The effect of various selected shade nets on field-grown bush tea's physicochemical and antioxidant properties (*Athrixia phylicoides* DC.)

By

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DECLARATION

I, Khuthadzo Ramenu of student number: 14006979, hereby declare that this dissertation for the Master of Science (MSc) degree in Agriculture (Horticultural Sciences) submitted to the department of Plant and Soil Sciences, Faculty of Science, Engineering and Agriculture, University of Venda, has not previously been submitted to any university for any other degree. I further declare that this is my own work in design and execution and that all reference material contained herein has been duly acknowledged.

Student: Ms K Ramenu	Ramenu	08/03/2023
	Signature	Date

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DEDICATION

Being young does not conclude that someone has no potential and unrevealed potentials are not dreams denied, I dedicate this dissertation to my late grandmother Masindi Ramenu who took education as priority to succeed in life, my father Thitevhelwi Edwin Ramenu who passed away during my study period for he always supported my goals, and to my mother Shonisani Maria Ramenu who always want to see me successful.

ABSTRACT

Bush tea (*Athrixia phylicoides* DC.) is an indigenous herbal tea plant in South Africa with medicinal potential. Various parts of this plant are used to make herbal infusions and have been used traditionally for different purposes, including treating other diseases owing to the presence of pharmacologically active metabolites and antioxidants that are believed to be the active ingredient in the plant. However, knowledge of the manipulation of agronomic practice to enhance the accumulation of physicochemical antioxidant properties is not yet present. Therefore, this research was aimed at evaluating the effects of various shade nets colours and light intensities on the physicochemical, polyphenols and antioxidant activities of field grown bush tea leaves. The field experiment was conducted during the 2018 and 2019 autumn seasons at the University of Venda, School of Agriculture. Three replicates of the experiment were conducted using a Randomized Complete Block Design (RCBD) with three distinct degrees of light intensity (i.e., 40%, 50%, and 80%), three shade nets (specifically, black, green, and white) and full sunshine (control, 100%). The individual plots were each 4.8 m x 4.8 m in size. There were 24 plants per plot, with each plot having four plant rows spaced 1.2 m apart and 0.75 m apart within each row. When necessary, a drip watering system was applied to irrigate the plots except during rainy periods. When necessary, weeding was done manually using a hand hoe throughout the cropping seasons. To protect the plants from termites, methamidophos was sprayed at a concentration of 10 mL per 20 L immediately following each weeding session. At the end of the season, the leaves were harvested by cutting the base of the stem and placed inside brown paper bags and stored in the shade at room temperature to allow shade drying process as it is the best method for retaining bioactive compounds and phytochemicals. The leaves were detached from the stems after drying, by the process of threshing and the leaves were ground using the Retsch miller machine. The parameters such as moisture, ash, dry matter, vitamin C, and colour attributes (a*, b*, c*, H° and ΔE) were analysed. In addition, parameters such as chlorophyll content a, chlorophyll content b, total chlorophyll content (TCC), total flavonoid content (TFC), total phenolic content (TPC), as well as antioxidant activities following ABTS and FRAP methods were analysed using a spectrophotometer. For each treatment, the samples were analysed three times. Analysis of variance was conducted in all the collected data using the statistical package SAS version 9.4 statistical software and student's t-LSDs (Least Significant Differences) were computed at 5%, comparing the means of substantial source effects at a considerable level. The analysis of Pearson's Correlation Coefficient was used to establish the association between the climatic factors and nutritional compositions, polyphenols, antioxidants activities, chlorophyll contents and colour attributes of bush tea.

The results revealed that different shade nets colours and light intensities significantly ($p < 0.0001$) influenced the nutritional compositions (moisture, ash, dry matter, vitamin C), polyphenols (TPC and TFC), antioxidants activities (ABTS and FRAP) and physicochemical properties (colour attributes, chlorophyll a, chlorophyll b, and TCC) of field grown bush tea in both years 2018 and 2019. Black shade net colour had a noteworthy influence on total chlorophyll content, chlorophyll a content, and chlorophyll b content. In contrast, green shade net influenced on H° values and the white shade net on TFC. In contrast, the use of shade nets colours had no effects on dry matter (92.86%), vitamin C (3.03 mg AA/100g), or colour attributes [b* (20.65) and c* (20.65)] values of bush tea as they were highly significant at control. The use of 80% light intensity, significantly influences the moisture content, chlorophyll a, chlorophyll b and total chlorophyll content and H°. However, control had a higher influence on dry matter, vitamin C, b*, c* and FRAP results. A very strong, strong, and moderate positive correlation was observed between the climatic factors and nutritional compositions, polyphenols, antioxidants, and chlorophyll contents of field-grown bush tea, under the influence of shade nets colours and light intensities during autumn 2018 and 2019. However, colour attributes: a*, b* and H° resulted in weak/ no correlations. This study is the first to demonstrate different shade nets colours and light intensities as the key factor of accumulating of chlorophyll contents and antioxidants in field-grown bush tea plants. The use of a black shade net together with 80% light intensity resulted in being the best treatment to promote the accumulation of antioxidants compared to other treatments. And it is evident that black shade net together with 80% light intensity was of significant influence after control (100% light intensity). Future studies will be to determine the regulatory effects of shade treatments on the biosynthetic pathway of flavonoids and the accumulation pathways of chlorophyll contents under the shade nets treatment.

Keywords*: bush tea, polyphenols, physicochemical, antioxidants, light intensity, shade net colour.*

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
CE	Catechin equivalent
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
TCC	Total Chlorophyll Content
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
Univen	University of Venda

CHAPTER ONE

1.1. Introduction

Bush tea belongs to the family Asteraceae and the genus Athrixia contains 14 species that can be found in Madagascar, Southern Africa, tropical Africa (Van Wyk and Gericke., 2000; Herman *et al*., 2000; Joubert *et al*., 2008). According to Leistner (2000), South Africa comprises nine of these species, which are still collected from the forest. However, because of the possible advantages that it may provide, primarily resulting from its traditional applications, studies have been concentrating on the indigenous species *Athrixia phylicoides* (Rampedi and Olivier, 2005). *Icholocholo*, *Itshelo* and *Umtshanelo* are frequent Zulu names for bush tea. *Mohlahlaishi* in Pedi, *Mutshatshaila* in Tshivenda, *Boesmans tee* in Afrikaans and in English bushman's tea (Lehlohonolo, 2013; Lerotholi, 2017).

Bush tea contains no caffeine but abundant antioxidants (McGaw *et al*., 2007). A desired feature of a healthy beverage is that it should not contain caffeine, as this implies the existence of antioxidants, which may have health advantages (McGaw *et al*., 2007). Compared to other teas, bush tea also has a low tannin level, preventing the bitter, astringent flavour (Chabeli *et al.,* 2008). Moreover, this gives bush tea a distinct flavour advantage over normal green and black tea derived from *camelia sinensis*. Furthermore, 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 (Mavundza *et al*., 2010) are found in bush tea leaves, along with tannins (Mudau *et al*., 2007a), antioxidants (Mogotlane *et al*., 2007), polyphenols (Mudau *et al*., 2006, 2007a), and has cytotoxic activities (McGraw *et al*., 2007).

Minimizing the intensity of solar radiation and altering other microclimatic parameters such as temperature, dew, frost, humidity and heat balance to mention few, shade nets may impact plant growth (Mahmood *et al*., 2018). Shade nets of different colours (white, green, black, pearl, red, blue, and black) are used in agriculture to determine their effects. Even though black nets are more frequently utilised, photo-selective nets are becoming more popular. Consequently, they can modify both the quality and amount of light; photo-selective nets may impact on plant growth, yield, and fruit quality (Arthurs *et al*., 2013; Fallik, 2009; Ilic *et al*., 2017). Shade nets decrease solar radiation regardless of net colour in relation to a decrease in air, plant, and soil temperatures.

Ilić *et al*., (2018) state that photo-selective nets or films are being used in horticulture to manipulate light quality to increase yield, quality, and phytochemical components. According to Huché-Thélier *et al*., (2016), mild shade could enhance photosynthesis, resulting in increased yields and quality due to a greater quantity of carbohydrates and more efficient water usage. A new emerging agronomic method that uses photo-selective shade netting technology that combines physical crop protection with various sunlight filtrations has been recently practiced. Al-Helal *et al*., (2010) also noted that the radiometry properties are determined by the porosity and colour of the nets, but that the net color affect light reflection. In addition, besides that the influence extends beyond how much sunshine is received; photo-selective netting can alter the radiation's direction by converting from direct to diffused light (Al-Helal *et al*., 2010) and entry into the plant canopy's interior. According to Stamps (2009) and Gu *et al*., (2002), photo-selective netting can be utilized for pest control, prevent greater heating or burning, and give a mild cooling benefit. Al-Helal *et al*., (2010) reported that harvesting season can be extended by using photo-selective colored net technology and agricultural crops' agronomic-economic performance in general, also more yield, and improved product quality. Fallik *et al*., (2008) researched the use of nets that are photo-selective regarding many vegetables, ornamentals (Fallik *et al*., 2008, Ada *et al*., 2008) and fruit trees (Shahak *et al*., 2004).

Biotic and abiotic variables have been found to affect the content of chemical compounds like total flavonoids and phenolics (Fine *et al*., 2006). Therefore, tea quality can be determined by the quantity of polyphenols, catechins, caffeine, flavour, taste, amino acids, and ash (Adnan *et al*., 2013). Tea with high ash levels, total polyphenols, total flavonoid content, and vital minerals will therefore have a high overall quality rating (Malongane *et al*., 2020).

1.2. Problem statement

Bush tea is a herbal tea that contains phytochemicals which influence the quality of the tea. Those phytochemicals have different antioxidant activities, giving the tea medicinal potential. Consequently, there has been a great demand for bush tea by both communities and researchers. It has been suggested that variations in seasonal temperatures and vapour pressure deficit may affect the quality and antioxidants of bush tea since it is harvested from the wild. Moreover, there is a limitation on modification of agronomic practices to enhance the accumulation of bush tea phytochemicals by manipulating the light quality using shade nets. Information about accumulation of phytochemicals by parameters like light quality, quantity and duration based on the shade net's colour and its factor on bush tea has yet to be reported.

1.3. Justification

Bush tea is an indigenous species with many potential benefits of which high antioxidants activities are one of the parameters which makes the researchers more interested in studying it further. The outcome of this study will provide both community and researchers with more knowledge about response of various selected shade nets on field-grown bush tea qualities. Shade nets help absorb direct light and enhance the amount of light passing into the plant canopy's interior. In addition, the colour of shade nets and its factors improves the nutritional value. It can result in various physiological responses in the plants, due to the type of plant, variety, and where the experimental farm is located.

1.4. Objectives

1.4.1. Main objective

Determination of the effect of various selected shade nets colours and light intensities on physicochemical and antioxidant properties of field-grown bush tea.

1.4.2. Specific objectives

1.4.2.1. To determine the effect of various selected shade nets colours and light intensities on nutritional composition (moisture, ash, and vitamin C) of field-grown bush tea.

1.4.2.2. To determine the effect of various selected shade nets colours and light intensities on physicochemical properties (total chlorophyll content, chlorophyll a content, chlorophyll b content and colour attributes) of field-grown bush tea.

1.4.2.3. To determine the effect of various selected shade nets colours and light intensities on polyphenols (total flavonoid and phenolic content) and antioxidant activities (following FRAP and ABTS assays) of field-grown bush tea.

1.5. Null Hypotheses (No treatment effect)

1.5.1. Various selected shade nets colours and light intensities have no effect on nutritional compositions of field-grown bush tea.

1.5.2. Various selected shade nets colours and light intensities have no effect on the physicochemical properties of field-grown bush tea.

1.5.3. Various selected shade nets colours and light intensities have no effect on the polyphenols and antioxidant properties of field-grown bush tea.

CHAPTER TWO: LITERATURE REVIEW

2.1. Introduction

The study investigates the effects of shade nets colours and light intensities on the accumulation of nutritional composition, polyphenols, antioxidants activities, and physicochemical properties that include colour attributes and chlorophyll contents of bush tea grown during autumn in year 2018 and 2019. This chapter contains the following: origin, classification and distribution of bush tea, plant description and morphology of bush tea; importance and uses of bush tea; antioxidants properties of herbal teas found in South Africa. The chemical/polyphenols compounds i.e., phenolic compounds and total phenolic contents, flavonoids and total flavonoid contents, the effect of shade drying, effects of shade nets on physicochemical and antioxidants properties, the effects of light on the physicochemical and antioxidants properties, and effects of light on the physicochemical and antioxidants properties.

2.2. Origin, classification, and distribution of Bush Tea

The genus *Athrixia* originated from the subtribe Athrixiinae, the tribe Inuleae from the family Asteraceae (Herman *et al.*, 2000; Mudau *et al*., 2007b). The phylicoides epithet particularly indicates its similarities to the Rhamnaceae family's Phylica genus, while the genus names Athrixia, which describes the leaves, derives from the Greek word thrix, which means hair (Mbambezeli, 2005). The genus *Athrixia* species endemic to southern Africa are *Athrixia angustissina*, *Athrixia elata*, *Athrixia ger-rardii*, *Athrixia hererophylla* and *Athrixia phylicoides* (Herman *et al*., 2000; Joubert *et al.*, 2008). According to Herman *et al*., (2000), Athrixia phylicoides is one of these species that is extensively dispersed in South Africa's eastern region (Figure 2.1), from the coast to the Drakensberg Mountain in KwaZulu-Natal and from the Soutpansberg mountain in Limpopo Province to Queenstown, King William's Town, and East London (Herman *et al*., 2000; Rampedi and Olivier, 2005).

In South Africa, bush tea adapts well to wide grassland and dense forest borders; these regions include KwaZulu-Natal, some portions of the Eastern Cape Province, Limpopo Province, the Free State Province, and the neighbouring country of Swaziland (Germishuizen *et al*., 2006; Mudau *et al.,* 2007b). According to Roberts (1990), good drainage, full sunlight, with adequate area for expanding plant branches are all necessary for good plant establishment.

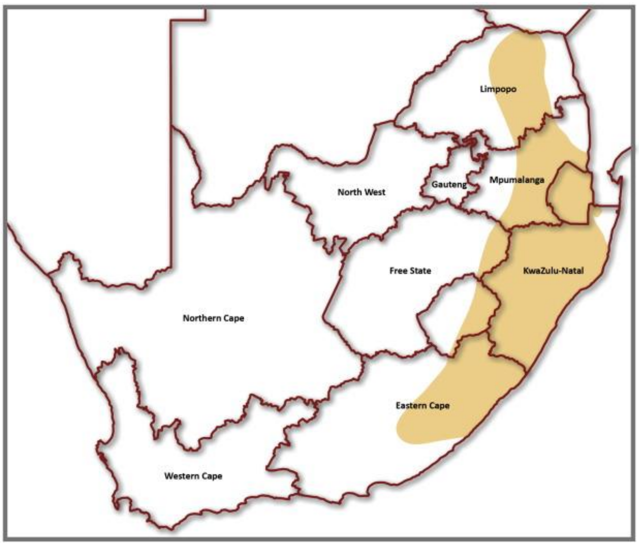


Figure 2.1. Distribution map of *Athrixia phylicoides* in South Africa Lerotholi *et al*. (2017)

2.3. Plant description and morphology of Bush Tea

Botanically, bush tea is a perennial, aromatised shrub with leaves and the plant can grow up to 1 m maximum height, with the appearance of woolly white, fluffy stems (Fox and Young, 1982; Joubert *et al*., 2008). The plant of bush tea (Figure 2.2) is described as grey-white and velvety below on stem, and fine, dark green and shining above (Roberts, 1990; Leistner, 2000). Characteristics such as tapering to a sharp point, simple and alternate linear to broadly lanceolate have been used to describe the bush tea leaves (Herman *et al*., 2000), with margins entirely or slightly revolute (Mudau *et al*., 2007b). Furthermore, the leaves at their base are often auriculate and shortly stalked (Roberts, 1990; Mudau *et al*., 2007b). The inflorescence head is sub sessile or sessile and terminal axillary in large sub corymbose panicles (Herman *et al*. 2000; Mudau *et al*. 2007b).

The bush tea flowers (Figure 2.3) are daisy-like; the petals differ from pink to all pink shades as well as pleasant purple colour and bright yellow centres or bright yellow disk florets, and due to region and edaphic factors; they can be available all year (Van Wyk and Gericke, 2000; Joubert *et al*., 2008). Bush tea consist of fruits with achenes of about 0.01 to 0.06 mm wider that are thin, cylindrical, and narrow (Araya, 2007). The seed uses 12 pappus for dissemination and the seed length is 4 mm (Araya, 2007). However, according to Lerotholi *et al*. (2017) the seed is 5 mm long and pappus are about 2 mm long (Figure 2.4). The literature needs to be more consistent regarding flowering in southern Africa. Carruthers (2000) found that bush tea plants produce flowers (Figure 2.3) from May to November, covering the autumn to summer seasons. Mbambezeli (2005) reported that flowers may bloom throughout the year, but March to May is when they do so most frequently. Yet, according to Roberts (1990), inland flowers begin to bloom in the middle of summer, while the blossoming period in coastal locations lasts from May to June this might have occurred because the coastal climates have wetter winters and drier summers. In contrast, the inland climates tend to have humid summers and drier winters. In addition, it can be concluded that bush tea flowering periods needs wetter or more humid conditions.

Figure 2.2. Roadside bush tea plants in Witvlag, Limpopo Province, South Africa (Tshikhudo *et al*., 2019).

Figure 2.3. *Athrixia phylicoides* flower (Lerotholi *et al*., 2017).

