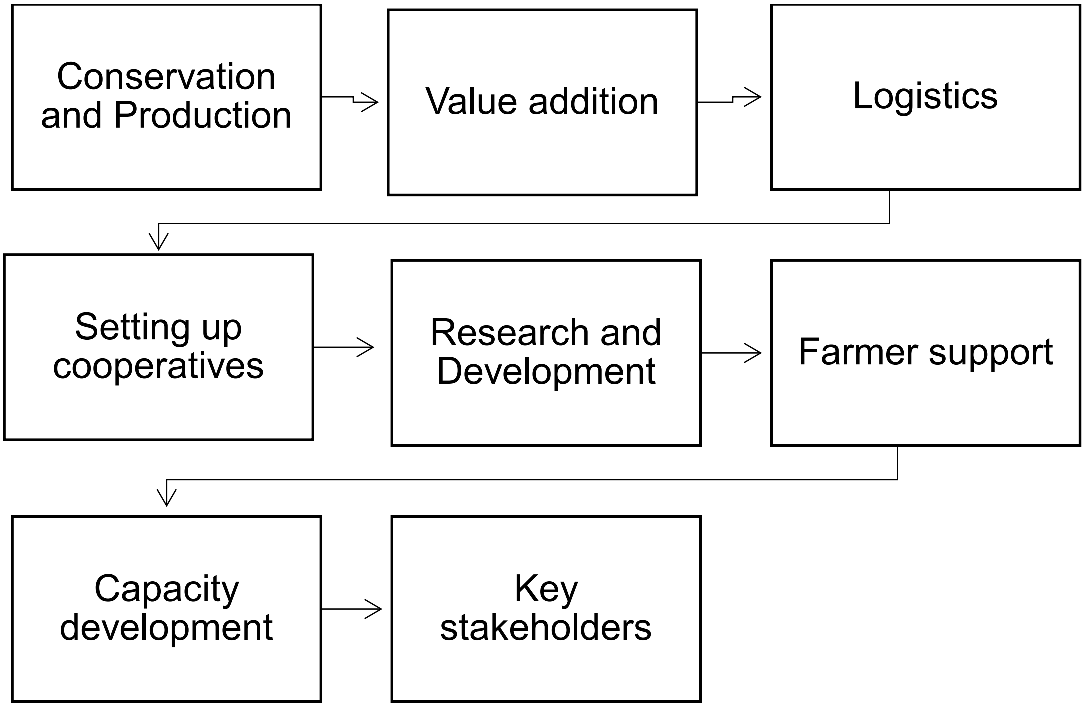

Figure 2.6: Production strategy for neglected and underutilised functional medicinal crops

2.4.2 Conservation and production

The initial point should be at the start-up point of the value chain, i.e., for successful and sustainable commercialisation of NUFMS to occur, the supply side has to be developed. This entails shifting from current practices of harvesting wild stocks, which are already showing signs of depletion and focusing on domestication and cultivation of medicinal plants. In this regard, the following is therefore proposed:

Short-term: In the short term, there is a need to introduce conservation strategies for medicinal plants, especially those flagged as vulnerable or near-endangered. These strategies will ensure consistent plant genetic resources to expand the medicinal plant industry. Therefore, collectors of NUFMS should be educated on sustainable harvest practices (Box 1). As part of the strategy, *in situ* conservation, which entails on-site conservation through improved forms of controlled use of naturally growing plants, should be explored.

Medium-term: *Ex-situ* conservation, i.e., off-site conservation, where part of the population is removed from a threatened habitat and placed in a new location, should be explored. This will allow for the regeneration of declining wild stocks and provide new material to meet demand while relieving wild stocks. This stage also involves setting up and/or establishing dedicated medicinal plant nurseries in areas where targeted farmers are located.

Long-term: As a long-term strategy for conservation and meeting demand, *in-domo* conservation should be explored. This would involve conservation by developing cultivation practices to conserve medicinal plant species. *In-domo* conservation will form the basis for promoting the cultivation of medicinal plants within smallholder farming communities across the country. This stage will require support from research and development, as well as targeted farmer advisory and technical support.

2.4.3 Value addition

Value addition is often the mid-point of the value chain and includes activities from the farm gate up to distribution. It includes activities such as post-harvest handling and storage, as well as agro-processing. The objective of this stage is to add to the value of the raw material and improve its utilisation. For developing countries and rural economic development, this is a key point of the value chain that remains underdeveloped. It may explain why farmers continue to earn less from their production. The following should be noted:

- Within the context of medicinal plants, agro-processing refers to the set of techno-economic activities carried out for the conservation, production and handling of plants to make them usable as herbal medicines, food supplements and/or industrial raw material;
- Agro-processing of these plants includes all operations from the stages of cultivation, harvesting and post-harvest handling until the material reaches the end-users in the desired quality;
- Developing agro-processing capacity for NUFMS will certainly have a significant impact on the sustainable supply and marketing of a wide range of plant products;

- There is a lack of local information describing the pharmacology, post-harvest handling and storage information for the range of plants currently utilised in South Africa;
- As a starting point, this report recommends the adoption of the World Health Organisation (WHO, 2003) guidelines on good agricultural and collection practices (GACP) and post-harvest processing for NUFMS;
- Due to the lack of robust and empirical information describing the pharmacology and post-harvest handling and storage of NUFMS, there is a need for research and development into this area; and
- While some knowledge already exists in indigenous knowledge, mostly held by traditional healers and herbalists, this IK remains poorly documented. Research and development should initially seek to document this IK and then complement it with scientific knowledge. The integration of IK and scientific knowledge will open new pathways that will allow traditional healers and herbalists to participate in the knowledge economy.

An Agri-hub should be set up to address the agro-processing aspect of the value chain. However, it is critical to note that the success of the Agri-hub depends largely on investments into the entry point of the value chain (i.e., conservation, propagation and propagation) as well as components of the mid-point value chain that have been discussed above.

2.4.4 Logistics

Logistics management is a cross-cutting aspect of value chains involved in organising, controlling and implementing operations along the value chain. It also manages the forward and backward interactions of the value chain. In this regard, logistics planning plays a critical role in the viability and sustainability of any value chain. Since value chains for medicinal plants are still underdeveloped, the following logistical considerations should form part of the strategy:

Pre-production: there is a need to establish nurseries for priority NUFMS plants within suitable sites. These will be used as a source of plant material for supplying identified farmers and encouraging cultivation.

Production: there is a need to invest in infrastructure such as tunnels/greenhouses to support the all-year-round production of NUFMS plants and thus guarantee the stability of supply for the plant.

Technology: there is a need to invest in irrigation to manage the risk posed by water availability, especially during drought periods. This could negatively impact supply during climate extremes. The huge costs of setting up irrigation infrastructure require that it be complemented by low-cost technologies such as rainwater harvesting and conservation. This will contribute to the overall resilience of production.

Input support: in the initial phase, there is a need to support identified farmers with inputs, i.e., planting material, fertilisers, herbicides and pesticides. This will ensure yield potential is realised and that quality material is also produced. Input support should also be complemented with farmer training so that, over time, farmers are capable of procuring their inputs, especially their planting materials.

Post-harvest: integrated pre- and post-harvest management practices should be developed to identify priority NUFMS. Like nurseries, adequate pack houses should be established. Low-cost technologies such as air and solar drying should be prioritised as these can easily be set up within the identified communities. This will ensure that the quality established during field production is maintained throughout the value chain.

2.4.5 Setting up co-ops

The logistical considerations also give rise to opportunities for setting up cooperatives to address various aspects of logistics as follows:

Primary co-ops: these will be related to managing logistics linked to the start-up/entry point of the value chain. These activities include the production of medicinal plants. As such, smaller cooperatives that offer geographic and economies of scale advantages in terms of land area could be explored as a starting point,

Secondary co-ops: could target common/shared interest areas within the appropriate location. Given that part of the strategy recommends setting up nurseries in target areas, this could be a point of formation combining the various primary co-ops in each of the areas to form a secondary co-op for managing the nurseries, and

Tertiary co-ops: this is a high-level and specialised co-op which will possibly manage the procurement of inputs and distribution logistics (refrigerated trucks and pack-houses),

2.4.6 Research and development for medicinal plants

Research and development activities in medicinal plants are still limited due to the infancy of commercial production of medicinal plants. Successful commercialisation of medicinal plants requires that research and development in NUFMS are focused on unlocking and supporting points in the value chain. In this regard, research on the chemical properties and value of plants and their extracts and the development of commercial, pharmaceutical, and nutritional products that may arise from chemical analysis is required. In addition, indigenous knowledge systems linked to plants and their uses and the ecological and economic considerations relating to production should be researched. Activities that can lead to achieving these goals are:

- Continuous research into the agronomic considerations and conservation of priority medicinal plants.
- Research on traditional knowledge and medicine systems.
- Research on agro-techniques, biodiversity, biotechnology and genetic improvement of medicinal plants. This includes biosynthesis and metabolic assays, tissue culture and propagation, phytochemical research, plant-derived agents for cancer, immune-related diseases and hepatotoxicity.
- Quality control assessment and research on active ingredients and substances of NUFMS.

2.4.7 Farmer support units

There is a strong case to provide logistical support for the farmers along the various points of the NUFMS value chain through a dedicated Farmer Support Unit (FSU) consisting of experts from various disciplines and traditional healers/herbalists. The role of the FSU will be as follows:

- To articulate and implement a research and development plan aimed at supporting all the role players in the NUFMS value chain;
- To provide monitoring and evaluation support for interventions and investments made in targeted areas;
- To provide technical support geared at propagation, production, post-harvest handling and storage;
- To support the establishment of production enterprises by providing information on best management practices for the cultivated medicinal plants, i.e. crop choice, planting date, fertilisers, harvesting and post-harvest; and
- to oversee capacity development of farmers by offering regular training and field visits and farmer field days. The FSU will initiate knowledge development concerning the production of NUFMS.
- The role of the FSU is both strategic and critical to the unpacking of any strategy. More so with the recommendation contained herein. As such, the formation of the FSU should precede the commencement of medicinal plants and other related activities in the value chain.

2.4.8 Capacity development

The lack of knowledge transfer between researchers and farmers, extension services, policymakers and lobbyists is widely recognised. This knowledge bottleneck may affect the successful commercialisation of medicinal plants. Researchers need to translate, package, and transmit their findings for information regarding medicinal plants to reach intended beneficiaries. Research – training – deployment constitutes a 3-dimensional capacity development approach that can increase knowledge, skills and experiences in the medicinal plant industry. Capacity development is how individuals and organisations obtain, strengthen and maintain the capabilities to set and achieve their development objectives over time. In this regard, capacity is about the growth of individuals and/or institutes in medicinal plants' knowledge, skills, and experience. For this to happen, the following is proposed:

Research: A database containing medicinal plants should be created and accessible to medicinal plant stakeholders. After that, research findings on medicinal plants should be repackaged so that all stakeholders can easily understand the findings. This would entail producing training guides, brochures, newsletters, policy briefs, seminars, and media coverage.

Training: Stakeholders will need to be trained on effective and efficient ways to execute their roles within the value chain. Using available information, regular training workshops should be done to increase capacity. The focus should be on extension officers and community heads who are centrally positioned within the support unit. Evaluations should be done based on changes in performance, based on the four main issues: institutional arrangements, leadership, knowledge, and accountability.

2.4.9 Key stakeholders

To fully support the commercialisation of medicinal plants, actors or key stakeholders who directly and indirectly participate in the value chain need to be identified and their roles clearly stated. Table 2.2 identifies key experts and institutes that may be required for the successful commercialisation of medicinal plants and outlines their role.

2.5 Prioritisation of functional medicinal plants

The report's initial sections highlighted a wide range of NUFMS plants with potential within the nutraceutical and pharmaceutical industries. However, to allocate resources and develop targeted interventions, there is a need to streamline these and develop a priority list for crops. The following key points should be noted:

1) the initial part of the report provided a list of NUFMS crops based on literature and was not exhaustive, but provided a basis for prioritisation, and
2) priority NUFMS should possess exceptional (Supplementary Table 1) defined in terms of
    a) *nutritional value*: this is regarded as part of food quality and is the measure of a well-balanced ratio of the essential nutrients, carbohydrates, fat,

protein, minerals, and vitamins in items of food or diet concerning the nutrient requirements of their consumer,

b) *Pharmaceutical value*: therapeutic indications, pharmacological effects, pharmacokinetic properties, physicochemical properties, and molecular mechanisms underlying the therapeutic benefits
c) *Cultural value*: from part of core principles and ideals upon which an entire community exists and protects and relies for existence and harmonious relationships,
d) *Environmental importance* – provide several regulatory and support services to ecosystems, which include, but are not limited to, photosynthesis, nutrient cycling, the creation of soils, and the water cycle, pollination, decomposition, water purification, erosion and flood control, and carbon storage and climate regulation, and
e) *Economic potential*: the crop's potential for economic development and growth and creation of surplus value.

The list of priority NUFMS crops provided below is provisional. It still needs to be verified by relevant stakeholders such as traditional healers/herbalists, pharmaceuticals and the targeted industry.

Cyclopia spp (Honeybush tea) Currently, approximately 200 ha, mostly of *C. genistoides* and *C. subternata* shrubs, are under cultivation, but to cater to demand, wild-harvesting, especially of C. intermedia, still contributes the major part of the annual production. It is estimated that 75% of honeybush tea is still harvested in the wild. Traditionally, leafy shoots and flowers were fermented and dried to prepare tea (Joubert et al., 2011). The increase in demand has placed natural populations that are not well-protected in jeopardy through unsustainable. Researchers have played a vital role in the 'rediscovery of this product and the development of the industry. Commercial cultivation and factory-based production have increased the access and value of honeybush tea. Extracts of tea are gaining more scientific attention due to their phenolic composition (Agapouda et al., 2020).

Athrixia phylicoides DC. (Bush tea) is an indigenous South African shrub commonly used as an anti-depressant and aphrodisiac (Ajao et al., 2019). This shrub naturally grows in semi-arid regions with limited canopy coverage, such as grassland and forest biomes of South Africa (Limpopo, Free State, KwaZulu-Natal and Eastern Cape Provinces) and Swaziland (Herman et al., 2000). Traditionally, the herb is greatly treasured as a traditional medicine to treat diabetes, heart disease and hypertension, acne, boils, colds, cuts, headaches, infected wounds, loss of voice and infected throat (Nchabeleng et al., 2012; Mathivha et al., 2019). It is also recommended as a potent blood cleanser (Mudau and Mariga, 2013) and is further substantiated by its high levels of total polyphenol content (Mudau et al., 2007a). The commercialisation of bush tea is a potential prospect for developing high-value products for the beverage and pharmaceutical industries. There is, however, a need for an alternative supply of plant material as wild plants are under extreme pressure from increased demands for local and export markets. Lerotholi et al. (2017) reported that the intensive harvesting of bush tea due to its increasing use has, in many places, resulted in overexploitation and is a serious threat to biodiversity in the region. The only option for the crop species is its cultivation largely for financial viability (Cunningham, 1993).

Brassica oleracea var. acephala (Kale) is a cruciferous vegetable characterised by leaves along the stem, which, in recent years, has gained great popularity as a 'superfood'. Consequently, it is listed as one of the healthiest vegetables in popular culture. Although kale has been cultivated for several centuries and has been included in many traditional meals, especially in the Mediterranean, it has become very popular in South Africa. However, its popularity is as dichotomous as its households' current food and nutrition security status. Kale is a superfood among the health-conscious upper-class, who primarily use it as an antioxidant booster. In the poor communities of SA, kale and its timely indigenisation and increased use support the notion of food crop globalisation (Liu et al., 2019; Traill, 1997; Ukonu, 2016). This notion has been supported by the southward migration of people from Africa's central and western regions, where these leafy vegetables are considered a staple, to South Africa in search of employment opportunities (NEPAD, 2018). These crops have become indigenised and remain underutilised, creating opportunities to develop new value and transformation of those already existing, supporting rural agricultural development and food and nutrition security (Mabhaudhi et al., 2019). Kale has become a very popular crop among organic farmers due to its good tolerance for the extreme temperature fluctuations caused by climate change in recent decades.

Colocasia esculenta (Taro) is a herbaceous perennial that grows to a height of 1-2m. The main stem is an edible starch-rich underground structure (Chivenge et al., 2015). It is called the corm, from which leaves grow upwards, roots grow downwards, while corms, daughter corms and runners grow laterally (Son et al., 2021). The root system is fibrous and confined mainly to the top layer of the soil (Sunitha et al., 2013). Corms in the dasheen type of taro are cylindrical and large. They are up to 30cm long and 15cm in diameter and constitute the main edible part of the plant (Modi and Mabhaudhi, 2013). In the eddoe types, the corm is small and globoid and surrounded by several corms and daughter corms. The corm and the daughter corms constitute a significant portion of the edible harvest of eddoe taro. Since taro is free of gluten and displays low protein, high-calorie content, and low-fat levels, taro consumption can benefit individuals with dietary restrictions, such as those presenting allergies, especially in children and gluten-intolerant individuals, contributing to reducing the risk of obesity and type II diabetes.

Moringa oleifera is a common tree native to India and cultivated throughout subtropical areas from West Africa to Fiji (Sreelatha and Padma, 2011). In South Africa, moringa grows in the Limpopo, Free State, Mpumalanga, KwaZulu-Natal and Gauteng provinces. The names are drumstick plant, kelor tree, and horseradish tree; they are sources of food and medicine (Sreelatha and Padma, 2011). According to traditional African and Indian (Ayurvedic) medicine, moringa has almost 540 compounds to treat or prevent 300 health issues (Aderinola et al., 2019; Freiberger et al., 1998; Rivas et al., 2013). In developing countries, the moringa leaf powder is commonly used as a medicinal herb rather than food, as in Asian populations. It is often taken as a supplement by HIV-infected people to enhance immunity and manage opportunistic infections. In SA, several flagship projects have been initiated due to growing interest. One example includes the Agricultural Research Council's Vegetable and Ornamental Plants group in Roodeplaat, researching moringa propagation, cultivation practices, processing, storage (shelf-life) and analysis of biological activities, safety and phytochemistry (Mashamaite et al., 2021).

2.6 Conclusion and recommendations

A wide range of NUFMS plants has potential within the nutraceutical and pharmaceutical industry. However, little attention has been paid to allocating resources and developing targeted interventions, as these crops have enormous promise as food and industrial crops. Since NUFMS plants can promote food security, their capacity to boost agricultural productivity should not be ignored. To effectively meet the requirements for nutraceutical and pharmaceutical development, there is a need to improve the supply of functional medicinal plant material. Their research and development areas should focus on unlocking and supporting points in the value chain for medicinal plants to be commercialized successfully. However, their chemical qualities/properties and values are the building blocks for developing commercial, medicinal, and nutritional products associated with these plants. It is imperative to identify the key role players and relevant stakeholders who can participate directly or indirectly in the value chain, with a clear description of their responsibilities, to support the proper commercialization of medicinal products. Therefore, it is recommended that knowledge transfer between researchers and farmers, extension services policymakers, and lobbyists focus on the standardized framework for designing systems for the nutraceutical and pharmaceutical industries.

3 REVIEW OF MORPHOLOGICAL PARAMETERS, ECO-PHYSIOLOGICAL PARAMETERS, AND QUALITY ATTRIBUTES OF BUSH TEA

Rumani, M, Ndou V, Mudau, FN, Mabhaudhi, T

3.1 Botany and ecology

3.1.1 Introduction

Athrixia phylicoides D.C (Bush tea) is an erectophile, herbaceous plant from the family *Asteraceae* (Mudau et al., 2007a). It grows wild in the semi-arid regions of South Africa, and it has great horticultural potential in cultivation, which is valued for its health benefits and nutritional components (Mudau et al., 2007a). It has been used traditionally as medicine, particularly among low-income groups, because rural populations have harvested it as tea for decades (Mudau, 2006). Throughout history, South Africans have mostly used it as a pharmaceutical tea for purifying or cleansing the blood and treating boils, headaches, infected wounds, and cuts. This solution can also be used to make a foam bath (Rakuambo, 2007). Similarly, the foam bath brew can be applied as a lotion for skin eruptions or as a cut (Rakuambo, 2007). Thus, the tea may also be used to treat coughs and colds and gargle for throat infections and voice loss (Rakuambo, 2007). According to different researchers, the *Athrixia phylicoides* D.C can be commercialized as a herbal beverage with its medicinal properties (Mudau, 2006; Nchabeleng et al., 2012; Rakuambo, 2007; Tshikhudo et al., 2016; Tshivhandekano et al., 2014; Van Wyk and Gorelik, 2017). However, nowadays, it is used commercially as an herbal beverage (Rakuambo, 2007).

Traditionally, various tribes have used bush tea differently; extracts from soaking roots and leaves have helped Venda people remove or fight parasitic worms, particularly human intestinal helminths (Van Wyk and Gorelik, 2017). Furthermore, Zulu people use fine twigs and leaves of bush tea as medicinal decorations and herbal teas (Tshivhandekano et al., 2013). Similarly, they also use roots to extract essence by utilizing or boiling as a wheeze cure (Tshivhandekano et al., 2013). Honey-bush tea and rooibos tea are two indigenous herbal teas produced in South Africa (Maudu et al., 2010). In South Africa, honey-bush, bush tea (*Athrixia phylicoides* D.C), and rooibos have all been used as therapeutic teas for decades (Mudau, 2006). The commercialization of the production system as a herbal beverage is required for domestication and preparation for market production of bush tea (Rakuambo, 2007). Therefore, one of the most significant factors in determining the price of herbal tea for domestic or export use is based on the quality (Maedza, 2015; Mashimbye et al., 2006; Mudau, 2006).

3.1.2 Botanical Aspects

3.1.2.1 Classification

The *Asteraceae* (daisy) family, tribe *Inuleae*, and subtribe *Anthrixiinae* include the genus *Athrixia* (Windvogel, 2019). According to Lehlohonolo et al. (2013), the genus name *Athrixia* is derived from the Greek word *thrix*, which means "hair," referring to the leaves. In contrast, the specific epithet *phylicoides* refers to its resemblance to Rhamnaceae. Traditionally, depending on various tribes, they call bush tea in different ways viz, Icholocholo, Itshelo, Umtshanelo, (Zulu), Mohlahlaishi (Pedi), Sephomolo (Sotho), Mutshatshaila (Venda), bush tea, Bushman's tea, Zulu tea (English), Boesmans tee (Afrikaans), Luphephetse (Swati) (Mudau, 2006).

Figure 3.1: Physical appearance of the Bush tea (Athrixia phylicoides DC.) plant (A), stems and leaves (B) flowers (Tshivhandekano et al., 2014).

3.1.2.2 Origin and distribution

Researchers have found that *Athrixia phylicoides* is widely distributed in these eastern areas viz, Soutpansberg mountains in Limpopo to Queenstown, King Williams Town, and East London, and throughout KwaZulu-Natal from the coast to the Drakensberg mountains (Joubert et al., 2008; Lehlohonolo et al., 2013; Marasha et al., 2013; Windvogel, 2019). However, A. *phylicoides* is ideally suited to a wide range of heights, from 600 to 2000m above sea level (Williams, 2013). Conversely, there are 14 species, the majority of which are found in tropical Africa and Madagascar, as well as Southern Africa, with 9 of them belonging to it (Mudau, 2006). Apart from this, *Athrixia Angushssina, A. elata, A. ger rardi, A. hererophylla*, and A. *phylicoides* are most abundant in South Africa (Joubert et al., 2008).

