Effects of different climatic and soil factors at different locations on chemical composition of bush tea (*Athrixia phylicoides* DC.)

by

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DECLARATION

I declare that the full-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Agricultural Management (Horticulture) has not previously been submitted by me for a degree at this or any other university; that this is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Surname, Initials (title) **Date**

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ABSTRACT

Surveys have shown that the consumption of bush tea (*Athrixia phylicoides*) is widespread and commercialization of its extracts holds economic potential. It is a shrub of about 1 m in height, with leafy stems throughout. This shrub flowers throughout the year depending on the climatic conditions as well as edaphic factors. The best flowering is from March to May. Flowers of this tea vary from pink to all shades of pink and purple colour, depending on the edaphic factors and geographical area.

Currently, there are no data that describe the effects of climatic (rainfall, temperature, altitude) and soil (N, P, K and soil pH) factors at different locations on chemical composition (polyphenols, tannins, antioxidants) of bush tea. This study was initiated to document data on the areas for good quality bush tea production in the Limpopo and Mpumalanga Provinces of South Africa.

A completely randomized design (CRD) experiment was laid out with 8 treatments replicated 6 times. The treatments, which were the areas where bush tea samples were collected, comprised of Khalavha, Louis Trichardt, Mudzidzidzi, Muhuyu, Haenertsburg, Hazyview, Barberton and Levubu. Other areas such as Kwazulu Natal Province, as well as Swaziland, would also have been evaluated. The limitation was due to funding. Wild bush tea samples were collected for the analysis of polyphenol, tannin and antioxidant contents.

Polyphenol content reached a maximum of 7.7 mg/100 g in bush tea samples from Haenertsburg whereas the lowest content of 3.6 mg/100 g was recorded in Levubu. The highest polyphenol content was reached when altitude was at 1 410 m in Haenertsburg. Tannin content was the lowest (0.05 mg/100 g) in Khalavha. The highest tannin content was observed in Levubu with a maximum of 9.8 mg/100 g. The amount of total antioxidant contents remained the same in all the locations at 35 µmol/g. A positive correlation (R^2 = 0.55) was observed between total polyphenol content and altitude. Rainfall, temperature, soil macro elements and soil pH did not have any influence on total polyphenol, total tannin and total antioxidant contents.

CHAPTER 1

1. BACKGROUND

1.1 General introduction

Bush tea (*Athrixia phylicoides* DC.) is a popular beverage used as a herbal tea and as a traditional medicinal plant. It is commonly known as bushman's tea (English), Boesmans tee (Afrikaans), Mutshatshaila (Venda), Mohlahlaishi (Pedi), Icholocholo, Itshelo and Umthsanelo (Zulu). Bush tea is a shrub of 1 m in height, with leafy stems throughout (Figure 1). This shrub is found in grassland, forest, bushveld, rocky and sloping habitats. Bush tea will flower throughout the year depending on the climatic and edaphic factors, but the best flowering time is from March to May (Mbambezeli, 2005).

Fig 1. A picture of bush tea sample collected from Hazyview

Surveys have shown that the consumption of bush tea is widespread and commercialization of the extracts holds economic and developmental potential (Chellan *et al.*, 2008). However, for bush tea to be commercially viable, its uses and properties must either outcompete or complement teas already on the market. These are green and black teas from *Camellia sinensis*, rooibos tea from *Aspalathus linearis* and honeybush tea from *Cyclopia intermedia*.

Traditional medicinal uses of bush tea, another indigenous South African plant with very limited localized use as herbal tea, include treatment of boils, acne, infected wounds and infected throats (Joubert *et al.*, 2008). Aside from its horticultural potential, this plant also has traditional economic uses. The Bushman used to make tea from the leaves of bush tea. Sotho and Xhosa people also chewed it to alleviate symptoms of sore throats and coughs (Roberts, 1990; Mbambezeli, 2005). This beautiful plant may be used as a filler plant in the open spaces in flowerbeds, though it works well as a specimen plant in the garden (Mbambezeli, 2005). The dried leaves and fine twigs of *A. phylicoides* have traditionally been used by the Khoi and Zulu people as a herbal tea and medicinal decoction (Van Wyk and Gericke, 2000).The Venda people are reported to use extracts from soaked roots and leaves as anthelmintics (Mbambezeli, 2005). It is used for cleansing or purifying the blood, treating boils, headaches, infested wounds and cuts (Roberts, 1990; Joubert *et al.*, 2008).

Bush tea is also used for acne (Joubert *et al.*, 2008); for washing and as a lotion on skin eruptions, for coughs and colds, for loss of voice and for infested throats as a gargle (Roberts, 1990). Mabogo (1990) also reported the aphrodisiac properties in bush tea. Bush tea contains 5-hydroxy-6,7,3,4′,5′-hexamethoxyflavon-3ol as a major flavonoid (Mashimbye *et al.*, 2006). The potential for development of bush tea as a healthy beverage alternative to caffeine-containing tea was reported by Mudau *et al.* (2007b). It has no phytoxicity (McGaw *et al.*, 2007). Aqueous extracts from bush tea were reported to have a potential benefit to type two diabetic people (Chellan, 2011).

Currently, rooibos and honey bush tea are produced for the herbal tea market while bush tea has been reported to have a potential for commercialization (Joubert *et al.*, 2008). The most important chemicals present in tea, which are of considerable pharmacological significance, are polyphenols and caffeine (Kuroda and Hara, 1999). Tannin content in tea leaves is the main potential indicator of medicinal potential due to its antioxidant activities (Hirasawa *et al.*, 2002).

Bush tea is found at different altitudes with different rainfall regimes which have been found to affect quality of tea in *C. sinensis*. There is no data that describe the effects of climatic and soil factors on wild bush tea quality. Therefore, the objective of this study was to determine the effect of climatic and soil factors at different locations on chemical composition of bush tea.

1.2 Problem statement

Although wild bush tea is predominantly found in many areas of South Africa that differ in climate and soil conditions, information on effect of climatic and soil factors at different locations on chemical composition of bush tea is not well established. The effects of climatic and soil factors at different locations on chemical composition of bush tea need to be determined.

1.3 Motivation for the study

Bush tea is used for various purposes in various communities. It is used by traditional Zulu people as a cough remedy and purgative. Traditional Venda people also use bush tea for aphrodisiac purposes. Bush tea is also used as a treatment for acne, lotion on boils or skin and loss of voice. Bush tea leaves contain 5 hydroxy-6, 7, 3′, 4, 5′-hexamethoxy flavon-3-o1 which is possibly responsible for bioactivity in the plants (Mashimbye *et al.*, 2006). It is evident that bush tea has a potential to be used commercially as a herbal beverage. Therefore, the results from this study will contribute to the better understanding on characteristics of areas (soil and climatic factors) which will yield good quality bush tea for its various uses.

1.4 Aim

To evaluate whether climatic (rainfall, altitude and temperature) and soil (N, P, K levels and soil pH) factors at different locations have an influence on chemical composition of bush tea.

1.5 Objectives of the study

General objective: To study the effects of climatic and soil factors on the chemical composition of bush tea.
Specific objective 1: To determine the effects of climatic factors (rainfall, altitude and temperature) at different locations on chemical composition of bush tea.
Specific objective 2: To determine the effect of soil macro elements (nitrogen, phosphorus and potassium) and soil pH at different locations on chemical composition of bush tea.
Specific objective 3: To determine the correlation between climatic, soil factors at different locations and key quality constituents of bush tea.

1.6 Hypotheses

1. Climatic factors at different locations have no influence on chemical composition of bush tea.
2. Soil macro elements (N, P, K levels) and soil pH at different locations have no effect on chemical composition of bush tea.
3. There is no correlation between climatic and soil factors at different locations, and key quality constituents of bush tea.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Introduction

The genus *Athrixia* belongs to the *Asteraceae* family and there are 14 species which are predominantly found in southern Africa (Herman *et al.*, 2000). *A. phylicoides* is an indigenous plant in South Africa and is botanically an attractive shrub, about 50 cm to 1 m in height. Its leaves are simple, alternate linear to broadly lanceoalate at the base, light grey-green, smooth on the upper surface and white wooly below. *A. phylicoides* leaves have margins entirely or slightly revolute (Herman *et al.*, 2000). Flowers vary from pink to all shades of pink and purple colour depending on the edaphic and geological area (Van Wyk and Gericke, 2000).

A. phylicoides has not yet been commercialized; however, a lively informal trade in tea and brooms is practiced especially in the Gauteng, Limpopo and Mpumalanga areas of South Africa (Rampedi and Olivier, 2005). Potential for commercialisation is high, as a survey among rural and urban communities in the northern parts of South Africa indicated a willingness to buy *A. phylicoides* products. In order to commercialise the tea, cultivation will be necessary (Joubert *et al.*, 2008).

The Haenertsburg grassland is very popular with rural women for collecting plants to make traditional tea, brooms and wild spinach (Anon., 2003). Average net annual incomes for producers and traders of bush tea were modest at ZAR 2,000 and ZAR 1,000 respectively (ZAR = South African Rand), although some were earning considerably more (Anon, 2003). While unlikely to provide a way out of poverty, the trade was critical in allowing diversification and in providing a safety net, assisting poor households to overcome adversity, meet several basic needs, and educate their children (Anon., 2003).

The extensive local and regional market for traditional, handcrafted twig and brooms made from *A. phylicoides* in the Bushbuckridge municipality, South Africa, provides an important means of livelihood security for several hundred poor households in the face of increasing economic hardship (Shackleton and Campbell, 2007). Many African plant products have been used over the centuries as herbal teas, as popular local drinks and as refreshing beverages, for their perceived health and medicinal attributes (Juliani *et al.*, 2009). With the regionalization and globalization of foods, beverages and natural products, the introduction of new drinks such as African teas into other countries and even regions in Africa can provide new herbal teas to a wide range of new consumers (Juliani *et al.*, 2009).

The trade in African medicinal plants is poorly regulated. The regulation includes three fundamental aspects: quality, safety and efficacy. The adverse effects of African traditional medicines are not well documented in the literature (Street and Staden, 2009). African herbal teas can also be exported outside the continent into Europe, North America and Asia and in doing so have potential to contribute to the economic development of those involved in the collection or production, processing, packaging and distribution of such products (Juliani *et al.*, 2009). Information on the quality, chemical analysis and health attributes, and consumer response to new products contribute to the commercialization and marketing success of any product (Juliani *et al.*, 2009).

Psychoactive plants have been used by humans for recreational, spiritual, and therapeutic purposes for millennia. Africa possesses an ancient tradition of medicinal plant use and has a rich tradition of using its indigenous plants for these purposes (Stafford *et al.*, 2009). Given Africa's high floristic diversity, and the strong connection between plants and the many African cultures and societies, relatively few African psychoactive plants have been investigated in detail when compared to the Americas (Stafford *et al.*, 2009).

To be able to understand the potential of bush tea response to variation in environmental conditions, the characteristics, uses and response of *Camellia sinensis*, *Aspalathus linearis* and *Cyclopia intermedia* to environmental conditions are reviewed below.

2.1.1 Tea

Tea (*Camellia sinensis*), one of the most popular beverages in the world, is an infusion of the leaves of the (*C. sinensis)* plant which belongs to the *Theaceae* family. Tea is grown in about 30 countries but is consumed worldwide, although at greatly varying levels. Hundreds of different teas are now produced, mainly in Southeast Asia and Central Africa, and exported throughout the world (Khokhar and Magnusdottir, 2002). Tea is popularly known for its taste and aroma (Khokhar and Magnusdottir, 2002). Although it has been consumed for centuries, tea is currently touted as a beverage that can promote health and help prevent a number of diseases (Higdon, 2002). Increased public awareness of the health protective characteristics of tea, which are generally considered to be associated with the high flavonoid content of the leaves and extracts, has contributed to the public's general favourable attitude towards the beverage (Khokhar and Magnusdottir, 2002).

Tea is the most widely consumed beverage aside from water (Graham, 1992). Black tea is one of the most widely consumed beverages in the world and it is prepared by the infusion of water with processed leaves of tea (*C. sinensis)*, which is cultivated in more than 30 countries, thus assuming a great socioeconomic importance (Lima *et al.*, 2009). The tea plant is kept as an evergreen shrub by pruning. Only the apical bud and the first few leaves are plucked for tea processing. In tropical countries, tea leaves are harvested all year around. In temperate countries, harvesting is seasonal. There are many different kinds of products of different quality arising from different cultivation practices, growing conditions and processing methods (Hara *et al.*, 1995).

Green and fermented or black teas are consumed worldwide and have been reported to possess various biological, pharmacological and anticancer effects (Joubert and Britz, 2001). Black tea is one of the most widely consumed beverages in the world and it is prepared by the infusion of water with processed leaves of *Camellia sinensis*. Green tea is an aqueous infusion of dried unfermented leaves of *C. sinensis* from which numerous biological activities have been reported including antimutagenic, antibacterial, hypocholesterolemic, antioxidant, antitumor and cancer preventive activities (Valcic *et al.*, 1996).

Traditional Chinese medicine has recommended tea (*C. sinensis)* for headaches, body aches and pains, digestion, depression, detoxification, as an energiser and, in general, to prolong life (Ferrara *et al.*, 2001). Tea contains volatile oils, vitamins, minerals, purines, polyphenols, particularly catechins (Ferrara *et al.*, 2001). Tea contains the active ingredient polyphenol, which has a subgroup known as catechins (Boehm *et al.*, 2009).

There are several major categories of tea which are distinguished by different processing methods and different concentrations of specific tea polyphenols (Higdon, 2002). The beneficial effects of tea are thought to be due to its polyphenolic components (Yang *et al.*, 2001). Epidemiological data and *in vitro* studies on cancer chemoprevention by tea polyphenols have gained attention recently from the scientific community, nutritionists, the pharmaceutical industry, and the public (Leone *et al.*, 2003). The tea plant spontaneously grows widely from tropical to temperate regions in Asia. At present, the tea plant is cultivated in more than 20 countries of Asia, Africa and South America as one of the most favourite horticultural plants (Yamamoto *et al.*, 1997). *Camellia sinensis* is a plant growing in India, Sri Lanka, Java, Japan and its properties were known 4000 years ago (Ferrara *et al.*, 2001). The Western Cape of South Africa is known for production of rooibos (*Aspalathus linearis*), a herbal tea that is commonly consumed in South Africa and also attracts interest from consumers elsewhere (Joubert and Britz, 2001).

Rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*) tea were reported to be free from caffeine, low in tannin and high in ascorbic acid content. Rooibos tea contains the antispasmodic, quercetin, and is said to have enough fluoride to inhibit the formation of cavities in teeth. Consumer tests indicate that rooibos tea may be an acceptable alternative to tea, coffee, cocoa and high-caffeine soft drinks (Morton, 2008; McKay and Blumberg, 2006). The principal polyphenols in honeybush include the xanthone mangiferin and the flavonones hesperitin and isokuranetin (McKay and Blumberg, 2006). Traditional medicinal uses of rooibos in South Africa include alleviation of infant colic, allergies, asthma and dermatological problems, while honeybush is used as a restorative and as an expectorant in pulmonary tuberculosis (Joubert *et al.*, 2008).