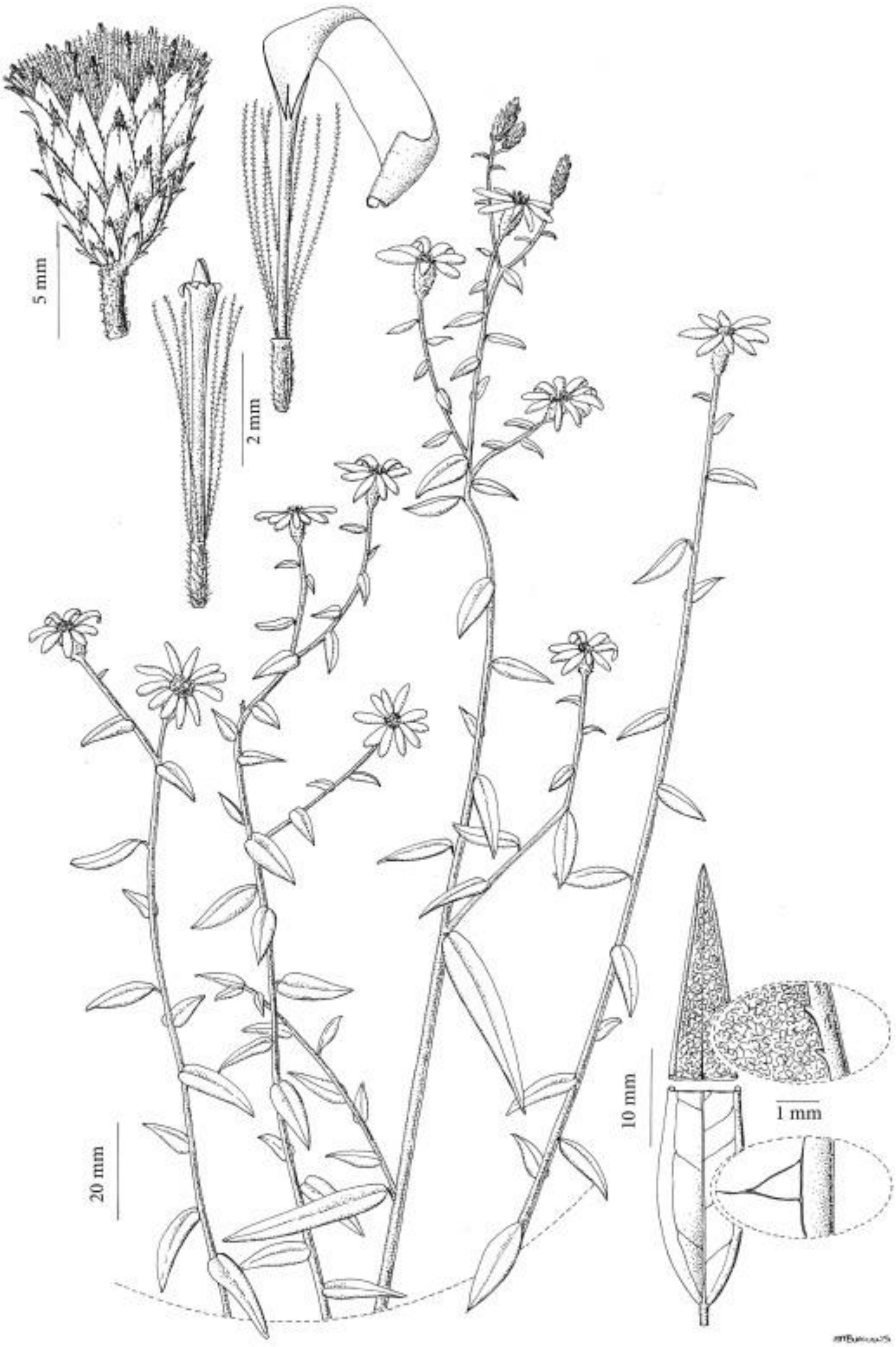


Figure 2.4. Botanical illustration of the aerial parts of Bush tea (Lerotholi *et al*., 2017).

2.4. Bush tea's importance and uses

In South Africa, bush tea leaves have been utilised for a long time as a traditional herbal medicine (van Wijk, 1986; Mudau *et al*., 2007b). Yet, one of the most popular uses for bush tea is the production of brooms, which involves the harvesting of long branches, the removal of their leaves, and the binding of the stems to create a broom handle that is comfortable to hold (Van Wyk and Gericke, 2000; Rampedi and Olivier, 2005). Large bundles of the cut branches are therefore bundled together, transported home, and either stored in a dry location or affixed to the roof beams. Bush tea hasn't gained as much recognition as other locally grown South African herbal teas with commercialization feasibility (Rampedi and Olivier, 2005). According to Mudau *et al*., (2007b), bush tea can be domesticated and developed into a marketable health beverage.

Various South African ethnic groups have historically utilised different bush tea parts for medical and domestic uses (Van Wyk and Gericke, 2000). Xhosa and Sotho tribes chew the leaves to treat coughing, colds and swelling throat (Mbambezeli, 2005; Roberts, 1990; Mudau *et al*., 2007b), and the strong brew is also used by Sothos as a soothing foot wash for painful feet (Roberts, 1990; Mudau *et al*., 2007b). The roots decoction of bush tea is used as purgative and remedy for coughing by Zulu people (Van Wyk and Gericke, 2000; Mbambezeli, 2005). The soaked leaves and roots form an extract used as an aphrodisiac by Venda people (Mabogo, 1990; Van Wyk and Gericke, 2000; Mudau *et al.,* 2007). The unmarried Venda boys are prohibited from consuming these root infusions that are said to arouse sexual cravings (Hutchings *et al*., 1996). Root and leaf extracts treat an anthelmintic in the Vhembe district. (Mabogo, 1990; Mbambezeli, 2005; Mudau *et al*., 2007b).

Decoctions are gargled to cure the loss of voice and prevent throat diseases (Roberts, 1990). For decoctions and infusions, leaves and twigs are boiled in water for about 5 minutes; after filtering, the delicious beverage is typically sipped without milk or sugar is added (Rampedi and Olivier, 2005). Rural people also frequently use decoctions to treat diabetes, diarrhoea, vomiting, hypertension, circulatory problems, and heart problems, while root decoctions are used as purgatives. The traditional cleansing of the womb, kidneys, veins, blood purification, and infusions to treat stomach aches, influenza, and leg wounds all point to the detoxifying properties of bush tea (Rakuombo, 2007). It is used to treat boils, headaches, infected wounds, and cuts (Roberts, 1990; Joubert *et al*., 2008; Mudau *et al*., 2007b). It is further used to cleanse the skin and is smeared as a lotion to treat skin rashes and lessen acne (Joubert *et al*., 2008). The bush tea uses are summarised in Figure 2.5 below.

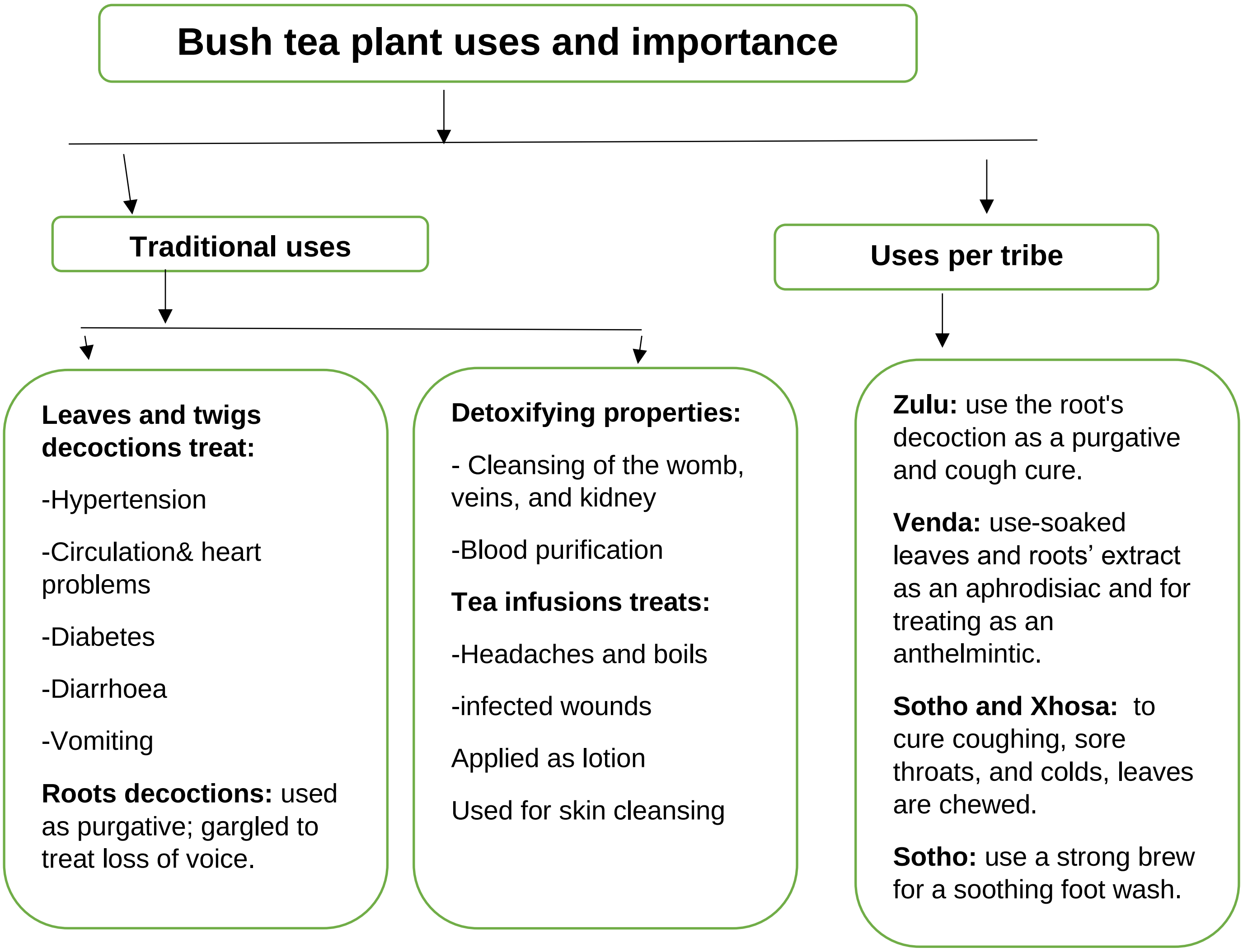


Figure 2.5. Summary of bush tea uses/ importance

2.5. Antioxidant properties of herbal teas found in South Africa

Reactive oxygen species (ROS) is a term used to describe oxygen-derived free radicals namely the nitric oxides, hydroxyl radicals and the superoxide anion that are accountable for degenerative illnesses. These ROS are known to be significantly neutralized by antioxidants (Malongane *et al*., 2017). All tea varieties and herbs carry bioactive compounds that are acknowledged to defend bodily tissue from oxidative stress, including free radicals made from oxygen and products of lipid peroxidation (Ryan and Petit, 2010). In addition, it is important to maintain balance of antioxidants so that they can interact with free radicals. The presence of antioxidants in teas is highly beneficial as compared to their presence in many plant species. Thus, the concentrations of antioxidants, the concentration of internal antioxidants and the availability of other antioxidants determine whether an antioxidant is harmful or beneficial

(Villanueva and Kross, 2012). Compounds with high antioxidant content have the potential to defend biological processes from the damaging effects of severe oxidization, which include the reaction of nitrogen and oxygen species (Mogotlane *et al*., 2007). Moreover, Mogotlane *et al.,* (2007) detailed that antioxidant content is a common criterion to characterise various materials for health benefits. Mathivha and Mudau (2017), found that bush tea had a low total antioxidant activity of which ABTS resulted (58. 34 µmol TE/g) and 77. 17 µmol TE/g of DPPH) compared to special tea which had 197. 46 µmol TE/g of ABTS; and a DPPH of 109. 13 µmol TE/g).

2.6. Chemical compounds of bush tea and other herbal teas found in South Africa

2.6.1. Phenolic compounds

Phenolic chemicals or compounds are a sizable group of diversely structured plant secondary metabolites. The structures can range from easy molecules such as phenolic acids, through polyphenols like flavonoids, to polymeric compounds (Kala *et al*., 2016). A structure of a simple phenolic molecule or a large high-molecular-weight polymer can be found in phenolic compounds, which have an aromatic ring with one or more hydroxyl groups (Ferrezano *et al*., 2011). Figure 6 shows diverse types of phenolic compounds. Bush tea leaves are a high source of polyphenols, which are possible quality indicators and significant antioxidants naturally (Mudau *et al*., 2007a). Bush tea has high polyphenol concentrations, the main identifier of a herbal teas' antioxidant potential (Mudau *et al*., 2007a). In comparison to the sun (6.42 mg/100 g) and oven (5.62 mg/100 g) dried samples of bush tea leaves, Mudau and Ngezimana (2014) discovered that total phenolics were greater in the shade (8.34 mg/100 g) and freeze (8.34 mg/100 g) dried bush tea. Determination of total flavonoid content and total phenolic content on different herbal teas found in South Africa namely bush tea, honeybush tea, special tea and rooibos has been done thus, Malongane *et al*. (2020) found that special tea had the highest TPC (1.10 mg GAE/g), followed by bush tea (0.75 mg GAE/g), rooibos (0. 60 mg GAE/g) and lastly honey bush tea (0. 48 mg GAE/g).

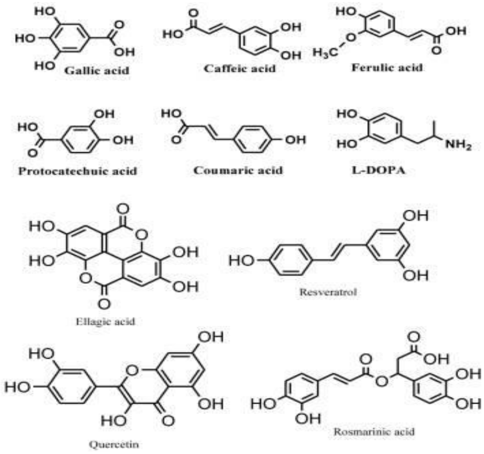


Figure 2.6. Common phenolic compounds in plants include an aromatic ring with one or more hydroxyl substituents, simple phenolic molecules, and compounds that have conducted significant polymerization (adapted from Velderrain-Rodrguez et al., 2014).

2.6.2 Flavonoids content

Throughout the plant kingdom, flavonoids that are polyphenolic compounds are produced as secondary metabolites. Moreover, these compounds are found in foods, ingredients, and medications made from plants, and have generated a great deal of interest due to their potential abilities to prevent a wide range of degenerative diseases (Nishiumi et al., 2011). Di[MS1]phenylpropanoids is a well-known group of flavonoids and is made up of one or more phenolic groups (Figure 2.7). Consequently, it has been determined that innumerable compounds found in plants' food are flavonoids, and they can be classified into different subclasses, together with flavones, flavanols, flavan-3-ols (catechins), flavanones, isoflavones, anthocyanidins, as well as some other minor components such as chalcones, aurones and coumarins (Nishiumi *et al*., 2011). Bush tea contains some of these flavonoids as shown in Figures 2.8 and 2.9 (Mavundza *et al.*, 2010; Mashimbye *et al.*, 2006). Apart from identifying compounds, other studies have been done to determine the total flavonoid content (TFC) of herbal teas found in South Africa thus the highest TFC value was found on special tea (12.311 mg QE/g), then rooibos (9.717 mg QE/g), honeybush tea (8.831 mg QE/g) and bush tea had the lowest value (7.820 mg QE/g) (Malongane *et al*., 2020).

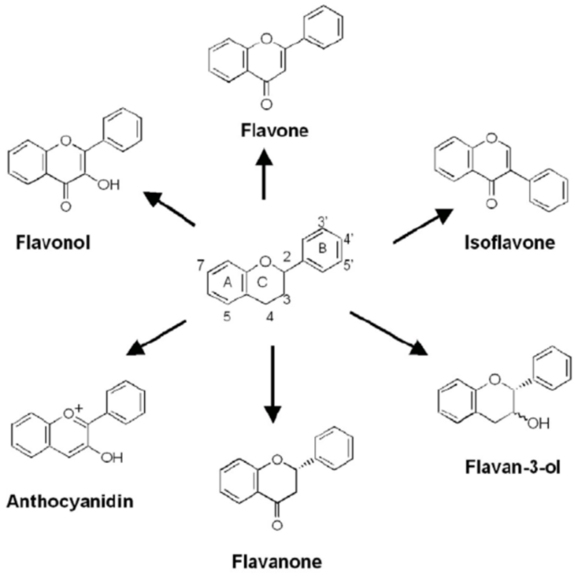


Figure 2.7. Structures of different Flavonoids (Nishiumi *et al*., 2011).

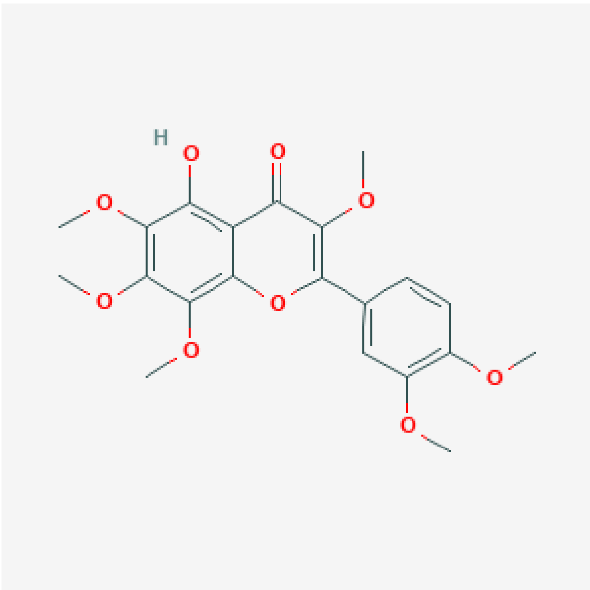


Figure 2.8. 5,6,7,8,3',4'-hexamethoxyflavone (Mavundza *et al.*, 2010).

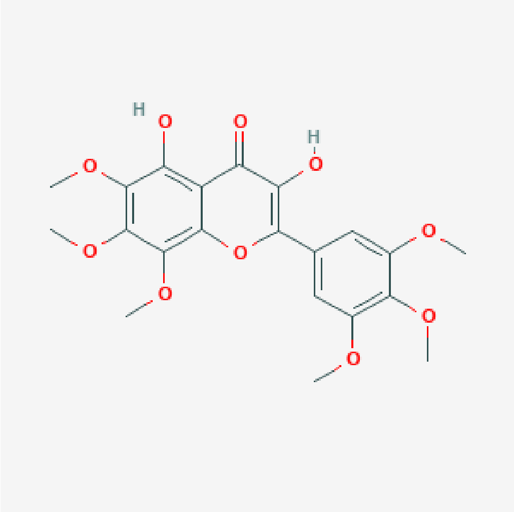


Figure 2.9. 5-hydroxy-6,7,8,3’,4’,5’-hexamethoxy flavon-3-ol (Mashimbye *et al.*, 2006).

