

CHAPTER 1

INTRODUCTION

Bush tea (*Athrixia phylicoides* DC.) has only recently received interest as another indigenous South African herbal tea with potential for commercialization (Rampedi and Olivier, 2005). It is also commonly known as bushman's tea, Boesmanstee (in Afrikaans), and Icholocholo, itshelo, or umthsanelo (in Zulu) (Mudau, Araya, du Toit, Soundy, and Olivier., 2007b). Bush tea leaves have been used for many years in South Africa as an African traditional medicinal herbal tea (van Wijk, 1986; Mudau et.al. 2007b). Various tribes and cultures of South Africa use bush tea in various ways for various purposes. For instance Vhavenda people use bush tea as an aphrodisiac (Mabogo, 1990; Mudau, Soundy and du Toit., 2007a), and in Vhembe district extracts from roots and leaves are used for treating anthelmintics (Mudau et.al. 2007b). Sothos and Xhosas chew bush tea to cure sore throats and coughs (Mudau et.al. 2007b), and Sothos also use strong brew as a calming wash for sore feet (Roberts, 1990; Mudau et.al. 2007b). Zulu people use bush tea decoction of the root as a cough remedy and purgative (Watt and Brandwijk, 1962,). Other traditional African people use of bush tea including cleansing or purifying of the blood, treating boils, headaches, and infested wounds (Mudau et.al. 2007a)

While bush tea has traditionally been harvested for medicinal purposes from the wild, van Wyk and Gericke (2000) reported the suitability of bush tea for domestication and development as a commercial health beverage (Mudau et.al. 2007b).

The success of domestication and commercialization of bush tea hinges on maintenance and/or enhancement of quality of bush tea as a herbal beverage. Herbal tea quality is one of the critical factors in commercialization that would determine the price of tea for sale and export (Mudau, Ngele, Mashela, and Soundy, 2007c). Factors that affect quality parameters are classified into four

categories *inter alia* cultivars, environmental conditions, cultural practices and seasonal variation (Chiu, 1989; Taylor, Baker, Owour, Orchard, and Othieno, 1992; Sud and Baru, 2000; Owour, Ng'etich, and Obanda. 2000; Mudau et.al. 2007c).

The aim of this study was to establish the effect of harvesting at various phenological stages, the effect of pruning at different height and the effect of applying gibberellins at different rates, on chemical composition of bush tea.

The objectives of this study were to:

- (i) determine the quality of tea harvested at various phenological stages for wild and cultivated bush tea,
- (ii) determine the effect of pruning on growth and chemical compositions of cultivated bush tea, and
- (iii) determine the effect of application of gibberellins on sprouting and quality of bush tea.

CHAPTER 2

LITERATURE REVIEW

2.1. BOTANICAL DESCRIPTION OF BUSH TEA

The genus *Athrixia* contains 14 species, found in southern Africa, tropical Africa and Madagascar (Joubert, Gelderblom, Louw and de Beer, 2008). Of these, *Athrixia phyllicoides* DC. (Bush tea) belongs to the family Asteraceae tribe Inucleae and subtribe Athrixiinae (Mudau et.al. 2007b)

Bush tea (*Athrixia phyllicoides* DC) is an aromatic, perennial, leafy shrub of up to 1m with woolly white stems (Fox and Young, 1982; Joubert et.al., 2008). The leaves of bush tea are simple, alternate linear to broadly lanceolate, tapering to a sharp point (Herman, Retief, Koekemoer and Welman, 2000), dark-green and shiny below and woolly white above (Joubert et.al.,2008), with margins entirely or slightly revolute (Mudau et.al. 2007b). The leaves are also often shortly-stalked and auriculate at the base (Mudau et.al. 2007b)

The inflorescence head of bush tea is sessile or sub-sessile and terminates axillarily in large subcorymbose panicles (Mudau et.al. 2007b). Flowers of bush tea are daisy-like with pink to purple petals and bright yellow centers, occurring throughout the year depending on the geographic area (Van Wyk and Gericke, 2000; Joubert et.al., 2008).

The fruits of bush tea consist of narrow, cylindrical and thin achenes that are approximately 0.01 to 0.06 mm wide (Araya, 2005). The seed is 4mm in length and has 2 pappuses that are used for dissemination (Araya, 2005). Bush tea adapts well in open grassland and in thick forest margins of South Africa, including Limpopo Province, Free State Province, Kwa-Zulu Natal and some parts of Eastern Cape province (Mudau et.al. 2007b). It can be successfully propagated by seeds and cuttings (Mudau et.al. 2007b).

2.2. CHEMICAL COMPOSITION AND QUALITY OF HERBAL TEAS

Quality of herbal tea is one of the critical factors as it determines the price of tea (Mudau et.al. 2007c). Chemical compounds have been reported to influence the quality of herbal teas (Owour, Ng'etich and Obanda, 2000). Compounds such as polyphenols, flavonols, and tannins are main indicators of the medicinal potential of herbal teas due to their antioxidant activities (Hirasawa, Takada, Makimura and Otake, 2002; Mudau et.al. 2007c). Green tea leaves (*Camellia sinensis*) have been reported to contain 10–30% (dry leaf weight) of polyphenols, including catechins, flavonols, flavanones, phenolic acids, glycosides and the aglycones of plant pigments (Pan, Niu, and Liu, 2003). Other chemical components such as amino acids, carbohydrates, organic acids, vitamins and volatile flavor compounds provide sensory quality attributes such as astringent taste, bitterness, sweetness and aroma (Mudau et.al. 2007c).

2.2.1. Antioxidant activities of herbal tea

Antioxidant content is widely used as a parameter to characterize different materials for health benefits (Mogotlane, Mudau, Mashela and Soundy, 2007). This activity is associated with compounds capable of protecting a biological system against the harmful effect of reactions that can cause excessive oxidation, involving reaction of oxygen and nitrogen species (Mogotlane et.al., 2007). Antioxidant properties of tea are a result of wide range of amphipathic molecules known as phenolic compounds (Ivanova, Gerova Chervenkov, and Yankova, 2004). The antioxidant activity of phenolics are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and/or metal chelators (Rice-evans, Miller, and Paganga, 1997). The most important chemicals which are common in bush tea leaves are polyphenols, tannins and flavonols (Ivanova et.al., 2004)

2.2.2. Polyphenolic compounds

Tea polyphenols are natural antioxidants and considered to be responsible for the anticarcinogenic and antimutagenic properties of tea (Reza, Gholam, and Mohammad, 2007). Herbal teas have a wide range of polyphenols (Owour et.al, 2000), which have beneficial biochemical and physiological properties for human health (Hirasawa et.al., 2002). The major polyphenol antioxidant reported in green tea is epigallocatechin-3-gallate (EGCG). EGCG is reported to reduce the amount of free radicals and inflammatory prostaglandins in skin cells (Katiyar and Mukhtar, 1996). Tea leaves are reported to be rich sources of polyphenolic substances (Benzie, Szeto, Strain, and Thomlinson, 1999), and account for one third of dry mass of dried leaves (Liang, Lu and Shang, 1996). According to Benzie et.al., (1999), the colour of the beverage and taste, especially astringency, is attributed to these polyphenolic compounds.

2.2.3. Flavonoids

Flavonols are potential quality indicators of herbal teas since they are antioxidant in nature (Mudau et.al. 2007b). Bush tea has been reported to contain 5-hydroxy-6,7,8,3',4,5'-hexamethoxy-flavon-3-ol as a major flavonoid (Mashimbye, Mudau, Soundy and van Ree, 2006). Flavonoids have a wide range of physiological and medicinal activity which makes their presence in any traditional remedy or beverage significant. Flavonoids are known among other things for cAMP diphosphoesterase inhibition activities, growth inhibition activities and cytotoxicity activities (Mashimbye et.al., 2006).

2.2.4. Tannins

Tannins are phenolic compounds that are typically astringent and found in a variety of herbal products. Tannins may be grouped into hydrolysable and condensed tannins (Bokuchova and Skobeleva, 1980). Condensed tannins are polymers of 2 to 5 or more flavonoid units that are joined by carbon-carbon

bonds, which are not susceptible to hydrolysis (van Wyk and Gericke, 2000). Hydrolysable tannins are hydrolyzed by weak acids or bases to produce carbohydrates and phenolic acids (Haslam, 1996). Tannins found in herbal teas are reported to prevent cancers and heart problems (Stensvold, Tversdal and Solvoll, 1992). They reduce the tendency of blood platelets to stick together (Stensvold et.al., 1992). Herbal teas are reported to generally contain low tannin levels with a lot of variation from one season to another. In the study of seasonal variation of tannins in wild bush tea, Mudau et.al. (2007c) found that the highest concentrations of condensed tannins were in autumn (4.8%) compared with winter (2.4%), spring (2.7%) and summer (3.0%). Hydrolysable tannins were on the other hand found to be lowest during summer (0.10%) compared to autumn (0.14%), winter (0.14%) and spring (0.13%) (Mudau et.al. 2007c). This suggests that, to obtain high tannin content, the best times to harvest bush tea is during autumn and winter.

2.3. CULTURAL PRACTICES AND SEASONAL VARIATION ON QUALITY OF HERBAL TEAS

Teas (including herbal teas) are cultivated in diverse environments causing yield and quality differences in their beverages. To maximize yields and quality, agronomic practices should be optimized (Owour, Kamau and Jondiko, 2009). Other factors that affect yield and quality parameters are seasonal variation (Sud and Baru, 2000) as well as cultivar type (Owour et.al., 2000). Cultural or agronomic practices that affect yield and quality of tea include plucking or harvesting, mineral nutrition, pruning and application of growth regulators.

2.3.1. Plucking

Plucking is one important agronomic practice that if incorrectly done, may reduce tea yields and/or quality (Owour et.al., 2009). As a result the process needs to be optimized for realization of high yields and quality.

Intensity of harvesting is an important parameter in the realization of high yields in tea production (Mouli, Onsando, and Corley, 2007). One way of ensuring high plucking intensity in tea production is through the use of short plucking intervals (Owour et.al., 2009). Owuor and Odhiambo, (1994) reported increased yield responses with short plucking intervals in Kenya. Long plucking intervals and shear plucking was reported to have reduced tea yields and produced coarser leaf than short plucking intervals and hand plucking respectively (Owour and Odhiambo, 2006).

