EFFECT OF NET SHADING AND SEASON ON PLANT GROWTH, PRODUCTIVITY AND QUALITY OF BUSH TEA (*ATHRIXIA PHYLICOIDES* DC.)

by

MAANEA LONIA RAMPHINWA

Submitted in accordance with the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in the subject

Agriculture

at the

UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: PROFESSOR F.N. MUDAU

CO-SUPERVISOR: PROFESSOR N.E. MADALA

: PROFESSOR G.R.A. MCHAU

18 APRIL 2023

DECLARATION

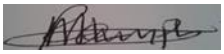
Name : Ms. Maanea Lonia Ramphinwa

Student number : 32342314

Degree : PhD in Agriculture

EFFECT OF NET SHADING AND SEASON ON PLANT GROWTH, PRODUCTIVITY AND QUALITY OF BUSH TEA (*ATHRIXIA PHYLICOIDES* DC.)

I declare that the above thesis is my own work and all the sources I have used or quoted have indicated and acknowledged by means of completed references.



18/04/2023

SIGNATURE DATE

ACKNOWLEDGEMENT

First and foremost, I would like to express my heartfelt appreciation to my supervisor, Prof Fhatuwani Nixwell Mudau, for his guidance, patience, expertise, and outstanding contributions throughout my PhD journey. He encouraged me to work hard every day, eventually bringing out abilities in me that I had no idea existed.

I would also like to express my special gratitude to my co-supervisor Prof Ntakadzeni Edwin Madala for his encouragement, insightful comments, and hard questions during my PhD endeavour.

To Prof G.R.A Mchau, thank you for the advice you've given me and for entrusting me with this project.

My special thanks go to my husband (Vhonani Theophilus Makuya), daughter (Phophi Princess Makuya), son (Muhali Makuya), Mom (Thizwilondi Sarah Ramphinwa), twin sister (Dzulani Juliet Ramphinwa), brothers (Tovhowani, Azwinndini, Given, Paris) and in-laws for the love, support and allowing me to pursue my PhD studies.

I would also like to express my gratitude to my colleagues Tshidi Theka, Fhatuwani Thovhogi, Petrus Malele, Mpho Mashau for helping me during the time when I was conducting my research activities.

I would like to express my Gratitude to the University of Venda, University of South Africa, National Research Fund for funding my project.

Above all, my greatest gratitude goes to my God Almighty, Jehovah Jireh, Jehovah Rapha, Jehovah Nissi. Everything is possible with God.

DEDICATION

This dissertation is dedicated to my late father Mr Mmberegeni Managa Frank Ramphinwa and my younger brother Matodzi Paris Ramphinwa who was unwell during my PhD studies.

ABSTRACT

Bush tea (*Athrixia phylicoides* DC.) is a South African native plant that grows naturally in different parts of South Africa that are characterized by different climatic conditions. The effects of agricultural practices on plant growth, yield and chemical composition of bush tea have been studied on pots experiment under controlled environment. However, the effect of shade net and season on field grown bush tea and its quality have not yet investigated. Hence, its quality is determined by the accumulation, partitioning and distribution of secondary metabolites which are affected by major abiotic and biotic factors which include environmental conditions, UV light, temperature, water availability, type and composition of soil. Currently, there is no recommended best production method and season for bush tea crop in Southern Africa that maximizes plant growth, yield, accumulation, partitioning and distribution of secondary metabolites. Therefore, this thesis evaluated the effect of the application of shade nets and season on plant growth, development and quality of bush tea. Net shade application reduced bush tea plant growth and yield when compared to plants exposed to direct sunlight during the summer season. Hydroxycinnamic acid recorded higher in 80% white shade net plots than the plants exposed to direct sunlight.

The study also evaluated the effect of UV-induced geometrical isomerization of hydroxyl-cinnamic acid-containing molecules of bush tea using ultra high-performance liquid chromatogram quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS). The study highlighted that light impacted the chemistry of plants which results in the formation of newly formed metabolites which are not naturally part of the plant. Secondary metabolites which have been responded to photo isomerization were discovered to be structurally related by the formation of a very tight molecular family using when molecular networking algorithm.

This study also investigated the metabolite diversification of bush tea through glucaric acid conjugation by cinnamic acid derivatives using UHPLC-QTOF-MS. The findings revealed that hydroxyl-cinnamic acids (HCAs) derivatives undergo photo-isomerization during post ultra-violet (UV) light exposure, evidenced by the emergence of photo-isomers. The study indicated the ability of conducive environment to promote plant growth, development, yield as well as enhancing quality of bush tea.

Future prospects will include investigation of the effect of different colours of ultraviolet light on bush tea extracts and response of molecular network of bush tea exposed to different types of shade nets using UHPLC-QTOF-MS.

LIST OF ABBREVIATION

%	Percent
α	Proportion of the intercepted radiation
$[M\text{-}H]^-$	Negative-ion electrospray mass spectrometric
µmm	Micromillimeter
ANOVA	Analysis of Variance
B40%	Black40%
B50%	Black50%
B80%	Black80%
BPI	Base Peak Intensity
C	Celsius
C	Control
CCL	Chlorophyll Content
CCM	Chlorophyll Content Meter
CGAS	Chlorogenic Acids
CID	Collision Induced Dissociation
CM	Centimeter
CQA	Caffeoylquinic acid
DA	Daltons

DC	Augustin Pyramus de Candolle
DICQA	Dicaffeoylquinic acid
DMRT	Duncan’s Multiple Range of Test
E	East
ESI	Electron Spray Ionization
EV	Electron Volts
G PLANT-1	Gram per plant
G	Gram
G40%	Green40%
G50%	Green50%
G80%	Green80%
GNPS	Global Natural Product Social Molecular Networking
HCAS	Hydroxyl-Cinnamic Acids
HIV-1INT	Human Immunodeficiency Virus 1 Intergrase
HR	Hour
IBA	Indole-3-butyric Acid
IR	Intercepted Radiation
K HA^{-1}	Potassium Per Hectare
KG HA^{-1}	Kilogram per hectare

KV	Kilo Volt
L	Litre
L/MIN	Litre per minute
L/MIN	Litre Per Minute
LC-MS	Liquid Chromatogram Mass Spectrometry
LC-QTOF-MS	Liquid Chromatogram Quadrupole Time of Flight Mass Spectrometry
M	Meter
MASL	Mass Above Sea Level
ML	Millilitre
ML/MIN	Millilitre per minute
MM	Millimeter
MM	Millimeter
MS	Mass of Square
MS(MS/MS)	Tandem Mass Spectrometry squared
MS/MS	Tandem Mass Spectrometry
MV	Multivariate
MZ	Mass-to-Charge Ratio
N	Nitrogen
NAI	Sodium Iodide

NPK	Nitrogen Phosphorus Potassium
NS	Non significance
OH	Hydroxide
$P\ HA^{-1}$	Phosphorus per hectare
P_A	Photosynthetically Active Radiation Above the Canopy
PAR	Photosynthetic Active Radiation
P_B	Photosynthetically Active Radiation Below the Canopy
PCOA	Coumaric acid
PH	Potential of Hydrogen
QA	Quinic Acid
QTOF	Quadrupole Time of Flight
RT	Retention Time
S	South
SPSS	Statistical Package for Social Sciences
SS	Sum of Square
TOF	Time of Flight
TRICQAS	Tricaffeoylquinic acid
UHPLC-QTOF-MS	Ultra High-Performance Liquid Chromatogram Quadrupole Time of Flight Mass Spectrometry
UV	Ultra-Violet

VV	Volume per Volume
W40%	White40%
W50%	White50%
W80%	White80%
XG	Times Gravity
µM	Micrometer
µW/cm	Milliwatts per centimeter

LIST OF TABLES

LIST OF FIGURES

LIST OF PUBLICATIONS

Ramphinwa, M.L., McHau, G.R.A., Madala, N.E., Nengovhela, N., Ogola, J.B.O., Mudau, F.N., 2022. Response of Plant Growth and Development, and Accumulation of Hydroxyl-cinnamoyl Acid Derivatives to Selected Shade Nets and Seasonality of Field-grown Bush Tea (Athrixia phylicoides DC). HortScience 57, 87–96. https://doi.org/10.21273/HORTSCI16171-21.

Ramphinwa, M.L., Madala, N.E., Mchau, G.R., Ramabulana, A.T. and Mudau, F.N., 2022. Effect of UV-induced geometrical isomerization of hydroxyl-cinnamic acid-containing molecules of bush tea (Athrixia phylicoides DC.) using UHPLC-QTOF-MS. Scientia Horticulturae, 301, 111124. https://doi.org/10.1016/j.scienta.2022.111124.

Ramphinwa, Maanea Lonia, Godwin Richard Ainamensa Mchau, Mpho Edward Mashau, Ntakadzeni Edwin Madala, Vimbayi Grace Petrova Chimonyo, Thembinkosi Albert Modi, Tafadzwanashe Mabhaudhi, Vuyisile Samuel Thibane, and Fhatuwani Nixwell Mudau. "Eco-physiological response of secondary metabolites of teas: Review of quality attributes of herbal tea." Frontiers in Sustainable Food Systems 7 (2023): 41.

SIGNIFICANCE CONFERENCE PUBLISHED ABSTRACT

Ramphinwa, M.L., Mchau, G.R.A., Mudau, F.N. Effect of net shading and season on plant growth, productivity and quality of bush tea (*Athrixia phylicoides* DC.). XI International Symposium on Protected Cultivation in Mild Winter Climates & I International Symposium on Nettings and Screens in Horticulture. Tenerife (Spain - Canary Islands), January 27-31, 2019.

Ramphinwa, M.L., Mchau, G.R.A., Madala, N.E., Mudau, F.N. To protected environment or not: revealing convincing evidence of UV-induced geometrical isomerization of cinnamic acid containing molecules in bush tea (*Athrixia phylicoides* DC.) using UHPLC-QTOF-MS.

24 – 26 January 2022.

TABLE OF CONTENTS

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

Bush tea is herbaceous plant which belongs to the Asteraceae family and genus *Athrixia* (Herman et al., 2000; Mudau and Makunga, 2018). Genus *Athrixia* consists of 14 species which are found in Southern Africa, Tropical Africa as well as Madagascar (van Wyk and Gericke, 2000; Lerotholi et al., 2017). Bush tea is a drought tolerant plant which is predominately found at different locations with different altitude that ranges from 600 to 2000 m above sea level in South Africa (Mbambezeli, 2005; Lerotholi et al., 2017). It is widely distributed from Northern part of Limpopo Province and Mpumalanga to the Eastern parts of South Africa which includes KwaZulu-Natal, Eastern Cape Provinces, Swaziland and Lesotho (Germishuizen et al., 2006; Lehlohonolo et al., 2013). Bush tea is known as Mutshatshaila (Venda), Mohlahlaishi (Pedi), Boesmans tee (Afrikaans), bushman’s tea (English), Umtshanelo, Icholocholo, Itshelo (Zulu) and Luphephetse (Swati) (Lehlohonolo et al., 2013).

Bush tea plant is characterized by small, dark green pointed leaves with white woolly backs and small pink daisy flowers which are tainted with bright yellow at the center (Robert, 1990; Mudau et al., 2006). The colour of its flowers differs from pink to all shades of pink and attractive purple due to geographical areas and soil type (van Wyk and Gericke, 2000; Lehlohonolo et al., 2013). The plant produces best flowers from March to May and also has the ability to produce flowers throughout the year depending on the climatic conditions (Mbambezeli, 2005). Bush tea bear fruits which are narrow, cylindrical as well as achenes that ranges between 0.01 to 0,06 mm wide (Araya, 2005; Mudau et al., 2006). Its seed’s length is about 4 mm and contains 2 pappus per seed which are used for dissemination of a seed as a parachute.

Herbal teas are gaining popularity in many countries due to the numerous benefits they provide to humans. High intake of herbal infusions as beverages has been discovered to reduce risk of chronic diseases due to antioxidant, antiatherosclerotic, antiflammatory, antimutagenic, antitumor, antiviral activities (Hertog et al., 1996; Malongane et al., 2018). Different ethnic groups in South Africa use bush tea for different purposes. For an example, bush tea is used by the Vhavenda people to make brooms (Rampedi and Olivier, 2005; Tshivhandekano et al., 2013), as an aphrodisiac (Mabogo, 1990; Rakuambo et al., 2007), and extracts from soaked roots and leaves are used as anthelmintics (Mbambezeli, 2005; Mathivha et al., 2020). The Zulu people use it as a cough remedy and purgative. The Sotho and Xhosa people chewed bush tea to reduce incidences of sore throat, loss of voice, colds and coughing. It is also used by other traditional people for cleansing and purifying blood, treating boils, infested wounds as well as a remedy for headache (Mudau, et al., 2007; Tshivhandekano et al., 2013).

Till date, bush tea is still harvested from wild for the above mentioned various medicinal purposes, while van Wyk (2000), Mudau et al. (2007) and Reichelt et al. (2012), stated the potential of domestication and development of bush tea as a commercial health benefit in South Africa. Bush tea has gained attention to be industrialized, since it is used as herbal infusion and for medicinal purposes to fight against chronic diseases such as cardiovascular disease and cancer (Nijveldt et al., 2001; Mudau et al., 2016).

Despite its multiple uses, there is hardly any bush tea production in Southern Africa. As a result, no significant studies have recommended best production method for bush tea crop in Southern Africa. Over the years, studies have focused mostly on the influence of cultural practices which involves plant density (Kigalu, 2007), mineral nutrition (Mudau et al., 2006; Tshivhandekano et al., 2018) and environmental conditions on the quality of tea (Tshivhandekano et al., 2013). The quality, economic value and health function of tea is determined by secondary metabolites which are present in tea plant such as flavonoids (or phenolic compounds), alkaloids, theanines and others (Tounekti et al., 2012). Zhang et al. (2014), reported that quality parameters of tea are significantly affected by environmental factors and management practices. In cultivated green tea leaves, photosynthetic rate (Haukioja et al., 1998), non-structural accumulation of carbohydrates (Wanyoka, 1983) as well as biosynthesis of carbon-based secondary metabolites (Haukioja et al., 1998; Chabeli et al., 2008) have been described to increase concentration of total polyphenols after the application of nitrogenous fertilizers. In *Labisia pumila* Blume plants, it has been reported that carbon-based secondary metabolites increase frequently when environmental conditions enhance the accumulation of non-structural carbohydrates (Ibrahim and Jaafar, 2011). Information about the effects of differing light intensity and season on plant growth, yield, and quality on bush tea is not yet documented. According to previous studies on bush tea, 50% restricted light intensity (standard method) in a black net shade with nitrogen application of less than 300 Kg ha-^{1} has been found to be a favorable condition for cultivating bush tea in order to maintain plants with optimal leaf polyphenol during the winter season (Mudau et al., 2006). Similarly, other high value crops such as lettuce cultivation in South African conditions under photo-selective pearl and yellow nets with 40% shading improved the fresh leaf mass and percentage of marketable yield at harvest (Ntsoane et al., 2016). Compared to the traditional method (black shade net), application of red, pearl (white) and yellow nets resulted into better yield and fruit quality, reduced infestation by pests and improved post-harvest quality (Stamps 2009; Goren et al. 2011; Shahak, 2014). The main objective of adopting different colored shade netting approach is to prolong or extend the harvesting period (maturation rate) while enhancing the carbon-based secondary metabolites that are associated with the quality herbal tea.

1.1.2. PROBLEM STATEMENT

Bush tea has been a popular crop over the years among local indigents in Southern Africa due to its healthy promoting properties. Currently, bush tea is harvested from the wild; however, demand exceeds supply, so large-scale commercial cultivation has yet to be established. As a result, no significant studies have recommended the best production method for bush tea crop in Southern Africa. It has been suggested that variation in seasonal temperatures and vapor pressure deficit may affect the quality and antioxidants of bush tea. Hardly any study has evaluated the effect of different net shading in terms of color and light intensities and variation in season on plant growth, yield, and quality of bush tea. To date, it is still unclear as to how secondary metabolites on bush tea crop respond to different types of net shading and different levels of light intensities. The main objective of adopting different colored shade netting approach is to prolong the harvesting period while enhancing the carbon-based secondary metabolites that is associated with quality of herbal tea.

1.1.3. AIMS

The study is designed to develop and determine the effect of net shading and season on the plant growth, productivity, quality of bush tea.

1.1.4. OBJECTIVES

Objective 1. To determine the effect of selected shade nets and seasonal variation on plant growth and development, and hydroxycinnamic acid content of field-grown bush tea.

Objective 2. To identify hydroxyl-cinnamic acid metabolites affected by UV-induced photo-isomerization of bush tea using UHPLC-QTOF-MS.

Objective 3. To enhance the levels of caffeoyl-D-glucaric acid derivatives through UV induced geometrical isomerization.

1.2. LITERATURE REVIEW

There is insufficient information to allow a balanced discussion of the literature review that describes the response of plant growth, productivity, and quality to net shading and season of bush tea.

1.2.1. Quality attributes of bush tea

Antioxidant activities associated with secondary metabolites such as polyphenols, flavonoids, and tannins are important indicators of herbal tea's medicinal potential (Hirasawa et al., 2002; Tshivhandekano et al., 2018). Secondary metabolites are chemical compounds that are synthesized from primary metabolites when plants interact with their environment in order to adapt or defend themselves (Ramakrishna et al., 2011; Mohale et al., 2018). These chemical compounds are used to combat environmental stress, herbivores and plant pathogen attacks.

Secondary metabolites found in bush tea leaves include 5- hydroxy-6,7,8,3',4',5'-hexamethoxy flavon-3-ol (Mashimbye et al., 2006), 3-0-demethyldigicitrin, 5,6,7,8,3',4'-hexamethoxyflavone and quecertin (Mavundza et al., 2010), tannins (Mudau et al., 2007; Chabeli et al., 2008), total polyphenols (Mudau et al., 2006; Maudu et al., 2012) and total antioxidants (Maudu et al., 2010; Mogotlane et al., 2007) compounds with antimicrobial activity (Mavundza et al., 2010; Lehlohonolo et al., 2013). Due to the antioxidants that have been reported on bush tea, the above mentioned active secondary metabolites can also be used as potential medicinal indicators (Tshivhandekano et al., 2018).

1.2.2. Different cultural practices on quality of bush tea

Herbal plants are grown in a variety of climates, with biotic and abiotic factors influencing growth, productivity, yield and quality (Maudu et al., 2010; Tshivhandekano et al., 2013). Mineral nutrition (Mudau et al., 2006; Tshivhandekano et al., 2018) and planting density (Kigalu, 2007), pruning (Maudu et al., 2010; Mohale et al., 2018), have been shown to have a significant impact on tea growth and productivity (Owuor, 1998; Ramphinwa et al., 2022). Therefore, agronomic practices should be optimized to maximize plant growth, yields and quality of bush tea (Maudu et al., 2010; Mohale et al., 2018).

1.2.2.1. Mineral nutrition

Nitrogenous fertilizer application has been reported to boost photosynthetic rates (Haukioja et al., 1998; Mudau et al., 2007), non-structural carbohydrate accumulation (Wanyoko, 1983), and the biosynthesis of carbon- and nitrogen-based specialized metabolites (Haukioja et al., 1998; Tshivhandekano et al., 2018), as well as tea leaf quality (Owour et al., 1990). These findings are consistent with those of Chabeli et al. (2008), who reported that nutrient application has been shown to increase carbohydrate accumulation for plant growth and photosynthetic rates, resulting in the biosynthesis of carbon-based secondary metabolites known as total polyphenols. Mudau et al., (2006), also confirmed that high nitrogen application levels of up to 300 kg/ha N improved the quality of potted bush tea grown under shade nets. Therefore, nitrogen fertilization has been revealed to have significant linear relationships between leaf tissue N and total polyphenol content, regardless of season, in a shaded nursery environment, implying a strong trade-off in nutrients channeled toward the production of phenolics.

1.2.2.2. Pruning

Pruning has been shown to increase tea productivity and quality (Yilmaz et al., 2004; Marasha et al., 2013), and it is widely used in tea plantations. It also involves raising tea branches to a predetermined height (Kumar et al., 2015). Tea plants are shrubs that can grow to a height of 1.5 m, so pruning is required to achieve the desired height (Mohale et al., 2018). Pruning bush tea plant is necessary to stimulate vegetative growth, support the plant by shaping and controlling its directions, and eliminate diseased, damaged, unproductive, unwanted, dead tissues, and crossing branches (Ravichandran, 2011; Mohale et al., 2018). Pruning bush tea had a significant effect on secondary metabolite accumulation, suggesting that it could improve bush tea quality (Maudu et al., 2010; Mohale et al., 2018), while reducing yields. These results concur with those of Maudu et al., (2012), who reported that at the new growth stage, both cultivated and wild bush tea had the highest total polyphenol content.

1.2.2.3. Plant growth regulators

It has also been reported that the use of growth hormones or regulators has an effect on the chemical composition of tea. Jibika, IAA, cycocel, thiourea, methanol, succinic acid, and sucrose were bioregulators found to have a significant effect on quality parameters such as polyphenol oxidase (PPO) activity, caffeine, crude protein, starch, nitrogen, carotenoid, and ascorbic acid (vitamin C) content of pruned *Camellia sinensis* (Maudu et al., 2010). These results concur with those of Liang et al. (1996), who found that gibberellins increased plant growth and chemical composition in green tea production.

1.2.3. Response of shading and photosynthetic active radiation (PAR) of of bush tea and other herbal teas

Tea is a shade-loving plant, therefore, shade trees with a canopy of moderate shade are required in tea production to maintain optimum growth and productivity (Janendra et al., 2007). Shade materials may influence the spectral distribution of radiation (i.e., diffuse/direct) in addition to reducing the amount of radiation (Makoka, 2007). The proportion of diffuse to direct radiation increases towards the bottom of the canopy due to greater penetration of diffuse radiation. Radiation is a major resource for plants and its competition under shade environment has been reported to affect their growth and development (Imaizumi and Kay 2006). The amount of light above and under the canopy of the plant respond differently with different planting densities (Kumar, 2013). Crop species and the degree of shading determines the performance of plants under shading environment (Kumar, 2013; Fini et al., 2010).

Previous studies have indicated that the application of shade nets in bush tea had impacted the plants by reducing plant growth and yield compared to the plants exposed to full sunlight during summer season (Ramphinwa et al., 2022). This could be due to the fact that shade had a potential to reduce photosynthetically active radiation (PAR), modifies spectral quality and affect plant photosynthesis as well as biomass and yield of the crop (Rao and Mitra 1998; Bell et al. 2000). Similar findings were reported by Kumar (2013), who found that Salvia sclarea had significantly higher total biomass/plant under open field conditions, which could be attributed to a significantly higher number of roots and length/plant under this condition, resulting in vigorous vegetative growth. These results are not in line with those of Barua and Gogoi (1979), who reported that when compared to shaded tea plants in Northeast India, the removal of shade reduced yield by 50%. It has also been established that higher total biomass in tea production, particularly tea shoots exposed to 35% shade, compared to plants exposed to full sunlight (Barua et al., 1969). These results could be attributed to the fact that inappropriate light to plants results to stunting growth and development that demonstrate significant of favorable condition that determines plant growth and yield of bush tea in any region.

Appropriate intercepted radiation and its utilization are of great importance in herbal tea production. Shade level of 25% in stevia plants significantly resulted to taller plants than those which were exposed to direct full sunlight conditions, and this may be due to long internodal lengths and thinner stems (Kumar et al., 2013). According to (Singh 1994), plants that their reproductive growth is not of great importance perform well when exposed to shades. Shading has a tendency of producing taller plants due to its shoot growth as the plants seeks light and finally activates phototropism responses that alters plant leaf distribution to reduce mutual shading (Takemiya, 2005). Higher shade levels determine length and width of leaves due to cell expansion. These findings concur with those of Moniruzzaman et al. (2009) who stated that plants exposed to lower light intensities resulted in higher apical dominance than those in higher light intensities. Ramphinwa et al., (2022), discovered that bush tea plants exposed to full direct sunlight tend to have taller plants than those exposed to shade levels. These results might have been attributed to higher canopy size as well as plant height due to higher proportion of intercepted radiation under the environmental condition that promoted plant growth and development (Muchow et al., 1990). Variations in microclimate through spacing and levels of shades had significant differences in growth and development resulted to faster growth and development in plants under full sunlight than those grown under shade (Kumar et al., 2013). Thakur (2019) also confirmed that higher shade level is capable of reducing PAR, altered light intensities, as well as impacting photosynthetic rate and biomass production. These findings concur with those of Kumar et al., 2013, who reported that different shade nets had the potential to influence micro-climatic parameters that determines plant growth and development. The response of total intercepted radiation in plants differs between treatments depending on days they take to reach their physiological maturity stage (Anwar et al., 2003).

1.2.4. Response of plant growth and development to different types of seasons of bush tea and other herbal teas

Bush tea plant growth parameters recorded higher during summer seasons under control than the plants exposed under different shade nets (Ramphinwa et al., 2022). The results might have been influenced by the larger canopy size exhibited in summer caused by the greater proportion of intercepted radiation recorded in summer. Challa et al. (1999) and Cockshull et al. (1992) revealed that the amount of intercepted radiation received by the plants during the cropping season had a great influence on plant growth, development, and total biomass. These findings contradict those of CTFIL (1995), who reported that plant growth, development and yield was limited during summer cropping season. It might have been influenced by higher temperatures experienced during summer which had impacted photosynthesis rate and respiration.

1.3. References

Anwar, M.R., B.A. McKenzie, and G.D. Hill. 2003. The effect of irrigation and sowing date on crop yield and yield components of Kabuli chickpea (*Cicer arietinum* L.) in a cool-temperate sub humid climate. J. Agric. 141:259-271, doi: 10.1017/S0021859603003617.

Araya, H.T. 2005. Germination and vegetative propagation of bush tea (*Anthrixia phylicoides*) (Doctoral dissertation, MSc Thesis, University of Pretoria, South Africa. 92.

Barua, D.N. and B.N. Gogoi. 1979. Effects of shade removal. Two and a Bud. 26:40-42.

Bell, G.E., T.K. Danneberger, and M.J. McMahon, 2000. Spectral irradiance available for turfgrass growth in sun and shade. Crop sci. 40:189-195, doi: 10.2135/cropsci2000.401189x.

Chabeli, P.M., F.N. Mudau, P.W. Mashela, and P. Soundy. 2008. Effects of nitrogen, phosphorus and potassium nutrition on seasonal tannin content of bush tea (*Athrixia phyliciodes* dc.). S. Afr. J. Plant Soil. 25:79–83, doi: 10.1080/02571862.2008.10639899.

Challa, H. and M.J. Bakker. 1999. Potential production within the greenhouse environment. In Ecosystems in the world: Vol. 20: Chapter 15: Greenhouse Ecosystems. 20:333-348.

Cockshull, K. E., C.J. Graves, and C.R. Cave. 1992. The influence of shading on yield of glasshouse tomatoes. Journal of Hortic. Sci. 67:11-24, doi: 10.1080/00221589.1992.11516215.

CTIFL, Centre Technique Interprofessionel des Fruits et des Legumes. 1995. Maitrise de la conduite climatique. Paris. CTIFL. 127.

Fini, A., F. Ferrini, P. Frangi, G. Amoroso, C. Giordano, and L. Bonzi. 2010. Growth, leaf gas exchange and leaf anatomy of three ornamental shrubs grown under different light intensities. Europ. J. Hort. Sci. 75:113-117.

Goren, A., S. Alkalai-Tuvia, Y. Perzelan, Z. Aharon, and E. Fallik. 2011. Photoselective shade nets reduce postharvest decay development in pepper fruits. Adv. Hortic. Sci. 25:26-31.

Haukioja, E., V. Ossipov, J. Koricheva, T. Honkanen, S. Larsson, and K. Lempa. 1998. Biosynthetic origin of carbon-based secondary compounds: Cause of variable responses of woody plants to fertilization. Chemoecology. 8:133–139, doi: 10.1007/s000490050018.

Herman, P.P.J., E. Retief, M. Koekemoer, and W.G. Welman. 2000. Seed plants of Southern Africa. O.A. Leister Editions, NBI. Pretoria, South Africa.

Hertog, M.G., E.J. Feskens, and P.C. Hollman. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the zutphen elderly study. Lancet. 342:1007-1011.

Hirasawa, M., K. Takada, M. Makimura, and. S.Otake. 2002. Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. Alt. Med. Rev. 37:433-438.

Ibrahim, M.H., J.Z.E Jaafar, A. Rahmat, and Z.A. Rahman. 2011. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of Kacip fatimah (*Labisia pumila* Blume). Int. J. Mol. Sci. 12:5238–5254, doi: 10.3390/ijms12085238.

Imaizumi, T. and S.A. Kay. 2006. Photoperiodic control of flowering: not only by coincidence. Trends Plant Sci. 11:550–558, doi.org/10.1016/j.tplants.2006.09.004.

Janendra, W. A., M. Costa1, J.A. Mohotti, and M.A. Wijeratne. 2007. Ecophysiology of tea. Braz. J. Plant Physiol. 19:299–332.

Kigalu, J.M. 2007. Effects of planting density and drought on the productivity of tea clones (*Camellia sinensis* L.): Yield responses. Phys. Chem. Earth. 32:1098-1106, doi: 10.1016/j.pce.2007.07.022.

Kumar, R., J.S. Bisen, M. Singh, and M. Bera. 2015. Effect of pruning and skiffing on growth and productivity of Darjeeling tea (*Camellia sinensis* L.). Int. J. Tech Res. Appl. 3:28-34.

Kumar, R., S. Sharma, and V. Pathania. 2013. Effect of shading and plant density on growth, yield and oil composition of clary sage (*Salvia sclarea* L.) in Northwestern Himalaya. J. Essent. Oil Res. 25:23-32, doi: 10.1080/10412905.2012.742467.

Lehlohonolo, N., I.K., Mariga, W. Ngezimana, and F.N. Mudau. 2013. Bush tea (*Athrixia phylicoides* dc.) Success stories in South Africa. A review. J. Crop Prod. 2:37–43.

Lerotholi, L., S.K. Chaudhary, S. Combrinck, and A. Viljoen, A., 2017. Bush tea (*Athrixia phylicoides*): A review of the traditional uses, bioactivity and phytochemistry. S. A. J. Bot. 110:4-17.

Liang, Y., J. Lu, and S. Shang. 1996. Effect of gibberellins on chemical composition and quality of tea (*Camellia sinensis* L). J. Sci. Food Agric. 72:411-414.

Mabogo, D.N.E. 1990. The Ethnobotany of Vhavenda. MSc Thesis, University of Pretoria, Pretoria.

Malongane, F., L.J. McGAW, and F.N. Mudau. 2017. The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: a review. J. Sci. Food Agric. 97:4679-4689. doi: 10.1002/jsfa.8472.

Marasha, N., I.K. Mariga, W. Ngezimana, and F.N. Mudau. 2013. Effect of pruning on carbohydrate dynamics of herbal and medicinal plant species: Prospects leading to research on the influence of pruning on productivity and biochemical composition of bush tea (*Athrixia phylicoides* DC). Afr. J. Agric. Res, 3528-3533.

Mathivha, P.L., T.A. Msagati, V.S. Thibane, and F.N. Mudau. 2020. Phytochemical analysis of herbal teas and their potential health, and food safety benefits: a review. J. Herb. Med. 281-301, doi: 10.1007/978-981-13-7248-3_20.

Maudu, M.E., F.N. Mudau, and I.K. Mariga. 2010. The effect of pruning on growth and chemical composition of cultivated bush tea (*Athrixia phylicoides* D.C). J. Med. Plants Res. 4:2353– 2358.

Maudu, M.E., F.N. Mudau, and I.K. Mariga. 2012. Quality Profiles of Cultivated and Wild Bush Tea (*Athrixia phylicoides*) Harvested at Various Phenological Stages. Int. J. Agric. Biol. Eng.14.

Mavundza, E.J., T.E. Tshikalange, N. Lall, A.A. Hussein, F.N. Mudau, and J.J.M. Meyer. 2010. Antioxidant activity and cytotoxicity effect of flavonoids isolated from *Athrixia phyllicoides*. J. Med. Plant Res. 4:2584-2587, doi: 10.5897/JMPR10.823.

Mbambezeli, G. 2005. *Athrixia phylicoides*. South African National Biodiversity Institute. Kirstenbosch NBG. 1-3.

Mohale, K.C., A.T. Hintsa, M.A. Emanuel, and F.N. Mudau. 2018. Metabolic profiling of cultivated bush tea (*Athrixia phylicoides* DC.) in response to different pruning types. HortSci. 53:993-998, doi: 10.21273/HORTSCI13023-18.

Mokoka, N.N. 2007. Indigenous knowledge of fever tea (*Lippia javanica*) and effect of shade netting on plant growth, oil yield and compound composition. Univ. of Pretoria, Pretoria, South Africa, Doctoral Diss.

Moniruzzaman, M., M.S. Islam, M.M. Hossain, T. Hossain, and M.G. Miah. 2009. Effects of shade and nitrogen levels on quality Bangladhonia production. Bangladesh J. Agri. Res. 34:205-213.

Muchow, R.C., T.R. Sinclair, and J.M. Bennett, 1990. Temperature and solar radiation effects on potential maize yields across locations. J. Agron. 82:338–342, doi: 10.2134/agronj1990.00021962008200020033x.

Mudau, F.N. and N.P. Makunga, 2018. Effect of Seasonal Variations and Growth Conditions on Carbohydrate Partitioning in Different Organs and the Quality of Bush Tea. HortSci. 53:999-1005, doi: 10.21273/HORTSCI12972-18.

Mudau, F.N., A.R. Mudau, M. Nkomo, and W. Ngezimana. 2016. Variation in carbohydrate reserves and dry matter production of bush tea (*Athrixia phylicoides*) grown under different environmental conditions. HortSci. 51:1537-1541, doi: 10.21273/HORTSCI11197-16.

Mudau, F.N., H.T. Araya, E.S. Du Toit, P. Soundy, and J. Olivier. 2007. Bush tea (*Athrixia phylicoides* DC.) as an alternative herbal and medicinal plant in southern Africa: Opportunity for commercialization. Med. Aromatic Plant Sci. Biotechnol. 1:74–76.

Mudau, F.N., P. Soundy, E. Du Toit, and J. Olivier. 2006. Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. S. Afr. J. Bot. 72:398–402.

Ntsoane, L.L., P. Soundy, J. Jifon, and D. Sivakumar. 2016. Variety-specific responses of lettuce grown under the different-coloured shade nets on phytochemical quality after postharvest storage. J. Hortic. Sci. Biotechnol. 91:520-528, doi: 10.1080/14620316.2016.1178080.

Owour, P.O. and M. Obanda. 1998. Clonal selection criteria for quality in Kenya black tea production: achievements, problems and perspectives: review. Tea. 19:49-58.

Owuor, P.O., S.O. Obaga, and C.O. Othieno. 1990. The effects of altitude on the chemical composition of black tea. J. Sci. Food Sci. 50:9-17.

Rakuambo, Z.J. 2007. Indigenous knowledge of bush tea (*Athrixia phylicoides*) and effect of fertigation frequency and growing medium on plant growth (Doctoral dissertation, University of Pretoria).

Ramakrishna, A. and G.A. Ravishankar. 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6:1720–1731, doi: 10.4161/psb.6.11.17613.

Rampedi, I. and J. Olivier. 2005. The use and potential commercial development of *Athrixia phylicoides*. Acta Acad. 37: 165-183.

Ramphinwa, M.L., G.R.A. McHau, N.E. Madala, N. Nengovhela, J.B.O. Ogola, and F.N. Mudau. 2022. Response of plant growth and development, and accumulation of hydroxyl-cinnamoyla acid derivatives to selected shade nets and seasonality of field-grown bush tea (*Athrixia phylicoides* DC). Hort Sci. 57:87–96, doi: 10.21273/HORTSCI16171-21.

Rao, L.J. and B.N. Mittra, 1998. Growth and yield of peanut as influenced by degree and duration of shading. J. Agron. Crop Sci. 160:260-265, doi: 10.1111/j.1439-037X.1988.tb00327. x.

Reichelt, K.V., Hoffmann-Lücke, P., Hartmann, B., Weber, B., Ley, J.P., Krammer, G.E., Swanepoel, K.H. 2012. Phytochemical characterization of South African bush tea (*Athrixia phylicoides* DC.). S. Afr. J. Bot. 83:1–8, doi: 10.1016/j.sajb.2012.07.006.

Robert, M. 1990. Indigenous healing plants. 1st edition. Southern Book Publishers. Halfway House.

Shahak, Y. 2008. Photoselective netting for improved performance of horticultural crops. A review of ornamental and vegetable studies carried in Israel. Acta Hortic. 770:161–168.

Singh, S. 1994. Physiological response of different crop species to low light stress. Ind J Plant Physiol. 37:147–151.

Stamps, R.H. 2009. Use of colored shade netting in horticulture. HortScience. 44:239-241, doi: 10.21273/HORTSCI.44.2.239.

Takemiya, A., S.I. Inoue, M. Doi, T. Kinoshita, and K.I. Shimazaki. 2005. Phototropins promote plant growth in response to blue light in low light environments. The Plant Cell. 17:1120-1127, doi.org/10.1105/tpc.104.030049.

Thakur, M., V. Bhatt, and R. Kumar. 2019. Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose (*Rosa damascena* Mill.) under mid hill conditions of Western Himalayas. Plos one (14) p.e0214672.

Tounekti, T., E. Joubert, I. Hernandez, and S. Munne-Bosch. 2013. Improving the polyphenol content of tea. Crit. Rev. Plant Sci. 32:192–215, doi: 10.1080/07352689.2012.747384.

Tshivhandekano, I., F.N. Mudau, P. Soundy, and W. Ngezimana. 2013. Effect of cultural practices and environmental conditions on yield and quality of herbal plants: Prospects leading to research on influence of nitrogen fertilization, planting density and eco-physiological parameters on yield and quality of field grown bush tea (*Athrixia phylicoides* DC.). J. Med. Plants Res. 7:2489–2493, doi: 10.5897/JMPR2013.4466.

Tshivhandekano, I., W. Ngezimana, T.E. Tshikalange, N.P. Makunga, and F.N. Mudau. 2018. Nitrogen application influences quality, pharmacological activities and metabolite profiles of *Athrixia phylicoides* DC. (Bush tea) cultivated under greenhouse and field conditions. Acta Agric. Scand. Sect. B Soil Plant Sci. 68:388–400, doi: 10.1080/09064710.2017.1412496.

Van Wyk, B.E. and N. Gericke. 2000. People's plants: A guide to useful plants of Southern Africa. 1st edition. Briza publications. 102.

Wanyoka, J.K. 1983. Fertilizer on tea: Nitrogen – a review. Tea (Nairobi). 4:28–35.

Yilmaz, G., N. Kandemir, and K. Kinalioglu. 2004. Effects of different pruning intervals on fresh shoot yield and some quality properties of tea (*Camellia sinensis* L. Kuntze) in Turkey. Pak. J. Biol. Sci. 7:1208–1212, doi: 10.3923/pjbs.2004.1208.1212.

Zhang, Q., Y. Shi, L. Ma, X. Yi, and J. Ruan. 2014. Metabolomic analysis using ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF MS) uncovers the effects of light intensity and temperature under shading treatments on the metabolites in tea. PLoS On.e 9:112572, doi: 10. 1371/journal.pone.0112572.

CHAPTER 2. ECO-PHYSIOLOGICAL RESPONSE OF SECONDARY METABOLITES OF TEAS: REVIEW OF QUALITY ATTRIBUTES OF HERBAL TEA

Published to Frontiers In Sustainable Food Systems. (See appendix A)

Abstract

Herbal teas are a rich source of secondary metabolites which are reputed to have medicinal and nutritional efficacy. These secondary metabolites are influenced by the abiotic and biotic stresses that improve the production of herbal teas in terms of biomass production, accumulation and partitioning of assimilates of compounds. In this study, various examples of herbal teas have been shown to respond differently to secondary metabolites affected by environmental factors. Thus, the meta-analysis of this study confirms that different herbal teas' response to environmental factors depends on the type of species, cultivar, and the degree of shade that the plant is exposed. It is also evident that the metabolic processes are also known to optimize the production of secondary metabolites which can thus be achieved by manipulating agronomic practices of crops used in herbal teas preparation. The different phenolic compound in herbal teas possesses the antioxidant, antimicrobial, antiatherosclerosis, anti-inflammatory, antimutagenic, antitumor, antidiabetic and antiviral activities that are important in managing chronic diseases associated with lifestyle. It can be precluded that more studies should be conducted to establish interactive responses of biotic and abiotic environmental factors on quality attributes of herbal teas.

Keywords: medicinal properties, polyphenolic compounds, health benefits, environmental factors

2.1. INTRODUCTION

In its 2014–2023 strategy, the World Health organisation (WHO) aims to promote utilisation of traditional medicines, including herbal medicine, with the goal of keeping populations healthy by providing access to efficient and reasonably priced alternatives to medicine and by offering healthcare options that are consistent with people's cultural practices (World Health organaisation, 2013). Therefore, there is a need to develop new herbal teas from the underutilised herbal plants if we are to achieve WHO strategy. The consumption of herbal teas is gaining attraction in recent years because most of them are rich in natural bioactive components such as alkaloids, carotenoids, coumarins, flavonoids, polyacetylenes and terpenoids (Chandrasekara and Shahidi, 2018).

A herbal tea is an aqueous infusion of different plant materials in hot or cold water to extract the phytochemical constituents (Poswal et al., 2019). It is made up from various parts of the plant which include flowers, roots, barks, and seeds, compared to the traditional leaves of black tea. Herbal teas exist in different taste, colour, and smell depending on the blend’s preparation of other plants (Phelan and Rees, 2003; Malongane et al., 2020) and are referred to as ‘tisanes’ (Sehgal, 2022). Its brewing process only takes 5-10 minutes (Omogbai and Aigbodion, 2013, Sharpe et al., 2016). Herbal tea has low calories and can be used as a relaxing drink.

A high intake of herbal tea as a beverage reduces the risk of chronic diseases due to antioxidant, antiatherosclerosis, anti-inflammatory, antimutagenic, antitumor, and antiviral activities (Jurendić et al., 2021). According to McGaw et al. (2007), bush tea is a healthier beverage as it does not contain caffeine and pyridoxine compared to tea.

Abiotic and biotic stresses such as light intensity, temperature, water availability, type, and soil composition have an impact on the productivity and the quality attributes of herbal teas (Ramakrishna and Ravishankar, 2011). Environmental stresses which include inappropriate radiation or temperature as well as drought trigger the over-production of reactive oxidative species (ROS), which involves superoxide (O_2 −), singlet oxygen (•O_2), hydroxyl ion (OH^-), and H_2O_2 (Smirnoff, 1993). Hence, plants can potentially divert their photosynthetic resources to defence mechanisms against detrimental environmental factors to promote vegetative (biomass) and reproductive stages (secondary metabolites) (Wahid et al., 2007; Pezzani et al., 2017). Therefore, herbal teas use secondary metabolites as defence mechanisms to scavenge and detoxify reactive oxygen species (ROS) using enzymes such as (Catalase) CAT, peroxidase or superoxide dismutase and decompose H_2O_2 to H_2O at different cellular locations.