The nutritional significance of tea is confined to certain vitamins and minerals. Tea is able to contribute riboflavin, folic acid, β-carotene, α-tocopherol and phylloquinone, and is also one of the more significant sources of fluoride in the United Kingdom diet. Beneficial effects attributed to tea polyphenols acting synergistically with ascorbic acid include the strengthening of capillary blood vessels and an anti-atherosclerosis action (Stagg and Millin, 2006). Tea, particularly green tea, has received a great deal of attention because tea polyphenols are strong antioxidants, and tea preparations have shown inhibitory activity against tumorigenesis (Anon., 2010). Studies have observed protective effect of tea against stroke, coronary heart diseases and cancer (Kohlmeier *et al.*, 1997). Reports have also suggested tea having many beneficial health effects, including the prevention of cancer and heart diseases (Yang *et al.*, 2004). Tea consumption has been shown to afford protection against chemical carcinogens including in the stomach, lung, oesophagus, duodenum, pancreas, liver, breast and colon (Katiyar and Mukhtar, 1997).

2.2 Tea medicinal uses

The most widely known health benefits of black tea relate to the polyphenols as the principal active ingredients in protection against oxidative damage and in antibacterial, antiviral, anticarcinogenic, and antimutagenic activities, but polyphenols in tea may also increase insulin activity (Anderson and Polansky, 2002). Several known compounds found in tea were shown to enhance insulin with the greatest activity due to epigallocatechin gallate followed by epicatechin gallate, tannins, and theaflavins (Anderson and Polansky, 2002).

Lindenmuth and Lindenmuth (2000) reported tea to have effective relieving of colds and flu symptoms at an early stage in a shorter period of time. Statistical findings showed an 11.2% lowering of the systolic blood pressure and a 10.7% decrease of the diastolic pressure in females and males of the ages between 52.6 twelve days after drinking sour tea (*Hibiscuss sabdariffa*), as compared with the first day (Haji-Faraji and Haji-Tarkhani, 1999). Oolong tea may be an effective adjunct to oral hypoglycemic agents in the treatment of type 2 diabetes (Hosoda *et al.*, 2003).

Green tea extracts contain a unique set of catechins that possess biologic activity in antioxidant, antiangiogenesis, and antiproliferative assays that are potentially relevant to the prevention and treatment of various forms of cancer (Cooper *et al.*, 2005; Doss *et al.*, 2005; Min and Lin, 2007). Habitual moderate strength green or oolong tea consumption significantly reduces the risk of developing hypertension (Pan and Dongmin, 2008) in the Chinese population (Yang *et al.*, 2004). Tea catechins reduce intestinal lipid absorption, prevent vascular inflammation, inhibit proliferation of vascular smooth muscle cells and suppress platelet adhesion (Pan and Dongmin, 2008).

2.3 Tea quality

To maximise yields and quality, agronomic practices should be optimized (Owuor *et al.*, 2009). Quality of tea depends on the cultivar and climatic conditions (Kottur *et al.*, 2010). The quality of tea is affected by a number of factors which can be classified under 4 major items, namely cultivars, environment, cultural practices and tea processing techniques (Chiu, 1990).

Cultivars: The fermentation ability, chemical components and agronomic characters vary with cultivars. For partially fermented tea, quality is more important than yield (Chiu, 1990). Although most clones are selected for quality and yields, there are clones in production with superior yields but producing inferior black tea quality. Some of these clones with superior yield give very poor quality black tea (Owuor and Obanda, 1999). Kottur *et al.* (2010) reported the overall quality to be positively correlated to the cultivars.

Environments: Soil and climate are two major factors affecting the quality of tea. High elevations are considered to be the most favourable for production of quality tea (Chiu, 1990). Climate influences yield, crop distribution and quality. Therefore, before cultivating tea in a new area, the suitability of the climate is the first point to be considered. Tea grows best on well-drained, fertile, acid soil on highlands (Anon., 2008). A superior quality genotype in one location is assumed to replicate the same attributes when planted in different regions, especially when climatic variations are minimal (Owuor *et al.*, 2010). (2008) reported rainfall ranges from 2 000-4 000 mm to influence tea quality.

Cultural practices: Tillage, weeding, fertility management, irrigation, plant protection and harvesting management, may affect the quality of tea though their effect on the yield may be more important. Among the management practices affecting the quality, leaf age and season of harvesting are the most significant (Chiu, 1990).

Processing technique: Parameters including fresh mass of green leaves (as yield), canopy area, soluble and insoluble ash, % tannin, % caffeine, theaflavins (TF), % thearubigins (TR), TR/TF ratio in black tea, total colour (TC) and brightness (BR) were measured. Black tea quality factors such as total theaflavins, thearubigins and total colour decreased with course of plucking (i.e., increasing yield quantity), while there was a positive relationship between increasing yield per bush and increasing canopy area of each bush (Amiri and Asil, 2007). Caffin *et al.* (2004) concluded from a study that tea leaves harvested in the warmer months, such as January, when processed, would contain the highest level of theaflavins, and produce a correspondingly higher quality black tea.

The quantity and quality (compounds which contribute to black tea quality) fluctuated by the seasonal growth (spring, summer and fall) and the variation of shoot length (Amiri and Asil, 2007). The variability in caffeine and tannin levels was not significant, whereas the variability in TF ranging from 0.89 to 1.8% and for TR ranging from 9.9 to 14.1% for black leaf tea, indicated a quality difference among the shoot lengths for plucking in spring flushing tea (Amiri and Asil, 2007). The brightness (BR) in black tea leaf ranged from 21.4 to 24.6% with an average of 24% in spring flushing, and total colour (TC) varied from 4.1 to 5.2% with an average of 4.5%, indicating that brightness, colour and flavour index decreased when TR/TF decreased (Amiri and Asil, 2007).

2.4 Tea polyphenols

Polyphenols are a group of compounds that exhibit numerous biochemical activities (Pan *et al.*, 2003). The popularity of tea is increasing on the global aspect because of its role as a significant source of phenolic compounds in human diet (Horžić *et al.*, 2009; Bravo, 2009). In recent years, there has been an increased interest in polyphenolic compounds found in plants. It is evident that some of these flavonoids may have beneficial effects on humans. They constitute one of the most common and widespread group of substances in plants

(Bennick, 2002). Green tea contains between 30 and 40% of water extractable polyphenols, while black tea contains between 3 and 10%. The amount of polyphenols depends on the genetic make-up and environmental factors such as light, rainfall, temperature, nutrient availability and leaf age (Kuroda and Hara, 1999). The level of total polyphenols of fresh tea shoots is important to black tea quality, and may be a reliable parameter for identifying and propagating potential high quality clonal tea plants (Obanda *et al.*, 1997).

The four primary polyphenols found in fresh tea leaves are epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate and epicatechin (Daniells, 2006). Wide ranges of the polyphenols are either being actively developed or currently sold as dietary supplements and/or herbal remedies (Ferguson, 2001). Polyphenolic compounds are common in the diet and have been suggested to have a number of beneficial health effects, including the prevention of cardiovascular diseases and diabetes (Yang *et al.*, 2008; Bravo, 2009). Beneficial effects attributed to tea polyphenols acting synergistically with ascorbic acid include the strengthening of capillary blood vessels and anti-atherosclerosis action (Stagg and Millin, 2006).

Many laboratory studies have demonstrated inhibitory effects of tea preparations and tea polyphenols against tumor formation and growth. These polyphenols may also inhibit carcinogenesis by blocking the endogenous formation of N-nitroso compounds, suppressing the activation of carcinogenesis and trapping of genotoxic agents (Yang and Wang, 1993). A high intake of polyphenols is likely to have beneficial effects on the cardiovascular system (Sahelian, 2005). Recent research has shown that the polyphenolic antioxidants in green tea possess cancer chemopreventive qualities (Ahmad and Mucktar, 2009).

2.5 Tea antioxidants

Green and black teas have 10 times the amount of antioxidants found in fruits and vegetables (Davis, 2009). Studies of humans and animals show that the antioxidants in black and green teas are highly beneficial to health. All teas from the *Camellia sinensis* tea plant are rich in polyphenols, which are a type of antioxidant (Davis, 2009). These wonder nutrients scavenge for cell-damaging free radicals in the body and detoxify them. Both black and green tea have more different types of antioxidants (thearubigins, epicatechins, and catechins) than fruits and vegetables. All are considered flavonoids, a type of antioxidant (Davis, 2009).

The detoxifying effect of these antioxidants protects cells from free radicals, the damage that can lead to blood clot formation, atherosclerosis and cancer (Davis, 2009). The tea flavonoids may be active as antioxidants in the digestive tract or in other tissues after uptake. Antioxidants may therefore be particularly important in protecting against chronic diseases (Rietveld and Wiseman, 2003). Nyholm (2009) further reported that the antioxidants in green tea have anti-flammatory power that helps to keep the body and the mouth healthy and that they are one of the key necessities for human nutrition.

Flavonoids, which are considered the major group of antioxidants in tea, reduce the risk of developing cardiovascular disease, cancers, cataracts, cognitive impairment, osteoporosis, Alzheimer and liver diseases. Flavonoids also have a favourable effect in changing intestinal bacterial growth (Bhosale, 2009). Mogotlane *et al.* (2007) further reported that for improved total antioxidant contents in cultivated bush tea leaves, 300N, 300P and 100K kg ha^{-1} were recommended.

2.6 Tannins in tea

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. Tea "tannins" are chemically distinct from other types of plant tannins such as tannic acid and tea extracts. Tannins can be divided into hydrolysable and condensed tannins (Hamilton-Miller, 1995). They are chemically defined as phenylpropanoid compounds often condensed to polymers of variable length. Tannins and phenolic compounds are widely distributed secondary metabolites in plants and play a prominent role in general defense strategies of plants, as well as contributing to food quality (Swanson, 2003). Plant polyphenols comprise a heterogeneous group of compounds. Tannic acid is a common tannin found in tea, coffee, immature fruits, etc. and it has also been used as a food additive (Nepka *et al.*, 1999).

Tannins are considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzymes and affect the utilization of vitamins and minerals (Chung *et al.*, 1998a). The tea plant (*C. sinensis*) is an example of a plant said to have naturally high tannin content (Hamilton-Miller, 1995). They are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Chung *et al.*, 1998b).

Tannins are abundant in the human diet and therefore may affect the growth of some intestinal bacteria and thus may have an impact on human health (Chung *et al.*, 1998c). An increasing body of experimental evidence supports the hypothesis that tannins exert anticarcinogenic activity in chemically induced cancers in animal models (Nepka *et al.*, 1999). The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation (Chung *et al.*, 1998ab). The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins (Chung *et al.*, 1998ab). Incidences of certain

cancers, such as oesophagus cancer, have been reported to be related to consumption of tannin-rich foods such as betel nuts and herbal teas, suggesting that tannins might be carcinogenic (Chung *et al.*,1998b). Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses (Chung *et al.*,1998b).
It is known that bush tea harvested from the wild has the highest concentrations of condensed tannins when collected during autumn (4.82%) compared with winter (2.44%), spring (2.66%) and summer (3.04%) (Mudau *et al.*, 2007a).

2.7 Factors affecting tea quality

Quality of herbal products depends on ecological conditions as well as agricultural and manufacturing practices (Myer *et al.*, 1999).

2.7.1 Tea processing techniques

***Fermentation time and temperature on tea quality*:** Temperature is the major environmental variable affecting the yield of tea (Squire, 1982). Quality of rooibos tea improved with increasing fermentation temperature whereas quality decreased with increasing drying temperature (Joubert and De Villiers, 1997). According to Owuor and Obanda (2001), processing of black tea at low fermentation temperatures improved quality, and there was no significant difference between fermenting at 15 and 20°C. Exposure of black tea samples to temperatures of 80°C was found to be necessary for quality development (Temple *et al.*, 2001). In both Kenya and Malawi black teas, aroma quality declined with a long duration of fermentation (Owuor *et al.*, 2008).

The compounds responsible for tea quality (theaflavins and thearubigins), were found to increase with fermentation time (Robertsons, 1983; Cloughley, 2006a; Muthumani and Senthil Kumar, 2007). Other seasonal variations in the formation of theaflavins and reduction in the levels of the catechins and their gallates showed no regular patterns throughout the black tea processing. This could be due to factors associated with tea processing (Caffin *et al.*, 2004). Hlahla *et al.* (2010) reported fermentation temperature to increase polyphenols at 30, 34 and 38°C whereas tannin contents showed a great reduction at 38 and 42°C. Increasing fermentation time to 60 and 90 minutes achieved a significant increase in both polyphenol and tannin contents. Antioxidant content was not influenced by either fermentation time or temperature (Hlahla *et al.*, 2010).

Du Toit and Joubert (1999a) reported that colour development increased with increasing fermentation temperature. Fermentation at 70°C for 60 hours and 90°C for 90 hours produced the best flavoured tea. Long fermentation duration and high temperature favoured production of more intense coloured black teas with high thearubigin level and lower theaflavin (Owuor and Obanda, 2001). Cloughley and Ellis (2006) reported that adjusting the pH of fermenting tea from the physiological value to within the range 4.5-4.8 resulted in an increase in theaflavin content, a reduction in thearubigin levels, and an increase in the organoleptic evaluation of black tea.

According to Du Toit and Joubert (1999b), the development of the desired dark-brown colour and honey-like flavour during fermentation was faster in hot and cold water treated material and gave a more uniform coloured product than with the bruising pretreatment. Liquor characteristics also improved with water pretreatment (Du Toit and Joubert, 1999b). A red-brown extract, compared with the light yellow-brown of untreated samples, was obtained with the water pretreatment. Hot water pretreatment inactivated the enzymes polyphenol oxidase (PPO) and peroxidase (POD), but did not terminate or impair fermentation (Du Toit and Joubert, 1999b).

Du Toit and Joubert (1999b) also reported that the inactivation of the enzymes, together with high temperature (>60°C) fermentation, resulted in a chemical oxidation process rather than an enzymatic reaction in honeybush tea fermentation. Owuor and Obanda (1999) were of the opinion that long fermentation decreased brightness and flavour index and increased volatile flavour compound levels in black tea. Teas produced at higher temperatures had more rapid colour development at 25 and 35°C. Report from Cloughley (2006b), stated that colour development was more rapid at temperatures between 25 and 35°C. Controlled drying at 40°C for 6 hours did not affect extract colour, aroma, taste and mouthfeel, but resulted in a darker leaf colour (Du Toit and Joubert, 1998).

According to Owuor and Obanda (1996), high temperature during withering process of black tea manufacture decreased the brightness and flavour index. Black teas manufactured with temperature above 30°C have high thearubigins and total colour levels but lack briskness (Owuor and Obanda, 1996). Sud and Baru (2000) reported that hot air circulation through the withering troughs increased the brightness and total colour of rainy season teas. Brightness and flavour index decreased, while therubigins, colour, and group I and II volatile flavour compound levels increased with long fermentation time in black teas (Owuor and Obanda, 1999).

