

# Seasonal Variation of Tannin Content in Wild Bush Tea

# Fhatuwani N. Mudau<sup>1\*</sup> • Asanda Ngele<sup>1</sup> • Phatu W. Mashela<sup>1</sup> • Puffy Soundy<sup>2</sup>

Department of Plant Production, University of Limpopo, Private Bag X1106, Sovenga, 0727, South Africa
Department of Plant Production and Soil Science, University of Pretoria, Pretoria, 0002, South Africa

Corresponding author: \* mudaufn@ul.ac.za

#### ABSTRACT

The objective of the study was to determine seasonal variation in the concentration of tannins in wild bush tea (*Athrixia phylicoides* L.) leaves. The BuOH-HCl\_Fe<sup>III</sup> reagent was used to analyze condensed tannins, whereas hydrolysable tannin was analyzed using potassium iodate. The absorbance was measured using a spectrophotometer. Significant seasonal variations in both condensed and hydrolysable tannins in leaf tissue occurred. The highest concentrations of condensed tannins were found in autumn (4.82%) compared with winter (2.44%), spring (2.66%) and summer (3.04%). The hydrolysable tannins were lowest during summer (0.10%) compared with autumn and winter (0.14%) and spring (0.13%). The data obtained from this study suggests that winter and autumn are the best times to harvest bush tea to maximize tannin contents of bush tea leaves.

Keywords: Athrixia phylicoides (L.), condensed tannin, hydrolysable tannin, season

### INTRODUCTION

Bush tea (Athrixia phylicoides L.) is a beverage used as a herbal tea and traditional African medicinal plant known for cleansing the blood, treating boils, headaches, infected wounds and cuts (van Wyk and Gericke 2000). Herbal teas contain low tannin content, which helps to prevent cancers and heart problems by lowering the tendency of blood platelets to stick together (Stensveld et al. 1992). Tannin content in tea leaves is the main potential indicator of medicinal potential due to its antioxidant activities (Hirasawa et al. 2002). Tannins are phenolic compounds that typically astringent found in a variety of herbal products and they may be grouped into hydrolysable and condensed tannins (Bokuchova and Skobeleva 1980). Condensed tannins are polymers of 2 to 5 or more flavonoid units that are joined by carbon-carbon bonds, which are not susceptible to hydrolyzation (van Wyk and Gericke 2000). Hydrolysable tannins are hydrolyzed by weak acids or bases to produce carbohydrates and phenolic acids (Haslam 1996).

Herbal tea quality is one of the critical factors determining the price of tea for export. It is currently measured by tea taster's scores from sensory evaluation, which is prompted to be subjective, depending upon the sensory tasting skills of the taster (Taylor *et al.* 1992). The sensory quality attributes are astringent taste, bitterness, sweatiness and aroma (Hu *et al.* 2001a). The other chemical components of tea quality parameters are total ployphenols (Ventakatesan *et al.* 2004), amino acids (Chen *et al.* 1985) carbohydrates (Sanderson *et al.* 1976), organic acids (Sanderson and Selvendran 1965), vitamins (Hu *et al.* 2001b), volatile flavour compounds and plant pigments (Taylor *et al.* 1992).

Factors that affect tea quality parameters can be classified into four major categories viz., cultivars (Owour *et al.* 2000), environmental conditions (Chiu 1989), cultural practices (Taylor *et al.* 1992) and seasonal variation (Sud and Baru 2000). Thus, climatic conditions in different countries and variation in seasons make it impractical to produce tea of the same quality throughout the year (Roberts and Smith 1963; Owour *et al.* 1997).