Bush tea (*Athrixia phyllicoides*)

(Tshikhudo et al., 2019)

Special tea (*Monsonia burkeana*)

(Nnzeru et al., 2019)

Fever tea (*Lippia Javanica*)

(Maroyi, 2017)

Rooibos (*Aspalathus linearis)*

(Joubert and de Beer, 2014)

Bitter gourd (*Mormodica charantia*)

(Wang et al., 2022)

Blackjack (*Bidens Pilosa*)

(Mtenga et al., 2022)

Figure 2.1. Pictures of selected herbal teas

Currently, the vast majority of herbal teas are still collected in the wild for various medicinal attributes and variation in quality characteristics is of great concern (Fig. 2.1). According to reports, seasonal variations as well as varying climatic conditions affect the quality of herbal teas throughout the year (Ghasemzadeh et al., 2010; Mudau et al., 2018). Carbon-based secondary metabolites have been discovered to increase in *Labisia pumila* plants only when climatic conditions promote the production, accumulation, and partitioning of non-structural carbohydrates (Ibrahim et al., 2011). Therefore, cultivation of herbal teas using microtool climate is crucial to maximize the quality of secondary metabolites (Mudau et al. 2006) throughout the season. Furthermore, domestication of herbal teas for commercialization as a healthy beverage could also protect the plants from becoming extinct (Mashimbye et al., 2006). Previous studies have documented four major factors that affect parameters that determine quality in herbal tea production, namely: environmental conditions (Tshivhandekano et al., 2013; MacAlister et al., 2020; Ramphinwa et al., 2022), cultural practices (Mudau et al., 2006; Mohale et al., 2018; Yilmaz et al., 2004; Bandara, 2011; Mphangwe, 2012; Hlahla et al., 2010), cultivars (Owuor et al., 2000) and seasonal variation (Mudau et al., 2018). Thus, environmental factors and agronomic practices have an impact on the quality parameters of tea (Zhang et al., 2014).

Although drinking black (fermented), green (unfermented), and oolong tea (semi fermented) (Zhao et al., 2019) (*Camellia sinensis*) has been linked to various health advantages, there is relatively little research on the effects of most herbal teas. Despite being particularly rich sources of polyphenols, recommendations for the intake of plant-based beverages such as herbal teas are still lacking. Recently, Malongane et al. (2017), claimed that polyphenolic compounds in herbal tea might have synergistic potential, which aids in managing medical conditions when used alongside conventional medicines. The present review gathers data on eco-physiological response of secondary metabolites of herbal tea to better understand the interrelationships between herbal tea and health considering the shift toward integrated medicine and increased interest in herbal tea and health. Drinking herbal tea has been linked to some therapeutic and preventative advantages and this is according to a new scoping review that compiled data from 21 studies, including observational studies (Poswal et al., 2019).

The overall main objective of the present work is to provide a review of production, health benefits and antioxidant properties and how eco-physiological parameters and environmental factors influence accumulation and distribution of secondary metabolites of herbal tea. Thus, the use of locally available herbal plants to develop new herbal tea or infusion might have a significant socio-economic impact on the herbal tea producers in terms of job creation, introducing the product into local and international market as well as improved nutrition and health of consumers since herbal tea is rich in secondary metabolites. Therefore, there is a need to develop strategies to optimize biotic and abiotic factors to promote effective secondary metabolites on a large scale of herbal tea production.

2.2. METHODOLOGY

The methodology of the current study is divided into four phases to achieve the research objectives, viz: definition of key terms, a mixed methods review to determine the current state of herbal teas and identify gaps in their mainstreaming, a review to quantify the amount of knowledge on how light, shading, season, soil composition, and temperature affect secondary metabolite accumulation in herbal teas. The following sections outline the details of four different stages.

Phase 1: definitions of key phrases

Four definitions have been used in this article to describe herbal tea's medicinal properties and functions. Key phrases to be defined include herbal teas, medicinal plants, antioxidants, and polyphenols. Original papers and review articles have been used to gather information to describe the key terms.

Phase 2: identifying medicinal properties and their functions in herbal teas. Google was used throughout the study to gather information.

Phase 3: identifying bioactive compounds and their functions in herbal teas.

Phase 4: a mixed-method review approach including quantitative and qualitative research has been used in this phase to gather information about production, processing, biological activities and how environmental factors affect the accumulation of secondary metabolites of herbal teas.

2.2.1. Search strategy

This section was created using a two-step approach to provide an in-depth assessment of herbal teas' nutraceutical, medicinal properties, and priorities.

First stage: planning the review

Research questions were developed in this stage and were as follows:

1. The current review aims to gather information about nutraceutical properties and environmental factors that influence the quality of herbal teas.

2. What are the medicinal and antioxidant characteristics of herbal teas?

3. Which bioactive compounds are related to the functional characteristics of herbal teas?

4. How do environmental factors affect the quality of herbal teas?

The second stage: a review

Information about the nutraceutical and medicinal benefits of herbal teas was collected through reviewing different research articles. The main objective was to identify herbal teas' medicinal properties and bioactive compounds. Therefore, it was significant to include nutraceutical and medicinal properties attributes of herbal teas. Different attributes identified included: *antimicrobial, antioxidant, hepatoprotection, anti-allergy, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, anti-diabetic, anti-inflammatory and antinociceptive, antifungal, toxicological, anti-diabetes, anti-cancer, antinociceptive, antimelanogenic, wound healing, memory enhancing, antibacterial, antimycotic, antitumor, antiviral, gastrointestinal protective, hepatoprotective and chemopreventive, anti-diarrhea, neuro-protective, anti-allergic, improves cardiac health antiseptic and stimulant, antimicrobial, sedative properties, anti-cholesterol, anti-hypertensive, analgesic, chemoprotective activities and anti-aging*.

2.3. RESULTS AND DISCUSSION

2.3.1. Definitions of key phrases

Herbal teas are an aqueous mixture of different plant materials in hot or cold water to extract the phytochemical constituents for an unspecified period. They are a popular worldwide beverage and are utilized as a therapeutic vehicle in different forms of traditional medicine (Poswal et al., 2019).

A medicinal plant contains active compounds or therapeutic properties in one or more organs that can benefit the human body pharmacologically (Rasool, 2012; Namdeo, 2018). Since prehistoric times, medicinal plants, also known as medicinal herbs, have been discovered and used in traditional medicine practices. These herbs are preferred by non-industrialized societies, owing to their lower cost compared to modern medications.

Antioxidants are groups of compounds that act in the cell to neutralize free radicals and reactive oxygen species (ROS) (Gocer et al., 2013; Cakmakci et al. 2015). A free radical is a highly charged and unstable carbon or oxygen atom with an unpaired electron. Lipids, proteins, and carbohydrates may all produce free radicals.

Polyphenols are secondary metabolites produced by higher plants that have potential health benefits for humans, primarily as antioxidants, anti-allergic, anti-inflammatory, anticancer, antihypertensive, and antimicrobial agents (Fraga et al., 2019). They are essential in plant-pathogen and animal defence, herbivore aggression, and stress response to a wide range of biotic and abiotic stressors.

2.3.2. Characteristics of searched literature

2.3.2.1. Diversity of functional herbal teas

The research study listed 26 herbal teas belonging to 17 different species sampled worldwide (Table 1). The regions were presented by the sampled articles which include South Africa (0.15%), Africa (0.04%), South America (0.04%), South-East America (0.04%), South East Asia (0.12%), Algeria (0.04%), Germany (0.04%), Asia (0.15%), Europe (0.08%), North America, India, South-East Europe, Asia, Mexico (0.04%), Euro Siberian (0.04%), Irano Siberian (0.04%), North Asia, Saudi Arabia, East Europe (0.04%), North Asia (0.04), China (0.04%), South India (0.04%) and Sri-Lanka (0.04%).

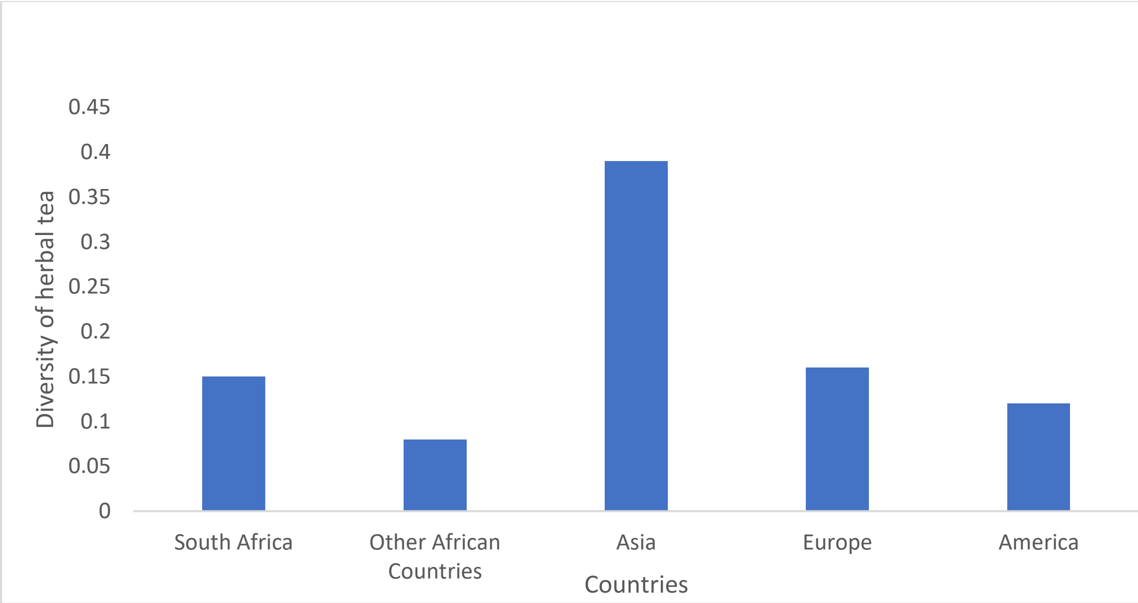


Figure 2.2. Diversity of herbal teas in different continents

2.3.2.2. Processing of herbal teas

Total concentration of polyphenol, antioxidants and tannin content of herbal tea have been reported to be determined by extraction conditions, variety, extracting solvent and processing methods (Villa-Rodriguez et al., 2018; Vural et al., 2020). Differences in chemical constituents and biological activity of plant products are caused by different processing methods and determining how to assess the impact of post-harvest treatment. Chemical and biological testing are essential for improving the quality control of herbal tea products (Chao et al., 2017). However, Turkmen et al. (2009), discovered the high concentration of polyphenols and antioxidants activity on black tea when extracted over extended period using aqueous acetone compared to lower concentration of polyphenols and antioxidant which were influenced by short period of time during extraction using absolute acetone.

Drying is a thermal process in the production of herbal teas which may result in significant losses of bioactive ingredients, reducing the health benefits and quality of the products (Mbondo et al., 2018; Monika et al., 2019). The content of the desired components in tea infusion may be affected significantly by the brewing conditions such as quality, volume, and temperature of water and brewing time (Nikniaz et al., 2016; Sharpe et al., 2016).

2.3.2.3. Polyphenols of herbal teas

Different polyphenols substances, such as flavonoids, tannins, phenolic acids, chlorogenic acids etc., have been reported in herbal teas; however, different parts of herbal teas react differently to different polyphenols (Table 2). Herbal teas have medicinal attributes due to their high concentration of polyphenols (Koch et al., 2020), which is well known to have a wide range of advantageous biochemical and physiological properties (Koch, 2020; Afrin et al., 2020). Herbal teas use these bioactive compounds as their defence against environmental stresses, herbivores, and pathogen attack. Hence, the nature of growing conditions and the amount of stress that the plant is subjected to, determine the concentration of secondary metabolites (Akula and Ravishankar, 2011; Mohale et al., 2018). Polyphenols of herbal teas are found on leaves, flowers, twigs, roots, and rhizomes.

Bush tea has been reported to contain 5-hydroxy-6,7,8,3′,4′,5′-hexamethoxy flavon-3-ol (Mashimbye et al., 2006), 3-0-demethyldigicitrin, 5,6,7,8,3′,4′-hexamethoxyflavone, and quercetin, as well as other polyphenols, tannins, and antioxidants (Mudau et al, 2006; Mudau 2007; Tshivhandekano et al., 2018). The special tea' leaves and fruits has been reported to have high concentration of total phenolic compound and total antioxidant activity (Mamphiswana et al., 2010; Suleman et al., 2015; Mfengu et al., 2021). The unfermented leaves of honey bush tea have been discovered to contain polyphenol compounds which include pinitol, shikimic acid, p-coumaric acid, 4-glucosyltyrosol, epigallocatechin gallate, the isoflavone orobol, flavanones hesperedin, narirutin, and eriocitrin, a glycosylated flavan, the flavones luteolin, 5-deoxyluteolin, and scolymoside, the xanthone mangiferin, and the flavonol C-6-glucosylkaempferol (Dube et al., 2017; Ajuwon et al., 2018). Rooibos has been characterized by a diverse range of phenolic compounds, including dihydrochalcones (aspalathin, aspalalinin, and nothofagin), flavones (orientin, iso-orientin, vitexin, isovitexin, chrysoeriol, and luteolin), flavonols (quercetin, isoquercitrin, hyperoside, and rutin), and phenolic (Ajuwon et al., 2018). Fever tea has been reported to have volatile oil as well as various monoterpenoids which include myrcene, linalool, p-cymene, caryophyllene and ipsdienone (Makoka et al., 2007; Endris et al., 2016).

Endris et al. (2015) and Chawafambira (2021), discovered that leaves of fever tea produce numerous polyphenolic compouds such as of palmitic, stearic, myristic, arachidic, oleic, behenic, triacontane alkanes and lignoceric acids. *Misai kucing* possess pharmacological properties due to the presence of different groups of phenolic acids including terpenoids (diterpenes and triterpenes) (Abdullah et al., 2020), flavonoids (Pang et al., 2017), and benzochromenes (Abdullah et al., 2020). Lemon grass possess different compounds as hydrocarbon terpenes, alcohols, ketones, esters due to different geographical origin (Majewska et al., 2019). Guajava contains significant biological activities caused by the presence of phenolic, flavonoid, carotenoid, terpenoid and triterpene (Angulo-López et al., 2021).

The high levels of antioxidant activity influenced by phenolic compounds contents as phenolic acids and flavonoids are found on the leaves of lemon myrite (Kim et al., 2017). Triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic compounds are the major compounds of bitter gourd which are responsible for antidiabetic activities (Grover and Yadav, 2004; Chung et al., 2018). Mascotek has been characterised by various phenolic compounds including flavonoids, saponins, tannins, steroids and terpenes (Nasma et al., 2018). Peppermint has been discovered to possess flavonoids such as flavanone aglycone eriodictyol and glycosides eriocitrin (eriodictyol-7-O-rutinoside), hesperidin (hesperetin-7-O-rutinoside) and naringenin-7-O glucoside, the flavone aglycone luteolin and the glycosides isorhoifolin (apigenin-7-O-rutinoside) and luteolin-7-O-glucoside (Bodalska et al., 2019). The extract of pegaga consist of bioactive compounds such as plant sterols, flavonoids, and other components with no known pharmacological activity (Nazmi and Sarbon, 2019).

Chamomile have been reported to contain numerous bioactive phenolic compounds which are coumarins: (herniarin, umbelliferone; phenylpropanoids: chlorogenic acid, caffeic acid; flavones: apigenin, apigenin, 7-O-glucoside, luteolin, luteolin-7-O-glucoside; flavonols: quercetin, rutin and flavanone: naringenin) and are found in chamomile extract (Gupta et al., 2010; Bayliak et al., 2021). The major compounds of oregano are caryophyllene, spathulenol, germacrene-D and aterpineol (Sahin et al., 2004). The volatile oil from *Cymbopogon citratus* consist of volatile oil which is characterised by monoterpene hydrocarbons (Adesegun, 2013; Oladejiet al., 2019). The fraction of monoterpene has been classified by a high percentage content of geranial (39.53%), neral (33.31%), myrecene (11.41%) and other sesquiterpene (0.78%) (Moreira et al, 2010; Wright et al., 2009).

Da-Costa-Rocha et al. (2014), discovered that the extracts of roselle consist of a high percentage of organic acids, including citric acid, hydroxycitric acid, hibiscus acid, malic and tartaric acids as major compounds, and oxalic and ascorbic acid as minor compounds. Goose berry extracts are rich source of polyphenols which are responsible for cytotoxic activity against cervical and ovarian cancer cells (De et al., 2013). Red raspberry possesses two major polyphenols such as anthocyanin and ellagitannins content (Singh et al., 2020). Ginger is a rich source of phenolic components which are important food material and can be served as cheap (Gbenga-Fabusiwa et al., 2018).

Table 2.1. Different types of herbal teas used globally

Family	Scientific name	Common name	Part used	Countries	References
Acanthaceae	*Thunbergia laurifolia and Orthosiphon aristatus*	Akar ketuau	Leaves	India	Boonyarikpunchai et al. (2014)
Asteraceae	*Athrixia phylicoides*	Bush tea	Leaves, roots, and flowers	South Africa	Mudau et al. (2006), Mohale et al. (2018), Ramphinwa et al, (2022)
Asteraceae	*Bidens Pilosa*	Blackjack	Leaves and stem	South America	Liang et al. (2016), Bilanda et al. (2017)
Asteraceae	*Matricaria recutita*	Camomile	Leaves	Southern and Eastern Europe	Gupta et al. (2010), Bayliak et al. (2021).
Apiaceae	*Centella asiatica*	Pegaga	Leaves	South-East Asia	Nazmi and Sarbon, (2019)
Cucurbitaceae	*Mormodica charantia,*	Bitter gourd	Leaves	Asian	Ramabulana et al. (2021), Muronga et al. (2021)
Fabaceae	*Aspalathus linearis*	Rooibos	Leaves	South Africa	Joubert and Schulz, (2012), Gaggia et al. (2018)
Fabaceae	*Cyclopia intermedia*	Honeybush	Shoots stems and leaves	South Africa	De Beer et al. (2021)
Geraniceae	*Monsonia burkeana*	Special teas	Roots and leaves	South Africa	Mamphiswana et al. (2010), Nnzeru et.

					(2016), Mfengu et al. (2021)
Lamiaceae	*Mentha piperita*	Peppermint	Leaves	Europe	Bodalska et al. (2019)
Lamiaceae	*Mentha spicata*	Mint	Leaves	Europe, North America and Asia	Yu et al. (2015)
Lamiaceae	*Origanum vulgare*	Oregano	Leaves, flowers, roots and stems	Mediterrenian, Euro-Siberian and Irano-Siberian	Han et al. (2017)
Lamiaceae	*Orthosiphon aristatus*	Misai kucing	Leaves	South-East Asia	Hui Gan et al. (2017)
Malvaceae	*Hibiscus sabdariffa*	Roselle	Leaves, flowers and seeds	India and Saudi Arabia	Da-Costa-Rocha, et al. (2014)
Moraceae	*Ficus deltoidei*	Mas cotek	Leaves	South-East Asia	Nasma et al. (2018)
Myrtaceae	*Psidium guajava*	Guajava	Leaves	Asia	Angulo-López et al. (2021)
Myrtaceae	*Backhousia citriodora*	Lemon myrite	Leaves	Germany	Kim et al. (2017)
Myrataceae	*Psidium guajava and Taona sinensis*	Guava	Leaves and roots	Mexico	Angulo-López et al. (2021)
Phyllanthaceae	*Phyllanthus amarus*	Indian gooseberry	Leaves	India	Zhao et al. (2014)
Poaceae	*Cymbopogon citratus*	Lemon grass	Leaves	Algeris	Olayemi, (2017)
Poaceae	*Cymbopogon citratus*	West Indian lemongrass	Leaves	South-East Asia	Kamaruddin, et al. (2021)
Rosaceae	*Rubus idaeus* L	Red rasberry	Leaves	Eastern Europe and North Asia	Singh et al. (2020)

Zingiberaceae	Zinger officinale Roscoe	Ginger tea	Rhizomes and leaves	Asia	Gbenga-Fabusiwa et al. (2018)
Zingiberaceae	Elettaria cardamomum	Cardamom tea	Seeds	South India and Sri-Lanka	Anwar et al. (2016)

2.3.2.4. Biological activities of herbal teas

Medicinal properties of herbal teas are summarised in Table 2 and has been reported by different researchers.

2.3.2.4.1. Antioxidant activity

Herbal teas, which are herb extracts, are popular because of their fragrance and antioxidative properties (Aoshima et al., 2007; Farzaneh and Carvalho, 2015; Jin et al., 2016). Antioxidants are substances that have been shown to significantly neutralize reactive oxygen species (ROS), which are oxygen-derived free radicals that cause degenerative diseases such as superoxide anion, hydroxyl radicals, and nitric oxides (Malongane et al., 2017). In vitro studies revealed that ethanolic bush tea extract has strong antioxidant activity and inhibition of DPPH was found to be 81.6% when the lowest concentration was used to test antioxidant activity (Mavundza et al., 2007). Moreover, Bahadori et al. (2018) demonstrated that *Stachys byzantina* and *Stachys iberica* exhibited DPPH scavenging activity ranging from 26 to 125 mg TE/g extracts.

2.3.2.4.2. Antidiabetic activity

According to Martínez-Solís et al. (2021), diabetes mellitus has been treated by herbal teas and infusions. Diabetes mellitus is a chronic condition in which the body's ability to control the amount of glucose in the blood is impaired (DeFronzo et al., 2015). This is due to pancreatic insulin resistance or a lack of insulin secretion. Phenolic compounds in herbal teas such as chlorogenic acid, 1,3-dicaffeoylquinic acid, and hydroxylcinnamic acid, have been linked to diabetes and obesity prevention (Joubert and Schulz, 2012). Chellan et al. (2012) reported that herbal teas could be used to treat metabolic irregularities associated with diabetes by increasing glucose utilisation in insulin-responsive tissues. Previous studies have also demonstrated anti-diabetic, antioxidant, antilipidemic, and antinociceptive effects using aqueous and alcoholic extracts of *Annona muricata*, *Annona squamosa*, *Annona stenophylla*, *Annona macroprophyllata*, and *Annona diversifolia* and this is attributed to the presence of phenolic compounds in their leaves (Martínez-Solís et al., 2021).

2.3.2.4.3. Antimicrobial activities

Herbal teas have been reported to have antimicrobial activities against gram-positive and -negative bacteria and yeast when they are used alone (Hacioglu et al., 2017). Depending on the antibiotic or type of tea, the synergistic, additive, or antagonistic effects of herbal teas with antibiotics were observed. Thus, using herbal teas alone or in combination with chemical antimicrobials may be a viable alternative treatment strategy for a wide range of pathogenic microorganisms. Generally, antimicrobial activities decrease with the extent of tea fermentation, implying that green tea is more active than black tea (Nibir et al., 2017). Green tea catechins, particularly epigallocatechingallate (EGCG) and epicatechingallate (ECG), have antibacterial properties against Gram-positive and Gram- bacteria negative bacteria (Bancirova et al., 2010; Ignasimuthu et al., 2019).

Green tea can help prevent tooth decay by inhibiting oral bacteria (Zayed et al., 2021). Xia et al. (2020) discovered that combining white tea and pepper mint resulted in synergistic antibacterial activity against four strains, two of which were gram-positive (*S. argenteus* and *B. halotolerans*) and two of which were gram-negative (*E. coli* and *P. aeruginosa*). Bush tea inhibits microorganisms such as *Staphylococcus aureus, Bacillus cereus, Enterecoccus, Escherichia coli,* and *Mycobacterium smegmatis*.

Table 2.2. Different types of herbal teas and their health functions

Herbal teas	Available antioxidants	Functions	References
Bush tea	Flavonoids, polyphenols and tannins	They protect against inflammation, cardiovascular disease, cancer, and the aging process. Tannins bind to iron and reduce non-heme iron absorption.	Mudau et al. (2006), Mudau, et al., (2018)
Blackjack	Aliphatics, terpenoids, tannins, alkaloids, hydroxycinnamic acid (HCA), and phenylpropanoids are all constituents of blackjack	It has anticancer, anti-inflammatory, and antihypertensive properties.	Ramabulana et al. (2020)
Bitter gourd	Cucurbitane triterpenoids, saponin glycosides, chlorogenic acids and flavonoids	It consists of anti-diabetes, anti-cancer, and anti-inflammatory properties	Dandawate et al. (2017)

Chamomile	Phenols and flavonoids	It has anti-inflammatory, anti-diarrhea, antioxidant, anti-cancer, neuroprotective, anti-allergic and antimicrobial properties and improves cardiac health	Ašimovićet al. (2022)
Fever tea	Monoterpenoids (myrcene, caryophyllene, linalool, p-cymene, and ipsdienone); stearic, palmitic, myristic, oleic, arachidic, behenic, and lignoceric acids, as well as triacontane alkanes and Iridoid glycosides, and highly toxic triterpenoids (acerogenins)	It has antimalarial, antiviral, cytostatic activities, and antimicrobial properties	Endris et al. (2016)

Ginger tea	Flavonoids and phenolics	It has antioxidant, anti-aging, and anti-cancer properties.	Huyen, (2020)
Guajava	Phenolic, flavonoid, carotenoid, terpenoid and triterpene	It has antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive properties(Joseph and Jini, 2013)	Guajava
Honeybush	Hesperidin and eriocitrin flavanones, as well as scolymoside flavone	They have anti-diabetic, anti-cancer, anti-obesity, antioxidant, and antimicrobial properties	De Beer et al. (2021)

Indian gooseberry	Phenolic compounds and Vitamin C	It has anti-inflammatory, antioxidant, and chemoprotective properties.	Zhao et al. (2014)
Lemongrass	Flavonoids and tannins	It contains antimicrobial activity properties	(Olayemi, 2017)
Lemon myrite	Phenolic acids and flavonoids	It serves as antimicrobial, antifungal and toxicological activities	Hayes and Markovic, (2002); Kim et al. (2017)
Mas cotek	flavonoids isovitexin, vitexin proanthocyanidins, flavan-3-ol monomers and flavones glycosides	It has anti-diabetic, antinociceptive ulcer healing, antioxidant, anti-inflammatory, antimelanogenic properties	Nasma et al. (2018)
Misai kucing	Phenolic acids, terpenoids (diterpenes and triterpenes), flavonoids and benzochromenes	It possesses antioxidant, anti-inflammatory, and antihyperglycemic	Pang et al. (2017), Abdullah et al. (2020)

	Phenolic compounds, such as caffeic acid, rosmarinic acid, sinensetin, and eupatorine		
Oregano	Coumarins and phenylpropanoids	Possesses antiseptic and stimulant	Han et al. (2017)
Pegaga	Polyphenol, flavonoid (3-carotene, tannin, vitamin C, and DPPH (2, 2-diphenyl-1-picrylhydrazyl).	It has antioxidant, anti-inflammatory, wound healing, memory-enhancing properties	Singh et al. (2010)
Peppermint	Phenolic and flavonoids	It has antioxidants, antiallergenic, antibacterial, anti-inflammatory,	Bodalska et al. (2019)
Rooibos	Polyphenols (aspalathin, nothofagin; free flavones and glycosides: orientin, iso-orientin, vitexin, isovitexin, luteolin,	It has antioxidant properties, antimutagenic properties, and the ability to lower blood glucose levels.	Joubert and Schulz, (2012), Gaggia et al. (2018)

	luteolin-7-O—D-glucoside) flavonols and glycosides (quercetin, hyperoside, rutin)		
Roselle	Flavonoid, tannin, and a phenolic compound	It contains anti-cholesterol, anti-diabetic and anti-hypertensive properties	Abdullah et al. (2020)
Special tea	Polyphenols and tannins	They serve as antioxidants and antimicrobial	Ngoepe et al. (2018)
West Indian lemongrass	Alkaloids, saponins, tannins,	Antimicrobial, anti-inflammatory, and sedative properties	West Indian lemongrass

2.3.2.4.4. Anti-mutagenic/anti-carcinogenic activities

The antimutagenic assay revealed that unfermented rooibos and honeybush tea extracts had a strong antimutagenic effect against both metabolically activated carcinogens, antimutagenic activity against 2-acetylaminofluorene (2-AAF) and (aflatoxin) AFB1 (Marnewick et al., 2000, Chaudhary et al., 2021). Carabajal et al. (2017), also discovered that antimutagenic activity of the freeze-dried plant infusions tested against a direct-acting mutagen (4-NPD), with at least three herbal mixtures resulting to a positive response.

2.3.3. Environmental factors that affect the production and accumulation of secondary metabolites of herbal teas

Abiotic and biotic factors, including ultraviolet irradiation, high light, temperature, pathogen attack, wounding, herbicide, and nutrient deficiency have been reported to determine the concentration of different metabolites in herbal plants (Akula and Ravishankar, 2011; Mohale et al., 2018). Plants are a rich source of natural compounds with diverse bioactivities (Masike et al., 2017). These organic compounds are divided into two categories: primary and secondary metabolites and used by plants as defence mechanisms against biotic and abiotic (Akula and Ravishankar, 2011; Mohale et al., 2018). The quality of herbal teas remains one of the constant critical aspects in determining tea’s price (Ravichandran and Parthiban, 1998; Tshivhandekano et al., 2018), industrialization, and exportation (Mudau et al., 2007). Herbal tea’s quality is determined by active secondary metabolites present, viz., flavonoids, polyphenols, and tannins (Mathivha et al., 2020). Thus, compounds such as polyphenols, flavonols, and tannins are the main indicators of the medicinal potential of herbal teas due to their antioxidant activities (Hirasawa et al., 2002; Mudau et al., 2007; Poswal et al., 2019).

2.3.3.1. Light

Light is a critical resource for plants (Ghasemzadeh et al., 2010; Rihan et al., 2020) and competition for light under shade affect growth and development of herbal teas (Kumar et al., 2013; Ramphinwa et al., 2022). Plant growth and development, photosynthetic rate, and production of both primary and secondary metabolites are all influenced by light (Zhang et al., 2014). Currently, the light requirements of herbal teas are not well documented. Although it is difficult to define details of the ideal range of shadow required by tea plants, 50% of diffused sunshine is generally necessary for optimal physiological activity of *Camellia sinensis* tea (Tshivhandekano et al., 2013; den Braber et al. 2010). Shade/low irradiance enhances flavonoid synthesis and other bioactive compounds in ginger production (Ghasemzadeh et al., 2010).

Photosynthetically Active Radiation (PAR) is a waveband that has a significant influence on plants' growth, development (Proutsos et al, 2022) and accumulation of secondary metabolites (Ghasemzadeh et al., 2010). It is also known as the intercepted radiation that ranges between 400 to 700-nanometre wavebands (Mubvuma et al., 2018; Rihan et al., 2020). Appropriate intercepted radiation and utilisation are of great importance in herbal tea production. Bush tea plants exposed to 80% white shade net accumulated more chlorogenic acid (CGAs) than plants exposed to other shade nets (i.e., 80% black, green shade nets and full sunlight) (Ramphinwa et al., 2022). These results could be attributed to the accumulation of CGAs caused by low temperatures and light intensities under 80% white shade net. The white shade net might have induced a higher number of chlorogenic acids than other shade nets due to the amount of light penetrating it. These findings are consistent with those of Karimi et al., (2013), who found that when different varieties of *Labisia pumila* Benth are exposed to high light intensities, tend to accumulate more phenolic compounds such as gallic acid, caffeic acid, and flavonoids such as quercetin, rutin, myricetin, kaempferol, and naringin. Contradictory, higher oxygenated monoterpene and sesquiterpene components such as citronellol, geranyl acetate, linalool and trans-rose oxide were recorded in an open field compared to 25% and 50% shade levels. Kumar et al. (2014) and Rezai et al. (2018), also reported that oxygenated monoterpenes and sesquiterpene components decreased with shade levels in *S. sclarea* and *T. minuta*. Light might have influenced these results as a critical component that plays a significant role in producing secondary metabolites that vary due to the plant's metabolic processes and physiology. Variation of light intensities revealed plant morphological and physiological changes, which significantly impacted the herb’s medicinal compounds (Idrees, et al., 2018). Therefore, different plants respond differently to light intensity, resulting in differences in secondary metabolite production (Ibrahim et al., 2011; Ni et al., 2011)

Herbal plants utilized in traditional therapeutic techniques are frequently produced in places with significant UV exposure, and there are no defined conventional agricultural practices (Makola et al., 2016a). In such conditions, photoisomerization reactions of bioactive compounds are expected to be simple, putting active molecules at risk. As a result, it is critical to investigate the environmental effects of plants with therapeutic properties. Plants naturally produce cinnamic acids in a trans-configuration, which changes to a *cis* configuration when exposed to UV light (Makola et al., 2016b). These results concur with those of Masike et al. (2017) and Nobela et al. (2018), who reported the increasing of the content of secondary metabolites containing cinnamic acids through UV light. Chrysanthemum's concentrations of flavonoids and phenolic acids increased in response to increased UV-B radiation (Ma et al., 2016). These results might have been attributed to an increase in UV radiation associated with the amount of solar radiation received by the plants (Naghiloo et al., 2012).

2.3.3.2. Shading

Plant shading is caused primarily by dense plant populations, intercropping, planting geometry, and excessive vegetative growth, and it has an impact on crop performance by reducing plant photosynthetic capacity (Kumar et al., 2013; Zaman et al., 2022). Irradiance is one of the crucial environmental factors that affect many physiological processes in plants, such as plant growth and development, reproduction, and distribution of secondary metabolites (Kumar et al., 2013; Zhang et al., 2015). Photoselective films, which regulate light conditions and influence medicinal plant growth and secondary metabolism, are important for optimising secondary metabolite accumulation (Khandaker et al., 2010; Grbic et al., 2016). Black tea exposed to artificial shade resulted in a higher concentration of theaflavin, lower concentration of thearubigin, higher flavour index and taster's evaluation compared to tea grown in an open field. This hypothesis holds that removing the shade from tea gardens results in a loss of quality (Owuor et al., 1988; Zaman et al., 2022).

These findings concur with those of Morita and Tuji (2002), who reported that shading produces high-quality Gukyo and Tencha green teas in Japan. The results might have been influenced by low light intensity under shades. Hence, shading could define tea's characteristics by slowing the photosynthesis process and thus increasing the chlorophyll content (Lehlohonolo et al., 2013). These might be due to the leaves that turn dark green, and the tannin content decreases, resulting in a sweeter flavour rather than the astringent taste common in green teas under shades. These results contradict with Kumar et al. (2013), who reported that shade regimes did not affect the accumulation of stevioside and Rebaudioside-A in the stevia plant. Thus, accumulation and partitioning of the secondary metabolite are impacted by plant species, different plant organs, and environmental conditions to which the plants are subjected (Eko, 2012; Mohd Yusof et al., 2021).

2.3.3.3. Seasons

Producing tea of the same quality is impractical throughout the year due to different climatic conditions and seasonal variations (Owour et al., 1998; Mudau et al., 2007; Mudau 2016). The results are in line with those of Lin-Wang et al. (2011), who reported that significant climatic changes throughout the cropping season have a significant impact on tea quality and value. These findings might have been influenced by harsh climatic conditions, which are unsuitable for producing high-quality herbal tea (Mudau et al., 2016). According to Mudau et al. (2006), variation in seasonal temperatures and vapour pressure deficit affect the quality and antioxidants in bush tea. The concentration of polyphenols in bush tea leaves collected from the wild has been recorded to be lowest in autumn, spring and highest in winter (Mudau et al., 2006; Nchabeleng et al., 2012).

Furthermore, hydrolysable tannins concentration was lowest during summer compared to autumn, spring and winter. The results might have been attributed due to drought stress (Hamilton et al., 2001; Lv et al., 2021) and low temperatures (Mudau et al., 2006) during autumn and winter. The results are in line with those of Caruso et al. (2020), who reported the highest value of hydrophilic antioxidant activity under unshaded fields, but there was no significant difference between autumn and winter cropping seasons; hence lowest was recorded in the last cropping season.

Turkmen et al. (2009) and Soni et al. (2015), further reported variation of catechin content and distribution in fresh tea leaves are influenced by harvesting season. Similar results were also discovered by Chou et al. (1999) and Salman et al. (2022), who reported that catechin content variation due to harvesting season and higher antimicrobial activity during summer cropping season resulted in a higher concentration of catechin. In Australia, higher epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) levels were recorded during warmer months (Yao et al., 2005; Kashchenko et al., 2021), while EGC recorded higher levels in cooler months. As a result, the warmer season is recommended for producing high-quality black tea (Lin et al., 1996; Hossain et al., 2017). Understanding the metabolic process of herbal teas will elucidate how the variation of seasons may impact the formation and its quality.

2.3.3.4. Temperature

The effect of temperature on plant biosynthesis and phytochemical accumulation is significant (Cheynier et al., 2013; Pola et al., 2020). The physiological and biochemical processes that result in the formation of plant metabolites are influenced by temperature (Tshivhandekano et al., 2013). Low temperature restricts the accumulation of alkaloids (morphinane, phthalisoquinoline and benzylisoquinoline) in dry *Papaver somniferum*) (Yang et al., 2018). Similarly, Dutta et al. (2007) and Jan et al. (2021), reported the reduction of vindoline and catharanthine levels in *Catharanthus roseus* leaves due to low temperatures. Contradictory, different cultivars of *Lupinus angustifolius* increased accumulation of alkaloid when exposed to high temperature. Thakur et al. (2019), also reported that higher temperatures promote leaf senescence and concentration of the secondary metabolite in the roots of *Panax quinquefolius*.

2.3.3.5. Soil type and composition

The production, accumulation, and partitioning of secondary metabolites in plants have been reported to rest on a few hypotheses: carbon nutrient balance (CNB) and growth differentiation. Verma and Shukla, (2015) discovered that plant growth and cell development take precedence over secondary metabolite production. Before distributing carbon and nitrogen to produce secondary metabolites, the growth requirement must be met. Nutrient-deficient in plants promotes a higher concentration of carbon-based secondary metabolites at the expense of plant growth and development than plants with enough nutrients (Radušienė et al., 2019). These findings imply that herbal tea could accumulate more secondary metabolites when plants are deprived of nutrients.

The most important determinant of the rate of secondary metabolite production is the collection of precursor molecules (Waterman et al., 2019). As a result, it suggests that the status of carbon-nutrient balance in plants, as determined by resource availability, has a significant influence on secondary metabolite allocation. Hence, the palatability and resistance of the plant to herbivores are affected by the allocation of secondary metabolites. The more nitrogen is invested in soil, the less the accumulation of phenolics and flavonoids in *Labisia pumila* Benth (Ibrahim et al., 2011). Contradictory, previous studies reported that N shortage increased the content of carbon-based secondary metabolites (CBSMs) (e.g., polyphenols, flavonoids, triterpenoids) (Ibrahim et al., 2013; Strzemski et al., 2021; Sun et al., 2021).

2.3.3.6. Water stress

Water stress is one of the most important environmental stresses affecting plant morphological growth and development and their biochemical properties (Asharaf et al., 2018). It can increase the accumulation of secondary metabolites in a wide range of plant species. Endogenous levels of plant secondary metabolites increased in response to drought stress in several medicinal plants, including *C. roseus*, *H. perforatum* and *Artemisia annua*. Drought stress, for example, increased phenolics and photosynthetic pigments while decreasing plant fresh and dry biomass in *T. ammi* (Azhar et al., 2011). Similar results were reported by Verma and Shukla (2015), that improved quality of secondary metabolites such as rutin, quercetin, and betulinic acid in *Hypericum brasiliense, Artemisinin* and *Artemisia* resulted in water stress. Contradictory, essential oil content (%) and yield decrease significantly with increasing the level of water stress in *Origanum vulgare* and *Melissa officinalis* (Said-Al Ahl and Hussein, 2010). Drought tolerance in plants differs from species to species (Thakur et al., 2019).

2.4. LIMITATION AND FUTURE PERSPECTIVE OF HERBAL TEAS

Over the past two decades, the safety and medicinal potency of herbal teas have been subject of interest. Results from different studies show that herbal teas contain phytochemicals that are beneficial while other are toxic to human health (Upadhya et al., 2004; Krushna et al., 2009; Gohil et al., 2010; Singh et al., 2013). There is a very limited information from the literature with regards to the safety of herbs and herbal teas, herb-herb and interconnections of herb-therapeutic drug (Chandrasekara and Shahidi, 2018). Therefore, there is a need that future research determines the toxicity and bioactivity of herbal teas.

Another challenge of herbal teas is that their market is unstructured and there is risk of poisonous products entering the market. For example, Jin et al. (2018) found 14 poisonous species used for herbal teas in the ethnobotanical surveys of traditional medicinal markets on the Dragon Boat Festival, China. Poor sourcing standards could result in such risk since there might be no quality measurement of original plant materials. Therefore, there is a need for sector to provide products that meet medical standard and not only concentrating on the amount of phytochemicals in the plant materials, but to guarantee quality and reassure consumers that the products are not contaminated (Booker et al., 2016). Moreover, authors indicated that there might be adulteration of herbal teas in the same market. A recent quality evaluation of marketed chamomile tea found that other plant materials are more likely to adulterate crude flowers than German chamomile tea bags (Guzelmeric et al., 2017).

The labelling and promotion claim for most of herbal teas are questionable, with claims of superior antioxidant activity compared with tea or coffee and the potential to treat various health disorders (McKay and Blumberg, 2007). Such claims should be supported by evidence that is sound and research based (Balentine et al., 1999). Most of these herbal teas have not been well documented especially for studies that involve human trials, therefore, such health benefit claims are not justifiable even though most of these teas have history of utilisation in some traditional medicines. Poswal et al. (2019) suggested that there might be relationships between the consumption of herbal teas and low risk of thyroid disease and liver. Therefore, there is a need to conduct further research on analytical and clinical trials to evaluate the phytochemicals of herbal beverages that act in reducing the risk of disease in human as well as verifying how they promote human health (Chandrasekara and Shahidi, 2018).

Other areas of interest include synergistic combination and microencapsulation of herbal teas. Moreover, non-thermal treatments, such as high-pressure processing should also be exploited. The synergistic effect of various combinations of herbal teas has been studied. The mixture of tea with other herbs has demonstrated to synergistically improve antioxidant activities and this leads to the development of various blends of tea blends. The antioxidant activity of the synergistic mixture might offer concomitant health benefit since polyphenolic compounds available in the tea and herbs play role in the prevention of cardiovascular diseases and type 2 diabetes mellitus (Malongane et al., 2017). Mathivha et al. (2019) determined the synergistic effect of South African herbal teas namely bush tea – *Athrixia phylicoides* DC and special tea – *Monsonia burkeana* Planch. ex Harv and reported that the mixture has high antioxidant activities that might play a role in managing lifestyle diseases such as diabetes (Etheridge and Derbyshire, 2020).

Microencapsulation is an emerging technique which leads to the protection of various components of food or functional ingredients against different processing methods since it covers them inside a polymeric or nonpolymeric material and allow their controlled release under specific conditions (Choudhury et al., 2021). Moreover, microencapsulation improves the organoleptic attributes of food by camouflaging the unpleasant smell and taste as well as preventing the growth of microorganisms (Hasanvand et al., 2015; Sengupta et al., 2001). Spray and freeze drying are the extensively used methods for microencapsulation. Pasrija et al. (2015) microencapsulated polyphenolic compounds of green tea with walls of maltodextrin, b-cyclodextrin and combination of both and determined its effect on the quality of bread. Green tea extract and encapsulates added bread maintained their quality characteristics with regards to loaf volume and crumb firmness and they were almost similar to control sample. The total polyphenolic content of extract of green tea and microencapsulated bread did not differ significantly.

