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Quality Profiles of Cultivated and Wild Bush Tea (*Athrixia phylicoides*) Harvested at Various Phenological Stages

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ABSTRACT

Bush tea (*Athrixia phylicoides* DC.) is a plant used in the processing of popular herbal beverages indigenous to South Africa. It is rich in antioxidants and contains no caffeine. Bush tea has lower tannin levels than green or black tea. The quality of tea may be influenced by phenological stage of the leaves. A trial to determine the quality of tea harvested at various phenological stages (namely new growth, older growth & whole plants) from wild and cultivated bush tea was conducted. This was to determine the best phenological stage to harvest bush tea of best quality for health benefits. In cultivated bush tea, harvested new growth and whole plants produced higher quality beverage owing to their higher polyphenol and tannin attributes, respectively. In wild bush tea, both new and older growth showed higher quality due to their higher total polyphenol content and higher total antioxidants, respectively. © 2012 Friends Science Publishers

Key Words: Bush tea; Phenological stages; Wild and cultivated plants; New growth; Older growth; Quality

INTRODUCTION

Bush tea (*Athrixia phylicoides* DC.) is a plant used in the processing of popular beverages indigenous to South Africa. There are 14 species of the genus *Athrixia*, nine of which are found in South Africa (Leistner, 2000). The plant has been used for many years by the indigenous people of South Africa for treating boils, cleansing or purifying blood, bad acne, skin eruption, and for bathing (Roberts, 1990). The Vhavenda in northern South Africa use it as an aphrodisiac while the Zulu in eastern part of South Africa use a decoction of the root as a cough remedy and a purgative. It has inhibitory effects against micro-organisms such as *Staphylococcus aureus, Bacillus coreus, Enterococcus and Esherichia coli and Mycobacterium smegmatis* (Negukhula, 2010). Although it is rich in antioxidants, it does not contain caffeine (McGaw *et al.*, 2007).

The absence of caffeine is a desirable feature of a healthy beverage, as is the presence of antioxidants which may have beneficial health benefits (McGaw *et al*., 2007). Boonchum *et al.* (2011) details the different pathways to generate free radicals in the human body and benefits of natural antioxidants. This gives bush tea a distinct advantage over regular green and black tea beverages made from *Camellia sinensis*. Bush tea also possesses low level of tannins thus bypassing the bitter, astringent taste experienced with many other teas (Hu *et al*., 2001a). Low tannin content of bush tea is an advantage for people with digestive problems who have difficulty with tannin-rich beverages as tannins bind iron and reduce the absorption of non-heme iron (Bukochova & Skobeleva, 1980). Tannins also precipitate protein, inhibit digestive enzymes and affect the utilization of vitamins and minerals (Tabasum *et al*., 2001). The usage of *Athrixia* tea has declined over time with the availability of commercially produced teas but the plant is considered to have economic potential as a herbal infusion.

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

Flush is followed by accumulation of carbohydrate reserves and these are channeled towards the production of total polyphenols (Roberts, 1990). In the fresh first tea flush there exists a wide variety of non-volatile compounds, including polyphenols, flavanols, flavones, phenolic acids and depsides, amino acids, chlorophyll and other pigments (Hart, 2009). *Camellia sinensis* total polyphenols in tea flush range from 20% to 35% (Hart, 2009). In *C. sinensis* there is maximum content of theaflavins, thearubigins and caffeine during early flush and gradual decline with

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progress in season, being minimum during main flush and slightly improved through backend flush (Gulati & Ravindranath, 1996).

Although some information on the effect of phenological stages on quality of certain other tea species is available, information on the effect of phenological stages specifically on bush tea quality is totally lacking. The aim of this study was to investigate the changes in the chemical composition (total polyphenols, tannins & antioxidants) of both cultivated and wild bush tea due to phenological stages. The distinctive phenological stages used for this study were the aerial new growth (leaves) and the older basal leaves of bush tea and whole plants.

MATERIALS AND METHODS

Collection of wild bush tea materials: Wild bush tea materials were collected from Mudzidzidzi Village in Limpopo province of South Africa[24^o 50' S 31^o 17E; Altitude 610 m above mean sea level (amsl)]; with subtropical-type climate of summer rainfall, and cold and dry winter. Thirty plants of bush tea were randomly collected for sampling at various phenological points of top new growth leaves, further down older leaves and whole plants. The collected materials were air dried in the shade for the determination of chemical composition assays.