2.7. Shade drying

In a shady area, shade drying utilises ambient air with ambient humidity and sufficient ventilation to conduct the UV-free drying process (Barek *et al*., 2015). In addition, due to the ability to save light-sensitive materials and lessen light-induced chemical processes like oxidation, the shade drying approach may offer advantages over sun drying (Thamkaew *et al*., 2021). However, shade drying takes longer to dry than sun drying (Pirbalouti *et al*., 2013). Furthermore, according to Abioye *et al*. (2014), shade drying of leaves takes longer than other natural drying processes. Still, it has the potential to retain most nutrients due to considerable variations in the concentrations of minerals, ascorbic acid, and proximate compounds. Nonetheless, shade drying is still well-known in rural areas or small businesses due to its cheap initial cost and high-quality dried goods (Janjai and Bala, 2012). Shade drying also demonstrated good bioactive compounds retention in dried herbs like misai kucing (*Orthosiphon aristatus*) (Abdullah *et al., 2012*) as well as in bush tea (*Athrixia phylicoides)* (Mudau and Ngezimana, 2014).

2.8. Effects of shade nets on physicochemical and antioxidants properties of teas

Using shade nets changes the light quality being received by plants; thus, changes may occur in crop biochemical and physiological processes, metabolite profiles, and quality. The polymerization of catechins and glycosylation of flavonol that are essential mechanisms of flavonoid biosynthesis in tea leaves, have been documented to be affected by shading treatment (Wang *et al*., 2012). High light intensity has a negative impact particularly on shade plants though, biosynthesis of phenolic compounds and formation of flavonoid depend on light intensity and density (Xie *et al*., 2013). When comparing open and shade cultured green tea, Ku *et al*., (2010) discovered that the antioxidant activity of the green tea samples was highly linked with their total flavonoid and total phenol contents. Shading describes the character of the tea by slowing photosynthesis, enhancing chlorophyll content, and gaining a high level of theanine, the leaves become dark green, and the tannin concentration is decreased, releasing out a sweeter flavor rather than the often-sour taste of green teas (Juneja *et al*., 1999). This phenomenon is related to a change in chlorophyll and other compounds when leaves are shaded from the sun by netting in the days or weeks before harvest (Goodwin, 2012). Manja and Aoun (2019), reported that selecting and managing shade trees are useful tools for effective pest management. Still, they depend on selection of components (species, varieties, provenances) and site characteristics (soil and climate), below-ground and above-ground traits of the trees and crops and management techniques. *Mycena citricolor and Phythphora palmivora* are commercially significant diseases and pests that may be more prevalent with increased shade while *Colletotrichum gloeosporioides* and *Cercospora coffeicola* may be less prevalent (Beer *et al.*, 1997). Black tea produced under artificial shade had higher theaflavin and decreased thearubigin concentrations as well as an outstanding flavor score and taste evaluation compared to tea produced in open field (Lehlohonolo *et al*., 2013).

2.9. Effects of light on the physicochemical and antioxidant properties of medicinal plants and teas

It has been documented that environmental parameters such light intensity, temperature, water availability, type, and soil composition affect the quality and productivity of medicinal plants Radušienė, 2012). According to Nchabeleng (2012), the sub-groups of flavonoids in tea might be affected differently depending on the light intensity. By photosynthesis, plants absorb sunlight and utilize it to generate increased amounts of oxygen and secondary metabolites (Ghasemzadeh *et al*., 2010). Stagnari (2014) discovered that the total phenolic compounds in red beet were altered by higher percentage of PAR's transmittance under the photo-selective coatings. However, Bergquist *et al*., (2007) demonstrated that the build-up of flavonoid concentrations in baby spinach was affected by the low-light transmittance under the shade nets. High blue and red-light ratios beneath pearl photo-selective nets over the winter season had an impact on the build-up of isorhamnetin, quercetin, and myricetin in the loose lettuce cultivars such as "Exbury," green loose lettuce "Ashbrook," and "Aquarell" (Mashabela *et al*., 2015). Also, after stored for 14 days, fresh-cut coriander, marjoram, and basil produced under red photo-selective nets had higher antioxidant, flavonoid (quercetin), and total phenolic compound scavenging activity (Ntsoane *et al*., 2016). According to Buthelezi (2015), red, blue, or UV-A light may induce the production of phenolic compounds and an increase in antioxidant activity.

2.10. Conclusion and summary

The current review has generally evaluated the crucial characteristics of bush tea and other herbal teas and factors that affect their growth along with quality that influences the accumulation of nutritional compositions, polyphenols, antioxidants activities, chlorophyll contents and colour attributes. In addition, the bush tea plant information has been reviewed into details. The review has further shown that environmental parameters such light intensity, temperature, water availability, shade netting, kind, and soil composition have been observed to influence the quality and yield of medicinal plants.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study site

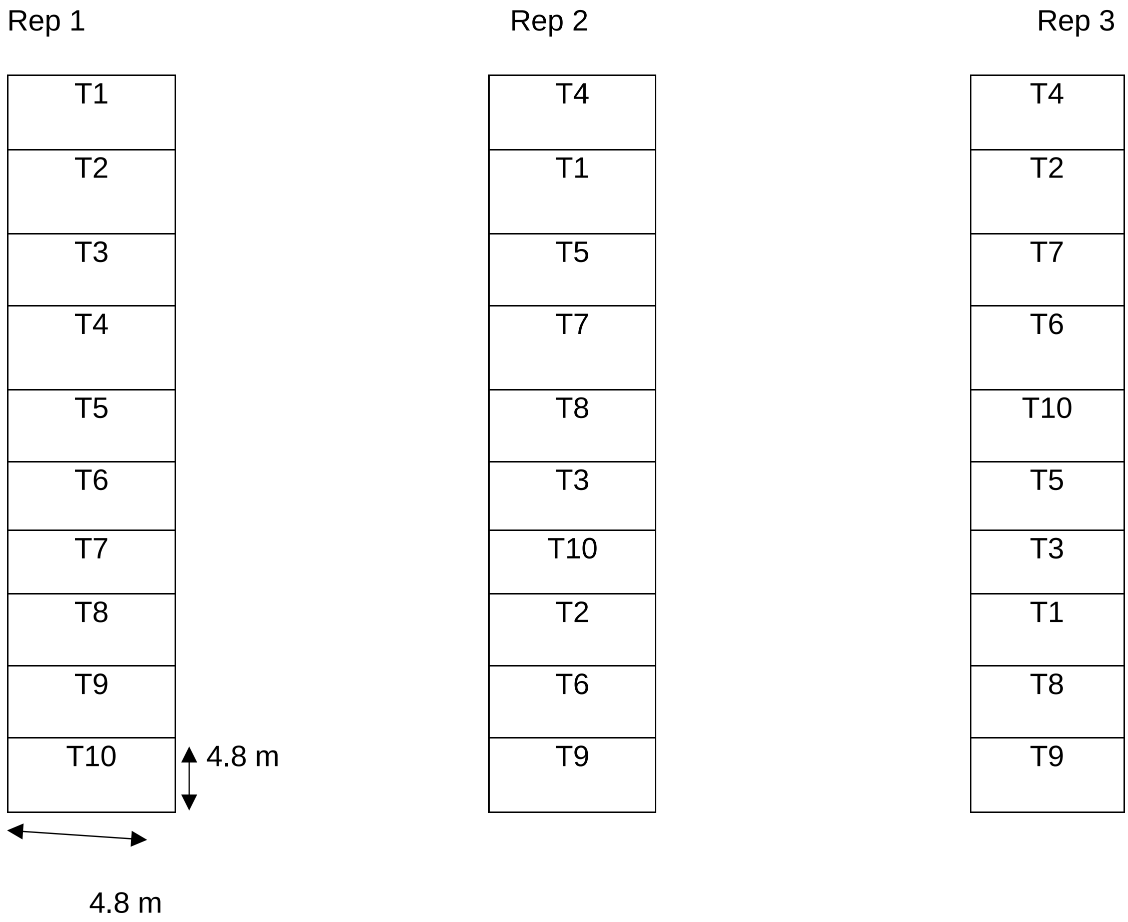
The study took place at Agriculture experimental farm at University of Venda in Thohoyandou, Vhembe district (Figure 3.1). The site is situated at longitude and latitude of 22°58.081 S and 30°26.411 E. With an annual rainfall of less than 500 mm and temperature variations between 10°C in the winter and 40°C in the summer, the climate is described as arid and semi-arid (receives precipitation below potential evapotranspiration). Moreover, Odhiambo (2010) found that the area has deep, well-drained clay soil. Average daily weather data by month for the following climatic factors: maximum temperature (°C), minimum temperature (°C), humidity (%), rainfall (mm) and wind speed (km/h) in Table 3.1 were provided by South African Weather Services (SAWS, 2021) for cropping season of 2018 and 2019.

Figure 3.1. Study area (Agricultural farm in Univen, Thohoyandou, Limpopo province, South Africa). Blue line indicates the university demarcation whereas the purple rectangle shows the site where the experiment was carried out.

Source: Mapcarta, 2022

3.2. Experimental lay out

Three replicates of the experiment were conducted using a Randomized Complete Block Design (RCBD) (Figure 3.2) with three replicates from three distinct degrees of light intensity (i.e., 40%, 50%, and 80%), three shade nets (specifically, black, green, and white) and full sunshine. The individual plots were each 4.8 m x 4.8 m in size (Figure 3.2). There were 24 plants per plot, with each plot having four plant rows spaced 1.2 m apart and 0.75 m apart within each row.



T1=Black 40 %, T2=Green 40 %, T3=White 40 %, T4=Control, T5=Black 50 %, T6=Green 50 %, T7=White 50 %, T8=Black 80 %, T9=Green 80 %, T10=White 80 %

Figure 3.2. Field experimental design

3.3. Cultural practices and experimental management protocols

The land was ploughed by disc plough connected to the Tractor. The field was established from the 3 months rooted stem cuttings and were supplemented with the nitrogen-phosphorus-potassium (N-P-K) fertilizer. Moreover, the application was done during transplanting and, also two weeks after transplanting and the application rates of N at 300 kg/ha, P at 300 kg/ha, and K at 200 kg/ha were done following Mudau *et al*. (2007). When necessary, a drip watering system was applied to irrigate the plots except during rainy periods. In addition, the irrigation pipes were checked and aligned monthly to allow quality irrigation. When necessary, weeding was done manually using a hand hoe throughout the cropping seasons. To protect the plants from termites, methamidophos was sprayed at concentration of 10 mL per 20 L immediately following each weeding session.

Table 3.1. Average of the daily maximum temperature (°C), minimum temperature (°C), humidity (%), rainfall (mm) as well as wind speed (km/h) by month during autumn season at year 2018 and 2019 at Thohoyandou (Univen) from South African Weather Service (SAWS, 2021).

	Autumn season							
	February		**March**		**April**		**Average (s)**	
Climatic factors	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
Daily maximum temp (°C)	28.9	30.1	28.9	30.0	27.6	27.7	28.5	29.3
Daily minimum temp (°C)	20.4	20.1	18.7	19.8	16.6	17.2	18.6	19.0
Humidity (%) at 08 am	85.0	82.0	83.0	84.0	84.0	86.0	84	84
Humidity (%) at 14 pm	62.0	59.0	57.0	54.0	54.0	56.0	57.7	56.3
Humidity (%) at 20 pm	75.0	72.0	76.0	71.0	79.0	78.0	76.7	73.7
Average daily Humidity (%)	74.0	71.0	72.0	69.7	72.3	73.3	72.8	71.3
Rainfall (mm)	287.2	182.8	18.8	22.6	44.8	28.0	116.9	77.8
Wind speed (km/h) at 08 pm	2.4	1.4	1.8	1.7	1.9	1.6	2.0	1.6
Wind speed (km/h) at 14 pm	2.5	2.4	2.2	2.5	2.1	2.0	2.3	2.3
Wind speed (km/h) at 20 pm	1.9	2.0	1.9	1.8	2.0	1.6	3.0	18
Average daily wind speed (km/h)	2.3	1.9	2.0	2.0	2.0	1.7	2.4	1.9

3.4. Materials that were used in the study

The Retsch miller machine (*SM 100)* was obtained at Univen, animal science laboratory. The Ultrasonic bath, Oven, Muffle furnace, Centrifuge machine (*Universal 320 R*), Spectrophotometer *(DR2800, Hach, USA)*, Lovibond colour machine (*LC 100 Spectro colorimeter*) was obtained at Univen, Food Science and Technology laboratory. The chemicals and reagents were purchased from Sigma-Aldrich SA (pty) ltd (Kempton Park, South Africa).

3.5. Sample collection, preparations, and analysis

The samples were collected from three distinct degrees of light intensity (i.e., 40%, 50%, and 80%), three shade nets (specifically, black, green, and white) and unshaded treatment (control, with100% light intensity). The samples were collected by cutting the base of the stem using the pruning shear and placed in brown paper bags and stored in the shade for drying process at room temperature and, the shade drying process was used as it is best in retention of bioactive compounds and phytochemicals. The leaves were detached from the stem after drying by process of threshing. Placed inside brown paper bags, and then dried under the shade at a room temperature for two months to allow drying before analysis. The leaves were ground using the Retsch (*SM 100*) Miller machine until 10 g of uniform powder was formed. The ground powder was poured inside polyethylene plastic bags and the plastics were sealed. Therefore, the polyethylene plastic bags were packed inside brown paper bags until the samples were used.

3.6. Nutritional composition of the bush tea leaf powder

3.6.1. Moisture content

The moisture content was evaluated using AOAC (2016): method 934.01. Dry coded, clean crucibles was placed in an oven drier for about 30 min, cooled and weighed. The weight of the samples was weighed into the crucibles. About 2 g of tea powder was separately weighed into crucibles in triplicate. The samples were dried at 105 ºC for 24 h. The percentage of moisture was calculated using equation 1.

$$Moisture\ (\%) = \frac{w2 - w3}{w2 - w1} \times 100 \qquad (1)$$

Where:

W1= weight of empty crucible

W2= weight of crucibles and tea powder sample before drying

W3= Final weight of crucibles and tea powder sample after drying

3.6.2. Ash content

AOAC (2016): Procedure 923.03 was used to measure the ash content. Two grams of powdered tea samples were individually weighed into triple crucibles, and the samples were then burned at 550°C for 24 hours in a muffle furnace until a pale grey ash was visible. The samples were weighed to get the ash content after cooling in the desiccator to prevent moisture absorption. The ash content was calculated using equation 2:

$$Ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100 \qquad (2)$$

3.6.3. Dry matter content

The dry matter had been calculated and expressed in percentage by equation below:

$$100 - moisture\% \qquad (3)$$

3.6.4. Vitamin C content

The extract of bush tea leaves powder was done following Kumar *et al*., (2013). About 4 g of powder tea sample was weighed into a 500 ml beaker, 100 ml of distilled water was poured, thereafter the beaker was shaken until the sample dissolves. The beakers containing sample were placed in ultrasonic bath (model:703) at 25°C for 30 min. Afterwards, the sample were transferred into centrifuge tubes and centrifuged for 10 min at a speed of 3000 rpm. The centrifuged samples were filtered using qualitative filter paper (150 mm) to get vitamin C extract. Vitamin C was measured by titration procedure with iodine solution according to Helmestine (2015). The burette was filled with 0.025 M iodine solution. Approximately 25 ml of bush tea extract was added into 250 ml beaker for each sample. Five drops of 1% starch were added, and titration took place using iodine solution in the burette until the tea extract changes to black colour. The samples' vitamin C concentration was reported as mg AA/100 g wet basis.

3.7. Determination of polyphenols and antioxidant activities of bush tea leaf powder

3.7.1. Extraction of bush tea leaf powder

About 2 g of finely ground sample was added into 500 ml beaker and 20 ml of 1% methanolic acid was added and then sonicated at 25°C for 15 min in an ultrasonic bath. The sample was poured into centrifuge tubes and at a speed of 3000 rpm, the samples were centrifuged for a period of 10 min and thereafter filtered using qualitative filter paper (150 mm) to obtain an extract.

3.7.2. Polyphenolics of bush tea leaf powder

3.7.2.1. Total Phenolic Content (TPC)

The TPC was evaluated spectrophotometrically using a process described by Lee *et al*. (2015). An extract of 0.5 ml was transferred into laboratory tubes. Folin-Ciocalteu reagent of about 1.5 ml was add to 0.5 ml of extract and left for 5 min at room temperature. After 5 min, 2 ml of 7.5% w/v of sodium carbonate was poured to the solution, therefore the laboratory tubes were covered by a foil paper and incubated in the dark place for 45 min with intermittent shaking to obtain blue colour. When the blue colour developed was too deep on the solution, it was diluted by distilled water of about 10 ml, thereafter the absorbance in different samples were read at 765 nm wavelength from a spectrophotometer (D*R2800, Hach, USA*). In the standard curve equation prepared from gallic acid. Therefore, the total phenolic content was calculated as gallic acid equivalents GAE/g on the standard curve of gallic acid (Siddiqui *et al*., 2017).

3.7.2.2. Total Flavonoid Content (TFC)

The TFC was established spectrophotometrically following procedure described by Meda *et al*. (2005) based on the formation of a complex flavonoid-aluminium. An extract of 0.3 ml was poured into a 50 ml test tube together with 0.15 ml of 0.5 M sodium nitrite, 3.4 ml of 30% methanol, and 0.15 ml of 0.3 M aluminium chloride hexahydrate and mixed thoroughly. The 1 ml of 1 M Sodium hydroxide was added after 5 min. The spectrophotometer *(DR2800, Hach, USA)* was used to detect the absorbance against the reagent blank at wavelength of 506 nm. The total flavonoids standard curve was prepared from (Y=0.0496X+0.0014; R^2=0.9942) catechin standard solution. The total flavonoids were calculated as milligrams of catechin equivalents per gram (mg CE/g) of dried fraction.

3.7.3. Antioxidant activities of powder made from bush tea leaves

3.7.3.1. Radical Scavenging Activity (ABTS)

The process outlined out by Arnao *et al*. (2001) for the ABTS determination test was followed. As stock solutions, 2,4 mM and 7 mM potassium persulfate solutions were used. The working solution was then made by blending equal portions (50 ml) of the two stock solutions, and they were allowed to react for 14 hours at room temperature in a dark environment. Dilution of the solution was done with 60 ml of methanol and 1 ml of ABTS solution to measure 0.706 ± 0.01 absorbance using a spectrophotometer at 734 nm, which came out to be. An entirely fresh ABTS solution was created for every assay or test. A spectrophotometer (DR2800, Hach, USA) was utilised to determine the absorbance at 734 nm after 7 minutes of allowing 1 ml of plant extract and 1 ml of ABTS solution to react (). As a result, the % of inhibition was computed using the following equation 4 below.

$$ABTS\ radical\ scavenging\ activity\ \% = \frac{Ac-As}{Ac} \times 100 \qquad (4)$$

Where:

Ac= the absorbance of the control (ABTS radical in methanol)

As= the absorbance of the tea sample combined with ABTS radical solution

3.7.3.2. Ferric reducing antioxidant power (FRAP) scavenging

Bush tea leaves were evaluated for their ability to reduce ferric using an approach developed by Benzie and Strain (1996). The mixture of 2.5 ml $FeCl_3.6H_2O$ solution, 25 ml of acetate buffer, and 2.5 ml TPTZ solution altogether made the working FRAP reagent. The Bush tea's sample extract (40 µL) and 2 ml of the FRAP reagent were combined in a test tube and incubated in dark area for 15 minutes and its absorbance was read at 593 nm on the spectrophotometer (*DR2800, Hach, USA*). The standard curve was prepared using ferrous sulphate solution. The FRAP results were expressed as micromole per gram (µmol/g).