High pressure processing (HPP) is applied to food to eliminate vegetative microorganisms, thereby warranting microbial safety and improved shelf life of food (Guerrero-Beltra´n et al. 2004). During HPP process, hydrostatic pressure varying from 100 to 800 MPa, is applied to beverages or food which leads to inactivation of microorganism by denaturing protein or cell injury (Guerrero-Beltra´n et al. 2005). Moreover, HPP also retains bioactive components such as polyphenols, antioxidant activity and it does not affect small molecules such as pigments, vitamins and volatile compounds (Patras et al., 2009; Medina-Meza et al., 2015; Marszalek et al. 2017).

Kieling et al. (2018) evaluated the quality characteristics of lemongrass- lime mixed beverage processed under the optimal HPP conditions during 8 weeks of storage at 4°C. The beverage was compared with the control and pasteurised sample. High pressure processed beverage retained its phenolic compounds, no significant losses of ascorbic acid observed, and physicochemical properties were closer to control sample compared with pasteurised sample. Moreover, HPP of 250 MPa for 1 min at 25°C resulted in microbiological safety, based on the inactivation tests with *Listeria innocua* as the target microorganism. This shows that HPP can be utilised as an alternative or substitute of thermal processing of herbal teas since it extended the shelf life of lemongrass-lime mixed beverage.

2.5. CONCLUSIONS

Herbal tea is a rich source of secondary metabolites. Environmental factors have been studied herein to determine their influence on the quality of herbal teas. Generally, abiotic and biotic stresses increase the production, accumulation and partitioning of secondary metabolites on plants at the expense of plant growth, development, and yield. In this study, various examples of herbal teas have been shown to respond differently to secondary metabolites affected by environmental factors. Therefore, this study confirms that different herbal teas' response to environmental factors depends on the type of species, cultivar, and the degree of shade that the plant is exposed to. Quality assurance is a critical step required to be achieved for the potential market for herbal teas. Hence, metabolic processes known to optimize the production of secondary metabolites can thus be achieved by manipulating agronomic practices on herbal teas. It is thus concluded that light, shading, season, soil composition, water stress and temperature play a significant role in cultivating herbal teas. The health benefits of herbal tea necessitate more effort to understand the complex interaction of biotic and abiotic environmental factors with the plant to produce secondary metabolites.

2.6. REFERENCES

Abdullah, F.I., L.S. Chua, S.P. Mohd Bohari, and E. Sari. 2020. Rationale of *Orthosiphon aristatus* for healing diabetic foot ulcer. Nat. Prod. Commun. 15:1-13, doi: 10.1177/1934578X20953308.

Adesegun, A.S., O.S. Folorunso, G.O. Raimi, and S.A. Funmilola. 2013. Antioxidant activity of the volatile oil of *Cymbopogon citratus* and its inhibition of the partially purified and characterized extracellular protease of Shigella sonnei. Department of Biochemistry, Faculty of Science, Lagos State University, Lagos, Nigeria.

Ajuwon, O.R., A.O. Ayeleso, G.A. Adefolaju. 2018. The potential of South African herbal tisanes, rooibos and honeybush in the management of type 2 diabetes mellitus. Molecule. 23:3207, doi: 10.3390/molecules23123207.

Akula, R., and G.A. Ravishankar. 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6:1720-1731, doi: 10.4161/psb.6.11.17613

Amina, E.M., E.C.C. Mara, C.A.N.O. Antonio, H.R. Josefa, L.A.M.A.R.T.I. Ahmed, and B.A. Marino. 2021. Comparative study of wild chamomile plants from the North-West of Morocco: Bioactive components and total antioxidant activity. J. Med. Plant Res. 5:431-441.

Angulo-López, J.E., A.C. Flores-Gallegos, C. Torres-León, K.N. Ramírez-Guzmán, G.A. Martínez, and C.N. Aguilar. 2021. Guava (*Psidium guajava* L.) fruit and valorization of industrialization by-products. Processes. 9:075, doi: 10.3390/pr9061075.

Anwar, F., A. Abbas, and K.M. Alkharfy. 2016. Cardamom (*Elettaria cardamomum* Maton) Oils. In Essential oils in food preservation, flavor and safety. 295-301. Editor(s): Victor R. Preedy, Academic Press, doi: 10.1016/B978-0-12-416641-7.00033-X.

Aoshima, H., S. Hirata, and S. Ayabe. 2007. Antioxidative and anti-hydrogen peroxide activities of various herbal teas. Food Chem. 103:617-622.

Ashraf, M.A., M. Iqbal, R. Rasheed, I. Hussain, M. Riaz, and M.S. Arif, M. 2018. Envinmental stress and secondary metabolites in plants: an overview. Plant metabolites and regulation under environmental stress. 153-167, doi: 10.1016/B978-0-12-812689-9.00008-X.

Ašimović, Z., E. Sirbubalo, L. Čengić, Š. Muminović, and J. Jurković. 2022. Phenolic Content and Antioxidant Activity Determination in Chamomile (*Matricaria Recutita*) and Sage (*Salvia Officinalis*) Teas. In: 10th Central European Congress on Food. CE-Food 2020. Springer, Cham, doi: 10.1007/978-3-031-04797-8_31.

Azhar, N., B. Hussain, M.Y. Ashraf, and K.Y. Abbasi. 2011. Water stress mediated changes in growth, physiology and secondary metabolites of desi ajwain (*Trachyspermum ammi* L.). Pak. J. Bot. 43:15-19.

Bahadori, M.B., G. Zengin, L. Dinparast, M. and Eskandani. 2020. The health benefits of three Hedgenettle herbal teas (*Stachys byzantina, Stachys inflata, and Stachys lavandulifolia*)- profiling phenolic and antioxidant activities. Eur. J. Integr. Med. 36:101134, doi: 10.1016/j.eujim.2020.101134.

Balentine, D.A., M.C. Albano, and M.G. Nair. 1999. Role of medicinal plants, herbs, and spices in protecting human health. Nutr. Rev. 57:41-45, doi: 10.1111/j.1753-4887. 1999.tb01806..x

Bancirova, M. 2010. Comparison of the antioxidant capacity and the antimicrobial activity of black and green tea. Food Res. Int. 43:1379-1382, doi: 10.1016/j.foodres.2010.04.020.

Bandara, S.N. 2012. Agronomy of irrigated tea in low elevation growing areas of Sri Lanka. PhD thesis, Univ. Adelaide School Agriculture, Food Wine, Adelaide, Australia.

Bayliak, M.M., T.R. Dmytriv, A.V. Melnychuk, N.V. Strilets, K.B. Storey, and V.I. Lushchak, 2021. Chamomile as a potential remedy for obesity and metabolic syndrome. EXCLI. J. 20:1261, doi: 10.17179/excli2021-4013.

Bilanda, D.C., P.D.D. Dzeufiet, L. Kouakep, B.F.O. Aboubakar, L. Tedong, P. Kamtchouing, and T. Dimo. 2017. *Bidens pilosa* ethylene acetate extract can protect against L-NAME-induced hypertension on rats. BMC Complement Altern. Med. 17:1-7, doi: 10.1186/s12906-017-1972-0.

Bodalska, A., A. Kowalczyk, M. Włodarczyk, and I. Fecka. 2019. Analysis of polyphenolic composition of a herbal medicinal product—peppermint tincture. Molecules. 25:69, doi: 10.3390/molecules25010069.

Booker, A. and Heinrich, M. 2016. Value chains of botanical and herbal medicinal products: A European perspective. HerbalGram. 112:40-45.

Carabajal, M.P.A., M.I. Isla, and I.C. Zampini. 2017. Evaluation of antioxidant and antimutagenic activity of herbal teas from native plants used in traditional medicine in Argentina. S. Afr. J. Bot. 110, 258-265, doi: 10.1016/j.sajb.2016.10.006.

Caruso, G., L. Formisano, E. Cozzolino, A. Pannico, C. El-Nakhel, Y. Rouphael, A. Tallarita, V. Cenvinzo, and S. De Pascale. 2020. Shading affects yield, elemental composition and antioxidants of perennial wall rocket crops grown from spring to summer in southern Italy. Plants. 9:933, doi: 10.3390/plants9080933.

Chandrasekara, A., and F. Shahidi. 2018. Herbal beverages: Bioactive compounds and their role in disease risk reduction-A review. J. Tradit. Complement. Med. 8:451-458, doi: 10.1016/j.jtcme.2017.08.006.

Chao, J., Y. Dai, H.Y. Cheng, W. Lam, Y.C. Cheng, K. Li, W.H. Peng, L.H. Pao, M.T. Hsieh, X.M. Qin, and M.S. Lee. 2017. Improving the concentrations of the active components in the herbal tea ingredient, *Uraria crinita*: The effect of post-harvest oven-drying processing. Sci. Rep. 7:1-16, doi: 10.1038/srep38763.

Chaudhary, S.K., M. Sandasi, F. Makolo, F.R. van Heerden, and A.M. Viljoen. 2021. Aspalathin: A rare dietary dihydrochalcone from *Aspalathus linearis* (rooibos tea). Phytochem Rev. 20:1161-1192.

Chawafambira, A. 2021. The effect of incorporating herbal (*Lippia javanica*) infusion on the phenolic, physicochemical, and sensorial properties of fruit wine. Food Sci. Nutr. 9:4539-4549, doi: 10.1002/fsn3.2432.

Chellan, P., K.M. Land, A. Shokar, A. Au, S.H. An, C.M. Clavel, P.J. Dyson, C.D. Kock, P.J. Smith, K. Chibale, and G.S. Smith. 2012. Exploring the versatility of cycloplatinated thiosemicarbazones as antitumor and antiparasitic agents. Organometallics. 31:5791-5799, doi: 10.1021/om300334z.

Chen, Y., S. Cheng, J. Dai, L. Wang, Y. Xu, X. Peng, X. Xie, and C. Peng, C. 2021. Molecular mechanisms and applications of tea polyphenols: A narrative review. J. Food Biochem. 45:p.e13910, doi: 10.1111/jfbc.13910.

Chou, C.C., L.L. Lin, and K.T. Chung. 1999. Antimicrobial activity of tea as affected by the degree of fermentation and manufacturing season. Int. J. Food Microbiol. 48:125-130, doi: 10.1016/S0168-1605(99)00034-3.

Choudhury, N., M. Meghwal, and K. Das. 2021. Microencapsulation: An overview on concepts, methods, properties and applications in foods. Food Frontiers. 2:426-442, doi: 10.1002/fft2.94.

Chung, I.M., K. Rekha, G. Rajakumar, and M. Thiruvengadam. 2018. Elicitation of silver nanoparticles enhanced the secondary metabolites and pharmacological activities in cell suspension cultures of bitter gourd. Biotech. 8:1-12. doi: 10.1007/s13205-018-1439-0.

Da-Costa-Rocha, I., B. Bonnlaender, H. Sievers. I. Pischel, and M. Heinrich. 2014. *Hibiscus sabdariffa* L.-A pharmacological and phytochemical. Food Chem. Elsevier Ltd. 165:424-443

Dandawate, P.R., D. Subramaniam, S.B. Padhye, and S. Anant. 2017. Physiology, I. City K., City K., City K. and Campus A. 913-945, doi: 10.1016/S1875-5364(16)60002-X.

De Beer, D., B.V.P. du Preez, and E. Joubert. 2021. Development of HPLC method for quantification of phenolic compounds in *Cyclopia intermedia* (honeybush) herbal tea infusions. J. Food Compos. Anal. 104:104154, doi: 10.1016/j.jfca.2021.104154.

De Wet, H., V.N. Nzama, and S.F. Van Vuuren. (2012). Medicinal plants used for the treatment of sexually transmitted infections by lay people in northern Maputaland, KwaZulu–Natal Province, South Africa. S. Afr. J. Bot. 78:12-20, doi: 10.1016/j.sajb.2011.04.002.

den Braber K., D. Sato, and E. Lee. 2011. Farm and forestry production and marketing profile for tea (*Camellia sinensis*). Specialty Crops for Pacific Island Agroforestry. Permanent Agriculture Resources. Hawai.1-33.

Dube, P., S. Meyer, and J.L. Marnewick. 2017. Antimicrobial and antioxidant activities of different solvent extracts from fermented and green honeybush (*Cyclopia intermedia*) plant material. S. A. J. Bot. 110:184-193, doi: 10.1016/j.sajb.2016.10.010.

Dutta, A., J. Sen, and R. Deswal. 2007. Downregulation of terpenoid indole alkaloid biosynthetic pathway by low temperature and cloning of a AP2 type C-repeat binding factor (CBF) from *Catharanthus roseus* (L). G. Don. Plant Cell Rep. 26:1869-1878, doi: 10.1007/s00299-007-0383.

Eko, H., W. Hardhina, H. Triana, and H.S. Bambang. 2012. Effect of shading, nitrogen and magnesium fertilizer on phyllanthin and total flavonoid yield of *Phyllanthus niruri* in Indonesia soil. J. Med. Plant Res. 6:4586-4592.

Endris, A., Asfaw, N., and Bisrat, D., 2016. Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Lippia javanica* leaves from Ethiopia. J. Essent. Oil Res. 28:221-226, doi: 10.1080/10412905.2015.1108880.

Etheridge, C.J. and E. Derbyshire. 2020. Herbal infusions and health: A review of findings from human studies, mechanisms and future research directions. Nutr. Food Sci. 50:969-985, doi: 10.1108/NFS-08-2019-0263

Farzaneh, V. and I.S. Carvalho. 2015. A review of the health benefit potentials of herbal plant infusions and their mechanism of actions. Ind. Crops Prod. 65:247-258, doi: 10.1016/j.indcrop.2014.10.057.

Fraga, C.G., K.D. Croft, D.O. Kennedy, and F.A. Tomás-Barberán. 2019. The effects of polyphenols and other bioactives on human health. Food Funct. 10:514-528, doi: 10.1039/c8fo01997e.

Gaggìa, F., L. Baffoni, M. Galiano, D.S. Nielsen, R.R. Jakobsen, J.L. Castro-Mejía, S. Bosi, F. Truzzi, F. Musumeci, G. Dinelli, and D. Di Gioia. 2018. Kombucha beverage from green, black and rooibos teas: a comparative study looking at microbiology, chemistry, and antioxidant activity. Nutrients. 11:1, doi: 10.3390/nu11010001.

Gbenga-Fabusiwa, F.J., E.P. Oladele, G. Oboh, S.A. Adefegha, and A.A. Oshodi. 2018. Polyphenol contents and antioxidants activities of biscuits produced from ginger-enriched pigeon pea–wheat composite flour blends. J. Food Biochem. 42:e12526, doi: 10.1111/jfbc.12526.

Ghasemzadeh, A., H.Z. Jaafar, A. Rahmat, P.E.M. Wahab, and M.R.A. Halim. 2010. Effect of different light intensities on total phenolics and flavonoids synthesis and antioxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). Int. J. Mol. Sci.11:3885-3897, doi: 10.3390/ijms11103885.

Göçer, H., A. Akıncıoğlu, N. Öztaşkın, S. Göksu, and İ Gülçin. 2013. Synthesis, Antioxidant, and Antiacetylcholinesterase Activities of Sulfonamide Derivatives of Dopamine-R elated Compounds. Arch. Pharm. 346:783-792, doi: 10.1111/ijfs.12637.

Gohil, K.J., J.A. Patel, and A.K. Gajjar. 2010. Pharmacological review on *Centella asiatica*: a potential herbal cure-all. Indian J. Pharm. Sci. 72:546, doi: 10.4103/0250-474X.78519.

Grbic, N., K. Paschko, I. Pinker, and M.H. Böhme. 2016. Effect of different light spectra by using coloured plastic films on growth, fresh and dry matter, nutrient solution uptake and secondary metabolites of *Perilla frutescens* (L.). Britt. Sci. Hortic. 210:93-98, doi: 10.1016/j.scienta.2016.07.016.

Guerrero-Beltrán, J.A., G.V. Barbosa-Cánovas, and B.G. Swanson. 2005. High hydrostatic pressure processing of fruit and vegetable products. Food Rev. Int. 21:411-425, doi: 10.1080/87559120500224827.

Gupta, V., P. Mittal, P. Bansal, S.L. Khokra, and D. Kaushik. 2010. Pharmacological potential of *Matricaria recutita*-A review. Int. J. Pharm Sci. Drug Res. 2:12-6.

Gutiérrez, R.M.P., S. Mitchell, and R.V. Solis. 2008. *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 117:1-27, doi: 10.1016/j.jep.2008.01.025.

Guzelmeric, E., P. Ristivojević, I. Vovk, D. Milojković-Opsenica, and E. Yesilada. 2017. Quality assessment of marketed chamomile tea products by a validated HPTLC method combined with multivariate analysis. J. Pharm. Biomed. Anal. 132:35-45, doi: 10.1016/j.jpba.2016.09.030.

Hacioglu, M., S. Dosler, A.S.B. Tan, and G. Otuk. 2017. Antimicrobial activities of widely consumed herbal teas, alone or in combination with antibiotics: an in vitro study. Peer J. 5:e3467, doi: 10.7717/peerj.3467.

Hamilton, J.G., A.R. Zangerl, E.H. DeLucia, and M.R. Berenbaum. 2001. The carbon–nutrient balance hypothesis: its rise and fall. Ecol. Lett. 4:86-95, doi:10. 1046/j.1461-0248.2001.00192.x.

Han, F., G.Q. Ma, M. Yang, L. Yan, W. Xiong, J.C. Shu, Z.D. Zhao, and H.L. Xu. 2017. Chemical composition and antioxidant activities of essential oils from different parts of the oregano. J. Zhejiang Univ. Sci. B. 18:79-84.

Han, K.H., A. Matsumoto, K.I. Shimada, K.M. Sekikawa, and M. Fukushima. 2007. Effects of anthocyanin-rich purple potato flakes on antioxidant status in F344 rats fed a cholesterol-rich diet. Br. J. Nutr. 98:914-921, doi: 10.1017/S0007114507761792.

Hasanvand, E., M. Fathi, A. Bassiri, M. Javanmard, and R. Abbaszadeh. 2015. Novel starch based nanocarrier for vitamin D fortification of milk: Production and characterization. Food Bioprod. Process. 96:264-277, doi: 10.1016/j.fbp.2015.09.007.

Hayes, A.J., and B. Markovic. 2002. Toxicity of Australian essential oil *Backhousia citriodora* (Lemon myrtle). Part 1. Antimicrobial activity and in vitro cytotoxicity. Food Chem. Toxicol. 40:535-543, doi: 10.1016/S0278-6915(01)00103-X.

Hirasawa, M., K. Takada, M. Makimura, S. Otake. 2002. Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. J. Periodontal Res. 37: 433-438, doi: 10.1034/j.1600-0765.2002.01640.x.

Hlahla, L.N. 2010. Effect of fermentation temperature and duration on chemical composition of Bush tea (*Athrixia phylicoides* DC.). PhD thesis, Univ. of Limpopo, Turfloop Campus, Mankweng, South Africa.

Hossain, M.F., M.T. Islam, M.A. Islam, and S. Akhtar. 2017. Cultivation and uses of stevia (*Stevia rebaudiana* Bertoni): A review. African J. Food, Agric. Nutr. Dev. 17:12745-12757, doi: 10.18697/ajfand.80.16595.

Hui Gan, S., T. Chai Tham, M. Xiang Ng, L Suan Chua, R. Aziz, M. Redza Baba, L. Chuah Abdullah, S. Pheng Ong, and C. Lim Law. 2017. Study on retention of metabolites composition in misai kucing (*Orthosiphon stamineus*) by heat pump assisted solar drying. J. Food Process. Preserv. 41:e13262, doi: 10.1111/jfpp.13262.

Huyen, T.T. 2020. Optimization of microwave-assisted extraction of phenolic compounds from ginger (Rosc.) using response surface methodology. Herba Pol. 66:19-27, doi: 10.2478/hepo-2020-0007.

Ibrahim, M.H., H.Z. Jaafar, A. Rahmat, and Z.A. Rahman. 2011. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of *Kacip Fatimah*

(*Labisia pumila* Blume). Int. J. Mol. Sci. 12:5238-5254, doi: 10.3390/ijms12085238.

Ibrahim, M.H., H.Z. Jaafar, E. Karimi, and A. Ghasemzadeh. 2013. Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of *Kacip Fatimah* (*Labisia pumila* Benth). Molecules. 18:10973-10988, doi: 10.3390/molecules180910973.

Idrees, M., B. Sania, B. Hafsa, S. Kumari, H. Khan, H. Fazal, I. Ahmad, F. Akbar, N. Ahmad, S. Ali, and N. Ahmad. 2018. Spectral lights trigger biomass accumulation and production of antioxidant secondary metabolites in adventitious root cultures of *Stevia rebaudiana* (Bert.). C. R. Biol. 341: 334-342, doi: 10.1016/j.crvi.2018.05.003

Ignasimuthu, K., R. Prakash, P.S. Murthy, and N. Subban, N. 2019. Enhanced bioaccessibility of green tea polyphenols and lipophilic activity of EGCG octaacetate on gram-negative bacteria. Lwt. 105:103-109, doi: 10.1016/j.lwt.2019.01.064.

Jan, R., S. Asaf, M. Numan, and K.M. Kim. 2021. Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. Agronomy. 11:968, doi: 10.3390/agronomy11050968.

Jansen, G., H.U. Jürgens, and F. Ordon. 2009. Effects of temperature on the alkaloid content of seeds of *Lupinus angustifolius* cultivars. J. Agron. Crop Sci. 195:172-177, doi: 10.1111/j.1439-037X.2008. 00356.x.

Jin, B., Y. Liu, J. Xie, B. Luo, and C. Long. 2018. Ethnobotanical survey of plant species for herbal tea in a Yao autonomous county (Jianghua, China): results of a 2-year study of traditional medicinal markets on the Dragon Boat Festival. J. Ethnobiol. Ethnomedicine. 14:58, doi:

10.1186/s13002-018-0257-0.

Jin, L., X.B. Li, D.Q. Tian, X.P. Fang, Y.M. Yu, H.Q. Zhu, H.Y.Y. Ge, G.Y. Ma, W.Y. Wang, W.F. Xiao, and M. Li. 2016. Antioxidant properties and color parameters of herbal teas in China. Ind. Crops Prod. 87:198-209, doi: 10.1016/j.indcrop.2016.04.044.

Joubert, E., and H. Schultz. 2012. Production and quality aspects of rooibos tea and related products. A review. J. Appl. Bot. Food Qual. 80:138-144.

Joubert, E. and D. de Beer. 2014. Antioxidants of rooibos beverages: role of plant composition and processing. In Processing and impact on antioxidants in beverages. Academic Press. 131-134, doi: 10.1016/B978-0-12-404738-9.00014-3.

Jurendić, T., and M. Ščetar. 2021. Aronia melanocarpa products and by-products for health and nutrition: A Review. Antioxidants. 10:1052. doi: 10.3390/antiox10071052

Kamaruddin, Z.H., R. Jumaidin, M.Z. Selamat, and R.A. Ilyas. 2021. Characteristics and Properties of Lemongrass (*Cymbopogan Citratus*): A Comprehensive Review. J. Nat. Fibers. 1-18. doi: 10.1080/15440478.2021.1958439.

Karimi, E., H.Z. Jaafar, A. Ghasemzadeh, and M.H. Ibrahim. 2013. Light intensity effects on production and antioxidant activity of flavonoids and phenolic compounds in leaves, stems and roots of three varieties of *Labisia pumila* Benth. Aust. J. Crop Sci. 7:1016.

Kashchenko, N.I., D.N. Olennikov, and N.K. Chirikova. 2021. Metabolites of Siberian raspberries: LC-MS profile, seasonal variation, antioxidant activity and, thermal stability of *Rubus matsumuranus* phenolome. Plants. 10:2317, doi: 10.3390/plants10112317.

Khandaker, L., A.M. Akond, M.B. Ali, and S. Oba. 2010. Biomass yield and accumulations of bioactive compounds in red amaranth (*Amaranthus tricolor* L.) grown under different colored shade polyethylene in spring season. Sci. Hortic. 123:289-294, doi: 10.1016/j.scienta.2009.09.012.

Kieling, D.D. and S.H. Prudencio. 2019. Antioxidants properties, physicochemical, and sensory quality attributes of a new soy-based beverage with lemongrass and lime juice. J. Culin. Sci. Technol. 17:103-117, doi: 10.1080/15428052.2017.1405859.

Kim, P.K., K.I Jung, Y.J. Choi, and S.W. Gal. 2017. Anti-inflammatory effects of lemon myrtle (*Backhousia citriodora*) leaf extracts in LPS-induced RAW 264.7 cells. J. Life Sci. 27:986-993, doi: 10.5352/JLS.2017.27.9.986.

Koch, W., W. Kukuła-Koch, M. Czop, P. Helon, and E. Gumbarewicz. 2020. The role of extracting solvents in the recovery of polyphenols from green tea and its antiradical activity supported by principal component analysis. Molecules. 25:2173, doi: 10.3390/molecules25092173

Krushna, N.S., C. Shiny, S. Dharanya, A. Sindhu, S. Aishwarya, and R.B. Narayanan. 2009. Immunolocalization and serum antibody responses to *Brugia malayi* pepsin inhibitor homolog (Bm-33). Microbiol. Immunol. 53:173-183, doi:10.1111/j.1348-0421.2009. 00114.x.

Kumar, R., S. Sharma, and V. Pathania. 2013. Effect of shading and plant density on growth, yield and oil composition of clary sage (*Salvia sclarea* L.) in Northwestern Himalaya. J. Essent. Oil Res. 25:23-32, doi: 10.1080/10412905.2012.742467.

Kumar, R., S. Sharma, K. Ramesh, and B. Singh. 2013. Effects of shade regimes and planting geometry on growth, yield and quality of the natural sweetener plant stevia (*Stevia* *rebaudiana* Bertoni) in North-Western Himalaya. Arch. Agron. Soil Sci. 59:963-979, doi: 10.1080/03650340.2012.699676.

Kumar, R., S. Sharma, K. Ramesh, V. Pathania, and R. Prasad. 2014. Irradiance stress and plant spacing effect on growth, biomass and quality of wild marigold (*Tagetes minuta* L.)–an industrial crop in western Himalaya. J. Essent. Oil Res. 26:348-358, doi: 10.1080/10412905.2014.935497.

Lehlohonolo, N., I.K. Mariga, W. Ngezimana, and F.N. Mudau. 2013. Bush tea (*Athrixia phylicoides* dc.) Success stories in South Africa A review. Crop Prod. 2:37-43.

Liang, Y.C., M.T. Yang, C.J. Lin, C.L.T. Chang, and W.C. Yang. 2016. *Bidens pilosa* and its active compound inhibit adipogenesis and lipid accumulation via down-modulation of the C/EBP and PPARγ pathways. Sci. Rep. 6:4285, doi: 10.1038/srep24285.

Lin-Wang, K.U.I., D. Micheletti, J. Palmer, R. Volz, L. Lozano, R. Espley, R.P. Hellens, D, Chagne, D.D. Rowan, M. Troggio, and I. Iglesias. 2011. High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. Plant Cell Environ. 34:1176-1190, doi: 10.1111/j.1365-3040.2011. 02316.x.

Lv, Z., C. Zhang, C. Shao, B. Liu, E. Liu, D. Yuan, Y. Zhou, and C. Shen. 2021. Research progress on the response of tea catechins to drought stress. J. Sci. Food Agric. 101:5305-5313, doi: 10.1002/jsfa.11330.

Ma, C.H., J.Z. Chu, X.F. Shi, C.Q. Liu, and X.Q. Yao. 2016. Effects of enhanced UV-B radiation on the nutritional and active ingredient contents during the floral development of medicinal chrysanthemum. J. Photochem. Photobiol. B, Biol. 158:228-234, doi:

10.1016/j.jphotobiol.2016.02.019.

MacAlister, D., A.M. Muasya, O. Crespo, J.B. Ogola, S.T. Maseko, A.J. Valentine, C.O. Ottosen, E. Rosenqvist, and S.B. Chimphango, 2020. Effect of temperature on plant growth and stress tolerant traits in rooibos in the Western Cape, South Africa. Sci. Hortic. 263:109137, doi: 10.1016/j.scienta.2019.109137.

Majewska, E., M. Kozlowska, E. Gruszczynska-Sekowska, D. Kowalska, and K. Tarnowska. 2019. Lemongrass (*Cymbopogon citratus*) essential oil: extraction, composition, bioactivity and uses for food preservation-a review. Polish J. Food Nutr. Sci. 69:327–341, doi: 10.31883/pjfns/113152.

Makola, M.M., I.A. Dubery, G. Koorsen, P.A. Steenkamp, M.M. Kabanda, L.L. du Preez, and N.E. Madala. 2016. The effect of geometrical isomerism of 3, 5-dicaffeoylquinic acid on its binding affinity to HIV-integrase enzyme: A molecular docking study. Evid. Based Complement. Alternat. Med. 2016:4138263, doi: 10.1155/2016/4138263.

Makola, M.M., P.A. Steenkamp, I.A. Dubery, M.M. Kabanda, and N.E. Madala. 2016a. Preferential alkali metal adduct formation by cis geometrical isomers of dicaffeoylquinic acids allows for efficient discrimination from their trans isomers during ultra-high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 30:1011-1018, doi: 10.1002/rcm.7526.

Malongane, F., L.J. McGAW, and F.N. Mudau. 2017. The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: a review. J. Sci. Food Agric. 97:4679-4689. doi: 10.1002/jsfa.8472.

Mamphiswana, N.D., P.W. Mashela, and L.K. Mdee. 2010. Distribution of total phenolics and antioxidant activity in fruit, leaf, stem and root of *Monsonia burkeana*. Afr. J. Agric. Res. 5:2570-2575.

Marnewick, J.L., W.C. Gelderblom, and E. Joubert. 2000. An investigation on the antimutagenic properties of South African herbal teas. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 471: 157-166, doi: 10.1016/S1383-5718(00)00128-5.

Maroyi, A., 2017. *Lippia javanica* (Burm. F.) Spreng.: traditional and commercial uses and phytochemical and pharmacological significance in the african and indian subcontinent. Evid. Based Complementary Altern. Med. 2017, doi:10.1155/2017/6746071.

Marszałek, K., L. Woźniak, S. Skąpska, and M. Mitek. 2017. High pressure processing and thermal pasteurization of strawberry purée: quality parameters and shelf-life evaluation during cold storage. J. Food Sci. Technol. 54:832-841, doi: 10.1007/s13197-017-2529-4.

Martínez-Solís, J., F. Calzada, E. Barbosa, and M. Valdés. 2021. Antihyperglycemic and antilipidemic properties of a tea infusion of the leaves from *Annona cherimola* miller on streptozocin-induced type 2 diabetic mice. Molecules. 26:2408, doi: 10.3390/molecules26092408.

Mashimbye, M.J., F.N. Mudaua, P. Soundy, and T. Van Ree. 2006. A new flavonol from *Athrixia phylicoides* (bush tea). S.A. J. Chem. 59:1-2.

Masike, K., F. Tugizimana, N. Ndlovu, E. Smit, L. du Preez, I, Dubery, and E. Madala. 2017. Deciphering the influence of column chemistry and mass spectrometry settings for the analyses of geometrical isomers of L-chicoric acid. J. Chromatogr. B Biomed. Appl. 1052:

73-81, doi: 10.1016/j.jchromb.2017.03.023.

Mathivha, P.L., T.A. Msagati, V.S. Thibane, and F.N. Mudau. 2020. Phytochemical analysis of herbal teas and their potential health, and food safety benefits: a review. J. Herb. Med. 281-301, doi: 10.1007/978-981-13-7248-3_20.

Mavundza, E.J., T.E. Tshikalange, F.N. Mudau, and J.J. Meyer. 2007. Evaluation of antibacterial and antioxidant activities in ethanol extract of wild bush tea (*Athrixia phylicoides* (DC.). Med. Aroma.t Plant Sci. Biotechnol. 1:240-242.

Mbondo, N.N., W.O. Owino, J. Ambuko, and D.N. Sila, 2018. Effect of drying methods on the retention of bioactive compounds in African eggplant. Food Sci. Nutr. 6:814-823, doi: 10.1002/fsn3.623.

McKay, D.L., and J.B. Blumberg. 2007. A review of the bioactivity of South African herbal teas: rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*). Phytother. Res. 21:1-16. doi: 10.1002/ptr.1992.

Medina-Meza, I.G., C. Barnaba, F. Villani, and G.V. Barbosa-Cánovas. 2015). Efects of thermal and high-pressure treatments in color and chemical attributes of an oil-based spinach sauce. LWT-Food Sci. Technol. 60:86-94, doi: 10.1016/j.lwt.2014.09.033.

Mfengu, M.O., M. Shauli, G.A. Engwa, H.T. Musarurwa, and C.R. Sewani-Rusike. 2021. *Lippia javanica* (Zumbani) herbal tea infusion attenuates allergic airway inflammation via inhibition of Th2 cell activation and suppression of oxidative stress. BMC Complement Altern. Med. 21:192, doi: 10.1186/s12906-021-03361-8.

Mkwanazi, M.V., S.Z. Ndlela, M. Chimonyo. 2021. Indigenous knowledge to mitigate the challenges of ticks in goats: A systematic review. Vet. Anim. Sci. 13:100190, doi: 10.1016/j.vas.2021.100190.

Mohale, K.C., A.T. Hintsa, M.A. Emanuel, and F.N. Mudau. 2018. Metabolic profiling of cultivated bush tea (*Athrixia phylicoides* DC.) in response to different pruning types. HortSci. 53:993-998, doi: 10.21273/HORTSCI13023-18.

Mohd Yusof, F.F., J.S. Yaacob, N. Osman, M.H. Ibrahim, W.A.A.Q.I. Wan-Mohtar, Z. Berahim, and N.A. Mohd Zain. 2021. Shading effects on leaf gas exchange, leaf pigments and secondary metabolites of *Polygonum minus* Huds, an aromatic medicinal herb. Plants. 10:608, doi: 10.3390/plants10030608.

Mokoka, N.N. 2007. Indigenous knowledge of fever tea *(Lippia javanica*) and effect of shade netting on plant growth, oil yield and compound composition. PhD thesis, Univ. of Pretoria, Pretoria, South Africa.

Monika, M. and S. Selvakumar. 2019. Recent developments in direct C–H functionalization of quinoxalin-2 (1H)-ones via radical addition processes. Synthesis. 1:4113-4136, doi: 10.1055/s-0037-161191.

Moreira, F.V., J.F. Bastos, A.F. Blank, P.B. Alves, and M.R. Santos. 2010. Chemical composition and cardiovascular effects induced by the essential oil of *Cymbopogon citratus* DC. Stapf, Poaceae, in rats. Rev. Bras. Farmacogn. 20:904-909, doi: 10.1590/S0102-695X2010005000012.

Morita, A. and M. Tuji. 2002. Nitrate and oxalate contents of tea plants (*Camellia sinensis* L.) with special reference to types of green tea and effect of shading. J. Soil Sci. Plant Nutr. 48:547–

553, doi: 10.1080/00380768.2002.10409237.

Mphangwe, N.I.K. 2012. Lung pruning: A review of practice. Tea Research Foundation of Central Africa (TRFCA) News. 18–23.

Mtenga, D.V. and A.S. Ripanda. 2022. A review on the potential of underutilized Blackjack (Biden Pilosa) naturally occurring in sub-Saharan Africa. Heliyon, p.e09586, doi: 10.1016/j.heliyon.2022.e09586.

Mubvuma, M.T. 2018. Planting date as an adaptive strategy to improve yield of Chickpea (*Cicer arietinum*) under under climate change condition in Southern Africa. PhD thesis, Department of Plant Production, School of Agriculture, University of Venda, Thohoyandou, South Africa.

Mudau, F.N. and N.P. Makunga, 2018. Effect of Seasonal Variations and Growth Conditions on Carbohydrate Partitioning in Different Organs and the Quality of Bush Tea. HortSci. 53:999-1005, doi: 10.21273/HORTSCI12972-18.

Mudau, F.N., A. Ngele, P.W. Mashela, and P. Soundy, 2007. Seasonal variation of tannin content in wild bush tea. Med. Aromat. Plant Sci. Biotechnol. 1:74-76.

Mudau, F.N., A.R. Mudau, M. Nkomo, and W. Ngezimana. 2016. Variation in carbohydrate reserves and dry matter production of bush tea (*Athrixia phylicoides*) grown under different environmental conditions. HortSci. 51:1537-1541, doi: 10.21273/HORTSCI11197-16.

Mudau, F.N., P. Soundy, and E.S. Du Toit. 2007. Effects of nitrogen, phosphorus, and potassium nutrition on total polyphenol content of bush tea (*Athrixia phylicoides* L.) leaves in shaded nursery environment. HortSci. 42:334-338, doi: 10.21273/HORTSCI.42.2.334.

Muronga, M., C. Quispe, P.P. Tshikhudo, T.A.M. Msagati, F.N. Mudau, M. Martorell, B. Salehi, A.F. Abdull Razis, U. Sunusi, R.M. Kamal, and J. Sharifi-Rad. 2021. Three selected edible crops of the genus momordica as potential sources of phytochemicals: biochemical, nutritional, and medicinal values. Front. Pharmacol. 12:625546, doi: 10.3389/fphar.2021.62554.

Naghiloo, S., A. Movafeghi, A. Delazar, H. Nazemiyeh, S. Asnaashari, and M.R. Dadpour. 2012. Ontogenetic variation of volatiles and antioxidant activity in leaves of *Astragalus compactus* Lam. (Fabaceae). EXCLI. J. 11:436.

Namdeo, A.G. 2018. Cultivation of medicinal and aromatic plants. Editor(s): Subhash C. Mandal, Vivekananda Mandal, Tetsuya Konishi, Natural Products and Drug Discovery. Elsevier. 525-553, doi: 10.1016/B978-0-08-102081-4.00020-4.525-553.

Nasma, A., N. Aishath, A. Azilah, and A.Z. Sulaiman. 2018. Optimization of vitexin and isovitexin compounds extracted from dried *Mas Cotek* leaves using one-factor-at-a-time (OFAT) approach in aqueous extraction. Int. Food Res. J. 25.

Nazmi, N.N.M. and. N.M. Sarbon. 2020. Characterization on antioxidant and physical properties of gelatin based composite films with incorporation of *Centella asiatica* (pegaga) extract. Food Res. 4:224-233.

Nchabeleng, L., F.N. Mudau, and I.K. Mariga. 2012. Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors. J. Med. Plants Res. 6:1662-1666, doi: 10.5897/JMPR11.1453.

Ngoepe, N.M., Z. Mbita, M. Mathipa, N. Mketo, B. Ntsendwana, and N.C. Hintsho-Mbita, 2018. Biogenic synthesis of ZnO nanoparticles using *Monsonia burkeana* for use in photocatalytic, antibacterial and anticancer applications. Ceram. Int. 44:16999-17006, doi: 10.1016/j.ceramint.2018.06.142.

Ni, Y.W., K.H. Lin, K.H. Chen, C.W. Wu, and Y.S. Chang. 2020. Flavonoid compounds and photosynthesis in Passiflora plant leaves under varying light intensities. Plants. 9:633, doi: 10.3390/plants9050633.

Nibir, Y.M., A.F. Sumit, A.A. Akhand, N. Ahsan, and M.S. Hossain. 2017. Comparative assessment of total polyphenols, antioxidant and antimicrobial activity of different tea varieties of Bangladesh. Asian Pac. J. Trop. Biomed. 7:352-357.

Nikniaz, Z., R. Mahdavi, S.J. Ghaemmaghami, N.L. Yagin, and L. Nikniaz. 2016. Effect of different brewing times on antioxidant activity and polyphenol content of loosely packed and bagged black teas (*Camellia sinensis* L.). Avicenna J. Phytomedicine. 6, 313.0.

Nnzeru, L.R., P.N. Tshikhudo, and F.N. Mudau. 2019. 1h Nuclear Magnetic Resonance Spectroscopy reveals secondary metabolic variations of special tea (*Monsonia burkeana* planch. ex harv) populations from three selected locations in South Africa. Appl. Ecol. Environ. Res. 17:14095-14103, doi: 10.15666/aeer/1706_1409514103.

Nnzeru, R.L., K. Ntushelo, and F.N. Mudau. 2016. Physical appraisal and attributes of *Monsonia burkeana* (special tea): the perspective of tea users. Indilinga African Journal of Indigenous Knowledge Systems. 15:111-122, doi: 10.15666/aeer/1706_1409514103.

Nobela, O., R.S. Renslow, D.G. Thomas, S.M. Colby, S. Sitha, P.B. Njobeh, L. du Preez, L., F. Tugizimana, and N.E. Madala. 2018. Efficient discrimination of natural stereoisomers of chicoric acid, an HIV-1 integrase inhibitor. J. Photochem. Photobiol. B, Biol. 189:258-266, doi: 10.1016/j.jphotobiol.2018.10.025.

Oladeji, O.S., F.E. Adelowo, D.T. Ayodele, and K.A. Odelade. 2019. Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. Sci. Afr. 6:e00137, doi; 10.1016/j.sciaf.2019.e00137.

Olayemi, R.F. 2017. Comparative study of root, stalk and leaf essential oils of *Cymbopogon Citratus* (lemon grass). Chem. Search. J. 8:20-28.

Omogbai, B.A. and M. Ikenebomeh. 2013. Microbiological characteristics and phytochemical screening of some herbal teas in Nigeria. Eur. Sci. J. 9.

Owour, P.O. and M. Obanda. 1998. Clonal selection criteria for quality in Kenya black tea production: achievements, problems and perspectives: review. Tea. 19:49-58

Owuor, P.O., T. Tsushida, H. Horita, and T. Murai. 1988. Effects of geographical area of production on the composition of the volatile flavour compounds in Kenyan clonal black CTC teas. Experimental Agriculture. 24:227-235, doi: 10.1017/S0014479700015970.

Pang, S.F., M.Z. Lau, M.M. Yusoff, and J. Gimbun. 2017. Microwave-irradiation induced fast simultaneous extraction of methoxylated and hydroxylated phenolic compounds from *Orthosiphon stamineus* leaves. Mater. Sci. Forum. 890:155-158. Trans Tech Publications Ltd. doi: 10.4028/www.scientific.net/MSF.890.155.

Pasrija, D., P.N. Ezhilarasi, D. Indrani, and C. Anandharamakrishnan. 2015. Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. LWT-Food Sci. Technol. 6:289-296, doi: 10.1016/j.lwt.2015.05.054.

Patras, A., N.P. Brunton, T.R. Gormely, and F. Butler. 2009. Impact of high-pressure processing on antioxidant activity, ascorbic acid, anthocyanins and instrumental colour of blackberry and strawberry puree. Innov. Food Sci. Emerg. Technol. 10:308-313, doi:10.1016/j.ifset.2008.12.004.

Pezzani, R., S. Vitalini, and M. Iriti. 2017. Bioactivities of Origanum vulgare L.: An update. Phytochem Rev. 1253-1268, doi: 10.1007/s11101-017-9535-z.

Phelan, J. and J. Rees, J. 2003. The erosive potential of some herbal teas. J. Dent. 31:241-246. doi: 10.1016/S0300-5712(03)00048-4.

Pola, W., S. Sugaya, and S. Photchanachai. 2020. Influence of postharvest temperatures on carotenoid biosynthesis and phytochemicals in mature green chili (*Capsicum annuum* L.). Antioxidants. 9:203, doi: 10.3390/antiox9030203.

Poswal, F.S., G. Russell, M. Mackonochie, E. MacLennan, E.C. Adukwu, and V. Rolfe. 2019. Herbal teas and their health benefits: a scoping review. Plant Foods Hum. Nutr. 74:266-276, doi: 10.1007/s11130-019-00750-w.

