Effects of Foliar Spray Application of Selected Micronutrients on the Quality of Bush Tea

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Abstract. Bush tea (*Athrixia phylicoides* DC.) is a herbal beverage and medicinal plant indigenous to South Africa. The aim of the study was to provide the baseline for foliar spray application of micronutrients to enhance the quality of bush tea. A trial was conducted to determine the effect of micronutrients on the quality of bush tea. Four separate trials for boron (B), iron (Fe), zinc (Zn), and copper (Cu) were laid out in a completely randomized block design. Treatments consisted of an unsprayed control and single foliar sprays of B, Fe, Zn, and Cu, with 10 replicates. The levels of each element were unsprayed control, 50 mL·L⁻¹, 100 mL·L⁻¹, and 150 mL·L⁻¹. Parameters recorded were leaf tissue B, Fe, Zn, and Cu, and total polyphenol, total antioxidant, total flavonoids, and total tannin contents. Results from this study demonstrated that foliar application of B did not induce significant response in terms of total polyphenol content after B application. There was a quadratic response for total flavonoids (167 mg·g⁻¹), with most of the total flavonoids reaching maximum at 100 mL·L⁻¹. Foliar application of Fe exhibited a quadratic response, with most of the total polyphenols (45.1 mg·g⁻¹) reaching maximum at 100 mL·L⁻¹. All treatments showed a linear response for total antioxidant, total flavonoids, and total tannin contents. Treatments elicited a quadratic response for total polyphenols (70.6 mg·g⁻¹), total antioxidants (78.3 mg·g⁻¹), total flavonoids (148.9 mg·g⁻¹), and total tannin contents (78.3 mg·g⁻¹) after foliar Zn application, reaching maximum at 100 mL·L⁻¹. Foliar application of essential elements in bush tea led to a significant increase in the Zn, Fe, Cu, and B content; application at 100 mL·L⁻¹ is recommended for improved chemical composition of bush tea. A further trial with treatment combinations is required to determine chemical responses of bush tea.

Traditionally, bush tea has been used for the treatment of various ailments such as boils, acne, infected wounds, cuts, headaches, colds, loss of voice and throat infection (Mudau et al., 2006; Nchabeleng et al., 2013), hypertension, heart disease, and diabetes (Nchabeleng et al., 2013). The extracts from soaked roots and leaves are reportedly used as an anthelmintic by the VhaVenda people (Mbambezi, 2005). It is also used for cleansing or purifying blood (Joubert et al., 2008; Roberts, 1990). Bush tea contains 5-hydroxy-6,7,8,3',4',5'-hexamethoxy-flavan-3-ol (Mashimbye et al., 2006), 3-O-demethylgigicitrin, 5,6,7,8,3',4'-hexamethoxyflavone and quercetin, polyphenols

(Mudau et al., 2007a), tannins (Mudau et al., 2007b), and antioxidants (Mogotlane et al., 2007). Tea flush has been known to be followed by carbohydrate accumulation reserves, which are channeled toward polyphenol production (Roberts, 1990). During tea flush, total polyphenols range from 20% to 35%. The clone being examined, soil type, leaf age, agronomic practices, and climatic conditions determine the chemical composition of the tea leaves (Hartmann et al., 1996).

Antioxidant content is associated with those compounds which are able to protect biological systems against the harmful effects of reactions of extreme oxidation, which involve nitrogen and oxygen species reaction (Mogotlane et al., 2007). Thus, to an extent, altering chemical compositions will also have an impact on compounds that facilitate the scavenging ability of reactive oxygen species.

Generally, microelements such as Zn, aluminium (Al), Cu, and B are key components in many biological compounds, and

play a significant role in increasing photosynthesis of the crop (Ibrahim et al., 2011) and the production of secondary metabolites (Ibrahim and Hawa, 2013). Previous studies showed that agronomic practices such as the application of macroelements such as nitrogen (N), phosphorus (P), and potassium (K) (Mudau et al., 2007a), pruning, and drying methods are cultural practices which influence the quality of bush tea.