According to Joubert (1990), extraction of the different phenolic groups from tea leaves increased significantly with increasing temperature. Joubert (1990) also reported that at extraction temperatures of 23 and 50°C, increasing mass ratio resulted in a significant increase in total polyphenol and flavonoid content. Polyphenol contents significantly decreased with increasing fermentation temperature (Du Toit and Joubert, 1999b). According to Cloughley (1983), the decrease in caffeine levels of black tea samples was related to the time and temperature. Flavonoids may act as antioxidants to inhibit free-radical mediated cytotoxicity and lipid peroxidation; as anti-proliferative agents to inhibit tumour growth; or as weak oestrogen agonists or antagonists to modulate endogenous hormone activity (Lyons-Wall and Samman, 1997).

At extraction temperatures of 23 and 50°C, increasing mass ratio resulted in a significant increase in total polyphenol and flavonoid content of the soluble solids.

The non-flavonoid phenol content increased significantly with increasing mass ratio at temperatures above 23°C in rooibos tea (Joubert, 2007). Results showed that different teas had widely different in-vitro antioxidant power and that the antioxidant capacity was strongly correlated (*r* = 0.956) with the total phenolics content of the tea (Benzie and Szeto, 1999). Mogotlane *et al.* (2007) reported total antioxidant contents of bush tea to be 100 percent during autumn, winter and spring and 93 percent during summer, suggesting a temperature dependant decline. Phenolic composition of green honey bush tea subjected to pre-drying for a period of 6 months at 0 and 30°C remained stable (Joubert *et al.*, 2010)

Expressed as μmol of antioxidant power/g of dried tea leaves, values ranged as 132−654 μmol/g for black ("fermented") teas, 233−532 μmol/g for Oolong ("semifermented") teas, and 272−1144 μmol/g for green ("nonfermented") teas (Benzie and Szeto, 1999). Fermentation at 70°C for 60 h and 90°C for 36 h produced the best flavored tea. Fermentation at 90°C for 36 h produced optimal development of the desired dark-brown leaf color and dark red-brown extract color. Longer fermentation times are necessary for fermentation at 60, 70 and 80°C (Du Toit and Joubert, 2007).

2.7.2 Environmental conditions

Geographical areas on tea quality: Tea quality parameters such as polyphenols were significantly improved by altitude (Owuor *et al.*, 2006b), and shading (Owuor *et al.*, 2006a). Catechin biosynthetic pathway is most diverse in China and least in Japan tea (Magoma *et al.*, 2003).

Tea on the mountain (900 m altitude) during the period of bud germination in spring was retarded and growth rate of shoots was remarkably slower because of lower temperature (Li *et al.*, 1988a). The length of shoots was longer, the shoots were tender, fairly big in size and heavy (Kovács *et al.*, 2009). Typical differences were also observed in the aroma patterns of volatile flavour contents between the tea from different altitudes and soils (Li *et al.*, 1988b). The tea made from the fresh leaves in high altitude had stronger aroma.

Black tea theaflavins varied significantly ($P \leq 0.05$) with geographical area of production, but other plain tea quality parameters did not vary with location (Owuor *et al.*, 2009). There were significant ($P \leq 0.05$) differences in the plain tea quality parameters with location of production. It is therefore not possible to produce tea of the same quality even from the same cultivars when the production location is varied. Regression coefficients (R^2) of linear correlations of the same parameters at different sites revealed low values that cannot be used to predict quality (Owuor *et al.*, 2010).

The contents of the volatile flavour compounds and the flavour index of black teas vary with localities (Owuor *et al.*, 1988). It was revealed that the changes of main chemical components in tea were probably influenced by soil conditions, and volatile flavour contents and aroma pattern were related to altitude (Li *et al.*, 1988b). The quality differences of teas grown at different altitudes and geographical location of growing regions were reported (Kovács *et al.*, 2009).

Kenyan black teas produced at regions of 450 m in altitude (Anon., 2009) were reported to have higher theaflavin content (Owuor *et al.*, 2006c). Kato and Omori (1998) reported that tea from Kenya showed to have high contents of polyphenols while high content of catechins was also reported in teas from Austria and Kenya. It was shown that quality (free amino acid, polyphenol, caffeine, theaflavin and thearubigin contents) of black tea was considerably increased in teas from China as compared to Austria and Kenya (Ruan and Härdter, 2002). These differences may be attributed to the differences in altitude. Production of black tea from the same vegetatively propagated (VP) cultivars, in Kenya and Malawi, showed variations in both chemical composition and quality due to different areas of production (Owuor *et al.*, 2008).

The total volatile flavour compounds (VFC), of Group I and II VFC and the flavour index were higher in black teas processed in Kenya, further demonstrating the fact that high altitude grown Kenyan teas are more flavoury. Changes in quality parameters of crush, tear and curl (CTC) black clonal teas due to variations in altitude within a radius of 10 km were determined and were found to be significant (P = 0.05) due to areas of production despite the short distance sampling radius. The VFC of group I increased with decrease in altitude whereas Group II VFC and flavour index (FI), theaflavins (TF), thearubigins (TR) (except in clone TN 14-3) and caffeine increased with an increase in altitude (Owuor *et al.*, 2006c).

Consequently, in Malawi the maximum amount of theaflavins formation takes shorter fermentation duration than in Kenya. Given ample fermentation duration, fermentation in Kenya produced a similar amount of theaflavins. This makes it necessary to optimize fermentation time, in different geographical regions even when the same cultivar is being processed (Owuor *et al.*, 2008). However, the pattern in the changes in the individual theaflavins or theaflavins digallate equivalent followed that of total theaflavins, suggesting that the flavan-3-ols patterns in tea leaf might not have been affected by the geographical area of production (Owuor *et al.*, 2008).

In a genotype × environment experiment at four sites with four tea (*Camellia sinensis*) clones in Kericho, Kenya, differences in ground cover and total dry matter production and partitioning were found between clones and between sites (Ng'etich and Stephens, 2001). The major contributor to these differences was the daily intercepted solar radiation that differed by as much as 30 percent between sites. The rate of dry matter production was affected by the prevailing weather conditions (Ng'etich and Stephens, 2001). Geochemical environment, landscape and climate conditions are important factors affecting the distribution and quality of agricultural products (Guohua *et al.*, 1995).

Methods and techniques of exploration geochemistry were applied to study geochemical rock and soil characteristics which affect the cultivation and quality of world famous Longjing tea. The study showed that soil type and its total and bioavailable element content directly affect the quality of Longjing tea. Geochemical (rock and soil characteristics) factors can be used in recommending the siting of Longjing tea plantations to obtain high quality tea, and to assist in tea-garden management (Guohua *et al.*, 1995). Environmental factors related to tea yield and quality in some high mountain areas in China were: geology, topography, climate, hydrology, soil, and vegetation (Shoubo, 1991).

Historical development and climatic conditions in countries such as China and Kenya were noted to have been key in determining the choice in the selection and adaptation of the different varieties within different regions (Mamati and Yuerong, 2004). China has both subtropical and temperate conditions in both elevated and low altitude areas in which tea is conditioned to grow (Mamati and Yuerong, 2004). Temperature is the major environmental variable affecting the yield of tea (Squire, 1982). The volatile flavour compounds were similar in all black teas manufactured from fine and coarse plucking of Assam tea and orthodox teas from three different altitudes except that geranoic acid was detected in Darjeeling teas. The different ecosystems of Assam and Darjeeling have been found to have a pronounced impact upon aroma concentration. However, major diferences between the volatiles of teas from the different altitudes at Darjeeling appear to be not so significant (Mahanta *et al.*, 2006).

2.7.3 Response of *Camellia sinensis* to fertilizer application

- **Mineral nutrition**

Agronomic aspects and environmental factors have been known to play important roles in the determination of tea quality (Hara *et al.*, 1997). Agronomic practices, such as application of N, P, and K fertilizers, were reported to have a pronounced effect on leaf total polyphenol of tea (Owuor *et al.*, 1991).

Fertilizers should be applied only when the tea bushes are ready to utilize them. As a general rule, the best time for fertilizer application is after the first rain in spring has moistened the soil to a depth of 45 cm and when there is some new growth in case of unpruned tea. In case of pruned and skiffed teas, the fertilizers should be applied after the bushes have produced two new leaves. A weed free clean ground is desirable at the time of manuring (Anon., 2008).

The amount of total antioxidants in bush tea leaves was reported to be highest at 0-300 N, 300 P and 100 K kg/ha^{-1} (Mogotlane et al., 2007). Mogotlane *et al.* (2007) further reported the total antioxidant contents of bush tea to be decreased by the higher N fertilizers between 300-500 kg/ha N. Most of the total antioxidant contents occurred between 0-100 kg ha^{-1} in a K trial (Mogotlane *et al.*, 2007). Fertilizer recommendations for North-East India are given in Table 1.

Table 1. Recommendation on NPK manuring in mature tea for plain districts of North East India

Yield range (cycle average) (kg/ha)	N kg/ha	P_2O_5 kg/ha	K_2O kg/ha		
			Soil available potash status		
			Low (< 60 ppm)	Medium (60-100 ppm)	High (> 100 ppm)
Up to 1500	Up to 90	20	Up to 90	Up to 70	Up to 50
1500-2000	90-110	20-30	90-110	70-80	50-70
2000-2500	110-140	30-50	110-140	80-120	70-100
2500-3000	140-165	50	140-165	120-140	100-120

Source: Anon, 2008

Nitrogen: Application of nitrogen fertilizers leads to an increase in tea production but reduced resultant black tea quality (Owuor, 1997). It was reported that nitrogen rate at 300 kg ha^{-1} enhanced the caratenoid content of green leaves of tea (Ravichandran, 2002).

Owuor *et al*. (1990a) reported that the fatty acid levels increased with an increase in nitrogen fertilizer and the flavour quality deteriorated with an increase in nitrogen rate in black tea samples. Results from a study by Anon. (2008), indicated that for sustaining a crop of 2300 kg, made tea in different regions, a dose of nitrogen not exceeding 140 kg ha^{-1} would suffice. The N application levels in the Darjeeling hills tea plantations of India should not exceed 110-120 kg ha^{-1} due to the cool temperatures which slow down the organic matter decomposition (Anon., 2008). Venkatesan and Ganapathy (2004) reported that the overall quality of tea was impaired when nitrogen was applied. Increasing N application rates had adverse effects on the liquor quality as assessed by theaflavin (TF) analysis (Cloughley, 2006c).

Increased levels of nitrogen were found to increase the chlorophyll content in black teas which led to an increase in grassy taste and a decrease in quality (Van Lelyveld *et al.*, 1990). A lower polyphenol content was recorded when tea samples were supplied with less or no nitrogen fertilizers. High rate of nitrogen fertilizers increased the caffeine content of black tea with minimum value of theaflavins produced at 235 kg ha^{-1} (Owuor *et al.*, 1987). According to Owuor *et al.* (2000), nitrogen application rate caused a significant decline in total colour of black tea at application on rate of 200-400 kg ha^{-1}. Increasing nitrogen treatments (120-400 kg N ha $^{-1}$) and different seasons had a significant influence on EGC, epicatechin (EPC) at Shinkiari (1000 m) and Battal (1500 m) localities of Pakistan (Ahmad *et al.*, 2003).

Addition of N fertilizer supplements resulted in significantly ($P \leq 0.001$) increased concentrations of total polyphenols in bush tea leaves in all seasons with the most prominent increase evident at application rates of 0 to 100 kg ha^{-1} in a shaded nursery (Mogotlane *et al.*, 2007). It was concluded that the most suitable conditions for cultivating bush tea to obtain plants with optimal leaf polyphenol content were those of reduced light intensity during winter with nitrogen application below 300 kg ha^{-1} (Mudau *et al.*, 2006a). Owuor *et al.* (2000) found that N fertilizer applications of 450 kg ha^{-1} improved plant yields, leaf polyphenol and amino acid contents of green tea leaves.

Potassium: Ruan *et al.* (1999) reported that polyphenol content in tea leaves was increased by potassium application, while theaflavin and thearubigin were decreased by potassium application of 150 kg ha^{-1}. Potassium improved liquor components such as theaflavins and thearubigins at higher levels (Venkatesan *et al.*, 2005). According to Ruan *et al.* (1999), potassium fertilizer application of 150 kg ha^{-1} increased the contents of free amino acids and caffeine content of various tea types. Total polyphenol contents were significantly increased with K applications at a maximum level of 150 kg ha^{-1} during spring and autumn (Ruan *et al.,* 1999). Maximum total antioxidant content was 83 percent during autumn, 100 percent during winter and spring and 63 percent during summer when K was applied at 0-100 kg ha^{-1} (Mogotlane *et al.*, 2007).

Phosphorus: Total polyphenols in bush tea leaves increased quadratically regardless of the season, with most of the total polyphenol response occurring when phosphorus fertilizer was applied at the rates between 0 and 300 kg ha^{-1} (Mudau *et al.*, 2007a). Increase in growth and productivity of bush tea leaves were also reported to be increased by phosphorus application of 0 to 300 kg ha^{-1} (Mudau *et al.*, 2007b).

According to Owuor *et al.* (1998), the levels of thearubigin and flavour index were generally high when phosphorus was applied at 250 kg ha^{-1}. For P trials, the highest total antioxidant contents were reported to be 100 percent for autumn, winter and spring and 93 percent during summer (Mogotlane *et al.*, 2007).

CHAPTER 3

RESPONSE OF POLYPHENOL, TANNIN AND ANTIOXIDANT CONTENTS OF BUSH TEA TO SOIL AND CLIMATIC FACTORS

3.1 Introduction

South Africa has over 19 500 indigenous plant species. Most of these plants are of ethnobotanical significance and are still used by local rural communities as medicine, food and for making beverages (Rampedi, 2010). Throughout history, herbs have had their usage changing very little as the centuries passed. Ancient cultures made plentiful use of herbs to improve the taste of food, or to make medicines and tea (Manteiga *et al.*, 1997). *Athrixia phylicoides* (bush tea) is a plant indigenous to the north-eastern mountain ranges of South Africa.

It has been harvested for generations by rural communities for use as a tea and a medicine as well as to make brooms (Rampedi and Olivier, 2005). Due to the popularity of these products, signs of over-exploitation of the plant have recently been noted. Commercial production of bush tea may be considered as a means of alleviating this pressure on the plant's natural abundance and its habitat, provided that there is sufficient demand for the plant and its products (Rampedi and Olivier, 2005).

Bush tea adapts well to open grassland and thick forest margins of South Africa (Roberts, 1990; Mbambezeli, 2005). It is widely distributed in the eastern part of South Africa (Rampedi and Olivier, 2005), from the Soutpansberg Mountains in Limpopo, including Mpumalanga, to Queenstown, King William's Town and East London and throughout KwaZulu-Natal from the coast to the Drakensberg Mountains (Herman *et al.*, 2000). Distribution of herbal teas of South Africa is shown in Figure 2.

