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Full Length Research Paper

Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors

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This study was conducted to determine the chemical composition of wild bush tea growing at locations differing in altitude, climate and edaphic factors. Samples were collected from 8 locations selected for their abundance of wild bush tea and replicated 6 times in the laboratory using a completely randomized design (CRD). The areas where bush tea samples were collected comprised of Khalavha, Louis Trichardt, Mudzidzidzi, Muhuyu, Haenertsburg, Hazyview, Barberton and Levubu, respectively. Bush tea samples were collected from the bush, and the total polyphenol content, tannin and total antioxidant contents were determined. 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 content 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. However, rainfall, temperature, soil macro elements and soil pH did not have any influence on total polyphenol, total tannin and total antioxidant contents.

Key words: Polyphenols, tannins, antioxidants, edaphic factors, climatic factors.

INTRODUCTION

Bush tea (*Athrixia phylicoides* DC*.)* is a popular beverage used as an 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. This shrub is found in grassland, forests, bushveld, rocky and sloping habitats. Bush tea flowers throughout the year depending on the climatic and edaphic factors, but the best flowering time is from March to May (Mbambezeli, 2005). Surveys have shown that the consumption of bush tea is widespread and commercialization of the extract holds economic and developmental potential (Chellan et al., 2008). However, for bush tea to be commercially viable, its uses and properties either have to outcompete or complement teas already on the market. These are green and black teas from *Camellia sinensis*, rooibos tea from *Aspalathus linearis* and honey bush tea (*Cyclopia intermedia*). Currently, rooibos and honey bush are produced for the herbal tea market, while bush tea has been reported to have a potential for commercialization (Joubert et al., 2008).

Traditional medicinal uses of *A. phylicoides* DC. or bush tea, an indigenous South African plant with very limited localized use as herbal tea include treatment of boils, acne, infected wounds and throat infection (Joubert et al., 2008). Aside from its horticultural potential, this plant also has traditional economic uses. The bush man makes tea from the leaves of *A. phylicoides*. Sotho and Xhosa people also chew it for sore throats and coughs

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(Mbambezeli, 2005; Roberts, 1990). This beautiful plant may be used as a filler plant in the open spaces in flowerbeds, and is best used when planted in a group, though it works well as a specimen plant in the garden (Mbambezeli, 2005). In addition, the dried leaves and fine twigs of *A. phylicoides* have traditionally been used by the Khoi and Zulu people as an herbal tea and medicinal decoction (Van Wyk and Gericke, 2000). The Venda people are reported to use extracts from soaked roots and leaves as antihelminthic (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-3-ol as a major flavonoid (Mashimbye et al., 2006). Bioassay-guided fractionation of ethanolic extract from aerial parts of *A. phylicoides* using silica and Sephadex column chromatography showed the isolation of other three known flavonoids, 3-0-demethyldigicitrin, 5,6,7,8,3',4'hexamethoxyflavone and quercetin (Mavundza et al., 2010). The potential for development of bush tea as a healthy beverage alternative to caffeine-containing tea was reported by Mudau et al. (2007b) with no phytotoxicity or presence of pyrrolizidine (McGaw et al., 2007). 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. These different sites have varying soil characteristics. There is, however, no data that describes the effects of altitude, climatic and edaphic factors at different locations on wild bush tea quality. Therefore, the objective of this study was to determine the chemical composition of wild bush tea (*A. phylicoides* DC) growing at locations differing in altitude, climate and edaphic factors. This study will provide the baseline of where bush tea in large commercial scale can be grown depending on altitude, climatic and edaphic fit.

MATERIALS AND METHODS

Study sites and bush tea sample collection

This study was carried out at the University of Limpopo Technology Station. Samples of bush tea were collected in August 2008 from the following bush tea abundance locations in South Africa; Hazyview and Barberton in Ehlanzeni district (Mpumalanga Province), Khalavha, Louis Trichardt, Mudzidzidzi, Muhuyu, Levubu in Vhembe district and Haenerstburg in Mopani district (Limpopo Province).

Experimental design and treatment details

Bush tea samples from 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.

Sample preparation

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 vortexed every ten min for 2 h. 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 vortex mixing every 5 min for 20 min. The tubes were again left to stand to achieve separation. The supernatant was then removed and the rinsing step was repeated. The supernatants were collected and stored in a freezer (-10°C) and analyzed after 24 h.

Polyphenol assay

Total polyphenol content was determined using the Waterman and Mole (1994) method. 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 and 10 ml) and the solvent which was methanol (10, 8, 6, 4, 2 and 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 to 8 min 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 h at a room temperature from the time when the Folin Ciocalteu 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.

Tannin assay

Prince et al. (1978) method was used in the determination of tannin. 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 bat h. 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 and 10 ml) and the solvent which was methanol (10, 8, 6, 4, 2 and 0 ml) were added to prepare a serial dilution. The resultant colour was read spectrophotometrically at 500 nm after incubating for 20 min. 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.

Antioxidant assay

Awika et al.'s (2004) method was used for the determination of total

Table 1. Total polyphenol contents in bush tea samples collected from different elevated locations of the Limpopo and Mpumalanga provinces in 2008.