Herbal tea quality is measured by metabolites such as tea polyphenols. Polyphenol-rich diets have been linked with prevention of several chronic and degenerative diseases (Neilson and Ferruzzi, 2011), including cancer (Neuhouser, 2004), cardiovascular disorders (Ding et al., 2006), obesity, and diabetes (Nagao et al., 2009; Thielecke and Boschmann, 2009), including neurodegenerative disorders (Mandel et al., 2005). Polyphenols are extensively altered during first-pass metabolism such that, the resulting metabolites are conjugates (e.g., sulfates and glucuronates) of the parent aglycone or conjugates of methylated parent aglycones (Barbosa, 2007). Tannins on the other hand are also involved in the formation of polyphenols and their astringent nature impacts on the taste of tea (Arbenz and Avérous, 2016), while the flavonoids indirectly influence antioxidant activity (Tan et al., 2016). However, micronutrients are involved in the metabolism by way of precursors from both the shikimate and the acetate-malonate pathways (Crozier et al., 2000; Urquiaga, and Leighton, 2000). In this study, the indirect effect of micronutrient foliar application on polyphenols, tannins, flavonoids, and antioxidants is that linked to tea quality. However, data that describe the effect of microelements on growth, productivity, and chemical composition of bush tea are lacking. Therefore, the aim of the study was to determine the effect of selected microelements (B, Fe, Zn, and Cu) on the quality of bush tea. The study was intended to provide the baseline for the foliar spray application of micronutrients to enhance quality of bush tea.

Materials and Methods

Experimental site. The study was conducted at the Science Campus of the University of South Africa, latitude S26°9.501 and longitude E27°54.113, during the 2013/2014 Summer and Winter seasons. The site is characterized by a humid subtropical climate (hot, usually humid summers, and mild to cool winters). Minimum temperatures of 13.1 to 15.0 °C and maximum temperatures of 29.1 to 32.1 °C, with average rainfall of 6 to 63 mm and 25 to 100 mm over the two seasons were recorded.

Experimental design and treatment details. Bush tea was planted on 10 Sept. 2013 according to the method described by Mudau et al. (2007b). A month thereafter, the plants were subjected to four separate foliar trials for B, Fe, Zn, and Cu. The levels of each micro-nutrient were 0 mL·L⁻¹ (unsprayed control), 50 mL·L⁻¹, 100 mL·L⁻¹, and 150 mL·L⁻¹

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laid in a randomized complete block design, sprayed every 2 weeks, with each treatment replicated 10 times. Fertilizer sources included ZnSO_4 , CuSO_4 , FeSO_4 , and $\text{Na}_2\text{B}_8\text{O}_{13}\cdot 4\text{H}_2\text{O}$ for Zn, Cu, Fe, and B, respectively, due to high content of selected microelements as these are used by farmers and readily available commercially. The macronutrient (N, P, and K) fertilizers were applied according to the recommended rates described by Mudau et al. (2007b), with slight modification by applying them in split twice for 2 months. At harvest, plants were freeze dried for leaf tissue analysis, and the measurement of total polyphenol, total antioxidant, total flavonoids, and tannin content.

Microelement analysis. Nutrients were analyzed using the method described by Zasoski and Burau (1977). One gram (1 g) of each sample was weighed and 3.5 mL of concentrated nitric acid was added. The sample was then placed on a heating block at 80 to 90 °C for an hour. After cooling, 1.5 mL of perchloric acid (70% to 72%) was added, and the sample was then placed on a heating block set at 180 to 200 °C until the denseness of the white fumes dissipated. Distilled water was then added to make a volume of 12.5 mL, and vortexed. After digestion, standards containing 5% perchloric acid were used as comparison in measuring the absorbance at a range of 360 to 460 nm.

Chemical composition. About 15 g of finely ground material was sieved (≤ 1.0 mm;

Endecotts test sieves) for 5 min. Acetone (5 mL) acetone was added to a 0.5 g sample of the sieved material and mixed for 2 h in a shaker, and then centrifuged for 5 min at 4000 rpm. The supernatant was carefully decanted and the extraction procedure was repeated three times on residues. Three supernatants were combined and a volume of 15 mL with 75% acetone was prepared. The residues were discarded.