Cultivated bush tea: The experiment on cultivated bush tea was carried out at Madzivhandila College of Agriculture in South Africa (22^o 56' 60S, 30^o 28' 60E), Altitude 709 m amsl. The planting materials made up of mature bush tea stock plants were collected from Mudzidzidzi village. Selection of the planting materials was made on the basis of true-to-name and type, free of disease and insects, and in a healthy physiological state. During cultivation, to stimulate rapid and prolific rooting of cuttings, plants were cut about 7-8 cm long and were treated with Seradix No.2 (0.3% IBA) (Bayer Pretoria, South Africa) and planted on seedling trays on a mist bed, supplied with a misting system operating through misting nozzles. The mist bed used was 3 m long, 1.5 m wide and 0.5 m high. Light irrigation was done 3 times a day every day except on rainy days.

Bush tea seedlings were allowed to grow on seedlings trays (Fig. 1) for 3 months. Wood rooted cuttings (seedlings) were ready and were transplanted directly into 20-L bags. The medium used during transplanting was pine bark and sand at a ratio of 2:1, respectively. In an attempt to achieve optimum growth, the growing bush tea plants in plastic bags were treated to a split application with N,P and K at rates 300, 300 and 200 kg/ha, respectively, as reported by Mudau *et al.* (2007) two weeks after transplanting.

Cultivated bush tea plants were allowed to grow in plastic bags (Fig. 2) in the nursery for 2 months before they were harvested. Harvesting of cultivated bush tea at different phenological stages (new leaves, older leaves & whole plants) was done eight weeks after transplanting.

Fig. 1: Bush tea seedlings growing on seedling trays

Fig. 2: Cultivated bush tea growing in the nursery

Fig. 3: Harvested bush tea material during the drying process in the shade

The harvested materials were air dried in the shade (Fig. 3) for the determination of chemical composition assays.

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

the sample were vortex mixed every 10 min for 2 h to improve extraction efficiency. The samples were then centrifuged at 3500 rpm for 10 min (25°C) using centrifuged tubes and decanted. Each sample residue was rinsed once with 20 mL of solvent, vortex mixed for 5 min, centrifuged as above, and decanted. Two supernatants were combined and used for analysis. The Folin Ciocalteau method (Singleton & Rossi, 1965), modified by Waterman and Mole (1994), was used to determine total phenols in tea extract. This method was based on the reducing power of phenolic hydroxyl groups (Hahn *et al.*, 1984), which react with the phenol reagent to form chromogens that can be detected spectrophotometrically. The methanol extract (0.5 mL) was added to a 50 mL volumetric flask containing distilled water and mixed. Folin Ciocalteau phenol reagent (2.5 mL) was then added and mixed, followed by 7.5 mL sodium carbonate solution (20 g/100 mL) within 1 to 8 min after addition of the Folin Ciocalteau phenol reagent. The contents were mixed and the flask made up to volume with distilled water and thoroughly mixed. Absorbance of the reactants was read after 2 h at 760nm using UV-visible Genesys 20 Spectrophotometer. Catechin was used as standard to prepare a standard curve and results were expressed as mg catechin equivalents/100 mg of samples on dry weight basis.

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

Determination of antioxidant activity: Antioxidant activity of the extracts was determined using Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Awika *et al.* (2004). TEAC is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the 2, 2'-azino-bis3-ethylbenzthiazoline-6-sulfonic (ABTS+) radical cation chromogen in relation to that of Trolox, the water soluble vitamin E analogue, which is used as an antioxidant standard. The $ABTS^+$ was produced by mixing equal volume of 8 mM ABTS with 3 mM potassium persulfates prepared in distilled water and allowed to react in the dark for at least 12 h at room temperature before use. The $ABTS^+$ solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH_2PO_4, 0.2 M $NaHPO_4$ and 150 mM NaCl in 1 L of distilled water, with pH adjustment using NaOH, where necessary. This solution was made fresh for each analysis. The $ABTS^+$ solution (2900 µL) was added to the methanol extracts of bush tea (100 µL) of Trolox in a test tube and mixed. Absorbances reading (at 734 nm) were taken after 30 min (for the samples) and 15 min (for the standard) of the initial mixing of the samples and standard respectively. The results were expressed as µM Trolox equivalents per g of sample on dry weight basis.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) using PROC GLM (General linear model) procedure of SAS version 8.0. (SAS Instutute Inc. 1999).