Level	Site	Altitude (m)	Total polyphenol content (mg/100 g)
High	Haenertsburg	1410	7.70[a]
	Louis Trichardt	944	6.08[b]
Medium	Barberton	816	5.30[c]
	Khalavha	870	5.17[c]
	Hazyview	855	5.04[c]
Low	Levubu	671	3.62[d]
	Mudzidzidzi	615	4.43[d]
	Muhuyu	610	4.28[d]
CV%			7.70

Means in a column followed by the same letter are not significantly different (P>0.05).

antioxidants content. The stock solution was prepared by 8 mM of *3*-ethylbenzthiazoline-6-sulfonic acid (ABTS) and 3 mM of potassium persulfate ($K_2S_2O_2$) solutions using distilled deionized water. Equal volumes of the 2 were mixed and left to react in the dark for at least 12 h at room temperature. The solution was prepared fresh every day. For the working solution, 5 ml of the stock 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. Adjust pH with NaOH if necessary. Trolox standard was prepared with 2900 µl working solution added to 100 µl of serial trolox dilutions, shaken and left to react for 15 min. The absorbances were measured at 734 nm. The absorbance readings should be between 0.1 to 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 left 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.

Statistical analysis

Data was subjected to analysis of variance (ANOVA) procedure using the Statistical Package for Social Sciences (SPSS).

RESULTS AND DISCUSSION

Polyphenols

Results in Table 1 showed an increase in total polyphenol contents with an increase in altitude. Ahmad et al. (2003) reported that there was correlation of higher content of total polyphenol with higher altitude at regions with different ecological conditions. Our results showed that Haenertsburg had the highest content (7.70 mg/100 g) of total polyphenols as compared to the other locations (Table 1). The lowest content (3.62 mg/100 g) of total polyphenol content was recorded in Levubu with the difference between the highest and the lowest at 4.09 mg/100 g. The lowest content of polyphenols was 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 content (7.70 mg/100 g) recorded in Haenertsburg was due to the high altitude area at 1 410 m.

A positive correlation was observed between altitude and total polyphenol content as compared to the other climatic and soil factors. Anon (2009) however, reported annual rainfall figures less than 1 300 mm to have a detrimental effect upon grown tea. The R^2 value of 55% in variation of total polyphenol content was explained by an increase in altitude.

Tannins

Results showed that Levubu had the highest concentration of total tannin content (9.80 mg/100 g), while the lowest was recorded at Khalavha (0.05 mg/100 g) (Table 2). The difference between the highest and the lowest was 9.8 mg/100 g. Although there was little correlation between total tannin content and an increase in altitude, different soil and climatic factors did not have any significant effect (P>0.5) on the total tannin content. Regardless of the variations in altitude, rainfall, temperature, soil macro elements and soil pH, tannin content was statistically not significant.

Chiu (1990) also reported maximum amounts of total tannin content in Pauchung tea to be obtained during summer months due to strong sunshine and higher

Table 2. Total tannin contents in bush tea samples collected from different locations of the Limpopo and Mpumalanga provinces in 2008.

Locations of sample collection	Total tannin contents (mg/100 g)
Khalavha	0.05[e]
Louis Trichardt	0.22[e]
Mudzidzidzi	0.92[e]
Muhuyu	4.28[d]
Haenertsburg	7.71[b]
Hazyview	6.04[c]
Barberton	6.27[c]
Levubu	9.80[a]
CV%	10.93

Means in a column followed by the same letter are not significantly different (P>0.05).

Table 3. Total antioxidant contents in bush tea samples collected from different locations of the Limpopo and Mpumalanga provinces in 2008.

Locations of sample collection	Total antioxidant contents (µmol/g)
Khalavha	35.08[a]
Louis Trichardt	35.05[a]
Mudzidzidzi	35.09[a]
Muhuyu	35.02[a]
Haenertsburg	35.05[a]
Hazyview	35.17[a]
Barberton	35.08[a]
Levubu	35.02[a]
CV%	0.59

Means in a column followed by the same letter are not significantly different (P>0.05).

temperatures. Total annual average temperature and rainfall means were different in all the locations from this study, but did not have any significant influence on tannin content. Therefore, variation in total tannin was not influenced by rainfall and temperature. In contrast, 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. Mudau et al. (2007a) reported tannin contents to be increased when bush tea samples were collected during autumn, which has the coolest temperatures. Results from this study showed minimum and maximum temperature to have a negative correlation with the changes in total tannin content.

Antioxidants

No significant difference was observed from the results (Table 3) of total antioxidant content when locations with different climatic and soil factors were used as treatments. Total antioxidant content (TAC) remained the same in all the locations at 35 µmol/ g. These results suggest that climatic and soil factors from different locations had no effect on the amount of total antioxidant content recorded respectively. Green tea grown in an area with high temperature, long sun exposure time and high rainfall had higher levels of thiamine, 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).

Conclusion

Based on the investigations from this study, high altitude areas had high significant difference on the amount of total polyphenols and a slight effect on tannin content, with no significant difference on the amount of total antioxidant content. Further studies still need to be conducted to determine the influence of soil and climatic factors at different location on chemical composition and sensory attributes of seasonal bush tea.

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