Method of plucking was also found to have an impact on quality of tea. Owour and Odhiambo (2006) observed that hand plucked teas had higher theaflavins (TF), caffeine, brightness, and flavour index. The authors also observed that black teas plucked from short plucking rounds were superior to those from long plucking rounds as assessed by theaflavins, caffeine, brightness, the chemical aroma quality parameters and sensory evaluation. Owour et.al., (2009) also reported that short plucking intervals ensure production of high quality tea.

Owuor, Obaga, and Othieno (1990) established that the quality of tea is affected by the growth rate of the pluckable shoots and improves as growth rate decreases .

2.3.2. Mineral nutrition

Mudau, Soundy, du Toit and Olivier (2006) reported that single application of 300 kg/ha N or P and 200 kg/ha K maximized shoot growth and total polyphenols whereas the combined applications of 300 kg/ha N or P and 200kg/ha K doubled growth and total polyphenol content of cultivated bush tea. Chabeli, Mudau,

Mashela and Soundy (2008) also reported that both condensed and hydrolysable tannins increased when N was applied at 300 kg/ha, when P was applied and increased quadratically reaching maximum concentrations when K was applied at 200 kg/ha. Mogotlane et.al., (2007) reported that the application of N, P and K fertilizers increased total antioxidant content with most of the increase occurring at 300 N, 300 P and 100 K kg/ha, regardless of season.

2.3.3. Pruning

Pruning has been identified as an essential agronomic practice in the production of leaves for the manufacture of black tea (Ravichandran, 2003). Satyanarayana, Sreedhar, Cox and Sharma (1994) reported that pruning leads to enhanced branching and hence a greater number of tender leaves. According to Satyanarayana et.al., (1994) unpruned tea plants produce more dormant buds than growing buds. Therefore, pruning prior to harvest has been considered to have great effects on plant productivity.

Besides having an effect on growth, pruning has an effect on quality of tea. In the study of effect of pruning and time from pruning on quality and aroma constituents of black tea, Ravichandran (2003) reported total polyphenols and catechin contents of green leaf showing an increase in the first year and decreasing thereafter. The green pigment (chlorophyll) content showed enhancement with time from pruning and the total carotenoids increased up to 2 years and started decreasing thereafter (Ravichandran, 2003). The lipoxygenase activity was found to decline with time from pruning. According to Ravichandran (2003) the gradual attainment of maturity in shoot components can be attributed to a higher accumulation of lipid, and decline in enzyme activity, resulting in desirable flavour in manufactured tea, with time from pruning.

Variation in growth rate as a result of pruning is expected to cause some changes in the green leaf constituents and hence the quality of made tea (Ravichandran and Parthiban, 2000).

2.3.4. Growth regulators

Application of growth hormones or regulators is also reported to have an effect on chemical composition of tea. Chandra and Pandey (1997) reported applied bioregulators, jibika, IAA, cycocel, thiourea, methanol, succinic acid and sucrose, having a significant effect on quality parameters such as polyphenol oxidase (PPO) activity, caffeine, crude protein, starch, nitrogen, carotenoid and ascorbic acid (vitamin C) content of pruned *Camellia sinensis*. Similarly, Liang et.al., (1996) reported the effect of gibberellins to cause improved growth and chemical composition of green tea. The same authors also reported that amino acids content increased by 9.8% while vitamin C content increased by 17.8% and tea catechins index increased by 12.9%. The content of tea polyphenols and ratio of tea polyphenols to amino acids decreased by 9.9% and 11.5%, respectively.

2.3.5. Seasonal variation

Agro-climatic conditions are reported as another factor that influences tea quality (Fernando and Roberts, 1984). Gulati and Ravindranath (1996) reported periodic seasonal variations in infusion quality of orthodox Kangra tea (*Camellia sinensis*) over various growth flushes. According to the authors, theaflavins, thearubigins and caffeine recorded maximum content during early flush and gradual decline with progress in season, showing a minimum during main flush and slight improvement through backend flush (Gulati and Ravindranath, 1996). Flavour profile analysis also revealed qualitative and quantitative seasonal variations in aroma complex of *Camellia sinensis* (Gulati and Ravindranath, 1996). Mudau, Soundy, du Toit and Olivier (2006) reported that concentrations of total polyphenols in leaves of wild bush tea plants were lowest during autumn (March and April) and spring (September) and highest during winter (June and July). The authors also reported highest condensed tannin contents in autumn (4.82%) compared to other seasons and lowest hydrolysable tannins during summer (0.01%).

2.4. SUMMARY AND CONCLUSIONS

This review has generally examined the important attributes of herbal teas and factors that affect their quality and growth. Herbal properties and qualities of herbal teas are determined or influenced by their active chemical compounds. These chemical compounds are indicators of the medicinal potential due to their antioxidant activities. The most important chemicals which are common in herbal teas include polyphenols, tannins and flavonols. Bush tea in particular contains 5-hydroxy-6,7,8,3',4,5'-hexamethoxy-flavon-3-ol as a major flavonoid.

The review has also revealed that the quality (as dictated by chemical composition) of herbal teas is influenced by cultural or agronomic and seasonal variations. Plucking of leaves and proper mineral nutrition enhanced total polyphenols. Hand and short-round plucking produced superior black teas than machine plucking and long round plucking. Nitrogen, phosphorus and potassium (N, P and K) application increased tannins and total antioxidant content respectively.

Pruning has been identified as another essential agronomic practice that influence growth and quality of tea. Pruning led to enhanced branching and number of tender leaves, while un-pruned tea plants produced more dormant buds than growing buds, thus having a greater effect on plant productivity. Variation in growth rate as a result of pruning caused some changes in the green leaf constituents.

Application of gibberellins led to a decrease in the content of polyphenols and ratio of polyphenols to amino acids in tea. Maximum content of theaflavins, thearubigins and caffeine was recorded during early flush but declined gradually with progress in season, showing a minimum during main flush and slight improvement through backend flush.

CHAPTER 3

QUALITY PROFILES OF CULTIVATED AND WILD BUSH TEA HARVESTED AT VARIOUS PHENOLOGICAL STAGES

3.1. INTRODUCTION

Phenological stage of tea can be described as tea growth flush. First spring leaf buds, called the first flush, are considered as the highest-quality leaves (MedlinePlus, 2009). When the first flush leaf bud is picked, another one grows, which is called the second flush, and this continues until an autumn flush. According to MedlinePlus (2009), the older leaves picked further down the stems are considered to be of poorer quality. Ellis and Grice (1983) reported that the finer the plucking standard, which involves plucking only the first two leaves and bud, the higher the quality.

Flush is followed by accumulation of carbohydrate reserves and are channeled towards the production of total polyphenols (Roberts, 1990). In the fresh first tea flush there exist a wide variety of non-volatile compounds, namely polyphenols, flavanols, flavones, phenolic acids and depsides, amino acids, chlorophyll and other pigments (Hart, 2009). Tea total polyphenols in tea flush range from 20% to 35% (Hart, 2009). Gulati and Ravindranath (1996) reported maximum content of theaflavins, thearubigins and caffeine during early flush and gradual decline with progress in season, showing a minimum during main flush and slight improvement through backend flush.

Although some information on the effect of phenological stages of certain other tea species is available, information on the effect of phenological stages specifically on bush tea is lacking. Therefore the aim of this experiment was to investigate the effect of phenological stages on chemical composition of both cultivated and wild bush tea. The distinctive phenological stages for this study

were the aerial new growth (leaves) and the older basal leaves of bush tea and whole plants.

3.2. MATERIALS AND METHODS

3.2.1. Collection of wild bush tea materials

Wild bush tea materials were collected from Mudzidzidzi Village (24° 50' 31° S 17E; Altitude 610m, summer rainfall and dry winter) (35 kilometers North-West of Thohoyandou); subtropical-type climate of summer rainfall, cold and dry winter. Thirty plants of bush tea were randomly collected for sampling at various phenological points of top new growth leaves, further down older leaves and whole plants. The collected materials were air dried in the shade for the determination of chemical composition assays.

3.2.2. Cultivated bush tea

The experiment on cultivated bush tea was carried out at Madzivhandila College of Agriculture (22° 56' 60S, 30° 28' 60E, Altitude 709m, summer rainfall and dry winter), The planting materials made up of mature bush tea stock plants were collected from Mudzidzidzi village. Selection of the planting materials was made on the basis of true-to-name and type, free of disease and insect damage, and in a healthy physiological state.

During cultivation, to stimulate rapid and prolific rooting of cuttings, plants were cut about 7-8cm long and were treated with Seradix No.2 (0.3% IBA) (Bayer Pretoria, South Africa) and planted on seedling trays on a mist bed, supplied with a misting system operating through misting nozzles. The mist bed used was 3m long, 1.5m wide and 0.5m high. Irrigation was done 3 times a day everyday except on rainy days.

Bush tea seedlings were allowed to grow on seedlings trays (Figure 3.1) for three months. Rooted cuttings (seedlings) were ready and were transplanted directly into 20-L bags on 15 December 2007. The medium used during transplanting was pine bark and sand at a ratio of 2:1, respectively.

In an attempt to achieve optimum growth, the growing bush tea plants in plastic bags were treated to a split application with NPK at rates 300kg/ha, 300kg/ha and 200kg/ha as reported by Mudau et.al. (2007b) two weeks after transplanting.



Figure 3.1: Bush tea seedlings growing on seedling trays

Cultivated bush tea plants were allowed to grow in plastic bags (Figure 2.2) in the nursery for about 2 months before they were harvested. Harvesting of cultivated bush tea at different phenological stages (of top new leaves, older leaves and whole plants) was done on 13 February 2008. The harvested materials were air

dried in the shade (Figure 3.3) for the determination of chemical composition assays.



Figure 3.2: Cultivated bush tea growing in the nursery

3.2.3. Data Collection

In this experiment, chemical composition analysis was done for total polyphenols, tannins and antioxidant content.