3.8. Determination of Chlorophyll content of powder made from bush tea leaves

Using the Lichtenthaler technique (1987), the concentrations of chlorophylls 'a' and 'b' were measured. One gram of powdered dry leaf samples was incubated with 50 ml of an 80% acetone (v/v) solution for 24 hours at room temperature to do the extraction. As the sample needed to be filtered, 150 mm qualitative filter paper was used, and 80% acetone (v/v) was added to the filtrate to make 100 ml. A spectrophotometer (DR2800, Hach, USA) was used to detect absorbance at 662 nm and 644 nm to establish the levels of chlorophylls 'a' and 'b,' respectively. The sum of chlorophylls 'a' and 'b' was used to determine the total chlorophyll concentration. On a dry weight basis, the amount of chlorophyll in each sample was measured in triplicate and represented as mg/g.

3.9. Colour measurement of powder made from bush tea leaves

Colour of powdered bush tea leaves was measured using Lovibond Colour Measurement (*LC 100 Spectrocolorimeter*) after calibration with tile. L*, a*, b*, c*, and H° Hunter value was used to express colour readings. L* is for lightness and measures to white from black (0–100); a* and b* stand for hue on the green (–) to red (+) and blue (–) to yellow (+) axis, respectively; c* stands for chroma; and h° stands for hue angle. Yellow, green, and blue hues are signified by angles of 90°, 180°, and 270°, respectively, while red is denoted by angles of 0° or 360°. Using equation 5, the total colour difference (ΔE) was computed:

$$\Delta E = \sqrt{(L - Lc)^2 + (a - ac)^2 + (b - bc)^2} \qquad (5)$$

where:

L = lightness, Lc = lightness of control sample

a = redness, ac = redness of control sample

b = yellowness, bc = yellowness of control sample.

3.10. Statistical analysis of the data

The statistical analysis was performed using SAS version 9.4 software (SAS, 1999). On the collected data, the suitable analysis of variance (ANOVA) was done. The Shapiro-test Wilk's was carried out on the standardized residuals to look for any abnormalities from normality. Where there was a noticeable difference from normalcy owing to skewness, outliers were eliminated up till the distribution was normal or symmetrical. Student's t-LSDs (Least Significant Differences) were computed at a 5% comparing the means of substantial source effects at a considerable level. The analysis of Pearson's Correlation Coefficient was used to establish the association among the climatic factors and nutritional compositions, polyphenols, antioxidants activities, chlorophyll contents and colour attributes. The model used was:

$Yijkl=\mu+Li+Sj+Dk+ (LS)ij+ (LD)ik+(SD)jk+(LSD)ijk+Eijkl$

Where:

μ=overall mean

Li=effects of the i^{th} shade nets colours

Sj=effects of the j^{th} year

Dk=effects of the k^{th} light intensities

(LS)ij=interaction of the i^{th} shade nets colours and j^{th} year

(LD)ik=interaction of the i^{th} shade nets colours and the k^{th} light intensities

(SD)jk=interaction of the j^{th} year and k^{th} light intensities

(LSD)ijk=interaction of the i^{th} shade nets colours, j^{th} year and k^{th} light intensities

Eijkl=residual

CHAPTER 4: RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1. The effects of shade nets colours and light intensities on nutritional compositions of bush tea during autumn season in year 2018 and 2019.

A significant difference of ($p \leq 0.0001$) between the bush tea leaves from different shade net colours and light intensities in terms of the moisture, ash, dry matter, and vitamin C contents was found (Table 4.1). The moisture content under the influence of shade nets colours was higher in 2019 (8.3% to 9.8%) than in 2018 (7.1% to 9.0%). The white shade net colour (9.0%) and green (9.8%) recorded higher moisture content in 2018 and 2019, than other shade net colours.

The moisture content of bush tea under the influence of light intensities was higher at 80% light intensities in both 2018 and 2019. However, the light intensities of 40% and 50% showed no significant difference in the years 2018 and 2019. The green shade net had the higher ash content (8.6%), while the white shade net had the lower ash content (8.0%). However, black net shade recorded higher ash content (9.0%), while lower ash content was recorded under control (8.3%) in the 2019 cropping season. The ash content was found to be highly influenced by 80% light intensity in both years, 2018 (8.4%) and 2019 (9.6%) with the lowest ash content found at 50% light intensity in both years 2018 (8.3%) and 2019 (7.8%).

There was a decrease in dry matter content when comparing year 2018 and 2019, as it ranged from 90.99% to 92.9% in 2018 and from 90.2% to 91.7% in 2019. The control (unshaded) had the highest dry matter content of 92.9% in 2018 and 91.8% in 2019. The 100% light intensity had the highest influence on dry matter in both 2018 (92.2%) and 2019 (91.5%). Control recorded higher vitamin C content in both years, starting from 1.3 (mg AA/100g) to 3.0 (mg AA/100g) in 2018, and from 1.7 (mg AA/100g) to 2.8 (mg AA/100g) in 2019. These results indicate that the amount of vitamin C was unaffected by shade nets. Vitamin C content ranged from 1.0 to 3.0 mg AA/100g in the year 2018, with the highest content found at 100% light intensity, followed by 40% then 50%, and the lowest content at 80% light intensity.

Table 4.1. Response of nutritional compositions of bush tea grown under various selected shade nets colours and light intensities during autumn season at year 2018 and 2019.

		Moisture (%)	Ash (%)	Dry matter (%)	Vitamin C (mg AA/100g)
2018					
Shade net colours	Control	7.84 ± 0.06^{cG}	8.38 ± 0.01^{cE}	92.86 ± 0.95^{aA}	3.03 ± 0.31^{aA}
	Black	8.40 ± 0.33^{bE}	8.47 ± 0.18^{bD}	91.60 ± 0.47^{cC}	1.99 ± 0.84^{cC}
	Green	7.14 ± 0.95^{dH}	8.58 ± 0.06^{aC}	92.16 ± 0.056^{bB}	2.25 ± 0.96^{bB}
	White	9.04 ± 0.52^{aC}	8.04 ± 0.34^{dF}	90.96 ± 0.46^{dE}	1.33 ± 0.39^{dE}
Light intensity (%)	Control	7.84 ± 0.06^{c}	8.38 ± 0.01^{aC}	92.16 ± 0.06^{aA}	3.03 ± 0.31^{aA}
	40	8.27 ± 0.37^{bD}	8.38 ± 0.24^{aC}	91.73 ± 0.48^{cC}	2.57 ± 0.59^{bB}
	50	8.07 ± 1.72^{bE}	8.27 ± 0.50^{bD}	91.93 ± 1.67^{bB}	1.97 ± 0.78^{cD}
	80	8.76 ± 0.50^{aB}	8.41 ± 0.12^{aC}	91.76 ± 0.55^{cC}	1.04 ± 0.15^{dE}
2019					
Shade net colours	Control	8.25 ± 0.75^{dF}	8.31 ± 0.09^{dE}	91.75 ± 0.74^{aC}	2.79 ± 0.30^{aA}
	Black	8.58 ± 1.07^{cD}	9.05 ± 0.31^{aA}	91.42 ± 1.03^{bD}	1.96 ± 0.35^{cC}
	Green	9.78 ± 0.06^{aA}	8.56 ± 0.99^{cC}	90.22 ± 0.06^{dF}	1.69 ± 0.30^{dD}
	White	9.15 ± 0.49^{bB}	8.83 ± 0.99^{bB}	90.85 ± 0.49^{cE}	2.12 ± 0.28^{bcBC}
Light intensities (%)	Control	8.52 ± 0.90^{cC}	8.31 ± 0.09^{cC}	91.48 ± 0.90^{aD}	2.79 ± 0.30^{aA}
	40	8.71 ± 1.09^{bB}	8.87 ± 0.16^{bB}	91.29 ± 1.04^{eE}	1.79 ± 0.39^{dD}
	50	8.76 ± 0.61^{bB}	7.79 ± 0.54^{dE}	91.24 ± 0.61^{cE}	1.96 ± 0.28^{cD}
	80	9.78 ± 0.055^{aA}	9.64 ± 0.27^{aA}	90.22 ± 0.055^{cE}	2.02 ± 0.37^{bC}

P-value (P<0.0001) significant; results are represented as mean± standard deviation. Note: different small superscripts letters in columns signify statistically significant differences in relative amounts of moisture, ash, dry matter, and vitamin C content of bush tea under different shade nets colours and light intensities during autumn season within year 2018 and 2019 and the capital superscripts letters in columns signify significant differences between year 2018 and 2019.

Furthermore, in 2019, vitamin C levels ranged from 1.9 to 2.8 mg AA/100g, and there was a positive relationship observed between light intensities and vitamin C content, as when light intensities decreased, vitamin C levels decreased as well. The results of moisture and ash content showed that there has been an increase from 2018 to 2019. However, there has been a decrease on dry matter and vitamin C content from year 2018 to 2019.

4.1.2. The interaction of shade net colours and light intensities on nutritional compositions of bush tea during autumn season in year 2018 and 2019.

The has been a significant effect in the interaction between the shade net colours and light intensities, however some parameters resulted not significant (Table 4.2). The moisture content ranged from 7.01% to 9.19%. The highest moisture content was found at interaction between white shade net colour and 40% light intensity and the lowest at interaction between green shade net colour and 50% light intensity. However, there was no variation between white shade net and 40% and 50% light intensity. The ash content was highly influenced by the combination of green shade net colour with 80% light intensity, whereas white shade net colour with 50% light intensity resulted lowest influence. The combination of the following shade net colours; black with 40% and 50% light intensity, green with 40%, and white with 40% showed no significant difference. The dry matter was highest at control and lowest at white shade net colour with 40% light intensity. However, several combinations of shade net colours and light intensities such as black with 80% and green with 40%; black with 40% and green with 50%; black with 50% and white with 50%, that resulted no significant difference on dry matter content. The results of vitamin C were found to be highest at control. There was no significant difference between combination of 80% light intensities with green and white shade net colours, as well as between green and white shade net with 40% light intensities.

Table 4.2. Response of nutritional compositions of bush tea under interaction of various selected shade nets colours and light intensities during autumn season at year 2018 and 2019.

	Moisture (%)	Ash (%)	Dry matter (%)	Vitamin C mg AA/g)
Control	8.81 ± 1.00^{d}	8.34 ± 0.07^{e}	93.00 ± 1.24^{a}	2.91 ± 0.32^{a}
Black x 40%	8.40 ± 0.60^{e}	8.67 ± 0.41^{d}	91.28 ± 0.63^{d}	2.44 ± 0.64^{b}
Black x 50%	9.07 ± 0.50^{b}	8.64 ± 0.08^{d}	90.93 ± 0.50^{ef}	2.00 ± 0.31^{c}
Black x 80%	7.97 ± 0.71^{f}	8.90 ± 0.50^{c}	92.20 ± 0.66^{b}	1.49 ± 0.53^{d}
Green x 40%	7.65 ± 0.23^{g}	8.62 ± 0.08^{d}	92.35 ± 0.23^{b}	2.09 ± 0.87^{c}
Green x 50%	7.01 ± 1.24^{h}	7.95 ± 0.66^{f}	91.19 ± 1.00^{d}	2.39 ± 0.61^{b}

Green x 80%	8.43±0.80^{e}	9.23±0.55^{a}	91.57±0.80^{c}	1.43±0.47^{d}
White x 40%	9.19±0.61^{a}	8.63±0.28^{d}	90.79±0.60^{f}	2.00±0.24^{c}
White x 50%	9.17±0.61^{a}	7.65±0.15^{g}	90.90±0.55^{ef}	1.50±0.38^{d}
White x 80%	8.91±0.18^{c}	9.03±1.00^{b}	91.09±0.18^{de}	1.68±0.75^{d}

Results are represented as mean± standard deviation. Note: different superscripts letters in columns signify statistically significant differences in relative amounts of moisture, ash, dry matter, and vitamin C content of bush tea under interaction of different shade nets colours and light intensities during autumn season at year 2018 and 2019.

4.1.3. The effects of shade nets colours and light intensities on polyphenolics contents and antioxidants activities of bush tea during autumn season in 2018 and 2019.

Table 4.3 displays the contents of TPC, TFC, ABTS and FRAP of bush tea at different shade nets colours and light intensities. There was a significant variation ($p \leq 0.0001$) in all the above-mentioned parameters. The TPC content was higher in 2018, ranging from 32.4 to 35.5 mg GAE/g and lower in 2019 (20. 4 to 32.3 mg GAE/g). Green colour shade net had significantly greater TPC in year 2018 compared to the control, black and white shade net colour. However, the white shade net colour recorded highest TPC compared to black and green shade net colour and control in 2019. The TPC ranged from 31.8 to 35.2 mg GAE/g, with the highest content at 40% light intensity (35.2 mg GAE/g), followed by 100% and 50% with the lowest content found at 80%. Moreover, during the year 2019, the TPC ranged from 20.4 to 31.8 (mg GAE/g) and there was a negative trend, as the TPC decreased as light intensities increased.

The TFC of bush tea in different shades net colours decreased from 2018 (2.8 mg CE/g) to 2019 (1.7 mg CE/g). The TFC in 2018 and 2019 was significantly greater at the white shade net colour, accompanied by green, then control and lowest was found at the black shade net colour. The highest (3.3 mg CE/g) and lowest (2.7 mg CE/g) TFC content in year 2018 was attained at 40% and 50% light intensities. However, in year 2019, 100% light intensity had the highest (2.4 mg CE/g) TFC and the lowest (2.0 mg CE/g) was attained at 80% light intensity.

The antioxidant activity under the influence of shade nets colours as measured by the ABTS scavenging activity was higher in 2018 (65.7 to 78.3%) compared to year 2019 (15.7 to 58.6%). Despite the decrease in scavenging activity, green shade net colour recorded higher antioxidant activity, and control had the lowest values in 2018 and 2019. In addition, the highest ABTS determination of antioxidant activity under the influence of light intensities decreased by

13.3% from 2018 to 2019 respectively. Nevertheless, the ABTS scavenging activities during 2019, ranged from 15.8% to 64.0%, and there was a negative relationship between the light intensities and ABTS activities as when the light intensities increase there was a decrease in ABTS scavenging activities.

Moreover, the FRAP's measurement of antioxidant activity in bush tea leaves was higher at control (1036.0 µmol/g) and white shade net colour (945.1 µmol/g) in 2018 and 2019. The FRAP activity on bush tea leaves under the influence of light intensities in year 2018 ranged from 605.5 to 1036.0 (µmol/g), and from 710.11 to 954.80 (µmol/g) in year 2019 and in both years, there was a positive relationship between the light intensities and FRAP activities as when the light intensities decrease the FRAP activities also decreased. When comparing year 2018 to 2019, there has been a decrease of TPC, TFC and ABTS, whereas FRAP resulted an increase.

Table 4.3. Response of polyphenolics content and antioxidant activities of bush tea grown under various selected shade nets colours and light intensities during autumn season in 2018 and 2019.

		TPC (mg GAE/g)	TFC (mg CE/g)	ABTS (%)	FRAP (µmol/g)
2018					
Shade net colours	Control	33.96±0.13^{bB}	2.92±0.02^{cB}	65.75±0.07^{cC}	1036.04±17.11^{aA}
	Black	32.73±0.97^{cC}	2.84±0.05^{dC}	74.21±4.48^{bB}	675.09±296.24^{dH}
	Green	35.45±1.14^{aA}	3.19±0.25^{bA}	78.29±3.54^{aA}	689.62±77.58^{cG}
	White	32.36±2.30^{dD}	3.20±0.91^{aA}	74.14±0.87^{bB}	854.43±181.98^{bD}
Light intensity (%)	Control	33.96±0.13^{bB}	2.92±0.02^{cC}	65.75±0.07^{dD}	1036.04±17.11^{aA}
	40	35.15±1.19^{aA}	2.66±0.47^{dD}	74.29±5.67^{cC}	605.51±257.06^{dH}
	50	33.61±1.65^{cC}	3.51±0.55^{aA}	77.32±2.10^{aA}	682.25±44.70^{cG}
	80	31.78±1.85^{dD}	3.07±0.25^{bB}	75.03±1.97^{bB}	931.38±125.84^{bC}
2019					
Shade net colours	Control	20.37±0.06^{cF}	2.42±0.01^{bE}	15.75±0.07^{dG}	883.81±0.56^{bC}
	Black	30.78±0.54^{bE}	1.72±0.12^{dF}	57.93±18.92^{bE}	806.90±219.63^{cE}
	Green	30.72±0.16^{bE}	2.41±0.48^{cE}	58.55±17.56^{aD}	766.70±70.08^{dF}

	White	32.34±1.05^{aD}	2.53±0.37^{aD}	48.25±31.79^{cF}	945.06±55.39^{aB}
Light intensity (%)	Control	20.37±0.06^{dG}	2.42±0.01^{aE}	15.75±0.07^{dH}	954.80±72.09^{aB}
	40	31.53±0.93^{bE}	2.28±0.61^{cF}	64.00±22.79^{aE}	710.11±162.52^{dF}
	50	31.78±1.24^{aD}	2.29±0.56^{bF}	61.58±12.89^{bF}	853.75±101.39^{bE}
	80	30.62±0.38^{cF}	2.08±0.30^{dG}	39.15±25.38^{cG}	883.81±0.56^{cD}

TPC: total phenolic content; TFC: total flavonoid content; ABTS: radical scavenging activities; FRAP: ferric reducing antioxidants power; GAE: gallic acid equivalent, CE: catechin equivalent; results are represented as mean± standard deviation. Note: different superscripts letters in columns signify statistically significant differences in relative amounts of TFC, TPC, FRAP and ABTS of bush tea under shade nets colours and light intensities during autumn season within year 2018 and 2019 and the capital superscripts letters in columns signify statistical differences between year 2018 and 2019.