Determination of antioxidant activity. Antioxidant activity of extracts was determined using Trolox equivalent antioxidant capacity (TEAC) assay as described by Awika et al. (2004). The chosen assay, TEAC, is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the 2, 2'-azino-bis(3-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 an 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 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 bush tea

methanol extracts, then 100 μL of Trolox in a test tube and mixed. Absorbance readings (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 a dry weight (DW) basis.

Determination of polyphenol content. Methanol extracts were used for the determination of total phenols. Duplicates of 2 g of bush tea were extracted using 30 mL of methanol. Ten milliliters of methanol were added to 2 g of sample in centrifuge tubes and vortexed every 10 min for 2 h to improve extraction efficiency. Samples were centrifuged at 3500 rpm for 10 min (25 °C) and decanted. Each sample residue was rinsed twice with 10 mL of solvent, vortexed for 5 min, centrifuged as above, and decanted. The two supernatants were pooled before being used for analysis. The Folin–Ciocalteu method (Singleton and Rossi, 1965), modified by Waterman and Mole (1994), was used to determine total phenols in all selected bush tea extracts. This method is 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.

Methanol extracts (0.5 mL) were added to a 50-mL volumetric flask containing distilled water and mixed. Folin–Ciocalteu phenol reagent (2.5 mL) was added and mixed, followed by 7.5 mL sodium carbonate solution

Fig. 1. Micronutrient content in the respective nutrient treatments of (A) boron (B), (B) iron (Fe), (C) zinc (Zn), and (D) copper (Cu).

Table 1. Response in terms of chemical composition to foliar spray applications of trace elements to bush tea leaves.

Treatment applied (mL·L ⁻¹)	Total polyphenol contents (mg·g ⁻¹)	Total antioxidant contents (mg·g ⁻¹)	Total flavonoids (mg·g ⁻¹)	Total tannin contents (mg·g ⁻¹)
<i>Foliar application of boron</i>				
Control	20.2	30.2	50.4	30.2
50	78.7	77.4	144.8	77.4
100	66.3	88.7	167.4	88.7
150	76.3	89.4	155.7	89.4
Response	NS	L*	Q*	L*
<i>Foliar application of iron</i>				
Control	36.4	36.4	72.8	36.4
50	38.0	54.4	99.5	54.4
100	45.1	67.0	102.2	67.7
150	35.2	89.0	123.2	89.3
Response	Q*	L*	L*	L*
<i>Foliar application of zinc</i>				
Control	34.3	34.3	68.6	34.3
50	60.3	98.5	158.8	98.5
100	70.6	78.3	148.9	78.3
150	69.7	34.3	104.0	34.3
Response	Q*	Q*	Q*	Q*
<i>Foliar application of copper</i>				
Control	41.1	41.1	82.2	41.1
50	68.4	89.3	157.7	89.3
100	76.5	97.4	163.3	87.4
150	67.4	87.3	154.7	87.3
Response	Q*	Q*	Q*	Q*

Linear (L*) or quadratic (Q*) effect at 1% level of significance of foliar application of microelements. NS = nonsignificant.

(20 g/100 mL) within 1 to 8 min after addition of the Folin–Ciocalteu phenol reagent. The contents were mixed and the flask was made up to volume with distilled water and thoroughly mixed. Absorbance of the reactants was read after 2 h at 760 nm using an ultraviolet-visible GENESYS 20 spectrophotometer (Germany). Catechin was used as a standard to formulate a standard curve, and results expressed as mg equivalents/100 mg of sample on a DW basis.

Determination of total flavonoids. The total flavonoids were measured using a modified colorimetric method described by Yoo et al. (2008). One milliliter of the extracts or standard solutions of catechin was added to a 10-mL volumetric flask. Distilled water was added to make a volume of 5 mL. At zero time, 0.3 mL of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 mL of 10% (w/v) AlCl₃ was added. Six minutes later, 2 mL of 1 M NaOH was also added to the mixture, followed by 2.1 mL of distilled water. The absorbance was measured against the blank at 510 nm after 15 min. The standard curve was prepared using different concentrations of catechin. Total flavonoids were expressed as mg catechin equivalents (CE) per 100 g of DW.