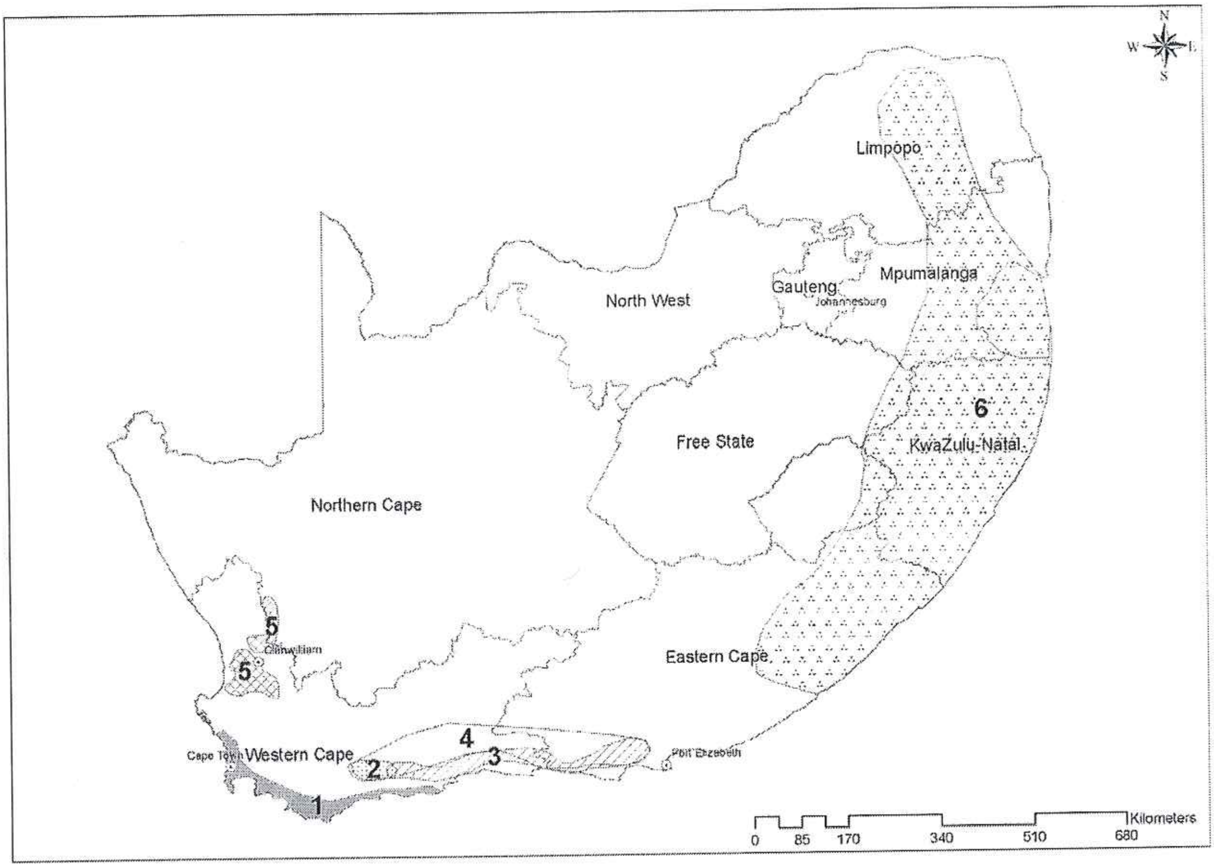

Fig. 2. Production area of *Aspalathus linearis* and natural distribution of *Cyclopia* species and *Athrixia phylicoides* Key: (1) *Cyclopia genistoides*; (2) *Cyclopia sessiliflora*; (3) *Cyclopia subternata*; (4) *Cyclopia intermedia*; (5) *Aspalathus linearis*; (6) *Athrixia phylicoides* (Rampedi and Olivier, 2005).

The plant needs well-drained soils with full sunlight and enough space for spreading their branches (Roberts, 1990). Harvesting time of wild bush tea was recommended to be during winter which contradicts with the way in which traditional people perceived that summer was the right time to harvest this crop (Mudau *et al.*, 2007b). Propagation of this tea is commonly by mature seeds, which are mostly collected at the end of summer (Roberts, 1990). Germination of bush tea seeds was reported to be highest (75.5%) at 20 and 25°C, followed by 64.5% at 15°C and lowest (47%) at 30 and 10°C (Araya, 2005). Germination percentage was further reported to be higher with continuous light than with alternate light (Araya, 2005). Better vegetative propagation and survival of bush tea can be attained from apical cuttings with Seradix No 2 hormone (Araya, 2005). Cool weather conditions were reported to maximize tannin contents of bush tea (Mudau *et al.*, 2007a). Regardless of season, N, P and K nutrition increased bush tea fresh and dry shoot mass, plant height, number of leaves, number of branches and leaf area (Mudau *et al.*, 2007c).

High-grown teas (*C. sinensis*) are found at elevations of 1 219 to 1 981 m and comprise approximately 25% of the harvest grown in Sri-Lanka. These teas have fragnant aromas and brisk full-bodied flavours. The perfect conditions found at these elevations provide the right environments for the tea (Heiss and Heiss, 2007). Bush tea in South Africa can be found at different locations of varying altitudes characterized by hot summers. Rainfall in these locations mainly occurs as thunderstorms in summer with dry and clear winters.

Only bush tea grown on a large scale will guarantee availability of the plant with consistency in quality (Mudau *et al.*, 2007b), therefore many studies still have to be conducted in order to document a lot of data on this crop. The objective of this study was to determine whether soil and climatic factors of different locations have any effects on chemical composition of bush tea.

3.2. Materials and Methods

Study sites and bush tea sample collection: The study was carried out at the University of Limpopo Technology Station. Samples of bush tea were collected in August 2008 from Hazyview and Barberton in Ehlanzeni district of Mpumalanga Province, South Africa; from Khalavha, Louis Trichardt, Mudzidzidzi, Muhuyu, Levubu in Vhembe district, and Haenertsburg in Mopani district of the Limpopo Province, South Africa.

General soil characteristics of Vhembe, Mopani (Limpopo Province) and Ehlanzeni (Mpumalanga Province) districts

Vhembe district: Generally acidic and Lithosolic soils are found in this area. This area generally consists of redish-brown, sandy-loam to loose sand and no water logging occurs in this area due to good drainage of the soil. Plant nutrients such as nitrogen and organic matter are extremely low due to high leaching occurring during rainy seasons (Macmillan, 1999). Soil pH ranges between 4 and 6 which represents fairly to slightly acidic conditions (table 1).

Ehlanzeni district: This area is characterized by highly erodible and permeable sandy soils. Soils are red-brown silty sand to silty clayed sand with gravel. It is more or less flat savanna of mixed bushveld with acacia woodland and broadleaved plants (Macmillan, 1999).

Mopani district: This area is characterized by deeper, high clay-loamy and good draining soils. The area has sub-tropical lowveld climate with summer rainfall and the rainfall occurring mainly in the form of thunderstorms. It is characterized by warm summers and mild winters (Macmillan, 1999).

Description of the Locations of sample collection

Khalavha is located in the Vhembe district, Limpopo Province, South Africa and its geographical coordinates are 22°55′0″ S, 30°18′0″ E at 892 m altitude with closed hills and mountains with moderate to high relief combined with lowlands, hills and mountains. It is characterized by red, yellow, loamy and clayed soils with summer rainfall (Macmillan, 1999).

Louis Trichardt (Makhado) is located in the Vhembe district, Limpopo Province, South Africa and its geographical coordinates are 23°0′3″ S, 29°54′0″ E and 935 m altitude. It is characterized by lowlands, hills and mountains with moderate to high relief, dystrophic red, yellow loam and clayed soils. This area is characterized by hot summers where winters are dry and clear. Night time temperature often drops to freezing point especially on the higher ground and the area receives summer rainfall (Macmillan, 1999).

Mudzidzidzi is located in the Vhembe district, Limpopo Province , South Africa and its geographical coordinates are 22°57′0″ S, 30°29′0″ E and 615 m altitude with closed hills and mountains with moderate to high relief combined with lowlands, hills and mountains. It is characterized by red, yellow, loamy and clayed soils with summer rainfall. Dry and clear winters are normally experienced at this location (Macmillan, 1999).

Muhuyu is located in the Vhembe district, Limpopo Province, South Africa and its geographical coordinates are 22°49'0" S, 30°29'0" E at 610 m altitude with closed hills and mountains with moderate to high relief combined with lowlands, hills and mountains. It is characterized by red, yellow, loamy and clayed soils with summer rainfall (Macmillan, 1999).

Haenertsburg is located in Mopani District and its geographical coordinates are 23°56′0″ S, 29°57′0″ E and 1402 m altitude. It is characterized by steep terrain with hills in the central areas and has rich clay-loamy soils. It has summer rainfalls, warm summers and mild winters and frost sometimes occurring in the low-lying areas (Macmillan, 1999).

Hazyview is located in Ehlanzeni district, Mpumalanga Province, South Africa and its geographical coordinates are 25°2′0″ S, 31°7′0″ E and 857 m altitude. Rocks such as biotite granite and migmatite can be found in this area. This area is characterized by deep slopes and closed hills with lowlands and high reliefs. Hot and dry summers are found here with most rainfall occurring mainly during midsummer (Macmillan, 1999).

Barberton is located in Ehlanzeni district, Mpumalanga Province, South Africa and its geographical coordinates are 25°47′0″ S, 31°3′0″ E and at 877 m altitude. It is characterized by hot summers and a summer rainfall. Sandstone, shale and conglomerate rocks are found in this area. Barberton's climate is subtropical and summer rainfall occurs with dry winters and loamy soils (Macmillan, 1999).

Levubu is located in the Vhembe district, Limpopo Province , South Africa and its geographical coordinates are 23°5′0″ S, 30°17′0″ E at 880 m altitude. Lowlands, hills and mountains with moderate to high relief, red, yellow and clay-loam soils can be found in this area. Hot summers and dry winters can be experienced with rainfall in summer (Macmillan, 1999).

3.3 Data collection

Leaves and branches of bush tea were collected for chemical composition (total polyphenol, tannin, and antioxidant contents). Soil samples were collected from each location at 0-30 cm and taken for analysis of N, P, K, cation exchange capacity and soil pH at the Johannesburg soil laboratory (Table 1). Weather data were retrieved from the South African Weather Service and the Agricultural Research Council. The results of the soil sample analysis are shown in table 1.

Table 2. Results of soil analysis for different locations of bush tea sample collection

Location	CEC (meq/100 grams)	pH	N %	P %	K %
Khalavha	2.1	5.6	2.9	0.9	2.8
Louis Trichardt	2.1	5.8	2.1	1	3.1
Mudzidzidzi	2.1	6.3	2.3	2.1	2.9
Muhuyu	2.1	5.6	3.8	1.7	2.1
Haenertsburg	2.1	4.9	2.8	0.5	2.5
Hazyview	2.1	6.1	2.8	1.1	3.1
Barberton	2.1	5.8	1.5	0.4	1
Levubu	2.1	5.3	2.8	1.1	3.1

Ten years (1999-2008) of temperature and rainfall data were used. Years with missing data were not included in the calculation of averages. The missing data were for Mudzidzidzi, Muhuyu from years 1998-2004 and therefore only data from 2005-2009 were used at those sites. Thohoyandou weather station data were used for Mudzidzidzi and Muhuyu. Weather data were retrieved from the South African Weather Service and the Agricultural Research Council. Rainfall data for the sites are given on an annual basis in Figure 3. Maximum and minimum temperatures are presented in Figures 4 and 5. Detailed temperature and rainfall data on a monthly basis are given in Table 2.

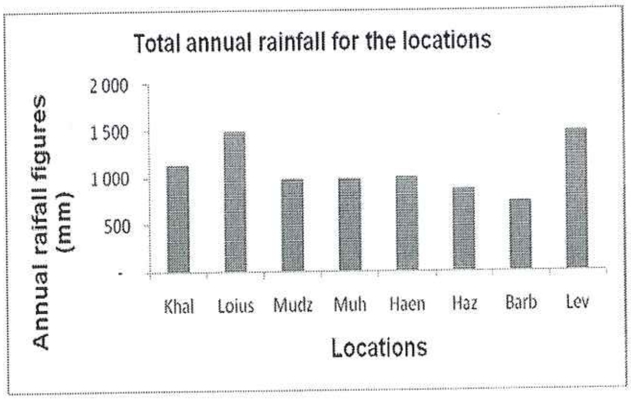

Fig 3. Annual rainfall data for the locations

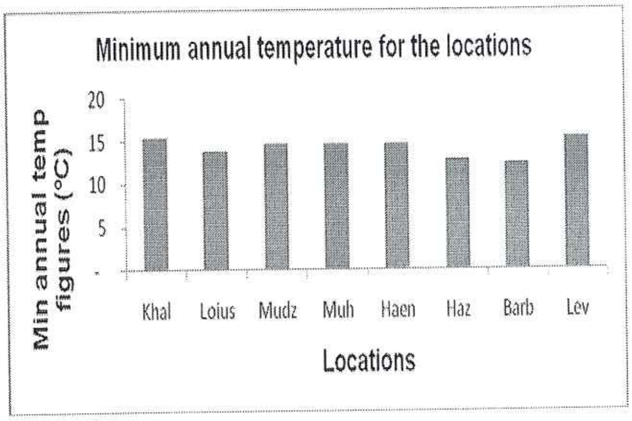

Figure 4. Minimum annual temperature data for the locations

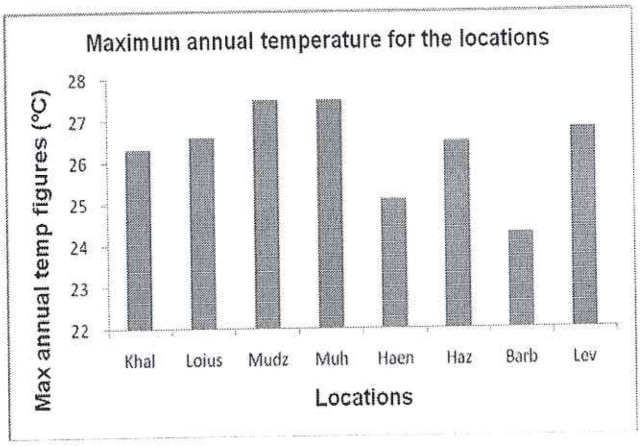

Figure 5. Maximum annual temperature data for the locations

Table 3. Mean monthly minimum and maximum temperatures, standard deviation and monthly average rainfall figures for the locations from 1999-2008

Location	Month	Min temp (°C)	Max temp (°C)	SD	Rainfall averages (mm)
Khalavha	Jan	18.75	28.94	7.21	18.63
	Feb	18.98	28.16	6.49	322.57
	Mar	18.11	26.73	6.10	183.83
	Apr	15.97	25.76	6.92	65.22
	May	12.76	24.38	8.22	14.76