Determination of total polyphenols content: Methanol was used as the extraction solvent for the determination of total phenols. Duplicates of 2g of tea were extracted using 40ml of the solvent as subsequently described. An amount of 20ml of methanol was added to 2g of sample in centrifuge tubes and the sample were vortex mixed every 10 minutes for 2hours to improve extraction efficiency. The samples were then centrifuged at 3500rpm for 10 minutes (25°C) using centrifuged tubes and decanted. Each sample residue was rinsed once with 20ml of solvent, vortex mixed for 5minutes, centrifuged as above, and

decanted. Two supernatants were combined and used for analysis. The Folin Ciocalteu method (Singleton and Rossi, 1965), modified by Waterman and Mole (1994), was used to determine total phenols in the black tea extract.



Figure 3.3: Harvested bush tea material during the drying process in the shade

This method was based on the reducing power of phenolic hydroxyl groups (Hahn, Rooney and Earp, 1984) which react with the phenol reagent to form chromogens that can be detected spectrophotometrically. In brief, methanol extract (0.5ml) was added to a 50ml volumetric flask containing distilled water and mixed. Folin Ciocalteu phenol reagent (2.5ml) was then added and mixed, followed by 7.5ml sodium carbonate solution (20g/100ml) within one to eight minutes after addition of the Folin Ciocalteu phenol reagent. The contents were mixed and the flask made up to volume with distilled water and thoroughly mixed. Absorbance of the reactants was read after 2 hours at 760nm using UV-visible genesys 20 Spectrophotometer. Catechin was used as standard to prepare a

standard curve and results were expressed as mg equivalents/100mg of samples on dry weight basis.

Determination of Tannins: The Vanillin HCL method of Prince, Scoyoc and Butler, (1978) was used for the determination of tannins. This method is based on the ability of flavoids to react with Vanillin in the presence of mineral acids to produce a red colour that is measured spectrophotometrically. The extracts and reagents were maintained at 30°C in a thermostat-controlled water bath before mixing the reactants. The methanolic extract (1ml) was added to 5ml vanillin reagent (4% HCL in methanol and 0.5ml vanillin in methanol) and mixed. Sample blanks were done with 4% HCL in methanol replacing vanillin reagent. The reactants were maintained at 30°C and absorbance read at 500nm after 20 minutes. Absorbance reading of the blanks was subtracted from those of the samples. Catechin was used as a standard and results were expressed as mg catechin equivalents/100mg sample on dry weight basis.

Determination of antioxidants activity: Antioxidants activity of the extracts was determined using Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika, Rooney and Waniska (2004). Trolox Equivalent Antioxidant Capacity (TEAC) is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the ABTS⁺ radical cation chromogen in relation to that of Trolox, the water soluble vitamin E analogue which is used as an antioxidant standard. The ABTS⁺ was produced by mixing equal volume of 8mM ABTS with 3mM potassium persulfates prepared in distilled water and allowed to react in the dark for at least 12 hours at room temperature before use. The ABTS⁺ solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH₂PO₄, 0.2 M NaHPO₄ and 150mM NaCl in 1 litre of distilled water, with pH adjustment using NaOH where necessary. This solution was made fresh for each analysis. The ABTS⁺ solution (2900µl) was added to the methanol extracts of tea (100 µl) of Trolox in a test tube and mixed. Absorbances reading (at 734nm) were taken after 30 minutes (for the samples)

and 15 minutes (for the standard) of the initial mixing of the samples and standard respectively. The results were expressed as μM Trolox equivalents /g of sample on dry weight basis.

3.2.4. Statistical analysis

Data was subjected to analysis of variance (ANOVA) using PROC GLM (General linear model) procedure of SAS version 8.0. (SAS, Institutes Inc. 1999). Mean separation for significant differences was done using the least significant difference (LSD) method.

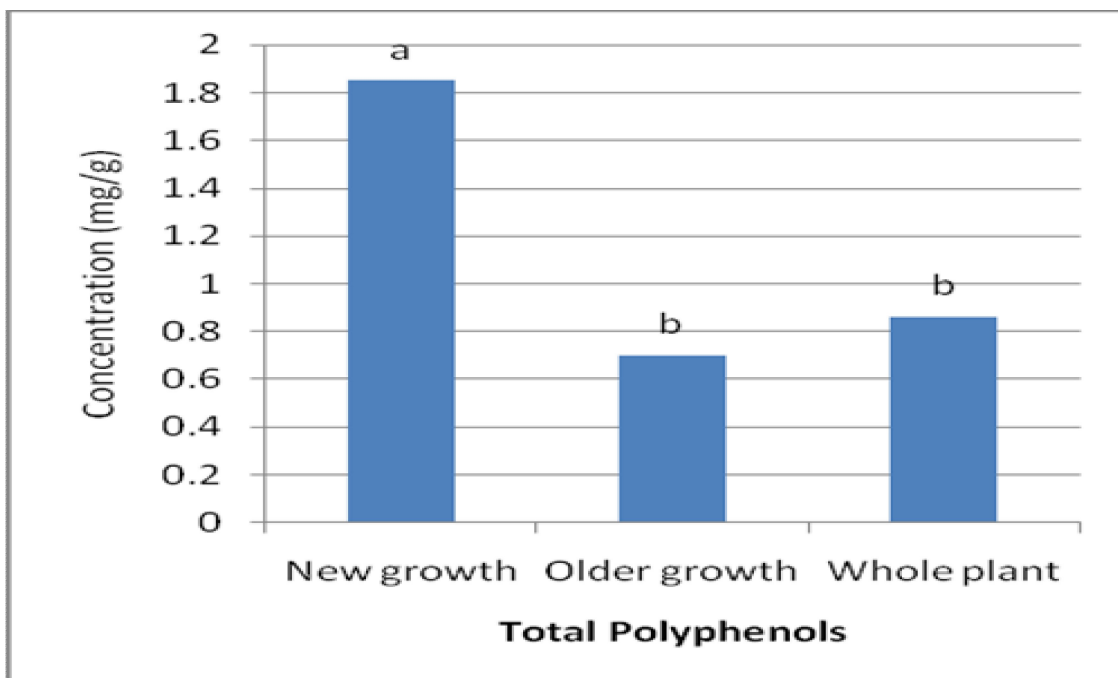
3.3. RESULTS AND DISCUSSIONS

3.3.1. Chemical concentrations in cultivated bush tea

Total Polyphenols.

Concentration of total polyphenols showed variation at different phenological stages of cultivated bush tea (Figure 3.4). The lowest total polyphenol concentrations were observed in older growth (0.7mg/g), while the highest concentrations were observed in new growth (1.85mg/g). The difference between the lowest and the highest concentration of total polyphenols was 1.15mg/g.

As higher total polyphenol concentration is an indication of higher quality in green tea (Hirasawa et.al., 2002), this results concurs with MedlinePlus (2009) findings that top new growth leaves are of higher quality while older leaves further down the stem of tea are of poorer quality. This was attributed to the distribution of polyphenols. Polyphenols are part of carbohydrate reserve or resource translocation to young leaves. Polyphenols are the primary nutritious constituents of bush tea.



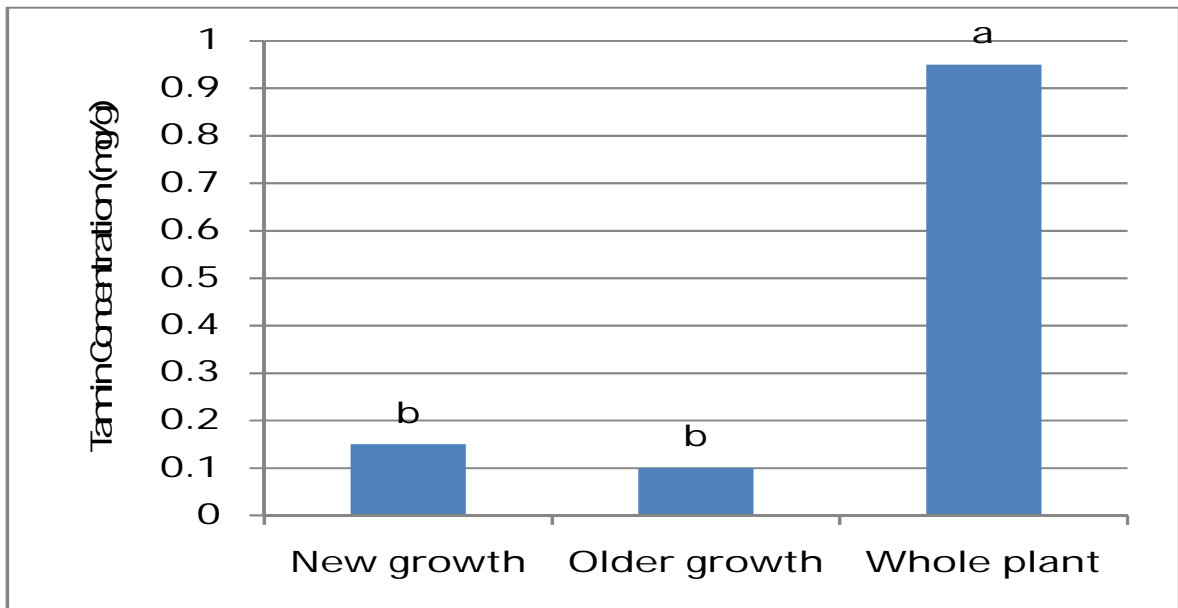
*Means denoted by the same letter are not significantly different at the 5% probability level

Figure 3.4: Total polyphenol concentrations of cultivated bush tea harvested at different Phenological stages.

The chief polyphenols are flavonoids such as catechin and proanthocyanidins, with the four major polyphenols being epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate. Most of the green tea catechins are oxidized during manufacture of black tea and converted into orange or brown products known as theaflavins and thearubigins but these still retain the basic C6-C3-C6 structure of flavonoids (Lakenbrink et al., 2000). The strong astringent flavor of tea is attributed to its polyphenol content (Hirasawa et al., 2002). Hlahla (2010) quotes literature on how polyphenols appear to thwart cancer by at least three methods : they shut off formation of cancer cells, turn up the body's natural detoxification defences and suppress cancer advancement. Thus from a health point of view, the results from this study suggest that new growth would be preferred to either older growth or use of whole plant as it has much higher concentration of polyphenols.