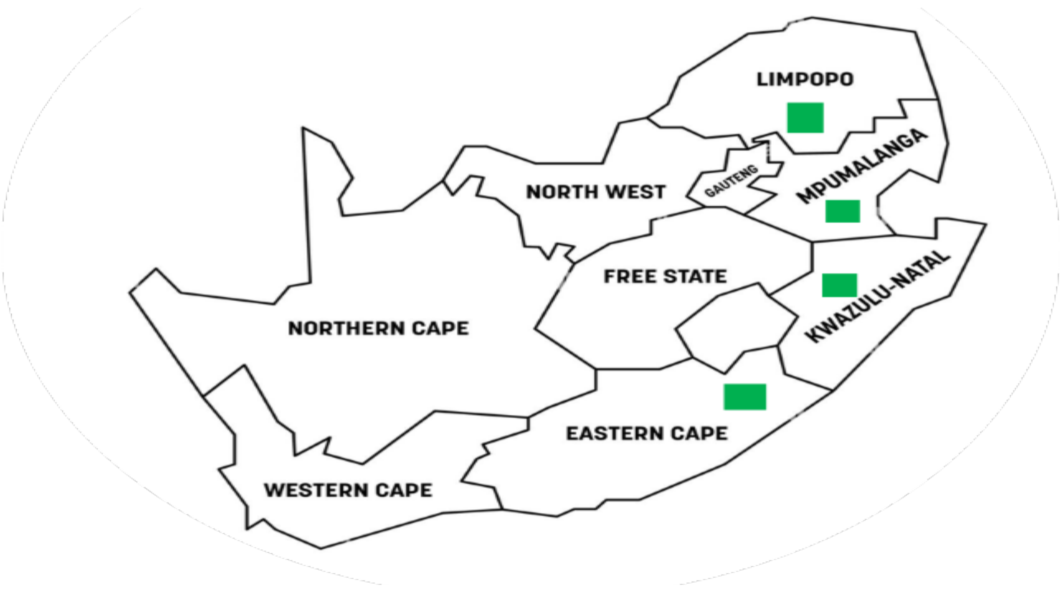

Figure 3.2: Bush tea (A phylicoides) distribution locations in South Africa.

3.1.2.3 Botanical information

Bush tea (*Athrixia phylicoides* D.C) is an aromatic herb, perennial, green shrub with woolly white stems that grows up to 1 m tall (Lerotholi et al., 2017). However, the stem seems fluffy and white. Conversely, bush tea leaves are simple, radicle, alternate linear (30 x 10 mm) to broadly lanceolate tapering to a sharp point, dark green and shiny below and woolly white above, with margins entirely or slightly revolute (Tshikhudo et al., 2016). Furthermore, the leaves are commonly auriculate at the base and have a short stalk. Nonetheless, bush tea has sessile or sub-sessile flower heads that end in huge sub-corymbose, large panicles axillary (Mudau et al., 2007a). Subsequently, bush tea flowers are daisy-like, with pink to purple petals and bright yellow centres, and bloom all year, depending on the region (Mudau et al., 2007a). Bush tea produces narrow, cylindrical, and thin fruits that are 0.01 to 0.06 mm broad. However, the seeds are 4 mm long and have two dispersal pappuses (Maedza, 2015). Bush tea thrives in South Africa's broad glass lands and heavy forest borders, including Limpopo, Free State, KwaZulu-Natal, and sections of the Eastern Cape (Maedza, 2015). Furthermore, seeds and cuttings are also effective ways to propagate them. At the end of summer, ripening seeds are propagated and mostly collected by this season (Marasha et al., 2013).

3.1.3 Tea consumption and medicinal use

Bush tea has been used as a medicinal tea by the indigenous inhabitants of South Africa for many years to cleanse or purify the blood, treat headaches, infected wounds, and cuts (Zaveri, 2006). In Venda culture, a bush tree is recognised as the tree that bachelors should not eat (Gedela et al., 2016). Meanwhile, Sotho and Xhosa people eat it to relieve sore throats and coughs (Mudau et al., 2007a). Bush tea extracts made from soaking roots and leaves are used to treat anthelmintics in the Vhembe district of Limpopo province (Mudau et al., 2007a). The tea can also be used as a gargle for throat infections and voice loss, as well as for coughs and colds (Chopade et al., 2008). Bush tea is traditionally made by boiling water with leaves for 10 to 15 minutes and then serving (Windvogel, 2019). Although younger generations added 2 – 3 teaspoons of sugar and fresh milk to bush tea, elders do not drink it with sugar and milk (Lerotholi et al., 2017). Additionally, herbalists dry leaf samples in the shade and give them to diabetic patients, as well as for blood cleansing and purification, curing boils, headaches, and contaminated wounds (Tshikhudo et al., 2016).

3.1.4 Medicinal importance of herbal teas

Herbal tea is an herbal beverage produced from the leaves, seeds, and or roots of a variety of plants (Zhao et al., 2013). Consequently, various herbal teas have been used for medicinal properties (Zhao et al., 2013). Moreover, herbal teas have played an essential part in everyday life in many lives throughout history, not only for their taste but for their therapeutic efficiency (Nookabkaew et al., 2006). Furthermore, coughs, sore throat, fever, pains, and headaches were all treated with herbal teas medicinally (Chopade et al., 2008). Conversely, some of them are ingested for their energetic effects, which can aid with relaxation, stomach or digestive disorders, and immune system strengthening (Windvogel, 2019). Therefore, black tea, green tea, chamomile, ginger tea, ginseng tea, peppermint tea, and cinnamon tea are some of the most popular herbal teas (Windvogel, 2019). On the other hand, herbal tea quality has been observed to be influenced by chemical components (Nchabeleng et al., 2012). Nevertheless, compounds, including polyphenols, flavanols, and tannins, are thought to be among the medicinal benefits found in herbal teas. Besides this, amino acids, carbohydrates, organic acids, vitamins, and volatile substances are also included in herbal teas, providing sensory quality features such as bitterness, sweetness, fragrance, and astringency (Gedela et al., 2016). Also, herbal teas' antioxidant content provides for a portion of their health advantages (Joubert et al., 2008). Thus, polyphenols, tannins, and flavanols are found in bush tea leaves (Maedza, 2015).

3.1.4.1 Medicinal value of herbal tea

Herbs have been utilised by humans for thousands of years as medicine, cosmetics, and culinary ingredients (Qasim et al., 2017). However, herbal preparations have also been used as an indulgence for physiological aches and pains for years (Mudau and Mariga, 2013). According to archaeological studies, herbs have a major role in every society and have been utilised throughout history (Marasha et al., 2013; Mudau and Mariga, 2013; Saeed et al., 2017; Zhao et al., 2013). Moreover, flowers and leaves are used to enhance the flavour, make tea, and make remedies (Islam, 2019). Herbal tea is one of the most common and long-lasting benefits of herbs (Qasim et al., 2017).

Furthermore, herbal tea has been used to keep people healthy for a long time (Lee and Lee, 2005). For many years, the indigenous civilisation of South Africa has utilised bush tea as a decontaminating tea (Özcan et al., 2009). However, in certain regions of South Africa, the roots are traditionally used as an aphrodisiac (Mudau and Mariga, 2013).

3.1.4.2 Phenological stage of herbal tea

Tea growth flush is the phenological stage of the herbal tea plant (Joubert et al., 2008). Moreover, the first flush of spring leaf buds, also known as the first flush, is the highest quality leaves (Ebrahimi et al., 2008). Besides this, when the first flush leaf bud is picked, a new sprout is in its place, which is known as the second flush, and so is until the autumn flush (Ebrahimi et al., 2008). According to several researchers, the greater the quality, the finer the plucking standards, which comprises just plucking the first two leaves and bud, following the flush, carbohydrates reserves are accumulated in preparation for the formation of total polyphenols (Gogoasa et al., 2013; Herman et al., 2000; Maedza, 2015; Mashimbye et al., 2006; Mudau, 2006; Özcan et al., 2009). Thus, there are non-volatile chemicals, including polyphenols, flavanols, phenolic acids, amino acids, chlorophyll, and other pigments, in the fresh initial tea flush (Nchabeleng et al., 2012). The chemical constitution of tea leaves is determined by leaf age, the clone under evaluation, soil, climatic and agronomic methods (Maudu et al., 2012). In addition to this, various research have found that the content of theaflavins and the arubigins was highest in the early flush and gradually decreased as the season progressed, with a minimum in the main flush and a marginal increase in black end flush (Lee and Lee, 2005; Lehlohonolo et al., 2013; Lerotholi et al., 2017; Maudu et al., 2012; Tshivhandekano et al., 2014).

3.1.4.3 Chemical composition of herbal teas

Polyphenols, flavanols and tannins are antioxidant chemicals that are used to determine the therapeutic benefits of herbal teas (Wüpper et al., 2020). Consequently, amino acids, carbohydrates, organic acids, vitamins, and volatile flavour molecules cause sensory quality attributes such as bitterness, fragrance, astringency, and sweetness (Tshivhandekano et al., 2014). Thus, antioxidants as the chemical composition of tea will be discussed.

3.1.4.3.1 Antioxidant

Free radicals are formed through oxidation processes; these radicals can cause cell damage by starting chain reactions in the cell (Özcan et al., 2009). Antioxidant molecules inhibit other molecules from oxidizing, preventing chain processes and inhibiting further oxidation reactions (Mashimbye et al., 2006). Antioxidants are usually reducing agents like thiols, ascorbic acids, or polyphenols, and they achieve this by becoming oxidized themselves (Qasim et al., 2017). Plants and animals maintain complex systems of many types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E, as well as enzymes such as catalase, superoxide dismutase, and different peroxidases, even though oxidation processes are necessary for survival (Qasim et al., 2017). Oxidative stress occurs because of a loss of oxidants or inhibition of antioxidant enzymes that could damage or kill cells (Özcan et al., 2009). Overly reactive oxygen-containing molecules and prolonged excessive inflammation cause oxidative stress, which damages cell structure and functioning (Maudu et al., 2012).

Antioxidants are used to treat strokes and neurological illnesses, and many human illnesses, including malignancies, appear to be exacerbated by oxidative stress (Maudu et al., 2012). In terms of being a measure to classify distinct materials, the antioxidant content is commonly or widely used for its health benefits (Singels et al., 2010). Antioxidant content refers to substances that can protect biological systems from the negative effects of severe oxidation processes involving nitrogen and oxygen species (Higdon and Frei, 2003). Tea's antioxidant properties are due to many phenolic chemicals discovered in or classified as amphipathic molecules (Higdon and Frei, 2003). The redox functions of phenolics, which allow them to be reduction agents and oxygen singlets, are primarily responsible for their antioxidant action (Mashimbye et al., 2006). Thus, polyphenols, tannins, and flavanols are the three most significant compounds found in bush tea leaves (Mashimbye et al., 2006).

3.1.4.4 Polyphenolic compounds

Polyphenols are a structural class of mostly natural and organic substances that include high numbers of phenol structural units (Lehlohonolo et al., 2013). Other than the hydroxyl group, polyphenol always features heteroatom substituents, and ester linkages are prevalent (Lehlohonolo et al., 2013). The addition of nitrogen, phosphorus, and potassium fertilizers to bush tea has been shown to boost total phenols, polyphenols, which are the key predictors of antioxidant capacity in herbal teas, are abundant in bush tea (Maedza, 2015). Tea polyphenols are natural antioxidants that have been chosen to regulate tea’s anticarcinogenic and antimutagenic properties (Ebrahimi et al., 2008). Polyphenols found in herbal teas have biochemical and physiological qualities that are beneficial to human health (Qasim et al., 2017). Epigallocatechin-3-gallate is the main polyphenol antioxidant found in green tea (Chopade et al., 2008). In skin cells, epigallocatechin-3-gallate has been shown to lower the number of free radicals and inflammatory prostaglandins (Chopade et al., 2008). Tea leaves are said to be high in polyphenolic compounds, accounting for about one-third of the dry mass of dried leaves (Lee and Lee, 2005). These polyphenolic components, according to researchers, are responsible for beverages' colour and flavour, particularly stringency (Lee and Lee, 2005; Lerotholi et al., 2017; Mashimbye et al., 2006).

3.1.5.4.1 Plant metabolites

Plant metabolites are tiny molecules with a low molecular weight that are either primary or secondary metabolites (Hong et al., 2016). Wherein primary metabolites are lipids, carbohydrates, and amino acids responsible for the growth and development of the plant, whereas secondary metabolites are for the survival of the plants under abiotic and biotic stress conditions (Hong et al., 2016). Polyphenols, particularly lignin and a phenolic component of suberin, show UV/V absorptivity due to aromatic structures with extensive conjugated systems of electron configurations; they also have inflorescence properties (Semenya and Maroyi, 2019). To characterize polyphenol oxidation products 2,2 amino-bis 3-ethylbenzothiazoline-6-sulphonic acid) might be utilized, polyphenols have a high affinity for proteins, which can result in the creation of soluble and insoluble protein polyphenol complexes (Özcan et al., 2009).

3.1.4.5 Flavonoids

Flavonoids are a kind of secondary metabolite found in plants (Junsi and Siripongvutikorn, 2016). Despite this, flavonoids were referred to as a vitamin probably because of the effect they had on the permeability of vascular capillaries (Junsi and Siripongvutikorn, 2016). They have a 15-carbon skeleton that comprises two phenyl rings (A and B) and a heterocyclic ring; flavonoids are the most significant plant pigments for floral colours, resulting in yellow or red/blue pigmentation in petals to attract pollination (Wüpper et al., 2020). Chemical properties, physiological regulators, and cell cycle inhibitors all play a significant role (Wüpper et al., 2020). Growth inhibition activities and pre-reserve activities are influenced by flavonoids by their cyclic adenosine monophosphate (camp) (Zaveri, 2006). In addition to this, flavonoids reduce the risk of hypertension, arterial blood pressure, and risk of atherosclerosis (Tshivhandekano et al., 2014). However, flavonoids improve endothelial and capillary function and modify blood lipid levels (Tshivhandekano et al., 2014).

3.1.4.6 Tannins

Tannins are astringent phenolic chemicals that can be found in a range of herbal products (Ebrahimi et al., 2008). Furthermore, tannins are divided into two categories: hydrolysed and condensed tannins (Ashok and Upadhyaya, 2012). Condensed tannins are polymers made up of 2 to 5 flavonoid units linked by carbon-carbon bonds that are resistant to hydrolysis (Ashok and Upadhyaya, 2012). Weak acids or bases hydrolyse hydrolysable tannins to generate carbohydrates and phenolic acids (Wang et al., 2019). Tannins present in herbal tea have been linked to the cure of cancer and heart disease, and they make blood platelets less likely to stick together (Wang et al., 2019). Herbal teas are said to have low tannin levels in general, with a lot of seasonal variances (Ukoha et al., 2011). Tannins can be used for the production of anti-corrosive primers (Ukoha et al., 2011). As a result, various researchers have found that in summer, herbal teas have the least number of hydrolysable tannins compared to autumn, winter, and spring seasons (Nchabeleng et al., 2012).

3.2 Environmental conditions

3.2.1 Water stress

Various studies were done to investigate the responses of tea (*Camellia sinensis* (L)) to low water availability, including drought stress and soil moisture content (Ahmed et al., 2014; Bhattacharya et al., 2014; Shirey and Lamberti, 2010). However, drought stress resulted in an increase in phenolic compounds and/or antioxidant activity in five of these investigations (Ahmed et al., 2014; Bhattacharya et al., 2014; Shirey and Lamberti, 2010; Upadhyaya et al., 2011). Moreover, two studies found a drop in these molecules, while two others found both an increase and a decrease in these compounds in response to drought (Upadhyaya and Panda, 2004; Chakraborty et al., 2002). Conversely, in drought-stressed tea plants, phenolics (including epicatechin and epigallocatechin gallate) accumulated (Chakraborty et al., 2002). Depending on the cultivar type, falling soil water content affected growth and total polyphenolic concentrations in tea shoots (Upadhyaya and Panda, 2004). Thus, it is worth noting that whereas phenolic secondary metabolites rise initially in response to drought, they fall in response to prolonged drought (Upadhyaya et al., 2011).

3.2.2 Light

Among all the ecological elements, light is the most important (Tshivhandekano et al., 2014). It is a source of energy for plants that have been shown to influence the photosynthetic rate and assimilate accumulation, as well as play a regulatory function in plant growth and development. Light is required for the biosynthesis of phenolics and flavonoids, whereas flavonoid production is completely dependent on light (Tshivhandekano et al., 2014). Shade treatment, light intensity, and light quality were all investigated in six studies on the impacts of light variables (Ahmed et al., 2014; Bhattacharya et al., 2014; Chakraborty et al., 2002; Ku et al., 2010; Zhang et al., 2014). Various studies found that shade-cultured green tea had lower levels of phenolic epigallocatechin and epicatechin compounds as well as higher levels of amino acids compared to unshaded green tea while having a higher level of phenolic epigallocatechin and epicatechin compounds and an inverse relationship between light and polyphenols (Ku et al., 2010; Zhang et al., 2014; Zheng et al., 2008).

Because of the differences in polyphenol profiles, shade-cultivated tea has a richer umami profile and is less astringent than unshaded tea (Ahmed et al., 2014; Ku et al., 2010; Zheng et al., 2008). Researchers discovered a non-linear association between shade levels (10% to 60% shade) and tea quality (Upadhyaya and Panda, 2004; Ku et al., 2010; Zhang et al., 2014). Three of the light experiments show that when light levels fluctuate, some terpenoids and other volatiles rise while others drop (Zhang et al., 2014). In general, greater light causes amino acid concentrations to drop (Zhang et al., 2014). According to Tshivhandekano et al. (2013), it is difficult to offer information on the ideal range of shadows required by the tea plant. However, roughly 50% diffused sunshine is typically necessary for maximum physiological activity of Camellia sinensis tea kinds. Furthermore, Camellia tea has been observed to require extended hours of sunlight, with an optimal day length of 1114 hours, for good vegetative development (Tshivhandekano et al., 2013). There has been no work done on light for bush tea.

3.2.3 Temperature

Based on catechins, phenolic secondary metabolites, and antioxidant compounds/activity, three of the four temperature studies found an inverse relationship between temperature and tea quality, while one study found that increased temperature resulted in increased catechin compounds (Lee et al., 2010; Tanongkankit et al., 2011; Wang et al., 2011; Yao et al., 2005). Higher catechin concentration was linked to decreased temperatures, according to Yao et al. (2005). Tea quality, as measured by major catechin components [(-)-epigallocatechin gallate, (-)-epicatechin gallate, and catechin gallate], was greater during warm months and lower during cold months, according to Yao et al. (2005), Lee et al. (2010), and Wang et al. (2011). Nchabeleng et al. (2012) found that variations in the total tannin content of bush tea cultivated at various elevations are negatively correlated with minimum and maximum temperatures. The influence of fermentation temperature and duration on the chemical makeup of bush tea was investigated in research (Nchabeleng et al., 2012). In a study conducted by Nchabeleng et al. (2012), temperature influences the growth of the plants. Because it is important in physical and chemical processes that regulate biological responses in plants (Tshivhandekano et al., 2013). Thus, the temperature has an important impact on crop physiological cycles, such as diffusion rate, liquid changes, substance solubility, and the equilibrium and stability of diverse systems, chemicals, and enzymes (Nchabeleng et al., 2012).

3.3 Cultivation practices

3.3.1 Propagation of bush tea

Plant propagation refers to the technique of growing new plants from a variety of sources, such as seeds, cuttings, bulbs, and other plant components (Marasha et al., 2013). The artificial or natural spread of plants is sometimes referred to as plant propagation (Araya, 2007). Cuttings are one of the most common methods for clonal regeneration in many horticultural crops, ornamental shrubs, and deciduous species, as well as wide and narrow-leaved evergreens (Araya, 2007). This type of propagation is said to be one of the most widely used and cost-effective methods of vegetative growth (Junsi and Siripongvutikorn, 2016). Cuttings, in contrast to other vegetative propagation techniques such as grafting, budding, and micropropagation, are a simple, affordable, and rapid method (Daniel et al., 2007). Thus, bush tea (*Athrixia phylicoides*) is usually propagated by collecting mature seeds at the end of the summer (Lee and Lee, 2005). However, early or late propagation might have an impact on the germination capacity of bush tea seeds and the effective bush tea transplanting capacity of bush tea seeds (Maedza, 2015). Nonetheless, effective bush tea transplanting survival may be achieved using apical cuttings, and cuttings with many roots are also necessary apical cuttings on pine bark with Seradix No 2 hormone, more rooting apical cuttings could be acquired (Maudu et al., 2012). Furthermore, the germination requirements of bush tea seed (light and temperature) are unknown (Araya, 2007).

3.3.2 Crop protection

3.3.2.1 Weed control

Weeds thrive in environments with more peat soils, and they are unsuitable for crops because they compete for space, light, moisture, and nutrients (Das et al., 2017). Weeds, on the other hand, act as hosts for diseases, insects, and nematodes (Das et al., 2017). A combination of pre-emergence and post-emergence herbicides, as well as land preparation, is the most effective weed control method (Walia et al., 2011). Moreover, weeds harm crops by reducing yield and quality (Walia et al., 2011). Furthermore, high plant densities and mulching are two methods for controlling weeds in bush tea plants (Liu et al., 2020). Nonetheless, weeding is highly recommended for bush tea, especially during the first 2 months after planting for healthy cultivation, as it is vital during the early phases of growth with a sparse leaf canopy and to minimize diseases on the leaves since its trifoliate leaves are utilised for medicinal properties and roots (Walia et al., 2011). Moreover, contamination of herbal teas is caused by a wide variety of weeds (Liu et al., 2020).

Weeds can also be found late in the season when the canopy has opened up due to older leaves withering (Walia et al., 2011). As a result, weed management is crucial for herbal tea during the early stages of vegetative growth as well as during the stages of starch accumulation and maturation (Liu et al., 2020). According to different researchers, weeds must be eradicated before planting because weeds can only be controlled by hand weeding once the honeybush tea has been established (Ediriweera, 2010; Habs et al., 2017; Rhoda et al., 2020). However, a systemic, broad-spectrum glyphosate herbicide, such as Roundup, should be used before planting to eliminate as many weeds as possible (Rhoda et al., 2020). Moreover, a slasher can be used to suppress weeds and other fynbos plants once they have been planted (Rhoda et al., 2020).

Weeds compete for nutrients, moisture, and light with tea (Coleman et al., 2020). Researchers discovered that uncontrolled weed growth removed half as much nitrogen, an equivalent amount of phosphate, and twice as much potash as tea during its most productive phase (Coleman et al., 2020; Das et al., 2017; Walia et al., 2011).

Weed control is considered an important part of tea tree crop management (Das et al., 2017). Within tea tree plantations, the most severe weed competition occurs during the first two months after planting (Coleman et al., 2020). When significant weed densities are present during crop establishment, including competition for light, nutrients, and moisture, yield losses of up to 97% can occur (Ediriweera, 2010). Herbicides applied before planting can help control weeds when they develop, according to experts (Walia et al., 2011). In tea tree plantations, herbicides are the most successful and cost-effective weed control method (Coleman et al., 2020).