	Jun	10.98	22.35	8.04	30.91
	Jul	10.48	22.65	8.61	14.83
	Aug	11.76	24.93	9.31	9.51
	Sept	13.64	26.63	9.19	33.96
	Oct	15.77	27.59	8.36	57.92
	Nov	17.68	28.51	7.66	162.71
	Dec	18.63	28.70	7.12	216.08
Louis Trichardt	Jan	18.71	29.07	7.33	215.6
	Feb	18.55	28.39	6.96	317.84
	Mar	17.16	27.70	7.45	261.5
	Apr	14.50	26.69	8.62	56.09
	May	10.84	24.99	10.01	29.47
	Jun	8.10	22.86	10.44	37.31
	Jul	7.77	22.82	10.64	37.21
	Aug	9.26	24.17	10.54	23.91
	Sept	12.04	27.49	10.92	43.1
	Oct	14.87	28.06	9.33	99.98
	Nov	16.28	28.27	8.48	165.49
	Dec	17.62	28.90	7.98	201.21
Mudzidzidzi	Jan	19.25	28.89	6.82	206.31
	Feb	19.14	29.95	7.64	97.08
	Mar	17.47	28.77	7.99	91.74
	Apr	14.45	26.93	8.82	104.7
	May	11.32	26.09	10.44	12.9
	Jun	9.11	24.16	10.64	17.8
	Jul	8.57	23.59	10.62	8.43
	Aug	9.99	25.61	11.05	8.12
	Sept	13.33	28.28	10.57	32.2
	Oct	16.58	29.14	8.88	43.19
	Nov	17.76	29.23	8.11	178.68
	Dec	19.18	29.76	7.48	181.72

Muhuyu	Jan	19.25	28.89	6.82	206.31
	Feb	19.14	29.95	7.64	97.08
	Mar	17.47	28.77	7.99	91.74
	Apr	14.45	26.93	8.82	104.7
	May	11.32	26.09	10.44	12.9
	Jun	9.11	24.16	10.64	17.8
	Jul	8.57	23.59	10.62	8.43
	Aug	9.99	25.61	11.05	8.12
	Sept	13.33	28.28	10.57	32.2
	Oct	16.58	29.14	8.88	43.19
	Nov	17.76	29.23	8.11	178.68
	Dec	19.18	29.76	7.48	181.72
Haenertsburg	Jan	18.64	27.39	6.19	139.6
	Feb	18.89	27.09	5.80	95.91
	Mar	18.05	26.55	6.01	97.16
	Apr	15.76	24.88	6.45	72.43
	May	14.04	23.60	6.76	40.06
	Jun	11.51	21.80	7.28	14.5
	Jul	11.11	21.57	7.40	2.78
	Aug	12.48	23.15	7.54	14.55
	Sept	14.61	25.64	7.80	13.44
	Oct	15.51	25.75	7.24	93.11
	Nov	16.92	26.70	6.92	190.81
	Dec	8.27	27.12	13.33	228.21
Hazyview	Jan	28.39	18.03	7.33	163.49
	Feb	29.45	17.14	8.70	207.68
	Mar	28.31	16.05	8.67	89.59
	Apr	25.93	12.57	9.45	31.30
	May	25.22	10.12	10.68	19.79
	Jun	23.35	6.76	11.73	14.53
	Jul	23.18	5.76	12.32	17.64

	Aug	25.15	7.95	12.16	8.26
	Sept	26.86	11.45	10.90	19.76
	Oct	26.51	13.73	9.04	61.95
	Nov	27.33	15.22	8.56	112.89
	Dec	28.38	17.01	8.04	122.01
Barberton	Jan	16.64	26.29	6.82	128.73
	Feb	14.98	26.66	8.26	97.28
	Mar	13.88	26.13	8.66	78.12
	Apr	12.34	24.29	8.45	40.24
	May	8.85	23.08	10.06	17.03
	Jun	6.12	21.24	10.69	9.22
	Jul	6.60	21.19	10.32	5.35
	Aug	9.28	22.83	9.58	11.20
	Sept	12.55	24.51	8.46	11.77
	Oct	14.69	24.98	7.28	68.78
	Nov	17.75	24.99	5.12	147.65
	Dec	15.44	26.01	7.47	125.16
Levubu	Jan	19.16	29.16	7.07	215.60
	Feb	19.22	28.85	6.81	317.84
	Mar	18.18	27.75	6.77	261.50
	Apr	18.18	26.74	6.05	56.09
	May	12.53	25.22	8.97	29.47
	Jun	10.46	23.15	8.97	37.31
	Jul	9.71	23.22	9.55	37.21
	Aug	11.81	25.37	9.59	23.91
	Sept	13.77	27.17	9.48	43.10
	Oct	15.60	27.77	8.61	99.98
	Nov	17.37	28.39	7.79	165.49
	Dec	18.22	28.99	7.62	201.21

Experimental design and treatment details: Bush tea samples from 8 locations (Khalavha, Louis Trichardt, Mudzidzidzi, Muhuyu, Haenertsburg, Hazyview, Barberton and Levubu) were used as treatments arranged in a completely randomized design (CRD) with six replications.

3.4 Data analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of Statistical Package for Social Sciences (SPSS).

3.5 Chemical analysis

3.5.1 Chemicals: Deionized water was used in all the chemical composition analyses. Folin-Ciocalteau, methanol, sodium carbonate (anhydrous), potassium persulfate, 2,2'Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) and 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (Steinheim, Germany). Tannic acid, catechin and sodium hydrogen orthophosphate were obtained from Saarchem-Holpro Analytical (Pty) Ltd (Johannesburg, South Africa). Vanillin and sodium dihydrogen orthophosphate (anhydrous) were obtained from Associative Chemicals Enterprise (Pty) Ltd (Johannesburg, South Africa).

3.5.2 Sample preparation for polyphenol, tannin and antioxidant extractions: Duplicates of 1 g sample were weighed into centrifuge tubes and 20 ml of the solvent (absolute methanol, 80% methanol, 1% HCl in methanol) was added. The extracts were votexed every ten minutes for 2 hours. The tubes were left to stand in order to achieve separation. The supernatant was removed and another 20 ml of the solvent was added to the residue and rinsed by votex mixing every 5 minutes for 20 minutes. The tubes were again let to stand to achieve separation. The supernatant was removed and the rinsing step was repeated. The supernatants were collected and stored in a freezer (-10°C) and analyzed after 24 hours.

3.5.3 Polyphenol assay: Total polyphenol content was determined using the method of Waterman and Mole (1994). In this method, approximately 10 ml of distilled water was added into each labeled 50 ml volumetric flask. Preparation of standards - a stock solution was prepared (0.1 g of tannic acid into a 100 ml methanol) and the stock solution (0, 2, 4, 6, 8, 10 ml) and the solvent which was methanol (10, 8, 6, 4, 2, 0 ml) were added to prepare a serial dilution. A sample standard or extract of 0.5 ml was added into the volumetric flasks. Folin reagent of 2.5 ml was added into the volumetric flasks and allowed to react for 1-8 minutes and then 7.5 ml of the sodium carbonate was added into the flasks. The flasks were filled with water to the mark of the flask, mixed well and allowed to react for 2 hours at room temperature from the time when the folin reagent was added. Absorbances were measured at 760 nm using UV-visible Genesys 20 spectrophotometer. A standard curve was plotted with concentration on the x-axis and absorbance on the y-axis. The R^2 should be above 0.995. Polyphenols were measured in mg catechin/100 g sample.

***3.5.4 Tannin assay*:** Prince *et al.* (1978) method was used. The reagents (methanol extracts of the sample: **Vanillin reagent** - 0.5% vanillin in methanol and 4% HCl in methanol; **Stock solution -** 0.1 g catechin dissolved in 100 ml of methanol) and extracts were maintained at 30°C in a thermostat-controlled water bath. Methanol extract of 1 ml was added to 5 ml of vanillin reagent. The sample blanks were prepared with 4% HCl in 100 ml of methanol replacing the reagent and the stock solution (0, 2, 4, 6, 8, 10 ml) and the solvent which was methanol (10, 8, 6, 4, 2, 0 ml) were added to prepare a serial dilution. The resultant colour was read spectrophotometrically at 500 nm after incubation for 20 minutes. The absorbance readings of the blanks were subtracted from those of the samples. A standard curve with concentration (x-axis) and absorbance (y-axis) was plotted. The results were then expressed as mg catechin equivalent/ 100 g sample.

3.5.5 Antioxidant assay: Awika *et al.* (2004) method was used for determination of total antioxidant content. Mother solution was prepared by 8 mM of ABTS and 3 mM of potassium persulphate ($K_2S_2O_2$) solutions using distilled deionized water. Equal volumes of the 2 were mixed and let to react in the dark for at least 12 hours at room temperature. The solution was prepared freshly every day. For the working solution, 5 ml of mother solution was added to 145 ml of pH 7.4 phosphate buffer solution (150 mM NaCl; mix 40.5 ml of 0.2 M Na_2HPO_4 (dibasic) with 9.5 ml 0.2 M Na_2HPO_4 (monobasic); add 0.877 g of NaCl, (top to 100 ml with distilled deionized water). Adjustment of pH with NaOH was done where necessary. Trolox standard was prepared with 2900 µl working solution added to 100 µl of serial trolox dilutions, shaken and let to react for 15 minutes. The absorbances were measured at 734 nm. The absorbance readings should be between 0.1-1.6 and the R^2 should be at least 0.995. A working solution of 2900 µl was added to 100 µl sample extract, shaken and let to react for exactly 30 minutes for the sample analysis. Absorbances were measured at 730 nm. Additional sample dilutions may be necessary if absorbance reading falls below 0.1. Antioxidant activity was measured in µmol trolox equivalents/ g sample.

CHAPTER 4

RESULTS

4.1 Polyphenols: Results in Table 3 showed an increase in total polyphenol contents with an increase in altitude. Ahmad *et al.* (2003) reported that there was a correlation of higher content of total polyphenol in tea with higher altitude at regions with different ecological conditions. Haenertsburg had the highest content (7.7 mg/100 g) of total polyphenols as compared to the other locations. The lowest content (3.6 mg/100 g) of total polyphenol content was recorded at Levubu with the difference between the highest and the lowest at 4.09 mg/100 g. The lowest contents of polyphenols were recorded between 600 and 700 m altitudes. Ahmad *et al*, (2003) further reported that at Battal location, at an altitude of 1 500 m, polyphenol content was higher than in tea samples collected from NTRI location, which is at 1 000 m altitude. It was evident from this study that the highest polyphenol contents (7.7 mg/100 g) recorded in Haenertsburg and (6.1 mg/100 g) in Louis Trichardt were due to the high altitude 900-1400 m.

A positive correlation was observed between altitude and total polyphenol contents as compared to the other climatic and soil factors. A significant correlation ($R^2 = 0.55$) was therefore observed between total polyphenol content and altitude. The relationship was moderate and positive which implies that 55% of the variation in polyphenol contents was explained by altitude. The analysis of variance in Appendix A1 showed total polyphenol concentrations to be significantly different from each other at 5% probability level.

Table 4. Total polyphenol content in bush tea samples collected from locations of different elevations in the Limpopo and Mpumalanga Provinces in 2008

Level	**Site**	**Altitude(m)**	**Total Polyphenol content (mg/100 g)**
High	Haenertsburg	1410	7.7^{a}
	Louis Trichardt	944	6.2^{b}
Medium	Barberton	816	5.4^{c}
	Khalavha	870	5.2^{c}
	Hazyview	855	5.04^{c}
Low	Levubu	671	3.6^{d}
	Mudzidzidzi	615	4.4^{d}
	Muhuyu	610	4.3^{d}
CV%			**7.7**

Means in a column followed by the same letter are not significantly different (P > 0.05) ns - Non significant difference at 5% probability level

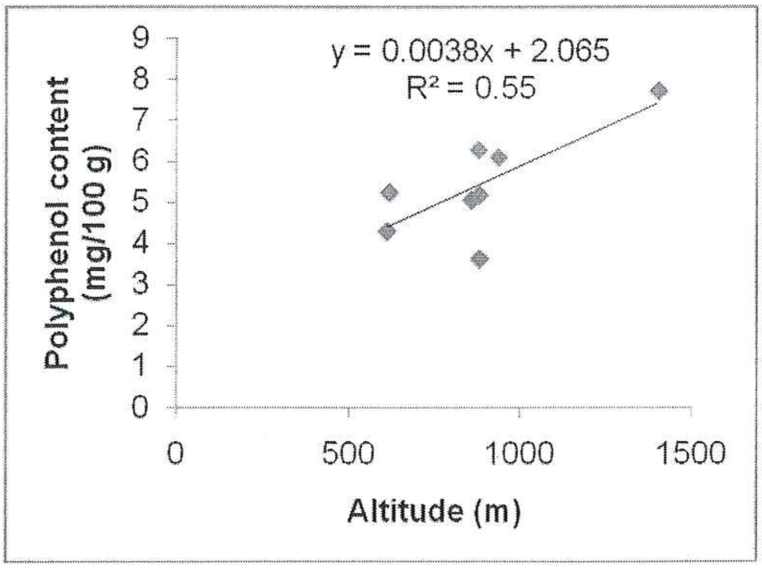

Figure 6: Correlation between altitude and polyphenol content

Annual average minimum temperature had a weak and negative influence on variation of total polyphenol content. Only 0.9% of the variation in concentrations of total polyphenol was explained by changes in minimum temperature. The R^2 value was 0.09 (fig 7).

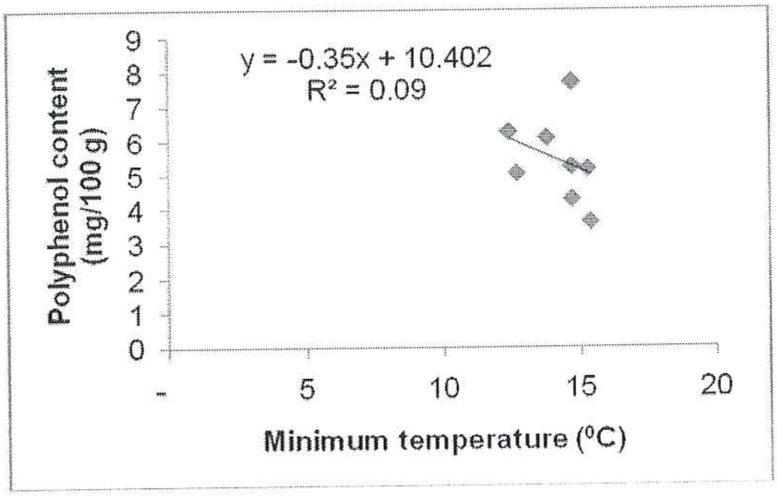

Figure 7: Correlation between annual average minimum temperature and polyphenol content

Maximum temperature had a moderate and negative influence on polyphenols where the R^2 was 0.48 (fig 8). This means that only 48% variation in polyphenol content was explained by the change in temperature. Polyphenol content decreased with an increase in temperature.