4.1.4. The interaction of shade net colours and light intensities on polyphenolics and antioxidant of bush tea during autumn season in year 2018 and 2019.

There has been a highly significant difference between the interaction of shade net colours and light intensities on TPC, TFC, ABTS and FRAP results (Table 4.4). The highest TPC was found on combination of white shade net colour with 40% light intensity (33.82 mg GAE/g) with lowest at control. The highest TFC was obtained at green shade net colour with 50% light intensity (3.26 mg CE/g) and lowest at interaction between black shade net colour and 50% light intensity (2.31 mg CE/g). The ABTS was highest at interaction between green shade net colour and 40% light intensity (81.61%) and lowest at combination of white shade net colour and 80% light intensity (40.97%). The FRAP activities were found to be highest at control (1023.63 µmol/g) and lowest at combination of black shade net colour and 40% light intensity.

Table 4.4. Response polyphenolics and antioxidant activities of bush tea under interaction of various selected shade nets colours and light intensities during autumn season at year 2018 and 2019.

	TPC (mg GAE/g)	TFC (mg CE/g)	ABTS (%)	FRAP (µmol/g)
Control	27.17±6.99^{i}	2.67±0.26^{e}	40.75±25.72^{j}	1023.63±15.37^{a}
Black x 40%	32.85±1.33^{d}	2.17±0.66^{j}	51.03±18.89^{h}	416.78±109.99^{i}

Black x 50%	31.88±0.97^{f}	2.31±0.62^{i}	77.00±0.71^{b}	813.26±111.82^{e}
Black x 80%	30.88±0.79^{g}	2.36±0.56^{h}	70.19±7.16^{d}	992.95±6.52^{b}
Green x 40%	33.57±3.28^{b}	2.72±0.45^{d}	81.61±0.41^{a}	655.98±71.48^{h}
Green x 50%	33.35±2.67^{c}	3.26±0.28^{a}	60.11±14.81^{g}	715.81±3.59^{g}
Green x 80%	32.33±1.83^{e}	2.42±0.56^{g}	63.55±18.01^{f}	812.70±51.97^{e}
White x 40%	33.82±1.36^{a}	2.53±0.49^{f}	74.80±1.59^{c}	900.67±11.35^{d}
White x 50%	32.85±0.63^{d}	3.14±1.10^{b}	67.82±6.59^{e}	774.95±166.78^{f}
White x 80%	30.39±0.70^{h}	2.94±0.50^{c}	40.97±37.55^{i}	959.93±79.19^{c}

TPC: total phenolic content; TFC: total flavonoid content; ABTS: radical scavenging activities; FRAP: ferric reducing antioxidants power; GAE: gallic acid equivalent, CE: catechin equivalent; results are represented as mean± standard deviation. Note: different superscripts letters in columns signify statistically significant differences in relative amounts of TFC, TPC, FRAP and ABTS of bush tea under interaction of shade nets colours and light intensities during autumn season at year 2018 and 2019.

4.1.5. The effects of shade nets colours and light intensities on chlorophyll contents of bush tea during the autumn season in 2018 and 2019.

The results in Table 4.5 show the response of chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll content of bush tea under the influence of different shade nets colours and light intensities. There was a significant variation ($p<0.0001$) in all the above-mentioned parameters. The chlorophyll ‘a’ content, chlorophyll ‘b’ content and total chlorophyll content were highly influenced by black shade net colour in both 2018 and 2019, compared to green and white shade net colours and control. The chlorophyll ‘a’ content, chlorophyll ‘b’ content and total chlorophyll content under the effects of light intensities, resulted in 80% light intensity highly increased the accumulation of all chlorophyll contents. The chlorophyll contents increased from year 2018 to 2019.

Table 4.5. Effect of shade nets colours and light intensities on chlorophyll content of field-grown bush tea during the autumn season in 2018 and 2019.

		Chlorophyll 'a' (mg/g)	Chlorophyll 'b' (mg/g)	Total chlorophyll content (mg/g)
2018				
Shade net colours	Control	6.18±0.01^{dH}	9.60±0.09^{cE}	10.82±0.192$_{dF}$
	Black	9.59±3.17^{aC}	13.02±4.76^{aA}	15.58±1.65^{aA}
	Green	8.72±1.86^{bE}	10.37±2.42^{bC}	14.14±4.07^{bB}
	White	8.63±2.67^{cF}	8.84±0.90^{dF}	12.27±0.99^{cD}
Light intensity (%)	Control	6.18±0.01^{dH}	9.60±0.09^{cE}	10.82±0.19^{dH}
	40	6.39±1.13^{cG}	9.98±2.29^{bC}	13.25±2.03^{cE}
	50	9.51±1.72^{bD}	8.95±1.63^{dF}	13.64±3.04^{bC}
	80	11.05±2.08^{aB}	13.31±4.45^{aA}	15.91±3.29^{aA}
2019				
Shade net colours	Control	8.27±0.022^{dG}	7.44±0.11^{dH}	11.36±0.02^{dE}
	Black	11.07±2.14^{aA}	10.99±1.70^{aB}	15.58±2.79^{aA}
	Green	10.13±4.99^{bB}	10.08±4.87^{bD}	14.15±6.91^{bB}
	White	9.03±1.21^{cD}	8.29±1.12^{cG}	12.43±1.69^{cC}
Light intensity (%)	Control	8.27±0.02^{dF}	7.44±0.11^{dH}	11.36±0.02^{dG}
	40	9.72±3.30^{bC}	8.83±3.06^{cG}	13.32±4.66^{bD}
	50	9.17±1.56^{cE}	9.85±1.53^{bD}	13.11±2.01^{cF}
	80	11.34±4.14^{aA}	10.66±3.98^{aB}	15.73±5.78^{aB}

($P<0.0001$) significant; results are represented as mean± standard deviation. Note: different superscripts letters in columns signify statistically significant differences in relative amounts of chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of bush tea under shade nets colours and light intensities during autumn season at year 2018 and 2019 and the capital superscripts letters in columns signify statistical differences between year 2018 and 2019.

4.1.6. The interaction of shade net colours and light intensities on chlorophyll contents of bush tea during autumn season in year 2018 and 2019.

The interaction of black shade net colour and 80% light intensity resulted highest chlorophyll a, b, and total chlorophyll content, with lowest contents being found at interaction of green shade net and 50% light intensity (Table 4.6).

Table 4.6. Response of chlorophyll contents of bush tea under combination of various selected shade nets colours and light intensities during autumn season at year 2018 and 2019.

	Chlorophyll a	Chlorophyll b	Total chlorophyll content
Control	7.23 ± 1.08^{h}	8.52 ± 1.12^{f}	11.09 ± 0.31^{g}
Black x 40%	9.51 ± 4.82^{e}	14.44 ± 1.60^{b}	17.53 ± 1.86^{b}
Black x 50%	10.33 ± 0.55^{c}	11.22 ± 0.19^{c}	15.85 ± 1.64^{c}
Black x 80%	15.28 ± 1.63^{a}	15.99 ± 3.56^{a}	21.35 ± 2.14^{a}
Green x 40%	7.10 ± 0.70^{i}	8.26 ± 2.63^{g}	10.68 ± 2.17^{h}
Green x 50%	7.18 ± 0.07^{hi}	7.55 ± 0.14^{i}	10.40 ± 0.12^{i}
Green x 80%	9.72 ± 0.29^{d}	8.82 ± 0.06^{e}	13.36 ± 0.22^{e}
White x 40%	7.55 ± 1.34^{g}	8.96 ± 1.18^{d}	11.64 ± 0.43^{f}
White x 50%	10.51 ± 0.07^{b}	9.04 ± 0.77^{d}	13.88 ± 0.73^{d}
White x 80%	$8.57\pm.87^{f}$	7.70 ± 0.50^{h}	11.16 ± 0.87^{g}

results are represented as mean± standard deviation. Note: different superscripts letters in columns signify statistically significant differences in relative amounts of chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll content of bush tea under interaction of shade nets colours and light intensities during autumn season at year 2018 and 2019.

4.1.7. The effects of shade nets colours and light intensities on colour attributes of bush tea during autumn season in 2018 and 2019.

The results in Table 4.7 show the response of colour attributes: lightness (L*), green (-) or red (+) (a*), blue (-) or yellow (+) (b*), chroma (c*), hue angle (H°) and colour difference (ΔE) of bush tea at different shade nets colours and levels of light intensities. There were highly significant ($p<0.0001$) differences between the shade nets colours and light intensities in all colour attributes. The shade net colours influence was observed, therefore the values of L* were highly influenced by control in 2018 and by white shade net colour in 2019. The a* values were higher at the white shade net colour in 2018 and in the control sample in 2019. Moreover, the control sample highly influenced the b* and c* values in both years 2018 and 2019. However, the H° were highly influenced by the green shade net colour than other colours of shade net in 2018 and 2019. The ΔE was influenced by white shade net colour (2018) and green colour (2019). The value of a* was found at the green terminal for the bush tea leaves grown at control, black and green shade net colour, whereas the tea found in the white shade net colour was at red terminal. However, in 2019 all a* values were found at the red terminal. The b* values were all found at the yellow terminal in 2018 and 2019.

The light intensity of 100% had a higher influence on values of L*, b* and C* in 2018, and for a*, b* and C* in 2019. The values of a* and ΔE were highly influenced by 40% light intensity in year 2018. The 80% light intensity resulted in higher values of H° during 2018, and H°, ΔE in year 2019. Fundamentally, the values of a* were found to be at the green terminal for samples found at 100 and 80% light intensities whereas 40 and 50% light intensities resulted at the red terminal; however, in 2019 the bush tea samples were found to be at the red terminal. The b* values at the yellow terminal in 2018 and 2019. The colour attributes; L*, b*, c*, H° and ΔE showed an increase from results obtained in year 2018 to 2019, except for an a* values

Table 4.7. The effects of shade nets colours and light intensities on colour attributes of bush tea during autumn season of 2018 and 2019.

		L*	a*	b*	c*	H°	ΔE
2018							
Shade net colours	Control	43.56±0.01^{aA}	-0.34±0.05^{cF}	20.65±0.01^{aA}	20.65±0.01^{aA}	90.76±0.08^{bB}	0.00±0.00^{dG}
	Black	41.63±1.39^{cC}	-0.17±0.54^{bE}	18.25±1.44^{dD}	18.27±1.44^{dD}	90.63±1.78^{bB}	3.17±1.50^{bB}
	Green	43.01±1.16^{bB}	-0.64±0.52^{dG}	18.99±1.05^{bB}	18.98±1.00^{bA}	92.00±1.62^{aA}	2.19±0.95^{cC}
	White	41.05±2.67^{dD}	0.96±2.19^{aD}	18.72±1.74^{cC}	18.88±1.62^{cC}	86.54±7.08^{cC}	3.97±3.22^{aA}
Light intensity (%)	Control	43.56±0.01^{aA}	-0.34±0.05^{c}	20.65±0.01^{aA}	20.65±0.01^{aA}	90.76±0.08^{bB}	0.00±0.00^{d}
	40	41.09±2.61^{dD}	0.91±1.98^{a}	18.16±1.36^{dD}	18.28±1.22^{dD}	86.81±6.70^{dD}	4.00±3.19^{a}
	50	42.67±1.94^{bB}	0.08±0.84^{b}	18.81±1.86^{cC}	18.82±1.85^{cC}	89.90±2.63^{cC}	2.78±1.73^{b}
	80	42.16±1.09^{cC}	-0.84±0.73^{d}	18.99±0.88^{bB}	19.02±0.89^{bB}	92.47±2.07^{aA}	2.55±0.89^{c}
2019							
Shade net colours	Control	34.04±0.04^{cG}	2.63±0.03^{aA}	13.18±0.22^{aE}	13.46±0.36^{aE}	77.33±0.09^{dG}	0.00±0.00^{dG}
	Black	33.24±1.05^{dH}	2.54±0.26^{bB}	11.63±0.31^{dH}	11.89±0.361^{dH}	77.69±1.02^{cF}	1.23±0.52^{cF}
	Green	34.90±1.54^{bF}	2.39±0.99^{cC}	11.84±0.01^{cG}	12.13±0.01^{cG}	79.86±4.05^{aD}	2.37±0.19^{aD}
	White	35.18±0.80^{aE}	2.40±0.29^{cC}	12.58±1.32^{bF}	12.79±1.32^{bF}	79.28±1.04^{bE}	1.63±1.24^{Be}
Light intensity (%)	Control	34.04±0.04^{cG}	2.76±0.60^{a}	13.18±1.00^{aE}	13.38±0.96^{aE}	77.33±0.09^{cH}	0.00±0.00^{d}
	40	34.33±1.62^{bF}	2.63±0.03^{b}	12.17±0.97^{bF}	12.47±1.07^{bF}	77.43±1.64^{cG}	1.76±0.76^{b}
	50	35.01±0.72^{aE}	2.35±0.27^{c}	12.05±0.67^{cG}	12.14±0.63^{cG}	78.99±1.35^{bF}	1.21±0.74^{c}
	80	33.98±1.68^{dH}	2.21±0.73^{d}	11.84±0.01^{dH}	12.13±0.01^{cG}	80.42±3.43^{aE}	2.25±0.98^{a}

P-value (P<0.0001) significant; results are represented as mean± standard deviation. Note: different superscripts letters in columns signify statistically significant differences in relative values of L* (lightness), a* (+redness) and (-greenness), b* (+yellowness) and (-blueness), c* (chroma), H° (hue angle) and ΔE (total colour difference) of bush tea under shade nets colours and light intensity during autumn season at year 2018 and 2019 and the capital superscripts letters in columns signify statistical differences between year 2018 and 2019.

4.1.8. The interaction of shade net colours and light intensities on colour attributes of bush tea during autumn season in year 2018 and 2019.

The colour attributes b* and c* were highly influenced by control, whereas the values of a* and ΔE were higher at interaction between white shade net colour and 40% light intensity. The lightness (L*) was found to be high at green shade net colour with 50% light intensity. The highest hue angle was found at interaction between green shade net colour and 80% light intensity with a value of 88.86 (Table 4.8).

Table 4.8. Response on colour attributes of bush tea under interaction of various selected shade nets colours and light intensities during autumn season at year 2018 and 2019.

	L*	a*	b*	c*	H°	ΔE
Control	38.80 ± 4.90^{d}	1.15 ± 1.53^{e}	17.18 ± 3.11^{a}	17.42 ± 3.89^{a}	84.05 ± 6.91^{f}	0.00 ± 0.00^{j}
Black x 40%	37.61 ± 5.86^{f}	1.26 ± 1.04^{d}	15.39 ± 4.44^{h}	15.50 ± 4.34^{g}	84.04 ± 5.53^{f}	1.69 ± 0.19^{g}
Black x 50%	35.77 ± 2.36^{j}	0.89 ± 1.92^{f}	13.94 ± 2.64^{j}	14.08 ± 2.51^{i}	85.09 ± 8.62^{c}	2.82 ± 2.36^{c}
Black x 80%	36.98 ± 4.50^{h}	1.41 ± 1.49^{c}	15.49 ± 3.81^{g}	15.66 ± 3.66^{f}	83.35 ± 7.12^{h}	2.10 ± 1.02^{f}
Green x 40%	39.30 ± 3.74^{c}	1.26 ± 2.49^{d}	16.03 ± 2.85^{d}	16.27 ± 2.62^{d}	84.24 ± 9.74^{e}	2.43 ± 0.18^{d}
Green x 50%	40.22 ± 4.66^{a}	$1.22\pm.28^{d}$	16.64 ± 4.05^{b}	16.39 ± 4.38^{c}	84.70 ± 5.63^{d}	1.63 ± 0.62^{h}
Green x 80%	37.35 ± 4.93^{g}	0.14 ± 1.20^{h}	15.59 ± 2.64^{f}	15.63 ± 2.62^{f}	88.86 ± 4.58^{a}	2.87 ± 0.52^{b}
White x 40%	36.23 ± 1.51^{i}	3.00 ± 0.49^{a}	14.07 ± 2.56^{i}	14.36 ± 2.59^{h}	78.07 ± 0.16^{i}	4.53 ± 4.06^{a}
White x 50%	38.23 ± 4.12^{e}	1.54 ± 0.53^{b}	15.71 ± 4.42^{e}	15.83 ± 4.38^{e}	83.54 ± 3.70^{g}	1.54 ± 0.85^{i}
White x 80%	39.89 ± 4.01^{b}	0.50 ± 2.32^{g}	16.24 ± 4.53^{c}	17.32 ± 3.03^{b}	87.12 ± 8.10^{b}	2.33 ± 1.05^{e}

Results are represented as mean± standard deviation. Note: different superscripts letters in columns signify statistically significant differences in relative values of L* (lightness), a* (+redness) and (-greenness), b* (+yellowness) and (-blueness), c* (chroma), H° (hue angle) and ΔE (total colour difference) of bush tea under interaction of shade nets colours and light intensity during autumn season at year 2018 and 2019.

4.2. Correlation between climatic factors and nutritional and physicochemical properties of bush tea

4.2.1. Correlation between climatic factors and nutritional compositions, polyphenolics, and antioxidants activities of bush tea leaves

Relationships/association between the climatic factors and nutritional compositions, total polyphenolics contents and antioxidant activities were calculated for leaves of bush tea (Table 4.9). The results revealed a weak (0.2-0.39), moderate (0.4-0.59), strong (0.6-0.79), and very strong (0.8-1) association between climatic factors and nutritional compositions, total polyphenolics, and antioxidants activities of bush tea. There was a very strong correlation between vitamin C and all climatic factors except for rainfall. Similar trend was observed between climatic factors and TPC. A strong relationship was observed between vitamin C and wind speed (0.784***), while a moderate association was observed between wind speed and dry matter (0.596 **). In contrast, no association was found between the wind speed and ash content (0.109ns). A strong relationship was observed between rainfall and ash content (0.752***), whereas a strong correlation was recorded between rainfall and ABTS (0.694**). There was no association observed between rainfall and TPC (0.024ns), TFC(0.033ns) and antioxidant activity FRAP (0.020ns). The relationship between humidity and FRAP was very strong (0.825***), but no relationship between humidity and ABTS (0.150ns).

Table 4.9. Association between climatic factors and nutritional compositions, polyphenolics, and antioxidants activities of bush tea leaves.