Mudau *et al.* (2006) found that concentrations of total polyphenols in wild leaves of wild bush tea plants were lowest in March and April (autumn), September (spring)

and highest in June and July (winter). However, data that describe the seasonal variation of tannin content, harvesting and production methods in wild bush tea leaves are lacking. The plant materials are only harvested in large-scale from the wild for medicinal and herbal tea. The objective of this study was to determine seasonal variation in the concentration of tannin in wild bush tea leaves harvested from the wild in an attempt to increase knowledge on chemical composition and relate to the best time to harvest wild bush tea. Therefore, the results of this study will be used in future to compare the extent to which different agronomic practices increase tannin content in cultivated bush tea.

## MATERIALS AND METHODS

#### Study site

The bush tea leaves were collected from Muhuyu Village ( $24^{\circ}$ N 50'E,  $31^{\circ}$ S17'E; alt 610 m; subtropical-type climate, i.e. summer rainfall, cold and dry winter). Two hundred (200) g of leaf (matured leaves i.e. 40-80 leaves from the shoot tip) on 20 plants of bush tea were randomly collected from end of each month i.e. January to December 2003. The leaves with the average moisture content of 42% were air dried in the shade. The annual average annual at this location is 650 mm per annum with temperatures ranging from 13-18°C in winter and 27-39°C in summer (Limpopo Province, South Africa). The season varies as follows i.e. autumn (March to May, winter (June to August), spring (September to November) and summer (December to February). The soil type is sandy loam with pH ranging from 5.6-7.4. The meteorological data were supplied by Provincial Department of Agriculture, Vhembe District and Limpopo Province, South Africa.

#### Assay for leaf tannin concentration

Ten (10) ml of 70% aqueous acetone was added to 0.2 g of finely ground bush tea leaves to pass a 20-mesh screen ( $\leq 1.0$  mm; Endocotts test sieves) in 25 ml glass beakers. The beakers were then suspended in an ultrasonic water bath (UMC-5, Ultrasonic manufacturing company, Pty Ltd) for 20 min to cool down at room temperature. The content of the beakers were subsequently centrifuged at 30,000 x g in a BHG Hermle Berthold Hermle centrifuge machine for 10 min. and the supernatants were stored on ice. The supernatant was carefully decanted and the extraction procedure

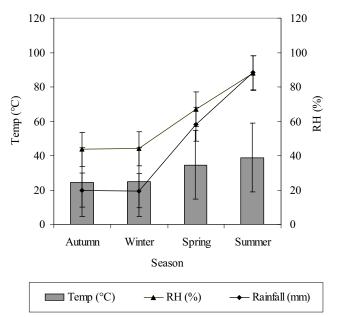
was repeated three times on residues. Three supernatants were combined and made-up a volume of 30 ml of the filtrate extracts. The residues were then discarded. The resulting suspension was assayed for condensed tannins according to the procedure descrybed by Porter *et al.* (1986), and hydrolysable tannins were determined by the method of Willis and Allen (1998).

#### Statistical analysis

Data were analyzed using GLM (General linear model) procedure of SAS version 8.0 (SAS Institute Inc., 1999). Mean separation was performed using Turkey's test procedure at 5% LSD.

#### **RESULTS AND DISCUSSION**

Presently, the seasonal variation of tannin content, harvesting and production methods in wild bush tea leaves is not well established. The results of this study attempted to increase knowledge on seasonal tannin content and relate to the best time to harvest wild bush tea. **Fig. 1** showed that the climatic conditions were unstable, with low temperature  $(24^{\circ}C)$ , rainfall (19.5 mm) and relative humidity (44%) during winter followed by gradual increase during spring and summer. The highest temperature (39.8°C), rainfall (88.3 mm) and relative humidity (88%) were during summer (**Fig. 1**).



**Fig. 1** Meteorological data with error bars of temperature (°C), relative humidity (%) and rainfall of Muhuyu Village, Vhembe District (Limpopo Province, South Africa).

Results in **Figs. 2** and **3** show that there was a differenttial seasonal variation in both condensed and hydrolysable tannin content. The highest concentration of condensed tannin was during autumn (4.8%) compared to the other seasons (**Fig. 2**), probably due to drought and low temperature stress during autumn and summer (**Fig. 1**). The differences between the highest and lowest condensed tannin content were 2.4 mg/g.