Before planting tea tree seedlings (pre-sowing pre-emergence) or immediately after planting, pre-emergent residual herbicides are applied to the soil surface to combat sprouting weeds (post-sowing pre-emergence) (Ediriweera, 2010). Post-emergent herbicides can be used to suppress weeds between crop rows (inter-row space) in an established tea tree crop, as long as they are directed away from tea tree plants with shielded spray equipment to avoid crop damage (Banerjee et al., 2006). Over-the-top spraying of some post-emergent selective herbicides is available to manage a specific range of weed species (Liu et al., 2020). Where few, if any, crop leaves are available to translocate the herbicide, non-selective herbicides can be sprayed over the top of the crop following harvest and before coppicing (re-growth) (Habs et al., 2017).

3.3.2.2 Pest and disease control

Pest and disease losses are estimated to be between 10-15% of the total yield (Banerjee et al., 2006). While the use of chemical pesticides has undoubtedly enhanced yields over the last 40 years, there have also been some negative consequences, such as the development of pesticide resistance, the accumulation of residues, and environmental damage (Habs et al., 2017). Pesticide use should be brought under control in an integrated pest management (IPM) system, with pesticides being used only when the pest population exceeds the tolerable economic threshold (Liu et al., 2020).

According to a review by Ediriweera (2010), money beetle and damping off are two pests and illnesses found in the honeybush. However, the sweet-smelling blossoms at the tips of the branches attract money bugs (Liu et al., 2020). Moreover, they are in charge of pollination (Walia et al., 2011). Furthermore, brown seeds grow in little pods that turn brown as they mature (Liu et al., 2020). Conversely, as the seed ripens, the pods dry out and break open within a few weeks (Ediriweera, 2010). A fungicide should be used to prevent damping off (Ediriweera, 2010). However, the availability of organic pest and insect control products is restricted, which may influence the decision to remain purely organic (Walia et al., 2011).

3.3.3 Harvesting and trading of bush tea

Bush tea is generally harvested throughout the blossom season between early October and mid-winter (Maedza, 2015). Flowers are considered to enhance sweetness on another harvesting when the leaves are dried in the shade. Thus, this is fundamentally ideal (Mudau et al., 2007a). There are several harvesting procedures, such as cutting new shoots as low as possible from the ground with the pruning shear or cutting other branches as low as possible from the ground (Mudau et al., 2007a). The cutting length promotes re-sprouting for further harvesting and prevents shoot dieback after harvesting (Lee and Lee, 2005). Because the stems of previously harvested bushes are softer, they provide superior resources for processing in the next season and also promote a more robust shoot growth (Lee and Lee, 2005).

Bush tea is traded based on the season for collecting the plant (Van Wyk, 2011). Bundles of bush tea stems are knotted neatly to produce brooms and sold on a small scale at vendors' markets in Limpopo, Mpumalanga, and KwaZulu-Natal provinces when the plant is not harvested for brewing (Van Wyk, 2011). Tea productivity, like any other crop, is determined by a variety of factors, including genotype, soil, and cultural techniques (Semenya and Maroyi, 2019). Apart from planting material, pruning, and harvesting patterns, fertilization is one of the most important drivers, including both macronutrients and micronutrients, to produce significant yields and high-quality products (Mudau et al., 2007a).

3.4 Nutrition and health

3.4.1 Mineral nutrition for plant production

Bush tea is produced on a large scale, ensuring product availability and quality consistency (Maudu et al., 2012). However, if the plant is harvested from the wild, the commercialization of bush tea is unlikely to be viable (Maudu et al., 2012). In agriculture, crop nutrition is a significant aspect of high yields and high-quality yields (Mudau, 2006). Despite that, plants get nutrients from the soil as well as external nutrient sources, including fertilizers and organic manure (Maedza, 2015). An adequate and proper supply of nutrients is a critical component of the bush tea production system, given the increased biomass synthesis (Maedza, 2015). Plant nutrition management aims to enhance crop yield sustainably and cost-effectively (Mashimbye et al., 2006). The most limiting factor limits plant development and, hence, ultimate production (Mashimbye et al., 2006). For example, when a specific nutrient shortage limits growth, growth response to watering will be modest (Lehlohonolo et al., 2013). Tea is a perennial plant that produces branches at regular intervals. Harvesting shoots depletes the plant's nutritional reserves (Mudau and Ngezimana, 2014). It is consequently important to use fertilizers to enhance soil nutrients (Mudau and Ngezimana, 2014). Various researchers found that Nitrogen (N), Phosphorus (P), and Potassium (K) enhanced bush tea plant height, fresh, dried shoot mass, and a number of branches in a study on the nutritional requirements of bush tea (Maedza, 2015).

Bush teas grow optimally when 300 kg of Nitrogen, 300 kg of Phosphorus, and 200 kg of Potassium are applied per hectare (Joubert et al., 2008). In a study of seasonal nutritional requirements of bush tea, total phenols increased in response to N, with the greatest level of 51.1 mg/g seen in winter at 200 kg/ha N (Mudau et al., 2007b). The findings of the Phosphorus study showed that at 300 kg/ha, total phenols increased to 46.8 mg/g in the winter (Mudau et al., 2007a). Total phenols reached 400 kg/ha K. However, most of the total polyphenols were found to be between 0 and 200 kg/ha K. These can be used as the conventional optimum values for the application of N, P, and K (Chabeli et al., 2008). In that investigation, they found that when N was applied at 300 kg/ha, condensed and hydrolysable tannins increased (Mudau et al., 2007b). There was a high value of 4.5% condensed tannins and 0.06% hydrolyzable tannins (Chabeli et al., 2008). At 300 kg/ha, tannins also increased in response to P application. 5% condensed tannins and 0.02% hydrolysable tannins were at the highest concentration (Chabeli et al., 2008). 5% condensed tannins and 0.041% hydrolysable tannins were found at 200 kg/ha K. applying N, P, and K fertilizer enhanced overall oxidant content, with the highest increase occurring at 300 N, 300 P, and 100 K kg/ha (Chabeli et al., 2008).

3.4.2 Dry matter content

Several studies have attempted to clarify the impact of various management practices and environmental variables on crop-specific gravity, dry matter, and nutritional content (Mudau, 2006; Ng'etich and Stephens, 2001). Another factor that affects dry matter is the planting date (Mudau, 2006). Nonetheless, polyphenols, proteins, carbohydrates, alkaloids, amino acids, pigment, vitamins, and minerals, along with many other things, make up the dry matter of tea, which provides nutritional value and health benefits (Mudau et al., 2016). Despite this, the traditional method for measuring dry matter content is a time-consuming task (Mudau et al., 2016). However, dry matter is a key factor in assessing the quantity and quality of tea throughout the production process (Mudau et al., 2016).

High dry matter content is the indication of high nutrient content present within the leaves of bush tea, which plays a significant role in determining the texture of the plant (Maudu et al., 2012). Previous studies have found that dry matter content has a strong correlation between the number of cuts and mean production g/tree in cultivated bush tea. In contrast, the least correlation was pronounced in the wild bush tea (Mudau et al., 2016). A study carried out in South Africa indicated that bush tea planted in autumn fall (March, April, May), and winter (June, July, August) had the lowest dry matter content compared with spring (September, October, November) and summer (December, January, February) (Mudau et al., 2016). This was ascribed to increased dry matter accumulation, particularly inside the leaves and vines of bush tea grown in hot climates (Mudau et al., 2016). However, other researchers reveal a similar observation for bush tea that was planted or grown in the field, showing the highest dry matter production in summer (December, January, February) with the lowest in winter (June, July, August); this was due to low temperatures (Mudau et al., 2016).

In addition, bush tea that was grown in the glasshouse shows a high dry matter content (Maudu et al., 2011). This is due to the high temperatures, especially the night temperature, which influences and reduces the risk of photosynthesis while increasing the rate of respiratory loss (Maudu et al., 2012). Researchers also reveal that the highest carbohydrate content was recorded in the field, and the lowest was recorded in the glasshouse conditions (Maudu et al., 2011; Mudau et al., 2016). This aims to show that the dry matter content of bush tea varies depending on the part of the plant (Tshivhandekano et al., 2018). Thus, the tea plant allocates more of the total dry matter percentage to the leaf blades and petioles during the early seasons (Tshivhandekano et al., 2018).

3.4.3 Starch content

Starch is a carbohydrate that has a significant number of glucose units as well as glycosidic bonds (Mudau et al., 2016). All green plants manufacture this polysaccharide in the form of energy storage on a regular basis (Marasha et al., 2013). Perchloric acid is a technique that is used to detect starch and soluble sugars in the samples (Marasha et al., 2013). A study that was done in South Africa under a bush tea stem revealed that the highest sugar content was observed in winter, whereas in summer, the sugar content was low (Mudau et al., 2016). Similarly, the highest reserve carbohydrate content on the stem of bush tea was during the winter season, followed by spring, with summer and autumn having the lowest (Mudau et al., 2016).

Furthermore, the carbohydrates in the seasonal response of bush tea leaf, winter had the lowest starch content in comparison with other seasons (Fhatuwani and Nokwanda, 2018). As a result, the highest soluble sugar content was observed in winter and summer and the lowest in autumn. In contrast, the total reserve carbohydrates were highest in winter, followed by summer and spring, and lowest in autumn (Fhatuwani and Nokwanda, 2018). Therefore, as previously stated, several environmental conditions impact the starch content of the crops, including extremely high day temperatures, which cause a drop in photosynthesis and an increase in respiration rate with lowering starch (Mudau and Nokwanda, 2018). Starch content, which is found within the stem, is converted to sucrose, causing sugar levels to rise. In contrast, dry matter levels fall in temperature, which affects mineral nutrient absorption and metabolism in plants by increasing the transpiration rate (Mudau et al., 2016).

3.4.4 Minerals and vitamins

The nutritional significance of tea is confined to certain vitamins and minerals (Gruenwald, 2009). This is because the composition of minerals and vitamins in tea differs throughout the parts of the plant (Gruenwald, 2009). One of the elements leading to the variance in nutritional content in tea production is genotype, plant age, and environmental conditions (Maudu et al., 2012). The overall composition of protein and vitamins for tea production is regarded as important since they play a major role in the human diet. Potassium (K) has been identified as one of the minerals found at considerable levels in previous studies of the mineral composition of tea (Maudu et al., 2012). Minerals like calcium (Ca), magnesium (Mg), and phosphorus (P) are also rich in tea (Maedza, 2015). Polyphenolic components (flavonoids and phenolic acid) found in teas are strong antioxidants that scavenge free radicles and thereby protect the body against diseases (Maedza, 2015).

A study that was conducted in South Africa reveals that significant amounts of Calcium (Ca) and Magnesium (Mg) are present in bush tea (Maedza, 2015). Consequently, based on nutritional observation, black tea leaves provide the richest sources of all minerals (Mudau, 2006). Bush tea leaves are known to be rich in phenolic antioxidants because they are the most popular indigenous tea consumed by rural communities (Mudau, 2006). Researchers have revealed that the dry leaf samples reveal that bush tea and coca tea had the highest total mineral content (Mudau, 2006). As a result, K levels are higher than any other minerals in all the teas compared to other teas (Ismail et al., 2000). Thus, bush tea is the most mineral-rich indigenous plant (Mudau, 2006). The mineral composition is known to determine the quality of the plant (Mudau, 2006).

3.4.5 Metabolomics

Metabolomics, as defined by Hasanpour et al. (2020) and Ch et al. (2020), is the large-scale study of tiny compounds, also referred to as metabolites, found inside cells, biofluids, tissues, and organisms. The term "metabolome" refers to this grouping of tiny molecules and how they interact with one another within a living organism. Additionally, according to Pirhaji (2016), metabolomics is the study of the metabolite makeup, or metabolome, of a particular cell type, tissue, organ, or organism. Metabolomics provides a "snapshot" of important biological processes by measuring global collections of low-molecular-weight metabolites, such as amino acids, organic acids, sugars, fatty acids, lipids, steroids, short peptides, and vitamins (Pirhaji, 2016). It offers a readout of the status of metabolic activity in connection to inherited traits, gene expression, or environmental factors (Pirhaji, 2016). Such external stimuli include infections and allergens.

A specific metabolome profile identifies the gene-environment interaction between the environmental agent and host molecules, such as Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, lipids, and other enzymes (Sánchez et al., 2020). Metabolomics is a powerful method for the analysis and quality assessment of pharmaceuticals manufactured from natural products (NP) (Lee et al. 2017). According to Glauser et al. (2013), the molecular network (MN) facilitates the identification of linked structural molecules. The spectrum library obtained from the molecular network (MN) has nonetheless demonstrated that comparable structural compounds have been discovered (Wang et al., 2021). Plants are valuable as food sources and plentiful supplies of natural products, according to the range of phytochemicals they contain (Glauser et al., 2013). According to Ramphinwa et al. (2022), bush tea contains chlorogenic acids, which help to improve cardiovascular functions. However, reviews on metabolomes for bush tea are limited.

3.4.5.1 Metabolomics experimental approach and pipelines

Untargeted (global) and targeted techniques are the general divisions of metabolomics experiments. These approaches vary in several ways, including the degree of quantitation, sample preparation complexity, experimental accuracy and precision, and number of metabolites identified (Carneiro et al., 2019). Untargeted metabolomics is an objective examination of the metabolite profile of a biological entity in a certain physiological state under predetermined environmental circumstances. It is important to keep in mind that it is hard to cover all metabolites objectively due to the limits of existing analytical platforms and the circumstances in which the samples are obtained and processed. Due to the fact that no metabolite was found before the sample analysis, an untargeted technique can still be considered objective. Although knowledge of metabolite classes of biological interest aids in choosing an appropriate analytical platform and the sample preparation method to enhance the detection of metabolites of interest, the hypothesis tested in this scenario is not related to a specific metabolite or group of metabolites (metabolome).

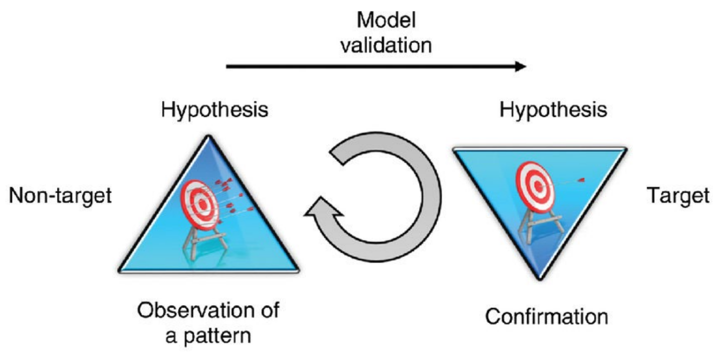

Figure 3.3: Metabolomics approaches (Carneiro et al., 2019).

The triple quadrupole mass spectrometer (TQMS) is one of the several methods used in molecular networking (Van Outersterp, 2023). The pipelines aid in sample preparation, data collecting, and data analysis (Henderson et al., 2016). The pipelines help to isolate and characterize various groups of metabolites in order to provide knowledge about a wide range of metabolic enzymes, their kinetics, end products, and the known biochemical pathways in which the set of metabolites participates Henderson et al., (2016)., as illustrated in Figure 3.4 below.

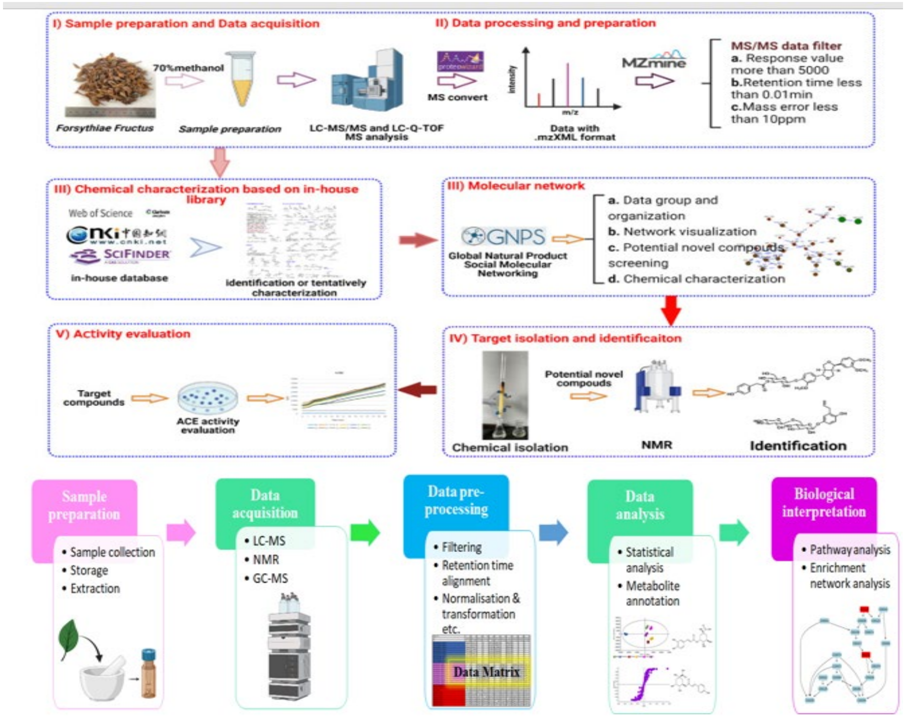

Figure 3.4: Metabolomics pipelines (Li et al., 2022).

3.5 Plant water relations

According to different researchers in all three East African study locations, the water relations of tea have been investigated (Carr, 2001). Measurements of stomatal behaviour, photosynthesis, transpiration, and xylem, water potential have been prioritized to understand better how the crop responds to its environment to inform field management practices and to find ways to identify drought-resistant cultivars early in the selection process (Carr, 2001). Thus, the nutritional water productivity will be discussed.

3.5.1 Nutritional water productivity (NWP)

Nutritional water productivity (NWP) is a tool for addressing the accessibility, availability, and utilisation aspects of food security (Ong et al., 2007). NWP has been discovered to be a beneficial technique for linking water, agriculture, and nutrition concerning food and nutrition security (Ong et al., 2007). Considering limited water resources and modified diets, nutritional water productivity (NWP) is defined as a measure of yield and nutrition yield per unit of water utilised (Wenhold et al., 2007). In contrast, linking NWP to health indicators might be more effective in maintaining the water-food-nutrition-health nexus operational and lowering nutrition issues in many regions of the world (Wenhold et al., 2007). Water nutritional productivity is measured in terms of energy, Calcium, fat, vitamin A, and iron production per unit of water input (Selvaratnam et al., 2003). Water use efficiency and water productivity are two newly developed measures that are increasingly being used to assess the impact of enhancing and growing food production, particularly in water-scarce areas which are prevalent in sub-Saharan Africa (Selvaratnam et al., 2003).

Nutritional water productivity is expressed to be a more useful tool, particularly in the semi-arid tropics, which include South Asia and sub-Saharan Africa, where water scarcity and food insecurity are on the rise daily (Wenhold et al., 2007). Water has been characterised as a limited and restricting natural resource component, particularly for increasing food and fibre production to feed a growing global population while also competing with other water consumer sectors such as industry, municipality, and the environment (Carr, 2001). Researchers also view nutritional water productivity as a starting point and acceptable index for analysing the influence of water consumption in conjunction with agriculture on global food and nutrition security (Carr, 2001).

In a plant density experiment in Tanzania, sap flow meters based on the stem heat flow method were successfully used to measure transpiration rates of individual plants in a plant density experiment (three years after field planting), expressing the yield through crop water productivity (Descheemaeker et al., 2010). Most rural farmers in Sub-Saharan Africa are unable to purchase inputs that can enhance yields, such as herbicides and insecticides (Descheemaeker et al., 2010). Water is so essential for providing food and nutrition security, as well as improved food nutrition and human health (Rockström et al., 2009). As a result, yields in Sub-Saharan African regions have lately been reported to be exceedingly low when compared to yields in the US and Europe, which are roughly 200% and 300% higher, respectively (Rockström et al., 2009). Moreover, irrigation system scientists have been working endlessly to enhance and develop more efficient irrigation systems. At the same time, breeders try to breed and improve water-efficient crops, and crop scientists continue to create water-solving farming systems in conjunction with better field management approaches (Carr, 2001). However, while these increases in water productivity have been regarded as beneficial since water shortage is not the only issue confronting the world, the problem stays unaddressed (Carr, 2001).

3.5.2 Crop water requirements

Crop water requirements (CWR) are the amounts of water needed to compensate for evapotranspiration losses in a cropped area during a certain period (Ali, 2010). Moreover, crop water requirements are commonly given in millimetres per day, month, or season (Chiarelli et al., 2020). Furthermore, they are also utilised for managerial purposes, such as irrigation water estimate irrigation and water delivery needs, as well as irrigation and water delivery schedules (Ali, 2010). Because both terms relate to the same amount of water, crop water requirements and crop evapotranspiration are strongly intertwined (Surendran et al., 2015). Nonetheless, there is a distinction between them (Mehta and Pandey, 2016).

While crop evapotranspiration shows the amount of water that should be given to account for these losses (i.e., a hydrological concept), crop water requirement specifies the amount of water that should be supplied to account for these losses (i.e., a hydrological phrase) (i.e., an irrigation management term) (Surendran et al., 2015). In reality, this amount of water is equivalent to the effective irrigation water supply required to achieve the highest yield (Gong et al., 2020). As a result, crop evapotranspiration is estimated first, followed by crop water requirements, with the latter commonly representing the values of crop evapotranspiration aggregated over time (Mehta and Pandey, 2016).

In reality, crop evapotranspiration should be converted into crop water requirements to estimate irrigation water requirements and, subsequently, irrigation water supply management (Gong et al., 2020). The amount of irrigation water efficiently delivered in the root zone and effective precipitation is then used to meet crop water requirements (Ali, 2010). With the construction of huge engineering works, it became essential to estimate the water amounts to be delivered to newly irrigated regions, and the idea of agricultural water requirements became crucial (Gong et al., 2020).

In general, there is a distinction to be made between crop water requirements for long-term planning purposes, where an average climate or a climate with a certain probability of occurrence can be used to estimate CWR, and crop water requirements for real-time management, where climatic data from the current season is used (Sawant et al., 2017). Because both phrases relate to the same amount of water, the processes for estimating agricultural water requirements are the same as those for estimating crop evapotranspiration (Surendran et al., 2015). As a result, under the argument "crop evapotranspiration" of this *Athrixia phylicoides*, further data about the measurement and estimation of crop water requirements are limited (Surendran et al., 2015).

3.5.2.1 Climate factors influencing crop water requirements

A crop planted in a sunny, hot area will require more water per day than a crop grown in a cloudy, cooler one (Li et al., 2020). Apart from sunlight and temperature, there are additional climatic elements that impact crop water requirements (Molua and Lambi, 2006). Nonetheless, humidity and wind speed are two of these variables (Savva and Frenken, 2002). Crop water requirements are higher when it is dry than when it is humid (Molua and Lambi, 2006). Crops in windy climates consume more water than crops in calm regions (Li et al., 2020). The largest crop water requirements are consequently found in hot, dry, windy, and sunny climates (Savva and Frenken, 2002). When the weather is chilly, humid, and gloomy with little or no breeze, the lowest readings are obtained (Li et al., 2020). From the foregoing, it is evident that crops cultivated in various climate zones would require varying amounts of water (Savva and Frenken, 2002).