Proutsos, N.D., A. Liakatas, S.G. Alexandris, I.X. Tsiros, D. Tigkas, and G. Halivopoulos. 2022. Atmospheric Factors Affecting Global Solar and Photosynthetically Active Radiation Relationship in a Mediterranean Forest Site. Atmosphere. 13:1207. doi: 10.3390/atmos13081207.

Radušienė, J., M. Marksa, L. Ivanauskas, V. Jakštas, Ö, Çalişkan, D. Kurt, M.S. Odabaş, and C. Çirak. 2019. Effect of nitrogen on herb production, secondary metabolites and antioxidant activities of *Hypericum pruinatum* under nitrogen application. Ind. Crops Prod. 139:111519, doi: 10.1016/j.indcrop.2019.111519.

Ralph, A.D., E. Ferrannini, L. Groop, R.R. Henry, W.H. Herman, J.J. Holst, F.B. Hu, C.R. Kahn, I. Raz, G.I. Shulman, and D.C. Simonson. 2015. Type 2 diabetes mellitus. Nat. Rev. Dis. Primers. 1:1-22, doi: 10.1038/nrdp.2015 19.

Ramabulana, A.T., D. Petras, N.E. Madala, and F. Tugizimana. 2021. Metabolomics and molecular networking to characterize the chemical space of four momordica plant species. Metabolites. 11:763, doi: 10.3390/metabo11110763.

Ramabulana, A.T., P. Steenkamp, N. Madala, and I.A. Dubery. 2020. Profiling of chlorogenic acids from *Bidens pilosa* and differentiation of closely related positional isomers with the aid of UHPLC-QTOF-MS/MS-based in-source collision-induced dissociation. Metabolites. 10: 178, doi: 10.3390/metabo10050178.

Ramphinwa, M.L., G.R.A. Mchau, N.E. Madala, N. Nengovhela, J.B.O. Ogola, and F.N. Mudau, 2022. Response of Plant Growth and Development, and Accumulation of Hydroxyl-cinnamoyl Acid Derivatives to Selected Shade Nets and Seasonality of Field-grown Bush Tea (*Athrixia phylicoides* DC.). HortSci. 57: 87-96, doi: 10.21273/HORTSCI16171-21.

Rasool Hassan, B.A. (2012). Medicinal plants (importance and uses). Pharmaceut Anal Acta. 3: 2153-2435, doi: 10.4172/2153-2435.1000e139.

Ravichandran, R. and R. Parthiban. 1998. Changes in enzyme activities (polyphenol oxidase and phenylalanine ammonialyase) with type of tea leaf and during black tea manufacture and the effect of enzyme supplementation of dhool on black tea quality. Food Chem. 62:277–281, doi: 10.1016/S0308-8146(97)00220-3.

Rezai, S., N. Etemadi, A. Nikbakht, M. Yousefi, and M.M. Majidi. 2018. Effect of light intensity on leaf morphology, photosynthetic capacity, and chlorophyll content in sage (*Salvia officinalis* L.). Korean J. Hortic. Sci. Technol. 36:46-57, doi: 10.12972/kjhst.20180006.

Rihan, H.Z., M. Aldarkazali, S.J. Mohamed, N.B. McMulkin, M.H. Jbara, and M.P. Fuller. 2020. A novel new light recipe significantly increases the growth and yield of sweet basil (*Ocimum basilicum*) grown in a plant factory system. Agronomy. 10:934, doi: 10.3390/agronomy10070934.

Şahin, F., M. Güllüce, D. Daferera, A. Sökmen, M. Sökmen, M. Polissiou, G. Agar, and H. Özer. 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. Food control. 15:549-557, doi: 10.1016/j.foodcont.2003.08.009.

Said-Al Ahl, H.A.H. and M.S. Hussein. 2010. Effect of water stress and potassium humate on the productivity of oregano plant using saline and freshwater irrigation. Ozean. J. Appl. Sci. 3:125-141.

Salman, S., G. Öz, R. Felek, A. Haznedar, T. Turna, and F. Özdemir. 2022. Effects of fermentation time on phenolic composition, antioxidant and antimicrobial activities of green, oolong, and black teas. Food Biosci. 49:101884, doi: 10.1016/j.fbio.2022.101884

Sehgal, A. 2022. An update on disease preventing potential of green tea in comparison with some tisanes. S. Afr. J. Bot. 144:92-96, doi: 10.1016/j.sajb.2021.08.027

Sengupta, A., K.E. Nielsen, G. Barinshteyn, and K. Li. 3M Innovative Properties Co, 2001. Encapsulation process and encapsulated products. U.S. Patent. 6:248-364.

Sharpe, E., F. Hua, S. Schuckers, S. Andreescu, and R. Bradley. 2016. Effects of brewing conditions on the antioxidant capacity of twenty-four commercial green tea varieties. Food chem. 192:380-387, doi: 10.1016/j.foodchem.2015.07.005.

Singh, R., S. De, and A. Belkheir, 2013. *Avena sativa* (Oat), a potential neutraceutical and therapeutic agent: an overview. Crit. Rev. Food Sci. Nutr. 53:126-144, doi: 10.1080/10408398.2010.526725.

Singh, S., T. Virmani, and K. Kohli. 2020. Phytochemicals and Medicinal Uses of Red Raspberry: A Review. J. Pharmaceut Res. 5:48-52.

Soni, U., S. Brar, and V.K. Gauttam. 2015. Effect of seasonal variation on secondary metabolites of medicinal plants. Int. J. Pharm. Sci. Res. 6:3654-3662.

Strzemski, M., K. Dzida, S. Dresler, I. Sowa, J. Kurzepa, G. Szymczak, and M. Wójciak. 2021. Nitrogen fertilisation decreases the yield of bioactive compounds in Carlina acaulis L. grown in the field. Ind. Crops. Prod. 170: 113698, doi: 10.1016/j.indcrop.2021.113698.

Suleman, Z., G.A. Engwa, M. Shauli, H.T. Musarurwa, N.A. Katuruza, and C.R. Sewani-Rusike. 2022. Neuroprotective effects of *Lippia javanica* (Burm. F.) Spreng. Herbal tea infusion on Lead-induced oxidative brain damage in Wistar rats. BMC Complement. Altern. Med. 22:4, doi: 10.1186/s12906-021-03471-3.

Sun, T., J. Zhang, Q. Zhang, X. Li, M. Li, Y. Yang, J. Zhou, Q. Wei, and B. Zhou. 2021. Integrative physiological, transcriptome, and metabolome analysis reveals the effects of nitrogen sufficiency and deficiency conditions in apple leaves and roots. Environ. Exp. Bot. 192: p.104633, doi: 10.1016/j.indcrop.2021.113698.

Thakur, M., V. Bhatt, and R. Kumar. 2019. Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose (*Rosa damascena* Mill.) under mid hill conditions of Western Himalayas. PloS one. 14:e0214672, doi: 10.1371/journal.pone.0214672.

Tshikhudo, P.P., K. Ntushelo, S.A. Kanu, and F.N. Mudau. 2019. Growth response of bush tea (Athrixia phylocoides DC.) to climatic conditions in Limpopo Province, South Africa. S. Afr. J. Bot. 121:500-504, doi: 10.1016/j.sajb.2018.12.012.

Tshivhandekano, I., W. Ngezimana, T.E. Tshikalange, N.P. Makunga, and F.N. Mudau. 2018. Nitrogen application influences quality, pharmacological activities and metabolite profiles of *Athrixia phylicoides* DC. (Bush tea) cultivated under greenhouse and field conditions. Acta Agric. Scand. - B Soil Plant Sci. 68:388-400, doi: 10.1080/09064710.2017.1412496.

Tshivhandekano, T., F.N. Mudau, P. Soundy, and W.N. Ngezimana. 2013. Effect of cultural practices and environmental conditions on yield and quality of herbal plants: Prospects leading to research on influence of nitrogen fertilization, planting density and eco-physiological parameters on yield and quality of field-grown bush tea (*Athrixia phylicoides* DC.). J. Med. Plants Res. 7:2489-2493.

Turkmen, N., S.A.R.I. Ferda, and Y.S. Velioglu. 2009. Factors affecting polyphenol content and composition of fresh and processed tea leaves. Akademik Gıda. 7:29-40.

Upadhya, C. 2004. A new transnational capitalist class? Capital flows, business networks and entrepreneurs in the Indian software industry. Econ. Political Wkly. 5141-5151.

Verma, N. and S. Shukla. 2015. Impact of various factors responsible for fluctuation in plant secondary metabolites. J. Appl. Res. Med. Aromat. Plants. 2:105-113, doi:

10.1016/j.jarmap.2015.09.002.

Villa-Rodriguez, J.A., A. Kerimi, L. Abranko, S. Tumova, L. Ford, R.S. Blackburn, C. Rayner, and G. Williamson. 2018. Acute metabolic actions of the major polyphenols in chamomile: An in vitro mechanistic study on their potential to attenuate postprandial hyperglycaemia. Sci. Rep, 1-14, doi:10.1038/s41598-018-23736-1.

Vural, N., Ö, Algan Cavuldak, M.A. Akay, and R.E. Anlı. 2020. Determination of the various extraction solvent effects on polyphenolic profile and antioxidant activities of selected tea samples by chemometric approach. J. Food Meas. Charact. 14:1286-1305, doi: 10.1007/s11694-020-00376-6.

Wahid, A., S. Gelani, M. Ashraf, and M.R. Foolad. Heat tolerance in plants: an overview. Environ. Exp. Bot. 61:199-223, doi: 10.1016/j.envexpbot.2007.05.011.

Wang, H., X. Shuai, S. Ye, R. Zhang, M. Wu, S. Jiang, Y. Li, D. Wu, and J. He. 2022. Recent advances in the development of bitter gourd seed oil: from chemical composition to potential applications. Cri. Rev. Food. Sci. Nutr. 1-13, doi: 10.1080/10408398.2022.2081961.

Waterman, P.G., and S. Mole. 2019. Extrinsic factors influencing production of secondary metabolites in plants. In Insect-plant interactions. 107-134. CRC press.

World Health Organisation, 2013. WHO traditional medicine strategy 2014–2023. *WHO*. https://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/. Accessed 11 June 2022.

Wright, S.C., J.E. Maree, and M. Sibanyoni. 2009. Treatment of oral thrush in HIV/AIDS patients with lemon juice and lemon grass (*Cymbopogon citratus*) and gentian violet. Phytomedicine.

16:118-124, doi: 10.1016/j.phymed.2008.07.015.

Xia, X., Z. Lin, K. Shao, X. Wang, J. Xu, H. Zhai, H. Wang, W. Xu, and Y. Zhao. 2021. Combination of white tea and peppermint demonstrated synergistic antibacterial and anti-inflammatory activities. J. Sci. Food Agr. 106:2500-2510, doi: 10.1002/jsfa.10876.

Yang, L., K.S. Wen, X. Ruan, Y. X. Zhao, F. Wei, and Q. Wang. 2018. Response of plant secondary metabolites to environmental factors. Molecules. 23:762, doi: 10.3390/molecules23040762.

Yao, L., N. Caffin, B. D'arcy, Y. Jiang, J. Shi, R. Singanusong, X. Liu, N. Datta, Y. Kakuda, and Y. Xu. 2005. Seasonal variations of phenolic compounds in Australia-grown tea (*Camellia sinensis*). J. Agr. food Chem. 53:6477-6483, doi: 10.1021/jf050382y.

Yilmaz, G., N. Kandemir, and K. Kinalioglu. 2004. Effects of different pruning intervals on fresh shoot yield and some quality properties of tea (*Camellia sinensis* L. Kuntze) in Turkey. Pak. J. Biol. Sci. 7:1208–1212, doi: 10.3923/pjbs.2004.1208.1212.

Yu, X., C. Liang, J. Chen, X. Qi, Y. Liu, and W. Li. 2015. The effects of salinity stress on morphological characteristics, mineral nutrient accumulation and essential oil yield and composition in *Mentha canadensis* L. Sci. Hortic. 197:579-583, doi: 10.1016/j.scienta.2015.10.023.

Zaman, S., J. Shen, S. Wang, Y. Wang, Z. Ding, D. Song, H. Wang, S. Ding, X. Pang, and M. Wang, 2022. Effects of Shading Nets on Reactive Oxygen Species Accumulation, Photosynthetic Changes, and Associated Physiochemical Attributes in Promoting Cold-Induced Damage in *Camellia sinensis* (L.) Kuntze. Hortic. 8:637, doi:

10.3390/horticulturae8070637.

Zayed, S.M., M.M. Aboulwafa, A.M. Hashem, and S.E. Saleh. 2021. Biofilm formation by Streptococcus mutans and its inhibition by green tea extracts. AMB Express. 11:1-10, doi: 10.1186/s13568-021-01232-6.

Zhang, L.X., Q.S. Guo, Q.S., Chang, Z.B. Zhu, L. Liu, and Y.H. Chen. 2015. Chloroplast ultrastructure, photosynthesis and accumulation of secondary metabolites *in Glechoma longituba* in response to irradiance. Photosynthetica. 53:144-153, doi: 10.1007/s11099-015-0092-7.

Zhang, Q., Y. Shi, L. Ma, X. Yi, and J. Ruan. 2014. Metabolomic analysis using ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF MS) uncovers the effects of light intensity and temperature under shading treatments on the metabolites in tea. PloS one. 9: e112572, doi: 10.1371/journal.pone.0112572

Zhao, C.N., G.Y. Tang, S.Y. Cao, X.Y. Xu, R.Y. Gan, Q. Liu, Q.Q. Mao, A. Shang, and H.B. Li. 2019. Phenolic profiles and antioxidant activities of 30 tea infusions from green, black, oolong, white, yellow and dark teas. Antioxidants. 8:215. doi: 10.3390/antiox8070215.

CHAPTER 3: RESPONSE OF PLANT GROWTH AND DEVELOPMENT, AND ACCUMULATION OF HYDROXYL-CINNAMOYL ACID DERIVATIVES TO SELECTED SHADE NETS AND SEASONALITY OF FIELD GROWN BUSH TEA (*ATHRIXIA PHYLICOIDES* DC.)

Published in HortScience Journal (See appendix B)

Abstract.

Horticultural practices and quality of bush tea (*Athrixia phylicoides* DC.) are critical for herbal tea industrialization. The objective of the current study was to determine the effect of selected shade nets and seasonal variation on plant growth and development, and hydroxycinnamic acid content of field grown bush tea. The trial was laid out in a randomized complete block design consisting of three shade nets (black, green, and white) and control or full sunlight with three different light intensities (40%, 50%, and 80%) replicated three times. Proportion of intercepted radiation by the canopy, chlorophyll content, plant height, fresh and dry mass were measured and hydroxycinnamic acid accumulation was determined. In addition, hydroxycinnamic acid composition was determined using liquid chromatography linked to mass spectrometry (LC-MS). The application of shade nets resulted in plant growth and yield reduction as compared to the plants exposed to full sunlight during summer followed by white shade net. The accumulation of hydroxycinnamic acid was higher in 80% white shade net plots compared to unshaded plants (control) and the other shade nets. Therefore, lack of shading provides a conducive environment to enhance plant growth and development of bush tea. The white shade net (80%) was an effective microclimate tool to enhance accumulation of caffeoylquinic acid (m/z 353), p-coumaric acids (m/z 337), dicaffeoylquinic acid (m/z 515) and tri-caffeoylquinic acids of bush tea. This study is the first to

demonstrate light as a determining factor for production of chlorogenates in bush tea plants. Future studies will be conducted to determine the effect of light on extracts of the bush tea using different solvents.

Key words: herbal tea, quality control, protected environment, light intensity, quality index.

3.1. Introduction

Bush tea (*Athrixia phylicoides* DC.) is a native plant of South Africa and it grows naturally in different climatic conditions of South Africa (Mavundza et al., 2010; Nchabeleng et al., 2012; and Van Wyk and Gericke, 2000). The plant grows to a height of about 0.5 m to 1 m, with branches of thin woolly stems (Mudau et al., 2006). It has simple alternate leaves that are described as linear, light grey-green above, and white-woolly below (Mudau et al., 2006). The leaf bases are broadly lanceolate, short stalked, taper to a sharp point, auriculate, smooth on the upper surface, and have margins that are entirely or slightly revolute (Mudau et al., 2006). The head of inflorescence is sessile or subsessile with terminal axillary in large sub corymbose panicles (Herman et al., 2000).

It is used traditionally as an important herbal and medicinal plant by South Africans to cleanse and purify blood, treat boils, heal wounds, and treat headaches (Fouché et al., 2006; Mudau et al., 2007). In some parts of the country, it is used as an aphrodisiac (Rakuambo, 2011). To date, bush tea is still harvested from the wild for a variety of medicinal purposes (e.g., to treat chronic diseases such as cardiovascular disease and cancer) and hence there is a huge potential for development of *A phylicoides* as a commercial health benefit herb in South Africa (Mudau et al., 2006). Therefore, domestication of bush tea is critical for commercial exploitation to protect the species from possible extinction.

Previous studies have documented the effects of cultural practices such as mineral nutrition (Mudau et al., 2006), pruning (Mohale, et al., 2018; Yilmaz et al., 2004), irrigation (Bandara, 2012) and harvesting methods (Mphangwe, 2012), as well as processing (Hlahla, 2010) and environmental conditions (Tshivhandekano et al., 2013) on chemical compositions of bush tea. The quality, economic value and health function of bush tea is determined by the content of secondary metabolites such as flavonoids (or phenolic compounds), alkaloids, and amino acids (Tounekti et al., 2013). Zhang et al. (2014) reported that quality parameters of tea are significantly affected by environmental factors and management practices. Application of N fertilizer increases total phenol concentrations in cultivated medicinal plants by enhancing photosynthetic rate (Haukioja et al., 1998) and accumulation of non-structural carbohydrates (Wanyoka, 1983).

However, it has been reported that, carbon-based secondary metabolites increase frequently when environmental conditions enhance the accumulation of non-structural carbohydrates in *Labisia pumila* plants (Ibrahim et al., 2011). Hydroxycinnamic acids (coumaric acid, ferulic acid, sinapic acid, and caffeic acid) are phenolic compounds that are found in plants either as free compounds or conjugated to other molecules such as quinic acid (QA), tartaric acid, citric acid, and sugars (Ncube et al., 2014). These derivatives are known as chlorogenic acids (CGAs) when they are conjugated/esterified to other molecules (Masike et al., 2017; Nobela et al., 2018; Roleira et al., 2018). CGAs are classified into two groups: mono- and di-caffeoylquinic acids (CQAs) (Ncube et al., 2014). Plants naturally produce and utilize chlorogenic acids to aid their protection against abiotic (Lallemand et al., 2012; Taofiq, et al., 2017) and biotic (Ramabulana et al., 2020) stresses, as they have been reported to be effective defence phytochemicals (Kundu and Vadassery, 2019). Several hydroxyl-cinnamic acid derivatives such as 3,5-dicaffeoylquinic acid (3,5-diCQA) have been shown to have anti-HIV-1 INT enzyme activity which is attributed to *trans-cis* isomerization (Masike et al., 2017).

Net shades improve the quality of tea leaves due to an increase in the concentration of amino acids with lower content of catechin in the plant (Ku et al., 2010) and it also prevents the concentration of flavonoids (Wang et al., 2012). Net shade has also been reported to provide 50% to 70% of diffused solar insolation to the tea cultivation area (Lehlohonolo et al., 2013). Shade netting has been used in normal tea plantation in major tea producing areas to enhance optimum growth and productivity (Janendra et al., 2007). In green tea production, the ratio of oxalate content to reduced N content was higher in the flushes exposed to shading environment than the flushes under full sunlight (Morita and Tuji, 2002). Despite the fact that several studies have documented plant growth and yield, as well as the quality response of bush tea to shade nets, the majority of the research has been conducted in pot experiments under controlled environments (de Beer et al., 2011; Mavundza et al., 2010; Mudau et al., 2007; Tshivhandekano et al., 2014). Moreover, information that describes the accumulation of hydroxycinnamic acids (HCAs) in bush tea grown in the field under different shade nets, as a standard agricultural horticultural practice to create conducive microclimate due to climate change, is still lacking. Therefore, the objective of the current study was to determine the effect of selected shade nets and seasons on plant growth and development, and hydroxycinnamic acid accumulation of field grown bush tea.

3.2. Materials and methods

3.2.1. Experimental site.

The field trial was established in Autumn 2018 at the University of Venda's experimental farm, which is situated at Thohoyandou (22°55.33'S, long. 30°18.218'E, and 595 m asl), Limpopo

Province, South Africa. The site is characterized by an annual rainfall of around 500 mm that falls mainly in summer, and average maximum and minimum temperature of 31 °C and 18 °C, respectively (Tadross et al., 2006). The type of the soils at the experimental site are characterised by deep, well-drained clays with slightly acidic pH (Soil classification working group, 1991). Weather data was supplied by South African weather service for 2018 and 2019 cropping seasons.

3.2.2. Annual and seasonal weather pattern during 2018 and 2019.

Thohoyandou had high maximum and minimum average temperatures throughout the year. Monthly average maximum and minimum temperatures ranged from 30 to 40 °C and 10 to 20 ºC, respectively in both years (Fig. 1a and b). Temperature > 40 ºC was recorded in December 2018 and September and October 2019 (Fig. 1c and d). Rainfall distribution was very poor with hardly any rainfall being recorded between May and October in both years (Fig 2).

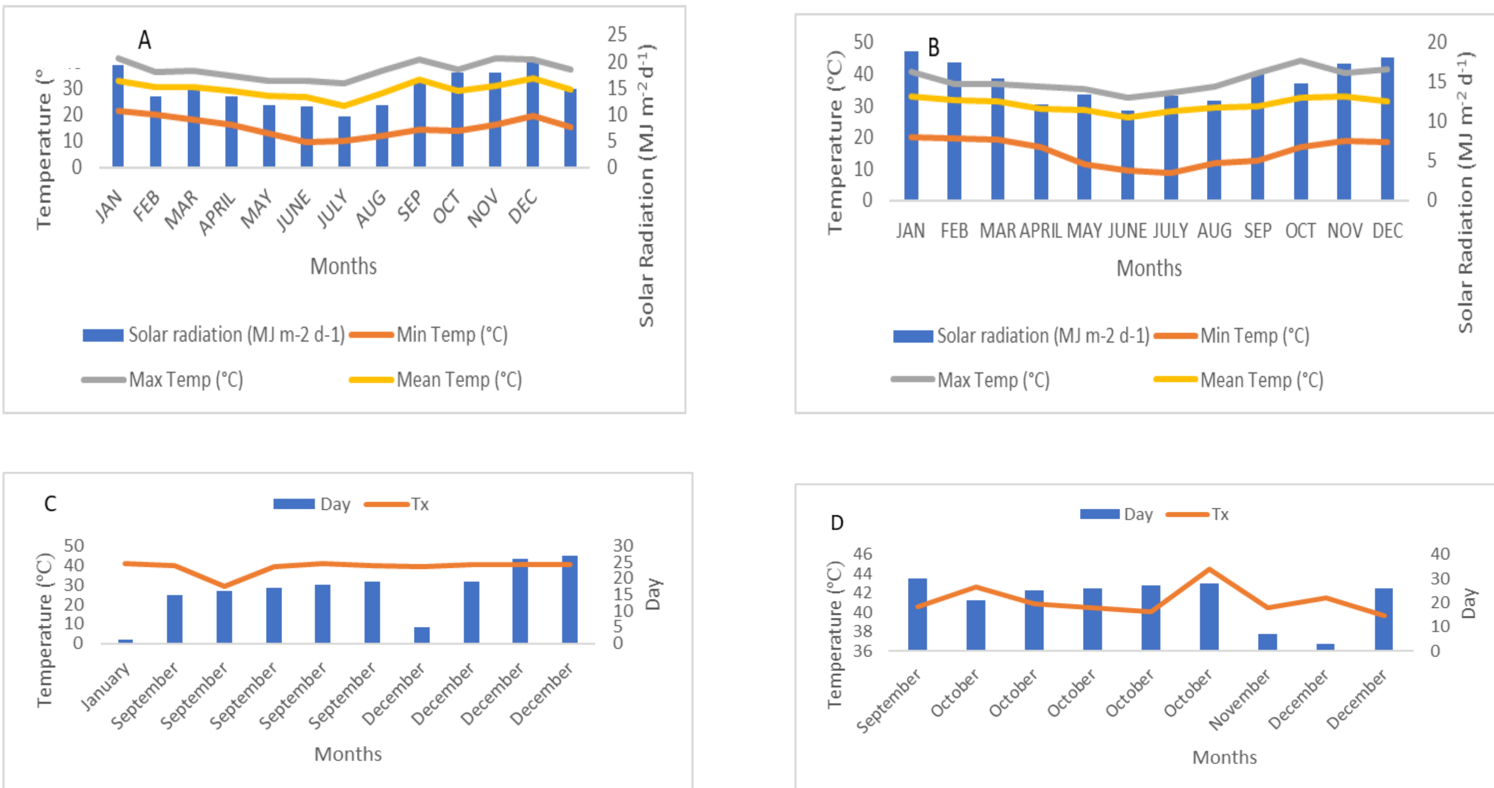


Figure 3.1 (a) Data denoting minimum and maximum temperatures ºC during 2018, (b) Data denoting minimum and maximum temperatures ºC in 2019 (c) Data denoting temperature > 40 ºC in 2018, (d) Data denoting temperature > 40 ºC in 2019. Tx: maximum temperature (South African weather service)

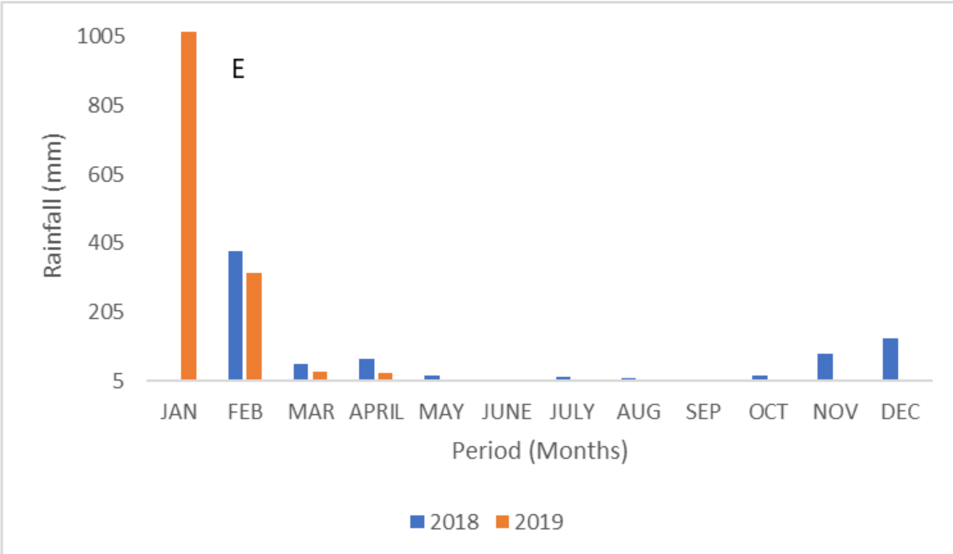


Figure 3.1 (b): The pattern of rainfall of Thohoyandou., South Africa, Limpopo Province during 2018 and 2019

3.2.3. Preparations of stem cuttings

The wild bush tea stem cuttings were collected from Tshivhulani Village (lat. 22° 55.331'S, long. 24º 50' 31º S 17E; Altitude 610 m), which is characterized by cold and dry winters. Planting materials of apical cuttings were cut at 7-8 cm long and dipped in Seradix No.2 hormone (0.3% IBA) to stimulate rapid and prolific rooting. 'True-to-name and type' material that was free of disease and insect damage was selected for planting. Stem cuttings were established on a 25 cm round plastic pot in a lath house at the University of Venda on 25 October 2017. Plants were irrigated daily except on rainy days. Rooted cuttings were transplanted into 1 L bags and placed into a net shade on 7 December 2017 for a period of 3 months. Planting materials, with approximately 25 leaves, were transplanted into four different types of environmental conditions (i.e., control, black, white, and green shade nets) on 12 March 2018. Data collection commenced three months after transplanting when the plants were well established. Growth/rooting media was a mixture of pine bark and sand at a ratio of 2:1. The initial media test chemical analyses were determined using Hanlon et al. (1994) procedure. NPK fertilizer was applied in two equal splits (at transplanting and two weeks after transplanting) at a rate 300 kg N ha^{-1}, 300 kg P ha^{-1}, and 200 kg K ha^{-1} based on previous studies (Mudau et.al., 2007).

3.2.4. Experimental description

The experiment was laid out in a randomized complete block design consisting of 3 shade nets (black, green, and white) and full sunlight with 3 different light intensities (40%, 50%, and 80%) replicated three times. The size of the individual plots was 4.8 m X 4.8 m. Each plot consisted of four plant rows, 1.2 m apart and the intra-row spacing was 0.75 m, giving a total of 24 plants per plot. The plots were watered, using a drip irrigation system, when necessary. Weeding was done manually throughout the cropping season to keep the experimental plots weed-free. Methamidophos was applied at a rate of 10 mL per 20 L after each weeding occasion to protect the plants against termites.

3.2.5. Growth parameters

Bush tea is a perennial crop that flowers throughout the year and is able to resprout after being cut. Data was collected only from regenerated plants for each season. Pruning was done after each harvest to avoid mineral resource competition between the previous and regenerated plants. In 2018, data collection began on 12th June for the winter season. Each net plot had 4 rows excluding the guard rows. 16 plants from the second inner rows of each experimental plot were harvested once per season. Plant height (from the soil surface to the tip of the topmost leaf) was determined a day before each harvest using a measuring tape. Fresh weight was recorded after harvest in each season each year using a weighing balance. The harvested plants were air-dried, under room temperature, for two months. The proportion of dry matter content was obtained by expressing the dry weight as a percentage of the fresh weight of the sample taken as shown in Equation 1.

$$\text{Dry matter (\%)} = \frac{Dry\ weight}{Fresh\ weight} \times 100 \qquad (1)$$

3.2.6 Chlorophyll content

Leaf chlorophyll content was determined, between 09h00 and 12h00 HR on each occasion, from five plants in each plot using chlorophyll content meter (CCM-200 Plus, Opti-Sciences, and Tyngsboro, Massachusetts). Measurements were taken weekly between 26 – 33 weeks (winter, 2018), 38 – 46 weeks (spring, 2018), 49 - 62 weeks (summer, 2018) and 71 -73 weeks (autumn,

2019) and 78 – 87 weeks (winter, 2019), 90 - 98 weeks (spring, 2019), 102 - 112 weeks (summer, 2019) and (118 – 120 weeks (autumn, 2020).

3.2.7. Proportion of intercepted radiation by the canopy cover

PAR (photosynthetically active radiation) measurements were taken at 7-days intervals, and the dates cover the vegetative and reproductive stages of plant growth in each season. The proportion of intercepted radiation was determined by measuring PAR above and below the canopy on various occasions, mostly at 7-day intervals, between 26 – 33 weeks (winter, 2018), 38 – 46 weeks (spring, 2018), 49 - 62 weeks (summer, 2018) and 71 -73 weeks (Autumn, 2019) and 78 – 87 weeks (winter, 2019), 90 - 98 weeks (spring, 2019), 102 - 112 weeks (summer, 2019) and 118 – 120 weeks (Autumn, 2020). The measurements were taken between 11h00 and 13h00 on clear, cloudless days using AccuPar LP-80 Ceptometer and the proportion of intercepted radiation (α) was calculated as shown in Equation 2.

$$\alpha = 1-(P_{A/}P_B) \qquad (2)$$

Where:

P_A is the Photosynthetically Active Radiation (PAR) above the canopy.

P_B is the Photosynthetically Active Radiation (PAR) below the canopy.

α is the proportion of the intercepted radiation.

3.2.8. Metabolites extraction

Metabolite extraction was accomplished by a method proposed by Makita et al. (2016). In brief, bush tea leaves were manually threshed from dried plants and ground as soon as the dry mass was weighed for the autumn season. They were ground to fine powder using hammer grinder, and two grams (2 g) of fine ground leaves was mixed with 20 mL (1:10 m/v) of 80% aqueous methanol. The homogenate was centrifuged at 5000 xg for 20 min to remove debris and finally, the supernatant was transferred to new clean tubes. The extracts were further diluted 1:1 (v:v) to final volume of 20 mL using methanol, followed by transfer of 10 mL of the diluted extracts into cylindrical quartz glass vials (2 x 10 cm). The samples were filtered into a 2 mL vial fitted with a 0.2 mL conical bottom glass insert using a syringe fitted with a 0.2 μmm filter.

3.2.9. Liquid Chromatography Mass Spectrometry analysis

Bush tea sample analysis was conducted on an LC-QTOF-MS, model LC-MS 9030 instrument with a Shim Pack Velox C18 column (100 mm × 2.1 mm with particle size of 2.7 μm) (Shimadzu, Kyoto, Japan), placed in a column oven set at 40°C. A binary solvent mixture, 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. 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 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.

3.2.10. MS/MS experiments

For tandem MS (MSMS) experiments, a mass calibration solution of sodium iodide (NaI) was used to obtain typical mass accuracies with a mass error below 1 ppm and a range of *m/z* 100-1000 was used for high resolution. Argon gas was used as a collision gas for MSMS experiments along with MS^E mode using collision energy ramp of 12 eV to 25 eV for generation of fragments.

3.2.11. Statistical analysis

All plant growth data were subjected to ANOVA using SPSS version 27. Means were separated using the Duncan's multiple range test (DMRT) when F-test indicated significant differences among the treatments. Correlation analysis was conducted to assess the relationships between the physiological parameters.

3.3. Results and discussion

3.3.1. Proportion of intercepted radiation (IR)

Shade nets and seasons affected ($p \leq 0.001$) the proportion of intercepted radiation by the crop canopy at all measurement dates in both 2018 and 2019 (Fig. 3 and 4). On average, the proportion of intercepted radiation was higher in control (57.8%) compared to white color (57.2%), green shade (50.2%) and black shade (52.4%) (Table 1). The higher proportion of intercepted radiation observed in control compared to other treatments could be attributed to the larger canopy size and plant height of the plants that were exposed to full sunlight (Muchow et al., 1990). Our findings are comparable to earlier observations that different shade nets can influence micro-climatic parameters that control plant growth and development (Kumar et al., 2013).

3.3.2. Total intercepted radiation.

Control had significantly ($p \leq 0.001$) higher total intercepted radiation than the other treatments in both years (Fig. 5 and 6) which is consistent with earlier findings that black shade nets tended to absorb more light compared to other shade nets (Mokoka, 2007). These findings are consistent with those of Anwar et al. (2003), who found that total intercepted radiation varies between treatments due to differences in days to physiological maturity. The interaction of shade nets and season also had a significant ($p \leq 0.001$) effect on total intercepted radiation in all the seasons.

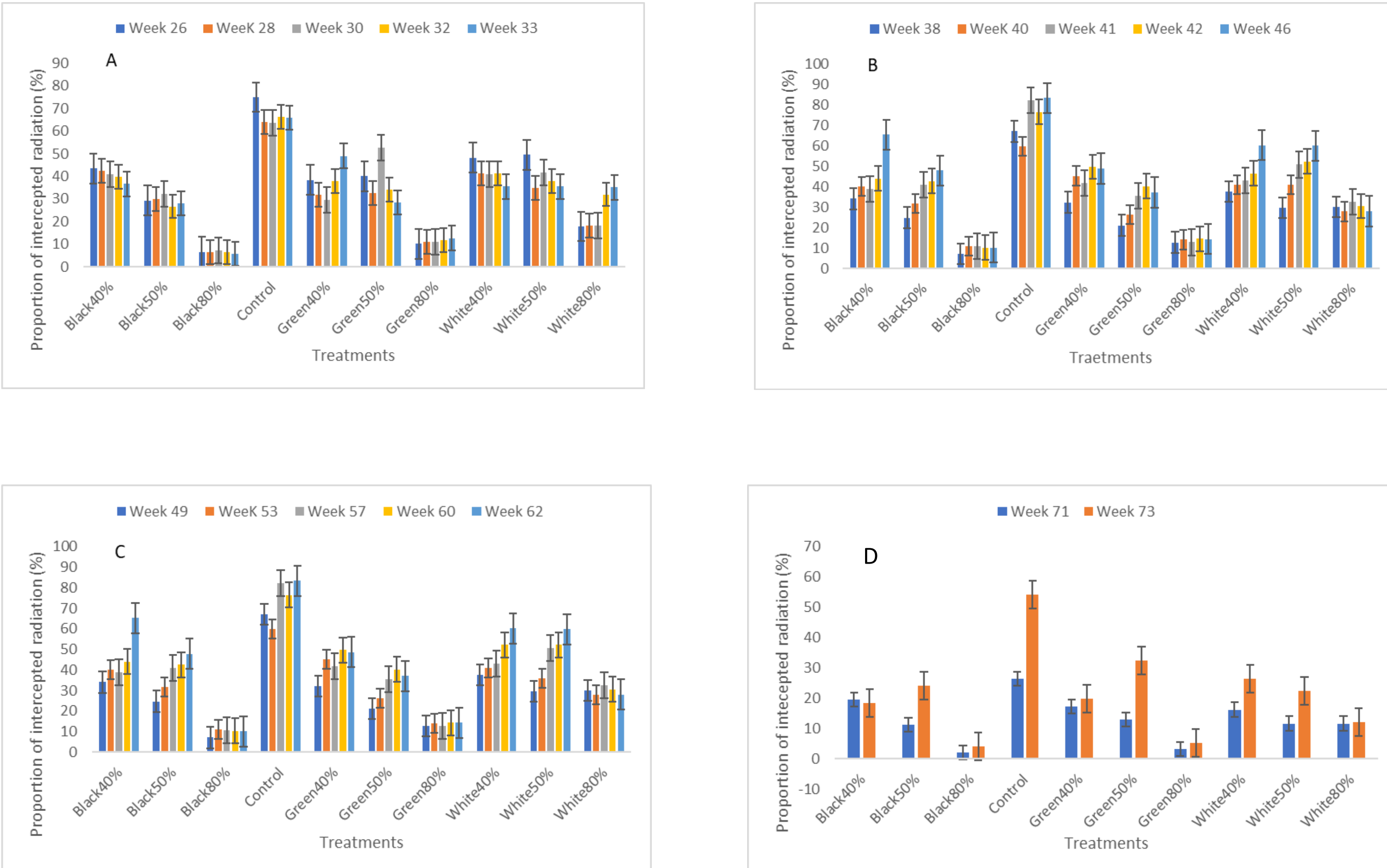


Figure 3.2. The effect of treatments on the proportion of intercepted radiation in 2018. A (winter 2018), B (spring 2018), C (summer 2018) and D (Autumn 2019)

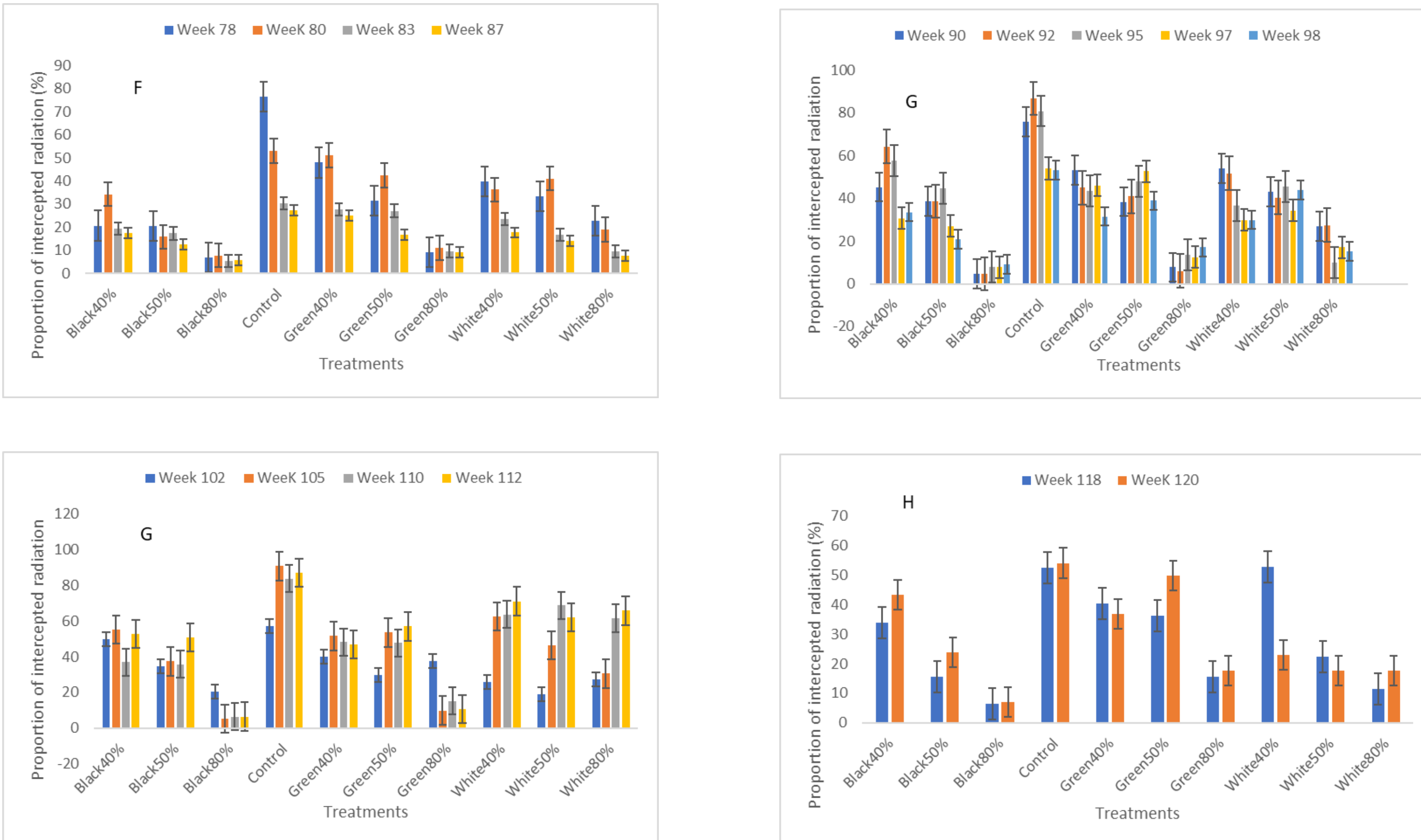


Figure 3.3. The effect of treatments on the proportion of intercepted radiation in 2019. A (winter 2019), B (spring 2019), C (summer 2019) and D (Autumn 2020)