The concentration of hydrolysable tannins were lowest during summer (0.10%) compared to autumn and winter (0.14%) and spring (0.13%); **Fig. 3**).

These differences may reflect a response of drought stress (Hamilton *et al.* 2001) during the dry autumn and winter months (**Fig. 1**). Mudau *et al.* (2006) also reported that the total polyphenols were high during winter and summer in wild bush tea. However, Owour (1992) reported that the highest concentrations total polyphenols (24.1 mg/g) in black tea seedlings growing in eastern highlands of Kenya occurred during midwinter in July. Chiu (1989) reported that Poachang tea yielded best quality tea in spring follow-

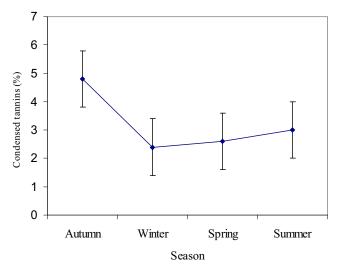


Fig. 2 Response of leaf condensed tannins ( $\pm$  standard errors) in wild bush tea to seasonal variations.

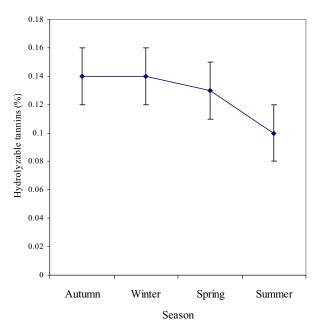


Fig. 3 Response of leaf hydrolyzable tannins ( $\pm$  standard errors) content in wild bush tea to seasonal variations.

ed by winter and autumn. Conversely, in other phenolic compounds such as caffeine and catechin content, high temperatures (38°C) and strong sunshine have been reported to produce tea with high caffeine and catechin content (Juan and Lee 1977). This was attributed to the low quality Poaching tea produced in summer characterized with astringency and bitterness taste (Chiu 1989). In a field trial conducted by Ruan *et al.* (1999) during spring and autumn, there were differential responses of total polyphenols content to seasons in green tea. However, besides seasons, other studies reported that agronomic practices such as plucking of leaves (Owour *et al.* 2000) and mineral nutrition (Owour and Obanda 1991) altered concentration of total polyphenols on sent tea.

The highest concentrations of condensed tannins were found during autumn (4.82%) compared to winter (2.44%), spring (2.66%) and summer (3.04%). The hydrolysable tannins were the lowest during winter (0.10%) compared to autumn and summer (0.14%). These data suggest that winter and autumn are the best times to harvest bush tea to maximize the tannin content of bush tea. Further research is necessary to determine the responses of agronomic practices on tannins concentration of cultivated bush tea.

#### ACKNOWLEDGEMENTS

The authors would like to acknowledge the National Research Foundation (NRF) for financial assistance.