Tannin Contents.

Results in Figure 3.5 show a significantly high tannin content (0.95mg/g) in harvested whole plants than in harvested new growth (0.15mg/g) and harvested older growth (0.1mg/g). There was however no significant difference in tannin content between harvested new growth and harvested older growth. Tannin content in tea leaves is the main potential indicator of the medicinal potential due to their anti-oxidant activities (Hirasawa *et al.*, 2002). Tannins help to prevent cancers and heart problems by lowering the tendency of blood platelets to stick together (Stansveld *et al.*, 1992). However, high level of tannins cause bitter, astringent taste in many types of teas. Low tannin content of bush tea is an advantage for people with digestive problems with tannin-rich beverages as tannins bind iron and reduce the absorption of non-heme iron (Bokuchova and Skobeleva, 1980). The results from this study suggest that new and older growth may make tastier drink but the whole plant could make healthier drink with increased antioxidant activities.

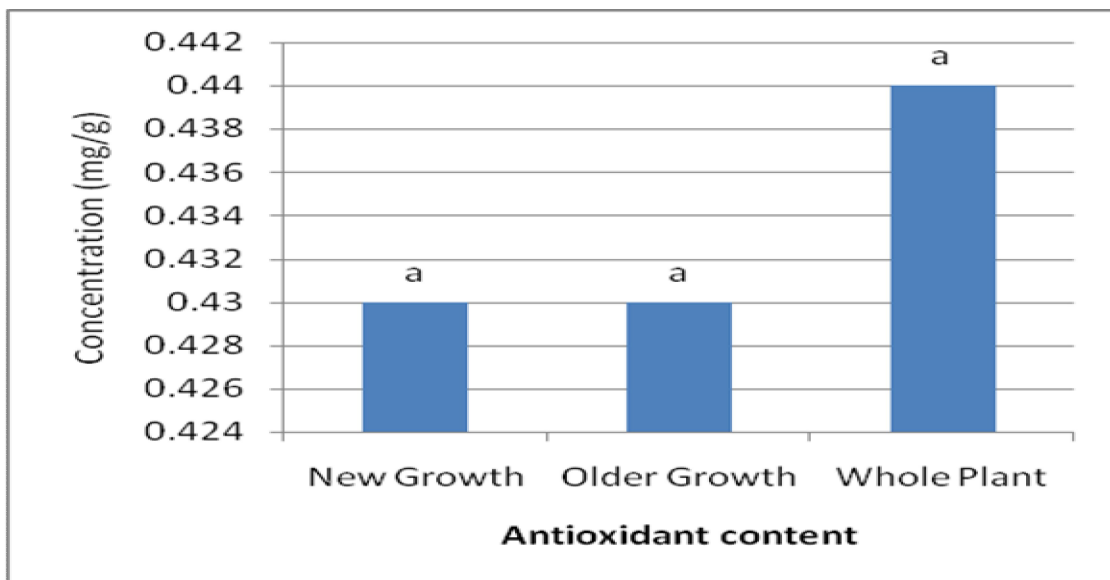


*Means denoted by the same letter are not significantly different at the 5% level probability

Figure 3.5: Tannin concentrations of cultivated bush tea harvested at different Phenological stages..

Total antioxidants.

No significant difference was observed between harvested new growth, harvested older growth and harvested whole plants of bush tea on antioxidant contents (Figure 3.6).



*Means denoted by the same letter are not significantly different at the 5% level probability

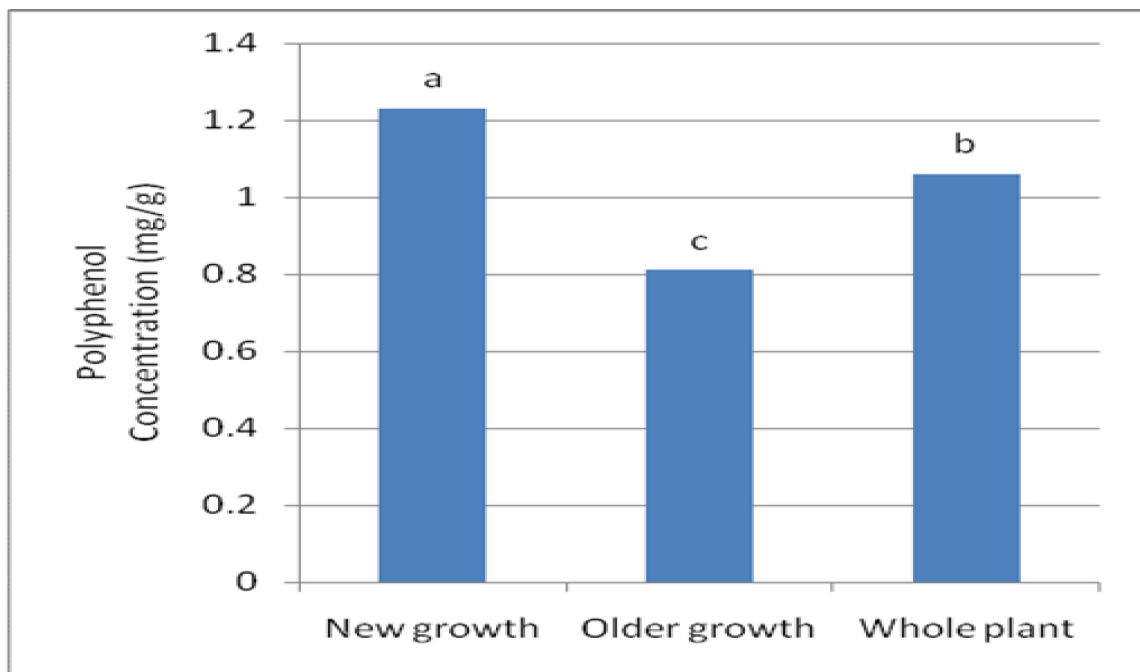
Figure 3.6: Total antioxidants concentrations of cultivated bush tea harvested at different Phenological stages..

These results show that anti-oxidant activity is linked to tannin content as whole plant samples (Figure 3.5) also have significantly higher tannin content than either new or older growth. Antioxidants are compounds that interact with harmful molecules (oxygen ions, free radicals, peroxide) in the body and may enhance body mechanism against diseases like cancer and coronary heart disease (Han et al., 2007). This study suggests that, despite potential bitter astringent flavor, whole plant samples make healthier bush tea than either new growth and older growth. Such tea may need to be blended with substances that can suppress the bitter astringent taste caused by high level of tannins.

3.3.2. Chemical concentrations in wild bush tea

Total Polyphenols

Concentration of total polyphenols showed variation at different phenological stages of wild bush tea (Figure 3.7). The lowest total polyphenols concentration were observed in older growth (0.81mg/g), while the highest concentrations were observed in new growth (1.23mg/g). Total polyphenol content in whole plant samples was intermediate between that of new growth and older growth. It is generally known that a higher total polyphenol concentration is an indication of higher quality. Similar results were reported by MedlinePlus (2009) who demonstrated that top new leaves were of higher quality while older leaves further down the tea plant were of poorer quality.



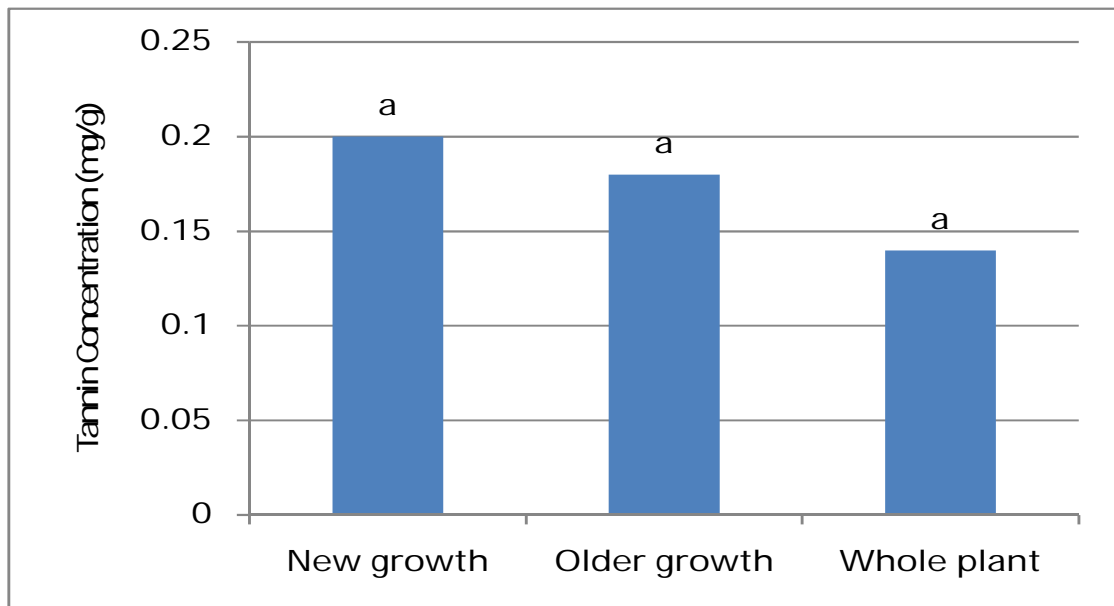
*Means denoted by the same letter are not significantly different at the 5% level probability

Figure 3.7. Total polyphenol concentrations of wild bush tea harvested at different Phenological stages.

The results are in line with earlier reports by Devlin and Witham (1983) and Arnold et.al., (2004) who reported a movement of carbohydrate resources or reserves from older aging leaves to young leaves. Similar pattern was seen in samples of cultivated bush tea and the results indicate better quality tea from new growth of bush tea.

Tannin Content

Figure 3.8 shows that there was no significant variation in tannin concentration of wild bush tea when harvested at new growth, old growth or whole plants. These results differed with those for cultivated tea where whole plant had significantly higher levels of tannins.