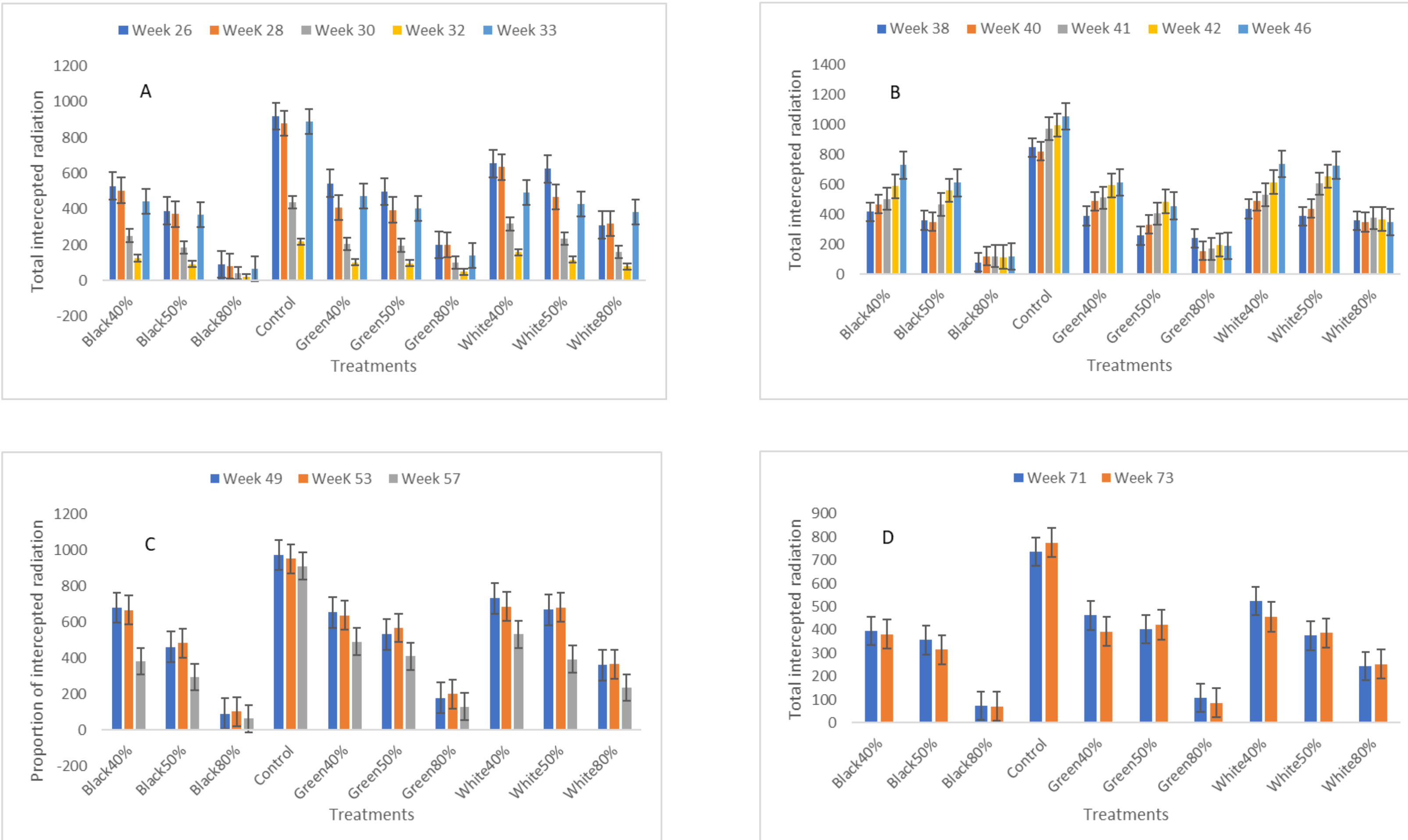


Figure 3.4. The effect of treatments on the Total radiation in 2018. A (winter 2018), B (spring 2018), C (summer 2018) and D (Autumn 2019)

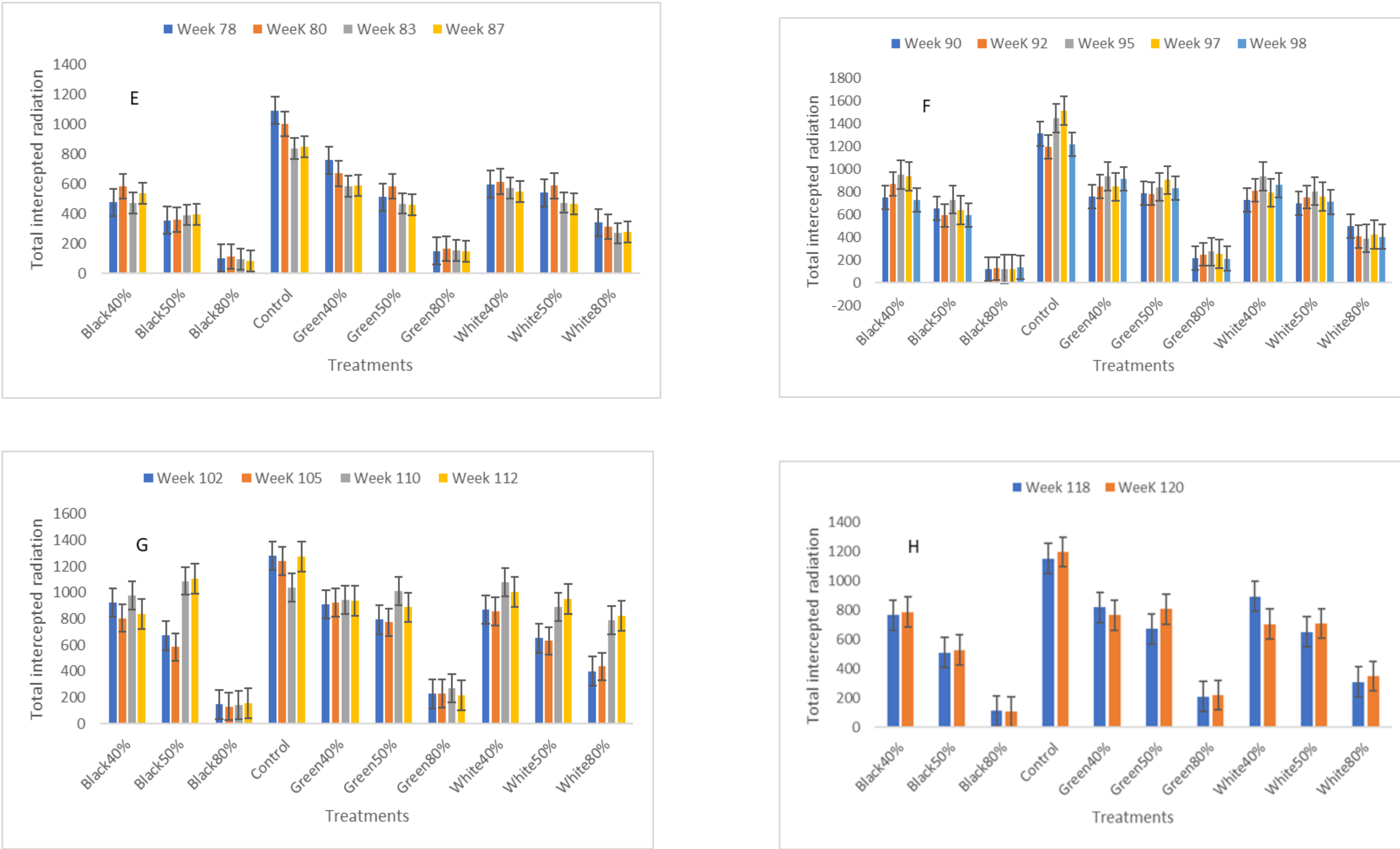


Figure 3.5. The effect of treatments on the Total radiation in 2019. A (winter 2019), B (spring 2019), C (summer 2019) and D (Autumn 2020)

3.3.3. The effect of shade nets on plant growth and development of bush tea

There was a significant difference among plant growth parameters (proportion of intercepted radiation, fresh mass, and dry mass) on bush tea plants grown under different shade nets. However, chlorophyll content and plant height did not vary with shade nets (Table 1).

3.3.3.1. Fresh and dry mass

Plants from unshaded plots recorded the highest fresh biomass (762.1g plant $^{-1}$) followed by white shade net treatments (533.6 g plant $^{-1}$), with the lowest fresh biomass (391.9 g plant $^{-1}$) being recorded in plants grown under black shade nets (Table 1). The complex interaction between quantity and quality of incident radiation determines the response of plant to shading conditions (Lee et al., 1997). The significant decrease of fresh mass on plants grown under black shade net might be due to insufficient light the plants had received which resulted in stunting growth which clearly demonstrate the importance of a favorable environment in determining plant growth and yield of bush tea in any region. Our results concur with earlier observations (Bell et al., 2000; Rao and Mintra, 1998) that tea grown under shade nets had an ability to reduce photosynthetically active radiation (PAR), change spectral quality, affect plant photosynthesis, dry matter production and yield of the crop. Similarly, Marchese and Figueira (2005) reported that plant growth and development was significantly influenced by environmental factors such as radiation, temperature, and photoperiod, and that increased biomass and essential oil was associated with higher photosynthetic rate of plants and higher radiation. Although plants exposed to low light intensity at the vegetative stage tend to increase their capacity to trap light by increasing the leaf area, this did not significantly contribute towards biomass accumulation in our study. Similar results were reported by Mokoka (2007), who observed that 55% black shade nets significantly reduced fresh shoot mass relative to plants grown under18% white shade nets in fever tea which is in line with reports that yield reduction due to shading was determined by crop species and degree of shading (Kumar, et al., 2013).

3.3.4. Effect of light intensity on plant growth and development of bush tea

There was a significant difference in plant growth parameters (i.e., IR, plant height, fresh mass and dry weight) of bush tea plants grown under different light intensities of shade nets. However, light intensity did not affect chlorophyll content (Table 1).

The unshaded control exhibited the highest proportion of intercepted radiation (57.7%) with the lowest (47.6%) being recorded in 80% shade net plots (Table 1). The response of plant height and fresh biomass to light intensity followed a similar trend with the highest (158 m and 772.3 g plant $^{-1}$) and lowest (144.2 m and 362.4 g plant $^{-1}$) plant height and fresh biomass recorded in unshaded control and 80% shade net plots, respectively (Table 1). The results of the current study are consistent with previous reports that changes in microclimate caused by spacing and shade levels result in significant differences in growth and development with faster growth and development exhibited in plants exposed to full sunlight compared to those grown under shade (Kumar et al., 2013). More recently, Thakur et el., (2019) concluded that higher shade level reduced photosynthetic active radiation, altered light intensities, and affected photosynthetic rate and yield production. In contrast to our results, Kumar et al. (2013) reported that shaded plants were taller than those exposed to full sunlight probably due to long internodal lengths and thinner stems in the plants grown under shade. The lower fresh and dry mass of bush tea plants in the shaded treatments compared to plants from unshaded plots that we observed in the current study may be attributed partly to a combination of low light and high air temperature which likely reduced available stored energy rapidly (Svenson, 2002). Similarly, Gregoriou et al., (2007) and Wei et al., (2005) reported that growth and productivity of plants were usually inhibited by lower light intensities through imbalances in gaseous exchange. In contrast, Barua and Gogoi (1979) reported that the yield in tea, especially tea shoots, subjected to light intensity under 35% were higher than in tea plants that were exposed to direct full sunlight which may suggest that unsuitable light intensities were usually capable of causing damage to the plant's photosynthetic system by interfering with photosynthesis and development (Szymborska-Sandhu et al., 2020). Clearly, the effect of light intensity on growth, development and yield varies greatly and may need further investigation.

3.3.5. The effect of season on plant growth and development

Bush tea plant growth parameters (IR, plant height, fresh mass, dry weight, and chlorophyll content) varied with seasons (Table 1). On average, the proportion of intercepted radiation (%) was higher in summer (64.6%) compared with winter (58.7%), autumn (54.9%) and spring (36.7%) (Table1), perhaps partly due to the larger canopy size that was exhibited in summer. Similarly, fresh biomass was higher in summer (636 g plant $^{-1}$) compared with the rest of the seasons (343.6 - 550.1 g plant $^{-}$) (Table 1). For plant height in contrast, the seasons were ranked as: autumn > summer > winter > spring (Table 1). The higher biomass accumulation in summer was associated with the greater proportion of intercepted radiation recorded in summer. The amount of solar radiation received during the cropping season influences plant growth, development, and yield (Challa and Bakker, 1999; Cockshull et al., 1992). Bush tea yield drops significantly under cloudy conditions, with heavy and continuous rainfall, just like it does when the weather is hot, dry, and sunny. In contrast to our findings, CTFIL (1995) reported that plant growth and yield was restricted during the summer season due to the effects of higher summer temperatures on photosynthesis and respiration.

3.3.6. Chlorophyll content (CCL)

There was a significant seasonal variation in chlorophyll content with the highest recorded in winter (17.1nm) and lowest in summer (11.4 nm) (Table 1). The results are in line with those of MacAlister et al., (2020), who reported that chlorophyll content was higher in winter compared to the summer season.

3.3.7. Interactive effect of color, light intensity, season and year on plant growth and development of bush tea cultivated under different conditions

There are no distinct research findings that have been reported and discussed on interactive effect of color, light intensity, season and year on plant growth and development of bush tea cultivated under different conditions. The interaction between light intensity and color of shade net affected the proportion of intercepted radiation ($p \leq 0.01$), plant height ($p \leq 0.05$), and fresh mass and dry mass, ($p \leq 0.001$) but not chlorophyll content (Table 2). In contrast, the interactive effect of light intensity and season as well as light intensity, shade net color, season and year was only significant on the proportion of intercepted radiation, and the 3-way interaction of light intensity, color of shade net and season did not affect any plant growth parameter (Table 2).

Table 3.1. Response of plant growth and development of bush tea cultivated under different shade nets colour, light intensity, and season

	Source of variation	IR(%)	Plant Height (m)	Fresh mass (g) Plant $^{-1}$	Dry mass (g) Plant^{-1}	Chlorophyll Content nm
Colour	Control	57.8^{a}	159.8^{a}	762.1^{a}	382.5^{a}	16.1^{a}
	Black	52.4^{b}	155.2^{a}	391.9^{c}	196.9^{b}	13.9^{a}
	Green	50.2^{b}	147.6^{a}	402.3^{c}	240.6^{b}	14.4^{a}
	White	57.2^{a}	151^{a}	533.6^{b}	262.2^{b}	13.2^{a}
Light intensity (%)	100	57.7^{a}	158^{a}	772.3^{a}	387.6^{a}	15.9^{a}
	40	56.9^{a}	152.5^{ab}	499.8^{b}	288.1^{b}	14.8^{a}
	50	55.2^{a}	157.2^{ab}	465.6^{b}	230.9^{bc}	11.9^{a}
	80	47.6^{b}	144.2^{c}	362.4^{c}	180.7^{c}	14.7^{a}
Season	Autumn	54.9^{c}	181.8^{a}	550.1^{b}	274.7^{ab}	12.1^{c}
	Spring	36.7^{d}	109.4^{d}	368.5^{c}	186.6^{bc}	15.5^{bc}
	Summer	64.6^{a}	173.4^{b}	636^{a}	314.5^{a}	11.4^{a}
	Winter	58.7^{b}	143.9^{c}	343.6^{c}	216.9^{c}	17.1^{b}

Means within a column followed by the same letter indicate no significant difference. Means within a column followed by different letter indicate significant difference

Table 3.2. Interactive effect of colour, light intensity, season and year on plant growth and development of bush tea cultivated under different conditions

	DF	IR(%)				Plant height (cm)				Fresh mass (g)				Dry mass (g)				Chlorophyll content (**nm**)			
		SS	MS	F-value	P-value	SS	MS	F-value	P-value	SS	Ms	F-value	P-value	SS	Ms	F-value	P-value	SS	Ms	F-value	P-value
Colour	3	2286.3	762.1	19	0.1ns	3679.9	1226.6	0.5	0.707ns	3103307.1	1035535.7	11784	0.000***	64065.3	2135514	4	0.008**	164.8	54.9	0.5	0.712ns
Light Intensity	4	4032.3	1008.1	2.5	0.041*	9737.6	2434.4	0.9	0.448ns	29998834	749708.5	8461	0.000***	911068.7	227767.2	4.4	0.002**	499.8	124.9	1.01	0.382ns
Season	3	26024.5	8674.8	28.7	0.000***	193529	64510	35.2	0.000***	3609893.9	1203297.9	14051	0.000***	592542.6	197514.2	3.7	0.01*	1340.6	446.9	3.9	0.010*
Year	1	282.6	282.6	7	0.406ns	33725	33725	13.5	0.000***	2406997.1	2406997.1	26.6	0.000***	338873.5	338873.5	6.3	0.013*	1028.2	1028	8.9	0.003**
L X C	4	1423.3	355.8	3.6	0.008**	14489	3622.2	3.4	0.01*	1626428.8	406607.2	9.8	0.000***	714233.3	178558.3	4.3	0.003**	196.3	49.1	0.4	0.79ns
L X S	6	1772.4	295.4	2.9	0.009**	3558.9	593.1	0.6	0.759ns	574197.6	95699.6	2.3	0.35ns	221508.5	36918.1	0.9	0.51ns	88.7	14.8	0.1	0.993ns
L X Y	2	1010.5	505.3	5.1	0.007**	3742.7	1871.4	1.9	0.172ns	19503.6	9751.8	0.3	0.789ns	74335.4	37167.7	0.9	0.414ns	6	3.7	0	0.974ns
C X S	6	252.8	42.1	0.4	0.863ns	943.5	157.2	0.5	0.989ns	480211.7	80035.3	1.945	0.77ns	414569.3	69094.9	1.7	0.137ns	611.4	101.9	0.9	0.507ns
C X Y	2	2792.6	1396.3	14	0.000***	1078.2	539.3	0.5	0.600ns	111308.2	55654.1	1352	0.262ns	97192.3	48596.2	1.2	0.316ns	145.6	72.1	0.6	0.533ns
S X Y	3	29840.7	9946.9	99.7	0.000***	165766	55255	52.4	0.000***	2612234.9	870744.9	21.2	0.000***	857282.3	285760.8	6.8	0.000***	2198.8	732.9	6.4	0.000***
L X C X S	12	1647.0	137.3	1.4	0.182ns	6977.6	581.5	0.6	0.877ns	522686.8	43557.2	1.1	0.399ns	573990.7	47832.6	1.1	0.330ns	388.1	32.3	0.3	0.992ns
L X C X Y	4	1299	324.8	3.3	0.013*	945.3	236.3	0.2	0.924ns	101301.1	25325.3	0.61	0.652ns	72485.6	18121.4	0.4	0.785ns	1314.6	328.6	2.9	0.026ns
L X S X Y	6	1385.3	230.9	2.3	0.036*	6093.9	1015.6	0.9	0.451ns	77875.6	12979.3	0.3	0.928ns	302745.3	50457.6	1.2	0.307ns	584.7	97.5	0.8	0.536ns
C X S X Y	6	1464.1	244	2.4	0.27*	1590.8	265.1	0.3	0.958ns	121491	20248.5	0.5	0.814ns	246825.6	41137.6	0.9	0.439ns	492.6	82.1	0.71	0.64ns
L X C X S X Y	12	1656.2	138	1.4	0.178ns	1866.8	155.6	0.2	1.00ns	150966.6	12580.6	0.3	0.988ns	579910.8	48325.9	1.2	0.321ns	851.5	70.9	0.71	0.826ns
ERROR	160	15956.6	99.7			168466.0	1052.9			6584500.2	41153.1			6701283.9	41883.0			18426.0	115.2		
TOTAL	240	789637.7				6179559.0				77870534.3				27978801.1				75781.1			

IR= Intercepted radiation, SS= Sum of square, MS=Means of square, *, **, *** and ns: significant at p≤ 0.05, p≤ 0.01, p≤ 0.001 and not significant

3.3.8. Identification of chlorogenic acid (CGA) in bush tea plants exposed to different shade nets and light intensities

The results revealed that there was greater production of chlorogenic acids viz: caffeoylquinic acid, coumaroylquinic acid, dicaffeoylquinic acid and *tri-caffeoylquinic acids (Tri-CQAs)* in plants that were exposed to white shade net at 80% level compared to plants that were exposed to full sunlight (Table 3). LC MS analysis was conducted on the leaf extracts of bush tea grown under different conditions. Only 4 major chlorogenic acid yielded under negative ionization and all fragmentation patterns were generated after negative ionization. MS^2 spectra (Fig. 8), achieved through collision induced dissociation (CID) based approaches, was found to be sufficient for characterization of these molecules.

Different shade nets and light treatments led to differential regulation of secondary metabolite production in bush tea, observed by presence and absence of certain metabolites (Table 3). Di-caffeoylquinic acids were identified equally in all treatments. More peaks of coumaroylquinic acids were detected in the combination of white shade nets and 80% light intensity compared to the other treatments. Tri-caffeoylquinic acids *(Tri-CQAs)* was detected only in the unshaded control and white shade net at both 50% and 80% light intensity. These CGA's are responsive towards light as their accumulation is subjected to light exposure. Chlorogenic acids enable plants to adapt in high light-intensity environments by absorbing excess light. Our findings are consistent with earlier observations that secondary metabolite accumulation is affected by plant species, different plant organs, and environmental conditions to which plants are exposed (Eko et al., 2012). These findings may be due to fact that accumulation of CGA's are related to light intensities. The results contradict with those of Karimi et al., (2013) who reported that three different varieties of

Labisia pumila Benth exposed to high light intensities results in an enhancement in phenolic compound such as gallic acid, caffeic acid and flavonoids compounds which includes quecetin, rutin myricetin, kaempferol and naringin.

3.3.8.1. Characterization of mono-acyl caffeoylquinic acids

Four peaks corresponding to a molecular formula of $C_{16}H_{18}O_9$ with precursor ion $[M-H]^-$ at *m/z* 353 (**1-4**) were detected in the chromatogram of all treatments of bush tea extracts. Two peaks **(1 & 4**) were detected as isomers of 3-CQA due to the presence of a peak with the product ions at m/z 191 and m/z 179 at 50% base peak (Fig. 8B). Similarly, a peak with the product ion at m/z 173 was identified as 4-CQA **(2).** A peak with a single product at m/z 191 was identified as 5CQA **(3).**

3.3.8.2. Characterization of p-coumaroylquinic acid

Five peaks with precursor ions at *m/z* 337 (**5-9**) suggesting a molecular formula of $C_{16}H_{18}O_8$, were detected on all treatments of bush tea extracts. These molecules were identified as *para*-Coumaroylquinic acids (*p*CoQA) based on accurate mass and accompanying fragmentation patterns (MS^2) patterns. Two peaks **(5 & 6)** were identified as isomers of 5-pCoQA due to the presence of the product ion at *m/z* 191 with additional peaks at *m/z* 161, and *m/z* 135. Another two peaks (**7 & 8)** with product ions of m/z 163, m/z 179 and m/z 191 were identified as 3-*p*CoQA (Fig. 8A). The presence of product ions at *m/z* 173 allowed the annotation of molecule **(9)** as 4-pCQA and it was only identified in white shade nets at both 50% and 80% light intensities.

3.3.8.3. Characterization of di-caffeoylglucosides

Three compounds with a precursor ion $[M-H]^-$ at *m/z* 515 were identified as di-caffeoylquinic acid (**10-12**) in all the treatments used in the study. A peak with product ions at m/z 353, m/z 173, m/z 191 and m/z 135 (Fig. 8C) was identified as 3,4-diCQA **(10).** In addition, a peak with product ions at m/z 353, m/z 173, m/z 191 and m/z 135 was identified as 3,5-diCQA **(11)**. Similarly, a peak with fragment ion at m/z 353 m/z, m/z 173, m/z 179 and m/z 135 was identified as 4,5-diCQA **(12)** at MS^2. These results concur with previous findings (Clifford et al., 2005; Masike et al., 2018) that elution order of di-CQA *regio*-isomers on a reverse-phase column was expected to be 3,4-di-CQA, 3,5-di-CQA, followed by 4,5-di-CQA.

3.3.8.4. Characterization of Tri-Caffeoylquinic acids (Tri-CQAs)

Compound **(13)** was annotated as 1,3,5-tri-CQA with the product ion of m/z 515, m/z 353, m/z 179 and m/z 173, corresponding to the molecular formula of $C_{34}H_{30}O_{15}$, and it was detected in unshaded control, as well as white shade net plots at both 50% and 80% light intensities (Fig. 8D).

Table 3.3. Characterization of chlorogenic acids (CGAs) of bush tea plants exposed to different net shade and light intensities

No	*m/z*	Rt (mn)	Molecular formula	Molecular formula	Abbr	Con	B40%	B50%	B80%	G40%	G50%	G80%	W40%	W50%	W8 %
1	353	3.279	$C_{16}H_{18}O_9$	3-O-Caffeoylquinic acid	191.05, 179,03, 135,04	✓	✓	✓	✓	✓	✓		✓	✓	✓
2	353	6.453	$C_{16}H_{18}O_9$	4-O-Caffeoylquinic acid	173.04,179.03,135.04,	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	353	6.985	$C_{16}H_{18}O_9$	5-O-Caffeoylquinic acid	191.05, 179.03	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	353	13.405	$C_{16}H_{18}O_9$	Cis-3-O-Caffeoylquinic acid	191.05, 179,03, 135,04	✓	✓	✓		✓	✓		✓		✓
5.	337	9.135	$C_{16}H_{18}O_8$	5-O-P-Coumaroylquinic acid	191.05, 161.02, 135.04	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6.	337	10.844	$C_{16}H_{18}O_8$	Cis-5-O-P-Coumaroylquinic acid	191.05161.02, 135.04	✓		✓	✓			✓	✓		✓
7.	337	5.208	$C_{16}H_{18}O_8$	3-O-P-Coumaroylquinic acid	163.03,179.03,191.05	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8.	337	14.297	$C_{16}H_{18}O_8$	Cis-3-O-P-Coumaroylquinic acid	163.03, 179.03, 191,05,	✓	✓	✓					✓	✓	✓
9.	337	9.154	$C_{16}H_{18}O_8$	4-O-P-Coumaroylquinic acid	191,05,173,03, 163,03									✓	✓
10.	515	13.113	$C_{25}H_{24}O_{12}$	3,4-Dicaffeoylquinic acid	353.08,173.03,191.05, 135.04	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
11.	515	13.365	$C_{25}H_{24}O_{12}$	3,5-Dicaffeoylquinic acid	353,08,191.05,179.03, 135.04	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	515	15.998	$C_{25}H_{24}O_{12}$	4,5-Dicaffeoylquinic acid	353,08, 173,04, 179.05, 135,04	✓	✓		✓	✓	✓	✓	✓	✓	✓
13.	677	15.775	$C_{34}H_{30}O_{15}$	1,3,5-Tri-Caffeoylquinic acid	515, 353, 179, 173	✓								✓	✓

Con: control, B40%: black 40%, B50%: black 50%, B80%: black 80%, G40%: green 40%, G50%: green 50%, G80%, green 80%, W40%: white 40%, W50%: white 50% and W80%: white 80%

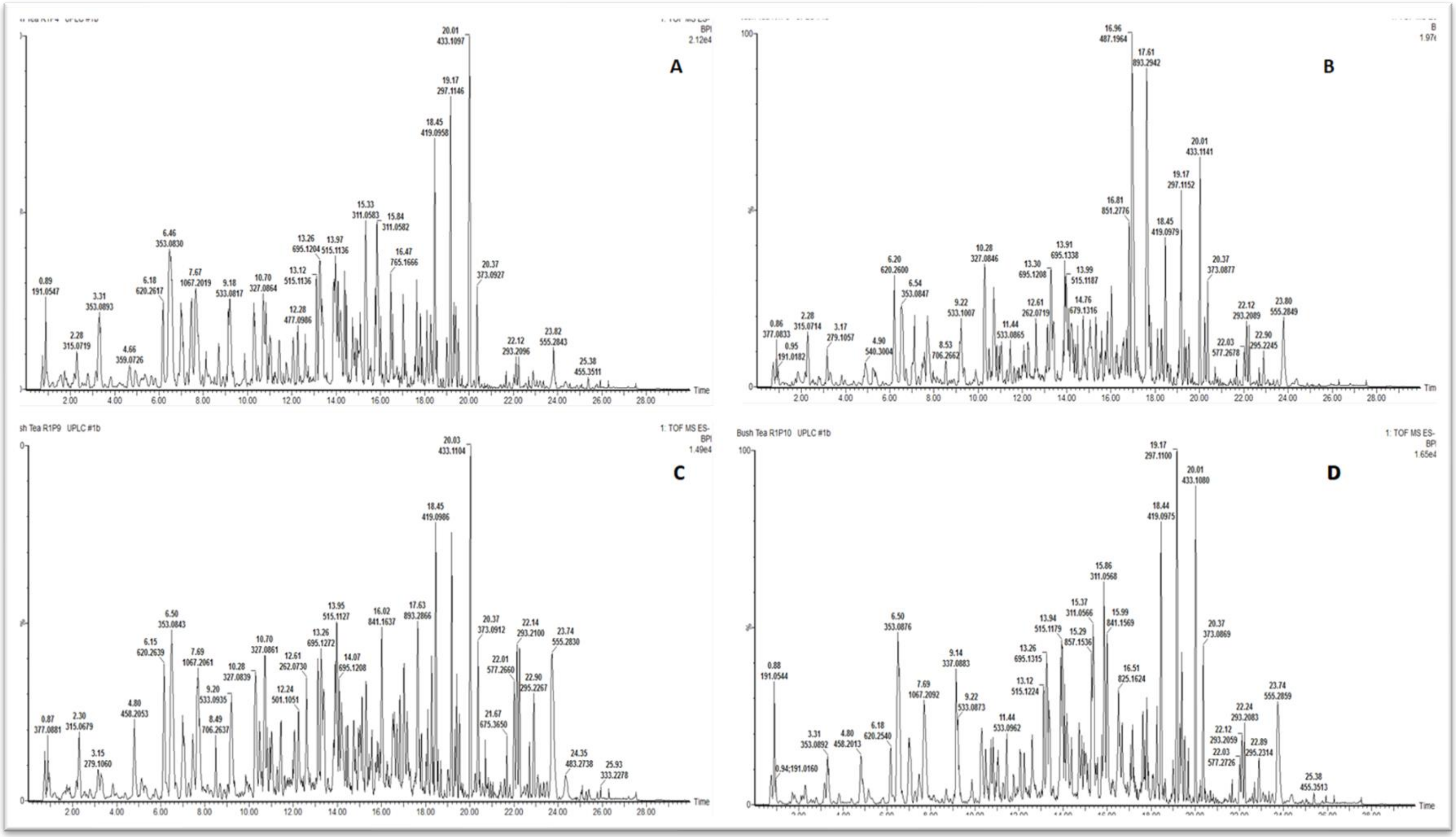


Figure 3.6. Representative UHPLC-QTOF-MS base peak intensity (BPI) chromatograms showing the separation of secondary metabolites in extracts of bush tea plants exposed to different [(A)control, (B)black, (C)green and (D) white] shade nets colours.

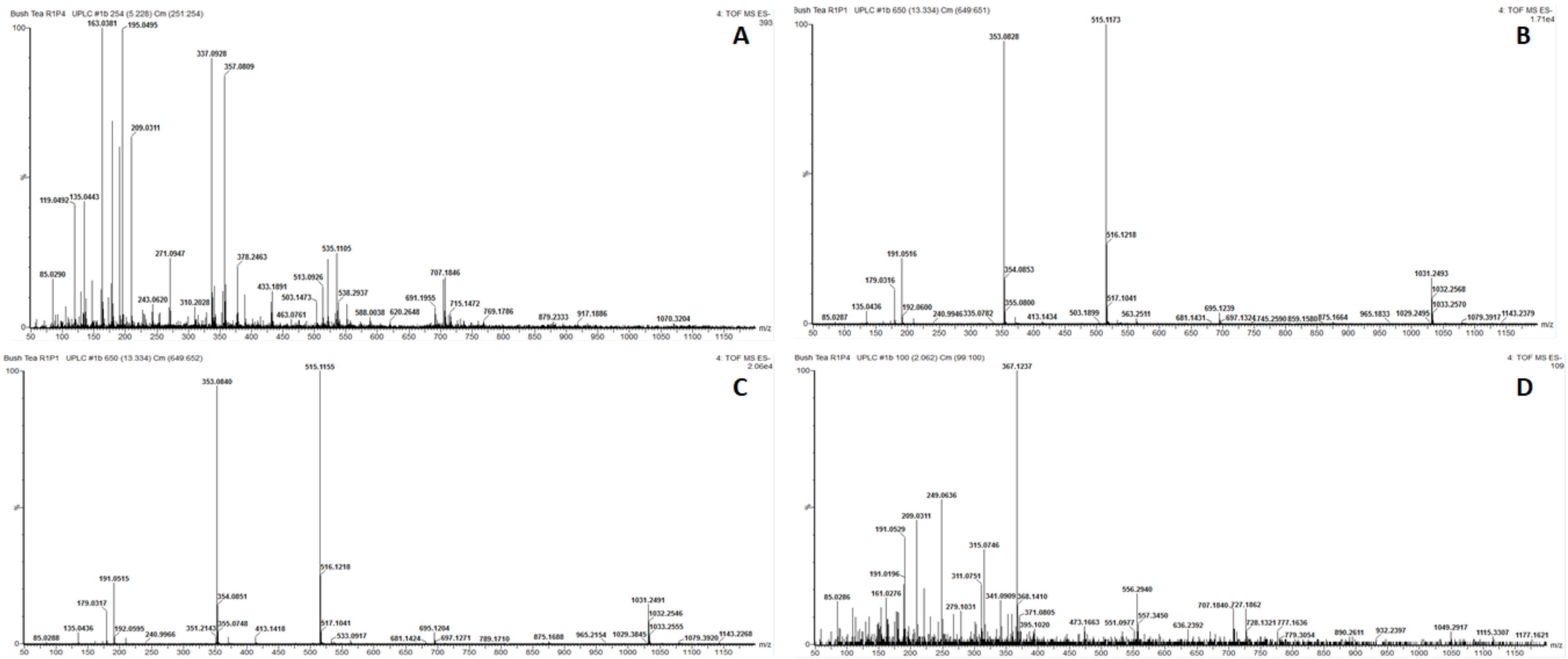


Figure 3.7: Representative ESI negative spectrum showing the fragmentation pattern of (A) coumaroylquinic acid (B) caffeoylquinic acid (C) di-caffeoylquinic acid and (D) tri-caffeoylquinic acid on bush tea sampes.

3.4. Conclusions and recommendations

The study revealed that bush tea plants grow best when they are exposed to full sun light, followed by white shade net and black shade net. Bush tea grown under full sun light improved plant growth and development and thus, tended to produce taller plants, as well as higher fresh and dry mass. The horticultural practices of using white (80%) shading led to greater accumulation of chlorogenic acid as compared to the plants exposed to full sun light. Therefore, a combination of white shade net and 80% light intensity may be the best microclimatic tools to enhance chlorogenic acids in bush tea production. However, we recommend further studies to investigate the response of bush tea extracts exposed to different color of the UV light.

3.5. References

Anwar, M.R., B.A. McKenzie, and G.D. Hill. 2003. The effect of irrigation and sowing date on crop yield and yield components of Kabuli chickpea (*Cicer arietinum* L.) in a cool-temperate sub humid climate. J. Agric. 141:259-271, doi: 10.1017/S0021859603003617.

Bandara, S.N. 2012. Agronomy of irrigated tea in low elevation growing areas of Sri Lanka. Univ. Adelaide School Agr, Food Wine, Doctoral Diss.

Barua, D.N. and B.N. Gogoi. 1979. Effects of shade removal. Two and a Bud. 26:40-42.

Bell, G.E., T.K. Danneberger, and M.J. McMahon, 2000. Spectral irradiance available for turfgrass growth in sun and shade. Crop sci. 40:189-195, doi: 10.2135/cropsci2000.401189x.

Challa, H. and M.J. Bakker. 1999. Potential production within the greenhouse environment. In Ecosystems in the world: Vol. 20: Chapter 15: Greenhouse Ecosystems. 20:333-348.

Clifford, M. N., S. Knight, and N. Kuhnert. 2005. Discriminating between the six isomers of dicaffeoylquinic acid by LC–MSn. J. Agric. Food Chem. 53:3821–3832, doi: 10.1021/jf050046h.

Cockshull, K. E., C.J. Graves, and C.R. Cave. 1992. The influence of shading on yield of glasshouse tomatoes. Journal of Hortic. Sci. 67:11-24, doi: 10.1080/00221589.1992.11516215.

CTIFL, Centre Technique Interprofessionel des Fruits et des Legumes. 1995. Maitrise de la conduite climatique. Paris. CTIFL, 127.

De Beer D., E. Joubert, C.J. Malherbe, and D.J. Brand. 2011. Use of counter current chromatography during isolation of 6-hydroxyluteolin-7-O-B-glucoside, a major antioxidant of *Athrixia phylicoides*. J. Chromatogr. 1218:6179–6186, doi: 10.1016/j.chroma.2010.12.096.

Eko, H., H. Wismarini, T. Hertiani, and B. Hendro Sunarminto. 2012. Effect of shading, nitrogen and magnesium fertilizer on phyllanthin and total flavonoid yield of *Phyllanthus niruri* in Indonesia soil. J. Med. Plant. Res. 6:4586-4592, doi: 10.5897/JMPR12.591.

Fouché, G., E. Khorombi, N. Kolesnikova, V.J. Maharaj, R. Nthambeleni, and M. Van der Merwe. 2006. Investigation of South African plants for anticancer properties. “Pharmacologyonline”. 3:494–500.

Gregoriou, K., K. Pontikis, and S. Vemmos. 2007. Effects of reduced irradiance on leaf morphology, photosynthetic capacity, and fruit yield in olive (*Olea europaea* L.). “Photosynthetica”. 45:172–181, doi: 10.1007/s11099-007-0029-x.

Hanlon, E.A., J.S. Gonzales, and J.M. Bartos. 1994. IFAS extension soil testing laboratory chemical procedures and training manual (Circular 812). Fla. Coop. Ext. Serv. Inst. Food and Agr. Sci., Univ. of Fla.

Haukioja, E., V. Ossipov, J. Koricheva, T. Honkanen, S. Larsson, and K. Lempa. 1998. Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization. "Chemoecology". 8:133-139, doi: 10.1007/s000490050018.

Herman, P.P.J., E. Retief, M. Koekemoer, and W.G. Welman. 2000. Seed plants of Southern Africa. O.A. Leister Editions, National Botanical Institute, Pretoria, South Africa.

Hlahla, L.N. 2010. Effect of fermentation temperature and duration on chemical composition of Bush tea (*Athrixia phylicoides* DC.) (Doctoral dissertation). Univ. of Limpopo, Turfloop Campus, Mankweng, South Africa.

Ibrahim, M.H., H.Z.E. Jaafar, A. Rahmat, and Z.A. Rahman. 2011. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of Kacip fatimah (*Labisia pumila Blume*). Int. J. Mol Sci. 12:5238–5254, doi: 10.3390/ijms12085238.

Janendra, W. A., M. Costa1, J.A. Mohotti, and M.A. Wijeratne. 2007. Ecophysiology of tea. Braz. J. Plant Physiol. 19:299–332.

Karimi, E., H.Z. Jaafar, A. Ghasemzadeh, and M.H. Ibrahim. 2013. Light intensity effects on production and antioxidant activity of flavonoids and phenolic compounds in leaves, stems and roots of three varieties of *Labisia pumila Benth*. Aust. J. Crop Sci. 7:1016.

Ku, K.M., J.N. Choi, J.K. Kim, L.G. Yoo, S.J. Lee, Y.S. Hong, and C.H. Lee. 2010. Metabolomics analysis reveals the compositional differences of shade grown tea (*Camellia sinensis* L.). J. Agric. Food Chem. 58:418-426, doi: 10.1021/jf902929h.

Kumar, R., S. Sharma, and V. Pathania. 2013. Effect of shading and plant density on growth, yield and oil composition of clary sage (*Salvia sclarea* L.) in Northwestern Himalaya. J. Essent. Oil Res. 25:23-32, doi: 10.1080/10412905.2012.742467.

Kundu, A. and J. Vadassery. 2019. Chlorogenic acid-mediated chemical defence of plants against insect herbivores. Plant Biol. 21:185–189, doi: 10.1111/plb.12947.

Lallemand, L.A., C. Zubieta, S.G. Lee, Y. Wang, S. Acajjaoui, J. Timmins, S. McSweeney, J.M. Jez, J.G. McCarthy, and A.A. McCarthy. 2012. A structural basis for the biosynthesis of the major chlorogenic acids found in coffee. Plant Physiol. 160:49–260, doi: 10.1104/pp.112.202051.

Lee, D.W., S.F. Oberbauer, B. Krishnapilay, M. Mansor, H. Mohamad, and S.K. Yap. 1997. Effects of irradiance and spectral quality on seedling development of two Southeast Asian Hopea species. Oecologi. 110:1-9.

Lehlohonolo, N., I.K. Mariga, W. Ngezimana, and F.N. Mudau. 2013. Bush tea (Athrixia phylicoides dc.) Success stories in South Africa A review. J. Crop prod. 2:37-43.

MacAlister, D., A.M. Muasya, O. Crespo, J.B.O. Ogola, S. Maseko, and A.J. Valentine. 2020. Effect of temperature on plant growth and stress tolerant traits in rooibos in the Western Cape South Africa. Sci. Hortic. 263:109137, doi: 10.1016/j.scienta.2019.109137.

Makita, C., L. Chimuka, P. Steenkamp, E. Cukrowska, and E. Madala. 2016. Comparative analyses of flavonoid content in *Moringa oleifera* and *Moringa ovalifolia* with the aid of UHPLC-qTOFMS fingerprinting. S. A. J. Bot. 105:116–122, doi: 10.1016/j.sajb.2015.12.007.

Marchese, J.A. and G.M. Figueira, 2005. Use of technologies before and after harvest and good agricultural practices in the production of medicinal and aromatic plants. Rev. Brasil. Plantas Me. 7:86-96.

Masike, K., F. Tugizimana, N. Ndlovu, E. Smit, L. du Preez, I. Dubery, and N.E. Madala. 2017. Deciphering the influence of column chemistry and mass spectrometry settings for the analyses of geometrical isomers of L-chicoric acid. J. Chromatogr. B. 1052:73-81, doi: 10.1016/j.jchromb.2017.03.023.

Masike, K., I., Dubery, P., Steenkamp, E., Smit and E. Madala. 2018. Revising reverse-phase chromatographic behavior for efficient differentiation of both positional and geometrical isomers of dicaffeoylquinic acids. J. Anal. Methods. Chem. doi: 10.1155/2018/8694579.

Mavundza, E.J., T.E. Tshikalange, N. Lall, A.A. Hussein, F.N. Mudau, and J.J.M. Meyer. 2010. Antioxidant activity and cytotoxicity effect of flavonoids isolated from *Athrixia phyllicoides*. J. Med. Plant Res. 4:2584-2587, doi: 10.5897/JMPR10.823.

Mohale, K.C., A.T. Hintsa, M.A. Emanuel, and F.N. Mudau. 2018. Metabolic Profiling of Cultivated Bush Tea (*Athrixia phylicoides* DC.) in Response to Different Pruning Types. Sci. Hortic. 53:993-998, doi: 10.21273/HORTSCI13023-18.

Mokoka, N.N. 2007. Indigenous knowledge of fever tea (*Lippia javanica*) and effect of shade netting on plant growth, oil yield and compound composition. Univ. of Pretoria, Pretoria, South Africa, Doctoral Diss.

Morita, A. and M. Tuji. 2002. Nitrate and oxalate contents of tea plants (*Camellia sinensis* L.) with special reference to types of green tea and effect of shading. J. Soil Sci. Plant Nutr. 48:547-553, doi: 10.1080/00380768.2002.10409237.

Mphangwe, N.I.K. 2012. Lung pruning: A review of practice. Tea Research Foundation of Central Africa (TRFCA) News. 18–23.