Climatic factors	Nutritional compositions				Polyphenolics		Antioxidant's activities	
	Moisture	Ash	Dry matter	vitamin C	TPC	TFC	ABTS	FRAP
Max temp	0.227ns	0.160ns	0.224ns	0.903***	0.980***	0.168ns	0.057ns	0.625**
Min temp	0.022ns	0.372*	0.021ns	0.844***	0.944***	0.011ns	0.020ns	0.297ns
Rainfall	0.008ns	0.752***	0.007ns	0.230ns	0.024ns	0.033ns	0.694**	0.020ns
Wind speed	0.597**	0.109ns	0.596**	0.784***	0.556**	0.663**	0.459*	0.916***
Humidity	0.425*	0.104ns	0.422*	0.891***	0.867***	0.370*	0.150ns	0.825***

*=P< 0.05, **=P<0.01, ***=P<0.001, ns=not significant, TPC=Total phenolic content, TFC=Total flavonoid content, ABTS=Radical scavenging activities, FRAP=Ferric reducing antioxidant power scavenging activity.

4.2.2. A relationship between climatic factors and physicochemical properties of bush tea

A relationship between climatic factors and physicochemical properties (chlorophyll contents and colour attributes) were determined for leaves of bush tea as shown in Table 4.10. The findings indicate a strong association between climatic factors and chlorophyll contents and a weak correlation between climatic factors and colour attributes. A very strong relationship was established between chlorophyll a, and rainfall (0.885***) with no association between rainfall and chlorophyll b. Moreover, the relationship between chlorophyll b and minimum temperature was very strong (0.962***) while there was no correlation between chlorophyll a, and minimum temperature. The relationship between rainfall and lightness (L*) was moderate (0.588**).

Table 4.10. Relationship between climatic factors and chlorophyll contents and colour attributes of bush tea leaves.

Climatic factors	Chlorophyll contents			Colour attributes					
	Chlorophyll a	Chlorophyll b	TCC	L*	a*	b*	c*	H°	ΔE
Max temp	0.165ns	0.737**	0.467*	0.051ns	0.003ns	0.217ns	0.248ns	0.000ns	0.090ns
Min temp	0.316ns	0.962***	0.702**	0.081ns	0.104ns	0.343ns	0.364*	0.077ns	0.001ns
Rainfall	0.885***	0.148ns	0.542*	0.588**	0.076ns	0.236ns	0.240ns	0.116ns	0.000ns
wind speed	0.201ns	0.300ns	0.293ns	0.081ns	0.254ns	0.009ns	0.003ns	0.290ns	0.495*
Humidity	0.140ns	0.548*	0.362*	0.005ns	0.031ns	0.079ns	0.102ns	0.044ns	0.255ns

*=P<0.05, **=P<0.01, ***P<0.001, ns= not significant, TCC=Total chlorophyll L* =lightness, a*=redness, b*=yellowness, c*=chroma, H°=hue angle, ΔE=total colour difference.

4.2.3. The relationship between nutritional compositions, polyphenolics, antioxidants activities of bush tea leaves.

The relationship between nutritional compositions, polyphenolics and antioxidants activities were analyzed and presented in Table 4.11. Generally, the results show a moderate relationship between the nutritional compositions and total polyphenolics contents. However, there was a weak relationship amongst the antioxidant's activities. A very strong relationship was found between the following: moisture and FRAP (0.818***), dry matter and FRAP (0.816***), however, TFC and FRAP (0.772***) resulted strong relationship. Nevertheless, a moderate association was found between vitamin C and FRAP (0.593**) and between TPC and FRAP (0.487*). The results show a weak relationship between chlorophyll contents and colour attributes. Nevertheless, a strong association was observed between the following parameters: chlorophyll 'a' and total chlorophyll content (0.842***), chlorophyll 'b' and total chlorophyll content (0.821***). However, a moderate relationship was observed between chlorophyll a and chlorophyll b (0.440*). There was a very strong correlation between lightness and chroma (0.866***), yellowness and chroma (0.998***). A strong relationship was observed between redness and chroma (0.645**).

Table 4.11. The relationship between nutritional compositions, polyphenolics, and antioxidants activities of bush tea leaves.

	Nutritional compositions				Polyphenols		Antioxidant activities		Chlorophyll contents			Colour attributes					
	Moisture	Ash	Dry matter	Vitamin C	TPC	TFC	ABTS	FRAP	Chlorophyll b	Chlorophyll a	TCC	L*	a*	b*	c*	H°	ΔE
Moisture	**1.000*** **																
Ash	0.106ns	**1.000*** **															
Dry matter	1.000***	0.107ns	**1.000*** **														
Vitamin C	0.180ns	0.381*	0.179ns	**1.000*** **													
TPC	0.127ns	0.188ns	0.125ns	0.860***	**1.000*** **												
TFC	0.914***	0.020ns	0.915***	0.204ns	0.077ns	**1.000*** **											
ABTS	0.195ns	0.257ns	0.196ns	0.244ns	0.019ns	0.461*	**1.000*** **										
FRAP	0.818***	0.002ns	0.816***	0.593**	0.487*	0.772***	0.271ns	**1.000*** **									
Chlorophyll a	0.030ns	0.959***	0.030ns	0.424*	0.169ns	0.002ns	0.452*	0.029ns	**1.000*** **								
Chlorophyll b	0.001ns	0.532*	0.001ns	0.762***	0.822***	0.001ns	0.020ns	0.158ns	0.440*	**1.000*** **							
TCC	0.013ns	0.886***	0.014ns	0.689**	0.507*	0.000ns	0.205ns	0.092ns	0.842***	0.821***	**1.000*** **						
L*	0.038ns	0.120ns	0.039ns	0.003ns	0.102ns	0.220ns	0.794***	0.019ns	0.256ns	0.059ns	0.024ns	**1.000*** **					
a*	0.557**	0.040ns	0.560**	0.005ns	0.037ns	0.766***	0.552**	0.295ns	0.000ns	0.172ns	0.049ns	0.562**	**1.00*** **				
b*	0.062ns	0.000ns	0.064	0.058ns	0.325ns	0.226ns	0.517*	0.001ns	0.026ns	0.326ns	0.046ns	0.868***	0.690**	**1.000*** **			
C*	0.041ns	0.000ns	0.043	0.073ns	0.357*	0.188ns	0.493*	0.000ns	0.027ns	0.337ns	0.047ns	0.866***	0.645**	0.998***	**1.000*** **		
H°	0.543*	0.017ns	0.546	0.015ns	0.025ns	0.773***	0.621**	0.313ns	0.005ns	0.132ns	0.024ns	0.609**	0.995***	0.699**	0.654**	**1.000*** **	
ΔE	0.946***	0.114ns	0.948***	0.089ns	0.028ns	0.960***	0.300ns	0.673**	0.025ns	0.037ns	0.038ns	0.148ns	0.772***	0.215ns	0.178ns	0.754***	**1.000*** **

*=P<0.05, **=P<0.01, ***=P<0.001, ns=not significant, TCC=total chlorophyll content, L* =lightness, a* =redness, b*=yellowness, c*= chroma, H°=hue angle, ΔE=total colour difference.

4.3. DISCUSSION

First time, this research showed how different net shade colours and light intensities affect the nutritional compositions, polyphenols, antioxidant activities, chlorophyll contents, and colour attributes of field grown bush tea, a native plant in South Africa's Limpopo Province. Indeed, net shade colours and light intensities of field grown bush tea were important determinants of nutritional compositions, polyphenols, antioxidant activities, chlorophyll contents, and colour attributes. The nutritional compositions, polyphenols, antioxidant activities, chlorophyll contents, and colour attributes were all significantly ($p \leq 0.0001$) higher under shade net colours and light intensities. The results show solid evidence that the leaves of field grown bush tea contain diverse nutritional compositions and antioxidants that may have health benefits. Moreover, all parameters measured resulted high contents influenced by different net shade except for parameters such as vitamin C, dry matter, b*, c* and FRAP results.

Significant practical experience and scientific research have proved that an increase of tea plants' overall internal and external quality has been influenced by shading (Chen *et al*., 2021). Moreover, the quality and yield of several crops under horticulture such as ornamental, tea, the vegetables, the herbs, and fruits has been published to be enhanced by shading (Hirai et al., 2008; Zhao *et al*., 2012; Liu., 2014; Abbasnia Zre., 2019; Díaz-Perez and John., 2019; Milenkovi'c., 2019; Sabir *et al*., 2020). However, the knowledge of how shade nets alter light quality and how these alterations affect the chemical content of leaves and other plant responses is currently limited (Kotilainen *et al*., 2018). Several mechanical, physical, and optical characteristics of shading nets permit changing amounts of light and temperature surrounding crops (Castellano *et al*., 2008). Shade nets can change the solar radiation's degree and quantity on nets like grey or black nets while leaving the light's spectral compositions unchanged (Ben-Yaki *et al*., 2008; Elad *et al*., 2008; Shahak, 2008).

The red, blue, pearl, and white photo-selective nets can maximize diffused radiation and regulate too-high light, temperature, humidity, and wind speed levels (Stamps, 2009), allowing for more effective vegetable production under protected production (Ferreira *et al*., 2012).In addition, photo-selective nets have reportedly been proven to improve the grade for the vegetables when harvested (Ili'c *et al*., 2018) and after the harvest stage (Mashabela *et al*., 2015; Selahle *et al*., 2015; Sivakumar and Jifon, 2018).

The moisture content that ranges from 2.5 to 6.5% has been reported to indicate the best tea quality (Adnan *et al*., 2013); nevertheless, the moisture content of bush tea in current research is higher. The results might have been influenced by the ability of coloured nets (white) to alter the type and amount of light, which could affect growth, yield, and fruit quality (Arthurs *et al*., 2013; Fallik, 2009; Ilic *et al*., 2017). Based on the investigation done by Malongane *et al*., (2020), bush tea resulted in an ash content of 8.01%; meanwhile, in this study the ash content ranged from 7.79 to 9.64% which might have been influenced using black and green shade nets colour and the reduced light intensity. The black shade nets are known to hold greater light than shade nets of other colours (Mokoka, 2007), which might have influenced the ash content accumulation. Moreover, vegetative growth is also promoted depending on the colour of the net and with black nets outperforming red nets (Manja and Aoun, 2019).

To maintain the standard of tea while being stored, the ash concentration of the tea should be less than 5.5%. (Adnan *et al*., 2013; Ismail *et al*., 2000; Rehman *et al*., 2002). On the other hand, a high ash concentration in teas indicates a high mineral content (Malongane *et al*., 2020). Therefore, this study revealed that bush tea had higher mineral content as per ash content results. Furthermore, rainfall and ash content resulted the strong positive correlation (0.752***), and the results concur with Boehm *et al*., (2016) who revealed that tea yield is influenced by increased rainfall.

The control sample influenced the dry matter and vitamin C accumulation. In vegetables, ascorbic acid has been found to be the major vitamin and with antioxidant properties (Lee and Kader, 2000) and on current study the vitamin C content of bush tea in control is higher compared to shade treatments. Thus, this could be one of the reasons for a positive correlation between this compound and light intensity, as per findings of Kosma *et al*. (2013). Caruso *et al*. (2020), found similar outcomes; that the ascorbic acid content (vitamin C) in the wall rocket's leaves planted in unshaded control was consistently higher than that of shade treatments and low vitamin C in shaded bush tea may be because of the reduced plant's photosynthetic performance. Although the dry matter and vitamin C levels were greater at 100% light intensity, vitamin C may be related to its function in avoiding abiotic plant damage (Ntsoane, 2015).

The TPC and TFC were both influenced by white shade net colour than black shade net and the reason for these results might be because light coloured (white) nets generally transmit a higher photosynthetically active radiation and increase light scattering compared with dark-coloured ones (red and black) (Cronje, 2020). In addition, according to Mditshwa *et al*. (2019), light-coloured nets also reduce air and soil temperature as well as increase relative humidity (depending on net density) more than dark-coloured nets, due to reducing the incoming radiant energy. Nevertheless, Wang *et al*. (2020) discovered that the use of shade profoundly affected TPC and TFC, as their contents were higher than that found on the control. Thus, their results agree with the results in this study as higher TPC and TFC were found in leaves produced under a white shade net colour than in leaves grown at full sunlight considered as control. Corresponding effects were found by Ili'c *et al*. (2017), wherein lettuce exposed to shade treatments had higher flavonoid content compared to unshaded treatment.

However, several biotic and abiotic factors or stress signals were reported to influence the concentration and secondary metabolism of phenolics and total flavonoids that make up chemical compounds (Fine *et al*., 2006; Pavarini *et al*., 2012). Research has revealed that light intensity may impact the buildup of amino acids and minerals (Riga *et al*., 2019; Stagnari *et al*., 2015; Zrig *et al*., 2016), and more light is considered to promote the formation of phenols and flavonoids to conserve the live plants (Liu *et al*., 2018; Riachi *et al*., 2018). The results of the study on bush tea are consistent with those found by Kumari *et al*. (2009), who reported that the production of total phenols caused by increased light intensity could be caused by higher light-triggered activation of the phenylalanine amino lysis enzyme in the synthesis pathway of phenolic acids. In addition, the expression of phenylalanine ammonia lyase is associated with the formation of flavonoids due to elevated light intensity (Graham, 1998; Saito *et al*., 2013).

According to Mogotlane *et al*. (2007), the antioxidant content is frequently utilized as a criterion to characterize various materials for health advantages. In this study, the antioxidant activities were investigated via ABTS and FRAP activities. The findings demonstrated that the radical scavenging activity of bush tea determined by ABTS was highly influenced by green shade net colour. In contrast, the ferric reducing antioxidant power (FRAP) exhibited high activity at white shade net colour. The light intensities that had the greater antioxidant activities were 100% in 2018 and 2019. Thus, the outcomes are in line with findings by Jin *et al*. (2009), who showed that a full light environment produced markedly increased antioxidant activity of rocket in comparison to lesser light intensity. Light is an important ecological factor that influences plant photosynthesis as well as the chemical make-up of plants (Fukuda, 2019; Kyriacou *et al*., 2016; Rouphael *et al*., 2018). It is generally indicated that light affects plant development, and that the intensity of the light has an immediate impact on the physiology and growth of plants (Chen *et al*., 2017; Shao *et al*., 2014). However, to be more precisely, the build-up of certain secondary metabolites is determined by light intensity. Contrariwise, using 80% shade nets created a microclimate with reduced amount of light being received by bush tea but increased the humidity. However, the higher antioxidant activity might have been caused by various variables like the age of plant, plant characteristics and the climatic conditions like humidity and wind speed as a positive correlation has been found. Environmental factors also have an impact on plant nutrients due to having an influence on their growth, development, and distribution (Siracusa and Ruberto, 2014; Tounekti and Khemira, 2015; Tounekti *et al*., 2010).

As chlorophyll functions as the primary pigment for photosynthesis in plants, its biosynthesis is a fundamental metabolic mechanism (Garrone *et al*., 2015). Moreover, Chlorophyll is available in two varieties: chlorophyll 'a,' the main pigment active in photosynthesis, and chlorophyll 'b', the accessory pigment that accumulates energy to transfer to chlorophyll 'a', thus both chlorophyll 'a' and 'b' are essential photosynthetic pigment for the process of photosynthesis, and they make up the total chlorophyll content. A significant environmental parameter that influences the production of plant chlorophyll is the modification of the light environment set about through shade (Fiorucci and Fankhauser, 2017). Moreover, the increase on the tea plant's chlorophyll levels were influenced by the shading treatments (Ku *et al*., 2010; Wang *et al*., 2012; Lee *et al*., 2013; Liu *et al*., 2018; Sano *et al*., 2018).

Further, the current results revealed considerable impact of black shade net than control on the build-up of total chlorophyll content, chlorophyll 'a', and chlorophyll 'b' throughout the cropping seasons. Moreover, the fact that black shade nets are not able to change the spectral spectrum of the light that plants receive may be the reason behind this increase (Arthurs *et al*., 2013). Similar results have been found in sweet peppers, when comparing the leaves produced under red, pearl, and white shade nets and the unshaded control, pepper leaves grown under black and blue shade nets were shown to have greater chlorophyll contents (Ili'c *et al*., 2017). In addition, a study conducted by Díaz-Pérez and John. (2019) on bell peppers, resulted in higher chlorophyll 'a' and chlorophyll 'b' concentrations in shaded leaves than unshaded leaves.

However, several studies have shown that shading has a considerable significant impact on the fresh leave's chlorophyll content, however, these increases are not influenced by the age of a leaf, the season to apply shading, and the quantity of shading treatments but rather by the shading's degree and duration (Lee *et al*., 2013; Sano *et al*., 2018; Yamashita *et al*., 2020) and the current findings, revealed that light intensity of 80% had a high significant to the accumulation of all the chlorophyll contents of bush tea, whereas 100% light intensity resulted to least significant in both year 2018 and 2019. Moreover, shade considerably reduces light intensity, the primary environmental component that stimulate the accumulation of chlorophyll in tea leaves (Chen *et al*., 2021). The changes in chlorophyll contents can be due to a reduction of its biosynthesis and or increase of its degradation caused by the quantity of light intensity which determines the amount of chlorophyll content that will be used for photosynthesis. Several studies on multiple species, have been conducted and revealed that temperature, quality of light as well as humidity have an impact on plant chlorophyll concentrations (Bourque and Naylor, 1971; López-Figueroa and Niell, 1990; Huang *et al*., 2017), and this has been proved by the results in this study as positive correlation has been found between the climatic factors i.e., temperature and humidity and the chlorophyll contents.

The research conducted by Sano *et al*. (2018), revealed that the leaves cultivated under the shade are greener, which expand the economic worth of the tea plant's dry leaves when compared with dry leaves grown in the sun. Thus, the lightness (L*), a*, b*, chroma (c*), H° and ΔE colour attributes in this study were significantly affected by shade nets (green colour) and light intensities (80%), and this may be because the green shade net colour attracts more sunlight and thereafter works like a filter and prevents the plants from receiving too much sunlight as it can control the heat around the plants with the 80% shading level as bush tea leaves were only receiving 20% of the sunlight. However, these findings differ from those of Ili'c *et al*. (2017) when comparing the effectiveness of photo-selective shade nets on lettuce's visual quality traits during a summer cycle, where in it was found that shade nets did not have an influence on the measured colour attributes L* and a*.