3.5.3 Crop management

Crop management is a process that begins with seed sowing, continues with crop maintenance during the growth and development phase, and concludes with crop harvest, storage, and distribution (Mudau et al., 2005). Crop management has long been known to have an impact on pests and their natural enemies in the soil environment (Mudau et al., 2005). According to Mudau et al. (2006), crop management operations impact the soil environment, influencing microbial development as well as the biodegradation process, which alters plant residues and pesticides applied to the soil. However, crop management activities such as soil management are one of the most important components in ensuring that sufficient nutrients are available for appropriate plant growth, which can lead to optimum yield (Mudau et al., 2005). Moreover, effective crop management, such as favourable environmental conditions with well-fertilized soil and sufficient drainage to support healthy plant development, has been recorded for herbal teas (Tshivhandekano et al., 2013).

3.6 Summary

Bush tea is considered an important herb for its medicinal benefits in different parts of South Africa. However, bush tea in South Africa is more practised by smallholder farmers in different regions of Limpopo, Kwazulu-Natal, and Mpumalanga provinces. Nonetheless, different researchers have revealed the required soil conditions and specific environmental conditions of tea, which include high humidity and acidic soil in a few areas, such as Asia and Africa. Consequently, previous studies have found that there is a gap in scientific knowledge research in South Africa, which is harming its output. As a result, the review concludes that little is known about bush tea's water use and nutritional water productivity in terms of yield and quality.

Furthermore, water nutritional productivity is an important new aspect being introduced, specifically to herbal plants, to improve human health when it comes to water use. There is still work to be done to better advise farmers on how to optimize their yields and crop quality when it comes to water use. Growers must observe planting dates, fertilizer application, cultivar, soil type, and climatic conditions while cultivating bush tea farmers. Furthermore, the study found that the planting date and fertilizer had a substantial influence on bush tea yield quality. Farmers need to follow pesticide dose guidelines, and the safe time between application and plucking must be rigorously followed because biological control will become more important.

Furthermore, a lack of knowledge and understanding of bush tea, as well as physiological aspects that may limit quality, are factors affecting higher yield production.

4 RESPONSE OF PHYSIOLOGICAL PARAMETERS, NUTRITIONAL WATER PRODUCTIVITY, AND PHYTOCHEMICAL PROFILES TO VARYING WATER REGIMES OF BUSH TEA

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4.1 Introduction

Bush tea is a South African nutritious herbal that is used as medicine by many households (Joubert et al., 2008). Traditionally, it is well-known as a herbal tea that purifies blood, treats boils, headaches, infected wounds, and cuts, and creates a foam bath (Mabogo, 1990; Mudau et al., 2006; Tshikhudo et al., 2020). Its roots are used as an aphrodisiac by the Vhavenda people (Rakuambo, 2007), while a decoction of the roots is used by the Zulu people as a cough suppressant and laxative (Tshivhandekano et al., 2013). Tshivhandekano et al. (2013) and Lerotholi et al. (2017) stated that plant tissues contain a significant number of bioactive compounds.

Despite its medicinal properties, the tea plant can grow naturally at various altitudes and with different rainfall and soil properties (Tshikhudo et al., 2019). Seasonal changes in temperature and vapour pressure will affect plant growth, yield, and bush tea quality (Ramphinwa et al., 2023). Gatabazi et al. (2022) reported that cultivation and climate change affect the life cycles of medicinal plant species and their distribution in different regions. Therefore, a controlled environment is one strategy to mitigate the changes in agriculture.

South Africa is recognized as a water-scarce nation (Chivenge et al., 2015). The annual average rainfall in the country is approximately 500 mm, which is lower than the world average of 860 mm (Mabhaudhi, 2012). In addition, Bendou (2022) found that one of the main variables limiting plant development and productivity in agricultural production for different crop species is dryness. In addition, drought affects plants at various stages of development, from fertilization through harvest, resulting in decreased plant development (Chrysargyris et al., 2018). Therefore, it is critical to determine the effect of cultural practices, such as varying water regimes under a controlled environment, to produce high-quality bush tea production.

Previous research on bush tea production has investigated plant growth, yield, and diverse cultural techniques such as nutritional management in a controlled and field environment (Mudau et al., 2007c), pruning (Mohale et al., 2018), harvesting approaches (Mphangwe, 2012), processing (Hlahla, 2010) and seasonal variation (Mudau et al., 2007c; Ramphinwa et al., 2022b). However, the impact of bush tea under different water regimes on growth, yield, and secondary metabolites in a controlled environment is still lacking. Irrigation is a significant factor that affects the growth, productivity, and quality of medicinal crops (Hassan and Ali, 2014). The tea plant, *Camellia Sinensis* (tea), responds well to irrigation and thrives well in dry land conditions (Carr, 2010). An important factor affecting crop yield and nutrient loss is the water used by crops during the growing season and water loss or vegetation (as evapotranspiration) (Dasila et al., 2016). Consequently, the study aimed to investigate the responses of physiological parameters, nutritional water productivity, and phytochemical analysis of bush tea grown under a controlled environment to varying water regimes.

4.2 Materials and methods

4.2.1 Experimental site

The protected environment experiment was conducted from the spring to summer season of the year 2022 in a tunnel situated at the University of KwaZulu-Natal's Controlled Environment Research Unit (CERU) (29.58° S, 30.42° E), in Pietermaritzburg, South Africa. Weather data was sourced from the University of KwaZulu-Natal (UKZN) agrometeorology weather service for the spring to summer season of the year 2022. The temperatures range from ~ 18/33 °C (day/night) and relative humidity (60-80%).

4.2.2 Plant material description

Bush tea stem cuttings were collected from the wild in Pietermaritzburg, Midlands (lat. 29˚.6168' S long. 30˚.3928' E), South Africa. A length of 7 to 8 cm of stem cuttings was dipped in Seradix No. 2 hormone (0.3% IBA) to stimulate the rapid and abundant root formation process. Cuttings were then grown on a 30 cm-long plastic bag in a tunnel. A daily irrigation system was used on the plants. To prevent transplanting shock, the rooted cuttings were transplanted into 1 L bags after 35 days for three months (Maedza, 2015). Thereafter, after being stored for 40 days, planting materials with 25 leaves were transplanted into long-raised beds. The experiment was conducted on raised beds with a set width of 0.75 m and a length of 11 m. Thereafter, uniform plants were chosen for the trial layout of water treatments. Planting beds were prepared before establishing a trial. Regular hand weeding was done to keep the experiment pest-free. Use karate (30 mL/15 L water) twice a week for eight weeks after planting to control the disease. The soil was submitted to the Institute for Commercial Forestry Research (ICFR) Analytical and Research Laboratory to determine chemical and physical properties. Therefore, the soil texture was clay (Table 4.1). However, soil tests (Table 4.1) show that the soil needs nitrogen fertilizer, which was applied in the soil with a concentration of 4 g of limestone ammonium nitrate (LAN) based on recommendations by Mudau et al. (2006). Weather data was sourced from the University of KwaZulu-Natal (UKZN) agrometeorology weather service for the spring to summer season of the year 2022.

Table 4.1: Physical and chemical properties of soil in the long-raised beds.

Clay (%)	**Organic C (%)**	**pH (KCl)**	**P (cmol/kg)**	**Ca (cmol/kg)**	**K (cmol/kg)**	**Mg (cmol/kg)**	**Na (cmol/kg)**	**N (%)**
38.5	2.70	5.19	539.62	1059	0.36	1.11	0.23	0.19

4.2.3 Experimental description

The experiment was laid out in a complete randomised block design (CRBD) with three water requirements (100%, 30%, and control (stress) replicated three times. Water flow was supplied with a filter, a pump, two solenoid valves, two water meters, a control box, Netafim inline drippers with four individual drippers, and a mainline that supplies water three times a day at 7, 11, and 3 a.m. The average rate per dripper was 2 Lhr^{-1}, while the drip rate was operating at 33 ml per min, whereas the system's maximum working pressure was set to 200 kPa. The discharge rate was set at 2 Lhr^{-1}. The irrigation event and irrigation interval were the same. However, water was provided twice daily at 7 and 11 a.m. with a discharge rate of a total of 2 L per day using a watering can for control (stress) for over 40 days after transplanting. Thereafter, irrigation was stopped to stress the bush tea. The spacing was 0.5 m x 0.5 m (inter and intra row spacing) (40 000 plants/ha^{-1} plant population). Because the crop factor for bush tea has not yet been identified, the irrigation plan was created using the daily crop water needed from the product of normal tea (unshaded) crop factors (K_c), as provided by Singh (2013). Evaporation data were collected from the agricultural meteorology automatic weather station (Table 4.2). All treatments were irrigated to the potential area before treatment application to reduce evaporation and runoff losses during peak sun hours and to ensure adequate soil moisture. Therefore, the crop water need was calculated using Eq. 4-1 based on Allen et al. (1998):

$$ET_c = ET_o \times K_c \qquad \text{Equation 4-1}$$

Table 4.2: Crop water requirements of bush tea in responses to varying water treatments

	K_c	ET_o	ET_c	Duration	Total water applied
		mm	mm	Days	mm
Initial	0.95	4.8	4.56	40	182.40
Mid-season	1.00	6.57	6.57	51	335.07
Late season	1.00	7.89	7.89	25	197.25
Total water applied (ET_a)					
• **100%**					714.72
• **30%**					214.416
• **0%**					0

Where: K_c = crop factor from tea based on Singh (2013). ETc= crop water requirement (mm), ET_o = reference evapotranspiration (mm).

4.2.4 Data collection

4.2.4.1 Gas exchange and chlorophyll fluorescence measurements

Gas exchange and chlorophyll fluorescence were performed by LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) integrated with an infrared gas analyser (IRGA) attached to a leaf chamber fluorometer (LCF) (640040B, 2 cm^2 leaf area, Licor Bioscience, Inc. Lincoln, Nebraska, USA). The leaf was maintained at a temperature of 25 ˚C. The relative humidity was kept at 43%, and the water flow rate was kept at 500 mol. In the cuvette, the leaf-to-air vapour pressure deficit was maintained at 1.7kPa to prevent stomatal closure from low air humidity. Between 8:00 and 11:00 a.m., measurements of the third partially developed leaf from the plant's tip were made by placing the leaf within the detector head while also applying 100%, 30%, and control (stress) water treatments. Three plants per replication were used for taking measurements. Stomatal conductance (gs), net CO_2

assimilation rate (A), transpiration rate (T), intercellular CO_2 concentration (Ci), and the ratio of intercellular and ambient CO_2 concentrations (Ci/Ca) were measured for gas exchange. The intercellular CO_2 concentration (A/Ci), which measures the net CO_2 assimilation rate. Calculating intrinsic water use efficiency (WUEi) and instantaneous water use efficiency (WUEins) was done using the ratio of A and gs and A and T, respectively. According to Evans (2009), estimated the following additional chlorophyll fluorescence measurements: Fv'/Fm'. The maximum quantum efficiency of photosystem II photochemistry, the effective quantum efficiency of photosystem II photochemistry (ΦPSII), photochemical quenching (qP), non-photochemical quenching (qN), and electron transport rate (ETR), The ratio of ETR and A was used to calculate a relative measure of electron transport to oxygen molecules. To determine a relative indicator of electron transport to oxygen molecules, the ratio of alternative electron sink (AES) and A was utilized. The ratio of the quantum efficiency of CO_2 assimilation (A) to the effective quantum efficiency of the photosystem.

4.2.4.2 Yield

Bush tea plants were harvested 153 days after planting. Bush tea yield components were determined by sampling 3 plants in each replicate of each treatment. The yield (fresh below-ground mass), number of roots per plant, and number of marketable roots were all recorded on a plot-by-plot basis (Ossom and Rhykerd, 2008). Marketable roots were defined as entire (undamaged), free of harvest wounds, pest damage, and disease damage. Actual yield (Y_a) was calculated using the fresh mass of marketable roots and then converted to $kg.ha^{-1}$. The percentage of marketable storage roots was calculated using Eq. 4-2:

$$\frac{Number\ of\ marketable\ roots/plot}{Total\ number\ of\ roots\ /plot} \times 100\% \qquad \text{Equation 4-2}$$

4.2.4.3 Determination of nutritional composition (NC)

Bush tea leaves were dried in an oven at 40 ˚C for 24 hours and then ground into powder using a mortar and pestle. The ground samples (0.5 g) were placed in a quartz crucible and charred in a wild Barfield muffle furnace at 650 ˚C for 120 minutes. Acid digestion was carried out according to Huang et al. (2007) with minor modifications. The ash was dissolved in 10 ml of diluted aqua regia (HNO_3 and HCL mixed in a ratio of 1:3) in a 25 ml volumetric flask. The contents of the flask were brought to volume by adding distilled H_2O and heated in a water bath for 1 hr at 85 ˚C. The samples were chilled to 25 ˚C before analysis using atomic absorption spectrometry (AAS) (Mandizvo and Odindo. 2019). The nutrients analysed per dry matter basis included micro-nutrients (calcium, zinc, iron, magnesium, and sodium).

4.2.4.4 Determination of nutritional water productivity (NWP)

The productivity of nutrient waters was calculated using the formula of Renault and Wallender (2000), which was calculated using Eq. 4-3:

$$NWP = \frac{Ya}{ETa} \times NC \qquad \text{Equation 4-3}$$

where: NWP is the nutrient productivity of water (nutritive value m^{-3} of water evapotranspiration), Y_a is the actual harvested yield (kg.ha-^{1}), ET_a is the water applied based on crop water requirement ($m^3.ha^{-1}$), and NC is the nutrient content per kg of product (nutritive value kg^{-1}).

4.2.4.5 Metabolite extraction

A method suggested by Makita et al. (2016) was used to determine the extraction of metabolites. The tea leaves were freeze-dried and then ground into a fine powder. The 2 grams of powder was extracted with 20 ml (1:10 m/v) of 80% aqueous methanol (Romil SpS, Cambridge, UK). The samples were rotated at 70 rpm in a digital rotisserie tube rotator for the whole night. Thereafter, the homogenate was centrifuged at 5000 x *g* to remove debris for 20 minutes. The supernatant was subsequently filtered using 0.22 µm nylon filters into glass vials with 500 µL inserts. For each sample group, at least 3 independent replicates were prepared; these were stored at 4 ˚C (Ramabulana et al., 2021). Equal quantities of each sample were mixed to generate quality control (QC) samples, which were used in conjunction with experimental samples to evaluate the quality of the data collected (Ramabulana et al., 2021).

4.2.4.6 Liquid Chromatography-Quadruple Time-of-flight Tandem Mass Spectrometry (LC-MS/MS).

A Liquid Chromatography-quadruple time-of-flight tandem mass spectrometry instrument (QTOF)–MS, model LC-MS 9030, Shimadzu Corporation, Kyoto, Japan). The chromatographic separation was performed on a Shim Pack Velox C18 column (100 mm × 2.1 mm with a particle size of 2.7 µm), which was used to analyse the prepared bush tea leaf extracts. The column oven was set at 40 ˚C as described by (Ramabulana et al., 2021). A binary solvent gradient consisting of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) was used at a constant flow rate of 0.4 mL/min. The gradient was set for 53 minutes, and the flow rate was set at 0.3 mL/min under the following separation conditions: The settings were maintained at 10% B for 3 min, 10%–60% B over 3–40 min, 60% B from 40–43 min, then the gradient increased to 90% B between 43–45 min and kept at 90% B for 3 min. The gradient was reset to its initial state 48–50 minutes later. This was followed by a 3-minute column re-calibration period. A mass spectrometer detector was used for monitoring analyte elations under the following conditions: ESI (electrospray ionization) negative modes; interface voltage of 3.5 kV; nitrogen gas was used as nebulizer at flow rate of 3 L/min, heating gas flow at 10 L/min; heat block temperature at 400 ˚C., CDL temperature at 250 ˚C, detector voltage of 1.70 kV and the TOF temperature at 42 ˚C. Tandem MS (MS/MS) experiments, typical mass accuracies with a mass error below 1 ppm were obtained using a mass calibration solution of sodium iodide (NaI). High resolution was obtained for MS and (MS/ MS) experiments using a mass-to-ratio (m/z) range of 100-1000 Da.

4.2.4.7 Molecular networking

For all datasets acquired as stipulated in Section 4.2.4.6, the network was constructed using the Wang et al. (2016) suggested technique and the online Global Natural Products Social Molecular Networking (GNPS) process (https://gnps.ucsd.edu). The data was filtered by removing all MS/MS fragment ions that were within +/- 17 Da of the m/z precursor. Only the top 6 fragment ions within the +/- 50 Da window were selected from the window-filtered MS/MS spectra. The MS/MS fragmentation ion tolerance (MS/MS) was set at 0.5 Da, while the mass tolerance for the precursor ion was 2.0 Da. Following the recommended procedure by Aron et al. (2020), a network with edges with cosines larger than 0.7 and more than 6 associated peaks was produced.

Additionally, edges detected between two nodes were preserved in the network when both nodes occurred in their 10 most similar nodes. The maximum molecular family size was eventually set at 100, and the lowest-ranked edges were removed from the molecular families until the molecular family size fell below this threshold. Then, the GNPS spectral libraries were used to search for network spectra. The library spectra were filtered in the same way as the input data. All matches between the library and lattice spectra are required to contain at least 6 matching peaks and a value larger than 0.7 (Aron et al., 2020).

4.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) using GenStat® version 20 (VSN International. UK). The least significant difference (LSD) was used to separate means at the 5% significance level. Principal component analysis (PCA) was performed for all metabolites, and the molecular network analysis was constructed on the GNPS. At the same time, the raw data were acquired from the Shimadzu LCMS-9030 QTOF of bush tea extracts under varying water regimes.

4.3 Results and discussion

4.3.1 Gas exchange and chlorophyll fluorescence measurements

It is important to understand the physiological processes that lead to the development of adaptation to water in the production of tea plants. Water level significantly ($P<0.01$) affected the gas exchange and chlorophyll fluorescence measurement of bush tea

(Tables 4.3 and 4.4). The highest photosynthetic rate was recorded under 30% ET_c, followed by 100% ET_c and control (stress) was recorded as the lowest. Similarly, stomatal conductance (gs), net CO_2 assimilation rate (A), transpiration rate (T), intercellular CO_2 concentration (Ci), ratio of intercellular and ambient CO_2 concentrations (Ci/Ca), intrinsic water use efficiency (WUEi) and instantaneous water-use efficiency (WUEins) increased under 30% ET_c compared to 100% ET_c and control (stress). The measured chlorophyll fluorescence measurements i.e., Fv'/Fm', maximum quantum efficiency of photosystem II photochemistry, effective quantum efficiency of photosystem II photochemistry (ΦPSII), photochemical quenching (qP), non-photochemical quenching (qN), and electron transport rate (ETR) varied significantly among the varying water treatments, with the highest values being recorded under 30% ET_c compared to 100% ET_c and control (stress). The results contradict those of Barros et al. (2023), who reported the reduction of net photosynthesis (A), stomatal conductance (gs), transpiration (E), and internal CO_2 concentration (Ci) due to water stress. It is critical to understand how and when to apply limited amounts of water to maximize the yield of high-quality plant products (Alderfasi and Alghamdi, 2010). Bush tea may thrive well when plants are exposed to 30% ET_c water stress.

The evaluated bush tea exhibited high water use efficiency under limited water application (30% ET_c). These results suggest that bush tea is an efficient water user, which is attributed to limited water application (Table 4.4), as the study exhibited maximum WUEi and WUEinst compared to other water treatments. The study revealed that bush tea plants are capable of reducing loss of water when exposed to water stress. Efficient PSII activity under limited water application protects the structure of chloroplasts against oxidative damage by preventing the creation of singlet oxygen (Levesque-Tremblay et al., 2009). Positive performance under limited water application is probably influenced by maintaining the effectiveness of PSII function and the antioxidant system components. The current study is the first to show that physiological measurements under limited water enhance the physiological development of bush tea under a protected tunnel environment.

Table 4.3: Analysis of variance showing mean squares and significant tests for leaf gas exchange and chlorophyll fluorescence measurements of bush tea evaluated under varying water treatments.

Source of variance	**d.f**	***gs***	***T***	***A***	***Ci***	***A/Ci***	***Ci/Ca***	***Wu$_i$***	***WUE$_{inst}$***
Water treatment	2	0.1401667**	10.5797**	3.47367**	61477.6**	6.63433**	376.711**	97.933**	175.599**
Time	1	0.14062678**	865.2420*	1509.6744**	535691.9**	1.82133**	2332.305**	2055.305**	18203.825**
Water treatment x time	2	0.00019671*	1.9300*	0.3664**	12523.9**	43.27496**	339.606**	2.060**	3.707**
Residual	12	0.0003317	0.1835	0.05408	255.2	0.06533	1.119	1.536	2.355

Source of variance	**d.f**	**Fo**	**Fm**	**Fv/Fm**	**$\Phi_{PS\,II}$**	**qP**	**qN**	**ETR**
Water treatment	2	197228**	390037**	0.1698479**	352.197**	71.56132**	78.52056**	1853447**
Time	1	232382**	570074*	0.1557303*	843.243**	119.91842**	81.06889**	6754541**
Water treatment x time	2	5079*	7197*	0.0007281*	76.338**	19.88921**	10.57056**	831555**
Residual	12	1514	3333	0.0003534	2.808	0.04054	0.07444	1342

d.f; degrees of freedom, gs; stomatal conductance, T; transpiration rate, A; net CO_2 assimilation rate, A/Ci; CO_2 assimilation rate/intercellular CO2 concentration, Ci; intercellular CO_2 concentration, C i/C a; ratio of intercellular and atmospheric CO 2, WUEi; intrinsic water use efficiency, WUEins; instantaneous water-use efficiency, Fv/Fm; maximum quantum efficiency of photosystem Ⅱ photochemistry, ΦPSⅡ; the effective quantum efficiency of PSⅡ photochemistry, qP; photochemical quenching, qN; non-photochemical quenching, ETR; electron transport rate, ETR/A; relative measure of electron transport to oxygen molecules, * and ** denote significant at 5 and 1% probability levels, respectively, Ns; non-significant.

Table 4.4: Means of leaf gas exchange and chlorophyll measurements of bush tea under varying water treatments.