Muchow, R.C., T.R. Sinclair, and J.M. Bennett, 1990. Temperature and solar radiation effects on potential maize yields across locations. J. Agron. 82:338–342, doi: 10.2134/agronj1990.00021962008200020033x.

Mudau, F.N, H.T. Araya, E.S. Du Toit, P. Soundy, and J. Olivier. 2007. Bush tea (*Athrixia phylicoides* DC.) as an alternative herbal and medicinal plant in southern Africa: opportunity for commercialization. Med. Aromatic Plant Sci. Biotechnol., 1:74-76.

Mudau, F.N., P. Soundy, E. Du Toit, and J. Olivier. 2006. Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. S. Afr. J. Bot. 72:398-402.

Nchabeleng, L., F.N. Mudau, and I.K. Mariga. 2012. Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors. J. Med Plants. Res. 6:1662–1666, doi: 10.5897/JMPR11.1453.

Ncube, E.N., M.I. Mhlongo, L.A. Piater, P.A. Steenkamp, I.A. Dubery, and N.E. Madala. 2014. "Analyses of chlorogenic acids and related cinnamic acid derivatives from Nicotiana tabacum tissues with the aid of UPLC-QTOF-MS/MS based on the insource collision-induced dissociation method," Chem. Cent. J. 8:1–10.

Nobela, O., R.S. Renslow, D.G. Thomas, S.M. Colby, S. Sitha, P.B. Njobeh, L. du Preez, F. Tugizimana, and N.E. Madala. 2018. Efficient discrimination of natural stereoisomers of chicoric acid, an HIV-1 integrase inhibitor. J. Photochem. Photobiol. B. Biol. 189:258-266, doi: 10.1016/j.jphotobiol.2018.10.025.

Rakuambo, Z.J. 2011. Indigenous knowledge of bush tea (*Athrixia phylicoides*) and effect of fertigation frequency and growing medium on plant growth. Univ. of Pretoria. Pretoria. South Africa. Doctoral Diss.

Ramabulana, A.T., P. Steenkamp, N. Madala, and I.A. Dubery. 2020. Profiling of chlorogenic acids from *Bidens pilosa* and differentiation of closely related positional isomers with the aid of UHPLC-QTOF-MS/MS-based in-source collision-induced dissociation. Metabolites. 10:178, doi: 10.3390/metabo10050178.

Rao, L.J. and B.N. Mittra, 1998. Growth and yield of peanut as influenced by degree and duration of shading. J. Agron. Crop Sci. 160:260-265, doi: 10.1111/j.1439-037X.1988.tb00327. x.

Roleira, F.M., C.L. Varela, S.C. Costa, and E.J. Tavares-da-Silva. 2018. Phenolic derivatives from medicinal herbs and plant extracts: anticancer effects and synthetic approaches to modulate biological activity. Stud. Nat. Prod. Chem. 57:115-156, doi: 10.1016/B978-0-444-64057-4.00004-.

Soil Classification Working Group. 1991. A Taxonomic System for South Africa. Department of Agricultural Development, Pretoria, South Africa, 257.

Svenson, S.E. 2002. Business Amer. Nurseryman. 19:23-28.

Szymborska-Sandhu, I., J.L. Przybył, E. Pióro-Jabrucka, A. Jędrzejuk, Z. Węglarz, and K. Bączek. 2020. Effect of Shading on Development, Yield and Quality of Bastard Balm Herb (*Melittis melissophyllum* L.). Molecules. 25:2142, doi: 10.3390/molecules25092142.

Tadross, M. A., W.J. Gutowski, B.C. Hewitson, C.J. Jack, and M. New. 2006. MM5 simulations of interannual change and the diurnal cycle of southern African regional climate. Theor. Appl Climatol. 86:63–80.

Taofiq, O., A.M. González-Paramás, M.F. Barreiro, and I.C.F.R. Ferreira. 2017. Hydroxycinnamic Acids and Their Derivatives: Cosmeceutical Significance, Challenges and Future Perspectives, a Review. Molecules, 22:281, doi: 10.3390%2Fmolecules22020281.

Thakur, M., V. Bhatt, and R. Kumar. 2019. Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose (*Rosa damascena* Mill.) under mid hill conditions of Western Himalayas. Plos one 14:p.e0214672, doi: 10 1371. /journal.pone.0214672.

Tounekti, T., E., Joubert, I., Hernández, and S. Munné-Bosch. 2013. Improving the polyphenol content of tea. Crit. Rev. Plant Sci, 32:192–215, doi: 10.1080/07352689.2012.747384.

Tshivhandekano, I., F.N., Mudau, P. Soundy, and W. Ngezimana. 2013. Effect of cultural practices and environmental conditions on yield and quality of herbal plants: Prospects leading to research on influence of nitrogen fertilization, planting density and eco-physiological parameters on yield and quality of field-grown bush tea (*Athrixia phylicoides* DC.). J. Med. Plant Res. 7:2489-2493, doi: 10.5897/JMPR2013.4466.

Tshivhandekano, I., K. Ntushelo, W. Ngezimana, T.E. Tshikalange, F.N. Mudau. 2014. Chemical compositions and antimicrobial activities of *Athrixia phylicoides* DC. (Bush tea), *Monsonia burkeana* (special tea) and synergistic effects of both combined herbal teas. Asian. Pac. J. Trop Med. 7:S448–S453, doi: 10.1016/S1995-7645(14)60273-X.

Van Wyk, B.E., and N. Gericke. 2000. People's plants: A guide to useful plants of Southern Africa. 1st edition. Briza publications. 102.

Wang, Y., L. Gao, Y. Shan, Y. Liu, Y. Tian, and T. Xia. 2012. Influence of shade on flavonoid biosynthesis in tea (*Camellia sinensis* (L.) O. Kuntze). Sci. Hortic. 141:7-16, doi: 10.1016/j.scienta.2012.04.013.

Wanyoka, J.K. 1983. Fertilizer on tea: nitrogen – a review. Tea. 4:28–35.

Wei, S.L., W.Q. Wang, X.H. Chen, S.Y. Qin, and X.T. Chen. 2005. Studies on the shade-endurance capacity of *Glycyrrhiza uralensis*. China Journal of Chinese Materia Medica. 30:100–104. pmid:15714809.

Yilmaz, G., N. Kandemir, and K. Kinalioglu. 2004. Effects of different pruning intervals on fresh shoot yield and some quality properties of tea (*Camellia sinensis* L. Kuntze) in Turkey. Pak. J. Biol. Sci. 7:1208–1212, doi: 10.3923/pjbs.2004.1208.1212.

Zhang, Q., Y. Shi, L. Ma, X. Yi, and J. Ruan. 2014. Metabolomic analysis using ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF MS) uncovers the effects of light intensity and temperature under shading treatments on the metabolites in tea. PloS one (9):112572, doi: 10.1371/journal.pone.0112572.

CHAPTER 4: EFFECT OF UV-INDUCED GEOMETRICAL ISOMERIZATION OF HYDROXYL-CINNAMIC ACID-CONTAINING MOLECULES OF BUSH TEA (*ATHRIXIA PHYLICOIDES* DC.) USING UHPLC-QTOF-MS

Published in Scientia Horticulturae (see appendix C)

Abstract

Bush tea (*Athrixia phyllicoides* DC.) is a herbal tea which contains bioactive compounds. Naturally, these metabolites aid plants to defend themselves against a wide spectrum of biotic and abiotic stresses. The objective of the study was to identify hydroxyl-cinnamic acids affected by UV (Ultraviolet) light exposure of bush tea through ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS). A randomized complete block experimental design was used consisting of control and 80% white shade net replicated three times and samples were analyzed in triplicates. The methanolic leaf extracts of bush tea was exposed to UV light at 254 nm for 24 hours and thereafter metabolites were measured and annotated through UHPLC-QTOF-MS. Hydroxyl-cinnamic acids (HCAs) have been shown to undergo photo-isomerization during post UV light exposure, evidenced by the emergence of photo-isomers. The study discovered that hydroxyl-cinnamic acids (HCAs) derivatives undergo photo-isomerization after being exposed to UV light, as evidenced by the formation of photo-isomers. Metabolites which underwent photo isomerization were found to be structurally related as they formed a very tight molecular family when molecular networking algorithm was used.

Key words: *Athrixia phyllicoides,* hydroxyl-cinnamic acids, isomerization, ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry, molecular network.

4.1. Introduction

Bush tea is one of the Asteraceae plants which contain antioxidants (Mogotlane et al., 2007), tannins (Mudau et al., 2007; Chabeli et al., 2008), and 5-hydroxy-6,7,8,3′,4′,5′ -hexamethoxy flavon-3-ol (Mashimbye et al., 2006), 5,6,7,8,3′,4′-hexamethoxyflavone, quercetin, 3-0-demethyldigicitrin, and other polyphenols (Mudau et al., 2006, 2007; Tshivhandekano et al., 2018). Other cinnamic acid containing molecules such as chlorogenic acids (CGAs) have been noted in this plant (de Beer et al., 2011). Plants absorb light from the sun and utilize it to produce high levels of oxygen and secondary metabolites through photosynthesis (Ghasemzadeh et al., 2010). However, light is also capable to regulate plant growth and development, plant photosynthesis rate and biosynthesis of both primary and secondary metabolites (Ramakrishna and Ravishankar, 2011, Radušienė et al., 2013). Due to excessive sunlight synonymous with areas where bush tea plant grows, it is expected that some of these metabolites (especially the hydroxyl-cinnamic acid-containing molecules) will form geometrical isomers (Clifford et al., 2008), thereby representing a chemical shift of which the biological consequence is unknown. Therefore, understanding plant chemistry is essential for identification of new bioactive compounds. The chemical constituent of an organism is referred to as a metabolome (Ncube et al., 2014). Metabolome complexity is governed by several factors such as isomerization (positional and geometrical) (Clifford et al., 2005; Masike et al., 2018) and differential chemical conjugation (Masike et al., 2017; Nengovhela et al., 2021). Hydroxyl-cinnamic acids derivatives are plant secondary metabolites which are capable to form conjugates with organic acids such as quinic acid (Clifford et al., 2003, 2005; Ncube et al., 2014; Khoza et al., 2016; Masike et al., 2017), isocitric acid (Masike et al., 2017) and tartaric acid (Lee and Scagel, 2013; Khoza et al., 2016; Masike et al., 2017) resulting in chemically diverse of hydroxyl-cinnamic acid (HCA) derivatives (Masike et al., 2017). The cinnamic acids are produced/synthesized with a *trans* configuration, however, upon UV light exposure they undergo geometrical isomerization to form the unnatural *cis* isomers (Clifford et al., 2008). Geometrical isomers were prominent in plant organs such as leaves and fruits that are exposed to the sun's UV rays and may exist in equal proportion to the *trans*-isomer, depending on the environmental conditions (Masike et al., 2018). Information about the data that describe the response of hydroxyl-cinnamic acid to UV-induced geometrical isomerization on bush tea plant are lacking. However, net shading in agricultural cultivation of plants has also been shown to have adverse effects in the plants (Mudau et al., 2006), mainly by filtering the light which is essential to the physiology of the plant and its chemistry (Ghasemzadeh et al., 2010). Therefore, the aim of the study was to identify hydroxyl-cinnamic acid metabolites affected by UV-induced photo-isomerization of bush tea using UHPLC-QTOF-MS.

4.2. Materials and methods

4.2.1. Experimental site

The field trial was established in 2018 at the University of Venda's experimental farm, which is situated at Thohoyandou (22° 58.081'S, 30° 26,411'E, and 595 m above sea level), Limpopo Province, South Africa. The site has an annual rainfall of around 500 mm, most of which falls in the summer, plus an average maximum and minimum temperatures of 31°C and 18°C, respectively (Mzezewa and van Rensburg, 2011). Thohoyandou has deep well drained clay type of soil classified as Rhodic Ferralsols (Fey, 2010). The South African weather service provided weather data for the 2018 and 2019 cropping seasons (http://www.weathersa.co.za).

4.2.2. Annual and seasonal weather pattern during 2018 and 2019

In 2018 and 2019, monthly average maximum and minimum temperatures ranged from 40°C to 30°C and 20 to 10 °C, respectively (Ramphinwa et al., 2022).

4.2.3. Preparations of stem cuttings

Preparation of stem cutting was conducted using a method proposed by Ramphinwa et al., (2022). The stem cuttings of wild bush tea were collected from Tshivhulani Village (24º 50' 31º S 17E; Altitude 610 m, which is distinguished by a cold and dry winter. Bush tea planting materials of apical cuttings were cut at approximately 7-8 cm length and dipped in Seradix No.2 hormone (0.3% IBA) to encourage prolific rooting of cutting. The planting materials were chosen based on their identity and type, as well as their resistance to disease and insect damage. On 25 October 2017, stem cuttings were planted in a 25 cm round plastic pot at the University of Venda's lath house. Except on rainy days, plants were irrigated daily. On 7 December 2017, rooted cuttings were transplanted into 1 L bags and placed in a net shade for 3 months. On 12 March 2018, planting materials containing approximately 25 leaves were transplanted into three shade nets (black, green, and white) and control or full sunlight with three different light intensities (40%, 50%, and 80%) replicated three times (Ramphinwa et al., 2022) and plants were allowed to establish themselves for three months prior to data collection. The growth or rooting media was made up from a 2:1 mixture of pine bark and sand. The harvested plants were kept at a room temperature for two months to dry.

4.2.4. Experimental description

The experiment was set up in a randomized complete block design with control and 80% white shade nets replicated three times and each sample per treatments per replication were analysed in triplicates. Control and 80% white shade net treatments were selected based on the findings of Ramphinwa et al., (2022), where it was shown that only plants grown under white shade accumulated more hydroxyl-cinnamic acids than other shade nets viz, black and green. Thus, for this trial the only data obtained under white shade nets are reported herein.

4.2.5. Metabolites extraction

The metabolites from bush tea plants were extracted using a solid-liquid phase extraction method described by Makita et al., (2016). Briefly, bush tea leaves for the summer season were thrashed manually from dried plants and ground as soon as the dry mass was weighed. The leaves were ground to a fine powder with a grinder, and 2 g of fine ground bush tea leaves were combined with 20 mL (1:10 m/v) of 80% aqueous methanol. The homogenate was centrifuged at 5000 g for 20 min to remove debris and the supernatant was finally transferred to new clean tubes. The extracts were further diluted 1:1 (v:v) to final volume of 20 mL using methanol, followed by transfer of 10 mL of the diluted extracts into cylindrical quartz glass vials (2 x 10 cm). Using a syringe fitted with a 0.2 µmm filter, bush tea samples were filtered into a 2 mL vial fitted with a 0.2 mL conical bottom glass insert.

4.2.6. UV irradiation

A 2 mg/mL solution of bush tea extracts was prepared with 80% methanol and placed in a Spectroline UV lamp operating at 254 nm with a 390 µW/cm intensity. UV irradiation was conducted for 24 h, and aliquots (100 µL) were taken at 0 h (before irradiation) and at 24 h post irradiation. UV treated and untreated samples were filtered in amber vials and subjected to UHPLC-QTOF-MS.

4.2.7. Ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry analysis

UV treated and untreated bush tea samples were analysed using UHPLC-QTOF-MS, model LC-MS 9030 with a Shim Pack Velox C18 column (100 mM × 2.1 mM with particle size of 2.7 µM) (Shimadzu, Kyoto, Japan), placed in a column oven set at 40°C) . A binary solvent mixture comprising of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) at a constant flow rate of 0.4 mL/min was used. Analyte elations were monitored using a mass spectrometer detector based on 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.

4.2.8. MS/MS experiments

Tandem MS (MS/MS) experiments, typical mass accuracies with a mass error below 1 ppm was 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. Argon gas was used as a collision gas for (MS/MS) experiments along with MS^E mode using collision energy ramp of 12 eV to 25 eV for generation of fragments.

4.2.9. Molecular network

Molecular network was accomplished by a method proposed by Wang et al., (2016). The network was constructed using an online workflow in the Global Natural Products Social Molecular Networking (GNPS, https://ccms-ucsd.github.io/GNPSDocumentation/) ecosystem. To filter the data, all MS/MS fragment ions within +/- 17 Da of the precursor *m/z* were removed. MS/MS spectra were window filtered throughout the spectrum by selecting the top 6 fragment ions only in the +/- 50 Da window. The precursor ion mass tolerance was set to 2.0 Da and (MS/MS) fragment ion tolerance of 0.5 Da. By following recommended procedure, a network was built, with edges having a cosine score greater than 0.7 and more than 6 matched peaks. Furthermore, edges detected between two nodes were kept in the network if both nodes appeared in each other's top 10 most similar nodes. The maximum size of a molecular family was eventually set to 100, and the lowest scoring edges were removed from molecular families until the molecular family size was less than this threshold. The spectral libraries of GNPS were then used to search the network's spectra. The library spectra were filtered similarly to the input data. All matches between network spectra and library spectra had to have a score greater than 0.7 and at least 6 matched peaks.

4.3. Results

Bush tea methanolic extracts from both treated and untreated samples were analysed by UHPLC-QTOF-MS. The data was acquired in (ESI) negative modes. Fig. 1(a) represents a complete chromatogram for the control while Fig 1. (b) represents a complete chromatogram for UV treated samples. The clear differences in peak intensities was shown by the presence and absence of some peaks through visual inspection of the base peak intensity (BPI) chromatograms (Fig. 1). These variations point to radiation-induced metabolic changes in the irradiated bush tea samples. The chlorogenic acids are the most abundant secondary metabolites found in both treated and untreated bush tea samples. As shown in Fig. 1 (a) and (b), treated bush tea samples contained more chlorogenic acid than untreated samples.

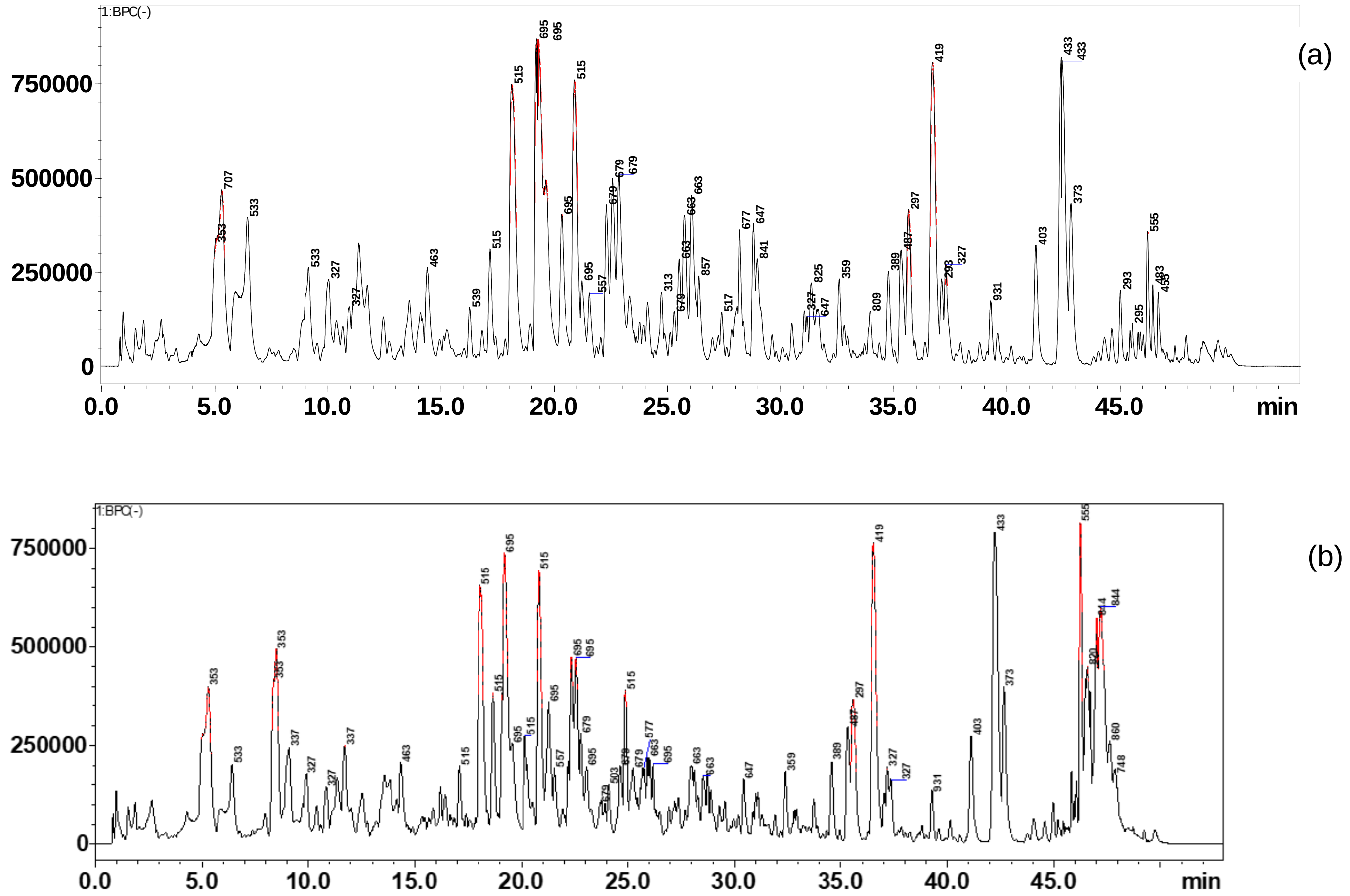


Figure 4.1. Comparison of UHPLC-QTOF-MS chromatograms of control (0 h) and UV treated (24 h) bush tea samples [(a) control and (b) UV treated bush samples using a biphenyl column].

In the current study, selected ion chromatogram of previously identified compounds in bush tea plant were generated (Fig. 2, 3 and 4) representing mono and di-acylated cinnamic acid derivatives. Interestingly, all the selected ions were shown to undergo geometrical isomerization post UV exposure regardless of the anchoring molecules where the cinnamic acid is acylated on. For instance, Fig. 2 and 3 represent mono and di-acylated cinnamic acid anchored on a quinic acid moiety.

It has been reported that different shade nets cause the formation of *cis* - isomers for HCAs on the bush tea plant (Ramphinwa et al., 2022). Previous research reported that when the bush tea plant is grown under 80% white shade net, the majority of HCAs were more pronounced than when the plant is exposed to a control or other shade nets. Therefore, in the current study, bush tea samples were exposed to UV light to assess the formation of *cis* - isomers due to UV light exposure. It is quite apparent that the formation of *cis* - isomers amplified the already complex metabolome of bush tea, due to UV exposure. The results herein clearly show that the UHPLC-QTOF-MS chromatograms of the untreated extracts (controls) were different from those generated using UV-irradiated extracts of bush tea (Fig. 2, 3 and 4). As shown in Fig. 3(b), mono-caffeoyl quinic acid levels were higher in treated bush tea samples than in untreated samples Fig. 3(a). Fig. 2(a) and (b) show that treated samples had higher levels of di-caffeoyl quinic acid than untreated samples. Di-caffeoyl glucarate acid levels were higher in treated samples Fig. 4(b) than in untreated samples Fig. 4(a). These results confirmed the fact that bush tea depends on UV light to form more of the *cis* - isomers. The separation of these molecules was also found to be efficient using a biphenyl stationary phase of a reverse phase column using a 30 min multiple gradient chromatographic method (Fig. 1, 2, 3 and 4). It can be precluded that UV irradiated bush tea samples resulted in the formation of more peaks compared to untreated control.

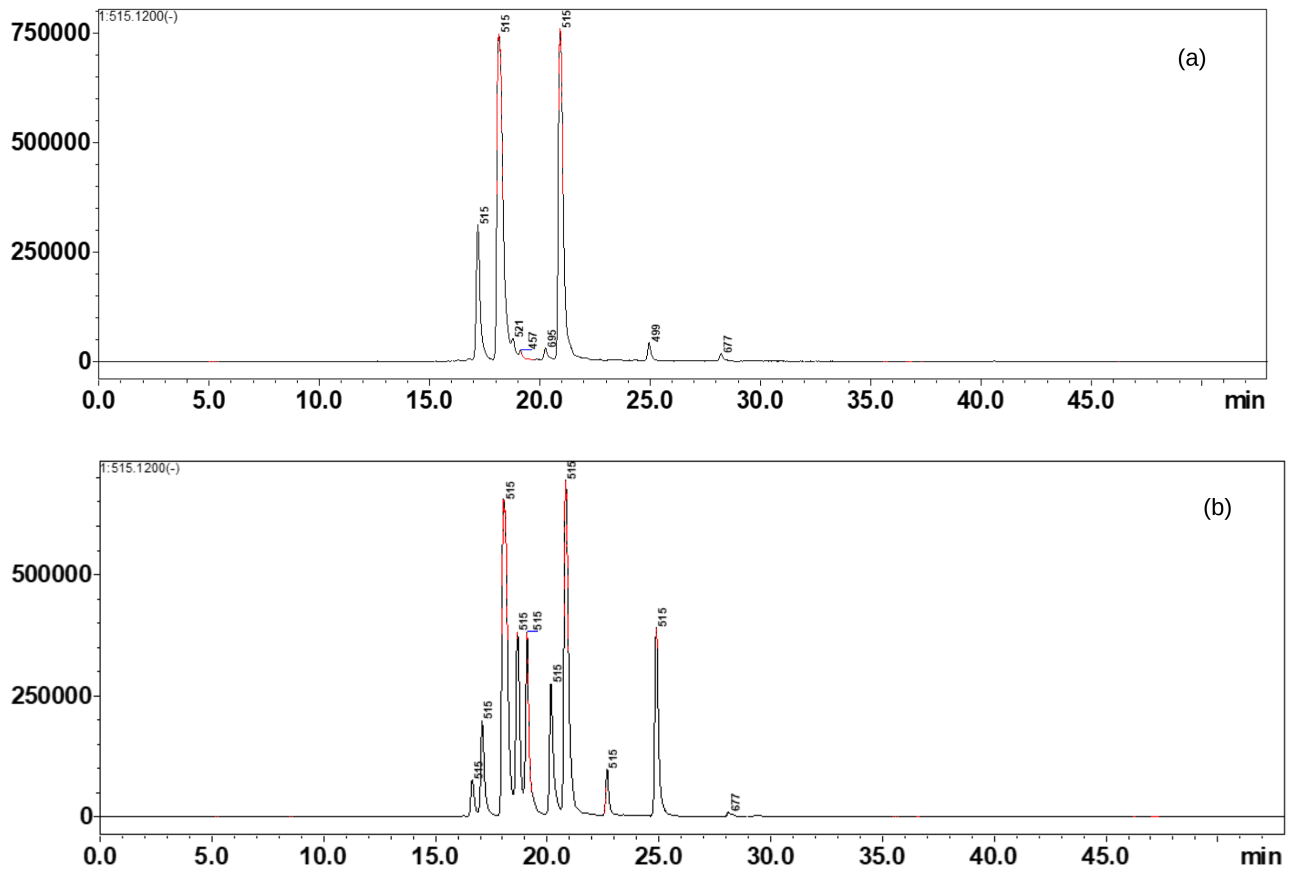


Figure 4.2. Comparison of UHPLC-QTOF-MS profile chromatograms of control (0h) and UV treated (24 h) bush tea samples at *m/z* 515 [(a) control and (b) UV treated bush tea sample using a biphenyl column].

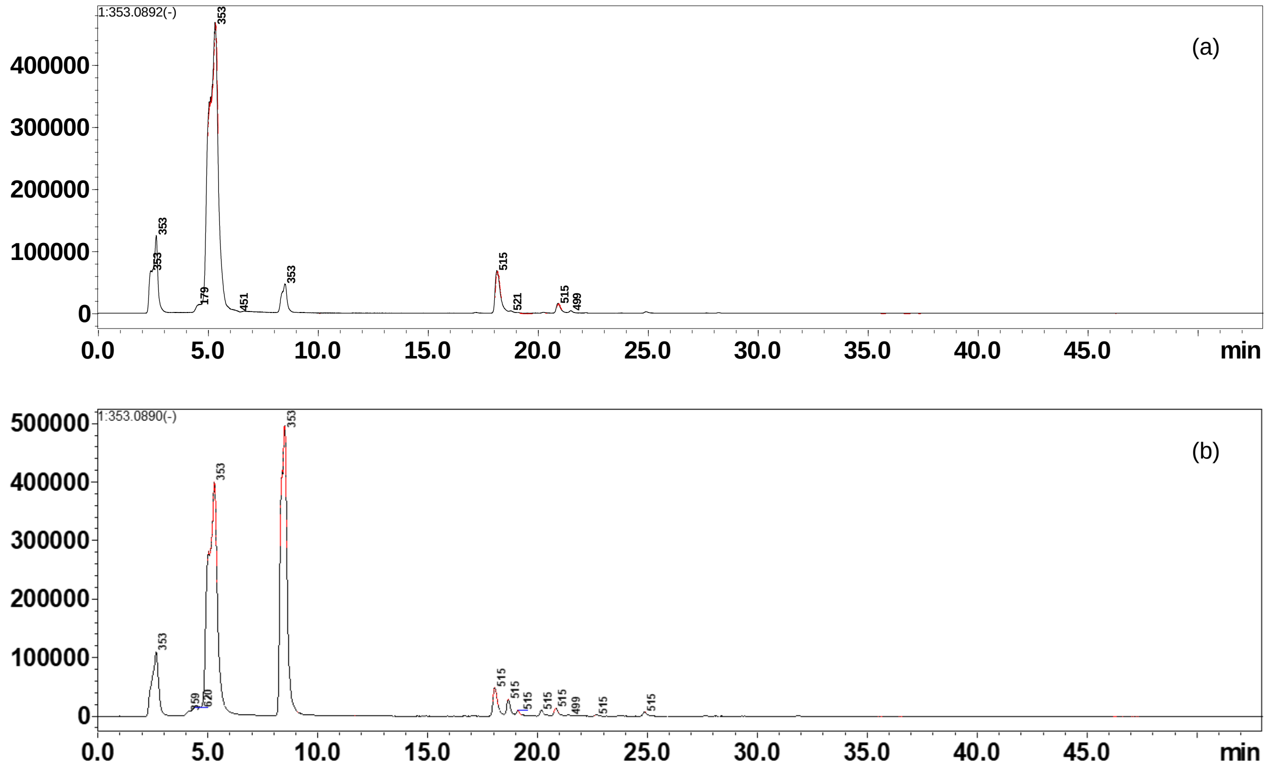


Figure 4.3. Comparison of UHPLC-QTOF-MS profile chromatograms of control (0h) and UV treated (24 h) bush tea samples at *m/z* 353 [(a) control and (b) UV treated bush tea samples using a biphenyl column].

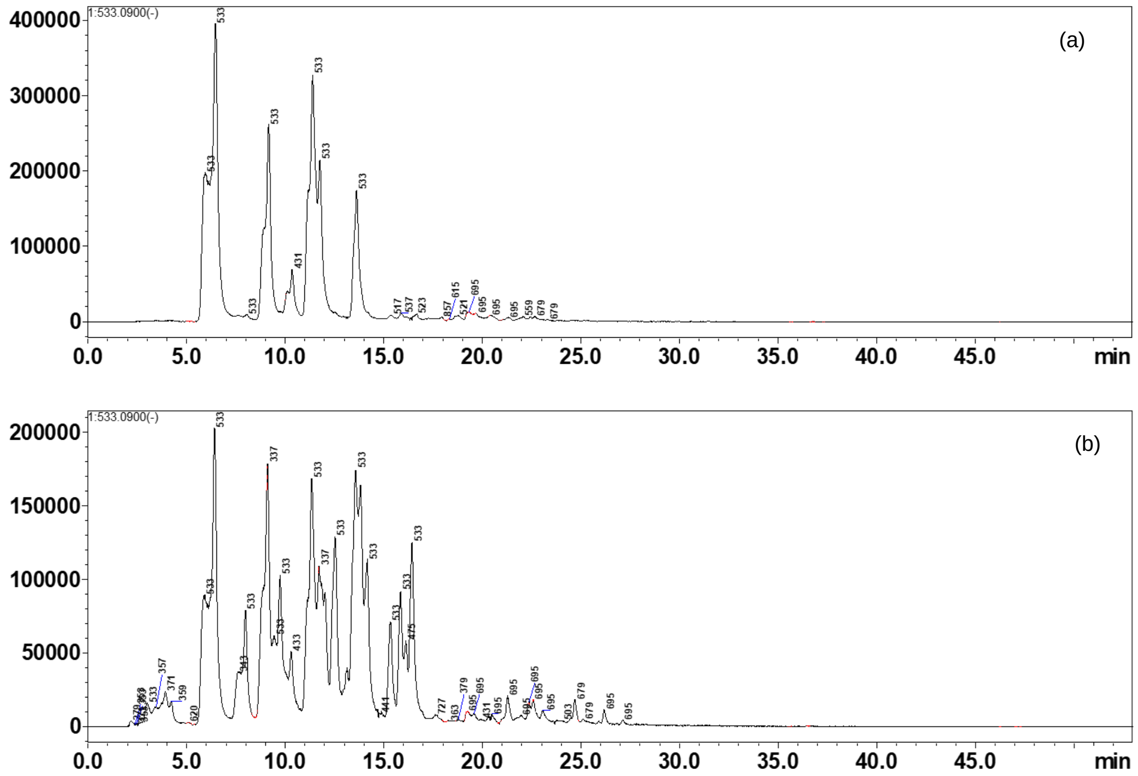


Figure 4.4. Comparison of UHPLC-QTOF-MS profile chromatograms of control (0h) and UV treated (24 h) bush tea samples at *m/z* 533 [(a) control and (b) UV treated bush tea samples using a biphenyl column].

4.3.1. Characterization of mono-caffeoylquinic acid

Chromatographic peaks with precursor ion at *m/z* 353 corresponding to a molecular formula of $C_{16}H_{18}O_9$ were identified as isomers of mono-acyl chlorogenic acid (Fig. 3, Table 1). Here, the product ion at *m/z* 191 was assigned to a deprotonated quinic acid after removal of the caffeic acid moiety through fragmentation (Ncube et al., 2014). The product ion at *m/z* 173 was synonymous with all metabolites with acylation at 4-OH of the quinic acid. Taken altogether with the already published hierarchical key schemes of CGA identification (Clifford et al., 2003), these metabolites were putatively identified as positional and geometrical isomers of caffeoyl-quinic acid (Table 1).

4.3.2. Characterization di-caffeoyl quinic acid

Chromatographic peaks with a precursor ion at *m/z* 515 and molecular formula of $C_{25}H_{24}O_{12}$ were detected (Fig. 2, Table 1) and identified as isomers of di-caffeoyl quinic acid. These peaks were identified based on the ratios between various products ions such as those appearing at *m/z* 135, 179, 191, 335, 353 (Ncube et al., 2014; Makola et al., 2016a).

4.3.3. Characterization of di-caffeoyl glucarate acid

Herein, the presence of di-caffeoyl glucarate acid was reported for the first time in bush tea plants (Fig. 4, Table 1). These compounds showed a consistent fragment appearing at *m/z* 209 (Table 1), indicating the presence of glucaric acid (Lorenz et al., 2012; Ruiz et al., 2013). Other product ions such as those appearing at 179 and 135, representing deprotonated caffeic acid and decarboxylated caffeic acid have been noted and taken altogether, this points towards acylation of glucaric acid by two caffeic acids resulting in multiple positional isomers. Interestingly, seven visually countable peaks corresponding to a molecular formula $C_{24}H_{22}O_{14}$

with precursor ion $[M-H]^-$ at *m/z* 533 were detected in untreated control samples whereas 15 visually countable peaks with the same molecular formula and precursor ion were detected on the UV treated samples**.** The current study is the first to show that geometrical isomers of these molecules exist on the bush tea plant.

Table 4.1. Characterization of chlorogenic acids (CGAs) of bush tea plants exposed to control and UV light

No	*m/z*	Rt (mn)	Molecular Formula	Molecular Formula	Fragmentation	Control	UV
1	353	2.455	$C_{16}H_{18}O_9$	3-O-Caffeoyl quinic acid	353.08,191.05, 179.,03, 135.04	✓	✓
2	353	2.699	$C_{16}H_{18}O_9$	4-O-Caffeoyl quinic acid	353.08,191.05, 161.0564	✓	✓
3	353	5.420	$C_{16}H_{18}O_9$	5-O-Caffeoyl quinic acid	353.08, 191.05, 179.03	✓	✓
4	353	8.475	$C_{16}H_{18}O_9$	Cis- 5-O-Caffeoyl quinic acid	353,08,191.05, 179,03	✓	
5	515	17.327	$C_{25}H_{24}O_{12}$	3,4-Di-caffeoyl quinic acid	353.08,173.04, 191.05, 135.04	✓	✓
6	515	16.630	$C_{25}H_{24}O_{12}$	3,4-Di-caffeoyl quinic acid	353.09, 173.04, 191.05, 135.04		✓
7	515	17.094	$C_{25}H_{24}O_{12}$	Cis 3.4-Di-caffeoyl quinic acid	353.08,173.04,191.05,135.04		✓
8	515	18.343	$C_{25}H_{24}O_{12}$	3.5-Di-caffeoyl quinic acid	353,08,191.05, 179.03, 135.04	✓	
9	515	18.160	$C_{25}H_{24}O_{12}$	3.5-Di-caffeoyl quinic acid	353.08, 191.05		✓
10	515	18.762	$C_{25}H_{24}O_{12}$	Cis-3.5-Di-caffeoyl quinic acid	353.05,191.01,179.03		✓
11	515	19,177	$C_{25}H_{24}O_{12}$	Cis-3.5-Di-caffeoyl quiic acid	353.08,191.05,179.03,135.04		✓
12	515	20.940	$C_{25}H_{24}O_{12}$	4,5-Di-caffeoyl quinic acid	353,08,173,04,135.04 191.05	✓	
13	515	20,336	$C_{25}H_{24}O_{12}$	4,5-Di-caffeoyl quinic acid	353.04, 135.04, 191.05		✓
14	515	20.939	$C_{25}H_{24}O_{12}$	Cis 4,5-Di-caffeoyl-quinic acid	353.08,173.04,191.05,135.04		✓
15	515	22.652	$C_{25}H_{24}O_{12}$	Cis 4,5-Di-caffeoyl quinic acid	353.08,173.04,191.05,135.04		✓
16	515	24.921	$C_{25}H_{24}O_{12}$	Cis 4,5-Di-caffeoyl quinic acid	353.08,173.04,191.05,135.04		✓
17	533	2.995	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (I)	371.06, 209.02, 191.01, 179.03	✓	
18	533	3.635	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (II)	371.06, 209.02,191.01, 179.03	✓	
19	533	4.105	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (III)	371.67, 209.02, 191.01, 173,00	✓	
20	533	5,135	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (IV)	371.05, 209.02, 191.01, 173.00	✓	

21	533	6.220	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (V)	371.05, 209.02, 191.01, 173.00	✓	
22	533	6.525	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (VI)	371.05, 209.02, 191.01, 173.00	✓	
23	533	6.880	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (VII)	371.05, 209.02, 191.01, 173.00	✓	
24	533	2.975	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (VIII)	371.06, 209.02,191.01, 179.03		✓
25	533	3.630	$C_{24}H_{22}O_{14}$	Di caffeoyl glucarate (IX)	371.06, 209.02,191.01, 179.03		✓
26	533	3.690	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (X)	371.06, 209.02,191.01, 179.03		✓
27	533	4.870	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XI)	371.06, 209.02,191.01, 179.03		✓
28	533	4.935	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XII)	371.06, 209.02,191.01, 179.03		✓
29	533	4.985	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XIII)	371.05, 209.02, 191.01, 173.00		✓
30	533	6.175	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XIV)	371.06, 209.02,191.01, 179.03		✓
31	533	6.195	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XV)	371.05, 209.02, 191.01, 173.00		✓
32	533	6.205	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XVI)	371.05, 209.02, 191.01, 173.00		✓
33	533	6.500	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XVII)	371.05, 209.02, 191.01, 173.00		✓
34	533	6.525	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XVIII)	371.06, 209.02,191.01, 179.03		✓
35	533	6.545	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XIX)	371.05, 209.02, 191.01, 173.00		✓
36	533	6.850	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XX)	371.06, 209.02,191.01, 179.03		✓
37	533	6.875	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XXI)	371.05, 209.02, 191.01, 173.00		✓
38	533	6.900	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (XXII)	371.06, 209.02,191.01, 179.03		✓

4.3.4. Molecular networking

The molecular network (MN) is a computational approach tool which classifies metabolites based on their structural similarities, thus molecules with similar structural moieties are grouped together to form a molecular family (Othibeng et al., 2021). During MN, tandem mass spectrometry data comprising of both unfragmented and collision induced dissociation (CID) data is used to create a network of metabolites, whereby metabolites with similar fragmentation patterns (especially due to common structural features) are connected to each other to form a molecular family. The results of the MN generated herein, support that, regardless of the anchoring molecules (either quinic acid or glucaric acid, in this case), almost all hydroxyl-cinnamic acid containing molecules are grouped together in a molecular family (Fig. 5), which further supports the identification made herein where the ions at *m/z* 533 were identified as di-caffeoyl glucarate (Table 1).