Fig. 2. Correlation and regression between (A) total polyphenols, (B) antioxidants, (C) flavonoids, and (D) tannins with leaf tissue boron.

Determination of tannin content. The vanillin HCl method of Prince et al. (1978) was used for the determination of tannins. This method is based on the ability of flavonoids to react with vanillin in the presence of mineral acids to produce a red color, which is measured spectrophotometrically. The extracts and reagents were maintained at 30 °C in a thermostat-controlled water bath before the reactants were mixed. The methanolic extracts (1 mL) were added to 5 mL vanillin reagent (4% HCl in methanol and 0.5 mL vanillin in methanol), then mixed. Sample blanks were prepared with 4% HCl in methanol replacing the vanillin reagent. The reactants were maintained at 30 °C and absorbance was read at 500 nm after 20 min. Absorbance readings of the blanks were subtracted from those of the samples. Catechin was used as a standard, and results were expressed as mg CE/100 mg sample on a DW basis.

Statistical analysis. Data were subjected to analysis of variance using the PROC GLM (General Linear Model) procedure of SAS version 8.0. Treatments, sum of squares were partitioned into linear and quadratic polynomial correlation in all microelements, and total polyphenol, total antioxidant, total

flavonoids, and total tannin contents were measured.

Results

Results in Fig. 1A showed a linear response in bush tea leaf B content with increasing foliar application levels. The highest B content was observed at 100 mL·L⁻¹ foliar sprays, reaching 586.3 mg·kg⁻¹. The difference between the highest and the lowest was 1183 mg·kg⁻¹. Leaf Fe and Zn content as shown in Fig. 1B and C showed quadratic responses to foliar application levels, respectively, both reaching a plateau at 100 mL·L⁻¹. Fig. 1D shows significant linear response to foliar Cu applied, with a highest Cu leaf content of 55.2 mg·kg⁻¹.

Not all treatments elicited a significant response in terms of total polyphenol content after B application (Table 1). However, there was a linear response after foliar spray application of B in terms of total antioxidants, with most of the antioxidant response to foliar B occurring between the unsprayed control and 50 mL·L⁻¹. Furthermore, there was a quadratic response in terms of total flavonoids (167 mg·g⁻¹); most of the total flavonoids response occurred between 0 and

50 mL·L⁻¹, but maximum was reached at 100 mL·L⁻¹. There was a linear response in terms of total tannins in bush tea leaves after foliar spray application of B, with most of the responses occurring between 0 and 50 mL·L⁻¹.

Foliar application of Fe elicited a quadratic response reaching maximum at 100 mL·L⁻¹, with most of the total polyphenols (45.1 mg·g⁻¹) occurring between 0 and 100 mg·g⁻¹ (Table 1). All treatments elicited a linear response in terms of total antioxidant, total flavonoids, and total tannin contents after Fe foliar application. There was a quadratic response in terms of total polyphenol (70.6 mg·g⁻¹), total antioxidant (78.3 mg·g⁻¹), total flavonoids (148.9 mg·g⁻¹), and total tannin contents (78.3 mg·g⁻¹) after foliar Zn application, reaching maximum at 100 mL·L⁻¹. The highest contents occurred between 0 and 50 mL·L⁻¹ (Table 1). With the application of Cu, all treatments elicited a quadratic response, reaching maximum at 100 mL·L⁻¹. The total polyphenols were 76.5 mg·g⁻¹, total antioxidant content was 97.4 mg·g⁻¹, total flavonoids were 163.3 mg·g⁻¹, and total tannin content was 87.4 mg·g⁻¹. With the application of B, no significant response was observed in terms of total polyphenol content, with the maximum reaching 78.7 mg·g⁻¹ at an application

Fig. 3. Correlation and regression between (A) total polyphenols, (B) antioxidants, (C) flavonoids, and (D) tannins with leaf tissue iron (Fe).