Leaf gas exchange measurements												**Chlorophyll Fluorescence measurements**					
Week1	**ET_a**	***Gs***	***T***	***A***	***C_i***	***A/C_i***	***C_i/C_a***	***WUEi***	***WUE_{inst}***	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***	***ETR/A***
	Control (no irrigation)	0.18a	32.48a	26.27a	318.0a	3.40a	0.85a	20.83a	52.30a	2676.4a	2437a	0.1219a	20.10a	0.137a	1.13a	663.5a	22.67a
	100%	0.37b	35.01b	28.30b	330.5b	3.56b	0.94b	26.51b	60.23b	2803.0b	2657b	0.250b	24.52b	2.10b	3.30b	943.6b	31.13b
	30%	0.3487c	36.18c	29.74c	423.9c	6.83c	1.59c	34.10c	64.22c	2983.6c	2951c	0.440c	28.96c	3.41c	6.13c	1036.6c	33.72c

Leaf gas exchange measurements										Chlorophyll measurements							Fluorescence
Week2	**ET_a**	***gs***	***T***	***A***	***C_i***	***A/C_i***	***C_i/C_a***	***WUEi***	***WUE_{inst}***	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***	***ETR/A***
	Control (no irrigation)	0.35a	47.63a	29.63a	570.9a	5.53a	11.99a	38.94a	117.68a	2837.8a	2848a	0.30a	25.56a	1.20a	2.367a	1205.7a	25.31a
	100%	0.56b	48.45b	30.45b	677.2b	8.67b	18.34b	40.86b	122.63b	3051.5b	2935b	0.42b	42.76b	8.50b	9.267b	2058.0b	42.47b
	30%	0.65c	49.19c	31.19c	877.2c	1.5c	41.35c	42.75c	127.25c	3255.4c	3330c	0.65c	46.32c	11.43c	11.667c	3055.5c	62.12c

d.f; degrees of freedom, gs; stomatal conductance, T; transpiration rate, A; net CO_2 assimilation rate, (µmol CO_2 m^{-2} s^{-1}), A/Ci; CO_2 assimilation rate/intercellular CO_2 concentration, Ci; intercellular CO_2 concentration, Ci/C a; ratio of intercellular and atmospheric CO_2, WUEi; intrinsic water use efficiency, WUEins; instantaneous water-use efficiency, Fv/Fm; maximum quantum efficiency of photosystem Ⅱ photochemistry, ΦPSⅡ; the effective quantum efficiency of PSⅡ photochemistry, qP; photochemical quenching, qN; non-photochemical quenching, ETR; electron transport rate, ETR/A; relative measure of electron transport to oxygen molecules. Varying upper-case letters within a column indicates significant differences among water treatments. gs; (mmol m^{-2} s^{-1}), T; (mmol H_2O m^{-2} s^{-1}), A; (µmol CO_2 m^{-2} s^{-1}), A/Ci; (µmol. mol m^{-1}), Ci; (µmol. mol m^{-1}), WUEi; (µmol (CO_2) m^{-2}); WUEins, (µmol. mol^{-1}), Fv/Fm; (ratio); ΦPSII, the effective quantum efficiency of PSII photochemistry; qP, photochemical quenching; qN, non-photochemical quenching; ETR, (µmol e^{-1} m^{-2} s^{-1}); ETR/A, (µmol e µmol−1 CO_2).

4.3.2 Yield

Varying water regimes had significantly affected ($P<0.01$) yield in bush tea production (Table 4.5). The yield metrics of bush tea plants subjected to various water regimes and grown in a controlled environment differed significantly. The higher yield was recorded under 100% ET_c (96 $kg.ha^{-1}$) compared to 30% ET_c (60.6 $kg.ha^{-1}$) and control (stress) (12.12 $kg.ha^{-1}$). The findings could imply that crop yield increases as irrigation water supply increases (Fereres et al., 2003). The results are like those reported earlier by Mabhaudhi et al. (2019), who also had similar water regimes on sweet potato production. A higher number of roots were recorded on bush tea plants that were exposed to 100% ET_c (46 kg) than plants from 30% ET_c and control (stress). The percentage of marketable roots is considered different between the water levels, with 100% ET_c recording the most marketable roots, followed by 30% ET_c, and with the lowest marketable roots recorded under control (stress condition). These results are similar to those of Osman (2000) and Hassan and Ali (2014), who found that more water leads to better growth and better outcomes compared to lower levels. Higher yield may be due to the full application of water to bush tea, which promotes vegetative growth, yield, and quality (Hassan and Ali, 2014). This result could have been attributed to the volume of water consumed by bush tea plants when exposed to 30% ET_c because WP depends on the amount of output (Ali and Talukder, 2008). Thus, the results of the current study are similar to the findings by Mabhaudhi et al. (2019), Mabhaudhi (2012) and Motsa et al. (2015).

Table 4.5: Yield and yield components of varying water treatments (100% ET_c, 30% ET_c, and control (no irrigation).

Water treatments (ET_c)	Yield (kg/ha)	Number of roots	Marketable roots (%)	WP (kg/m^3)
Control	12.12a	15.33a	13.33a	0a
30%	60.61b	32.67b	29b	95.62b
100%	95.62c	45.67c	41.67c	202.03c
LSD$_{(p < 5\%)}$	0.02	0.04	0.02	0.01

Note: Water productivity (WP) represents the total biomass. The yield, number of roots, marketable roots and WP values are statistically significant with p-values < 0.001, according to the LSD (5% level).

4.3.3 Elemental composition

Nutrient components of bush tea exposed to (30%, 100% ET_c, and control (stress) of the crop water requirements differed significantly ($P<0.05$). The highest nutrient compositions (calcium, copper, iron, potassium, magnesium, manganese, and zinc) were recorded under 30% ET_c, followed by 100% ET_c, with the lowest elemental composition recorded under control (stress) (Table 4.6). The study revealed that bush tea may generate more nutrients when exposed to 30% ET_c.

Table 4.6: Elemental composition of bush tea with varied water treatments (30% ET_c, 100% ET_c and control (no irrigation) of crop water requirements).

Water treatments (ET_c)	Ca (mg/L)	Cu (mg/L)	Fe (mg/L)	K (mg/L)	Mg (mg/L)	Mn (mg/L)	Zn (mg/L)
Control (no irrigation)	155a	0.52a	8.96a	134a	42a	1.19a	1.14a
100%	359a	0.59a	10.41a	669a	215a	2.4b	1.88b
30%	731a	0.72a	16.97b	1512b	608b	3.61c	2.6c
LSD $_{(P=0.05)}$	0.20	0.31	0.01	0.01	0.003	0.005	0.05

4.3.4 Nutritional water productivity

For the micronutrients and macronutrients (calcium, copper, iron, potassium, magnesium, manganese, and zinc) examined in this study, nutritional water productivity changed considerably ($P<0.05$) between various water treatments (Table 4.7). Interestingly, high NWPca, NWPcu, NWPfe, NWPMn, and NWPZn were observed under 30% ET_c relative to 100% ET_c (Table 4.7). However, 100% ET_c high NWPk and NWPMg (Table 4.7). This only demonstrates that potassium and magnesium, which have a common function in the functioning and contraction of muscles, are stimulated extremely effectively with the full application of water (Mills et al., 2000; Maughan and Shirreffs, 2019). The results of the study concur with the observations by Sibiya (2015) and Mabhaudhi et al. (2019), who also used similar varying water treatments in maize and sweet potato production.

Table 4.7: Macro and micronutrients determined on a dry mass basis of bush tea under varying water treatments (30% ET_c, 100% ET_c and control (no irrigation) of crop water requirements).

Water treatment	**NWP_Ca (mg/L)**	**NWP_Cu (mg/L)**	**NWP_Fe (mg/L)**	**NWP_K (mg/L)**	**NWP_Mg (mg/L)**	**NWP_Mn (mg/L)**	**NWP_Zn (mg/L)**
Control (no irrigation)	0a	0a	0a	0a	0a	0.0a	0.0a
100%	72463b	119.67b	2104b	135228b	43b	484.2b	379.8b
30%	69014b	68.41b	1619c	143589b	5784b	343.7b	247.9c
LSD (P=0.05)	0.06	0.005	0.01	0.008	0.001	0.001	0.001

NWP_ micro and macro-nutrients (mg/L): This denotes the analysis of variance concentration of micro or macronutrients in parts per million/ milligrams per litre of water.

4.3.5 Multivariate data analysis

Principal component analysis (PCA) was performed for all metabolites in each varying water regime. PCA model (score), including tea tree extract, showed that PC1 and PC2 explained 38.7% and 20.8% of the variance, respectively (Figure 4.1). The PCA model showed a clear separation of the three different water states, as shown in Figure 4.1. This shows that there are significant differences in metabolic nutrients and their distribution in tea plant tissue in different water sources. This observation also reflects changes in the utilization of isolated metabolites (Ramabulana et al., 2020). Similarly, PCA score plots were computed and validated for bush tea plant extracts under varying water regimes, as indicated in Figure 4.1. The similarities of molecule groups were represented through score plots, as shown in Figure 4.1, which indicated the general clustering within the datasets of bush tea plant extracts under varying water regimes.

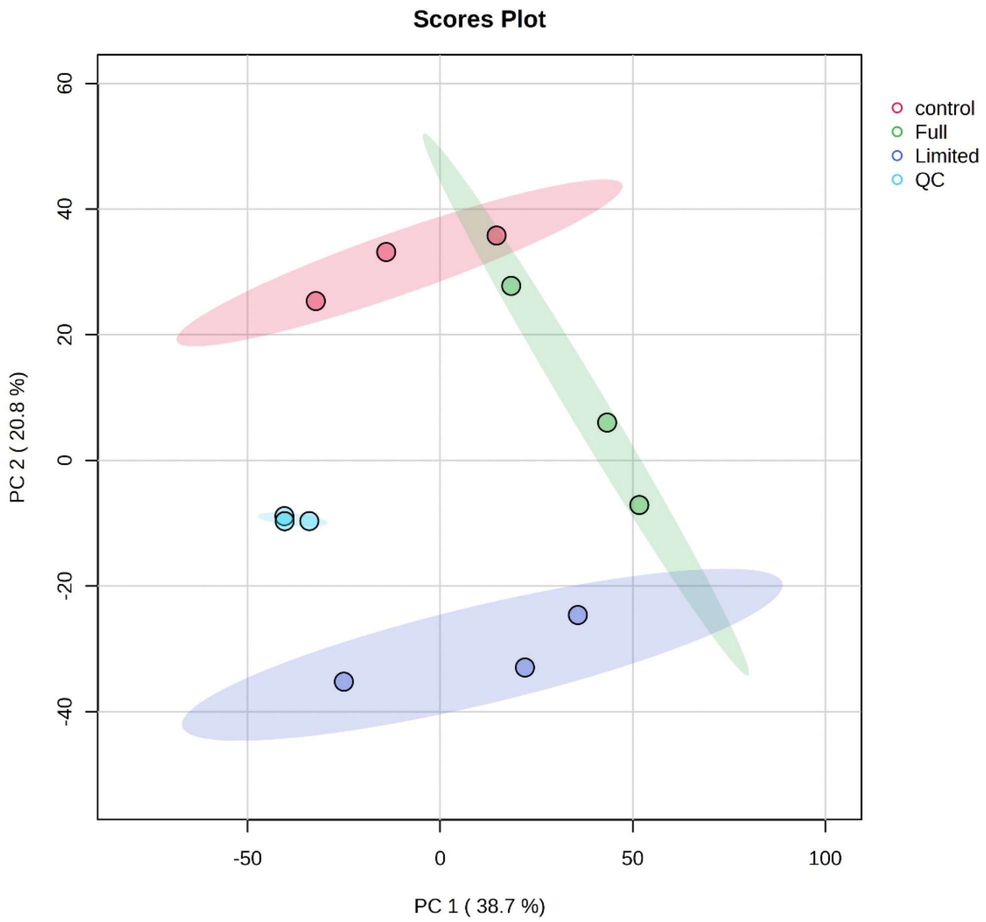

Figure 4.1: Multivariate data analysis of bush tea plant extracts under varying water regimes. A principal component analysis (PCA) scores scatterplot dataset obtained from LC-MS experiments with PCA 1 and PCA 2 explaining 38.7% and 20.8% of the variation indicated the general clustering within the datasets of bush tea plants of varying water regimes.

4.3.6 Molecular network

In negative MS mode, a significant number of metabolites were found. However, mass spectra were obtained in both ESI modes (+/-). Ultra-high performance liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-QTOF-MS/MS) base peak intensity (BPI) chromatography of bush tea leaves under varying water regimes contained 60 compounds, all were confirmed by their retention time, mass, and molecular formula as listed in Table 4.8. The presence and absence of specific metabolites revealed that different water treatments led to variable regulation of secondary metabolite formation in bush tea (Table 4.8). The identified chlorogenic acids (CGA) were found under varying water regimes with significant differences in retention time. The significant presence of chlorogenic acids (CGA) might be due to varying water regimes on bush tea plants. Our findings support prior findings by Zhang et al. (2018) that all herbal teas contain multiple bioactive compounds, including chlorogenic acids (CGA). Similarly, Meinhart et al. (2018) reported that chlorogenic acids (CGA) have various health benefits and can cure diabetes, cardiovascular disease, cancer, and obesity. This study is the first to show that different water regimes are vital for producing chlorogenic acids (CGA) in extracts of bush tea leaves.

4.3.7 Major chemical classes of bush tea metabolomics

Molecular networking (MN) was used to arrange MS/MS spectra into a network-shaped map based on spectral similarity, which shows structural similarity between molecules, as part of the data analysis workflow for untargeted MS/MS-based metabolomics studies (Ramabulana et al., 2021). The MS-Cluster algorithm was used for evaluating similarities between and within sample spectra (Frank et al., 2011). Ions were grouped into consensus spectra, which are represented as nodes within a predefined mass tolerance (Frank et al., 2011). Based on their similarity scores (cosine scores ≥ 0.7), structurally related compounds that have comparable gas phase chemistries were classified into molecular families. In the computed molecular networking (Figure 4.2), 1148 consensus spectra (nodes) were generated, of which 808 were clustered and grouped into 130 independent molecular families (with a minimum of two nodes connected by an edge) using GNPS spectral matching. Self-loop nodes at the bottom of the network were used to represent spectra that were not classified into molecular families. Figure 4.2 shows the computed MN; 81 of the nodes were allegedly annotated through an automated library spectral matching, providing some insight into the chemical identities of the bush tea leaf extracts but also alluding to the metabolome complexity of these plants and the lack of comprehensive spectral libraries. The study of the chemical interactions between each MS/MS spectrum and the visualization of the complete metabolome found in a sample revealed structurally linked molecular families in bush tea leaf extracts under varying water regimes.

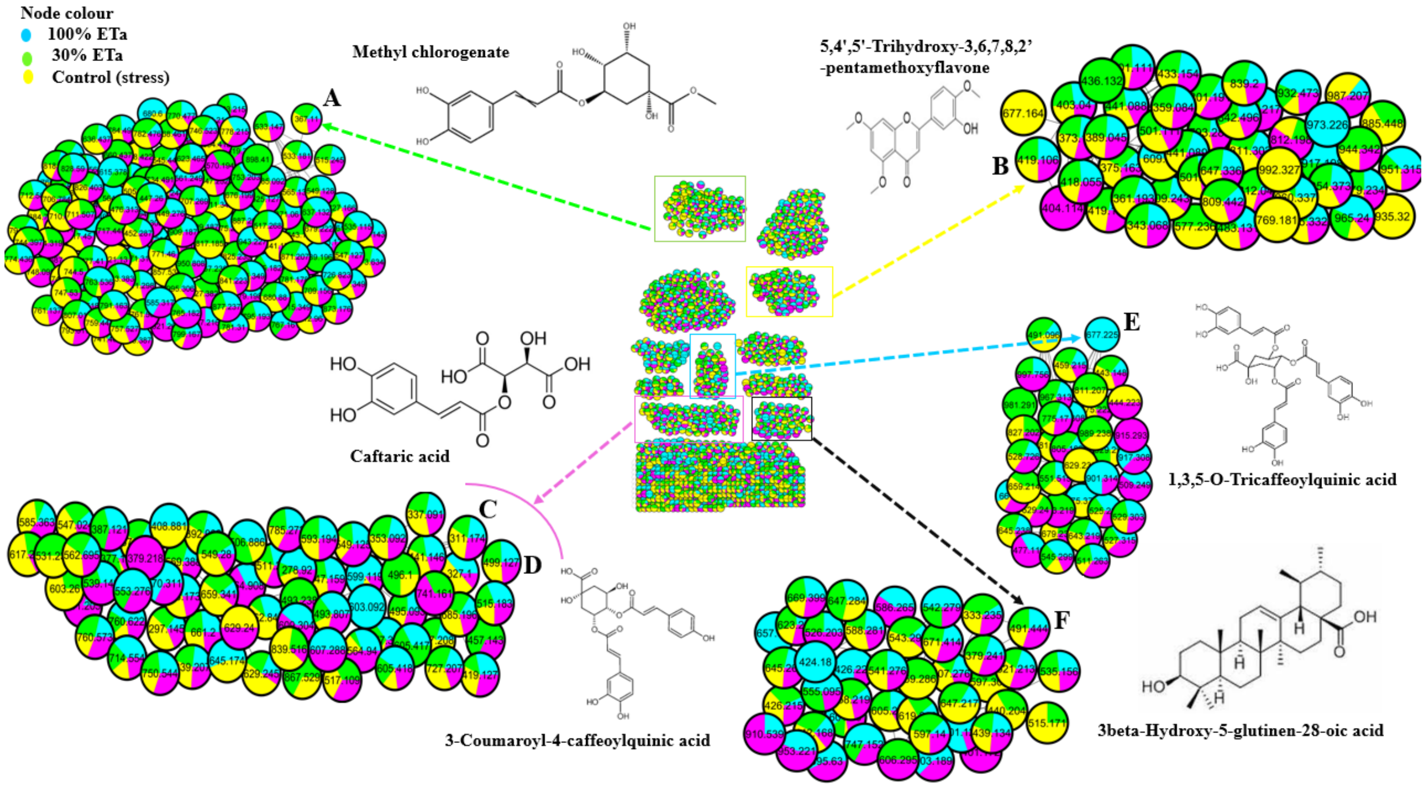

Figure 4.2: Molecular network of bush tea leaf extracts under varying water regimes analysed by liquid chromatography-tandem mass spectrometry using electrospray ionisation in negative mode, with a molecular family (centre). Identified highlighted nodes are bioactive compounds consisting of (A) methyl chlorogenate, (B) flavonoids, (C) tartaric acid, (D and E) caffeoylquinic acid, and (F) glutinane. Node colours represent the varying water regimes of bush tea leaf extracts and the respective MS2 spectral counts indicating the presence and absence of metabolites.

4.3.8 Impact of water on Communic acid

Communic acid compounds, are tiny amino acids called cysteine bioactive compounds that can be obtained in several organic foods, such as vegetables, grains, and legumes (McIntyre et al., 2022). However, it has not yet been identified in herbal teas. According to Table 4.8, a parent ion at m/z 301 and base peak fragments at m/z 164 and 225 are used to classify communic acid. These compounds were only detected under full water application. The results could suggest that the communic acid compound is present when the bush tea plant is irrigated fully compared to when the plant is stressed or when it is half irrigated.

4.3.9 Impact of water on Caftaric acid

Hydroxycinnamoyl-tartaric acid esters, which have been demonstrated to have a variety of positive health impacts, including antioxidant activities, are physiologically active substances (Mathatha et al., 2022). Based on the fragmentation patterns Rt described in Table 4.8 and the chemical structure as shown in Figure 4.2C, Caftaric acid was identified by its parent ion, [M-H]-, at m/z 311. Four tartaric acids were identified in all water treatments of bush tea leaf extracts. This could suggest that under different watering regimes, tartaric acid esters will still be biosynthesized in bush tea plants. These tartaric acids have not yet been identified in the leaves of bush tea.

4.3.10 Impact of water on Mono-Acyl Chlorogenic acids

Pei et al. (2016) found that the benefits of p-Coumaric acid include antioxidant, anti-inflammatory, and anti-cancer properties. The metabolites (7-11) with the molecular ion [M-H]-, at m/z 337, have been determined as 5-p-Coumaroylquinic acid based on their fragmentation patterns Rt, presented in Table 4.8. All these compounds were identified under varying water regimes. The results could reveal that the water levels that have been used in this study integrate bioactive compounds. These findings support earlier research by Ramphinwa et al. (2022b), who stated that bush tea contains these compounds in selected shades in relation to stress.

4.3.11 Impact of water on Caffeoylquinic acids

Caffeoylquinic acids are naturally occurring and are thought to be good for human health (Alcázar Magaña et al., 2021). Chan et al. (2010) and Genet (2020) reported that Caffeoylquinic acids are strong antioxidants with various beneficial effects. In this study, metabolites (12-25) were identified as caffeoylquinic acids by their parent ions (m/z 353) inferred from the fragmentation patterns Rt shown in Table 4.8. The caffeoylquinic acids were found to be present under all varying water regimes of bush tea leaf extracts. The findings may be used to demonstrate how water use, whether limited or extensive, as well as stressful environments, affect the build-up of caffeine-quininic acids. Interestingly, our results are consistent with earlier observations by Ramphinwa et al. (2022b), who reported that these compounds are present in bush tea plants under different shades in relation to stress.

4.3.12 Impact of water on Methyl chlorogenate acids

Methyl chlorogenate acids are a highly fascinating bioactive compound found in plants, with a biological function and acting as an antioxidant (Szymborska et al., 2022). Salem et al. (2022) and Zhang et al. (2018) reported that methyl chlorogenate has anti-inflammatory properties; however, it has not been identified in bush tea. Nonetheless, Methyl chlorogenate acids were identified by their parent ion, [M-H], at m/z 367, which fragmented to generate product ions at m/z 179, 119, 191, and 135, as presented in Table 4.8 and Figure 4.2A. According to the results of the current study, they are only present when the bush tea plant is irrigated with limited water (30% ET_a) because, under full application of water and water stress, these compounds were not present. This could suggest limited water as a recommended water application level in bush tea plants to synthesize methyl chlorogenate acid compounds. The current study is the first to demonstrate that the geometric isomers of methyl chlorogenate acids exist on bush tea leaf extracts under varying water regimes, where the 30% ET_a had the most significant effect compared to other water regimes.

4.3.13 Impact of water on flavonoids

Flavonoids contain several kinds of health properties, including anti-cancer and anti-inflammatory properties, and they reduce the risk of chronic diseases (Gutiérrez-Grijalva et al., 2017). Table 4.8 and Figure 4.2B display the impact of water on flavonoids such as 5,4',5'-Trihydroxy-3,6,7,8,2'-pentamethoxyflavone based on their parent ions at m/z 419 with the product ions at m/z 149 and 317. These compounds were detected only in 30% ET_a of bush tea leaf extracts. The results could suggest that when the bush tea plant is irrigated under limited water, it penetrates these compounds. Bush tea leaf extracts were found to contain 5,4',5'-Trihydroxy-3,6,7,8,2'-pentamethoxyflavone molecule for the first time.

Metabolites (28) and (29) were identified as 5'-Hydroxycudraflavone A, based on the parent ions at m/z 433, based on their fragmentation patterns Rt shown in Table 4.8. These 5'-Hydroxycudraflavone A derivatives were found to be only present under full water application of bush tea leaf extracts. The findings may show that these compounds won't be present in bush tea leaf extracts when there is limited water application and stress conditions.

4.3.14 Impact of water on triterpenoid and 3-Coumaroyl-4-caffeoylquinic acid

A pentacyclic triterpenoid was identified as the 3beta-Hydroxy-5-glutinen-28-oic acid. These compounds are derived from a natural anticancer botulin, which has pharmacological properties, including cholesterol reduction (Téné et al., 2021). Metabolites (30-31), based on their fragmentation patterns, Rt presented in Table 4.8, were discovered by their parent ion at m/z 491. Figure 4.2F shows the chemical structures in detail. Two of these molecules were identified in extracts of bush tea leaves with various water regimes. The current study's findings may indicate that different water treatments differentially regulate the formation of secondary metabolites in bush tea leaf extracts, as evidenced by the presence and absence of certain metabolites. The present investigation is the first to demonstrate that bush tea leaf extracts under various water regimes include geometric isomers of 3beta-Hydroxy-5-glutinen-28-oic acid.