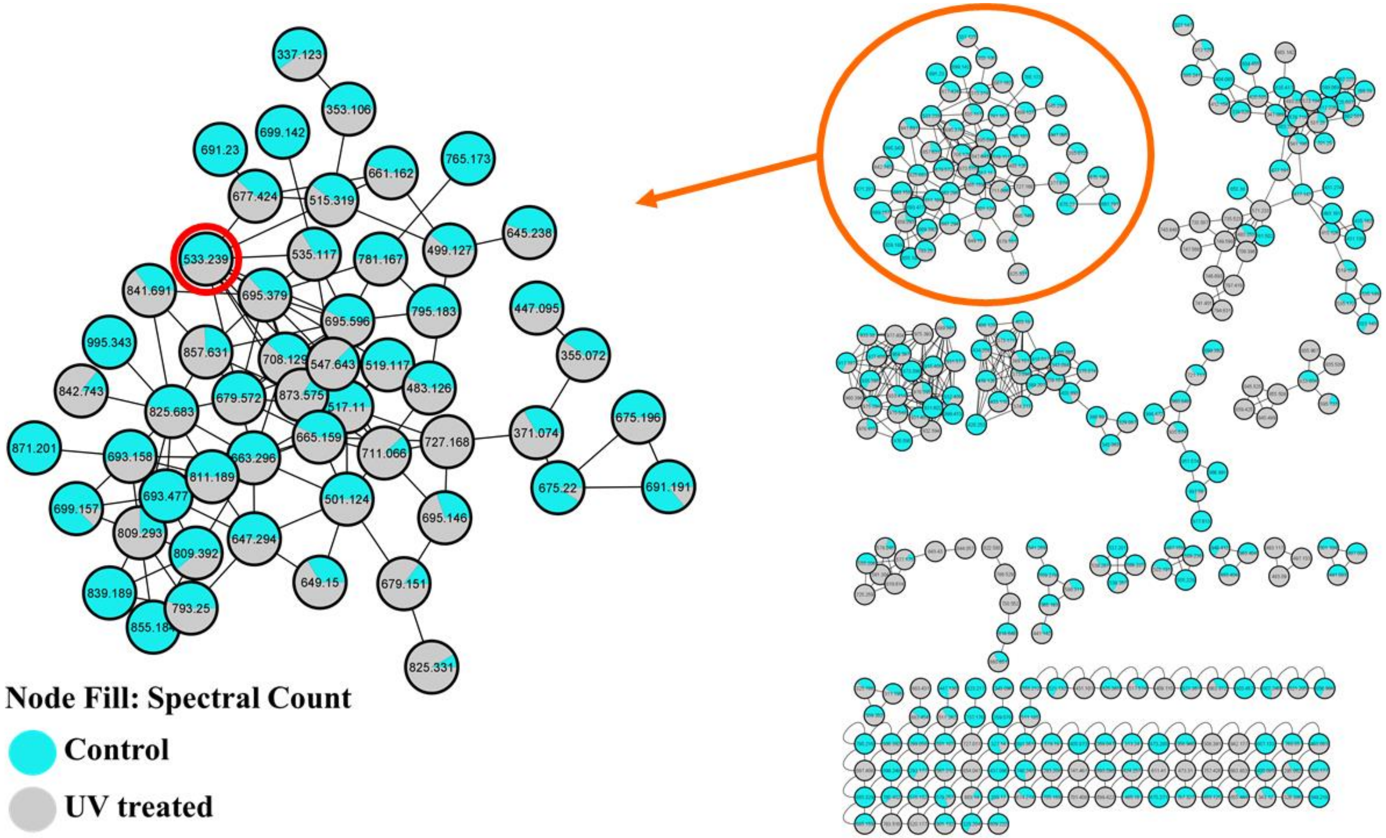


Figure 4.5. Molecular network of treated (UV) and untreated (control) bush tea extracts analysed by UHPLC-QTOF-MS with a molecular family (left) indicating grouped hydroxyl-cinnamic acid derivatives based on shared gas phase structural similarities. Highlighted in red (*m/z* 533.239), di-caffeoyl glucarate is shown to share fragmentation relatedness to other hydroxy-cinnamic acid derivatives

4.4. Discussion

The hypothesis of the current study was that accumulation, distribution and partitioning of hydroxyl-cinnamic acid containing molecules in bush tea might increase through UV-induced geometrical isomerisation. Therefore, in this study we aimed to investigate response of hydroxyl-cinnamic acid to UV light on bush tea plant through UHPLC-QTOF-MS. Acylation of hydroxyl-cinnamic acids to various organic acid result in diverse chemical space characterised by highly isomerizing molecules such as mono-caffeoyl quinic acid, di-caffeoyl quinic acid and di-caffeoyl glucarate and herein all these molecules have been detected in large quantity in bush tea samples which were exposed to UV light compared to non-exposed samples (Fig. 2, 3, and 4). Similar results were reported by Masike et al., (2017) and Nobela et al., (2018) who reported that UV light increases the content of secondary metabolites containing cinnamic acid. Taken altogether, the results of the current study together with those published elsewhere (Masike et al., 2017; Nobela et al., 2018) show that geometrical isomerization of metabolites is a plausible, non-enzymatic approach used by plants to maximize the metabolite composition and can serve multiple purposes as discussed below. For instance, geometrical isomers in *Moringa oleifera* Lam. were found to confer the plant an enhanced defensive capacity when exposed to highly oxidative stress caused by gamma radiation (Ramabulana et al., 2016). This phenomenon was found to contribute to a state of readiness which gives a plant an added advantage when faced by an eminent attack (various stressors). Geometrical isomerization has also been shown to have nutraceutical effects, for instance Makola et al., (2016b) showed that all four geometrical isomers of 3,5 di-caffeoylquinic acid binds to HIV-integrase enzymes through complementing binding strategies, where all these isomers bind through contrasting poses, thereby occupying a wider space of the active site . It is therefore expected that plants with extra geometrical isomers will exhibit enhanced nutraceutical attributes.

The new compounds are formed because of light-induced geometrical isomerization of hydroxyl-cinnamic acid containing molecules, a phenomenon which happen naturally and can also be augmented *in vitro* (Clifford et al., 2008; Mhlongo et al., 2016). Plants normally produce cinnamic acid with *trans* configuration which ultimately changes to *cis* configuration upon UV exposure (Makola et al., 2016a). The most notable characteristics of this isomerization process is that the newly formed compounds elute at different retention times on a chromatographic space (Clifford et al., 2008), an indication of an overwhelming polarity switching associated with geometrical isomerization. Through LC-MS technique, it is highly impossible to accurately identify each isomer with high degree of confidence, mainly because geometrical isomers produce similar MS signals (Masike et al., 2017). Even though, chromatographic elusion order is a premise of distinguishing between these closely related molecules, the elution order between the *trans* and *cis* isomers is normally used as an orthogonal characteristic for identification, more especially in combination with mass spectrometry-based fragmentation patterns (Masike et al., 2017). The findings of the current study resulted in more questions to be attended to in the future by dedicated studies such as those which uses sophisticated technologies such as ion mobility mass spectrometry (Zheng et al., 2017), to accurately distinguish isomers, especially those formed by conjugation with glucaric acid because they haven't been characterized as yet.

The changes in polarity have been explained through computational modelling of the presumed structures (Clifford et al., 2008) and as recently noted with another cinnamic acid containing molecule namely clovamide, the changes in chromatographic elution post UV-induced isomerization could be due to formation of additional intra-molecular forces such as hydrogen bonding which can ultimately affect the polarity thereof (Madala and Kabanda, 2021). The isomerization and polarity switching observed herein, is important because this could affect the bioavailability of the isomerized metabolites, especially when it is known that bioavailability is affected by polarity (Gurley, 2011). Bioavailability of plant-derived compounds is not easily predictable because many phytochemicals are highly lipophilic, and this is mainly associated with hydrophobic phytochemicals which the extraction is mainly achieved through organic solvents. However, this problem is also seen with highly water-soluble phytochemicals, which are easily extracted through water-based extractions, but exhibit poor oral bioavailability (Gurley, 2011). To circumvent these problems associated with bioavailability, several methods which include the use of drug delivery machineries has been implemented (Ajazuddin and Saraf, 2010; Musthaba et al., 2011). It is therefore imperative that other alternative means of increasing bioavailability of phytochemicals are needed and as presented herein mimicking a natural phenomenon associated with UV-induced geometrical isomerization has shown some promising results. The advantage of this approach is that it does not alter the natural molecular weight of the natural molecule which may affect the absorption across lipophilic cell membranes via simple passive diffusion processes (Gurley, 2011).

The variation in biomass yield and metabolite content of cultivated plants in agricultural production setting is influenced by shading (Morita and Tuji, 2002; Hajiboland et al., 2011). However, very little attention is given to the actual identity of the metabolites which the concentration is perturbed by the shading. Therefore, the results of the current study set a new perspective where it has been adequately demonstrated that 80% white shade net in bush tea resulted in accumulation of more HCAs than other shade nets (Ramphinwa et al., 2022), and this is due to isomerization of the natural contingency of these metabolites as presented herein. This result might also be influenced by the amount of UV light that penetrates the white shade net in comparison to other shade net colors. As indicated above, the metabolite content is determined by varying the concentration rather than the type of metabolite, a phenomenon which is being challenged by the findings of the current study which highlight the important of intraclass variation amongst the metabolites. To further, explain and substantiate the importance of this intraclass variation (isomerization) due to the presence of hydroxyl-cinnamic acid moiety in this context, molecular networking approach which classify metabolites based on the fragmentation patterns (Ramabulana et al., 2021) was used. The results of the molecular network allowed for info graphical display of all metabolites which the levels have been affected by UV-light treatment. Molecular network also enabled the identification of new metabolites of which the levels were perturbed by UV light and their association with other known hydroxyl-cinnamic acid containing molecules through formation of molecular family has enable their annotation. For instance, the identification of the ions with precursor ions at *m/z* 533 was based on accurate mass and through a novel metabolite dereplication approach which is based on molecular network algorithm, and this allowed for identification of these molecules in this plant for the first time. It is quite evidence that combining technology and computational methods is becoming attractive towards establishing smart agricultural practices.

4.5. Conclusion

The results of the current study show that bush tea contains hydroxyl-cinnamic acid that undergo photo-isomerization by the formation of *cis* geometrical isomers due to UV-light exposure. The newly formed metabolites underwent polarity switching, a phenomenon which might influence the bioavailability of these metabolites. Light has adverse effects on the chemistry of plants which results in the formation of newly formed metabolites which are not naturally part of the plant. These hydroxyl-cinnamic acid were also found to be structurally related as they formed a very tight molecular family when molecular networking algorithm was used. The formation of isomers was also seen to be accompanied by changes in polarity of the affected metabolites, a phenomenon which could have adverse effects on the bioavailability of these metabolites. Therefore, the current study presents UV-geometrical isomerization through agronomic practices viz., net shading and artificial light exposure as a feasible approach to enhance the metabolite composition of plants which could have, yet to be investigated, nutraceutical effects. The use of data inclined technologies as demonstrated herein to assess the effects of agricultural practices on the plant chemistry is a commendable approach towards smart agricultural practices. The prospects of the study are to investigate molecular network of bush tea under different types of shade nets and to evaluate which metabolites are affected by which type of light intensities.

4.6. Declaration of competing interest

None

4.7. Author contribution

MLR, and ATR contributed on the designing of the experiment, data acquisition, analysis and data interpretation and drafting of article. GRAM, ENM and FNM contributed on supervision, methodology, revision, editing and approval of submission of article.

4.8. Acknowledgements

This study was funded by the University of South Africa, University of Venda and National Research Foundation (Thuthuka grant – UID 118069).

4.9. References

Chabeli, P.M., F.N. Mudau, P.W. Mashela, and P. Soundy. 2008. Effects of nitrogen, phosphorus and potassium nutrition on seasonal tannin content of bush tea (*Athrixia phyliciode*s dc.). S. Afr. J. Plant Soil. 25:79–83, doi: 10.1080/02571862.2008.10639899.

Clifford, M.N., K.L. Johnston, S. Knight, and N. Kuhnert. 2003. Hierarchical scheme for LC-MSn identification of chlorogenic acids. J. Agric. Food Chem. 51:2900–2911, doi: 10.1021/jf026187q.

Clifford, M.N., J. Kirkpatrick, N. Kuhnert, H. Roozendaal, and P.R. Salgado. 2008. LC-MSn analysis of the cis isomers of chlorogenic acids. Food Chem. 106:379–385, doi: 10.1016/j.foodchem.2007.05.081.

Clifford, M.N., S. Knight, and N. Kuhnert. 2005. Discriminating between the six isomers of dicaffeoylquinic acid by LC-MSn. J. Agric. Food Chem. 53:3821–3832, doi: 10.1021/jf050046h.

de Beer, D., E. Joubert, C.J. Malherbe, and D. Jacobus Brand. 2011. Use of countercurrent chromatography during isolation of 6-hydroxyluteolin-7-o-β-glucoside, a major antioxidant of *Athrixia phylicoides*. J. Chromatogr. A. 1218:6179–6186, doi: 10.1016/j.chroma.2010.12.096.

Fey, M.V. 2010. Soils of South Africa. Cape Town: Cambridge University Press. 287.

Ghasemzadeh, A., H.Z.E. Jaafar, A. Rahmat, P.E.M. Wahab, and M.R.A. Halim. 2010. Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale Roscoe*). Int. J. Mol. Sci. 11:3885–3897, doi: 10.3390/ijms11103885.

Gurley, B.J. 2011. Emerging Technologies for Improving Phytochemical Bioavailability:

Benefits and Risks. Clin. Pharmacol. Ther. 89:915–919, doi: 10.1038/clpt.2011.51.

Hajiboland, R., S. Bastani, and S.B. Rad. 2011. Effect of light intensity on photosynthesis and antioxidant defense in boron deficient tea plants. Acta Biol. Szeged. 55:265–272.

Khoza, B.S., S. Gbashi, P.A. Steenkamp, P.B. Njobeh, and N.E. Madala. 2016. Identification of hydroxylcinnamoyl tartaric acid esters in *Bidens pilosa* by UPLC-tandem mass spectrometry. South African J. Bot. 103:95–100, doi: 10.1016/j.sajb.2015.08.018.

Lee, J. and C.F. Scagel. 2013. Chicoric acid: Chemistry, distribution, and production. Front. Chem. 1:1–17, doi: 10.3389/fchem.2013.00040.

Lorenz, P., J. Conrad, J. Bertrams, M. Berger, S. Duckstein, U. Meyer, and F.C. Stintzing. 2012. Investigations into the Phenolic Constituents of Dog's Mercury (*Mercurialis perennis* L.) by LC-MS/MS and GC-MS analyses. Phytochem Anal. 23:60–71, doi: 10.1002/pca.1325.

Madala, N.E. and M.M. Kabanda. 2021. LC-MS based validation and DFT investigation on the antioxidant properties of clovamide: •OH and •OOH scavenging and Cu(II) chelation mechanisms. J. Mol. Struct. 1236:130349, doi: 10.1016/j.molstruc.2021.130349.

Makita, C., L. Chimuka, P. Steenkamp, E. Cukrowska, and E. Madala. 2016. Comparative analyses of flavonoid content in *Moringa oleifera* and *Moringa ovalifolia* with the aid of UHPLC-qTOF-MS fingerprinting. South African J. Bot. 105:116–122, doi: 10.1016/j.sajb.2015.12.007.

Makola, M.M., P.A. Steenkamp, I.A. Dubery, M.M. Kabanda, and N.E. Madala. 2016a. Preferential alkali metal adduct formation by cis geometrical isomers of dicaffeoylquinic acids allows for efficient discrimination from their trans isomers during ultra-high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. Rapid

Commun. Mass Spectrom. 30, 1011–1018, doi: 10.1002/rcm.7526.

Makola, M.M., I.A. Dubery, G. Koorsen, P.A. Steenkamp, M.M. Kabanda, L.L. Du Preez, and N.E. Madala. 2016b. The Effect of Geometrical Isomerism of 3,5-Dicaffeoylquinic Acid on Its Binding Affinity to HIV-Integrase Enzyme: A Molecular Docking Study. Evidence-based Complement. Altern. Med. 2016:1–10, doi: 10.1155/2016/4138263.

Mashimbye, M.J., F.N. Mudau, P. Soundy, and T. Van Ree. 2006. A new flavonol from *Athrixia phylicoides* (Bush Tea). South African J. Chem. 59:1–2.

Masike, K., I, Dubery, P. Steenkamp, E. Smit, and E. Madala. 2018. Revising Reverse-Phase Chromatographic Behavior for Efficient Differentiation of Both Positional and Geometrical Isomers of Dicaffeoylquinic Acids. J. Anal. Methods Chem. 2018, doi: 10.1155/2018/8694579.

Masike, K., F. Tugizimana, N. Ndlovu, E. Smit, L. du Preez, I. Dubery, and E. Madala. 2017. Deciphering the influence of column chemistry and mass spectrometry settings for the analyses of geometrical isomers of L-chicoric acid. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1052:73–81, doi: 10.1016/j.jchromb.2017.03.023.

Mhlongo, M.I., L.A. Piater, N.E. Madala, P.A. Steenkamp, and I.A. Dubery. 2016. Phenylpropanoid defences in Nicotiana tabacum cells: Overlapping metabolomes indicate common aspects to priming responses induced by lipopolysaccharides, chitosan and flagellin-22. PLoS One. 11:1–21, doi: 10.1371/journal.pone.0151350.

Mogotlane Daniel, I., F.N. Mudau, P.W. Mashela, and P. Soundy. 2007. Seasonal Responses of Total Antioxidant Contents in Cultivated Bush Tea (*Athrixia phylicoides* L .) Leaves to Fertilizer Rates. Med. Aromat. Plant Sci. Biotechnol. 1:77–79.

Morita, A. and M. Tuji. 2002. Nitrate and oxalate contents of tea plants (*Camellia sinensis* L.)

with special reference to types of green tea and effect of shading. Soil Sci. Plant Nutr. 48:547–553, doi: 10.1080/00380768.2002.10409237.

Mudau, F.N., A. Ngele, P.W. Mashela, and P. Soundy. 2007. Seasonal Variation of Tannin Content in Wild Bush Tea.1:2–4.

Mudau, F.N., P. Soundy, E.S. du Toit, and J. Olivier. 2006. Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. South African J. Bot. 72:398–402, doi: 10.1016/j.sajb.2005.10.007.

Musthaba, S.M., S. Baboota, S. Ahmed, A. Ahuja, and J. Ali. 2011. Musthaba et al. - 2009 - Status of novel drug delivery technology for phytotherapeutics. 6:625–638.

Mzezewa, J. and L.D. van Rensburg. 2011. Effects of tillage on runoff from a bare clayey soil on a semi-arid ecotope in the limpopo province of South Africa. Water SA. 37:165–172, doi: 10.4314/wsa.v37i2.65862.

Ncube, E.N., M.I. Mhlongo, L.A. Piater, P.A. Steenkamp, I.A. Dubery, and N.E. Madala. 2014. Analyses of chlorogenic acids and related cinnamic acid derivatives from *Nicotiana tabacum* tissues with the aid of UPLC-QTOF-MS/MS based on the in-source collision-induced dissociation method. Chem. Cent. J. 8:1–10, doi: 10.1186/s13065-014-0066-z.

Nengovhela, N., P.A. Steenkamp, and N.E. Madala. 2021. LC-MS Based Metabolite Fingerprinting of Coccinia Plants Reveals Glycoisomerization as a Structual Diversification Strategy in Flavanoid Chemical Space. Natl. Acad. Sci. Lett. 44:209–213, doi: 10.1007/s40009-020-00990-4.

Nobela, O., R.S. Renslow, D.G. Thomas, S.M. Colby, S. Sitha, P.B. Njobeh, L. du Preez, L., F. Tugizimana, and N.E. Madala. 2018. Efficient discrimination of natural stereoisomers of chicoric acid, an HIV-1 integrase inhibitor. J. Photochem. Photobiol. B Biol. 189:258–

266, doi: 10.1016/j.jphotobiol.2018.10.025.

Othibeng, K., L. Nephali, A.T. Ramabulana, P. Steenkamp, D. Petras, K. Kang, H. Bin, Opperman, J. Huyser, and F. Tugizimana. 2021. A metabolic choreography of maize plants treated with a humic substance-based biostimulant under normal and starved conditions. Metabolites 11, doi: 10.3390/metabo11060403.

Radušienė, J., B. Karpavičienė, and Ž. Stanius. 2013. Effect of External and Internal Factors on Secondary Metabolites Accumulation in St. John's Worth. Bot. Lith. 18:101–108, doi: 10.2478/v10279-012-0012-8.

Ramabulana, A.T., D. Petras, N.E. Madala, and F. Tugizimana. 2021. Metabolomics and molecular networking to characterize the chemical space of four momordica plant species. Metabolites. 11:11–14, doi: 10.3390/metabo11110763.

Ramabulana, T., R.D. Mavunda, P.A. Steenkamp, L.A. Piater, I.A. Dubery, and N.E. Madala. 2016. Perturbation of pharmacologically relevant polyphenolic compounds in *Moringa oleifera* against photo-oxidative damages imposed by gamma radiation. J. Photochem. Photobiol. B Biol. 156:79–86, doi: 10.1016/j.jphotobiol.2016.01.013.

Ramakrishna, A. and G.A. Ravishankar. 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6:1720–1731, doi: 10.4161/psb.6.11.17613.

Ramphinwa, M.L., G.R.A. Mchau, N.E. Madala, N. Nengovhela, J.B.O. Ogola, and F.N. Mudau. , 2022. Response of Plant Growth and Development, and Accumulation of Hydroxyl-cinnamoyl Acid Derivatives to Selected Shade Nets and Seasonality of Field-grown Bush Tea (*Athrixia phylicoides* DC). HortScience. 57:87–96, doi: 10.21273/HORTSCI16171-21.

Ruiz, A., C. Mardones, C. Vergara,, I. Hermosín-Guti´errez,, D. von Baer, P. Hinrichsen, R.

Rodriguez, D. Arribillaga, and E. Dominguez. 2013. Analysis of hydroxycinnamic acids derivatives in calafate (*Berberis microphylla* G. Forst) berries by liquid chromatography with photodiode array and mass spectrometry detection. J. Chrom. 1281:38–45, doi: 10.1016/j.chroma.2013.01.059.

Saraf, S. 2010. Applications of novel drug delivery system for herbal formulations. Fitoterapia. 81:680–689, doi: 10.1016/j.fitote.2010.05.001.

Tshivhandekano, I., W. Ngezimana, T.E. Tshikalange, N.P. Makunga, and F.N. Mudau. 2018. Nitrogen application influences quality, pharmacological activities and metabolite profiles of *Athrixia phylicoides* DC. (Bush tea) cultivated under greenhouse and field conditions. Acta Agric. Scand. Sect. B Soil Plant Sci. 68:388–400, doi: 10.1080/09064710.2017.1412496.

Wang, M., J.J. Carver, V.V. Phelan, L.M. Sanchez, N. Garg, Y. Peng, D.D. Nguyen, J. Watrous, C.A. Kapono, T. Luzzatto-Knaan, C. Porto, A. Bouslimani, A.V. Melnik, M.J. Meehan, W.T. Liu, M. Crüsemann, P.D. Boudreau, E. Esquenazi, M. Sandoval-Calderón, R.D. Kersten, L.A. Pace, R.A. Quinn, K.R. Duncan, C.C. Hsu, D.J. Floros, R.G. Gavilan, K. Kleigrewe, T. Northen, R.J. Dutton, D. Parrot, E.E. Carlson, B. Aigle, C.F. Michelsen, C. Jelsbak, P. Sohlenkamp, A. Pevzner, J. Edlund, J. McLean, B.T. Piel, L. Murphy, L. Gerwick, C.C. Liaw, Y.L. Yang, H.U. Humpf, M. Maansson, R.A. Keyzers, A.C. Sims, A.R. Johnson, A.M. Sidebottom, B.E. Sedio, A. Klitgaard, C.B. Larson, C.A.P. Boya, D. Torres-Mendoza, D.J. Gonzalez, D.B. Silva, L.M. Marques, D.P. Demarque, E. Pociute, E.C. O'Neill, E. Briand, E.J.N. Helfrich, E.A. Granatosky, E. Glukhov, F. Ryffel, H. Houson, H. Mohimani, J.J. Kharbush, Y. Zeng, J.A. Vorholt, K.L. Kurita, P. Charusanti, K.L. McPhail, K.F. Nielsen, L. Vuong, M. Elfeki, M.F. Traxler, N. Engene, N. Koyama, O.B. Vining, R. Baric, R.R. Silva, S.J. Mascuch, S. Tomasi, S. Jenkins, V. Macherla, T.

Hoffman, V. Agarwal, P.G. Williams, J. Dai, R. Neupane, J. Gurr, A.M.C. Rodríguez, A. Lamsa, C. Zhang, K. Dorrestein, B.M. Duggan, J. Almaliti, P.M. Allard, P. Phapale, L.F. Nothias, T. Alexandrov, M. Litaudon, J.L. Wolfender, J.E. Kyle, T.O. Metz, T. Peryea, D.T. Nguyen, D. VanLeer, P. Shinn, A. Jadhav, R. Müller, K.M. Waters, W. Shi, X. Liu, L. Zhang, R. Knight, P.R. Jensen, B. Palsson, K. Pogliano, R.G. Linington, M. Gutiérrez, N.P. Lopes, W.H. Gerwick, B.S. Moore, P.C. Dorrestein, N. Bandeira. 2016. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. Nat. Biotechnol. 34:828–837, doi: 10.1038/nbt.3597.

Zheng, X., R.S. Renslow, M.M. Makola, I.K. Webb, L. Deng, D.G.Thomas, N. Govind, Y.M. Ibrahim, M.M. Kabanda, I.A. Dubery, H.M. Heyman, R.D. Smith, N.E. Madala, E.S. Baker. 2017. Structural Elucidation of cis/trans Dicaffeoylquinic Acid Photoisomerization Using Ion Mobility Spectrometry-Mass Spectrometry. J. Phys. Chem. Lett. 8:1381–1388, doi: 10.1021/acs.jpclett.6b03015.

CHAPTER 5: ENHANCING THE LEVELS OF CAFFEOYL-D-GLUCARIC ACID DERIVATIVES FROM BUSH TEA (*ATHRIXIA PHYLLICOIDES* DC) THROUGH UV-INDUCED GEOMETRICAL ISOMERIZATION

Manuscript has been submitted to Environmental control in biology and is under review with minor corrections (See appendix D)

Abstract

The cultivation of bush tea (*Athrixia phylicoides* DC.) has been shown to be affected by light availability which has encouraged the use of different net shading to filter the amount of light on the plant. The objective of this study was to enhance the levels of caffeoyl-D-glucaric acid derivatives through UV induced geometrical isomerization**.** The experiment was laid out in a randomized complete block design consisting of control and white 80% shade net and analysis were done in triplicates. Bush tea methanolic leaf extracts were placed under UV light lamp at 254 nm for the duration of 24 h and new metabolites which formed due to UV exposure were detected through ultra-high-liquid chromatography quadrupole time-of-fight mass spectrometry (UHPLC-QTOF-MS). Hydroxyl-cinnamic acids (HCAs) derivatives have been shown to undergo photo-isomerization during post ultra-violet (UV) light exposure, evidenced by the emergence of photo-isomers.

Keywords: Asteraceae, environmental factors, hydroxyl-cinnamic acid, isomers, UHPLC-QTOF-MS

5.1. INTRODUCTION

Bush tea is scientifically described as *Athrixia phylicoides* DC., and it is also a member of Genus *Athrixia* under Asteraceae family. The genus *Athrixia* consists of 14 species which are found in Southern Africa, Tropical Africa as well as Madagascar (Van Wyk., 2000). *A. phylicoides* has been reported to possess various bioactive compounds such as flavonoids (Mudau et al., 2007), tannins (Mudau et al., 2007), antioxidants (Mogotlane et al., 2007) and chlorogenic acids (de Beer et al., 2011; Reichelt et al., 2012; Ramphinwa et al., 2022). Therefore, different parts of the plant are utilized differently in South Africa for its different medicinal attributes (Fouche et al., 2006; Mudau et al., 2007).

However, environmental factors such as light intensity, temperature, water availability, type and composition of soil have been reported to have a great influence on productivity and quality attributes of medicinal plants in general (Ramakrishna et al., 2011). A broad response known as oxidative stress is brought on by harmful environmental variables such as biotic and abiotic stresses (Demidchik, 2014). Hence, plants are capable to divert their photosynthetic resources (carbon dioxide, water, and sunlight) to mechanisms that would confer a tolerance in order to address biotic and abiotic stresses at the expense of growth and reproduction (Wahid et al., 2007). According to previous reports, when environmental conditions are favorable for the accumulation of total non-structural carbohydrates, an increase in carbon-based secondary metabolites frequently occurs (Ibrahim et al., 2011). These findings are consistent with those of (Ramphinwa et al., 2022), who found that using an 80% white shade net as one of the horticultural practices increases hydroxyl-cinnamic acid production compared to bush tea plants exposed to direct sunlight. Elsewhere, it has been noted that accumulation of structurally diverse chlorogenic acids in plants is pre-formed chemical defense strategy where plants enter into a state of readiness in order to withstand the oxidative stress caused by either biotic or abiotic stress (Ramabulana et al., 2016). Moreover, chlorogenic acids (the most abundant cinnamic acids derivatives in plants) are known to play a role against sunlight exposure (Ramabulana et al., 2016; Moyo et al., 2022). These molecules undergo geometrical isomerization (trans/*cis* photoisomerization), a phenomenon which also increases the pool of these compounds in plants (Clifford et al., 2008), thereby increasing a chance of the plant to withstand any future stress associated with oxidative stress (Ramabulana et al., 2016). The formation of these isomers can be regarded as an important, non-enzymatic anti-oxidative defensive strategy (Mbedzi et al., 2022).

Recently, it has been shown that chemical modifications associated with cinnamic acid molecules are essential in ensuring the qualitative outcomes of UV-induced geometrical isomerization in plants extracts (Moyo et al., 2022). For instance, cinnamic acid can be conjugated to various organic acids (Nengovhela et al., 2022), such as quinic acid (Ncube et al., 2014), citric/isocitric acid (Masike et al., 2017) and glucaric acid (Ramphinwa et al., 2022). Interestingly, caffeoylquinic acid and other hydroxyl-cinnamic acid derivatives have been shown to have anti-HIV-1 INT enzyme activity (Makola et al., 2016) and, as such, agricultural means to grow plants with enhance levels or means to diversify these compounds should be developed. As stated above, UV light exposure is one such option to increase or modify the chemistry of plant extracts (Ncube et al., 2014; Mbedzi et al., 2022). Other than UV light, other means to form *cis* isomers has been reported and they include mechanical processing of coffee beans, and an exposure to high electric field during MS data acquisition (Xie et al., 2011). These newly photochemical produced products (*cis* isomers) add to the already complex metabolome of the plant (Masike et al., 2018) and as shown elsewhere, their levels are controlled by the chemical modification associated with their anchoring molecules (Moyo et al., 2022).

Currently, two unique caffeoyl-D-glucaric acid derivatives, di-caffeoyl glucaric acids and tri-caffeoyl glucaric acids were identified in the genus *Gnaphalium* and *Leontopodium alpinum*, both of which are from the *Asteraceae* family (Schwaiger et al., 2005; Schwaiger et al., 2006; Cicek et al., 2012). Up to date, there is no information documented yet about caffeoyl-D-Glucaric Acid in bush tea plant. Due to the stereochemistry associated with glucaric acid, the number of possible isomers that could be formed due to positional and geometrical isomerism present an interesting scientific question. Therefore, their presence in a plant could serve an important premise of which the metabolite composition of a plant could be artificially altered through light exposure, various horticultural means and this is expected to improve the quality of such nutraceutical formulation. Therefore, the main objective of the study was to enhance levels of caffeoyl-D-glucaric acid derivatives through UV induced geometrical isomerization and use LC-MS based phytochemical fingerprinting to do a direct comparison with the cinnamic acid conjugated to another organic acid, quinic acid in this case.

5.2. MATERIALS AND METHODS

5.2.1. Experimental site

The wild bush tea stem cuttings were collected from Tshivhulani Village (lat. 22°55.331'S, long. 30°18.218'E; Altitude 610 m, which is characterized by cold and dry winters. The cuttings were propagated at the University of Venda’s experimental farm. Bush tea leaves used in the experiment were harvested from the University’s experimental farm. Dr Khathutshelo Magwede, a botanist, assisted in authenticating the plants.

5.2.2. Metabolites extraction and UV-irradiation

Extraction of metabolites from bush tea leaves was done according to a protocol reported elsewhere (Makita et al., 2016). Bush tea leaves were manually thrashed from dried plant which were harvested during the summer season and ground to fine powder using a hammer grinder as soon as the dry mass was weighed. Furthermore, finely ground leaf materials (2 g) of bush tea were weighed and mixed with 20 mL (1:10 m/v) of 80% methanol and allowed to shake at 70 rpm for 24 h to aid extraction. Debris of bush tea leaves extract were removed through centrifugation for 20 min at 5000 xg at 4°C and finally transferred to clean tubes. The extracts were then diluted 1:1 (v:v) in methanol to give a volume of 20 mL, and diluted sample of about 10 mL were transferred to quartz tubes (2 x 10 cm) and exposed to UV light using a Spectroline UV lamp which operates at 245 nm with a 390 µW/cm intensity for 24 h following an experimental setup presented elsewhere (Nobela et al., 2018; Moyo et al., 2022), whilst the other 10 mL was left un-exposed in darkness. The control and UV-irradiated extracts were filtered into a 2 mL vials through 0.22 µmm nylon filter using a 1mL syringe and transferred into a conical bottom glass insert of about 0.2 mL and placed inside an amber LC/GC vial capped with centre-hole caps. The extracts were kept at - 4°C before UHPLC-QTOF-MS analyses.

5.2.3. Ultra-High-Perfomance Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry analysis

The control and UV-treated bush tea samples were analysed by ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS), LC-MS 9030 model instrument using shim Pack Velox C_{18} column (100 mm × 2.1 mm and 2.7 µm particle size) (Shimadzu, Kyoto, Japan) and finally placed in a column oven set at 40 °C. To aid separation, a binary gradient method using the following solvent system: 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) was used with a flow rate of 0.4 mL/min using a 53 min gradient method presented elsewhere (Ramabulana et al., 2022). The following MS conditions were used to monitor analyte elution using a QTOF mass spectrometer detector: interface voltage of 3.5 kV; ESI (electrospray ionization) was at negative modes; with a dry gas flow of 3 L/min flow rate, nitrogen gas was used as a nebulizer; heating gas flow at 10 L/min and the heat block temperature of 400 °C, and the DL temperature of 280 °C; detector voltage of 1.70 kV were used. To monitor mass accuracy, a solution of sodium iodide was used as a mass calibrant. In MS/MS experiments, pseudo-molecular ions of specific *m/z* were selected and specifically monitored between a mass range of 100-1000 Da. Argon gas was used as a collision gas during CID experiments.

5.3. RESULTS AND DISCUSSION

The chemical structures of new glucaric acid derivatives which were detected on bush tea are reported in Fig. 1. In this study, positional/regional isomers of hydroxycinnamic acids were profiled through UHPLC-QTOF-MS before and after exposure of bush tea extracts to UV light radiation. Naturally, plants produce hydroxyl-cinnamic acids conjugates with *trans* configuration (Nobela et al., 2018) and upon UV exposure *cis* isomers are known to emerge (Clifford et al., 2008). The formation of geometrical isomers (*cis* isomers) is characterised by a dramatic polarity shift (Makola et al., 2016; Masike et al., 2018; Madala et al., 2021). Thus, during chromatographic analyses, the *cis* isomers are known to occupy a different retention time, suggesting an adverse change in overall hydrophobicity (Clifford et al., 2008). Upon, visual inspection of the chromatograms obtained herein, the presence of two major compounds in bush tea plant appearing at *m/z* 533 and 695 were noted and the selected ion chromatograms (SIC) of these ions were also generated (Fig. 3). Further scrutiny through informatic based approaches such as library searches through the SIRIUS software (Dührkop, et al., 2019) revealed these compounds to be isomers of dicaffeoyl glucaric acids and tri-caffeoyl glucaric acids. Bush tea plant has also been reported to produce conjugates of quinic acids such as di-caffeoylquinic acid and tri-caffeoylquinic acids with precursor ions at *m/z* 515 and *m/z* 677 respectively (Fig. 2 and 3) (Ramphinwa et al., 2022). It is important to note that negative ionization MS mode was used as it has been preciously shown to be efficient in discriminating isomers of cinnamic acid containing molecules (Clifford et al., 2008; Ncube et al., 2014).

To further substantiate the existence of cinnamic acid derivatives on these molecules, the extracts of this plant were exposed to UV light. Interestingly, more compounds were detected when bush tea samples were exposed to UV light. The new compounds that emerged from either the di-acylated or tri-acylated glucaric acid post UV-exposure are the corresponding *cis* isomers thereof (Clifford et al., 2008). The un-exposed bush tea extracts (controls) had only four peaks of di-caffeoylquinic acid, whereas eight peaks were detected after UV light exposure of the same compound (Fig. 2A and B). One peak of tri-caffeoylquinic acid was detected in the control samples, while two peaks were observed in the UV-exposed samples (Fig. 2C and D). In the chromatogram of bush tea extracts, five peaks of di-caffeoyl glucaric acid were detected under control and at least fifteen peaks were detected from the UV exposure (Fig. 3E and F). Under control conditions, four peaks of tri-caffeoyl glucaric acid were detected, whereas eight peaks were observed in samples exposed to UV light (Fig. 3G and H). Notably, it can be seen from the above observation that hydroxyl-cinnamic acid (HCA) derivatives conjugated to glucaric acid produces more geometrical isomers than those conjugated to quinic acid. Structurally, both quinic acid and glucaric acid have equal number of OH group, thereby suggesting that they could attach the same number of HCA derivatives (Fig. 1). However, from Fig. 2 and 3 is much clearer that glucaric acid derivatives produces more isomers. Noteworthy, the control plant extracts showed minor presence of *cis* isomers, which suggest that geometrical isomerization does take place *in vivo*, but their levels are augmented upon UV light exposure shown by the current results and these results agree with what has been reported recently in a *Viscum* plant (Moyo et al., 2022). In a natural setting (in *planta*), the formation of geometrical isomers is an evolutionary defensive strategy to mitigate the biological consequences associated with excessive sunlight exposure (Moyo et al., 2022). As seen from the results herein, the initial structural configuration of cinnamic acid containing molecules is crucial for the formation of geometrical isomers both quantitatively and qualitatively. The number of the cinnamic acid containing molecule has been shown to be important in the establishment of the state of readiness against light induced stress through a "better be ready than sorry" phenomenon (Ramabulana et al., 2016). As already mentioned above, cinnamic acids conjugated to glucaric acid result in more isomers (qualitatively) than when they are conjugated to quinic acid. A simple explanation could be due to the stereochemistry of glucaric acid which allows for diversification of same molecule. Recently, it has been shown that, the stereochemistry of tartaric acid allows for efficient discrimination of chicoric acids isolated from related plants (Nobela et al., 2018). Naturally, chicoric acid exist as L-chicoric acid [(-)-chicoric acid, 2,3-dicaffeoyl-L-tartaric acid, 2,3-*O*-dicaffeoyltartaric acid, 2R,3R-*O*-dicaffeoyltartaric acid, or di-*E*-caffeoyl-(2R-3R)-(-)-tartaric acid] but another stereoisomer known as *meso*-chicoric acid (i.e., dicaffeoyl-*meso*-tartaric acid or di-*E*-caffeoyl-(2R-3S)-(-)-tartaric acid) has been found in other plants (Lee and Scagel., 2013). Due to this contrasting stereochemistry, the *meso*-chicoric acid was known to produce four geometrical isomers upon UV exposure whilst only three isomers are noted with the L-chicoric acid (Nobela et al., 2018). The above can also be said for glucaric acid-HCA derivatives which were shown to produce more geometrical isomers when exposed to UV light (Fig. 3).

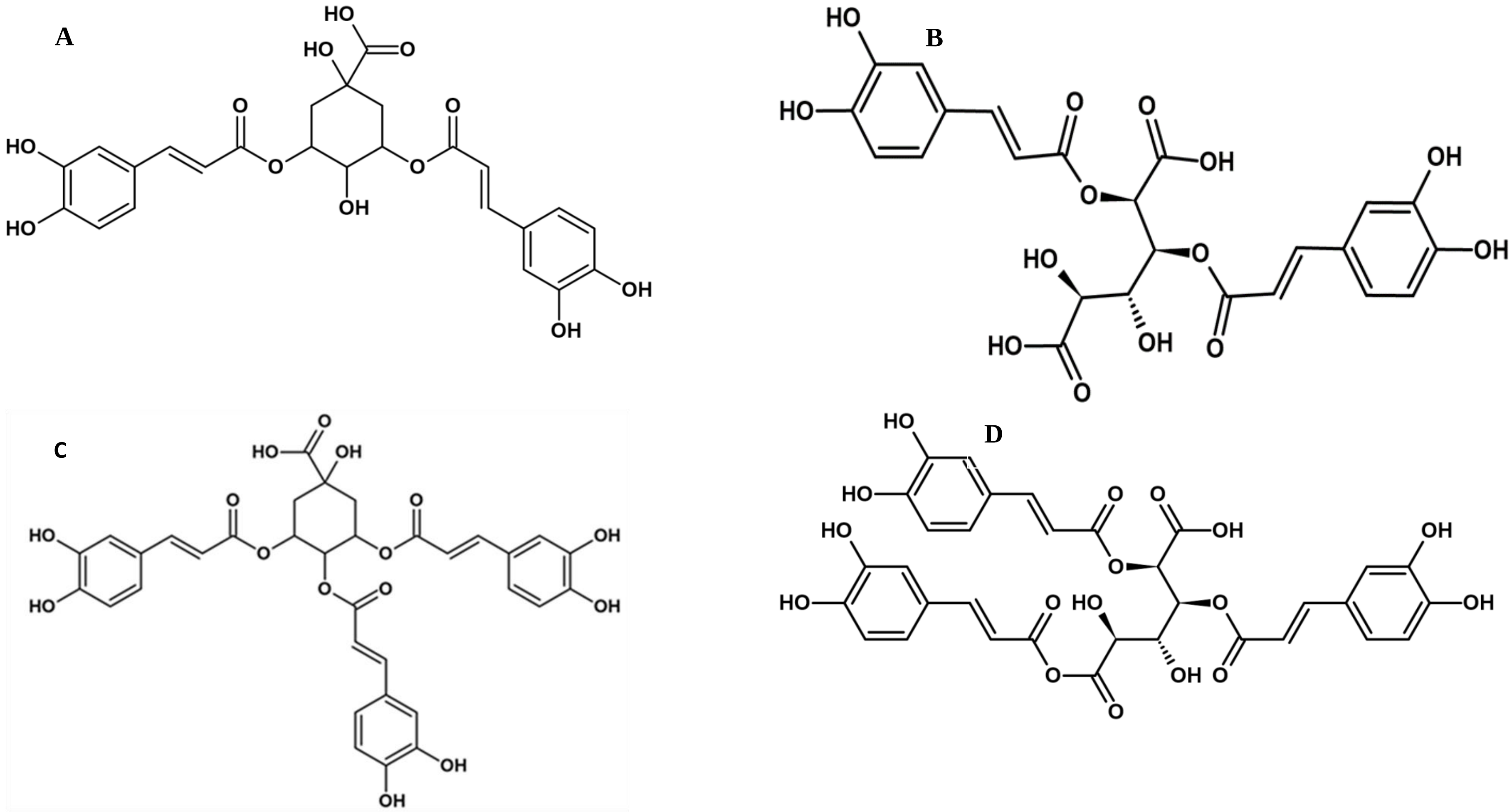


Figure 5.1. Representative of chemical structures showing dicaffeoyl quinic acid (A), dicaffeoyl glucaric acid (B), tricaffeoyl quinic acid (C), and tricaffeoyl glucaric acid

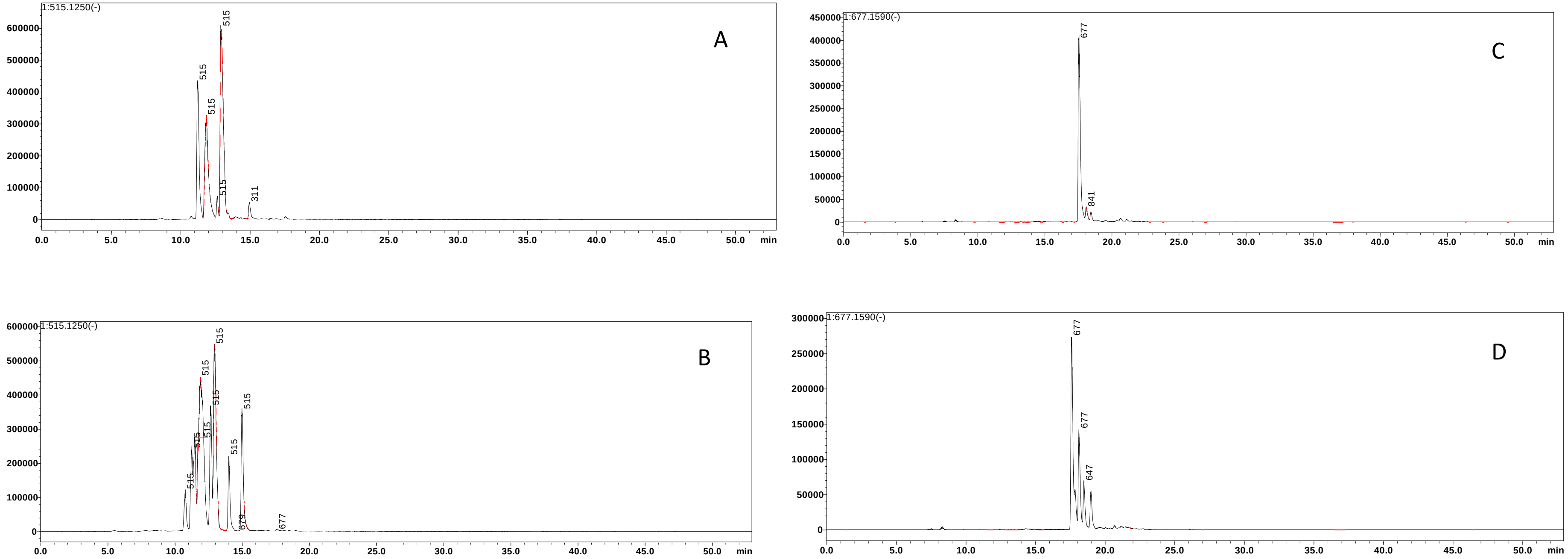


Figure 5.2. UHPLC-QTOF-MS profile chromatograms of untreated (0h) and treated (24 h) bush tea samples of di-caffeoyl glucaric acids and tri-caffeoylquinic acids

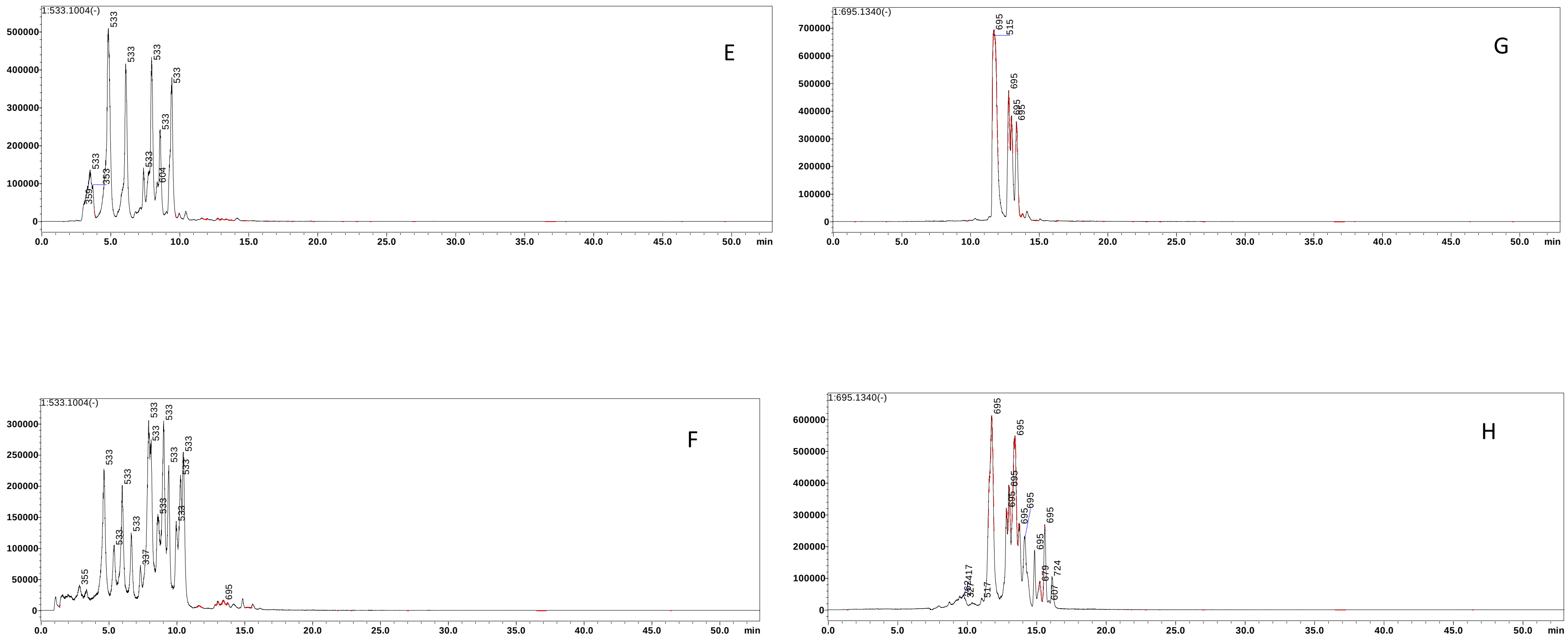


Figure 5.3. UHPLC-QTOF-MS profile chromatograms of untreated (0h) and treated (24 h) bush tea samples of di-caffeoyl glucaric acids and tri-caffeoyl glucaric acids

5.4. CONCLUSION

The existence of structurally diverse HCA metabolites in bush tea is an important factor towards mitigating photo-oxidative damages associated with excessive light exposure in natural environment. The di-acyl and tri-acyl HCA derivative attached to glucaric acid are reported for the first time in this plant. These compounds were found to undergo geometrical isomerization post UV exposure, producing structurally diverse *cis* isomers. The glucaric acid conjugates produced more *cis* geometrical isomers than the quinic acid derivatives. The results of the current study suggested that the existence of these compounds and their ability to amplify numbers post UV exposure could be an evolutionary strategy of plant to protect itself from excessive sunlight which may result in oxidative stress. This observation can further be an encouraging factor for consuming this plant (bush tea) as an alternative source of structurally diverse nutraceuticals, of which the composition can be manipulated using light.