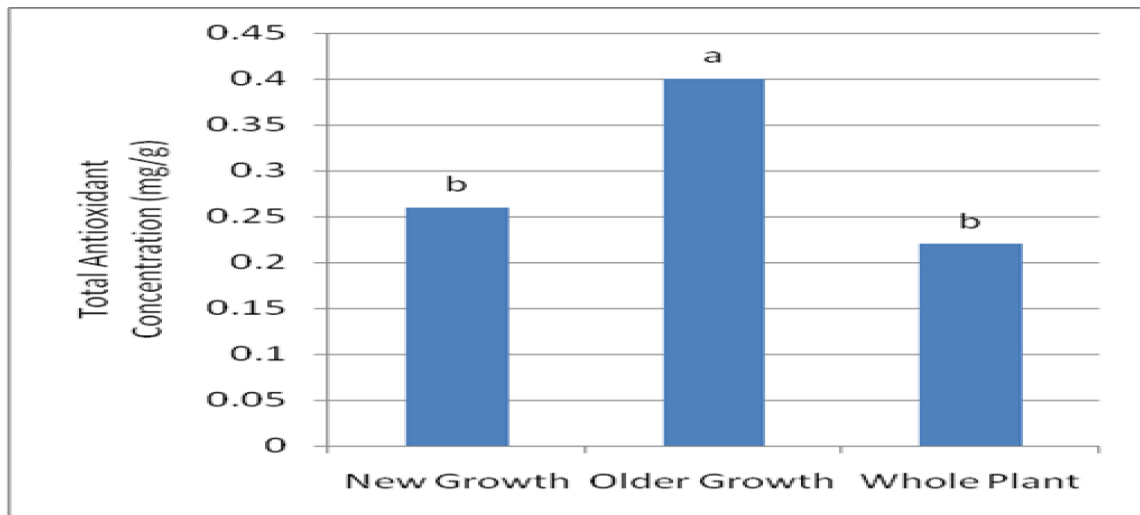
*Means denoted by the same letter are not significantly different at the 5% level probability

Figure 3.8: Tannin concentrations of wild bush tea harvested at different Phenological stages.

Total antioxidants

Results in Figure 3.9 showed that the concentration of total antioxidants varied between harvested new growth, older growth and harvested whole plant of wild bush tea. Harvested older growth recorded the highest concentration of 0.4mg/g

while harvested whole plants recorded the lowest antioxidant content of 0.22mg/g which was not significantly different from the recorded 0.26mg/g total antioxidant content of harvested new growth. The difference between the highest and the lowest total antioxidant concentration was 0.18mg/g.



*Means denoted by the same letter are not significantly different at the 5% level probability

Figure 3.9: Total antioxidants concentrations of wild bush tea harvested at different Phenological stages.

The results are a total contradiction to MedlinePlus (2009) findings that older leaves further down the stem were of poorer quality, as in this study older leaves showed to be of highest quality due to high concentration of total antioxidants.

3.4. CONCLUSIONS

In cultivated bush tea, harvested new growth or as whole plants proved to be of higher quality owing to their higher polyphenol and tannin attributes respectively, and therefore may preferably be harvested. Harvested older leaves proved to be

of lower quality as shown by lower levels of both total polyphenols, tannins and total antioxidants.

In wild bush tea, both new growth and older growth proved to be of good quality. Harvested new growth proved to be of better quality owing to the higher total polyphenol content while older growth proved to be of better quality owing to the higher total antioxidants. There was however no significant differences between new growth, older growth or whole plant as far as tannins were concerned.

These results suggest that people harvesting wild bush tea can harvest both new and older leaves and get comparative health benefits.

CHAPTER 4

THE EFFECT OF PRUNING ON GROWTH AND CHEMICAL COMPOSITIONS OF BUSH TEA

4.1. INTRODUCTION

Pruning is one of the most important operations, next to plucking, which directly determines the productivity of tea bushes (Tocklai Tea Research Association [TTRA], 2008). Tea plants are pruned to obtain a given table form and height, to eliminate unnecessary and diseased branches, to rejuvenate the tea plants, and to obtain healthier and better quality tea plants (Yilmaz, Kandemir and Kinalioglu, 2004). In spite of huge crop losses that result from pruning, it is a necessity that it be carried out periodically (TTRA, 2008). Yilmaz et.al., (2004) reported less yields in tea harvested 50cm above the ground in the first year, with yields increasing in the subsequent second and third year. Thus, pruning increases tea yields in the long term.

If pruning is not done or is delayed, the size and weight of growing shoots on plucking surface decreases, with loss of vigour of growing apices in the long run (TTRA, 2008). TTRA (2008) distinguished between the following types of pruning: light pruning where tea bushes are pruned 4-5cm at the top, medium pruning, where tea bushes are cut 45-60cm above ground, heavy pruning where tea bushes are cut 15-45cm above ground followed by collar pruning where all above ground parts of the tea bushes are cut. Light prune helps to renew the wood, regulate crop distribution and maintain ideal frame height of the bushes (TTRA, 2008). Medium prune helps in rejuvenating the tea bushes that have become old and their yields have started declining (TTRA, 2008). Heavy and collar prune are necessary for complete renewal of tea frame (TTRA, 2008). Collar prune however needs to be carried out only when the root system is strong enough to withstand the shock and initiate new growth, otherwise heavy mortality

will result (TTRA, 2008). TTRA, (2008) also reported a crop loss of 30-35% after light pruning and 60-70% after medium pruning.

Ravichandran (2003) reported that pruning is an essential agronomic practice in the production of leaves for the manufacture of black tea as it leads to enhanced branching and hence a greater number of tender leaves (Satyanarayana et.al., 1994). Asil (2008) reported that in comparative plucking method of black tea based on three lengths (*viz.* 5, 10 and 15 cm), the best result of green leaves yield and quality were obtained in the 5 cm treatment in spring flush, followed by 10 cm and 15 cm, respectively. Plucking of leaves increased the concentration of total polyphenols and total antioxidants in green tea (Owour et.al., 2000).

Pruning was also found to affect quality of tea. Medhi, Mahanta and Baruah (2006) reported that all the pigment contents of black tea, except chlorophyll, were found to be higher in pruned tea leaf than unpruned tea, thus enhancing the quality of tea. Ravichandran (2003) also reported that the precursors responsible for tea quality, such as polyphenols, were found to increase in the first year and thereafter declined in content with time from pruning. According to Ravichandran (2003), the green pigment content and ash content increased and the lipoxygenase activity declined progressively with time from pruning.

Data that describe the effect of pruning on growth (yield) and chemical composition in bush tea is lacking. Therefore this study was undertaken to determine the effect of pruning at different heights on growth and quality (chemical composition) of bush tea.

4.2. MATERIALS AND METHODS

4.2.1. The experimental site and planting materials

The planting materials for the experiment were made up of mature bush tea stock plants were collected from Mudzidzidzi village (about 35 kilometers North-West of

Thohoyandou) on 27 November 2007 and planted at Madzivhandila College (22° 56' 60S, 30° 28' 60E, Altitude 709m, summer rainfall and dry winter), in a 40% material shaded nursery. Selected planting materials were true-to-name and type, free of disease and insect damage, and in the proper physiological state. During cultivation, to stimulate rapid and prolific rooting of cuttings, plants were cut to about 7-8cm long and were treated with Seradix No.2 (0.3% IBA) (Bayer Pretoria, South Africa) and planted on seedling trays on a mist bed, supplied with a misting system operating through misting nozzles. The mist bed was 3m long, 1.5m wide and 0.5m high. Irrigation was done 3 times a day everyday except on rainy days.



Fig 4.1: Bush tea seedlings growing on a mist bed

Rooted cuttings (seedlings) were transplanted directly into 20-L bags after two and a half months on 11 January 2008. The medium used during transplanting was pine bark and sand at a ratio of 2:1 respectively.

In an attempt to achieve optimum growth, the growing bush tea plants in plastic bags were treated with a split application of NPK at rates 300kg/ha, 300kg/ha and 200kg/ha (Mudau et.al. 2007b) two weeks after transplanting.

On 14 February 2008 (a month after transplanting), pruning treatments, namely zero or no pruning (control), top-branch pruning, middle pruning and basal pruning were done. On 03 April 2008, the experimental plants were harvested for chemical analysis.

4.2.2. Experimental design and treatments

Treatments consisted of zero or no pruning (control), top-branch pruning, middle pruning and basal pruning arranged in a randomized complete block design (RCBD) with 10 replicates. Zero or no pruning involved leaving individual bush tea plants intact with no pruning from the start to end of the experiment. Top-branch pruning involved cutting or pruning of all top branches and stems of individual bush tea plants at the top up to 15cm length. Middle pruning involved cutting or pruning of all branches and stems of individual plants right in the middle of individual plants. Basal pruning involved cutting or pruning individual plants at the base just above the soil (media) surface.

4.2.3. Data collection

Parameters measured included growth parameters in the form of plant height, number of branches, biomass, and leaf area; and quality parameters such as polyphenols, tannins, and antioxidant activities.

Determination of total polyphenols content: Methanol was used as the extraction solvent for the determination of total phenols. Duplicates of 2g of tea were extracted using 40ml of the solvent as follows. An amount of 20ml of methanol was added to 2g of sample in centrifuge tubes and the sample were

vortex mixed every 10 minutes for 2 hours to improve extraction efficiency. The samples were then centrifuged at 3500rpm for 10 minutes (25°C) using centrifuged tubes and decanted. Each sample residue was rinsed once with 20ml of solvent, vortex mixed for 5 minutes, centrifuged as above, and decanted. Two supernatants were combined and used for analysis. The Folin Ciocalteu method (Singleton and Rossi, 1965), modified by Waterman and Mole (1994), was used to determine total phenols in the black tea extract. This method was based on the reducing power of phenolic hydroxyl groups (Hahn et.al., 1984) which react with the phenol reagent to form chromogens that can be detected spectrophotometrically. In brief, methanol extract (0.5ml) was added to a 50ml volumetric flask containing distilled water and mixed. Folin Ciocalteu phenol reagent (2.5ml) was then added and mixed, followed by 7.5ml sodium carbonate solution (20g/100ml) within one to eight minutes after addition of the Folin Ciocalteu phenol reagent. The contents were mixed and the flask made up to volume with distilled water and thoroughly mixed. Absorbance of the reactants was read after 2 hours at 760nm using UV-visible genesys 20 Spectrophotometer. Catechin was used as standard to prepare a standard curve and results were expressed as mg equivalents/100mg of samples on dry weight basis.