RESULTS

Chemical Concentration in Bush Tea

Total polyphenols: Concentration of total polyphenols showed variation at different phenological stages of both cultivated and wild bush tea (Fig. 4). The lowest total polyphenol concentration in cultivated bush tea was observed in older growth (0.7 mg catechin equivalents/100 mg), while the highest concentration was observed in new growth (1.85 mg catechin equivalents/100 mg). The concentration of total polyphenols for the whole plant was only slightly higher than that for older growth. In wild bush tea the lowest total polyphenol concentration was observed in older growth (0.81 mg catechin equivalents/100 mg), while the highest concentration was observed in new growth (1.23 mg catechin equivalents/100 mg). Total polyphenol content in whole plant samples of wild and cultivated bush tea were intermediate between that of new growth and older growth (Fig. 4). New growth of both wild and cultivated bush tea contained significantly higher levels of total polyphenols than in older growth and whole plant. The two types of bush tea had similar levels of total polyphenols in older growth but whole plants of wild bush tea had significantly higher level of total polyphenols.

Tannin content: Results in Fig. 5 show a significantly higher tannin content (0.95 mg catechin equivalents/100 mg) in harvested whole plants than in harvested new growth (0.15 mg catechin equivalents/100 mg) and harvested older growth (0.1 mg catechin equivalents/100 mg) for cultivated bush tea. There was however no significant difference in tannin content between harvested new growth and harvested older growth of both wild and cultivated bush tea. Fig. 5 also shows that there was no significant variation in tannin concentration of wild bush tea in harvested new growth, old growth and harvested whole plants. These results differed with those for cultivated tea where whole plant had significantly higher levels of tannins.

Total antioxidants: No significant difference was observed between harvested new growth, harvested older growth and harvested whole plants of cultivated bush tea

Fig. 4: Total polyphenol concentration of wild and cultivated bush tea harvested at different phenological stages

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

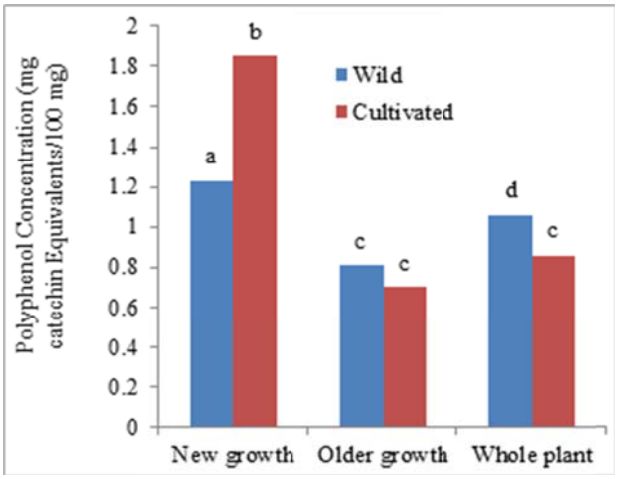
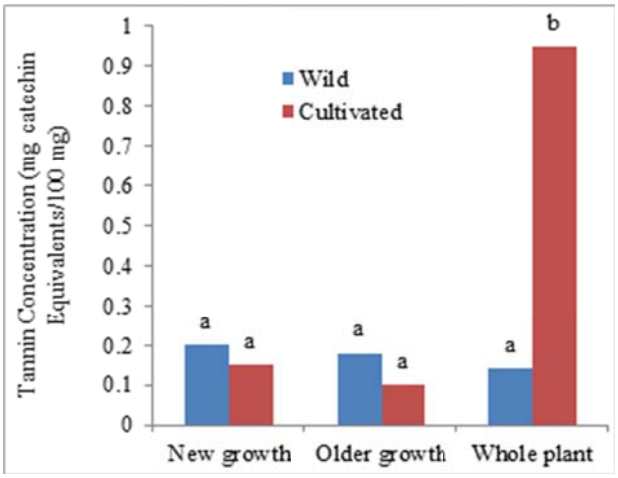


Fig. 5: Tannin concentration of wild and cultivated bush tea harvested at different phenological stages

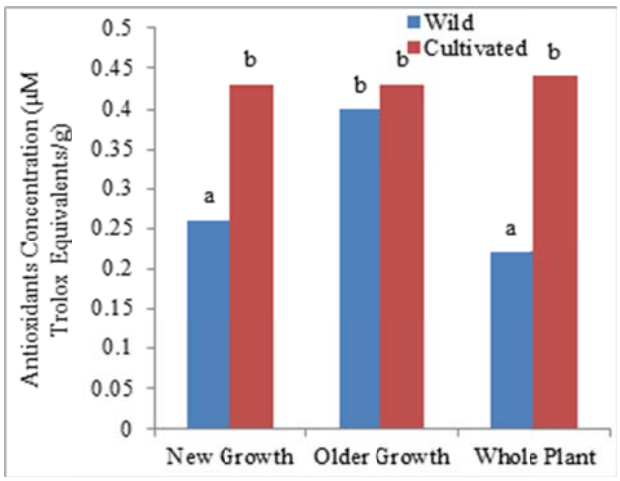
*Means denoted by the same letter are not significantly different at 5% probability level