Metabolites (32) were annotated as 3-Coumaroyl-4-caffeoylquinic acid (Table 4.8) and identified by a parent ion [M-H]- at m/z 499 with the chemical structure as illustrated in Figure 4.2D. According to Nunes et al. (2021), 3-Coumaroyl-4-caffeoylquinic acid exhibits antioxidants, and it is considered a beneficial compound for human health. This compound was only observed under stress conditions. The findings could show that these compounds can only be detected under zero water application because the absence of water will not limit the accumulation of these compounds on bush tea plants.

4.3.15 Impact of water on Feruloyl-Caffeoylquinic Acids

The feruloyl-caffeoylquinic acid is a soluble phenolic compound present in many angiosperms (Dias et al., 2012). Metabolites (46) were identified by their parent ion at m/z 529 with fragmentation ions at m/z 179 and 173, and the chemical structures, as shown in Figure 4.2. Feruloyl-caffeoylquinic acids were detected only under limited water application. These findings may be because of the limited water that the plant has received compared to the stress and full application. The current study is the first to detect feruloyl-caffeoylquinic acids in bush tea leaf extracts under varying water regimes. However, it has been identified in other medicinal plants (Ramabulana et al., 2020).

4.3.16 Impact of water on Tri-Caffeoylquinic Acids

Tri-caffeoylquinic acids are specialized bioactive metabolites and are protective against biotic and abiotic stress in plants. (Uleberg et al., 2022). Based on their fragmentation patterns Rt, Table 4.8 displays the identified tri-caffeoylquinic acids with parent ions [M-H]-, at m/z 677, and their chemical structures are shown in Figure 4.2E. On bush tea leaf extracts, these tri-caffeoylquinic acid derivatives were only found to be present when they were grown under water stress. These results might suggest that tri-caffeoylquinic acids enable bush tea plants to adapt under stress conditions with zero application of water. This study is the first to indicate the presence of tri-caffeoylquinic acids on bush tea leaf extracts under varying water regimes.

4.3.17 Impact of water on di-caffeoylglucosides and di-caffeoyl glucarate acid

Caffeoylglucosides are a powerful antioxidant and are used to treat cancer (Ștefănescu et al., 2019). Based on their fragmentation patterns Rt shown in Table 4.8 with varied intensities, thirteen compounds containing a precursor ion, M-H]-, at m/z 515 were recognized as di-caffeoylquinic acid (33-45) under all the different water regimes utilized in the investigation. The findings could indicate that bush tea accumulates these naturally occurring compounds even in the absence of water, under restricted water, and under full water application. These findings are similar to those reported by Ramphinwa et al. (2022a,b), who discovered the same mass to charge 515 on bush tea plants in relation to stress. Di-caffeoyl glucarate acid (47-57), which was identified by its precursor ion [M-H]- at m/z 533 and based on its fragmentation patterns Rt shown in Table 4.8, was identified using a similar technique under all varying water regimes of bush tea leaf extracts. A similar identification of these compounds supports Ramphinwa et al. (2022b), who identified m/z 533 on bush tea plants to stress.

Table 4.8: Classification of various bioactive compounds consisting of derivatives of quinic acid (QA), flavonoids, hydroxycinnamic acid (HCA), and tartaric acid from bush tea leave extracts under varying water regimes of bush tea leaf extracts

No	Mass to charge (m/z)	Retention time (mn)	Fragmentation ion	Molecular formula	Compound name	Water treatments (ET_a)		
						30%	100%	Control (no irrigation)
1	301,2226	21,494	164, 225	C20H30O2	Communic acid		•	
2	311,039	16,745	179, 135	C13H12O9	Caftaric acid	•		
3	311,039	10,54	187, 231, 267	C13H12O9	Cis-Caftaric acid	•		
4	311,035	10,318	132, 135	C13H12O9	Caftaric acid			•
5	311,039	10,491	129, 191, 209	C13H12O9	(-)-3,5-Dicaffeoyl quinic acid	•	•	•
6	311,038	16,348	163	C13H12O9	Caftaric acid	•	•	•
7	337,0988	13,311	143,191, 209	C16H18O8	4-p-Coumaroylquinic acid		•	
8	337,0991	5,42	191	C16H18O8	5-Coumaroylquinic acid	•		
9	337,0992	2,188	153, 191	C16H18O8	5-p-trans-Coumaroylquinic acid	•		
10	337,0992	8,103	138, 153, 191	C16H18O8	5-p-Coumaroylquinic acid			•
11	353,0942	7,02	191	C16H18O9	Caffeoylquinic acid		•	

12	353,0942	5,515	191, 175, 173, 135	C16H18O9	3-O-Caffeoylquinic acid	•		
13	353,0945	7,049	353, 191, 179	C16H18O9	4-Caffeoylquinic acid	•		
14	353,0946	5,144	191,129, 209	C16H18O9	3-O-Caffeoyl-muco-quinic acid			•
15	353,0945	4,945	191	C16H18O9	trans-4-Caffeoylquinic acid	•	•	•
16	353,0946	4,747	191	C16H18O9	(+)-5-Caffeoyl quinic acid	•	•	•
17	367,08	3,694	119, 191, 135	C17H20O9	Chlorogenic acid methyl ester	•	•	•
18	419,1063	13,419	149, 317	C20H20O10	5,4',5'-Trihydroxy-3,6,7,8,2'-pentamethoxyflavone	•		
19	433,1222	14,441	133, 161, 241	C25H22O7	5'-Hydroxycudraflavone A		•	
20	491,3500	24,423	116, 299, 433	C33H48O3	3beta-Hydroxy-5-glutinen-28-oic acid		•	
21	491,3508	24,336	152, 279	C33H48O3	3beta-Hydroxy-5-glutinen-28-oic acid	•	•	•

22	499,1346	10,2	353, 337, 191, 357	C25H24O11	3-Coumaroyl-4-caffeoylquinic acid			•
23	515,1295	20,345	191, 355, 533	C25H24O12	Dicaffeoylquinic acid		•	
24	515,1290	9,781	353, 191	C25H24O12	Dicaffeoylquinic acid 1	•		
25	515,1302	9,732	353, 191	C25H24O12	Dicaffeoylquinic acid 1			•
26	515,1296	9,723	133, 161, 191, 353	C25H24O12	Dicaffeoylquinic acid 1			•
27	515,1299	9,269	191, 353, 417	C25H24O12	Dicaffeoylquinic acid	•	•	•
28	515,1295	9,381	129, 191, 357	C25H24O12	Dicaffeoylquinic acid 1	•	•	•
29	529,13	17,39	179,173	C26H26O12	Dicaffeoylquinic acid 1	•		
30	533,1042	5,033	108, 167, 191	C24H22O14	Dicaffeoylquinic acid 1		•	
31	533,1042	8,329	191	C24H22O14	Dicaffeoylquinic acid 1	•		
32	533,1045	7,051	371, 209, 173, 135	C24H22O14	Dicaffeoylquinic acid 1			•
33	533,1045	9,091	191, 375	C24H22O14	Di-caffeoyl glucarate (VII)	•	•	•
34	677,16	10,44	191, 353	C34H30O15	Tricaffeoylquinic acid 1			•
35	677,12	23,199	255, 329	C34H30O15	Tricaffeoylquinic acid 1			•

*Shaded squares indicate the presence of metabolites under varying water regimes of bush tea leaf extracts.

4.4 Conclusions and recommendations

Varying water regimes affect the physiological parameters of bush tea under a controlled environment. Gas exchange and chlorophyll fluorescence measurements were recorded higher in bush tea plants exposed to 30% ET_c compared to other treatments, while 100% ET_c recorded a higher yield. Therefore, households in water-scarce regions may grow bush tea without compromising its nutritional benefits. However, full application yielded more compared to other water treatments. The horticultural practices of using 30% ET_c of crop water requirements caused a larger buildup of several bioactive compounds as compared to water stress and full application. Therefore, we recommend limited water application and water stress to enhance bioactive compounds in bush tea production. However, prospects led to investigations of more varying water regimes on maturity stages, nutritional water productivity, nutrient content, and phytochemical analysis on the stem and roots of bush tea extracts.

5 EVALUATION OF BUSH TEA (*ATHRIXIA PHYLICOIDES* DC.) GROWTH, DEVELOPMENT, NUTRITIONAL WATER PRODUCTIVITY AND QUALITY IN RESPONSE TO VARYING WATER REGIMES

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5.1 Introduction

Bush tea (*Athrixia phylicoides* DC.) is a perennial, aromatic, indigenous shrub cultivated in South Africa, well-regarded for its traditional use as an herbal tea and medicinal herb (Lerotholi et al., 2017; Tshikhudo et al., 2019). The plant is known to possess pharmacological properties, and its leaves and stems are rich in bioactive compounds that hold significant medicinal value, including the treatment of heart disease, headaches, and infected wounds (Mudau et al., 2007b; Lobo et al., 2010). Additionally, its compounds have been associated with potential benefits in managing high blood pressure, infected throats, and blood purification (Rakuambo et al., 2009). Notably, the edible parts of the plant contain notable levels of polyphenols, tannins, antioxidants, quercetin, and flavonoids, contributing to its therapeutic significance (Tshivhandekano et al., 2018). Despite its valuable attributes, bush tea plants are still primarily gathered from the wild, prompting efforts for their domestication and commercialization (Reichelt et al., 2012; Lerotholi et al., 2017).

Current research has emphasized the influence of cultural practices, mineral nutrition (Mudau et al., 2007b; Tshivhandekano et al., 2018), pruning (Mohale et al., 2018), and environmental conditions (Tshivhandekano et al., 2013; Ramphinwa et al., 2022b) on the growth, development, and quality of the bush tea plant. Bush tea has exhibited some water stress tolerance compared to other tea cultivars. Hence, the investigation into the impact of varying water regimes on its production and quality remains limited (Ponmurugan et al., 2016). Water is considered a crucial resource promoting vegetative growth and reproductive stages, particularly in arid and semi-arid regions, where fluctuations in rainfall can significantly affect plant growth and quality (Rostamza et al., 2011; Chivenge et al., 2015). Prior research has highlighted the influence of water stress on various plant species, indicating its significance in plant biomass, leaf area, and secondary metabolite production (Chiappero et al., 2019; Gatabazi et al., 2019).

Environmental conditions significantly impact the growth and development of bush tea. Furthermore, Ramphinwa et al. (2022a) found that the application of shade nets in tea cultivation areas has been documented to enhance tea leaf quality through increased amino acid concentration, decreased catechin content, and prevention of flavonoid accumulation, ultimately leading to improved plant growth and productivity. Bush tea is harvested from the wild, and while the domestication of this crop is now critical due to its commercial potential, the investigation into the impact of varying water regimes on its growth, development and yield is imperative. Therefore, the main objective of this study was to assess the response of bush tea to different water regimes, particularly focusing on plant growth, development, and yield.

5.2 Materials and methods

5.2.1 Plant material, site description and experimental design

A field experiment was conducted from the spring to summer season in the year 2022 at the Controlled Environment Research Unit (CERU), located at the University of KwaZulu-Natal (UKZN) in Pietermaritzburg, South Africa (29˚37' S; 30˚16' E). The region experiences an annual rainfall of 400 mm, with 45% occurring during rainy days from June to August, typical of its humid, sub-tropical climate. Temperature fluctuations are significant, ranging from a maximum of 33 °C during summer to a minimum of around 26 °C in winter, as detailed in Table 5.1. The soil from the experimental site was sampled randomly at the beginning of the trial from a depth of 0-30 cm using a soil auger. Thereafter, the soil samples were submitted to the Institute for Commercial Forestry Research (ICFR) Analytical and Research Laboratory to determine chemical and physical properties. The type of soil at the experimental site is predominantly characterized as loam (Table 5.1). The bush tea stem cuttings used in the study were sourced from the Agricultural Research Council (ARC) in Nelspruit, Mpumalanga province, South Africa (25˚.4518' S, 30˚.9697' E). Thereafter, two propagation media, sand and pine bark, were randomly assigned to seedling trays with 5 x 3 x 4.5 cm (width, breadth, and depth) cells. Cuttings were treated with Seradix® No.2 and planted in trays with a 2:1 ratio of pine bark to sand, each containing 45 cuttings. The trays were initially placed in shade netting and later transferred to a greenhouse, with irrigation adjusted based on solar radiation and plant size to maintain soil moisture. Bush tea plants were transplanted to the experimental field 80 days after the cuttings were set (Maedza, 2015).

Table 5.1: Physical and chemical characteristics of soil in the field

Clay (%)	Organic C (%)	pH (KCl)	P (cmol/kg)	Ca (cmol/kg)	K (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	N (%)
21.1	2.70	5.20	15.75	8.16	0.68	1.49	0.05	0.19

The experiment was laid out in a Complete Randomised Block Design (CRBD) with three treatments (100%, 30%, and control (stress) of crop water requirement (ET_a)), replicated three times. The total plot area was 100 m^2, and the plant population was kept at 6944 plants/ha. The size of the individual plots was 10 m × 10 m. Each plot consisted of two plant rows, 1.2 m and 0.75 m (inter- and intra-row spacing), giving a total of 12 plants per plot. Weeding was done by hand before crop establishment and then weekly until the crop reached full maturity. The irrigation schedule was determined by the daily crop water requirements from the product of normal tea (unshaded) crop factors (K_c), according to Singh (2013) (Table 5.2). The monthly average reference evapotranspiration (ET_o) was determined daily throughout the experimental period, enabling an accurate, timely response to the plant's water needs. The on-site automatic weather station of the Discipline of Agrometeorology was used to collect the evapotranspiration reference data. All treatments were irrigated to reach field capacity before starting the treatments. This practice was adopted to minimize potential losses from evaporation and drainage and to ensure that the soil maintained adequate moisture levels during the critical peak periods of the day. As a result, the crop water requirement ET_a was calculated using Eq. 5-1 per Allen et al. (1998):

$ET_c = ET_o \times K_c$ Equation 5-1

Table 5.2: Crop water requirements of bush tea in response to varying water regimes

		ET_o	**ETc**	**Duration**	**Total water applied**
	K_c	mm	mm	Days	mm
Initial	0.95	10.2	9.96	40	408
Mid-season	1.00	8.61	8.61	61	525.21
Late season	1.00	12.2	12.2	28	341.60
water applied (ET_a)					
• **100%**					1274.81
• **30%**					382.44
• **0%**					0

K_c = crop factor based on Singh (2013), ET_o = reference evapotranspiration, ET_c = crop water requirement, which was measured during the spring to summer season of the year 2022.

5.2.2 Data collection

Weather data was obtained from an automated weather station (AWS) using an honest observer by onset (HOBO) data logger sensors (Onset Computer Corporation, USA) (Table 5.3). The data was measured during the spring and summer cropping season, from 30th August to 1st November 2022. Daily meteorological data considered included minimum (T_{min}) and maximum (T_{max}), air temperature (˚C), solar radiation, relative humidity, rainfall and reference evapotranspiration (mm) was calculated using the Food and Agriculture Organization (FAO) calculator. The soil water content was determined through gravimetric sampling. Six soil samples were collected at a depth of 30 cm from all water treatments in each plot and sealed in zip-lock bags to prevent moisture loss. Subsequently, the gravimetric water content using Eq. 5-2:

$(\boldsymbol{\theta g}) = (\frac{\theta wet - \theta dry}{\theta dry}) \times \mathbf{100}$ % Equation 5-2 where:

Θ_g = Gravimetric moisture content (%),

Θ_{Wet} = wet soil (g) and

Θ_{Dry} = dry soil (g).

Eq. 5-3 was used to calculate volumetric water content from gravimetric water content:

$$(\boldsymbol{\theta v}) = \boldsymbol{\theta g} \times (\frac{Psoil}{Pwater}) \qquad \text{Equation 5-3}$$

where:

Θ_v = Volumetric moisture content (%),

Θ_g = Gravimetric moisture content (%),

P_{soil} = the bulk density of that given soil (g/cm^3) and

P_{water} = water density (g/cm^3).

Crop water use was then determined using Eq. 5-4.

$$\boldsymbol{ET}\mathrm{c} = \boldsymbol{P} + \mathrm{I} - \mathrm{RO} - \mathrm{D} \pm \Delta\boldsymbol{SWC} \qquad \text{Equation 5-4}$$

where:

ET_a = actual evapotranspiration (mm), P = Precipitation (mm), I = irrigation (mm), RO = runoff (mm), D = drainage and ∆SWC = changes in soil water content (mm). As the field is flat (slope less than 3) and irrigation was in split events, RO and D were assumed to be zero.

Table 5.3: Rainfall and temperature data for the duration of the experimental period from the UKZN CERU field

	June	**July**	**August**	**September**	**October**	**November**
Rainfall mm	11	9	30	42	95	110
$Temp_{min}$ (°C)	10	9	30	11	13	16
$Temp_{max}$ (°C)	22	24	26	28	23	25

$Temp_{min}$ and $Temp_{max}$ represent the minimum and maximum temperatures, respectively, recorded during the growing season.

5.2.3 Physiological measurements

Leaf gas exchange and chlorophyll measurements were measured using LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) integrated with an infrared gas analyser (IRGA) attached to a leaf chamber fluorometer (LCF) (640040B, 2 cm2 leaf area, Licor Bioscience, Inc. Lincoln, Nebraska, USA). Measurements were taken on the third half-fully formed leaf from the plant's tip between 08.30 and 11.30 a.m. by clamping the leaf inside the sensor head, with analysis conducted from the vegetative stage to harvesting (Figure 5.1), with three plants from each treatment, replicated three times.

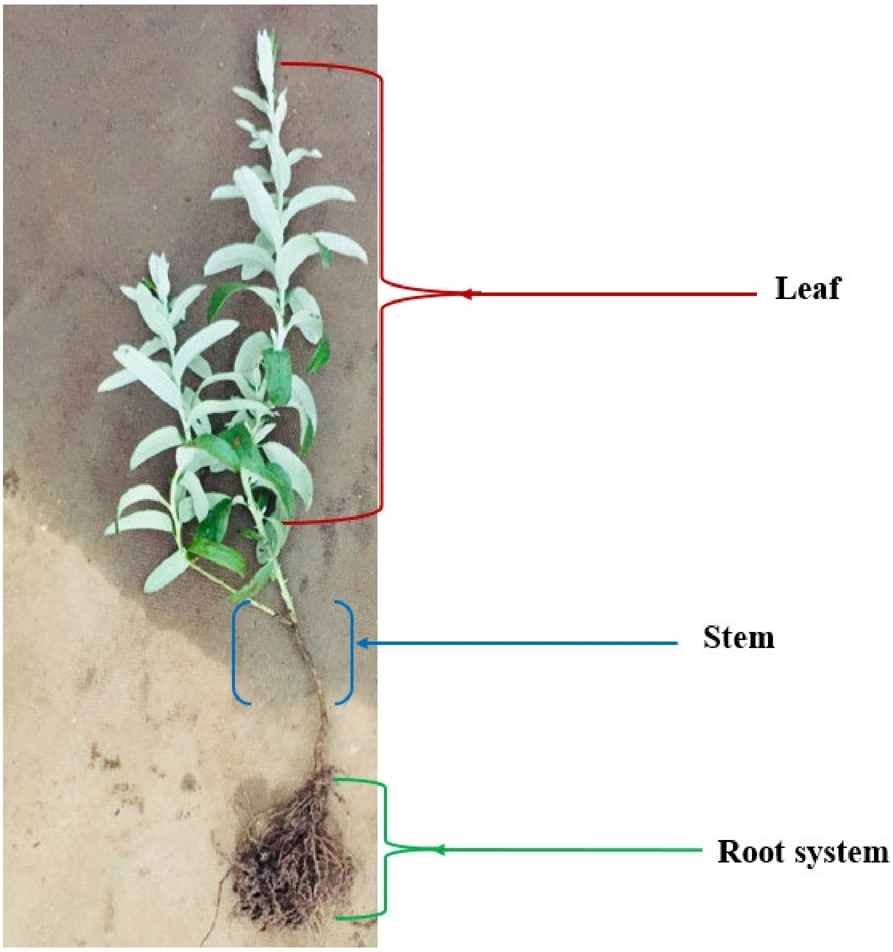


Figure 5.1: Bush tea plant at harvest.

5.2.4 Yield

The assessment of yield included the measurement of biomass, with the collection of bush tea samples taking place 119 days after planting. A comprehensive assessment of bush tea yield components was conducted by sampling three plants for each treatment. The measurements recorded on a plot basis included the yield, focusing on the fresh above-ground mass.

5.2.5 Determination of water productivity

Water productivity was calculated using Eq. 5-5:

$$WP = Y_a / ET_c \qquad \text{Equation 5-5}$$

where: WP is water productivity (kg/m^3), Y_a is actual yield, ET_c is the water applied based on crop water requirement.

5.2.6 Determination of nutritional composition

The aerial parts of the bush tea plant were freeze-dried using a model RV3 vacuum freeze drier (Edwards, United States of America) immediately after yield determination to preserve nutrients and avoid further metabolic reactions. Thereafter, samples were ground using mortar and pestle and analysed for nutritional content. The nutrients analysed per dry matter basis included macro-nutrients (calcium, magnesium and potassium) and micro-nutrients (copper, zinc, iron, manganese, and sodium).

5.2.7 Determination of nutritional water productivity (NWP)

Nutritional water productivity was calculated using Eq. 5-6:

$$NWP = (Y_a / ET_c) \times NC \qquad \text{Equation 5-6}$$

where: NWP is the nutritional water productivity (nutrition m^{-3} of water evapotranspired), Y_a is the actual harvested yield (kg/ha), ET_c is the water applied based on crop water requirement (m^3/ha), and NC is the nutritional content (kg^{-1}).

5.2.8 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using GenStat® version 20 (VSN International, UK). The least significant difference (LSD) was used to separate means at the 5% significance level.

5.3 Results and discussion

5.3.1 Soil water content

The data in Figure 5.2 displays the gravimetric sampling of soil water content (SWC) under different water regimes (ET_c levels) over five weeks. The results illustrate significant differences in soil moisture levels among the water treatments. Notably, the 30% ET_c water treatment exhibited the highest SWC, peaking at 15% during the study period, followed by the 100% ET_c treatment with a maximum of 12% and the control group with a peak SWC of 9%. These findings underscore the critical role of soil water content in influencing various aspects of plant development, productivity, and soil temperature, as well as the overall water dynamics within the soil (Deutsch et al., 2010). Moreover, the presence of higher moisture content in the unsaturated root zone indicates that bush tea plants prefer moderately moist soil conditions rather than excessively wet environments (Choi and Jacobs, 2007). These observations may be attributed to the complex interplay of factors such as soil texture and pore size distribution, which impact water depletion rates in different plant species (Gatabazi et al., 2022).