Determination of Tannins: The Vanillin HCL method of Prince et.al., (1978) was used for the determination of tannins. This method is based on the ability of flavoids to react with Vanillin in the presence of mineral acids to produce a red colour that is measured spectrophotometrically. The extracts and reagents were maintained at 30°C in a thermostat-controlled water bath before mixing the reactants. The methanolic extract (1ml) was added to 5ml vanillin reagent (4% HCL in methanol and 0.5ml vanillin in methanol) and mixed. Sample blanks were done with 4% HCL in methanol replacing vanillin reagent. The reactants were maintained at 30°C and absorbance read at 500nm after 20 minutes. Absorbance reading of the blanks was subtracted from those of the samples. Catechin was used as a standard and results were expressed as mg catechin equivalents/100mg sample on dry weight basis.

Determination of antioxidants activity: Antioxidants activity of the extracts was determined using Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika et.al., (2004). Trolox Equivalent Antioxidant Capacity (TEAC) is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the ABTS⁺ radical cation chromogen in relation to that of Trolox, the water soluble vitamin E analogue which is used as an antioxidant standard. The ABTS⁺ was produced by mixing equal volume of 8mM ABTS with 3mM potassium persulfates prepared in distilled water and allowed to react in the dark for at least 12 hours at room temperature before use. The ABTS⁺ solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH₂PO₄, 0.2 M NaHPO₄ and 150mM NaCl in 1 litre of distilled water, with pH adjustment using NaOH where necessary. This solution was made fresh for each analysis. The ABTS⁺ solution (2900µl) was added to the methanol extracts of tea (100 µl) of Trolox in a test tube and mixed. Absorbances reading (at 734nm) were taken after 30 minutes (for the samples) and 15 minutes (for the standard) of the initial mixing of the samples and standard respectively. The results were expressed as µM Trolox equivalents /g of sample on dry weight basis.

4.2.4. Data analysis

Data was then subjected to analysis of variance (ANOVA) using PROC GLM (General linear model) procedure of SAS version 8.0. (SAS Institute Inc.1999). Mean separation for significant differences was done using the least significant difference (LSD) method.

4.3. RESULTS AND DISCUSSION

4.3.1. Response of bush tea growth to pruning

Results in Table 4.1 showed a decrease in both plant height, number of branches fresh and dry biomass from zero or no pruning, to apical pruning, to middle pruning up to basal pruning. This results concur with TTRA (2008) observation of increasing crop loss at every degree of pruning from light pruning to medium pruning to heavy pruning. Fresh biomass figures reflect the same 30% loss at apical pruning equivalent to light pruning (TTRA, 2008), and 60% loss at middle pruning equivalent to medium prune as reported by Tocklai Tea Research Association (TTRA) (2008).

Basal pruning which is equivalent to collar pruning (TTRA, 2008) showed the lowest means in all the growth parameters of plant height, number of branches, leaf area and biomass. This is attributable to high mortality rate that resulted from the treatment. This suggests that the bush tea plant roots on which the treatment was applied were not strong enough to initiate new growth, as is the case with *Camellia sinensis*.

Tab.4.1. Growth characteristics of bush tea as affected by pruning at different heights

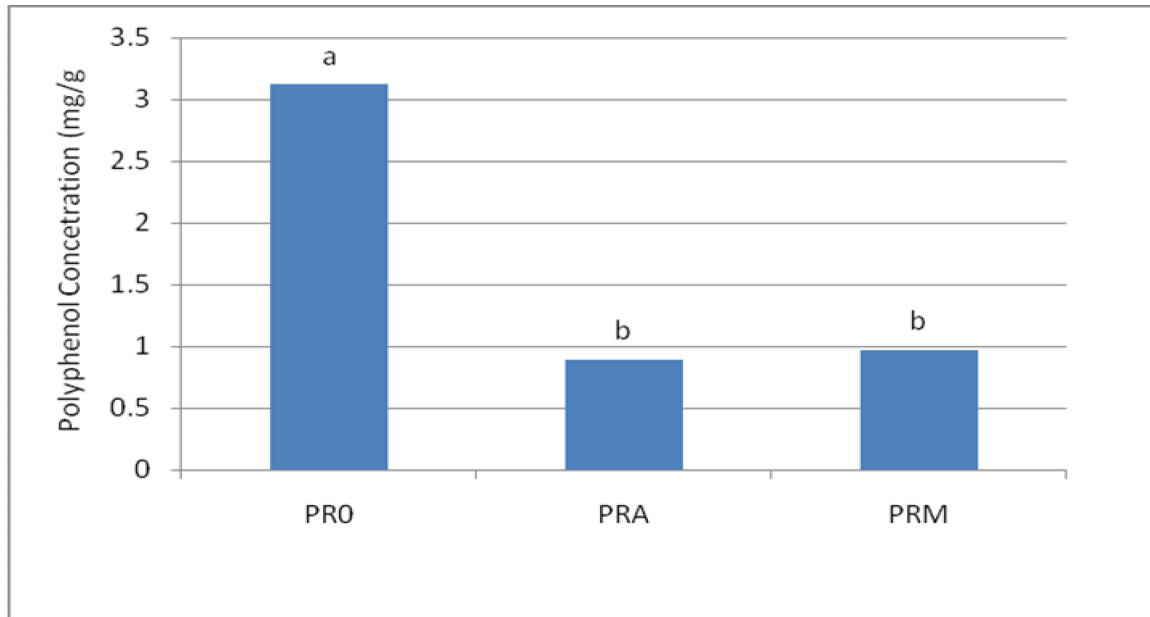
Treatment (Pruning methods)	Plant height Cm	Number of branches/plant	Leaf area/ plant cm ²	Fresh biomass/plant g	Dry biomass/plant g
No or 0 pruning	38.4 ±2.03a	31.8 ±3.16a	306.1 ±29.05a	27.43 ±2.92a	10.97 ±1.26a
Apical pruning	30.54 ±2.03b	28.5 ±3.16a	199.0 ±29.05b	18.73 ±2.92b	8.25 ±1.26a
Middle pruning	24.48 ±2.03c	16.5 ±3.16b	213.4 ±29.05b	10.88 ±2.92b	4.17 ±1.26b
Basal pruning	3.81 ±2.03d	4.1 ±3.16c	114.8 ±29.05c	1.98 ±2.92c	0.46 ±1.26c

*Means in columns followed by the same letter are not significantly different at 5% probability level

4.3.2. Effect of pruning on chemical composition

Concentration of total polyphenols:

There was variation in the concentration of total polyphenols between unpruned (PR0), apically pruned (PRA) and middle pruned (PRM) tea plants as shown in Figure 4.2. The highest total polyphenol concentration was observed in unpruned (PR0) plants with 3.12mg/g, while the lowest was observed in apically pruned (PRA) plants with 0.89mg/g. The difference between the highest and the lowest total polyphenol concentration was 2.23mg/g.



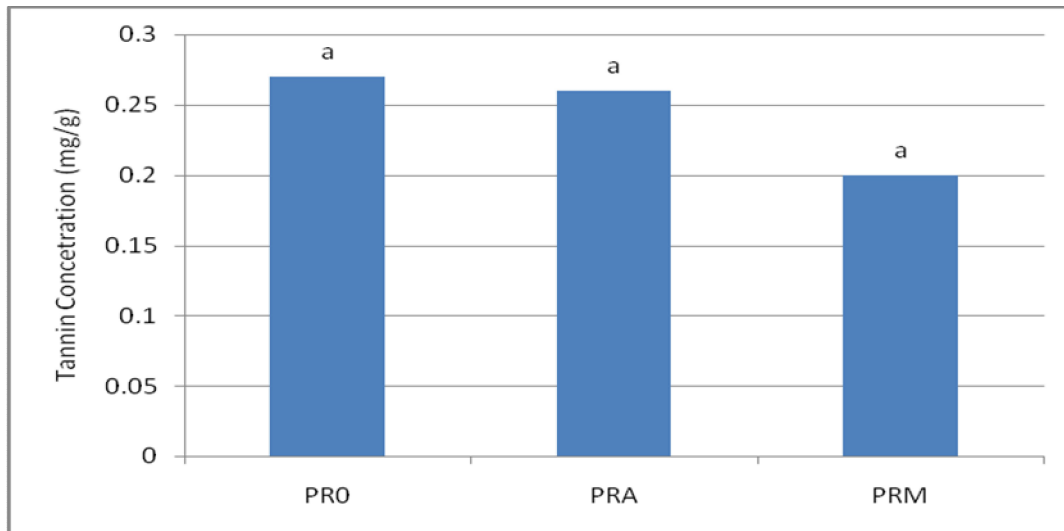
*Means denoted by the same letter are not significantly different at the 5% level probability

Figure 4.2: Total polyphenol concentrations of bush tea pruned at different heights.

Significantly higher total polyphenol in unpruned (PR0) than in pruned (PRA and PRM) tea contradicts Medhi et.al., (2006) who reported higher phenolic contents in pruned black tea than in unpruned black tea enhancing the quality of pruned tea made.

Tannin concentration:

Although there was no significant difference in tannin content between unpruned (PR0), apically pruned (PRA) and middle pruned (PRM) as Figure 4.3 shows, PRO plants yielded the highest tannin concentration of 0.27mg/g; 0.07 higher than the lowest tannin concentration of 0.2mg/g yielded by middle pruned (PRM) tea plants.