in antioxidant contents. Furthermore, concentration of total antioxidants varied between harvested new growth, older growth and harvested whole plant of wild bush tea (Fig. 6). Harvested older growth in wild bush tea recorded the highest concentration of 0.4 µM Trolox equivalents/g while harvested whole plants recorded the lowest antioxidant content of 0.22 µM Trolox equivalents/g which was not significantly different from the recorded 0.26 µM Trolox equivalents/g total antioxidant content of harvested new growth. New growth and whole plant samples of cultivated bush tea had significantly higher antioxidant levels than in wild bush tea. Older growth of wild bush tea had similar levels of antioxidants as new growth, older growth and whole plant samples of cultivated bush tea.

Fig. 6: Total antioxidant concentration of wild and cultivated bush tea harvested at different phenological stages

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



DISCUSSION

As higher total polyphenol concentration is an indication of higher quality in green tea (Hirasawa *et al.*, 2002), results from this study concur with the assertion by MedlinePlus (2009) that top new growth leaves are of higher quality while older leaves further down the stem of tea are of poorer quality. This was attributed to the distribution of polyphenols. Polyphenols are part of carbohydrate reserve or resource translocation to young leaves. Polyphenols are the primary nutritious constituents of bush tea. The chief polyphenols are flavonoids such as catechin and proanthocyanidins, with the four major polyphenols being epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate. Most of the green tea catechins are oxidized during manufacture of black tea and converted into orange or brown products known as theaflavins and thearubigins but these still retain the basic C6-C3-C6 structure of flavonoids (Lakenbrink *et al.*, 2000). The strong astringent flavor of tea is attributed to its polyphenol content (Hirasawa *et al.*, 2002). Polyphenols appear to thwart cancer by at least three methods: they shut off formation of cancer cells, turn up the body's natural detoxification defences and suppress cancer advancement (Hlahla, 2010). Thus from a health point of view, the results from this study suggest that new growth of bush tea would be preferred to either older growth or use of whole plant on the basis that it has much higher concentration of polyphenols.

Tannin content in tea leaves is the main potential indicator of the medicinal potential due to their anti-oxidant activities (Hirasawa *et al.*, 2002). Tannins help to prevent cancers and heart problems by lowering the tendency of blood platelets to stick together (Stenveld *et al.*, 1992). However, high level of tannins causes bitter, astringent taste

in many types of teas. Tannin levels reported in this study are low and similar to those reported for green tea but lower than for black tea and coffee (Tabasum *et al.*, 2001). Low tannin content of bush tea is an advantage for people with digestive problems with tannin-rich beverages as tannins bind iron and reduce the absorption of non-heme iron (Bokuchova & Skobeleva, 1980). The results from this study (Fig. 5) suggest that new and older growth may make tastier drink but the whole plant could make healthier drink with increased antioxidant activities.

Antioxidants are compounds that interact with harmful molecules (oxygen ions, free radicals, peroxide) in the body and may enhance body mechanism against diseases like cancer and coronary heart disease (Han *et al*., 2007). The results also show that anti-oxidant activity is linked to tannin content as whole plant samples (Fig. 5) also had significantly higher tannin content than either new or older growth. This study suggests that, despite potential bitter astringent flavor, whole plant samples make healthier bush tea than either new growth or older growth. Such tea may need to be blended with substances that can suppress the bitter astringent taste caused by high level of tannins.

It is generally known that a higher total polyphenol concentration is an indication of higher quality. Similar results were reported by MedlinePlus (2009) who demonstrated that top new leaves are of higher quality, while older leaves further down the *C. sinensis* plant are of poorer quality. The results are in line with those of Devlin and Witham (1983) and Arnold *et al.* (2004) who reported a movement of carbohydrate resources or reserves from older aging leaves to young leaves. Similar pattern was seen in samples of cultivated bush tea and the results indicate better quality tea from new growth.

The results on total antioxidants in wild bush tea from this study are a total contradiction to MedlinePlus (2009) who reported that older leaves further down the stem are of poorer quality, as in this study older leaves showed to be of highest quality due to high concentration of total antioxidants.

It can be concluded that in cultivated bush tea new growth or whole plants have higher tea quality (polyphenols, tannins & antioxidants) than older leaves while in wild bush tea harvesting both new and older growth has comparative health benefits due to higher total polyphenols and antioxidants.

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