of 50 mL·L⁻¹. Linear responses were observed in terms of tannin and antioxidant content, with a maximum level of 89.4 mg·g⁻¹ at 150 mL·L⁻¹ for both parameters. A quadratic response was seen in the case of total flavonoids, and the maximum level of 167.4 mg·g⁻¹ was observed at 100 mL·L⁻¹. Results in Fig. 2 also demonstrated a linear relationship between leaf tissue B and total polyphenol (2A), total antioxidant (2B), total flavonoids (2C), as well as total tannin content (2D) of bush tea. No linear relationships were observed between leaf tissue Fe and all variables measured (Fig. 3A–D). There was no relationship between foliar application of Cu and all variables measured (Fig. 4A–D). There was a clear relationship between leaf tissue Zn and all chemical compositions recorded (Fig. 5B–D), with the exception of total polyphenols (Fig. 5A).

Discussion

Foliar application of essential elements led to a significant increase in the Zn, Fe, Cu, and B content of bush tea. The results of the study conducted indicated that 50 mL·L⁻¹ to 100 mL·L⁻¹ were sufficient range applications for most of the foliar elements applied.

Increases in the various elements within the plants significantly improved the chemical compositions. Fertilization with Zn and Fe beyond 100 mL·L⁻¹ did not improve the leaf concentrations of the microelements, whereas plants fertilized with B and Cu displayed a linear concentration response at such levels. Manenji et al. (2015) reported that foliar spray of 1% to 2% ZnSO₄ increased nitrate reductase activity, and resulted in a 15% to 20% increase in N and protein content in tea shoots.

Nadim et al. (2012) reported that B concentrations (2.68–37.54 mg·kg⁻¹) increased photosynthesis and the formation of chlorophyll in the leaf tips and leaves. However, in most crop plants, the range between deficient and toxic levels of some micronutrients is narrow (Mengel and Kirkby, 1982; Ngezimana and Agenbag, 2014; Yu and Rengel, 1999), making management critical. In *Camellia sinensis*, total contents of Cu, Fe, Mn, and Zn were found to vary depending on several aspects, including the age of the tea leaves, soil conditions, rainfall, and altitude (Street et al., 2006).

In addition to the functions they fulfil within the plant, the various microelements

also have both curative and preventive properties in terms of combating diseases within the human body. It was possible to ascertain from the study that applying foliar micronutrients to bush tea has a significantly positive effect on the levels of microelements within the plant, and hence on their availability through tea infusions. According to various researchers, herbal tea products contain high concentration of these micronutrients (Bello et al., 2004; Chizzola and Franz, 1996; Lavilla et al., 1999; Nookabkaew et al., 2006).

Herbal teas contain a wide range of polyphenols (Owour et al., 2000), which have favorable biochemical and physiological effects on human health (Hirasawa et al., 2002). Applications of the various micronutrients had an effect on the nutrient concentrations within bush tea plants, and also exerted an influence on levels of total polyphenols, tannins, and total flavonoids. The health benefits of herbal tea are related to antioxidant content (Mogotlane et al., 2007), which is accounted for by the various polyphenols, tannins, and flavonols (Nchabeleng et al., 2013). Tea flush has been known to be followed by an accumulation of carbohydrate reserves, which are

Fig. 4. Correlation and regression between (A) total polyphenols, (B) antioxidants, (C) flavonoids, and (D) tannins with leaf tissue copper (Cu).

Fig. 5. Correlation and regression between (A) total polyphenols, (B) antioxidants, (C) flavonoids, and (D) tannins with leaf tissue zinc (Zn).

then channeled toward the production of polyphenols (Roberts, 1990).

In the study, responses in terms of total polyphenols, tannins, and total flavonoids to the various foliar fertilizer application treatments were varied. Levels of total polyphenols, tannins, and total flavonoids tended to increase with increasing levels of some macronutrients. In studies of *C. sinensis*, tea quality tended to improve with fertilization with microelements (Manenji et al., 2015; Njogu et al., 2014). Venkatesan et al. (2005) also reported a high and significant positive correlation between polyphenol content and Zn levels of mature leaves of tea. Zn and Cu in tea leaves have been reported to increase the levels of polyphenol oxidase and flavins. These elements are important in the formation of theaflavins and thearubins during fermentation of black tea; this could explain their significant effect on quality. Moreover, they are vital components of polyphenol oxidase. A positive correlation between total phenol content and Cu was found in samples of Nigerian tea; this improves tea quality (Ogunmoyela et al., 1994).