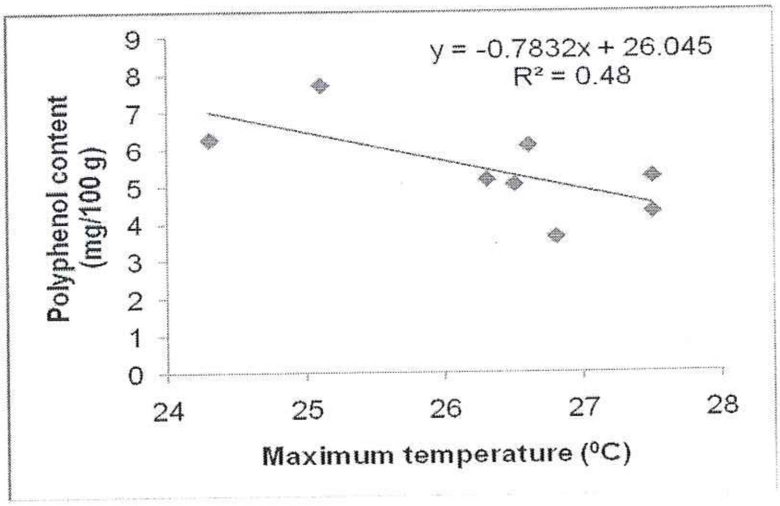

Figure 8: Correlation between annual average maximum temperature and polyphenol content

The relationship between rainfall and polyphenols was very weak and negative. Only 0.9% of the variation in concentrations of total polyphenol is explained by rainfall (fig 9). Polyphenols and rainfall had no relation at all because the correlation was less than 1. The R^2 was 0.09.

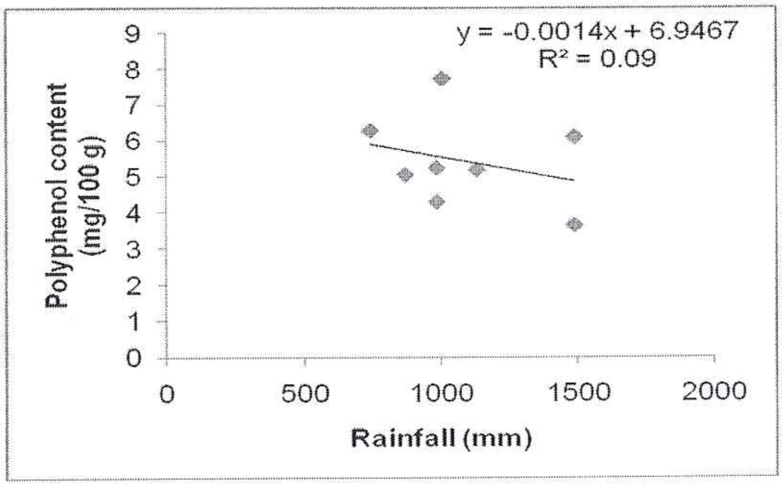

Figure 9: Correlation between total annual rainfall and polyphenol content

Soil pH content had a very weak and negative correlation with the amount of total polyphenol content (fig 10). The R^2 value was 0.08 and only 0.8% variation in polyphenol content was explained by the change in soil pH. This explains that there was no relation between soil pH content and bush tea polyphenols.

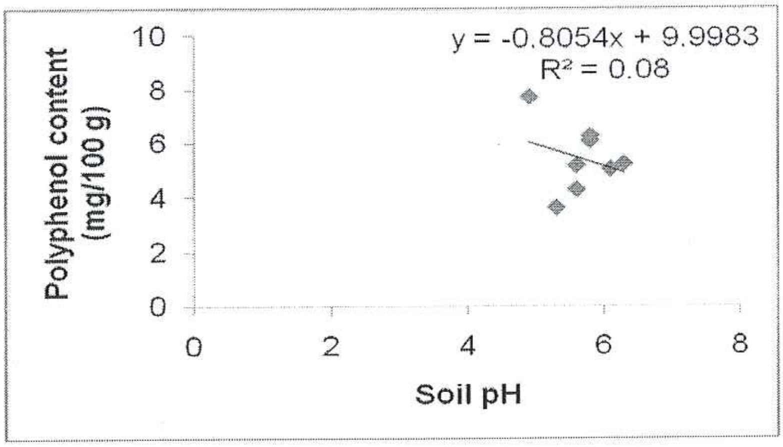

Figure 10: Correlation between soil pH and polyphenol content

The correlation between the amount soil nitrogen and total polyphenol content was weak and negative (fig 11). About 19% variation in concentration of total polyphenol content was explained by the soil nitrogen status. The R^2 value was 0.19.

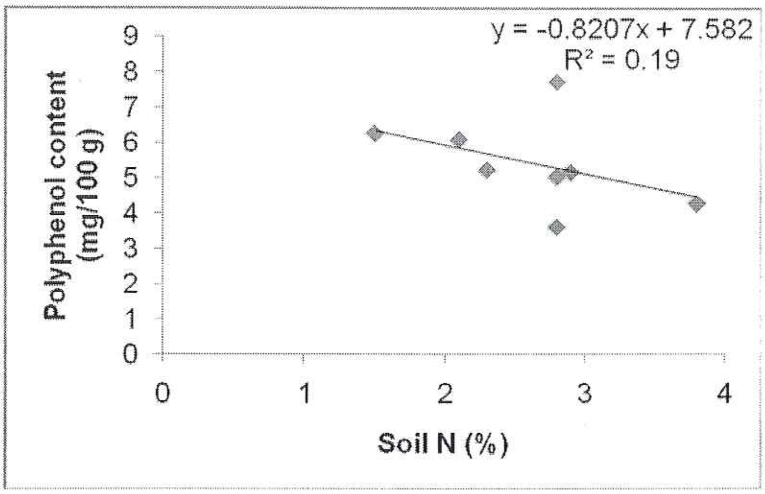

Figure 11: Correlation between soil N and polyphenol content

The correlation between the amount soil phosphorus and total polyphenol content was moderate and negative (fig 12). About 32% variation of polyphenol content was explained by the soil phosphorus status. The R^2 value was 0.32

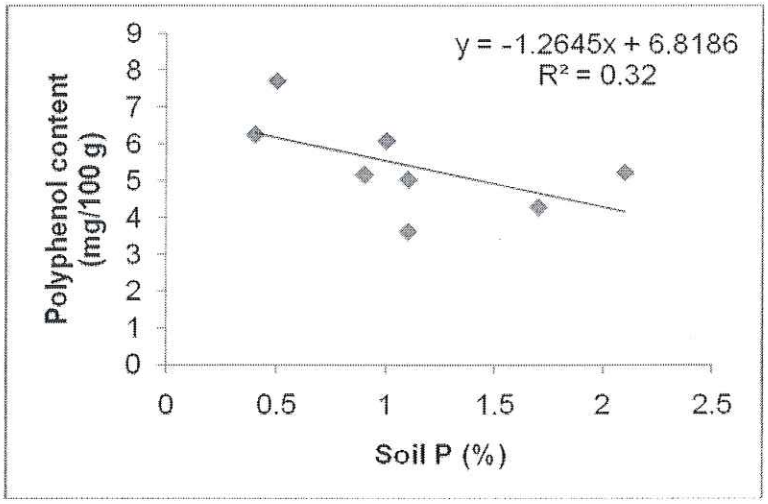

Figure 12: Correlation between soil P and polyphenol content

The correlation between the amount of soil potassium and total polyphenol content was very weak and negative (fig 13). About 0.7% variation was explained by the soil Potassium status. The R^2 value was 0.07.

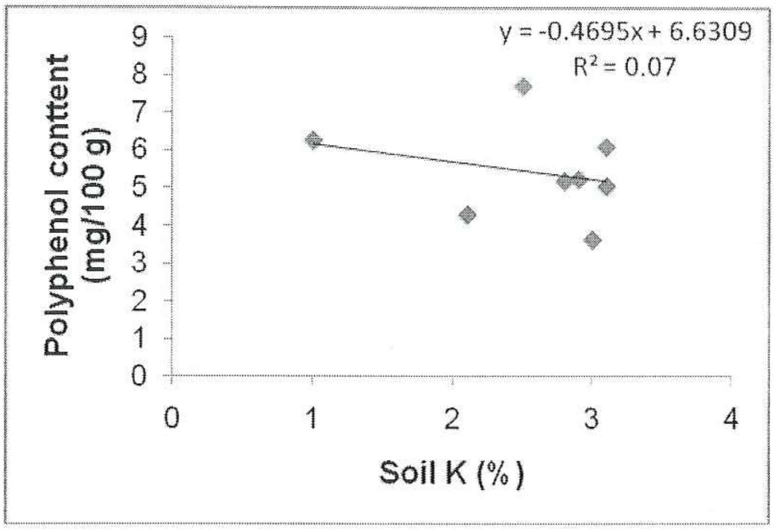

Figure 13: Correlation between soil K and polyphenol contents

4.2 Tannins**:** Results in Table 4 showed that Levubu had the highest concentration of total tannin content (9.78 mg/100 g) and the lowest recorded at Khalavha (0.1 mg/100 g). The difference between the highest and the lowest was 9.8 mg/100 g. There was little correlation between total tannin content and an increase in altitude. Different soil and climatic factors also did not have any significant difference on the total tannin content. The difference in the amount of tannin content was however not statistically significant and not influenced by the climatic and soil factors. However the positive and weak correlation was observed between tannins and altitude. Regardless of the variations in altitude, rainfall, temperature, soil macro elements and soil pH, tannin content was statistically not significant. The analysis of variance in Appendix A2 showed a difference in means of tannin content, however the difference was not due to the variation in climatic and soil factors.

Table 5. Total tannin content in bush tea samples collected from locations of different elevations in Limpopo and Mpumalanga provinces in 2008

Level	**Site**	**Altitude (m)**	**Total Tannin Content (mg/100 g)**
High	Haenertsburg	1410	7.7^{b}
	Louis Trichardt	944	0.2^{e}
Medium	Barberton	816	6.3^{c}
	Khalavha	870	0.1^{f}
	Hazyview	855	6.04^{c}
Low	Levubu	671	9.8^{a}
	Mudzidzidzi	615	0.9^{e}
	Muhuyu	610	4.3^{d}
CV%			**10.93**

Means in a column followed by the same letter are not significantly different ($P > 0.05$) ns - Non significant difference at 5% probability level

Tannin content and altitude had a positive but weak relationship (fig 14). The R^2 was 0.15 which implies that only 15% of the variation in tannin content was explained by altitude.

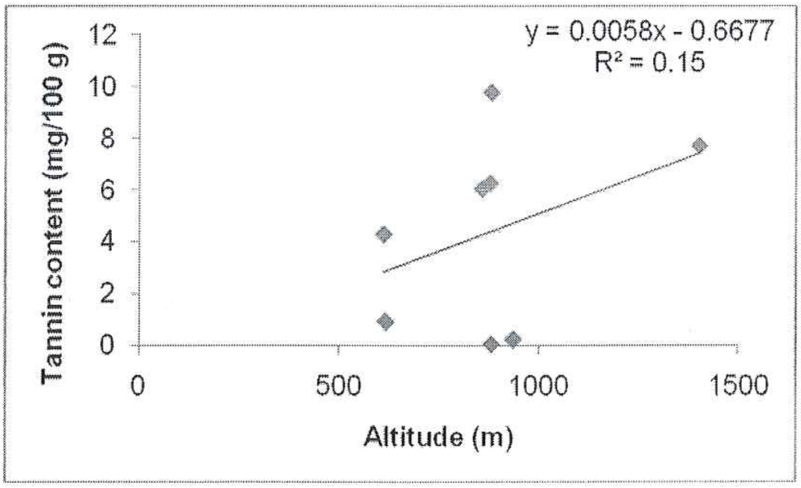

Figure 14: Correlation between altitude and tannin content

There was a very weak and negative relationship between annual average minimum temperature and tannin content (fig 15). Only 0.6% variation of total tannin content was explained by the change in minimum temperature. The R^2 was 0.06.

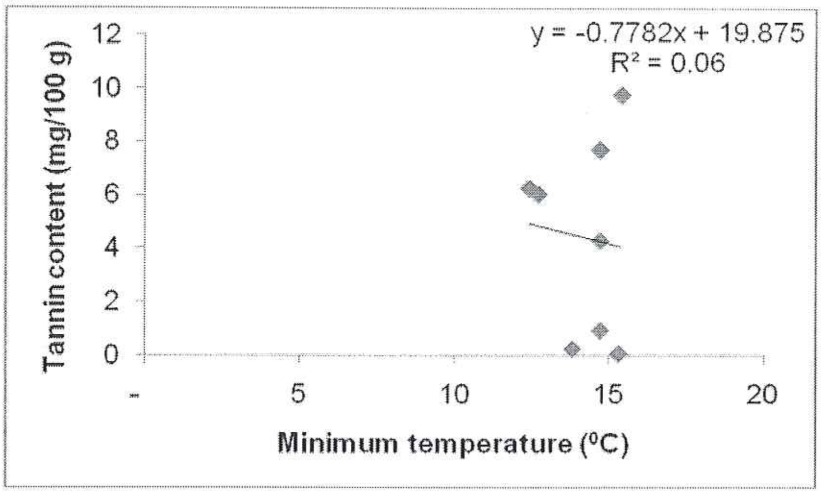

Figure 15: Correlation between annual average minimum temperature and tannin content

There was a weak and negative relationship between annual average maximum temperature and tannin content (fig 16). Only 13% variation of total tannin content was explained by the change in maximum temperature. The R^2 was 0.13.

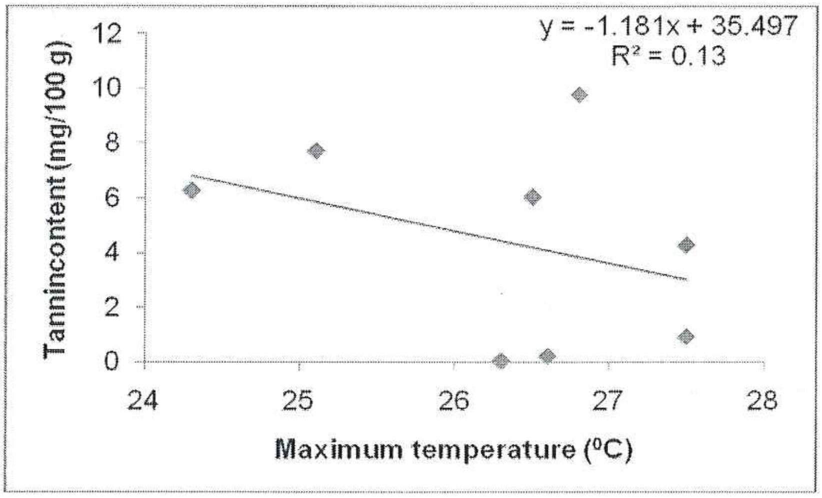

Figure 16: Correlation between annual average maximum temperature and tannin content

Total annual rainfall had a very weak and negative influence on the amount of total tannin content (fig 17). Only 0.1% of the variation in concentration of total tannin content was explained by the difference in rainfall changes. The R^2 value was 0.01.