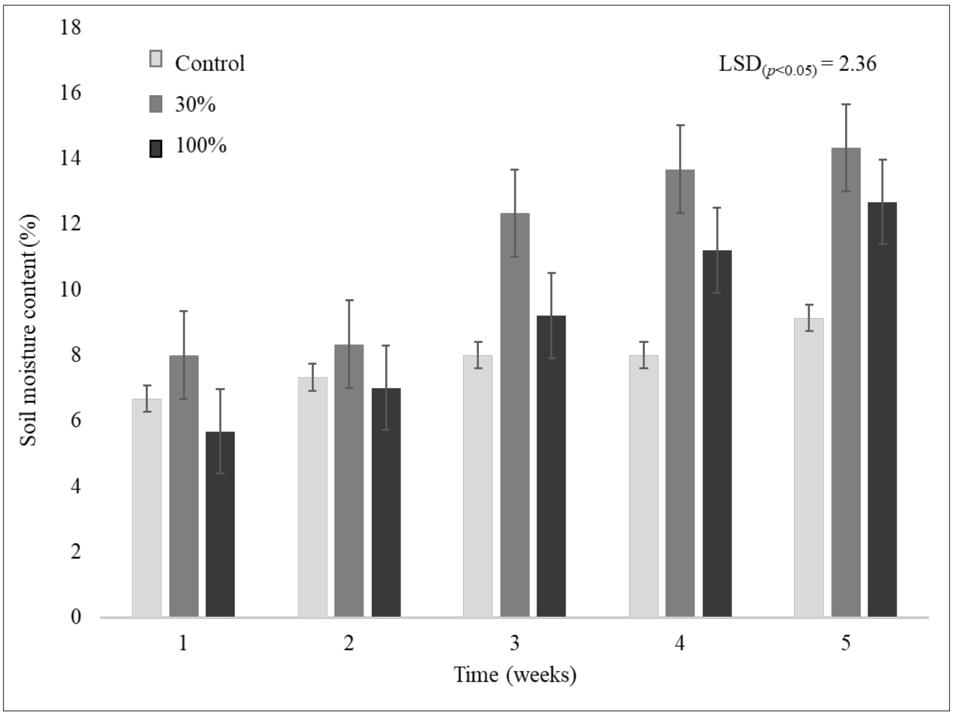


Figure 5.2: Soil water content (%) under varying water regimes of bush tea over five weeks.

5.3.2 Yield

The study revealed significant differences in yield parameters, specifically in yield and water productivity, among varying water treatments for bush tea plants (Table 5.4). The highest recorded yield was observed in the 100% water treatment, amounting to 259.1 kg/ha, surpassing the 30% treatment, which yielded 171.2 kg/ha. Conversely, the control treatment exhibited the lowest yield at 68.2 kg/ha. This reduction in yield under the control treatment could be attributed to water stress, a known factor limiting plant growth and crop yield across various agricultural settings (Gatabazi et al., 2015). These findings align with the observations made by Keshavarz Mirzamohammadi et al. (2021), who noted that water stress during the vegetative phase can impede cell division and expansion, ultimately leading to reduced plant growth and development.

Additionally, water-deficient plants tend to close stomata and restrict nutrient uptake in the root system, culminating in decreased dry matter accumulation (Keshavarz and Khodabin, 2019). Furthermore, drought-induced reductions in the leaf area index (LAI) during the growing season can limit biological yield by diminishing photosynthetic rates (Yang et al., 2018). In terms of water productivity, water treatments also exerted a significant influence ($P < 0.05$). The 100% water treatment exhibited the highest water productivity, with a yield of 570.5 kg/ha, outperforming other treatments. These results are consistent with those presented by Rahil (2022), who emphasized the direct relationship between the volume of water applied and the observed results. This outcome was likely influenced by the full application of water in the 100% treatment. The current study's results are in line with those reported by Mabhaudhi et al. (2019) and Motsa et al. (2015), who employed similar water treatments in their research on sweet potato production.

Table 5.4: Yield components of bush tea under varying water treatments (30% ET_c, 100% ET_c and stress)

Water treatments (ET_c)	**Yield (kg/ha^1)**	**WP (kg/m^3)**
Control (stress)	68.2a	0a
30%	171.2b	0.571b
100%	259.1c	0.259c

Note: Water productivity (WP) represents the total biomass, including leaves. The yield and WP values are statistically significant, according to the LSD (5% level).

5.3.3 Nutritional composition

The results indicated that the varying water regimes significantly influenced the nutritional composition of bush tea plants. Notably, the 30% ET_c treatment demonstrated the highest nutritional composition, with increased levels of essential elements such as calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), and zinc (Zn) compared to the 100% ETc and water stress treatments (Table 5.5). The observed lower nutritional composition under water stress conditions could be attributed to the adverse effects of drought on nutrient uptake in plants, resulting in altered ion concentrations within plant tissues (Corell et al., 2012). These findings are consistent with the research of Gunes et al. (2006), who similarly reported decreased nutrient uptake and mineral nutrient concentrations in crops under drought stress conditions. In addition, the optimized water treatment level is approximately 88.09% ET_c. This means that, according to the quadratic regression model, the nutritional composition of bush tea (specifically, calcium concentration in parts per million) is predicted to be maximized when the water treatment level is around 88.09% of the reference evapotranspiration (ET_c) rate.

Table 5.5: Nutritional composition of bush tea under three water treatments (30% ET_a, 100% ET_a and stress)

Water treatments (ET_c)	Ca (mg/L)	Cu (mg/L)	Fe (mg/L)	K (mg/L)	Mg (mg/L)	Mn (mg/L)	Zn (mg/L)
Control (stress)	127.3a	0.207a	9.60a	137a	43.4a	2.133a	2.173a
30%	624.7c	2.433c	18.07c	1175.3c	817.0c	10.20c	7.933c
100%	422.0b	1.523b	13.60b	870.0b	501.0b	4.803b	3.40b
LSD$_{(p<0.05))}$	4.5	0.23	2.3	1.78	1.73	1.60	6.90

5.3.4 Nutritional water productivity

The results obtained from the study demonstrate a significant difference in the micronutrient and macronutrient parameters, including Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), and Zinc (Zn), under varying water treatments (Table 5.6). Notably, the NWP_Ca (78694 m-3) was found to be higher under the 30% ET_c water treatment compared to the other treatments. The optimised water treatment level is approximately 54.44% ET_c. This means that, according to the quadratic regression model, the nutritional water productivity of bush tea (specifically, calcium concentration in parts per million per unit of water) is predicted to be maximised when the water treatment level is around 54.44% ET_c rate. These results provide valuable insights into the relationship between water treatment levels and both the nutritional composition and nutritional water productivity of bush tea. They suggest that an optimal water treatment level exists for maximizing these parameters, emphasising the importance of water management in tea cultivation to achieve desired nutritional outcomes while optimising water use efficiency. Similarly, the NWP_K (1175.3 m^{-3}) was observed to be higher under the 30% ET_c treatment in comparison to the 100% ET_c (870.0 m^{-3}) and stress conditions (0 mg/L). The interaction between NWP and micronutrients and macronutrients in water treatments was also found to be significantly different (P < 0.01). These results can be attributed to the varying responses of plants to water stress, influenced by the intensity and duration of the stress (Hosseinzadeh et al., 2018). Additionally, the findings align with previous observations on yield, water productivity (WP), and nutritional content (Mabhaudhi et al., 2019), highlighting the potential of bush tea to provide nutrition even under limited water conditions.

Table 5.6: Nutritional water productivity of bush tea under three water treatments (30% ET_c, 100% ET_c and stress)

Water treatments	NWP_Ca (mg/L)	NWP_Cu (mg/L)	NWP_Fe (mg/L)	NWP_K (mg/L)	NWP_Mg (mg/L)	NWP_Mn (mg/L)	NWP_Zn (mg/L)
Control (stress)	0a	0a	0a	0a	0a	0a	0a
30%	78694c	317.9c	18.07c	1175.3c	817.0c	10.2c	1018.7c
100%	17296b	62.8b	13.60b	870.0b	501.0b	4.80b	789.7b
LSD (p<0.05)	5.68	6.01	2.0	1.78	1.63	1.60	6.90

Note: NWP_ micro and macro-nutrients (ppm/mg/L): This denotes the analysis of variance concentration of micro or macronutrients in milligrams per litre of water.

5.3.5 Leaf gas exchange and chlorophyll fluorescence measurements

The findings from this study reveal a significant effect of varying water regimes on the physiological parameters, including leaf gas exchange and chlorophyll fluorescence in bush tea (Table 5.7). Photosynthetic rates were significantly influenced by varying water regimes, showing distinct differences among the treatments. Notably, the 30%

ET_c treatment exhibited the highest rates, with photosynthesis values of 27.43 and 34.77 µmol CO_2 m^{-2} s^{-1} during weeks 1 and 2, respectively. Additionally, the 100% ET_c treatment displayed improved photosynthesis rates, recording values of 18.10 and 28.56 µmol CO_2 m^{-2} s^{-1}. In contrast, the control treatment exhibited the lowest photosynthesis rates, with values of 4.01 and 18.56 µmol CO_2 m^{-2} s^{-1} for weeks 1 and 2, respectively. These findings suggest that the 30% ET_c treatment provided the most conducive conditions for photosynthesis in bush tea, resulting in significantly higher rates compared to the control and 100% ET_c treatments. The results emphasise the plant's ability to efficiently utilise water, even under limited water conditions, thereby enhancing photosynthetic activity and fostering sustained growth and productivity.

Notably, the control treatment displayed significantly lower stomatal conductance (0.11 and 0.37 mmol m^{-2} s^{-1}) compared to the other water treatments. This reduction in stomatal conductance is often associated with water stress, which affects various physiological and metabolic processes, leading to decreased gas exchange and inhibited photosynthesis (Shao et al., 2008). These results align with previous observations on Citron melon production under stress conditions (Mandizvo et al., 2022), emphasising the impact of reduced stomatal conductance and transpiration rate on water conservation and plant physiological functioning (Mandizvo et al., 2021). Moreover, the control (stress) treatment exhibited high water use efficiency, indicating that it efficiently utilised water through stress mechanisms such as reduced gas diffusion and transpiration rate. The 30% ET_c treatment displayed the highest water use efficiency indices (WUEi) of 38.25 and WUEinst of 113.4 compared to the 100% ET_c treatment with WUEi of 34.19 and WUEinst of 107.2. This suggests that the 30% ET_c treatment effectively maintained tissue water status, minimised water loss, and produced stable yields, highlighting the potential of bush tea crops to thrive even with limited rainfall.

Furthermore, chlorophyll fluorescence measurements indicated a higher proportion of photosynthesis under the 30% ET_c treatment compared to other water treatments, which could be attributed to the relatively lower water received by the bush tea plants under stress conditions and full irrigation. The study also observed an increase in the alternative electron sink (AES) for all bush tea water treatments, with the 100% and

30% ET_a treatments showing a significantly higher increase in the alternative sink. These findings further support the idea that bush tea can thrive well under limited water conditions, even in water-scarce regions, as demonstrated in provinces like KwaZulu-Natal (Mudau et al., 2015).

Table 5.7: Analysis of variance showing mean squares and significant tests for leaf gas exchange and chlorophyll fluorescence measurements of bush tea under three water treatments (30% ET_a, 100% ET_a and control)

Leaf gas exchange measurements									
Source of variance	**d.f**	***gs***	***T***	***A***	***Ci***	***A/Ci***	***Ci/Ca***	***Wu_i***	***WUE_{inst}***
Water treatment	2	0.0672744**	99.730**	236.4336**	444986.0**	1.37390**	86.49**	54.6706**	512.482**
Time	1	0.1734605**	614.302*	1679.5086**	628587.7**	103.43761**	789.04**	1232.8113**	14192.359**
Water treatment x time	2	0.0056122*	32.338*	70.7924**	24220.3**	1.25047**	75.24**	5.4087**	222.444**
Residual	12	0.0005902	2.505	0.4742	828.5	0.03739	12.44	0.9491	3.111
Leaf gas exchange measurements									
Source of variance	**d.f**	***Fo***	***Fm***	***Fv/Fm***	***$\Phi_{PS\,II}$***	***qP***	***qN***	***ETR***	
Water treatment	2	212670.4**	264909**	0.0364162**	39.9027**	6.9390**	0.85000**	3487.627**	
Time	1	395624.4**	2004*	0.0799728*	1120.0131**	1.6563**	1.21607**	11604621**	

Water treatment x time	2	55781.1*	584*	0.0142603*	3.4191**	0.1244**	0.84353**	1722146**
Residual	12	739.4	9033	0.0006282	0.3031	0.2717	0.01148	16521

d.f; degrees of freedom, gs; stomatal conductance, T; transpiration rate, A; net CO_2 assimilation rate, A/Ci; CO_2 assimilation rate/intercellular CO_2 concentration, Ci; intercellular CO_2 concentration, C i/C a; ratio of intercellular and atmospheric CO_2, WUEi; intrinsic water use efficiency, WUEins; instantaneous water-use efficiency, Fv/Fm; maximum quantum efficiency of photosystem Ⅱ photochemistry, ΦPSⅡ; the effective quantum efficiency of PSⅡ photochemistry, qP; photochemical quenching, qN; non-photochemical quenching, ETR; electron transport rate, ETR/A; relative measure of electron transport to oxygen molecules, * and ** denote significant at 5 and 1% probability levels, respectively, Ns; non-significant.

Table 5.8: Means of leaf gas exchange and chlorophyll fluorescence measurements of bush tea under three water treatments (30% ETa, 100% ETa and control)

Chlorophyll fluorescence measurements																	
Week 1	ET_a	***gs***	***T***	***A***	***C_i***	***A/C_i***	***C_i/C_a***	***WUEi***	***WUE_{inst}***	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***	***ETR/A***
	Control	0.11a	29.27a	17.64a	229.31a	1.73a	0.27a	17.47a	41.94a	2573.0a	2233a	0.05a	15.59a	0.03a	0.95a	533a	30.43a

	30%	0.38c	33.31c	29.57c	276.02c	3.43c	0.87c	21.63c	48.09c	2769.8c	2669c	0.30c	19.20c	2.20b	2.37a	981c	27.11c
	100%	0.27b	30.74b	22.79b	254.31b	1.93b	0.71b	20.04b	46.31b	2641.2b	2444b	0.16b	17.86b	1.23ab	1.23a	849b	37.30b
Chlorophyll fluorescence measurements																	
Week 2	ET_a	***gs***	***T***	***A***	***C_i***	***A/C_i***	***C_i/C_a***	***WUEi***	***WUE_{inst}***	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***	***ETR/A***
	Control	0.37a	35.86a	24.19a	462.82a	7.06a	8.50a	32.35a	84.33a	2055.50a	2264a	0.22a	29.69a	0.83a	0.99a	1050a	25.48a
	30%	0.53c	47.69b	36.18c	757.11c	7.17a	22.22c	38.25c	113.45c	2607.52c	2667b	0.78c	36.24c	2.94b	1.00b	3640c	76.49c
	100%	0.45b	45.59b	26.80b	661.03b	7.25a	10.85b	34.19b	107.21b	2431.40b	2478ab	0.35b	34.04b	1.50ab	0.99a	2491b	54.40b

d.f; degrees of freedom, gs; stomatal conductance, T; transpiration rate, A; net CO_2 assimilation rate, (μmol CO_2 m^{-2} s^{-1}), A/Ci; CO_2 assimilation rate/intercellular CO_2 concentration, Ci; intercellular CO_2 concentration, Ci/Ca; ratio of intercellular and atmospheric CO_2, WUEi; intrinsic water use efficiency, WUEins; instantaneous water-use efficiency, Fv/Fm; maximum quantum efficiency of photosystem Ⅱ photochemistry, ΦPSⅡ; the effective quantum efficiency of PSⅡ photochemistry, qP; photochemical quenching, qN; non-photochemical quenching, ETR; electron transport rate, ETR/A; relative measure of electron transport to oxygen molecules, AES; alternative electron sinks. Varying upper-case letters within a column indicates significant differences among water treatments.

gs; (mmol m^{-2} s^{-1}), T; (mmol H_2O m^{-2} s^{-1}), A; (µmol CO_2 m^{-2} s^{-1}), A/Ci; (µmol. mol m^{-1}), Ci; (µmol. mol m^{-1}), WUEi; (µmol (CO_2) m^{-2}); WUEins, (µmol. mol^{-1}), Fv/Fm; (ratio); ΦPSII, the effective quantum efficiency of PSII photochemistry; qP, photochemical quenching; qN, non-photochemical quenching; ETR, (µmol e^{-1} m^{-2} s^{-1}); ETR/A, (µmol e $µmol^{-1}$ CO_2).

5.4 Conclusions and Recommendations

The result of the study highlights the significant influence of different water regimes on the growth and yield of bush tea plants. The highest yield was observed in plants subjected to 100% ET_a, with a subsequent decrease in those under 30% ET_a and water stress treatments. Notably, the 30% ETa treatment positively impacted soil water content, nutritional composition, and water productivity, highlighting the importance of optimising water application in bush tea cultivation. Under control (stress) conditions, a decrease in stomatal conductance was noted, but water use efficiency increased, indicating the adaptability of bush tea to water-scarce environments. This emphasises the plant's resilience and potential to thrive in challenging conditions. The study underscores the crucial role of varying water regimes in influencing plant physiology, nutritional composition, leaf gas exchange, and chlorophyll fluorescence. The adaptability of bush tea to stress conditions suggests its potential as a valuable resource for food and nutritional security in arid regions. Future studies should delve into a more detailed analysis of nutritional water productivity and water use efficiency of bush tea. This is especially important under diverse water regimes and in varying environmental conditions. These in-depth investigations will contribute to a comprehensive understanding of the plant's adaptive mechanisms and shed light on its potential. Such insights are crucial for advancing sustainable agriculture practices and promoting further research in the realm of natural products, ultimately contributing to the broader field of agriculture and enhancing our understanding of the plant's potential applications.

6 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The result of the study highlights the significant influence of different water regimes on the growth and yield of bush tea plants. The highest yield was observed in plants subjected to 100% ETa, with a subsequent decrease in those under 30% ETa and water stress treatments. Notably, the 30% ETa treatment positively impacted soil water content, nutritional composition, and water productivity, highlighting the importance of optimizing water application in bush tea cultivation. Under control (stress) conditions, a decrease in stomatal conductance was noted, but water use efficiency increased, indicating the adaptability of bush tea to water-scarce environments. This emphasizes the plant's resilience and potential to thrive in challenging conditions. The results also showed that the 30% ETa enhanced plant growth, nutrient content, and nutritional water productivity compared to other water treatments. Nevertheless, 100% ETa yielded more (95.62 kg ha-1) than 30% ETa (60.61 kg ha-1) and control (12.12 kg ha-1). The chlorogenic acid accumulation was higher under 30% ETa compared to 100% ETa and control.

The research revolves around investigating the potential of varying water regimes to enhance bush tea's growth, development, and quality while facilitating its production for medicinal purposes. However, water resource scarcity has significantly impacted contemporary global concerns, particularly in Africa, as the region's population continues to grow (Kotir, 2011; Chappell and LaValle, 2011). Significant issues caused by population growth and climate change have increased pressure on food security, especially affecting countries with water problems, such as South Africa (Kotir, 2011). Although the South African state is committed to ensuring food security, food insecurity continues to increase in the country (de Cock et al., 2013; Chakona and Shackleton, 2019). As the problem of food security increases with diminishing resources and population growth (Chakraborty, 2011), small-scale agriculture is also increasing to meet the increasing demand (Lenkabula, 2006; Makunga et al., 2008).

Research on bush tea is considered a solution to the problem of malnutrition in agriculture (Chivenge et al., 2015; Mudau et al., 2022). This approach promises to improve current food practices. In countries facing water scarcity and malnutrition, as studied by Bain et al. (2013), bush tea cultivation turns out to be a beautiful plant with the potential to create new markets for the rural economy and contribute to the conservation of plant biodiversity and the search for plant-derived compounds for new medicines. It can also be a way to generate income for rural communities. The production of bush tea in SA is limited due to its growth, especially in tropical regions where water and soil fertility are inadequate (Mudau et al., 2022). However, it must be acknowledged that the availability of water and nutrients may cause problems in achieving good growth and good characteristics in bush tea. This study provides evidence of improved bush tea yield, NWP and nutrient yield, and nutrient water efficiency in water-limited regions. This study's main idea is that different water sources do not affect bush tea's growth, production, growth, yield, and nutritional content. To investigate this proposal, we conducted two experiments at the UKZN in 2022, one in a controlled environment and the other in a field environment.

The initial experiment, detailed in Chapter Four, was designed to assess the responses of physiological parameters, nutritional water productivity, and comprehensive quality analysis, including examining bush tea metabolites under different water regimes. Subsequently, the second experiment, presented in Chapter Five, aimed to assess plant growth and development, nutritional water productivity, and phytochemical analysis under varying water conditions for bush tea. The outcomes from both experiments consistently demonstrated that limited water application at 30% ET_a outperformed full water application at 100% ET_a and the control, which received no water (under water stress conditions). Nevertheless, it is noteworthy that the 100% ET_a treatment yielded satisfactory results, likely due to the substantial water input compared to the other water application levels. Furthermore, despite exhibiting reduced photosynthetic efficiency under water stress, bush tea is likely attributable to its natural habitat in arid environments (Mudau et al., 2022). As a result, the initial hypothesis was ultimately rejected.

Challenges:

The model aims in the objective

- The preliminary trials failed because they were conducted during the winter season; however, the trial was then set up in the spring and summer seasons and was successful.
- Transplanting shock occurs in winter; however, this experiment was conducted from spring to summer.
- Aphids, termites, and mealy bugs attacked the bush tea plant species in the controlled tunnel facility and the field experiments. Nevertheless, their population was effectively managed through weekly applications of insecticides.

6.2 Recommendations

The following recommendations are based on the findings made from the investigation:

- These findings offer valuable insights for future research, suggesting exploring diverse environments and seasons, both within and outside the province, for cultivating bush tea. This approach could improve its nutritional water productivity and overall yield. It would be interesting to investigate the interaction of bush tea with other herbal medicinal teas in future studies.
- Future studies should investigate the water utilisation and the water productivity of different medicinal plants since this study only focused on bush tea, both in a controlled environment and in a field experiment.
- To achieve a comprehensive understanding, future studies should consider exploring additional experimental variables, such as agronomic management practices like planting density. This will help in the scientific and systematic evaluation of their impact on the yield and quality of many bush tea varieties.
- Quantitative research of bush tea harvested under varying water regimes using comparative assessments through questionnaires in different locations to provide quality assessments.

- A molecular network on the stems and roots of bush tea is highly recommended to give more insight into the compounds available in bush tea plants under varying water treatments and in different locations and seasons.

The Literature Review (Chapter Two) showed that bush tea is one of the herbal teas with a significant number of nutrients (Olivier et al., 2012), which makes it a unique multi-purpose plant (Rakuambo, 2007). Its medicinal purposes make it an important plant to maintain during its growth stages to enhance its production (Lerotholi et al., 2017). Therefore, this study emphasised the measurements relating to its physiological (growth, development, and productivity) and its Nutritional Water Productivity (NWP), yield, and quality.

Chapter two provides a comprehensive overview of the insights offered by numerous researchers concerning herbal teas, with a primary focus on bush tea, to show the importance of cultivating bush tea under diverse conditions to stimulate its growth and enhance its quality within agricultural production. It also contributes to a deeper understanding of water utilization, yield potential, and nutritional attributes.