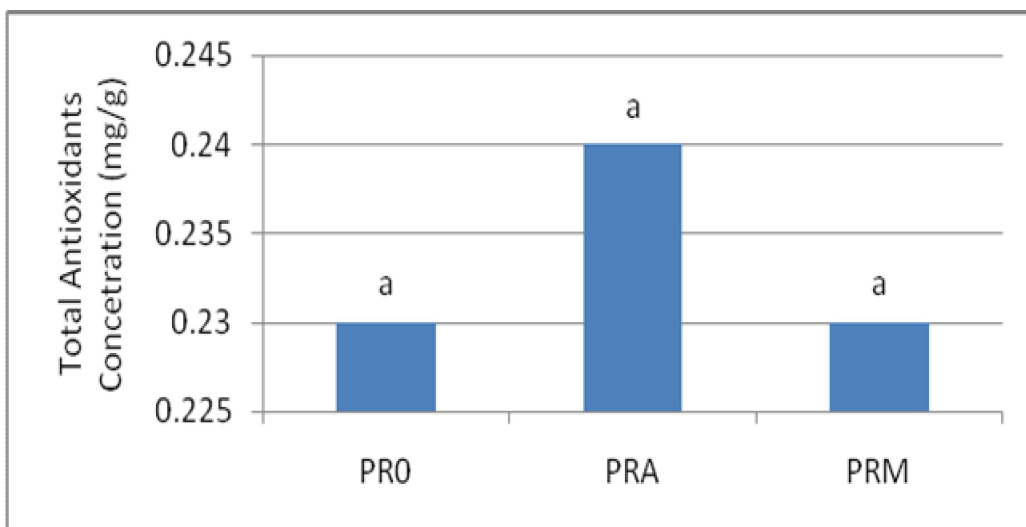
*Means denoted by the same letter are not significantly different at the 5% level probability

Figure 4.3: Tannin concentrations of bush tea pruned at different heights.

Lack of significant difference between unpruned (PR0) and pruned (PRA and PRM) tea contradicts Medhi et.al., (2006) who reported higher pigment contents in pruned black tea than in unpruned black tea as enhancing the quality of tea made. This maybe because bushes of black tea are much bigger than those of bush tea and can translocate large reserves to growing points after pruning.

Total antioxidant contents:

There were no significant differences in total antioxidant between PR0, PRA and PRM plants as shown in Figure 4.4. However, PRA plants yielded highest total antioxidants at 0.24mg/g, 0.01mg/g higher the lower yielding PRO and PRM plants.



*Means denoted by the same letter are not significantly different at the 5% level probability

Figure 4.4: Total antioxidant contents of bush tea pruned at different heights.

Lack of significant difference between unpruned (PR0) and pruned (PRA and PRM) tea contradicts Medhi et.al., (2006) who reported higher pigments of carotenoid and anthocyanin content in pruned black tea than in unpruned black tea enhancing the quality of tea made.

4.4. CONCLUSIONS

The experiment showed that pruning of bush tea largely led to crop losses. Compared to apically, middle and base pruned plants, unpruned bush tea plants remained the tallest plants, with higher number of branches, bigger leaf area and a larger biomass.

The experiment has also proved that pruning at different height has little or no effect on quality of bush tea. While only total polyphenols remained higher in unpruned tea plants, no significant difference were observed in tannin and total antioxidant content in both unpruned, apically pruned, middle pruned and base pruned tea plants.

CHAPTER 5

THE EFFECT OF GIBBERELLINS ON SPROUTING AND QUALITY OF BUSH TEA

5.1. INTRODUCTION

Gibberellins are a family of endogenous growth regulators in plants that are involved in nearly all stages of plant growth and development (Phillips, 1998). Gibberellins are implicated in germination, leaf expansion, bolting, flower induction, flower development, seed set and fruit development (Davies, 1995). Liang et.al., (1996) reported gibberellins to be effective in stimulating flushing of tea plant and increasing tea leaf yield. Kagira (1975) reported Gibberellic acid to have stimulated auxillary bud activity, inhibited extension growth of the apical meristems and to have an effect on tea leaf chemical composition and quality.

Liang et.al., (1996) reported that application of gibberellins was beneficial to green tea quality. They also reported that application of gibberellins improved content of amino acids, vitamin C and tea catechins index. These authors also reported that gibberellins reduced the content of tea polyphenols and the ratio of tea polyphenols to amino acids.

However the information on the effect of gibberellins on growth and chemical composition of bush tea is lacking. Therefore the objective of this study was to investigate the effects of gibberellins on sprouting and quality of bush tea.

5.2. MATERIALS AND METHODS

5.2.1. The experiment site and planting materials

The trial was conducted at Madzivhandila College of agriculture. The planting materials made up of mature bush tea stock plants for the experiment were

collected from Mudzidzidzi village, next to Tshatshingo Potholes on 27 November 2007 and planted at Madzivhandila College in a 40% net material shaded nursery on 28 November 2007. Selection of the planting materials was made on the basis of true-to-name and type, free of disease and insect damage, and in the proper physiological state.

During cultivation, to stimulate rapid and prolific rooting of cuttings, plants were cut about 7-8cm long and were treated with Seradix No.2 (0.3% IBA) (Bayer Pretoria, South Africa) and planted on a seedling trays on a mist bed, supplied with a misting system operating through misting nozzles. The mist bed used was 3m long, 1.5m wide and 0.5m high. Irrigation was done 3 times a day everyday except on rainy days.

Rooted cuttings (seedlings) were ready and were transplanted directly into 20l bags after two and half months on 11 January 2008. The medium used during transplanting was pine bark and sand at a ratio of 2:1, respectively.

In an attempt to achieve optimum growth, the growing bush tea plants in plastic bags were treated with NPK at rates 300kg/ha, 300kg/ha and 200kg/ha (Mudau et.al. 2007b) two weeks after transplanting.

5.2.2. Treatments and experimental design

The experiment was conducted under a 40% shade net with treatments laid in a Randomized complete block design (RCBD) with light/shading as a gradient. Treatments consisted of Gibberellins (Progibb 40%) applied at various rates as follows: 0% gibberellins, 1% gibberellins, 2% gibberellins, 3% gibberellins and 4% gibberellins arranged in a randomized block design and effected on 18 February 2008. Treatments were applied until run-off and the experimental plants were harvested for analysis three months after transplanting

5.2.3. Data collected and analysis

Data recorded: Growth and quality parameters were recorded. Growth parameters recorded were plant height, number of branches, biomass, as well as leaf area. Total polyphenols, tannins, and total antioxidants were analysed. The methodology used for Extraction of chemicals for analysis and recording was described in Chapter 2 previously.

Statistical analysis: Data was subjected to analysis of variance (ANOVA) using PROC GLM (General linear model) procedure of SAS version 8.0. (SAS Institute Inc. 1999). Mean separation for significant differences was done using the least significant difference (LSD) method.

5.3. RESULTS AND DISCUSSION

5.3.1. Effect of gibberellins on sprouting (growth) of bush tea

Results in Table 5.1 showed that there was no linear or quadratic response on plant height, number of branches, leaf area, fresh and dry biomass after the application of gibberellins at different rates. However, there was a tendency of high concentration of gibberellins at 3 % to increase plant height and fresh biomass. The number of branches were also increased at 4% gibberellins. The leaf area increased at 3% application of gibberellin concentration.

Tab.5.1. Growth characteristics of bush tea in response to different rates of gibberellins application

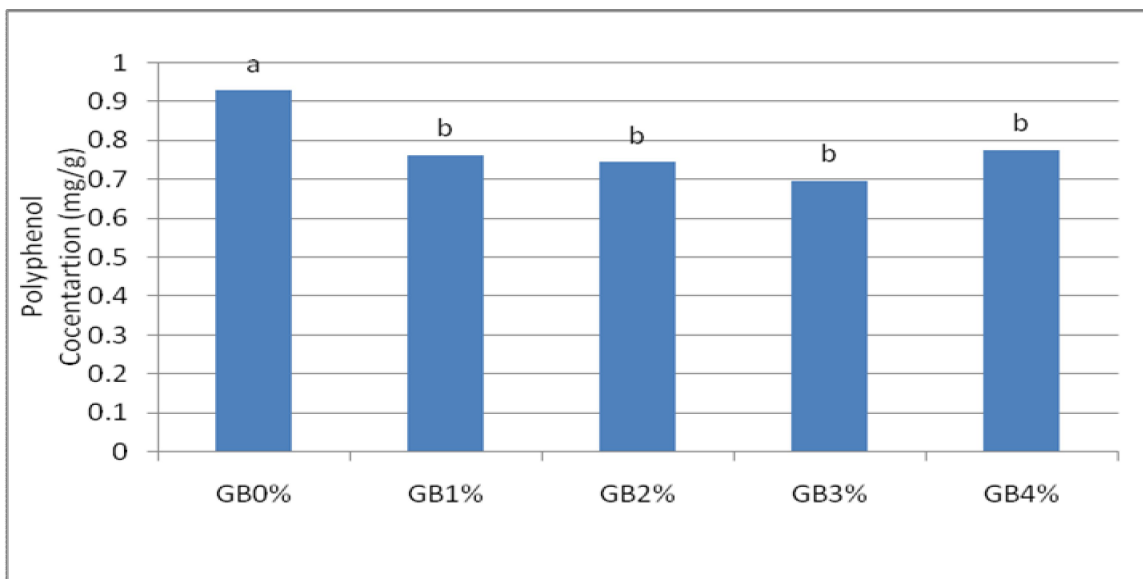
Applied gibberellins %	Plant height Cm	Number of branches/plant	Leaf area/plant cm ²	Fresh biomass/plant g	Dry biomass/plant g
0	32.5 ±2.35b	26.2 ±9.47c	261.8 ±22.96ab	26 ±3.95ab	10 ±5.11a
1	36.99 ±2.35ab	63.0 ±9.47ab	231.4 ±22.96abc	23.27 ±3.95ab	19.911 ±5.11a
2	31.6 ±2.35b	36.5 ±9.47bc	222.6 ±22.96bc	16.887 ±3.95b	7.19 ±5.11a
3	40.03 ±2.35a	57.4 ±9.47ab	293.1 ±22.96a	30.361 ±3.95a	14.157 ±5.11a
4	39.32 ±2.35a	66.8 ±9.47a	186.2 ±22.96c	29.9 ±3.95a	13.091 ±5.11a

*Means followed by same letters are not significantly different at 5% probability level

Increasing rates of gibberellins application enhanced plant growth as shown by resultant increase in many growth parameters measured. For plant height, number of branches, leaf area and fresh biomass best results were recorded at 3% and 4% application rates. Similar results were reported by Philips (1998) and Liang et.al., (1996) who reported gibberellins to be effective in promoting leaf expansion and stimulating flushing of tea plant and increasing tea leaf yield, respectively.