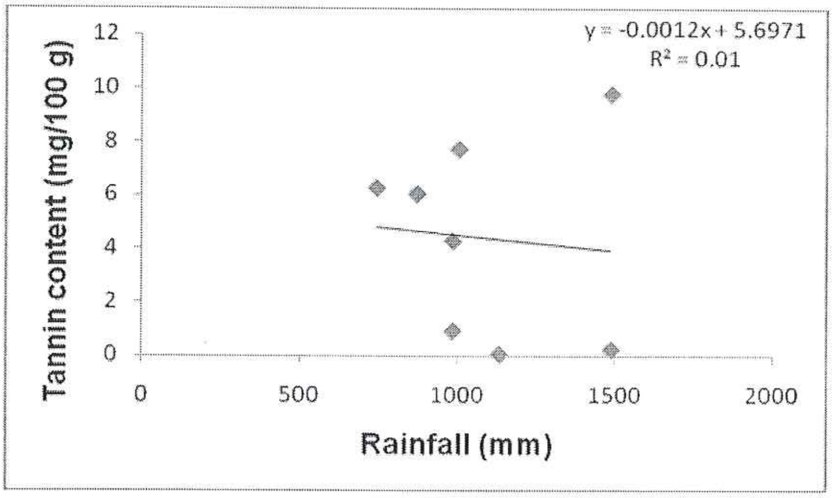

Figure 17: Correlation between total annual rainfall and tannin content

Soil pH status had a moderate and negative effect on tannin content (fig 18). The R^2 value was 0.28 and only 28% of variation in tannin content was explained by the soil pH status.

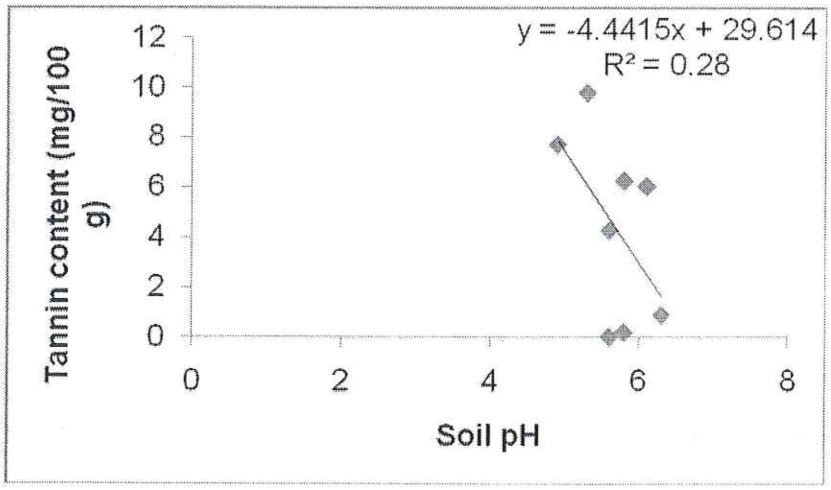

Figure 18: Correlation between soil pH and tannin content

Soil N had a very weak and positive effect on total tannin content (fig 19). The 0.1% variation in tannin content was explained by the soil N status. The R^2 value was 0.01

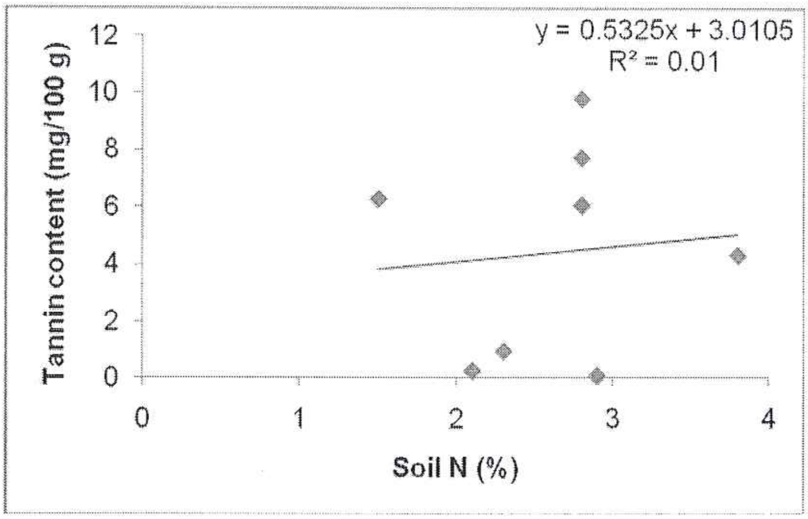

Figure 19: Correlation between soil N and tannin content

Soil P had a weak and negative effect on tannin content (fig 20). Only 14% of variation in tannin content was explained by soil phosphorus. The R^2 value was 0.14.

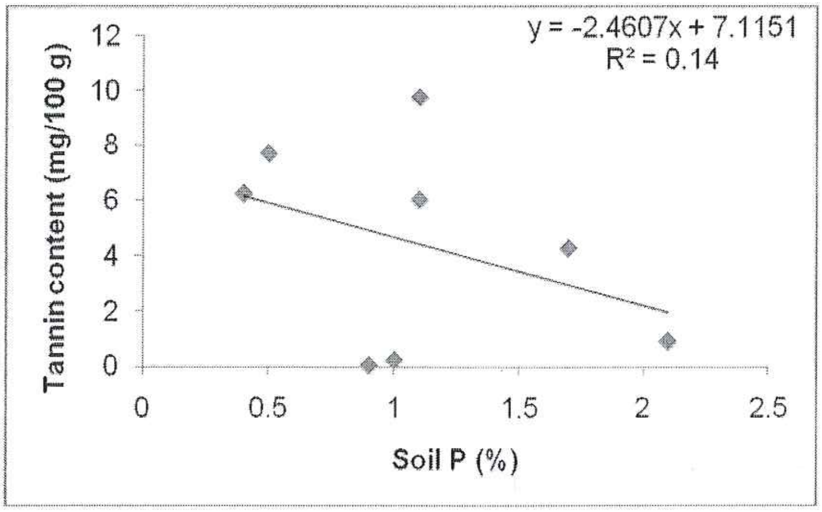

Figure 20: Correlation between soil P and tannin content

Soil K had a weak and negative effect on tannin content (fig 21). The 0.5% variation in concentrations of tannin content was explained by soil Potassium status. The R^2 value was 0.05.

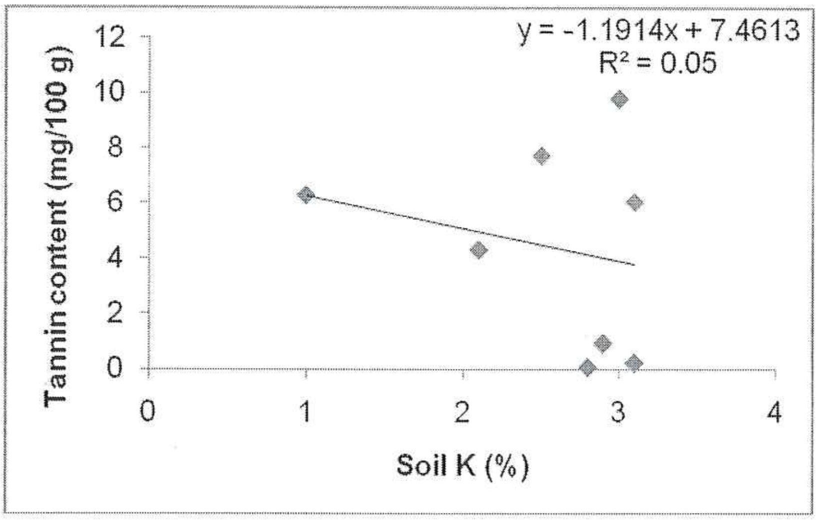

Figure 21: Correlation between soil K and tannin content

4.3 Antioxidants: No significant difference was observed from the results of total antioxidant contents when locations with different climatic and soil factors were used as treatments (Table 5). Total antioxidant content (TAC) remained slightly the same in all the locations at 35 µmol/g, regardless of the differences in rainfall, temperature, altitude, soil macro elements and soil pH figures. These results suggest that climatic and soil factors from different locations had no effects on total antioxidant content. The analysis of variance (Appendix A3) clearly shows that total antioxidant content from different locations were not significantly different from each other.

Table 6. Total antioxidant contents in bush tea samples collected from different locations of the Limpopo and Mpumalanga provinces in 2008

Level	Site	Altitude (m)	Total antioxidant content (µmol/ g)
High	Haenertsburg	1410	35.05 ^{a}
	Louis Trichardt	944	35.05 ^{a}
Medium	Barberton	816	35.1^a
	Khalavha	870	35.1 ^{a}
	Hazyview	855	35.2^a
Low	Levubu	671	35.02^a
	Mudzidzidzi	615	35.09 ^{a}
	Muhuyu	610	35.02 ^{a}
CV%			**0.59**

Means in a column followed by the same letter are not significantly different (P > 0.05) ns - Non significant difference at 5% probability level

Altitude had a weak and negative effect on antioxidant content (fig 22). The 0.08% in antioxidant content was explained by altitude status. The R^2 value was 0.008.

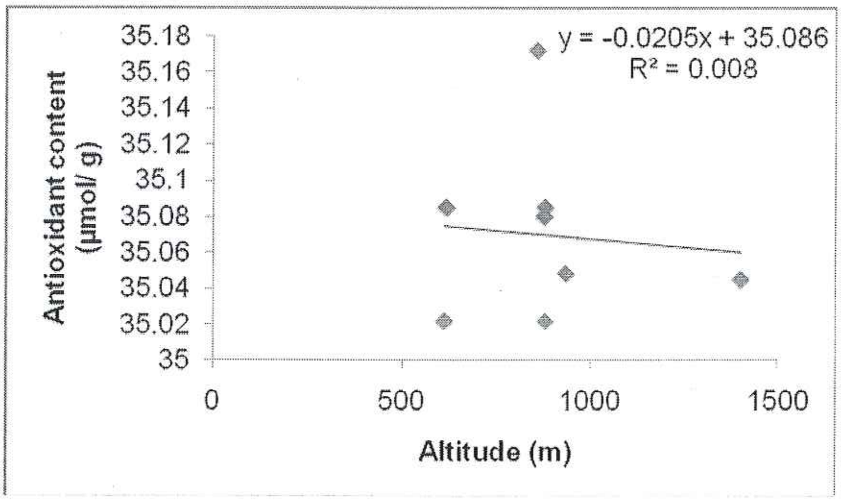

Figure 22: Correlation between altitude and antioxidant content

Annual average minimum temperature had a moderate and negative effect on antioxidant content (fig 23). Only 36% of antioxidant content was explained by the change in minimum temperature. The R^2 value was 0.36.

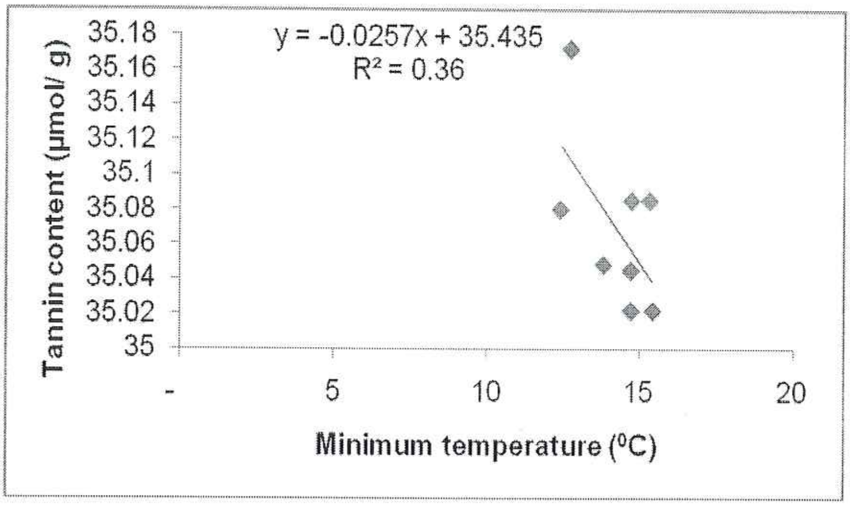

Figure 23: Correlation between annual average minimum temperature and antioxidant content

Annual average maximum temperature had a very weak and negative effect on antioxidant content (fig 24). Only 0.1% of antioxidant content was explained by the change in maximum temperature. The R^2 value was 0.01.

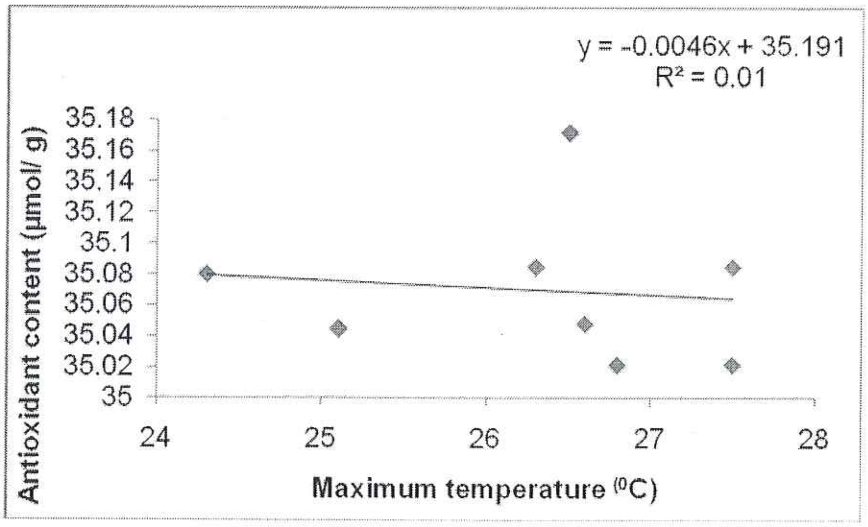

Figure 24: Correlation between annual average maximum temperature and antioxidant content

Total annual rainfall had a moderate and negative effect on antioxidant content (fig 25). Only 26% of antioxidant content was explained by the change in rainfall figures. The R^2 value was 0.26.

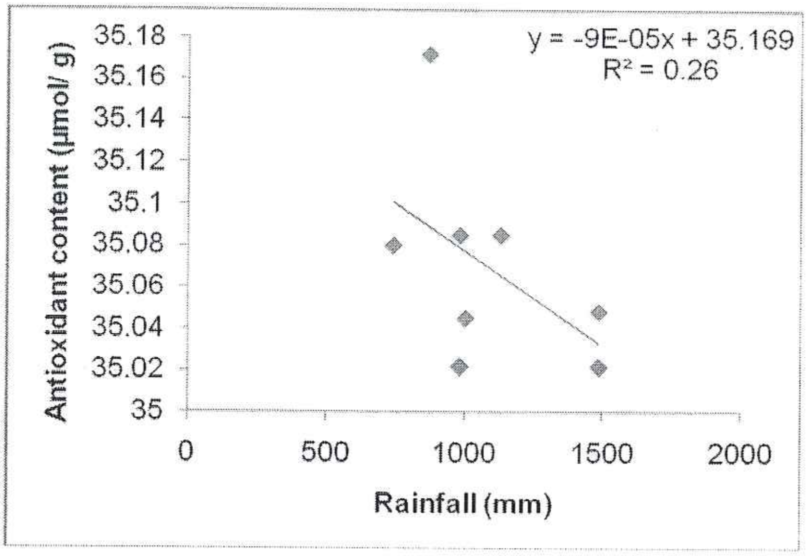

Figure 25: Correlation between total annual rainfall and antioxidant content

Soil pH status had a moderate and positive effect on antioxidant content (fig 26). Only 37% of variation in antioxidant content was explained by the change in soil pH status. The R^2 value was 0.37.