The climatic conditions might affect the development and proliferation of plant (Beg *et al*., 2002; Steffen, 2008; Sáenz-Romero *et al*., 2010). For instance, soil moisture has a direct impact on plant development and is more readily available to plants in cold and wet climates (Opio *et al*., 2017). Plant water interactions and growth are impacted by relative humidity (Mortensen, 1986). Reduced transpiration rates are caused by high relative humidity and temperatures that are low (Schippers *et al*., 2015). However, the degree to which these three environmental factors; rainfall, relative humidity, and temperature affect the development and proliferation of plant may vary (Bareja, 2011). To support this a positive correlation has been found in this study (Table 4.9 and 4.10) except for a*, b* and H°. In addition, according to Fiorucci and Fankhauser, (2017), shading would alter the microclimate of tea growth as well as other environmental parameters, since they typically lower the temperature and sunlight, alter the quality of light, and raise humidity of the environment.

In Limpopo Province, precipitation occurrences are extremely sporadic and strong, frequently linked to thunderstorms with convection (SARDC, 2002). Considering the major convective rainfall of Limpopo Province is significantly correlated with high temperatures, it is probable that temperature was automatically associated with the growth of vegetative parts by convective rain. The El Nino incident, which occurs when Pacific Ocean surface temperatures rise abnormally and cause precipitation to drastically drop and the dry season to stretch, is another risk that Limpopo Province faces (Mulungisi, 2015). This may help to explain why both maximum and minimum temperatures increased while the rainfall decreased from year 2018 to year 2019 (Table 3.1). Thus, the effect has been revealed in the accumulation of nutritional compositions and physicochemical properties of bush tea grown at Agriculture experimental farm, University of Venda, Limpopo Province.

CONCLUSION

The utilisation of herbal tea has risen during the recent decades owing to its health benefits; therefore, it is crucial to choose the finest treatment that is related to tea's quality and improved nutritious compositions. The current study presented the best shading nets and light intensities in respect to different characteristics such as moisture, ash, dry matter, vitamin C, TPC, TFC, antioxidants activities using ABTS and FRAP assays, total chlorophyll content, chlorophyll 'a' , and chlorophyll 'b' contents and colour attributes of bush tea and the treatments were found to be effective in enhancing the accumulation of the mentioned characteristics during autumn season in year 2018 and 2019. The black shade net colour was more efficient to accumulate the total chlorophyll content, chlorophyll a, and chlorophyll b contents of bush tea, whereas, dry matter content, vitamin C, yellowness (b*) and chroma (c*) were influenced positively by control sample. The 80% light intensity had higher influence on the contents of chlorophyll a content, moisture content, total chlorophyll content, and chlorophyll b content. However, vitamin C, dry matter, b*, c*, FRAP were influenced by 100% light intensity. These findings showed that shade nets application seem to be environmentally friendly method for changing agricultural microclimate characteristics. Moreover, those characteristics control both yield, quality as well as phytochemicals or antioxidants of field grown bush tea which can be used as a basic dietary intake.

REFERENCES

Abbasnia Zare, S. K., Sedaghathoor, S., Padasht Dahkaei, M. N. and Hashemabadi, D., 2019. The effect of light variations by photoselective shade nets on pigments, antioxidant capacity, and growth of two ornamental plant species: Marigold (*Calendula officinalis* L.) and violet (*Viola tricolor*). *Cogent Food and Agriculture*, 5(1), 1650415.

Abdullah, S., Shaari, A.R. and Azimi, A., 2012. Effect of drying methods on metabolites composition of misai kucing (*Orthosiphon stamineus)* leaves. *Apcbee Procedia*, *2*, 178-182.

Abioye, V.F., Adejuyitan, J.A. and Idowu, C.F., 2014. Effects of different drying methods on the nutritional and quality attributes of baobab leaves (*Adansonia digitata*). *Agriculture and Biology Journal of North America*, *5*(3),104-108.

Ada, N.L., Farkash, L., Hamburger, D., Ovadia, R., Forrer, I., Kagan, S. and Michal, O.S., 2008. Light-scattering shade net increases branching and flowering in ornamental pot plants. *The Journal of Horticultural Science and Biotechnology*, *83*(1), 9-14.

Adnan, M., Ahmad, A., Ahmed, A., Khalid, N., Hayat, I. and Ahmed, I., 2013. Chemical composition and sensory evaluation of tea (*Camellia sinensis)* commercialized in Pakistan. *Pakistan Journal of Botany*, 45(3), 901-907.

Al-Helal, I.M. and Abdel-Ghany, A.M., 2010. Responses of plastic shading nets to global and diffuse PAR transfer: Optical properties and evaluation. *NJAS: Wageningen Journal of Life Sciences*, *57*(2),125-132.

Association of Official Analytical Chemists (AOAC)., 2016. Official Methods of Analysis, 20th ed.; AOAC International: Arlington, VA, USA.

Araya, H.T., 2007. *Seed germination and vegetative propagation of bush tea (Athrixia phylicoides)* (Doctoral dissertation, University of Pretoria, Pretoria, South Africa).

Arnao, M.B., Cano, A. and Acosta, M., 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry*, *73*(2), 239-244.

Arthurs, S.P., Stamps, R.H. and Giglia, F.F., 2013. Environmental modification inside photo selective shade houses. *HortScience*, *48*(8),975-979.

Bareja, B. G., 2011. Climatic factors can promote or inhibit plant growth and development. *Crops Review. http://www.cropsreview.com/climatic-factors. html. Accessed on July 10,2022,* 2010-2016.

Barek, M.L., Hasmadi, M., Zaleha, A. and Fadzelly, A.M., 2015. Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah Snake Grass (*Clinacanthus nutans* Lind.) leaves. *International Food Research Journal,* 22**,** 661.

Beer, J., Muschler, R., Kass, D. and Somarriba, E., 1997. Shade management in coffee and cacao plantations. *Agroforestry Systems*, *38*(1),139-164.

Beg, N., Morlot, J. C., Davidson, O., Afrane-Okesse, Y., Tyani, L., Denton, F. and Rahman, A. A., 2002. Linkages between climate change and sustainable development. *Climate Policy*, 2(2-3), 129-144.

Ben-Yakir, D., Hadar, M. D., Offir, Y., Chen, M. and Tregerman, M., 2008. Protecting crops from pests using OptiNet (R) screens and ChromatiNet (R) shading nets. *Acta Horticulturae*, 770, 205-212.

Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76.

Bergquist, S.Å., Gertsson, U.E., Nordmark, L.Y. and Olsson, M.E., 2007. Ascorbic acid, carotenoids, and visual quality of baby spinach as affected by shade netting and postharvest storage. *Journal of Agricultural and Food Chemistry*, *55*(21),8444-8451.

Boehm, R., Cash, S. B., Anderson, B. T., Ahmed, S., Griffin, T. S., Robbat Jr, A. and Orians, C. M., 2016. Association between empirically estimated monsoon dynamics and other weather factors and historical tea yields in China: results from a yield response model. *Climate*, 4(2), 20.

Bourque, D. P. and Naylor, A. W., 1971. Large effects of small water deficits on chlorophyll accumulation and ribonucleic acid synthesis in etiolated leaves of jack bean (*Canavalia ensiformis* [L.] DC.). *Plant Physiology*, 47(4), 591-594.

Buthelezi, N.M.D., 2015. Effect of photo-selective netting on postharvest quality and bioactive compounds in three selected summer herbs *(coriander, marjoram and oregano)* (Doctoral dissertation, Tshwane University of Technology). Pretoria west, South Africa.

CARRUTHERS V., 2000. Die natuurlewe van Suider-Afrika – ‘n veldgids tot die diere en plante van die streek. Struik Uitgewers. Kaapstad, Suid-Afrika.

Caruso, G., Formisano, L., Cozzolino, E., Pannico, A., El-Nakhel, C., Rouphael, Y. and De Pascale, S., 2020. Shading affects yield, elemental composition and antioxidants of perennial wall rocket crops grown from spring to summer in southern Italy. *Plants*, 9(8), 933.

Castellano, S., Mugnozza, G. S., Russo, G., Briassoulis, D., Mistriotis, A., Hemming, S. and Waaijenberg, D., 2008. Plastic nets in agriculture: a general review of types and applications. *Applied Engineering in Agriculture*, 24(6), 799-808.

Chabeli, P.M., Mudau, F.N., Mashela, P.W. and Soundy, P., 2008. Effects of nitrogen, phosphorus and potassium nutrition on seasonal tannin content of bush tea (*Athrixia phylicoides* DC.). *South African Journal of Plant and Soil*, *25*(2),79-83.

Chen, C., Luo, X., Jin, G., Cheng, Z., Pan, X., Zhu, G. and Tang, N., 2017. Shading effect on survival, growth, and contents of secondary metabolites in micro propagated Anoectochilus plantlets. *Brazilian Journal of Botany*, 40(3), 599-607.

Chen, J., Wu, S., Dong, F., Li, J., Zeng, L., Tang, J. and Gu, D., 2021. Mechanism underlying the shading-induced chlorophyll accumulation in tea leaves. *Frontiers in Plant Science*, 12, 779819.

Chen, J. M., Wu, S. H., Zeng, L. T., Dong, F. and Gu, D. C., 2021. Research progress on the influence of shading on tea (*Camellia sinensis*) quality and yield. *China Tea*, 5, 1-10.

Cronje, R., 2020. Shade netting: a possible answer to the climatic challenges facing the SA litchi industry. *Subtropical Journal*, 29(3), 18.

Díaz-Pérez, J. C. and John, K. S., 2019. Bell Pepper (*Capsicum annum* L.) under colored shade nets: Plant Growth and physiological responses. *HortScience*, 54(10), 1795-1801.

Elad, Y., Messika, Y., Brand, M., David, D. R. and Sztejnberg, A., 2007. Effect of colored shade nets on pepper powdery mildew (*Leveillula taurica*). *Phytoparasitica*, 35(3), 285-299.

Fallik, E., Alkalai-Tuvia, S., Parselan, Y., Aharon, Z., Elmann, A., Offir, Y., Matan, E., Yehezkel, H., Ratner, K., Zur, N. and Shahak, Y., 2008. September. Can colored shade nets maintain sweet pepper quality during storage and marketing? In *IV Balkan Symposium on Vegetables and Potatoes, 830* ,37- 44.

Ferrazzano, G.F., Amato, I., Ingenito, A., Zarrelli, A., Pinto, G. and Pollio, A., 2011. Plant polyphenols and their anti-cariogenic properties: a review. *Molecules*, *16*(2), 1486-1507.

Ferreira, T. A. P. C., Valadares, K. O., Souza, M. J. F., Santana, J. Q., Balbino, M. P. and Ferreira, R. C., 2012. Yellow and red sweet pepper quality under photoselective screens and field crop conditions. In *VII International Symposium on Light in Horticultural Systems, 956*, 473-479.

Fiorucci, A. S. and Fankhauser, C., 2017. Plant strategies for enhancing access to sunlight. *Current Biology*, 27(17), R931-R940.

Fine, P.V., Miller, Z.J., Mesones, I., Irazuzta, S., Appel, H.M., Stevens, M.H.H., Sääksjärvi, I., Schultz, J. C. and Coley, P.D., 2006. The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology*, *87*(sp7), S150-S162.

Fox, F.W. and Young, M.E.N., 1982. Food from the veld; edible wild plants of Southern Africa. Delta Books (Pty) Ltd., Johannesburg, South Africa (1982),119-120.

Fukuda, N., 2019. Plant growth and physiological responses to light conditions. In *Plant factory using artificial light*. Elsevier. 71-77.

Garrone, A., Archipowa, N., Zipfel, P. F., Hermann, G. and Dietzek, B., 2015. Plant protochlorophyllide oxidoreductases A and B: catalytic efficiency and initial reaction steps. *Journal of Biological Chemistry*, 290(47), 28530-28539.

Germishuizen, G., Meyer, N.L., Steenkamp, Y. and Keith, M., 2006. A Checklist of South African Plants. Southern African Botanical Diversity Network Report No. 41. *City of Tshwane: SABONET*.

Ghasemzadeh, A., Jaafar, H.Z. and Rahmat, A., 2010. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale Roscoe*). *Molecules*, *15*(6),4324-4333.

Goodwin, L. 2012. Factors influencing caffeine levels in tea. www.About.com (Accessed on 15 October 2021).

Graham, T.L., 1998. Flavonoid and flavonol glycoside metabolism in Arabidopsis. *Plant Physiology and Biochemistry*, *36*(1-2), 135-144.

Gu, L., Baldocchi, D., Verma, S.B., Black, T.A., Vesala, T., Falge, E.M. and Dowty, P.R., 2002. Advantages of diffuse radiation for terrestrial ecosystem productivity. *Journal of Geophysical Research: Atmospheres*, *107*(D6), 223-302.

Helmenstine, A. M., 2015. Use Iodine Titration to determine the amount of vitamin C in food. https://www.thoughtco.com/vitamin-c-determination-by-iodine-titration-606322 . Accessed 10 June 2021.

Herman, P.P, Retief, J., Koekemoer, E. and Welman W.G., 2000. Seed Plants of Southern Africa: O. A. Leister Editions. National Botanical Institute, Pretoria, South Africa, 24.

Hirai, M., Yoshikoshi, H., Kitano, M., Wakimizu, K., Sakaida, T., Yoshioka, T. and Maki, T., 2008. October. Production of value-added crop of green tea in summer under the shade screen net: Canopy microenvironments. In *International Workshop on Greenhouse Environmental Control and Crop Production in Semi-Arid Regions,* 797, 411-417.

Huang, W., Ma, H. Y., Huang, Y., Li, Y., Wang, G. L., Jiang, Q. and Xiong, A. S., 2017. Comparative proteomic analysis provides novel insights into chlorophyll biosynthesis in celery under temperature stress. *Physiologia Plantarum*, 161(4), 468-485.

Huché-Thélier, L., Crespel, L., Le Gourrierec, J., Morel, P., Sakr, S. and Leduc, N., 2016. Light signaling and plant responses to blue and UV radiations—Perspectives for applications in horticulture. *Environmental and Experimental Botany*, *121*, 22-38.

Hutchings, A., Scott, A.H., Lewis, G. and Cunningham., 1996. A Zulu Medicinal Plants: An Inventory University of Natal Press, Pietermaritzburg, South Africa.

Ilić, Z. S., Milenković, L., Šunić, L., Barać, S., Mastilović, J., Kevrešan, Ž. and Fallik, E., 2017. Effect of shading by coloured nets on yield and fruit quality of sweet pepper. *Zemdirbyste-Agriculture*, 104(1), 53-62.

Ilić, Z. S. and Fallik, E., 2017. Light quality manipulation improves vegetable quality at harvest and postharvest: A review. *Environmental and Experimental Botany*, 139, 79-90.

Ilić, S. Z., Milenković, L., Dimitrijević, A., Stanojević, L., Cvetković, D., Kevrešan, Ž. and Mastilović, J., 2017. Light modification by color nets improve quality of lettuce from summer production. *Scientia Horticulturae*, 226, 389-397.

Ilić, Z. S., Milenković, L., Šunić, L. and Manojlović, M., 2018. Color shade nets improve vegetables quality at harvest and maintain quality during storage. *Contemporary Agriculture*, 67(1), 9-19.

Ismail, M., Manickam, E., Danial, A. M., Rahmat, A. and Yahaya, A., 2000. Chemical composition and antioxidant activity of Strobilanthes crispus leaf extract. *The Journal of nutritional biochemistry*, *11*(11-12), 536-542.

Janjai, S. and Bala, B.K., 2012. Solar drying technology. *Food Engineering Reviews*, *4*(1), 16-54.

Jin, J., Koroleva, O. A., Gibson, T., Swanston, J., Magan, J., Zhang, Y. A. N. and Wagstaff, C., 2009. Analysis of phytochemical composition and chemoprotective capacity of rocket *(Eruca sativa* and *Diplotaxis tenuifolia*) leafy salad following cultivation in different environments. *Journal of Agricultural and Food Chemistry*, 57(12), 5227-5234.

Joubert, E., Gelderblom, W.C.A., Louw, A. and de Beer, D., 2008. South African herbal teas: Aspalathus linearis, Cyclopia spp. and Athrixia phylicoides—A review. *Journal of ethnopharmacology*, *119*(3),376-412.

Juneja, L.R., Chu, D.C., Okubo, T., Nagato, Y. and Yokogoshi, H., 1999. L-theanine—a unique amino acid of green tea and its relaxation effect in humans. *Trends in Food Science and Technology*, *10*(6-7),199-204.

Kala, H.K., Mehta, R., Tandey, R., Sen, K.K. and Mandal, V., 2016. Ten years of research on phenolics (2005–2015): A status report. *Pacific Science Review A: Natural Science and Engineering*, *18*(1), 1-4.

Kosma, C., Triantafyllidis, V., Papasavvas, A., Salahas, G. and Patakas, A., 2013. Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season. *Emirates Journal of Food and Agriculture*, 25 (12),974-979.

Kotilainen, T., Robson, T. M. and Hernandez, R., 2018. Light quality characterization under climate screens and shade nets for controlled-environment agriculture. *PLoS One*, 13(6), e0199628.

Ku, K.M., Choi, J.N., Kim, J., Kim, J.K., Yoo, L.G., Lee, S.J., Hong, Y.S. and Lee, C.H., 2010. Metabolomics analysis reveals the compositional differences of shade grown tea (*Camellia sinensis* L.). *Journal of Agricultural and Food Chemistry*, *58*(1), pp.418-426

Kumar, V.G, Kumar, A., Raghu, K., Patel, G.R and Manjappa, S., 2013." Determination of Vitamin C in some Fruits and Vegetables in Davanagere City (Karanataka)- India", *International Journal of Pharmacy and Life Sciences*, 4 (3) ,2489-2491

Kumari, R., Singh, S. and Agrawal, S.B., 2009. Combined effects of Psoralens and ultraviolet-B on growth, pigmentation, and biochemical parameters of Abelmoschus esculentus L. *Ecotoxicology and Environmental safety*, *72*(4), 1129-1136.

Kyriacou, M.C., Rouphael, Y., Di Gioia, F., Kyratzis, A., Serio, F., Renna, M., De Pascale, S. and Santamaria, P., 2016. Micro-scale vegetable production and the rise of microgreens. *Trends in food science & technology*, *57*, 103-115.

Lee, S. K. and Kader, A. A., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, 20(3), 207-220.

Lee, L. S., Choi, J. H., Son, N., Kim, S. H., Park, J. D., Jang, D. J. and Kim, H. J., 2013. Metabolomic analysis of the effect of shade treatment on the nutritional and sensory qualities of green tea. *Journal of Agricultural and Food Chemistry*, 61(2), 332-338.

Lee, Y.H., Choo, C., Watawana, M.I., Jayawardena, N. and Waisundara, V.Y., 2015. An appraisal of eighteen commonly consumed edible plants as functional food based on their antioxidant and starch hydrolase inhibitory activities. *Journal of the Science of Food and Agriculture*, *95*(14),2956-2964.