5.3.2. The effect of gibberellins on quality of bush tea

Gibberellins application tended to decrease the concentration of total polyphenols compared to zero application of gibberellins application (Figure 5.1). The highest concentration at 0% application rate was 0.928 mg/g, making the difference between the highest total polyphenol concentration at 0% rate and lowest total polyphenol concentration at 3% application rate to be 0.233mg/g.

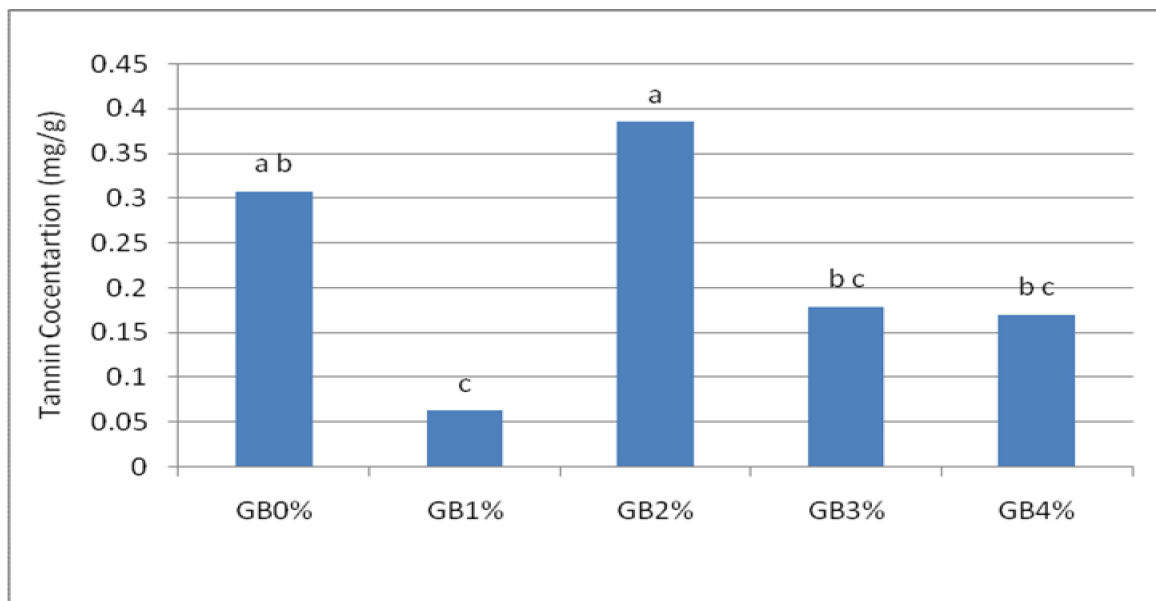


*Means denoted by the same letter are not significantly different at the 5% level probability

Fig. 5.1: Total polyphenol concentrations of bush tea at different rates of gibberellins application.

A decrease in tea polyphenols was also reported by Liang et.al., (1996) who reported a significant effect of gibberellins on *Camellia sinensis* by improving content of both amino acids, vitamin C and tea catechins index, and reducing the content of tea polyphenols. Their findings suggested that gibberellins stimulated the synthesis and accumulation of amino acids but inhibited accumulation of polyphenols and caffeine in tea plants.

Tannin concentration showed neither quadratic nor linear response to gibberellins application as shown in Figure 5.2. The highest concentration (0.385 mg/g) was realized at 2% application rate and the lowest concentration (0.0627 mg/g) at 1% gibberellins application rate. The difference in tannin concentration between 2% and 1% gibberellins application rates was 0.322mg/g.



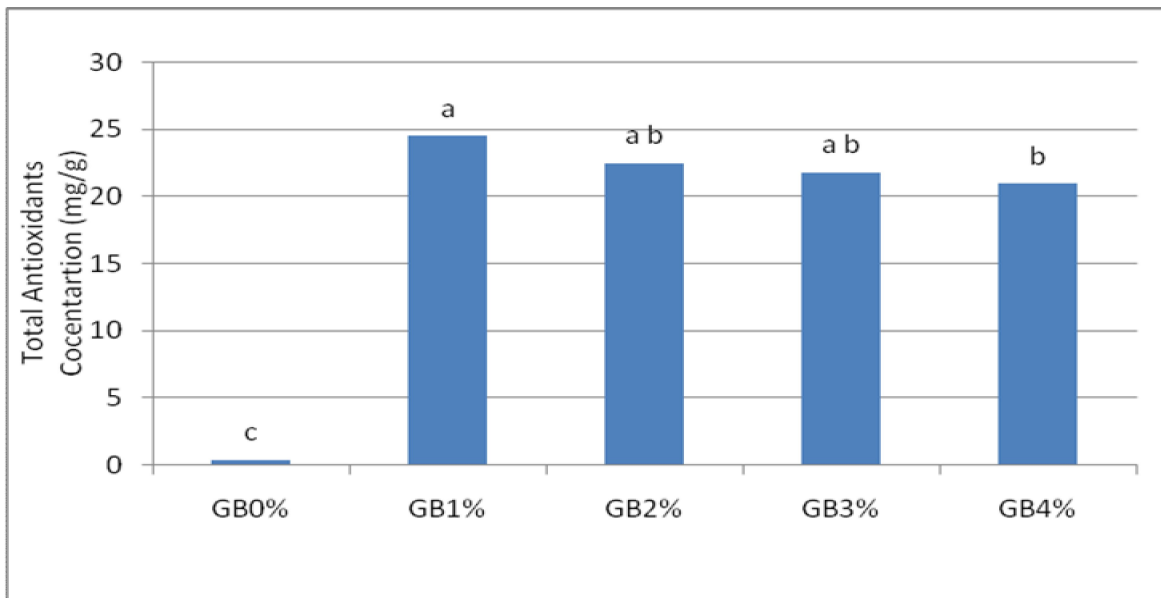
*Means denoted by the same letter are not significantly different at the 5% level probability

Fig. 5.2: Tannin concentrations of bush tea at different rates of gibberellins application.

The fact that the different rates of gibberellin application resulted in non-linear response to tannin concentration may be a proof that gibberellins have a

sporadic effect on tannin concentration in tea. Past studies in the relationship between tannins and gibberellins prove that it is the tannins that were antagonist to gibberellins (Green and Corcoran, 1975). According to Green and Corcoran, (1975) the tannins were particularly inhibitory against GA4 and GA14 where a ratio of 10:1 (tannins:GA by weight) resulted in up to 85% growth reduction. Inhibition could be completely reversed by increasing the amount of gibberellins in all combinations studied.

Figure 5.3 shows that application of gibberellins increased total antioxidants as compared to zero gibberellins application (GB0%). The lowest concentration was 0.419 mg/g at GB0% application rate and the highest content was 24.45 mg/g at GB1% application rate. Difference in total antioxidant content between the highest and lowest was 24.031mg/g.



*Means denoted by the same letter are not significantly different at the 5% level probability

Figure 5.3: Total antioxidant concentrations of bush tea at different rates of gibberellins application..

The general increase in total antioxidants from zero application is a proof to general assertion that biostimulants, including gibberellins, promote antioxidant production.

5.4. CONCLUSION

Experimental results have generally shown favourable response of bush tea growth to increased rates of gibberellins application. For most of the growth parameters, including plant height, number of branches, leaf area and fresh biomass, best results were recorded at 3% and 4% gibberellins application rates. Chemical attributes in the form of total polyphenols, and total antioxidants however, tended to decline with increasing gibberellin application rate. Tannin content however tended to peak at 2% gibberellins application rate. A good balance needs to be struck where a rate of gibberellins application will improve growth without compromising quality of bush tea to a greater extent.

GENERAL CONCLUSIONS AND FUTURE RECOMMENDATIONS

In herbal teas, quality is best determined by the presence of certain chemical compounds in the leaves. Chemicals such as polyphenols and tannins are known to be potential indicators of quality as they are antioxidant in nature. The quality as determined by these and other compounds are important as they determine the ultimate price of tea (Mudau, Ngele, Mashela and Soundy, 2007) if and when commercialized.

An experiment (Chapter 2) was conducted to determine the quality of tea harvested at various phenological stages for wild and cultivated bush tea. Results in this study indicated that, the best and ideal phenological stage to harvest both cultivated and wild bush tea is at the top new growth or new leaves. Both cultivated and wild bush tea proved to have the highest total polyphenol content at the new growth.

Pruning has been known to improve productivity of tea in general over time. In addition to yield, pruning has also been found to influence the quality of tea. An experiment (Chapter 3) was conducted to determine the effect of pruning on growth and chemical composition of cultivated bush tea. Results in this study showed that pruning reduced yields. Unpruned tea plants remained the tallest plants, with higher number of branches, bigger leaf area and a larger biomass than pruned tea at different heights. However, this experiment has only been conducted over short period of time in one year of planting. While Yilmaz et.al., (2004) reported the same trend of less yields in tea in the first year, they also indicated the trend of yields increasing in the subsequent second and third years. It is therefore recommended to test the impact of pruning bush tea in the long run stretching over two and/or more years. To allow for accumulation of starch reserves in stems and roots.

The results also showed that pruning at different heights have no favourable effect on quality of bush tea. While total polyphenols remained higher in unpruned tea plants, no significant differences were observed in tannin and total antioxidant content in pruned tea at different heights. Like yields, the prospect is that quality is expected to improve with time as tea is pruned periodically. It is also recommended that the impact of pruning on bush tea be examined over time for two years and more.

An experiment (Chapter 4) to determine the effect of gibberellins on growth and quality of cultivated bush tea was conducted. The experiment results showed that gibberellin application to have a favourable effect on growth of bush tea, with 3% and 4% showed the best favourable results.

The results also indicated a declining total polyphenol and antioxidant content with increasing gibberellin application rate, while tannins peaked at 2% application rate.

Viable bush tea commercialization will require that bush tea be cultivated or grown on a large scale. Only bush tea grown on a large scale will guarantee the availability of the plant with the consistency in quality (Mudau et.al. 2007b). While many trials on agronomic practices and bioactivity were conducted in a nursery setup, it is recommended that in future this trials be reciprocated on a field setup. This will help assess the performance of bush tea in a field environment, and if successful will guarantee the large scale production of tea imperative to commercialization of bush tea.

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