The cultivation of bush tea holds substantial promise for improving food production across much of sub-Saharan Africa while preserving its nutrient-rich composition. Chapter Four assessed the responses concerning bush tea's growth, development, productivity, yield, nutritional water productivity, and quality attributes. This evaluation involved the utilisation of varying water regimes within a controlled environmental facility. Findings show that water scarcity and stress impact plant growth, yield, and quality. In particular, bush tea growth and productivity were significantly affected compared to the optimal water conditions within a controlled environment. These results are also reflected in the growth and development pattern of the tea plant, which is more effective in unrestricted water, especially at 30% ET_c, compared to other water treatments. An additional noteworthy observation about the enhanced nutritional water productivity observed in bush tea under conditions of restricted water application, specifically at the 30% ET_c of crop water requirements. Drip irrigation effectively reduced soil evaporation losses due to its ability to get a smaller surface area. Considering that bush tea typically exhibits shallow root systems, adopting a regular irrigation schedule that ensures continuous moistening of the root zone can offer advantages. Consequently, the crop could access water within its root zone even under limited water availability. The investigation yielded evidence suggesting that implementing drip irrigation methods could enhance crop yields. Furthermore, the results indicated that a higher concentration of bioactive compounds was detected under limited water availability and water stress, as opposed to full water application. This observation underscores the potential for cultivating bush tea in regions characterised by water scarcity without encountering constraints related to nutrient availability. Another objective pursued in this study was the comprehensive assessment of bush tea's growth, development, productivity, nutritional water productivity, yield, and quality within field conditions, as detailed in Chapter Five.

The findings presented in this chapter demonstrate the presence of statistically significant distinctions between limited and full water application and rainfed conditions. These research outcomes, particularly concerning agronomic management practices, hold considerable potential for introducing novel crops into the prevailing agricultural system, thereby contributing to enhanced livelihoods. This initiative aims to address food insecurity and hunger challenges while enhancing the yield attributes and quality aspects of bush tea production, thus augmenting the overall value of food production in South Africa. Furthermore, the results unveiled a positive response of various identified compounds to limited water conditions and stress, exhibiting diverse mass-to-charge characteristics. Among these compounds are chlorogenic acids, flavanols, and terpenoids, all present in plants and possessing medicinal applications. These findings suggest that using these compounds can reduce malnutrition and mitigate food insecurity within the livelihoods of poor rural communities.

7 APPENDIX I: RESEARCH DISSEMINATION REPORT

A. Peer-reviewed articles (Total = 3)

1. Mudau FN, Chimonyo VG, Modi AT and Mabhaudhi T. 2022. Neglected and Underutilised Crops: A Systematic Review of Their Potential as Food and Herbal Medicinal Crops in South Africa. Frontiers in Pharmacology, 12, 809866. https://doi.org/10.3389/fphar.2021.809866
2. Rumani M, Mabhaudhi T, Ramphinwa ML, Ramabulana AT, Madala NE, Magwaza LS and Mudau FN. 2024. Response to Various Water Regimes of the Physiological Aspects, Nutritional Water Productivity, and Phytochemical Composition of Bush Tea (*Athrixia phylicoides* DC.) Grown under a Protected Environment. Horticulturae 10, 590. https://doi.org/10.3390/horticulturae10060590
3. Rumani M, Mabhaudhi T, Mandizvo T, Ramabulana AT, Madala NE, Ramphinwa ML, Magwaza LS and Mudau FN. 2024. Investigating the influence of varying water regimes on bush tea's growth and development and nutritional water productivity (*Athrixia phylicoides* DC.). Urban Agriculture & Regional Food Systems 9 (1): e20073. https://doi.org/10.1002/uar2.20073

B. Other publications

- **In preparation**

Author(s)	**Title**
Vhuhwavho Ndou, Tafadzwa Mabhaudhi, Mzamo Shozi, Tshepiso Papo, Maanea Ramphinwa Fhatuwani N. Mudau	Response of plant growth and quality of bush tea (Athrixia phylicoides) as affected by deficit irrigation and mulching

C. Conferences and Webinars

Presenters	Title	Event
Fhatuwani N Mudau Vhuhwavho Ndou, Tafadzwa Mabhaudhi, Mzamo Shozi, Tshepiso Papo, Maanea Ramphinwa	Response of plant growth and quality of bush tea (Athrixia phylicoides) as affected by deficit irrigation and mulching	Annual Conference 28 July - 1 August 2025, New Orleans, Louisiana

8 APPENDIX II: CAPACITY BUILDING

Provision was made in the budget to fund two full-time students over the project's three-year period. A summary of capacity building is presented in the following sections.

8.1 Post-graduate capacity building

The project has met the capacity development targets. The contractual obligation was to train two MSc students and one PhD (Cancelled the registration due to work commitments). The project engaged the following M.Sc students:

Ms Vhuhwavho Ndou (Submitted intention to submit)

Ms Rumani Muneiwa (Completed M.Sc Agric)

8.2 Project objectives

This study aimed to evaluate water use, nutritional water productivity (NWP), and the quality of bush tea in contrasting environments.

The following were the objectives of the project:

1. To determine the effect of different water levels on bush tea growth and productivity, water use, and nutritional water productivity under field and tunnel environmental conditions.
2. To determine the biochemical properties and phytochemical properties of bush tea.

8.2.1 General objective

To develop a framework for informing and guiding medicinal plant production, focusing on bush tea for poor rural communities.

8.2.2 Specific objective

Specific objective 1: To measure water use and nutritional water productivity of bush ta grown in contrasting environments.

Specific objective 2: To determine the pharmaceutical value of bush tea.

Specific objective 3: To model water use and map suitable areas for bush tea.

Specific objective 4: To formulate best management practices for maximising water use and nutritional value for bush tea.

All the contractually stated objectives were achieved and form the basis of this research report.

9 APPENDIX III: SUPPLEMENTARY MATERIALS

Supplementary Table 1: Search scope for Nutraceutical and pharmaceutical properties of neglected and underutilised crops

Query string	CODE	HITS		
Title, Abstract, Keywords		SCOPUS	WOS	PubMed
The terms nutraceutical OR pharmacological				
(nutraceutical OR pharmacological OR phytochemical OR pharmaceutical or medicinal)	#1	801 235	573 011	419 998
Nutraceutical and pharmacological characteristics				
("Anti- "AND (oxidant OR fungal OR bacterial OR viral OR mutagenic OR hepatoxic OR inflammatory OR histaminic OR immunomodulatory OR hypolipidemic OR diabetic OR convulsant OR carcinogenic OR hypolipidemic OR acetylcholinesterase OR neuropathic OR hypertensive OR analgesia OR malaria) OR (Lactogenic OR aphrodisiac OR diuretic OR hepatoprotective OR hypotensive OR carminative))	#2	805 921	337 149	466 116
(Antifungal OR antibacterial OR antiviral OR antimutagenic OR Antihepatoxic OR Anti-inflammatory OR Antihistaminic OR antiimmuno-modulatory OR antihypolipidemic OR antidiabetic OR anticonvulsant OR anticarcinogenic OR antihypolipidemic OR antiacetylcholinesterase OR antineuropathic OR antihypertensive OR antianalgesic OR lactogenic OR aphrodisiac OR diuretic OR hepatoprotective OR hypotensive OR carminative)	#3	958 943	527 836	547 661

(Anti-fungal OR anti-bacterial OR anti-viral OR anti-mutagenic OR Anti-hepatoxic OR Anti-inflammatory OR Anti-histaminic OR anti-immuno-modulatory OR anti-hypolipidemic OR anti-diabetic OR anti-convulsant OR anti-carcinogenic OR anti-hypolipidemic OR anti-acetylcholinesterase OR anti-neuropathic OR anti-hypertensive OR anti-analgesic OR lactogenic OR aphrodisiac OR diuretic OR hepatoprotective OR hypotensive OR carminative)	#4	578 390	272 043	248 294
Nutraceutical and Pharmaceutical characteristics [#2 OR #3 OR #4]	#5	248 028	822 204	752 433
Neglected and underutilised crops				
(indigenous OR neglected OR traditional OR orphan OR native OR underutili?ed OR future OR medicinal) PRE/2 crop*)	#6	5 222	3 998	406
Nutraceutical And pharmaceutical properties of NUS				
To address RQ1 -The mention of Nutraceutical and Pharmaceutical properties in NUS [#1 AND #6]	#7	361	364	60
To address RQ2 -Nutraceutical and Pharmaceutical characteristics in NUS [#5 AND #6]	#8	78	52	6
Combined search [#7 OR #8]	#9	384	383	62
Remove duplicates	339			
Removal of articles that were not in English	329			
Removal of articles where full texts could not be downloaded	223			
Removal of irrelevant articles (no explicit mention of Nutraceutical and Pharmaceutical properties of an underutilised crop)	106			
Total	106			

Supplementary Table 2: Scoring of pharmaceutical (P), nutraceutical (N), cultural (C), environmental (En) and economic (Ec) potential for neglected and underutilised functional medicinal crop species. The scores used were (*) low, (**) medium and (***) high

Common name	Scientific name	P[1]	N	C	En	Ec
Honeybush	*Cyclopia* (Vent.) *spp*	***	***	***	***	***
Bush tea	*Athrixia phylicoides* DC.	***	***	***	***	***
Tigernut	*Cyperus esculentus* (L.)	***	**	*	*	***
Ground Bean	*Macrotyloma geocarpum* (Harms) Maréchal & Baudet	**	***	*	*	**
Winged bean	*Psophocarpus tetragonolobus* (L.) D.C.	**	**	*	**	*
Sword bean	*Canavalia gladiate* (Jacq.) DC.	**	**	*	**	*
Sunn hemp	*Crotalaria juncea* (L.)	***	**	*	**	**
Lablab	*Dolichos lablab* (L.) *or Lablab purpureus* (L.) Sweet	***	**	*	**	***
Pigeon pea	*Cajanus cajan* (L.) Millsp.	***	**	*	**	***
Winged bean	*Psophocarpus tetragonolobus* (L.) D.C.	**	**	**	**	**
Bambara groundnut	*Vigna subterranea* (L.) Verdc.	**	***	**	**	***
Velvet bean	*Mucuna pruriens* (L.) DC. var utilis	**	**	*	**	*
Grass pea	*Lathyrus sativus* (L.)	**	***	*	*	*
Clusterbean	*Cyamopsis tetragonoloba* (L.) Taub.	**	**	*	*	*
Broad bean	*Vicia faba* (L.)	***	**	*	**	**
African yam bean	*Sphenostylis stenocarpa* (Hochst. ex A.Rich.) Harms	**	**	*	**	***
Black gram	*Vigna mungo* (L.) Hepper	**	**	*	*	*
Drumstick	*Moringa oleifera* (L.)	***	***	***	***	***

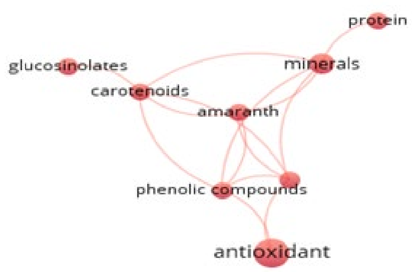
Baobab/ African Baobab	*Adansonia digitate* (L.)	***	***	***	**	**
Breadfruit	*Artocarpus altilis* (Parkinson) Fosberg	**	***	*	**	*
African fan palm	*Borassus aethiopum* (Mart.)	**	*	*	*	*
Blackberry	*Rubus fruticosus* (L.)	**	***	*	*	*
Mulberry	*Morus alba* (L.)	**	**	**	**	*
Physic nut	*Jatropha curcas* (L.)	***	***	*	*	*
Jackfruit	*Artocarpus heterophyllus* (Lam.)	*	**	*	***	*
Velvet tamarind	*Dialium guineense* (Willd.)	***	*	*	*	*
Marvel of Peru or four o'clock flower	*Mirabilis jalapa* (L.)	**	**	*	**	*
Chocolate weed	*Melochia corchorifolia* (L.)	***	*	**	**	***
Cannabis	*Cannabis sativa* (L.)	**	***	**	**	***
Hibiscus or Roselle	*Hibiscus sabdariffa* (L.)	**	**	*	**	**
Cape periwinkle; graveyard plant	*Catharanthus roseus* (L.) G.Don	**	**	*	***	*
Cassava	*Manihot esculenta* (Crantz)	**	**	*	**	**
Donkey berry	*Grewia flavescens* (Juss)	**	**	**	**	***
Carob	*Ceratonia silique* (L.)	**	**	*	**	**
Cancer bush	*Sutherlandia frutescens* (L.) R.Br. *or* *Lessertia frutescens* (L.) Goldblatt & J.C.Manning	**	**	**	**	**
Ethiopian eggplant	*Solanum aethiopicum* (L.)	**	**	*	**	**
African eggplant	*Solanum macrocarpon* (L.)	**	**	*	**	*
Bitter eggplant	*Solatium insanum,* (L.)	**	**	**	**	***
Miracle fruit	*Synsepalum dulcificum* (Schumach. & Thonn.) William Freeman Danielfer	**	**	*	**	*

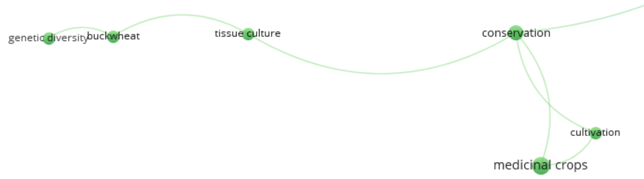
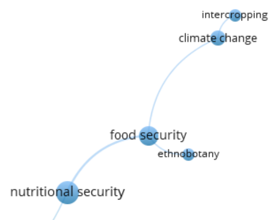
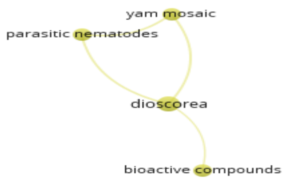
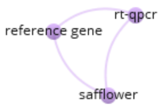
Cactus pear	*Opuntia robusta* J.C.Wendl. ex Pfeiff.	**	**	**	**	***
Pea eggplant	*Solanum torvum* (Sw.)	*	**	**	**	***
Quinoa	*Chenopodium quinoa* C.L. Willdenow (Willd.)	**	**	***	**	***
Buck wheat	*Fagopyrum esculentum* (Moench)	**	***	***	**	***
Amaranth	*Amaranthus spinosus* (L.)	**	**	*	**	*
Amaranth	*Amaranthus tricolor* (L.)	**	**	*	**	*
Elephant foot yam	*Amorphophalus campanulatus* (Dennst.) Nicolson	**	**	*	**	*
Up yam	*Dioscorea bulbiferaa* (L.)	**	**	*	**	*
Lesser yam	*Dioscorea esculenta* (Lour.) Burkill	***	***	**	***	**
Taro	*Colocasia esculenta* (L.) Schott	**	***	***	***	*
Greater yam	*Dioscorea alata* (L.)	**	**	*	**	*
Yam	*Dioscorea dumetorum* (Kunth) Pax	**	***	**	**	**
Sweet potato	*Ipomea batatas* (L.) Lam.	**	***	**	***	*
Giant taro	*Alocasia macrorrhiza* (L.) G.Don	**	***	**	**	**
Ethiopian potato	*Plectranthus edulis* (Vatke) A.J.Paton	**	***	**	**	*
Wild ginger	*Siphonochilus aethiopicus* (Schweinf.) B.L.Burtt	**	***	**	**	***
Tannia	*Xanthosoma sagittifolium* (L.) Schott	**	***	**	**	*
White seed melon	*Cucumeropsis manni* (Naudin)	**	***	**	**	*
Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	**	***	**	**	*
Bottle gourd	*Lagenaria siceraria* (Molina) Standl.	**	***	**	**	*
Bitter gourd	*Momordica charantia* (L.)	**	***	**	***	***
Wax gourd	*Benincasa hispida* ((Thunb.) Cogn.)	**	***	***	**	**

Bitter melon	*Momordica charantia* (L.)	**	***	**	**	*
Pumpkin	*Cucurbita pepo var. styriaca* (L.)	*	***	***	**	**
Napier grass	*Pennisetum purpureum* (Schumach)	*	***	***	**	**
Pearl millet	*Cenchrus americanus* (L.) Morrone	*	***	**	**	*
Barley	*Hordeum vulgare (*L.)	*	***	**	**	*
Proso millet	*Panicum miliaceum* (L.)	*	***	**	**	*
Fonio millet	*Digitaria exilis* (Kippist) Stapf	*	***	**	**	*
Foxtail millet	*Setaria italica* (L.) P. Beauvois	**	**	***	**	***
Finger millet	*Eleusine coracana* (L.)	**	***	**	**	*
Sorghum	*Sorghum bicolor* (L.)	**	**	***	**	***
Maize	*Zea mays* (L.)	**	**	***	**	***
Scarlet pimpernel	*Chenopodium album* (L.)	**	**	***	**	***
Sweet clover	*Melilotus officinalis* (L.) Pall.	**	***	*	**	**
Lemongrass	*Cymbopogon flexuosus* (Nees ex Steud.) W.Watson	**	***	**	**	*
Rapeseed/ Sarson	*Brassica napus* (L.)	**	***	**	**	***
Water hyssop	*Bacopa monnieri* (L.) Pennell	**	***	**	**	***
Safflower	*Carthamus tinctorius* (L.)	**	***	***	**	**
Fennel flower	*Nigella sativa* (L.)	**	***	*	**	*
Ladies' fingers or Okra	*Abelmoschus esculentus* (L.) Moench	**	***	*	**	*
Plantain	*Plantago major* (L.)	**	***	*	**	*
Toothache plant	*Acmella oleracea* (L.) R.K.Jansen	**	***	*	***	*
Creeping woodsorrel	*Oxalis corniculata* (L.)	**	***	***	***	*
Chinese water chestnut	*Eleocharis dulcis* (Burm.f.) Trin. ex Hensch.	**	**	*	***	*
Lamb’s quarters	*Chenopodium album (*L.)	**	**	***	***	**
False sesame	*Ceratotheca sesamoides* (Endl.)	**	**	***	***	**

Black sesame	*Sesamum radiatum* (Schumach. and Thonn)	**	**	***	***	**
	Crassocephalum rubens (Juss. and Jacq.) S.	*	**	***	***	*
Spiderflower	*Cleome gynandra* (L.)	**	**	**	***	*
Jute mallow	*Corchorus olitorius* (L.)	**	**	**	***	*
Thickhead, redflower ragleaf	*Crassocephalum crepidioides* (Benth.) S.Moore	**	**	***	***	*
Wild mustard	*Brassica juncea* (L.)	**	**	**	***	*
Kales	*Brassica oleracea* (L.)	***	***	***	**	***
Common dandelion	*Taraxacum officinale* F. H. Wigg	***	**	***	**	*
Purslane	*Portulaca oleracea* (L.)	**	**	**	**	*
Hyacinthus	*Hyacinthaceae*	**	**	**	**	*

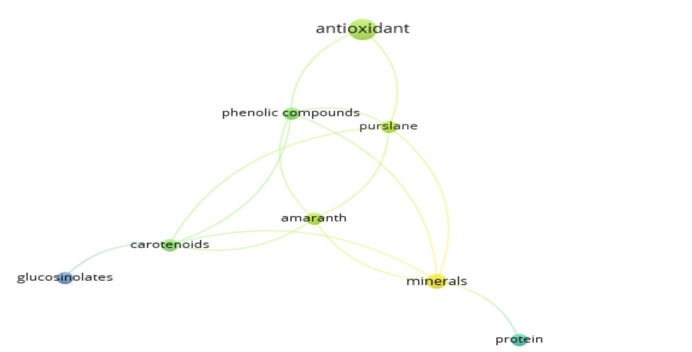
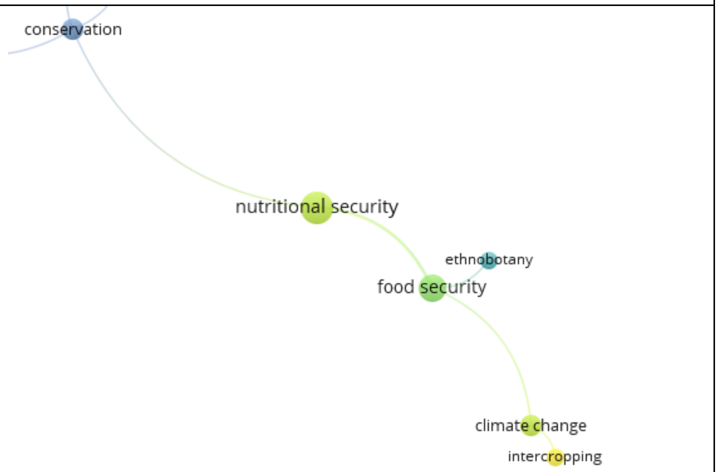
Supplementary Table 3: Clustering of keywords identified in Vosviewer on NUFMS

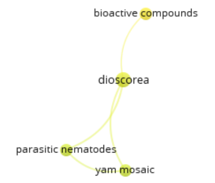
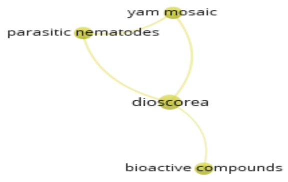
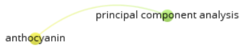
Colour	**Keywords**	**Cluster**
Red	Amaranth	
	Antioxidant	
	Carotenoids	
	Glucosinolates	
	Minerals	
	Phenolic compounds	
	Protein	
	Purslane	
Green	Buckwheat	
	Conservation	
	Cultivation	
	Genetic diversity	

	Medicinal crops	
	Tissue culture	
Blue	Climate change	
	Ethnobotany	
	Food security	
	Intercropping	
	Nutritional security	
Lime	Bioactive compounds	
	Dioscorea	
	Parasitic nematodes	
	Yam mosaic	
Purple	Reference gene	
	Rt-qpcr	
	Safflower	
Sky blue	Bioinformatics	
	Cajanus cajan	
Orange	Anthocyanin	
	Principal component analysis	
Brown	Aloe vera	
Lilac	Germination	
Peach	Grewia flavescens	
Light green	i-dopa	

Navy blue	Rapd	

Supplementary Table 4: Timeline of research on NUFMS

		Timeline of published articles according to cluster
Colour	**Keywords**	
Cluster 1	Amaranth	
	Antioxidant	
	Carotenoids	
	Glucosinolates	
	Minerals	
	Phenolic compounds	
	Protein	
	Purslane	
Cluster 2	Buckwheat	
	Conservation	
	Cultivation	
	Genetic diversity	
	Medicinal crops	
	Tissue culture	
Cluster 3	Climate change	
	Ethnobotany	
	Food security	
	Intercropping	

	Nutritional security	
Cluster 4	Bioactive compounds	
	Dioscorea	
	Parasitic nematodes	
	Yam mosaic	
Cluster 5	Reference gene	
	Rt-qpcr	
	Safflower	
Cluster 6	Bioinformatics	
	Cajanus cajan	
Cluster 7	Anthocyanin	
	Principal component analysis	
Cluster 8	Aloe vera	
Cluster 9	Germination	
Cluster 10	Grewia flavescens	
Cluster 11	i-dopa	
Cluster 12	Rapd	

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