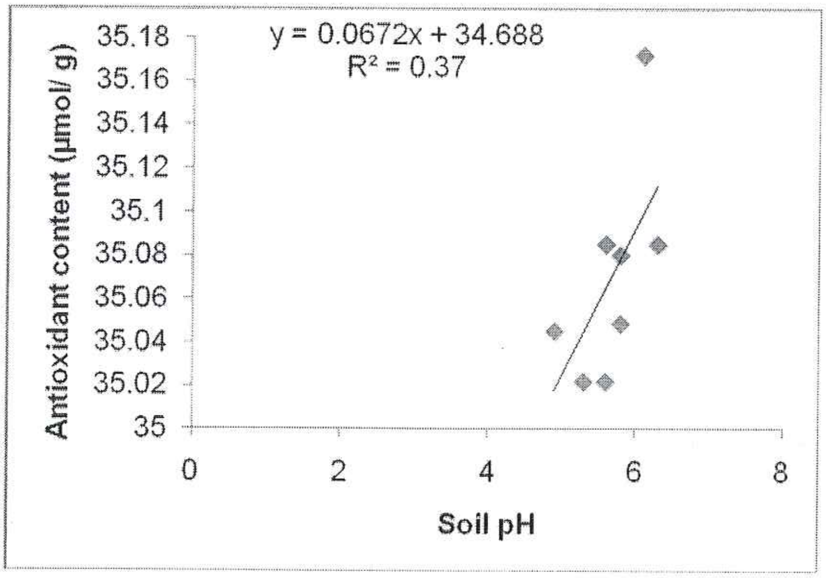

Figure 26: Correlation between soil pH and antioxidant content

Soil nitrogen status had a very weak and negative effect on antioxidant content (fig 27). Only 0.5% of variation in antioxidant content was explained by the change in soil N status. The R^2 value was 0.05.

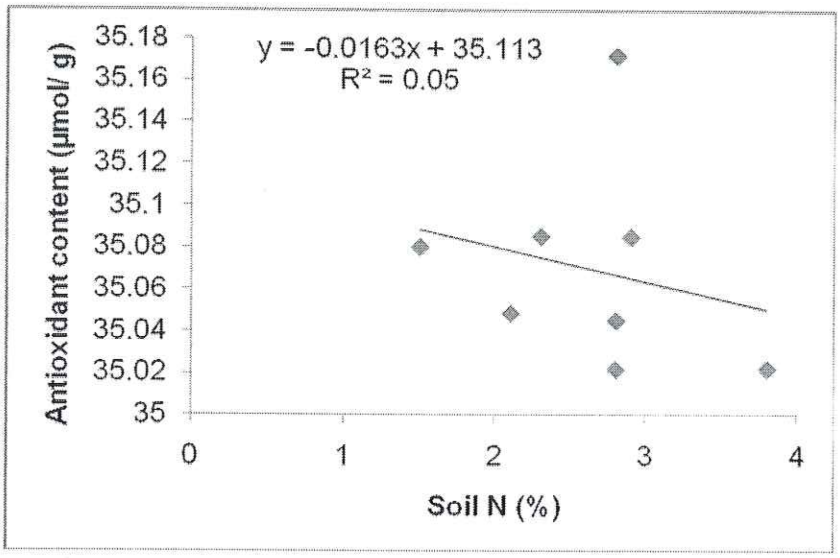

Figure 27: Correlation between soil N and antioxidant content

Soil phosphorus status had no effect on antioxidant content (fig 28). Only 0.01% variation in antioxidant content was explained by soil P status. The R^2 value was 0.001.

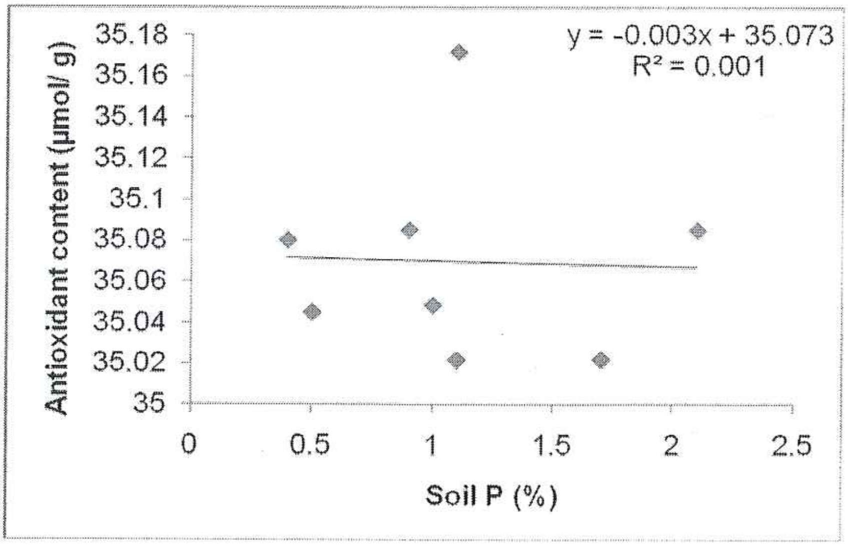

Figure 28: Correlation between soil Phosphorus and antioxidant content

Soil potassium status had a weak and negative effect on antioxidant content (fig 29). Only 0.3% variation in antioxidant content was explained by soil P status. The R^2 value was 0.03.

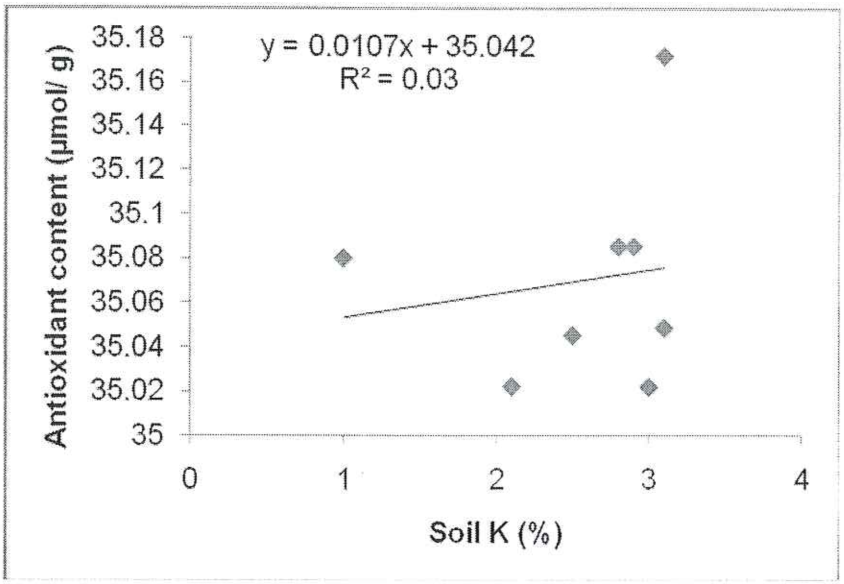

Figure 29: Correlation between soil Phosphorus and antioxidant content

CHAPTER 5:

DISCUSSION

5.1 Polyphenols

Bush tea polyphenol content correlated positively to the changes in altitude but showed no correlation with rainfall, temperature, soil macro elements and soil pH. Though there was a difference in the means of total polyphenols, the variations were not of statistical significance. Rainfall, temperature, soil macro elements and soil pH were different at most of the locations, but the difference did not have any significance influence on the changes in total polyphenols between the locations. It was also reported that the contents of epigallocatechin gallate (EGCG), catechin gallate (CG), and total catechins (TC) in the Oolong teas grown at a high altitude were significantly higher than those grown at a low altitude (Chen *et al.*, 2010). These results concur with the results from this study that bush tea polyphenols increased with an increase in altitude with the highest content (7.7 mg/100 g) in Haenertsburg which was at 1 410 m and the lowest content (3.6 mg/100 g) in Levubu which was at 671 m.

The relative levels of theaflavins, thearubigins and flavonol glycosides in black teas for different regions and countries of origin were compared and the statistical analysis results highlighted the characteristic differences in phenolic substance levels which contributed most powerfully to the discrimination between origins (McDowell *et al.*, 1995). An increase in altitude from 30 to 1859 m significantly increased ($p<0.001$) the number of harvested shoots in Sri Lankan TRI 2025 tea clone (Jin *et al.*, 2008). Correlation between temperature and polyphenols was negative. The results from this study suggested that variation in total polyphenol content at all 8 locations was not explained by the changes in minimum and maximum temperature figures. However, in a study where shoot production was examined for two clones at four sites in western Kenya, differing by up to 300 m in altitude and from 16.4 to 18.1°C in mean air temperature, the duration of the shoot growth cycle increased with altitude, and the rate of shoot extension decreased, mainly because of the temperature differences (Squire *et al.*, 1993).

Roberts (1990) also reported vigorous shoots in bush tea leaves when plants were exposed to lower (24°C) temperatures during winter and higher (38°C) during summer. Growth properties of rooibos tea showed a significant correlation with altitude, mean annual temperature, mean annual precipitation, slope, latitude and longitude. Most correlation was observed on altitude, temperature and precipitation (Jürgens and Schmiedel, 2010).

Ten commercial teas from various countries were analyzed to determine their mineral composition and the study showed that the variations of mineral composition, polyphenols and flavonoids are linked to different origins of the plant (Ferara *et al.*, 2001). However, variations in total polyphenol, total tannin and total antioxidant from the results of this study were not correlated to the locations of bush tea origin. Concentration of total polyphenols in leaves of wild bush tea plants were lowest in March (11.8 mg/ g), April (10.8 mg/ g) and September (10.8 mg/g) but highest in June (35.5 mg/ g) and July (35.9 mg/ g), with nitrogen applications between 0 to 300N kg ha^{-1} (Mudau *et al.*, 2006a; Mudau *et al.*, 2007c). Change in total polyphenol content was only caused by the differences in altitude from the results of this study, in which samples were collected in August.

5.2 Tannins

Tannin content of bush tea was correlated slightly with altitude as compared to other climatic and soil factors. However, the differences in tannin content were not significantly influenced by the changes in rainfall, temperature, soil macro nutrients and soil pH figures. Results from this study showed minimum and maximum temperatures to have a negative correlation, with slight changes in total tannin content. Mudau *et al.* (2007a) reported tannin content to be increased when bush tea samples were collected during autumn, which has cool temperatures.

The analysis of variance (Appendix A2) showed the concentrations of total tannin at different locations to be significantly different from each other, but the difference was not explained by soil and climatic factors with the highest content (9.8 mg/100 g) in Levubu and lowest content (0.5 mg/100 g) in Khalavha. In one study, concentrations of condensed tannins were lowest (0.10 %) during summer compared to autumn (0.14 %) and spring (0.13%) due to drought stress (Hamilton *et al.*, 2001). Samples for this study were collected in August and the means in tannin content where observed to be different with no statistical correlation between tannins and climatic and soil factors.

Winter and autumn are the best times to harvest bush tea to maximize tannin content (Mudau *et al.*, 2006a). Chabeli *et al.* (2008) reported hydrolysable tannin content to be maximum (0,020 %) during summer months. Chiu (1990) also reported maximum amounts of total tannin contents in Pauchung tea to be obtained during summer months due to strong sunshine and higher temperatures. Total annual average temperature and rainfall means were different in all the locations but did not have any significant influence on tannin content. Therefore variation in total tannin was not influenced by rainfall and temperature. Results from a study by Mossi *et al.* (2009), showed that average annual temperature and climate have significant effect on tannin content at a 95% confidence level.

5.3 Antioxidants

Antioxidant content of bush tea did not have any correlation with altitude, rainfall, temperature, soil macro elements and soil pH. Means of antioxidants in all 8 locations remained slightly the same regardless of variations in soil and climatic factors. Green tea showed stronger antioxidant activity when harvested in April and July months (Ku *et al.*, 2010). Green tea grown in an area with high temperature, long sun exposure time and high rainfall had higher levels of theanine but lower levels of isoleucine, leucine, valine, alanine, EC, EGC, EGCG and caffeine than green tea grown in areas with relatively low temperatures, short exposure time and low rainfall (Lee *et al.*, 2010).

Theaflavin (TF), thearubigin (TR) and total and extractable caffeine contents fluctuated following climatic variations, decreasing after rainy periods in Argentinean black tea (Malec, 1988). Total antioxidant content in all the locations remained the same regardless of the differences in latitudes and altitudes of sample collection areas. Tea cultivated in Argentina between 26° and 28° South latitudes was observed to be very good in quality (polyphenols and antioxidants) when compared to teas from other sources (Anesini *et al.*, 2008).

Results showed that total antioxidant activity were significantly different in tea samples from Kenya, Japan and China. Green, black and white tea products from Kenyan tea cultivars had significantly higher antioxidant activities than green tea processed from Japan and China (Karori *et al.*, 2007). Changes in climatic and soil factors did not have any effect on antioxidant content of bush tea samples from different locations of the Limpopo and Mpumalanga Provinces in this study.

6. GENERAL CONCLUSIONS AND RECOMMENDATIONS

Based on the investigations from this study, high altitude areas significantly influenced the amount of total polyphenols and a slight effect on tannin content with no significant influence on the amount of total antioxidant content. It was also evident that the amount of total polyphenols subsequently increased with an increase in elevation and declined with a decrease in elevation. Total tannin content was observed to be the highest (7.7150 mg/100 g) at Haenertsburg and lowest (3.6250 mg/100 g) at Levubu. Tannin and antioxidant contents were negatively correlated to climatic and soil factors. Change in the amount of TT content slightly correlated with the change in soil and climatic factors from different locations with maximal (9.7750 mg/100 g) content from Levubu and minimal (0.0467 mg/100 g) content recorded from Khalavha.

Further studies still need to be conducted to determine the influence of soil and climatic factors at different locations on chemical composition of seasonal bush tea. This will therefore further explain the results from this study that only altitude had a highly significant influence on TP content as compared to the other climatic and soil factors. It may be concluded from the results of this study that there is still a need to further investigate the influence of climatic and soil factors at different locations and seasons on sensory attributes and chemical composition of bush tea leaves, in order to document more data for the commercialization of bush tea.

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Appendix A1: Analysis of variance for total polyphenol content on bush tea collected from different locations with different soil and climatic factors

Source of variation	**df**	**Mean squares**[z]		**F tab**
			Total polyphenol content (mg/g)	5%
Treatments (Locations)	7	0.004	7.240*	2.365
Error	40	0.008		
Total	47			

[z] F-values significant (*), highly significant (**) or not significant (NS) at 5% probability level

Appendix A2: Analysis of variance for total tannin content on bush tea collected from different locations with different soil and climatic factors

Source of variation	**df**	**Mean squares**[z]		**F tab**
			Total tannin content (mg/g)	5%
Treatments (Locations)	7	0.004	30.139*	2.365
Error	40	0.008		
Total	47			

[z] F-values significant (*), highly significant (**) or not significant (NS) at 5% probability level

Appendix A3: Analysis of variance for total antioxidant content on bush tea collected from different locations with different soil and climatic factors

Source of variation	**df**	**Mean squares**[z]		**F tab**
			Total antioxidant content (μmol/g)	5%
Treatments (Locations)	7	0.004	0.522^{ns}	2.365
Error	40	0.008		
Total	47			

[z] F-values significant (*), highly significant (**) or not significant (NS) at 5% probability level