Significantly high tannin and total flavonoids concentrations manifested in bush tea fertilized with Cu. In bush tea leaves, the

most common important chemicals are flavonols (Ivanova et al., 2004). Mashimbye et al. (2006) found that 5-hydroxy-6,7,8,3',4,5'-hexamethoxy-flavan-3-ol is a major flavonoid in bush tea. Flavonoids are known for their growth inhibition activities, cytotoxicity activities, and cyclic adenosine monophosphate diphosphoesterase reserve activities (Mashimbye et al., 2006). In herbal teas, flavonoids are potential quality indicators, since in nature they are antioxidants (Mudau et al., 2007b).

In conclusion, micronutrient foliar applications influenced the growth and quality of bush tea. Response to the various foliar nutrients varies with foliar nutrient and application levels. The results of this study suggest that foliar application of B did not elicit a significant response in terms of total polyphenol content. Total flavonoids reached maximum at 100 mL.L⁻¹. As a foliar application, Fe elicited a quadratic response reaching maximum at 100 mL.L⁻¹ with most of the total polyphenols (45.1 mg.g⁻¹). With foliar applications of Zn and Cu, total polyphenol, total antioxidant, total flavonoids, and total tannin contents of bush tea reached maximum at 100 mL.L⁻¹. For maximum chemical composition of bush tea, foliar applications

of the microelements B, Fe, Zn, and Cu at a foliar spray rate of 100 mL.L⁻¹ are recommended. A further trial with treatment combinations is required to determine chemical responses of bush tea.

Literature Cited

- Arbenz, A. and L. Avérous. 2016. Tannins: A resource to elaborate aromatic and biobased polymers, p. 97–148. In: S. Kalia and L. Avérous (eds.). Biodegradable and biobased polymers for environmental and biomedical applications. Scrivener Publishing, Salem, MA.
- Awika, J.M., L.W. Rooney, and R.D. Waniska. 2004. Anthocyanins from black sorghum and their antioxidant properties. Food Chem. 90:293–301.
- Barbosa, D.S. 2007. Green tea polyphenolic compounds and human health. J. Verbraucherschutz Lebensmittelsicherheit 2:407–413.
- Bello, M.O., A.O. Ibrahim, I.A. Ogunwande, and N.O. Olawore. 2004. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chem. 85:67–71.
- Chizzola, R. and C.H. Franz. 1996. Metallic trace elements in medicinal and aromatic plants from Austria. J. Appl. Biol. 70:52–56.
- Crozier, A., J. Burns, A.A. Aziz, A.J. Stewart, H.S. Rabiasz, G.I. Jenkins, C.A. Edwards, and M.E.J. Lean. 2000. Antioxidant flavonols from fruits, vegetables and beverages: Measurements and bioavailability. Biol. Res. 33:79–88.