5.5. REFERENCES

Cicek, S.S., C. Untersulzner, S. Schwaiger, and C. Zidorn. 2012. Caffeoyl-D-glucaric acid derivatives in the Genus *Gnaphalinum* (Asteraceae: Gnaphalieae). Rec. Nat. Prod. 6:311–315.

Clifford, M.N., J.O. Kirkpatrick, N. Kuhnert, H. Roozendaal, P.R. Salgado. 2008. LC MSn analysis of the cis isomers of chlorogenic acids. Food Chem. 106:379-385, doi: 10.1016/j.foodchem.2007.05.081.

De Beer, D., E. Joubert, C.J. Malherbe, D.J. Brand, D.J. 2011. Use of counter current chromatography during isolation of 6-hydroxyluteolin-7-O-B-glucoside, a major antioxidant of *Athrixia phylicoides*. J. Chromatogr. 1218:6179–6186, doi: 10.1016/j.chroma.2010.12.096.

Demidchik, V. 2014. Mechanisms of oxidative stress in plants: from classical chemistry to cell biology, Environ. Exp. Bot. 109:212–228, doi: 10.1016/j.envexpbot.2014.06.021.

Dührkop, K., M. Fleischauer, M. Ludwig, A.A. Aksenov, A.V. Melnik, M. Meusel, P.C. Dorrestein, P.C., J. Rousu, and S. Böcker. 2019. Sirius 4: turning tandem mass spectra into metabolite structure information. Nat methods. 16:299-302, doi: 10.1038/s41592-019-0344-8.

Fouche, G., E. Khorombi, N. Kolesnikova, V.J. Maharaj, R. Nthambeleni, and M. Van der Merwe. 2006. Investigation of South African plants for anticancer properties. Pharmacologyonline. 3:494–500.

Ibrahim, M.H., H.Z.E. Jaafar, A. Rahmat, and Z.A. Rahman. 2011. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of Kacip fatimah (*Labisia pumila* Blume). Int. J. Mol. Sci. 12:5238–5254, doi: 10.3390/ijms12085238.

Lee, J. and C.F. Scagel. 2013. "Chicoric acid: chemistry, distribution, and production," Front Chem. 1:40, doi: 10.3389/fchem.2013.00040.

Madala, N.E. and M.M. Kabanda. 2021. LC-MS based validation and DFT investigation on the antioxidant properties of clovamide: • OH and• OOH scavenging and Cu (II) chelation mechanisms. J. Mol. Struct**.** 1236:130349, doi: 10.1016/j.molstruc.2021.130349.

Makita, C.L., L. Chimuka, P. Steenkamp, E. Cukrowska, and E. Madala. 2016. Comparative analyses of flavonoid content in *Moringa oleifera* and *Moringa ovalifolia* with the aid of UHPLC-qTOFMS fingerprinting. S.A. J. Bot. 105:116–122, doi: 10.1016/j.sajb.2015.12.007.

Makola, M.M., I.A. Dubery, G. Koorsen, P.A. Steenkamp, M.M. Kabanda, L.L. du Preez, and N.E. Madala. 2016. The effect of geometrical isomerism of 3, 5-dicaffeoylquinic acid on its binding affinity to HIV-integrase enzyme: A molecular docking study. Evid. -Based Complement. Altern. Med. 1-9, doi: 10.1155/2016/4138263.

Makola, M.N., P. Steenkamp, I.A. Dubery, M.M. Kabanda, and N.E. Madala. 2016. Preferential alkali metal adduct formation by *cis* geometrical isomers of dicaffeoylquinic acids allows for efficient discrimination from their trans isomers during ultra-high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 30:1011–1018, doi: 10.1002/rcm.7526.

Masike, K., I. Dubery, P. Steenkamp, E. Smit, and E. Madala. 2018. Revising reverse-phase chromatographic behavior for efficient differentiation of both positional and geometrical isomers of dicaffeoylquinic acids. J. Anal. Methods Chem. 2018:1-11, doi: 10.1155/2018/8694579.

Masike, K., M.I. Mhlongo, S.P. Mudau, O. Nobela, E.N. Ncube, F. Tugizimana, M.J. George, and N.E. Madala. 2017. Highlighting mass spectrometric fragmentation differences and similarities between hydroxycinnamoyl-quinic acids and hydroxycinnamoyl-isocitric acids. Chem. Cent. J. 11:1-7, doi: 10.1186/s13065-017-0262-8.

Mbedzi, D.T., L.M. Mathomu M.I. Mlongo, and N.E. Madala. 2022. Ultraviolet (UV) light-induced geometrical isomerization of cinnamic acid containing molecules: A plausible, non-enzymatic approach to modify metabolite composition of plant extracts. S. Afri. J. Bot. 150:845-850.

Mogotlane, I.D., F.N. Mudau, P.W. Mashela, P. Soundy. 2007. Seasonal responses of total antioxidant contents in cultivated bush tea (*Athrixia phylicoides* L.) leaves to fertilizer rates. Med. Aromatic Plant Sci. Biotech. 1:77-79.

Moyo, B., N.T. Tavengwa, N.E. Madala, N.E. 2022. Diverse chemical modifications of the chlorogenic acid composition of *Viscum combreticola* Engl.: A premise for the state of readiness against excessive sunlight exposure. J. Photochem. Photobiol. B. 233:112501, doi: 10.1016/j.jphotobiol.2022.112501.

Mudau, F.N., H.T. Araya, E.S. Du Toit, P. Soundy, J. Olivier. 2007. Bush tea (*Athrixia phylicoides* DC.) as an alternative herbal and medicinal plant in southern Africa: opportunity for commercialization.

Mudau, F.N., P. Soundy, E. Du Toit, J. Olivier. 2006 Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. S. Afr. J. Bot. 72:398-402, doi: 10.1016/j.sajb.2005.10.007.

Ncube, E.N., M.I. Mhlongo, L.A. Piater, P.A. Steenkamp, I.A. Dubery, N.E. Madala. 2014. “Analyses of chlorogenic acids and related cinnamic acid derivatives from Nicotiana tabacum tissues with the aid of UPLC-QTOF-MS/MS based on the insource collision-induced dissociation method,” Chem. Cent. J. 8:1–10.

Nengovhela, N., D.T. Mbedzi, A.R. Ndhlala, L.M. Mathomu, M.I. Mhlongo, N.E. Madala. 2022. LC-MS based metabolite profiling reveals hydroxylcinnamoyl conjugation as a discriminatory chemical factor between two closely related Coccinia species. S. Afri. J. Bot. 145:199-206, doi: 10.1016/j.sajb.2021.04.034.

Nobela, O., R.S. Renslow, D.G. Thomas, S.M. Colby, S. Sitha, P.B. Njobeh, L. du Preez, F. Tugizimana, N.E. Madala. 2018. Efficient discrimination of natural stereoisomers of chicoric acid, an HIV-1 integrase inhibitor. J. Photochem. Photobiol. B. Biol. 189:258-266, doi: 10.1016/j.jphotobiol.2018.10.025.

Ramabulana, T., Mavunda, R.D., Steenkamp, P.A., Piater, L.A., Dubery, I.A., Madala, N.E. 2016. Perturbation of pharmacologically relevant polyphenolic compounds in Moringa oleifera against photo-oxidative damages imposed by gamma radiation. J. Photochem. Photobiol. B. 156:79-86, doi: 10.1016/j.jphotobiol.2016.01.013.

Ramakrishna, A., Ravishankar, G. A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6:1720-1731, doi: 10.4161/psb.6.11.17613.

Ramphinwa, M.L., Madala, N.E., Mchau, G.R.A., Ramabulana, A.T., Mudau, F.N. 2022. Effect of UV-induced geometrical isomerization of hydroxyl-cinnamic acid-containing molecules of bush tea (*Athrixia phylicoides* DC.) using UHPLC-QTOF-MS. Scientia Horticulturae. 301:111124, doi: 10.1016/j.scienta.2022.111124.

Ramphinwa, M.L., Mchau, G.R., Madala, N.E., Nengovhela, N., Ogola, J.B., Mudau, F.N., 2022. Response of Plant Growth and Development, and Accumulation of Hydroxyl-cinnamoyl Acid Derivatives to Selected Shade Nets and Seasonality of Field-grown Bush Tea (*Athrixia phylicoides* DC.). HortScience. 57:87-96, doi: 10.21273/HORTSCI16171-21.

Reichelt, K.V., Hoffmann-Lücke, P., Hartmann, B., Weber, B., Ley, J.P., Krammer, G.E., Swanepoel, K.H. 2012. Phytochemical characterization of South African bush tea (*Athrixia phylicoides* DC.). S. Afr. J. Bot. 83:1–8, doi: 10.1016/j.sajb.2012.07.006.

Schwaiger, S., Cervellati, R., Seger, C., Ellmerer, E.P., About, N., Renimel, I., Godenir, C., André, P., Gafne, F., Stuppner, H. 2005. Leontopodic acid – a novel highly substituted glucaric acid derivative from Edelweiss (*Leontopodium alpinum* Cass.) and its antioxidative and DNA protecting properties, Tetrahedron. 6:4621-4630, doi: 10.1016/j.tet.2005.03.002.

Schwaiger, S., Seger, C., Wiesbauer, B., Schneider, P., Ellmerer, E.P., Sturm, S., Stuppner, H. 2006. Development of an HPLC-PAD-MS assay for the identification and quantification of major phenolic edelweiss (*Leontopodium alpium* Cass.) constituents, Phytochem. Anal. 17:291-298, doi: 10.1002/pca.917.

Van Wyk, B.E., Gericke, N. 2000. People's plants: A guide to useful plants of Southern Africa. 1st edition. Briza publications. 102.

Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R. 2007. Heat tolerance in plants: an overview. Environ. Exp. Bot. 61: 199–223, doi: 10.1016/j.envexpbot.2007.05.011.

Xie, C., Yu, K. Zhong, D. Yuan, T. Ye, F. Jarrell, J-A. Millar, A. Chen, X. 2011. Investigation of isomeric transformations of chlorogenic acid in buffers and biological matrixes by Ultra Performance Liquid Chromatography coupled with hybrid quadrupole/ion mobility/orthogonal acceleration time-of-flight mass spectrometry. J. Agr. Food Chem. 59:11078–11087, doi: 10.1021/jf203104k.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSION AND FUTURE PROSPECTS

6.1. General discussion

Bush tea (*Athrixia phylicoides* DC.) is a traditional herbal plant from South Africa that contains antioxidants (Mogotlane et al., 2007), tannins (Mudau et al., 2007; Chabeli et al., 2008), and 5-hydroxy-6,7,8,3′,4′,5′ -hexamethoxy flavon-3-ol (Mashimbye et al., 2006), 5,6,7,8,3′,4′-hexamethoxyflavone, quercetin, 3-0-demethyldigicitrin, and other polyphenols (Mudau et al., 2006, 2007; Tshivhandekano et al., 2018). However, several studies have suggested that seasonal variation has an impact on plant growth, yield, and quality of bush tea (Mudau et al., 2007; de Beer et al., 2011; Mavundza et al., 2010; Tshivhandekano et al., 2014), but the majority of the research has been done in controlled pot experiments. As a result, net shades have been reported as one of the microclimate tools that maximizes the quality of the tea leaves due to an increased concentration of amino acids with a lower concentration of catechin in the plant (Ku et al., 2010) and prevents the concentration of flavonoids (Wang et al., 2012). The effect of the net shading and season on plant growth, productivity and quality of field grown bush tea has never been investigated. The study revealed that net shades reduce plant growth and yield of bush tea plant compared to the plants exposed to the direct sunlight. Thus, this species requires full sunlight to maximize its plant growth and yield. In this study, the higher biomass accumulation of field grown bush tea was recorded during summer season due to the greater proportion of intercepted radiation recorded in summer cropping season which influences plant growth, development, and yield (Challa and Bakker, 1999; Cockshull et al., 1992), (See chapter 3). Bush tea plants have also been found to maximize hydroxyl-cinnamic acid when exposed to white 80% shade nets than other shade nets. The amount of solar radiation that penetrates the white shade net in comparison to other shade net colours might be the factor attributed to such kind of results. Therefore, the use of shade nets reduced plant growth and yield when compared to plants exposed to full sunlight during the summer, followed by the use of a white shade net.

Generally, abiotic, and biotic stresses increase the production, accumulation and partitioning of secondary metabolites on plants at the expense of plant growth, development, and yield (Wahid et al., 2007). These secondary metabolites aid the plants naturally to defend themselves against pathogen attack, wounding, ultraviolet irradiation (UV), high light, temperature, herbicides, and nutrient deficiencies (Ramakrishna and Ravishankar, 2011, Mohale et al., 2018). Plants normally produce cinnamic acid with a *trans* configuration, which eventually changes to a *cis* configuration when exposed to UV light (Makola et al., 2016). One of the most notable features of this isomerization process is that the newly formed compounds elute at different retention times on a chromatographic space (Clifford et al., 2008), indicating that geometrical isomerization causes an overwhelming polarity switching. This study significantly found that hydroxyl-cinnamic acids of bush tea undergo photo-chemical isomerization, which is characterized by the formation of isomeric molecules with *cis* geometry through UHPLC-QTOF-MS. Due to the high levels of sunlight in areas where the bush tea plant grows, it is expected that some of these metabolites particularly those containing hydroxyl-cinnamic acid will form geometrical isomers (Clifford et al., 2008), representing a chemical shift with unknown biological consequences. As a result, when the molecular networking algorithm was used, these photo isomerized metabolites were discovered to be structurally related, forming a very tight molecular family (see chapter 4).

The study also revealed the presence of two major compounds, the di-acyl and tri-acyl HCA derivative attached to glucaric acid for the first time in this plant using UHPLC-QTOF-MS. The presence of structurally diverse HCA metabolites in bush tea is a critical factor in mitigating the photo-oxidative damage described herein. In addition, these compounds were discovered to undergo geometrical isomerization upon UV exposure, resulting in structurally diverse *cis* isomers. As a result, more glucaric acid conjugates produced more *cis* geometrical isomers than quinic acid derivatives (see chapter 5).

6.2. Conclusion

This study concluded that direct sunlight and summer cropping season are the best recommended method for bush tea production because it increases plant growth and yield. When compared to plants exposed to full sun light, horticultural practices of using white (80%) shading resulted in higher chlorogenic acid accumulation. As a result, a white shade net and a light intensity of 80% may be the best microclimatic tools for increasing chlorogenic acids in bush tea production. Due to UV light exposure, bush tea contains hydroxyl-cinnamic acid, which is photo-isomerized by the formation of *cis* geometrical isomers. Therefore, light has an adverse effect on plant chemistry, resulting in the formation of newly formed metabolites that are not naturally present in the plant. It is clear that the formation of *cis* - isomers amplified the already complex metabolome of bush tea due to UV light exposure.

6.3. Future prospects

Hydroxycinnamic acids are bioactive phenolic compounds found in various plants, playing a crucial role in responding to biotic and abiotic stresses. These molecules possess numerous spatial and geometric isomers, making their identification and analysis via traditional LC-MS methods challenging in bush tea. Co-elution during chromatographic separation and near-identical spectra during MS analyses further complicate their characterization. Therefore, following future prospects are suggested based on the study's findings and conclusions: investigate the effect of net shade and water regime on plant growth, yield and quality of bush tea as well as to determine effect of different UV light intensities on bush tea extracts and the molecular network of bush tea under different types of shade nets.

6.4. References

Chabeli, P.M., Mudau, F.N., Mashela, P.W, and P. Soundy. 2008. Effects of nitrogen, phosphorus and potassium nutrition on seasonal tannin content of bush tea (*Athrixia phyliciodes* dc.). South African J. Plant Soil. 25:79–83, doi: 10.1080/02571862.2008.10639899.

Challa, H., and M.J. Bakker. 1999. Potential production within the greenhouse environment. In Ecosystems in the world: Vol. 20: Chapter 15: Greenhouse Ecosystems. 20:333-348.

Clifford, M.N., J. Kirkpatrick, N. Kuhnert, H. Roozendaal, and P.R. Salgado. 2008. LC-MSn analysis of the cis isomers of chlorogenic acids. Food Chem. 106:379–38, doi: 10.1016/j.foodchem.2007.05.081.

Cockshull, K. E., C.J. Graves, and C.R. Cave. 1992. The influence of shading on yield of glasshouse tomatoes. Journal of Hortic. Sci. 67:11-24, doi: 10.1080/00221589.1992.11516215.

De Beer D., E. Joubert, C.J. Malherbe, and D.J. Brand. 2011. Use of counter current chromatography during isolation of 6-hydroxyluteolin-7-O-B-glucoside, a major antioxidant of *Athrixia phylicoides*. J. Chromatogr. 1218:6179–6186, doi: 10.1016/j.chroma.2010.12.096.

Ku, K.M., J.N. Choi, J.K. Kim, L.G. Yoo, S.J. Lee, Y.S. Hong, and C.H. Lee. 2010. Metabolomics analysis reveals the compositional differences of shade grown tea (*Camellia sinensis* L.). J. Agric. Food Chem. 58:418-426, doi: 10.1021/jf902929h.

Makola, M.M., P.A. Steenkamp, I.A. Dubery, M.M. Kabanda, and N.E. Madala. 2016. Preferential alkali metal adduct formation by cis geometrical isomers of dicaffeoylquinic acids allows for efficient discrimination from their trans isomers during ultra-high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 30:1011–1018, doi: 10.1002/rcm.7526.

Mavundza, E.J., T.E. Tshikalange, N. Lall, A.A. Hussein, F.N. Mudau, and J.J.M. Meyer. 2010. Antioxidant activity and cytotoxicity effect of flavonoids isolated from *Athrixia phyllicoides*. 4:2583-2586, doi: 10.5897/JMPR10.823.

Mohale, K.C., A.T. Hintsa, M.A. Emanuel, and F.N. Mudau. 2018. Metabolic Profiling of Cultivated Bush Tea (*Athrixia phylicoides* DC.) in Response to Different Pruning Types. Sci. Hortic. 53:993-998, doi: 10.21273/HORTSCI13023-18.

Mudau, F.N., H.T. Araya, E.S. Du Toit, P. Soundy, and J. Olivier. 2007. Bush tea (*Athrixia phylicoides* DC.) as an alternative herbal and medicinal plant in southern Africa: opportunity for commercialization. Med. Aromatic Plant Sci. Biotechnol. 1:74-76.

Ramakrishna, A. and G.A. Ravishankar. 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6:1720–1731, doi: 10.4161/psb.6.11.17613.

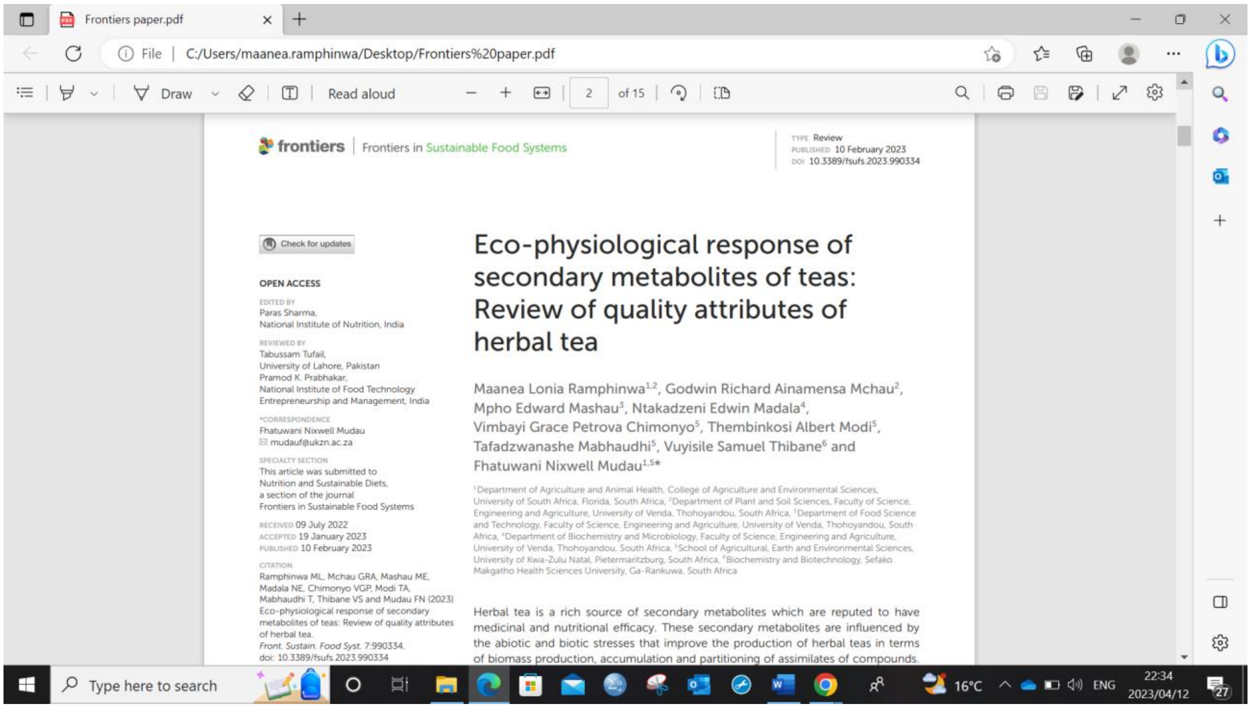
Tshivhandekano, I., K. Ntushelo, W. Ngezimana, T.E. Tshikalange, and F.N. Mudau. 2014. Chemical compositions and antimicrobial activities of *Athrixia phylicoides* DC. (Bush tea), *Monsonia burkeana* (special tea) and synergistic effects of both combined herbal teas. Asian. Pac. J. Trop Med. 7:S448–S453, doi: 10.1016/S1995-7645(14)60273-X.

Wahid, A., S. Gelani, and M. Ashraf, and M.R. Foolad. 2007. Heat tolerance in plants: an overview. Environ. Exp. Bot. 61:199–223, doi.org/10.1016/j.envexp.

Wang, Y., L. Gao, Y. Shan, Y. Liu, Y. Tian, and T. Xia. 2012. Influence of shade on flavonoid biosynthesis in tea (*Camellia sinensis* (L.) O. Kuntze). Sci. Hortic. 141:7-16, doi: 10.1016/j.scienta.2012.04.013.

APPENDICES:

Appendix A: Published paper.



frontiers | Frontiers in Sustainable Food Systems

TYPE Review
PUBLISHED 10 February 2023
DOI 10.3389/fsufs.2023.990334

Check for updates

OPEN ACCESS

EDITED BY
Paras Sharma,
National Institute of Nutrition, India

REVIEWED BY
Tabussam Tufail,
University of Lahore, Pakistan
Pramod K. Prabhakar,
National Institute of Food Technology Entrepreneurship and Management, India

*CORRESPONDENCE
Fhatuwani Nixwell Mudau
mudauf@ukzn.ac.za

SPECIALTY SECTION
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Sustainable Food Systems

RECEIVED 09 July 2022
ACCEPTED 19 January 2023
PUBLISHED 10 February 2023

CITATION
Ramphinwa ML, Mchau GRA, Mashau ME, Madala NE, Chimonyo VGP, Modi TA, Mabhaudhi T, Thibane VS and Mudau FN (2023) Eco-physiological response of secondary metabolites of teas: Review of quality attributes of herbal tea. *Front. Sustain. Food Syst.* 7:990334.
doi: 10.3389/fsufs.2023.990334

Eco-physiological response of secondary metabolites of teas: Review of quality attributes of herbal tea

Maanea Lonia Ramphinwa[1,2], Godwin Richard Ainamensa Mchau[2], Mpho Edward Mashau[3], Ntakadzeni Edwin Madala[4], Vimbayi Grace Petrova Chimonyo[5], Thembinkosi Albert Modi[5], Tafadzwanashe Mabhaudhi[5], Vuyisile Samuel Thibane[6] and Fhatuwani Nixwell Mudau[1,5]*

[1]Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Florida, South Africa, [2]Department of Plant and Soil Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Thohoyandou, South Africa, [3]Department of Food Science and Technology, Faculty of Science, Engineering and Agriculture, University of Venda, Thohoyandou, South Africa, [4]Department of Biochemistry and Microbiology, Faculty of Science, Engineering and Agriculture, University of Venda, Thohoyandou, South Africa, [5]School of Agricultural, Earth and Environmental Sciences, University of Kwa-Zulu Natal, Pietermaritzburg, South Africa, [6]Biochemistry and Biotechnology, Sefako Makgatho Health Sciences University, Ga-Rankuwa, South Africa

Herbal tea is a rich source of secondary metabolites which are reputed to have medicinal and nutritional efficacy. These secondary metabolites are influenced by the abiotic and biotic stresses that improve the production of herbal teas in terms of biomass production, accumulation and partitioning of assimilates of compounds.

Appendix B: Published paper.

HortScience 57(1):87–96. 2022. https://doi.org/10.21273/HORTSCI16171-21

Response of Plant Growth and Development, and Accumulation of Hydroxyl-cinnamoyl Acid Derivatives to Selected Shade Nets and Seasonality of Field-grown Bush Tea (*Athrixia phylicoides* DC.)

Maanea L. Ramphinwa
Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Private Bag X6, Florida, 1710, South Africa; and Department of Plant and Soil Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Private Bag X5050, Thohoyandou, 0950, South Africa

Godwin R.A. Mchau
Department of Plant and Soil Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Private Bag X5050, Thohoyandou, 0950, South Africa

Ntakadzeni E. Madala and Ndamulelo Nengovhela
Department of Biochemistry and Microbiology, Faculty of Science, Engineering and Agriculture, University of Venda, Private Bag X5050, Thohoyandou, 0950, South Africa

John B.O. Ogola
Department of Plant and Soil Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Private Bag X5050, Thohoyandou, 0950, South Africa

Fhatuwani N. Mudau
Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Private Bag X6, Florida, 1710, South Africa; and School of Agricultural, Earth and Environmental Sciences, University of Kwa-Zulu Natal, Cabbis Road, Scottsville, Pietermaritzburg, 3209

Additional index words. herbal tea, quality control, protected environment, light intensity, quality index

***Abstract.* Horticultural practices and quality of bush tea (*Athrixia phylicoides* DC.) are critical for herbal tea industrialization. The objective of the current study was to determine the effect of selected shade nets and seasonal variation on plant growth and development, and hydroxycinnamic acid content of field-grown bush tea. The trial was laid out in a randomized complete block design consisting of three shade nets (black, green, and white) and control or full sunlight with three different light intensities (40%, 50%, and 80%) replicated three times. Proportion of intercepted radiation by the canopy, chlorophyll content, plant height, and fresh and dry mass were measured, and hydroxycinnamic acid accumulation was determined. In addition, hydroxycinnamic acid composition was determined using liquid chromatography linked to mass spectrometry (LC-MS). The application of shade nets resulted in plant growth and yield reduction as compared with the plants exposed to full sunlight during summer followed by white shade net. The accumulation of hydroxycinnamic acid was higher in 80% white shade net plots compared with unshaded plants (control) and the other shade nets. Therefore, lack of shading provides a conducive environment to enhance plant growth and development of bush tea. The white shade net (80%) was an effective microclimate tool to enhance accumulation of caffeoylquinic acid (m/z 353), p-coumaric acids (m/z 337), dicaffeoylquinic acid (m/z 515), and tricaffeoylquinic acids of bush tea. This study is the first to demonstrate light as a determining factor for production of chlorogenates in bush tea plants. Future studies will be conducted to determine the effect of light on extracts of the bush tea using different solvents.**

Bush tea (*A. phylicoides* DC.) is a native plant of South Africa and it grows naturally in different climatic conditions of South Africa (Mavundza et al., 2010; Nchabeleng et al., 2012; Van Wyk and Gericke, 2000). The plant grows to a height of ≈0.5 m to 1 m, with branches of thin woolly stems (Mudau et al., 2006). It has simple alternate leaves that are described as linear, light gray-green above, and white-woolly below (Mudau et al., 2006). The leaf bases are broadly lanceolate, short stalked, taper to a sharp point, auriculate, smooth on the upper surface, and have margins that are entirely or slightly revolute (Mudau et al., 2006). The head of the inflorescence is sessile or subsessile with terminal axillary in large sub corymbose panicles (Herman et al., 2000).

It is used traditionally as an important herbal and medicinal plant by South Africans to cleanse and purify blood, treat boils, heal wounds, and treat headaches (Fouché et al., 2006; Mudau et al., 2007). In some parts of the country, it is used as an aphrodisiac (Rakuambo, 2011). To date, bush tea is still harvested from the wild for a variety of medicinal purposes (e.g., to treat chronic diseases such as cardiovascular disease and cancer) and hence there is a huge potential for development of *A. phylicoides* as a commercial health benefit herb in South Africa (Mudau et al., 2006). Therefore, domestication of bush tea is critical for commercial exploitation to protect the species from possible extinction.

Previous studies have documented the effects of cultural practices such as mineral nutrition (Mudau et al., 2006), pruning (Mohale et al., 2018; Yilmaz et al., 2004), irrigation (Bandara, 2012) and harvesting methods (Mphangwe, 2012), as well as processing (Hlahla, 2010) and environmental conditions (Tshivhandekano et al., 2013) on chemical compositions of bush tea. The quality, economic value, and health function of bush tea is determined by the content of secondary metabolites such as flavonoids (or phenolic compounds), alkaloids, and amino acids (Tounekti et al., 2013). Zhang et al. (2014) reported that quality parameters of tea are significantly affected by environmental factors and management practices. Application of nitrogen (N) fertilizer increases total phenol concentrations in cultivated medicinal plants by enhancing photosynthetic rate (Haukioja et al., 1998) and accumulation of nonstructural carbohydrates (Wanyoka, 1983).

However, it has been reported that carbon-based secondary metabolites increase frequently when environmental conditions enhance the accumulation of nonstructural carbohydrates in *Labisia pumila* plants (Ibrahim et al., 2011). Hydroxycinnamic acids (coumaric acid, ferulic acid, sinapic acid, and caffeic acid) are phenolic compounds that are found in plants either as free compounds or conjugated to other molecules such as quinic acid, tartaric acid, citric acid, and sugars (Ncube et al., 2014). These derivatives are known as chlorogenic acids (CGAs) when they are conjugated/esterified to

Appendix C: Published paper

Scientia Horticulturae 301 (2022) 111124

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effect of UV-induced geometrical isomerization of hydroxyl-cinnamic acid-containing molecules of bush tea (*Athrixia phylicoides* DC.) using UHPLC-QTOF-MS

Maanea L. Ramphinwa [a,b], Ntakadzeni E. Madala [c,*], Godwin R.A. Mchau [b], Anza T. Ramabulana [d], Fhatuwani N. Mudau [a,e,*]

[a] *Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Private Bag X6, Florida, 1710, South Africa*

[b] *Department of Plant and Soil Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, Private Bag X5050, Thohoyandou, 0950, South Africa*

[c] *Department of Biochemistry and Microbiology, Faculty of Science, Engineering and Agriculture, University of Venda, Private Bag X5050, Thohoyandou, 0950, South Africa*

[d] *Research Centre for Plant Metabolomics, Department of Biochemistry, University of Johannesburg, Auckland Park, 2006, South Africa*

[e] *School of Agricultural, Earth and Environmental Sciences, University of Kwa-Zulu Natal, Cabbis Road, Scottsville, Pietermaritzburg, 3201, South Africa*

ARTICLE INFO

Keywords:
Athrixia phyllicoides
Hydroxyl-cinnamic acids
Isomerization
Ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry
Molecular network

ABSTRACT

Bush tea (*Athrixia phyllicoides* DC.) is a herbal tea which contains bioactive compounds. Naturally, these metabolites aid plants to defend themselves against a wide spectrum of biotic and abiotic stresses. The objective of the study was to identify hydroxyl-cinnamic acids affected by UV (Ultraviolet) light exposure of bush tea through ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS). A randomized complete block experimental design was used consisting of control and 80% white shade net replicated three times and samples were analyzed in triplicates. The methanolic leaf extracts of bush tea were exposed to UV light at 254 nm for 24 h and thereafter metabolites were measured and annotated through UHPLC-QTOF-MS. Hydroxyl-cinnamic acids (HCAs) have been shown to undergo photo-isomerization during post UV light exposure, evidenced by the emergence of photo-isomers. The findings showed that hydroxyl-cinnamic acids containing molecules to undergo photo-chemical isomerization, characterized by formation of isomeric molecules with *cis* geometry. Metabolites which underwent photo isomerization were found to be structurally related as they formed a very tight molecular family when molecular networking algorithm was used.

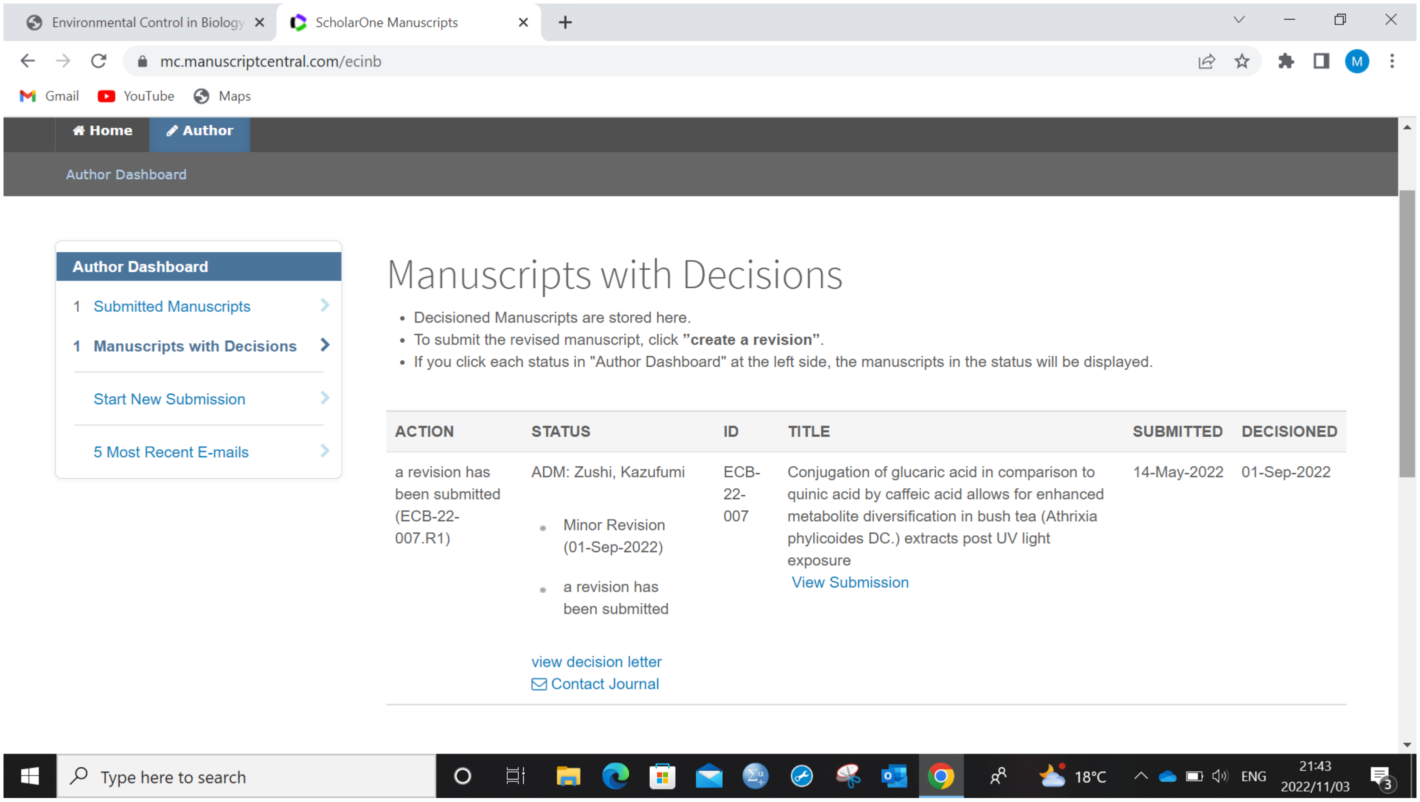
1. Introduction

Bush tea is one of the Asteraceae plants which contain antioxidants (Mogotlane et al., 2007), tannins (Mudau et al., 2007; Chabeli et al., 2008), and 5-hydroxy-6,7,8,3′,4′,5′ -hexamethoxy flavon-3-ol (Mashimbye et al., 2006), 5,6,7,8,3′,4′-hexamethoxyflavone, quercetin, 3-0-demethyldigicitrin, and other polyphenols (Mudau et al., 2006, 2007; Tshivhandekano et al., 2018). Other cinnamic acid containing molecules such as chlorogenic acids (CGAs) have been noted in this plant (de Beer et al., 2011). Plants absorb light from the sun and utilize it to produce high levels of oxygen and secondary metabolites through photosynthesis (Ghasemzadeh et al., 2010). However, light is also capable to regulate plant growth and development, plant photosynthesis rate and biosynthesis of both primary and secondary metabolites (Ramakrishna and Ravishankar, 2011, Radušienė et al., 2013). Due to excessive sunlight synonymous with areas where bush tea plant grows, it is expected that some of these metabolites (especially the hydroxyl-cinnamic acid containing molecules) will form geometrical isomers (Clifford et al., 2008), thereby representing a chemical shift of which the biological consequence is unknown. Therefore, understanding plant chemistry is essential for identification of new bioactive compounds. The chemical constituent of an organism is referred to as a metabolome (Ncube et al., 2014). Metabolome complexity is governed by several factors such as isomerization (positional and geometrical) (Clifford et al., 2005; Masike et al., 2018) and differential chemical conjugation (Masike et al., 2017; Nengovhela et al., 2021).

* Corresponding authors.
E-mail addresses: Ntakadzeni.madala@univen.ac.za (N.E. Madala), mudauf@ukzn.ac.za (F.N. Mudau).

https://doi.org/10.1016/j.scienta.2022.111124
Received 28 September 2021; Received in revised form 18 March 2022; Accepted 7 April 2022
Available online 12 April 2022

Appendix D: Manuscript has been submitted to Environmental control in biology and is under review with minor corrections.



Appendix E: Field experiment at the University of Venda's experimental farm situated in Thohoyandou (Vhembe district)