#### REFERENCES

- Bokuchova MA, Skobeleva NI (1980) The biochemistry and technology of tea manufacture. Critical Review in Food Science and Nutrition 12, 303-370
- Chen Q, Ruan Y, Wang Y, Liu W, Zhu H (1985) Chemical evaluation of green tea taste. *Journal of Tea Science* 5, 7-17
- Chiu WTF (1989) Factors affecting the production and quality of partially fermented tea in Taiwan. Taiwan Tea Experimental Station 2, 99-102
- Fernando V, Roberts GR (1984) The effects of process parameters on seasonal development of flavour in black tea. *Journal of the Science of Food and Agriculture* 35, 71-76
- Hamilton J, Zangerl AR, DeLucia EH, Berenbaum MR (2001) The carbonnutrient hypothesis: its rise and fall. *Ecology News Letters* 4, 86-95
- Haslam E (1996) Natural polyphenols (vegetable tannins) as drug: possible mode of action. *Journal of Natural Products* 59, 205-215
- Hirasawa M, Takada K, Makimura M, Otake S (2002) Improvement of periodontal status by green tea catechin using a local delivery system: A clinical pilot study. *Alternative Medicine Review* 37, 433-438
- Hu Q, Pan G, Zhu J (2001a) Effect of selenium on green tea preservation quality and amino acid composition of tea protein. *Journal of Horticultural Science and Biotechnology* **76**, 344-346
- Hu Q, Xu J, Pan G (2001b) Effect of selenium spraying on green tea quality. Journal of the Science of Food and Agriculture 81, 1387-1390
- Juan IM, Lee SJ (1977) A study on the variation of the caffeine content according to the different seasons and parts of tea young shoot. *Taiwan Tea Experimental Station* 76, 105-106
- Mudau FN, Soundy P, du Toit ES, Olivier J (2006) Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. *South African Journal of Botany* 72, 398-402
- **Owour PO, Ng'etich, KW, Obanda M** (2000) Quality response of clonal black tea to nitrogen fertilizer, plucking interval and plucking standard. *Journal of the Science of Food and Agriculture* **80**, 439-446
- Owour PO, Othieno CO, Odhiambo HO, Ng'etich WK (1997) Effects of fer-

tilizer levels and plucking intervals of clonal tea (Camellia sinensis L.). Tropical Agriculture 74, 184-191

- **Owour PO** (1992) Changes in tea quality parameters of commercial black seedling tea due to the time of the in the Eastern Highlands of Kenya. *Food Chemistry* **45**, 119-124
- **Owour PO, Obanda M** (1991) Clonal selection criteria for quality in Kenya black tea production: achievements, problem and perspectives. *Tea Review* **19**, 49-58
- Porter LJ, Hrstich LN, Chan BG (1986) Analysis of phenolic plant secondary metabolites. *Phytochemistry* 25, 223-230
- Raun J, Wu X, Ye Y, Härdter R (1999) Effects of potassium and magnesium nutrition on the quality components of different types of tea. *Journal of the Science of Food and Agriculture* 79, 47-52
- Robert EAH, Smith RF (1963) Phenolic substances of manufactured teas. IX. Spectrophometric evaluation of tea liquors. *Journal of the Science of Food* and Agriculture 14, 689-700
- Sanderson GW, Selvendran RR (1965) The organic acids in tea plants: a study of non volatile organic acids separated by silica gel. *Journal of the Science of Food and Agriculture* **16**, 251-258
- Sas Institute Inc. (1999) User's Guide, Version 8.0. (5th Edn, Vol 2) Cary, N.C.
- Stensveld I, Tversdal S, Solvoll K (1992) Tea comsumption. Relationship to cholesterol, blood pressure, and coronary and total mortality. *Preventive Medicine* 21, 546-553
- Sud RG, Baru A (2000) Seasonal variation in theflavins, thearubigins, total colour and brightness of Kangra orthodox tea (*Camellia sinensis* (L.) O Kuntze) in Himachal Pradesh. *Journal of the Science of Food and Agriculture* 80, 1291-1299
- Taylor S, Baker D, Owuor P, Orchard J, Othieno C, Gay C (1992) A model for predicting black tea quality from the carotenoid and chlorophyll composition of fresh green tea leaf. *Journal of the Science of Food and Agriculture* 58, 185-191
- van Wyk B-E, Gericke N (2000) People Plants. A Guide to Useful Plants of Africa, Briza Publication, Pretoria, 102 pp
- Venkatesan S, Murugesan S, Ganapathy MNK, Verma DP (2004) Long-term impact of nitrogen and potassium fertilizers on yield, soil nutrients and biochemical parameters of tea. *Journal of the Science of Food and Agriculture* 84, 1939-1944
- Willis RB, Allen PR (1998) Improved methods for measuring hydrolysable tannins using potassium iodate. *Analyst* 123, 435-439