Leistner, OA. 2000. Seeds plants of southern Africa: families and genera. Strelitzia*, 10. National Botanical Institute,* Pretoria,125

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

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

Lichtenthaler, H.K., 1987. [34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In *Methods in enzymology* (Vol. 148, pp. 350-382). Academic Press.

Liu, M., Xie, F., Cao, R., Qi, X. and Chen, X., 2014. Effect of different cover cultivations in later summer on aroma constituents of autumn tea (*Camellia sinensis* L.). *Journal of Agriculture Chemistry and Environmental*, 3, 1-6.

Liu, Y., Fang, S., Yang, W., Shang, X. and Fu, X., 2018. Light quality affects flavonoid production and related gene expression in *Cyclocarya paliurus*. *Journal of Photochemistry and Photobiology B: Biology*, 179, 66-73.

Liu, L., Li, Y., She, G., Zhang, X., Jordan, B., Chen, Q. and Wan, X., 2018. Metabolite profiling and transcriptomic analyses reveal an essential role of UVR8-mediated signal transduction pathway in regulating flavonoid biosynthesis in tea plants (*Camellia sinensis*) in response to shading. *BMC Plant Biology*, 18(1), 1-18.

López-Figueroa, F. and Niell, F. X.,1990. Effects of light quality on chlorophyll and biliprotein accumulation in seaweeds. *Marine Biology*, 104(2), 321-327.

Mabogo, DNE.1990. The Ethnobotany of Vha-Venda. MSc Thesis: University of Pretoria. Pretoria, South Africa.2

Mahmood, A., Hu, Y., Tanny, J. and Asante, E.A., 2018. Effects of shading and insect-proof screens on crop microclimate and production: A review of recent advances. *Scientia Horticulturae*, *241*,241-251.

Malongane, F., McGaw, L.J. and Mudau, F.N., 2017. The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: a review. *Journal of the Science of Food and Agriculture*, *97*(14), 4679-4689.

Malongane, F., McGaw, L.J. and Mudau, F.N., 2020. Topic: chemical compositions and mineral content of four selected South African herbal teas and the synergistic response of combined teas. *British Food Journal*,122(9), 2769-2785.

Manja, K. and Aoun, M., 2019. The use of nets for tree fruit crops and their impact on the production: A review. *Scientia Horticulturae*, *246*,110-122.

Mapcarta.2022.https://mapcarta.com/W460936140. Accessed 20 March 2022.

Mashabela, M.N., Selahle, K.M., Soundy, P., Crosby, K.M. and Sivakumar, D., 2015. Bioactive compounds and fruit quality of green sweet pepper grown under different coloured shade netting during postharvest storage. *Journal of Food Science*, *80*(11),2612-2618.

Mashimbye, M.J., Mudau, F.N., Soundy, P. and Van Ree, T., 2006. A new flavonol from Athrixia phylicoides (bush tea). *South African Journal of Chemistry*, *59*, 1-2.

Mathanghi, S.K. and Sudha, K., 2012. Functional and phytochemical properties of finger millet (*Eleusine coracana* L.) for health. *International Journal of Pharmaceutical, Chemical and Biology Sciences*, 2(4), 431-438.

Mathivha, P.L. and Mudau, F.N., 2017. Response of total phenolic content and antioxidant activities of bush tea and special tea using different selected extraction solvents. *Journal of Consumer Sciences*, 45(1),27-33.

Mavundza, E.J., Tshikalange, T.E., Lall, N., Hussein, A.A., Mudau, F.N. and Meyer, J.J.M., 2010. Antioxidant activity and cytotoxicity effect of flavonoids isolated from *Athrixia phylicoides*. *Journal of Medicinal Plants Research*., 4: 2584–2587

McGaw, L.J., Steenkamp, V. and Ellof, J.N., 2007. Evaluation of Athrixia bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. *Journal of Ethnopharmacology*, *110*(1), 16-22.

Mbambezeli, G., 2005. Distribution and habitat of Athrixia phylicoides. http://pza.sanbi.org/athrixia-phylicoides. Accessed 09 June 2021.

Mditshwa, A., Magwaza, L. S. and Tesfay, S. Z., 2019. Shade netting on subtropical fruit: Effect on environmental conditions, tree physiology and fruit quality. *Scientia Horticulturae*, 256, 108556.

Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G., 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3),571-577.

Milenković, L., Stanojević, J., Cvetković, D., Stanojević, L., Lalević, D., Šunić, L. and Ilić, Z. S., 2019. New technology in basil production with high essential oil yield and quality. *Industrial Crops and Products*, 140, 111718.

Mogotlane, I. D., Mudau, F. N., Mashela, P. W., and Soundy, P., 2007. Seasonal responses of total antioxidant contents in cultivated bush tea (*Athrixia phylicoides* DC.) leaves to fertiliser rates. *Medicinal and Aromatic Plant Science and Biotechnology*. 1(1),77-79.

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 Dissertation, University of Pretoria, Pretoria, South Africa.

Mudau, F.N., Soundy, P., Du Toit, E.S. and Olivier, J., 2006. Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. *South African Journal of Botany*, *72*(3),398-402.

Mudau, F.N., Soundy, P. and Du Toit, E.S., 2007. Effects of nitrogen, phosphorus, and potassium nutrition on total polyphenol content of bush tea (*Athrixia phylicoides* L.) leaves in shaded nursery environment. *Horticultural Science*, *42*(2), 334-338.

Mudau, F.N., Ngele, A., Mashela, P.W. and Soundy, P., 2007a. Seasonal variation of tannin content in wild bush tea. *Medicinal and Aromatic Plant Science and Biotechnology*, *1*(1),74-76.

Mudau, F.N., Araya, H.T., Du Toit, E.S., Soundy, P. and Olivier, J., 2007b. Bush tea (*Athrixia phylicoides* DC.) as an alternative herbal and medicinal plant in southern Africa: opportunity for commercialization. *Medicinal and Aromatic Plant Science and Biotechnology*, 1,70-73.

Mudau, F.N. and Ngezimana, W., 2014. Effect of different drying methods on chemical composition and antimicrobial activity of bush tea (Athrixia phylicoides). *International Journal of Agriculture and Biology*, *16*(5).

Mulungisi, A., 2015. The impacts of heavy rains on the vegetation cover in the Limpopo Province of South Africa. Doctoral dissertation, North-West University, Mafikeng, South Africa.

Nchabeleng, L., Mudau, F.N. and Mariga, I.K., 2012. Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors. *Journal of Medicinal Plants Research*, *6*(9),1662-1666.

Nishiumi, S., Miyamoto, S., Kawabata, K., Ohnishi, K., Mukai, R., Murakami, A., Ashida, H. and Terao, J., 2011. Dietary flavonoids as cancer-preventive and therapeutic biofactors. *Frontier in Bioscience*, *3*(3),1332-1362.

Ntsoane, M.L., 2015. Effect of photoselective nettings on plant growth, quality at harvest and after postharvest storage in lettuce varieties. Master of Science, Thesis, Tshwane University of Technology., Pretoria, South Africa.

Ntsoane, L.L., Soundy, P., Jifon, J. and Sivakumar, D., 2016. Variety-specific responses of lettuce grown under the different-coloured shade nets on phytochemical quality after postharvest storage. *The Journal of Horticultural Science and Biotechnology*, *91*(5),520-528.

Odhiambo, J.J., 2010. Decomposition and nitrogen release by green manure legume residues in different soil types. *African Journal of Agricultural Research*, *5*(1), 090-096.

Opio, A., Jones, B. M., Kansiime, F. and Otiti, T., 2017. Response of Cyperus papyrus productivity to changes in relative humidity, temperature, and photosynthetically active radiation. *African Journal of Plant Science*, 11(5), 133-141.

Pavarini, D. P., Pavarini, S. P., Niehues, M. and Lopes, N. P., 2012. Exogenous influences on plant secondary metabolite levels. *Animal Feed Science and Technology*, 176(1-4), 5-16.

Pirbalouti, A.G., Mahdad, E. and Craker, L., 2013. Effects of drying methods on qualitative and quantitative properties of essential oil of two basil landraces. *Food Chemistry*, *141*(3), 2440-2449.

Radušienė, J., Karpavičienė, B. and Stanius, Ž., 2012. Effect of external and internal factors on secondary metabolites accumulation in St. John's worth. *Botanica Lithuanica*, *18*(2),101-108.

Rakuambo, J.Z. 2007. Indigenous knowledge of bush tea (*Athrixia phylicoides*) and effect of fertigation and growing medium on plant growth. MSc Dissertation. University of Pretoria, Pretoria, South Africa.

Rampedi, I. and Olivier, J., 2005. The use and potential commercial development of *Athrixia phylicoides*. *Acta Academica*, *37*(3), 165-183.

Rehman, S. U., Almas, K., Shahzadi, N., Bhatti, N. and Saleem, A., 2002. Effect of time and temperature on infusion of tannins from commercial brands of tea. *International Journal of Agriculture and Biology*, *4*(2), 285-287.

Riachi, L. G., Simas, D. L. R., Coelho, G. C., Marcellini, P. S., da Silva, A. J. R. and de Maria, C. A. B., 2018. Effect of light intensity and processing conditions on bioactive compounds in maté extracted from yerba mate (*Ilex paraguariensis* A. St.-Hil.). *Food Chemistry*, 266, 317-322.

Riga, P., Benedicto, L., Gil-Izquierdo, Á., Collado-González, J., Ferreres, F. and Medina, S., 2019. Diffuse light affects the contents of vitamin C, phenolic compounds, and free amino acids in lettuce plants. *Food Chemistry*, 272, 227-234.

Roberts, M.1990. Indigenous healing plants. First Edition. *Southern book publishers*: Halfway house (1),56-57

Ryan, L. and Petit, S., 2010. Addition of whole, semi skimmed, and skimmed bovine milk reduces the total antioxidant capacity of black tea. *Nutrition Research*, *30*(1),14-20.

Rouphael, Y., Kyriacou, M.C., Petropoulos, S.A., De Pascale, S. and Colla, G., 2018. Improving vegetable quality in controlled environments. *Scientia Horticulturae*, *234*, 275-289.

Sabir, A., Sabir, F. and Jawshle, A. I. M., 2020. Quality changes in grape berry as affected using different colored shade nets proposed to alleviate the adverse effects of climate change. *Asian Journal of Agriculture and Food Sciences*, 8(1), 1-5.

Sáenz-Romero, C., Rehfeldt, G. E., Crookston, N. L., Duval, P., St-Amant, R., Beaulieu, J. and Richardson, B. A., 2010. Spline models of contemporary, 2030, 2060 and 2090 climates for Mexico and their use in understanding climate-change impacts on the vegetation. *Climatic Change*, 102(3), 595-623.

Saito, K., Yonekura-Sakakibara, K., Nakabayashi, R., Higashi, Y., Yamazaki, M., Tohge, T. and Fernie, A.R., 2013. The flavonoid biosynthetic pathway in Arabidopsis: structural and genetic diversity. *Plant Physiology and Biochemistry*, *72*, 21-34.

SAS. 1999. SAS/STAT User's Guide, Version 8, 1st printing, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

SAWS., 2021. Climatic data for autumn season for year 2018 and 2019.

Schippers, P., Sterck, F., Vlam, M. and Zuidema, P. A., 2015. Tree growth variation in the tropical forest: understanding effects of temperature, rainfall, and CO_2. *Global Change Biology*, *21*(7), 2749-2761.

Selahle, K. M., Sivakumar, D., Jifon, J. and Soundy, P., 2015. Postharvest responses of red and yellow sweet peppers grown under photo-selective nets. *Food Chemistry*, *173*, 951-956.

Shahak, Y., Gussakovsky, E. E., Gal, E. and Ganelevin, R., 2004, March. Color nets: crop protection and light-quality manipulation in one technology. In *VII International Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition 659* ,143-151.

Shahak, Y., 2006. August. Photo-selective netting for improved performance of horticultural crops. A review of ornamental and vegetable studies carried out in Israel. In *XXVII International Horticultural Congress-IHC2006: International Symposium on Cultivation and Utilization of Asian, 770*, 161-168.

Shahak, Y., Ratner, K., Zur, N., Offir, Y., Matan, E., Yehezkel, H., Messika, Y., Posalski, I. and Ben-Yakir, D., 2008, April. Photoselective netting: an emerging approach in protected agriculture. In *International Symposium on Strategies Towards Sustainability of Protected Cultivation in Mild Winter Climate 807*, 79-84.

Shao, Q., Wang, H., Guo, H., Zhou, A., Huang, Y., Sun, Y. and Li, M., 2014. Effects of shade treatments on photosynthetic characteristics, chloroplast ultrastructure, and physiology *Anoectochilus roxburghii*. *PloS One*, 9(2), e85996.

Shapiro, S. S. and Wilk, M.B. 1965. An Analysis of Variance Test for Normality (complete samples), *Biometrika*, 52, 591-611.

Siddiqui, N., Rauf, A., Latif, A. and Mahmood, Z., 2017. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata Benth*). *Journal of Taibah University Medical Sciences*, *12*(4),360-363.

Siracusa, L. and Ruberto, G., 2014. Chapter II: plant polyphenol profiles as a tool for traceability and valuable support to biodiversity. In: Watson R.R., (ed). Polyphenols in plants: isolation Purification and Extract Preparation. Academic Press, 15-33.

Sivakumar, D. and Jifon, J., 2018. Influence of photoselective shade nettings on postharvest quality of vegetables. Academic Press. In *Preharvest modulation of postharvest fruit and vegetable quality*, 121-138.

Song, B. H., Lee, D. H., Kim, B. C., Ku, S. H., Park, E. J., Kwon, I. H. and Kim, K. J., 2014. Photodynamic therapy using chlorophyll-a in the treatment of acne vulgaris: a randomized, single-blind, split-face study. *Journal of the American Academy of Dermatology*, 71(4), 764-771.

Southern African Research and Documentation Centre (SARDC)., 2002. Limpopo River Basin Factsheet, Southern African Research and Documentation Centre (SARDC), Harare, Zimbabwe. Accessed at: http://www.sardc.net/IMERCSA/Limpopo/pdf/Limpopo1. pdf. Accessed on: August 18, 2022.

Stagnari, F., Galieni, A., Cafiero, G. and Pisante, M., 2014. Application of photo-selective films to manipulate wavelength of transmitted radiation and photosynthate composition in red beet (*Beta vulgaris var. conditiva Alef.). Journal of the Science of Food and Agriculture*, *94*(4),713-720.

Stagnari, F., Galieni, A. and Pisante, M., 2015. Shading and nitrogen management affect quality, safety, and yield of greenhouse-grown leaf lettuce. *Scientia Horticulturae*, 192, 70-79.

Stamps, R.H., 2009. Use of colored shade netting in horticulture. *HortScience*, *44*(2),239-241.

Steffen, W., 2008. Working group 1 report of the IPCC fourth assessment—an editorial. *Global Environmental Change*, 1(18), 1-3.

Thamkaew, G., Sjöholm, I. and Galindo, F.G., 2021. A review of drying methods for improving the quality of dried herbs. *Critical Reviews in Food Science and Nutrition*, *61*(11),1763-1786.

Tshikhudo, P.P., Ntushelo, K., Kanu, S.A. and Mudau, F.N., 2019. Growth response of bush tea (*Athrixia phylicoides* DC.) to climatic conditions in Limpopo Province, South Africa. *South African Journal of Botany*, *121*, 500-504.

van Wijk Y.,1986. *The Practical Book of Herbs: Growing and Using Herbs in South Afric*a, Chameleon Press C.C., Cape Town, South Africa, 22.

Tounekti, T. and Khemira, H., 2015. NaCl stress-induced changes in the essential oil quality and abietane diterpene yield and composition in common sage. *Journal of Intercultural Ethnopharmacology*, *4*(3), 208-216.

Tounekti, T., Munné-Bosch, S., Vadel, A.M., Chtara, C. and Khemira, H., 2010. Influence of ionic interactions on essential oil and phenolic diterpene composition of Dalmatian sage (Salvia officinalis L.). *Plant Physiology and Biochemistry*, *48*(10-11), 813-821.

van Wyk B.E., Gericke, N., 2000. People's Plants: *A Guide to Useful Plants of South Africa* Briza Publication, Pretoria, (1), 102.

Velderrain-Rodríguez, G.R., Palafox-Carlos, H., Wall-Medrano, A., Ayala-Zavala, J.F., Chen, C.O., Robles-Sánchez, M., Astiazaran-García, H., Alvarez-Parrilla, E. and González-Aguilar, G.A., 2014. Phenolic compounds: their journey after intake. *Food and Function*, *5*(2),189-197.

Villanueva, C. and Kross, R.D., 2012. Antioxidant-induced stress. *International journal of Molecular Sciences*, *13*(2), 2091-2109.

Wang, Y., Gao, L., Shan, Y., Liu, Y., Tian, Y. and Xia, T., 2012. Influence of shade on flavonoid biosynthesis in tea (Camellia sinensis (L.) O. Kuntze). *Scientia Horticulturae*, *141*,7-16.

Wang, Y., Gao, S., He, X., Li, Y., Zhang, Y. and Chen, W., 2020. Response of total phenols, flavonoids, minerals, and amino acids of four edible fern species to four shading treatments. *Peer Journal, Plant Biology*, 8, e8354.

Xie, Y., Ho, S.H., Chen, C.N.N., Chen, C.Y., Ng, I.S., Jing, K.J., Chang, J.S. and Lu, Y., 2013. Phototrophic cultivation of a thermo-tolerant *Desmodesmu*s sp. for lutein production: effects of nitrate concentration, light intensity, and fed-batch operation. *Bioresource Technology*, *144*,435-444.

Yamashita, H., Tanaka, Y., Umetsu, K., Morita, S., Ono, Y., Suzuki, T., Takemoto, T., Morita, A. and Ikka, T., 2020. Phenotypic markers reflecting the status of overstressed tea plants subjected to repeated shade cultivation. *Frontiers in Plant Science*, *11*, 556476.

Zhao, D., Hao, Z. and Tao, J., 2012. Effects of shade on plant growth and flower quality in the herbaceous peony (*Paeonia lactiflora Pall.). Plant Physiology and Biochemistry*, 61, 187-196.

Zrig, A., Tounekti, T., Hegab, M. M., Ali, S. O. and Khemira, H., 2016. Essential oils, amino acids, and polyphenols changes in salt-stressed Thymus vulgaris exposed to open–field and shade enclosure. *Industrial Crops and Products*, 91, 223-230.