- Ding, E.L., S.M. Hutfless, X. Ding, and S. Girotra. 2006. Chocolate and prevention of cardiovascular disease: A systematic review. *Nutr. Metab.* 3:1.
- Hahn, D.H., L.W. Rooney, and C.F. Earp. 1984. Tannins and phenols of sorghum. *Cereal Foods World* 29:776–779.
- Hartmann, H.T., D.E. Kester, F.T. Davies, Jr., and L.R. Geneve. 1996. *Plant propagation: Principles and practices*. 6th ed. Prentice-Hall, Englewood Cliffs, NJ.
- Hirasawa, M., K. Takada, M. Makimura, and S. Otake. 2002. Improvement of periodontal status by green tea catechin using a local delivery system: A clinical pilot study. *Altern. Med. Rev.* 37:433–438.
- Ibrahim, A.K., A. Usman, B. Abubakar, and U.H. Aminu. 2011. Extractable micronutrients status in relation to other soil properties in Billiri local government area. *J. Soil Sci. Environ. Mgt.* 3:282–285.
- Ibrahim, M.H. and Z.E.J. Hawa. 2013. Absciscic acid induced changes in production of primary and secondary metabolites, photosynthetic capacity, antioxidant capability, antioxidant enzymes and lipoxygenase inhibitory activity of *Orthosiphon stamineus* Benth. *Molecules* 18: 7957–7976.
- Ivanova, D., D. Gerova, T. Chervenkov, and T. Yankova. 2004. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J. Ethnopharmacol.* 96:145–150.
- Joubert, E., W.C. Gelderblom, A. Louw, and D. De Beer. 2008. South African herbal teas: *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylicoides*: A review. *J. Ethnopharmacol.* 119: 376–412.
- Lavilla, I., A.V. Filgueiras, and C. Bendicho. 1999. Comparison of digestion methods for determination of trace and minor metals in plant samples. *J. Agr. Food Chem.* 47:72–77.
- Mandel, S.A., Y. Avramovich-Tirosh, L. Reznichenko, H.L. Zheng, and O. Weinreb. 2005. Multifunctional activities of green tea catechins in neuroprotection: Modulation of cell survival genes, iron-dependent oxidative stress and PKC signaling pathway. *Neurosignals* 14: 46–60.
- Manenji, B.T., M. Chandiposha, and A. Mashingaidze. 2015. Effects of foliar fertilisers containing magnesium, copper and zinc on yield and quality of mature tea. *Scholars J. Agr. Vet. Sci.* 2:256–261.
- Mashimbye, M.J., F.N. Mudau, P. Soundy, and T. Van Ree. 2006. A new flavonol from *Athrixia phylicoides* (bush tea). *S. J. Chem.* 59:1–2.
- Mbambezeli, G. 2005. *Athrixia phylicoides*. South African National Biodiversity Institute, Pretoria, South Africa.
- Mengel, K.K. and E.E. Kirkby. 1982. *Principles of plant nutrition*. Alemania International Potash Institute, Bern, Switzerland.
- Mogotlane, I.D., F.N. Mudau, P.W. Mashele, and P. Soundy. 2007. Seasonal responses of total antioxidant contents in cultivated bush tea (*Athrixia phylicoides* DC.) leaves to fertiliser rates. *Med. Arom. Plant Sci. Biotechnol.* 1:77–79.
- Mudau, F.N., A. Ngele, P.W. Mashele, and P. Soundy. 2007b. Seasonal variation of tannin contents in wild bush tea (*Athrixia phylicoides* DC). *Med. Arom. Plant Sci. Biotechnol.* 1:74–76.
- Mudau, F.N., H.T. Araya, E.S. Du Toit, P. Soundy, and J. Olivier. 2007a. Bush tea (*Athrixia phylicoides* DC.) as an alternative herbal and medicinal plant in Southern Africa: Opportunity for commercialisation. *Med. Arom. Plant Sci. Biotechnol.* 1:70–73.
- Mudau, F.N., P. Soundy, E.S. Du Toit, and J. Olivier. 2006. Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. *S. J. Bot.* 72:398–402.
- Nadim, M.A., I.U. Awan, M.S. Baloch, E.A. Khan, K. Naveed, and M.A. Khan. 2012. Response of wheat (*Triticum aestivum* L.) to different micronutrients and their application methods. *J. Anim. Sci.* 22:113–119.
- Nagao, T., S. Meguro, T. Hase, K. Otsuka, and M. Komikado. 2009. A catechin-rich beverage improves obesity and blood glucose control in patients with type 2 diabetes. *Obesity* 17:310–317.
- Nchabeleng, L., F.N. Mudau, and I.K. Mariga. 2013. Effects of chemical composition of wild bush tea (*Athrixia phylicoides* DC.) growing at locations differing in altitude, climate and edaphic factors. *J. Med. Plants Res.* 6:62–66.
- Neilson, A.P. and M.G. Ferruzzi. 2011. Influence of formulation and processing on absorption and metabolism of flavan-3-ols from tea and cocoa. *Annu. Rev. Food Sci. Technol.* 2:125–151.
- Neuhouser, M.L. 2004. Flavonoids and cancer prevention: What is the evidence in humans? *Pharm. Biol.* 42:36–45.
- Ngezimana, W. and G.A. Agenbag. 2014. Nitrogen and sulfur effects on macro- and micronutrient content in canola (*Brassica napus* L.) grown on acidic soils of the Western Cape province of South Africa. *Commun. Soil Sci. Plant Anal.* 45:1840–1851.
- Njogu, R.N.E., D.K. Kariuki, D.M. Kamau, and F.N. Wachira. 2014. Effects of foliar fertilizer application on quality of tea (*Camellia sinensis* L.) grown in the Kenyan highlands. *Amer. J. Plant Sci.* 5:2707–2715.
- Nookabkaew, S., N. Rangkadilok, and J. Satayavivad. 2006. Determination of trace elements in herbal tea products and their infusions consumed in Thailand. *J. Agr. Food Chem.* 54:39–44.
- Ogunmoyela, O.A., C.R. Obantu, and M.T. Adetunji. 1994. Effect of soil micronutrient status on the fermentation characteristics and organoleptic quality of Nigerian tea. *Afr. Crop Sci. J.* 1:87–92.
- Owour, P.O., K.W. Ng'etich, and M. Obanda. 2000. Quality response of clonal black tea to nitrogen fertilizer, plucking interval and plucking standard. *J. Sci. Food Agr.* 70:47–52.
- Prince, M.L., S. Van Scoyoc, and L.G. Butler. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agr. Food Chem.* 26:1214–1218.
- Roberts, M. 1990. *Indigenous healing plants*, p. 56–57. 1st ed. Southern Book Publishers, Halfway House, South Africa.
- Singleton, V.L. and J.A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Amer. J. Enol. Viticult.* 16:144–158.
- Statistical Analysis System Institute Inc. 2012. *The statistical procedure manual*, version 8.0. SAS publishing, Cary, NC.
- Street, R., J. Szakova, O. Drabek, and L. Mladkova. 2006. The status of micronutrients (Cu, Fe, Mn and Zn) in tea and tea infusions in selected samples imported to the Czech Republic. *J. Sci. Food Czech* 24:62–71.
- Tan, L.H., D. Zhang, G. Wang, B. Yu, S.P. Zhao, J.W. Wang, L. Yao, and W.G. Cao. 2016. Comparative analyses of flavonoids compositions and antioxidant activities of Hawk tea from six botanical origins. *Ind. Crops Prod.* 80:123–130.
- Thielecke, F., and M. Boschmann. 2009. The potential role of green tea catechins in the prevention of the metabolic syndrome—a review. *Phytochem.* 70:11–24.
- Urquiaga, I.N.E.S. and F. Leighton. 2000. Plant polyphenol antioxidants and oxidative stress. *Biol. Res.* 33:55–64.
- Venkatesan, S., S. Murugesan, V.K. Senthur Pandian, and M.N.K. Ganapathy. 2005. Impact of sources and doses of potassium on biochemical and green leaf parameters of tea. *Food Chem.* 90:535–539.
- Waterman, P.G. and S. Mole. 1994. *Analysis of phenolic plant metabolites*. Blackwell Scientific Publications, Oxford, UK.
- Yoo, K.M., C.H. Lee, H. Lee, B. Moon, and C.Y. Lee. 2008. Relative antioxidant and cytoprotective activities of common herbs. *Food Chem.* 106:92–96.
- Yu, Q. and Z. Rengel. 1999. Micronutrient deficiency influences plant growth and activities of superoxide dismutases in narrow-leafed lupins. *Ann. Bot. (Lond.)* 83:175–182.
- Zasoski, R.J. and R.G. Burau. 1977. A rapid nitric-perchloric acid digestion method for multi-element tissue analysis. *Commun. Soil Sci. Plant Anal.* 8:425–436.