

RESPONSE OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF BUSH TEA AND SPECIAL TEA USING DIFFERENT SELECTED EXTRACTION SOLVENTS

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ABSTRACT

The positive health benefits associated with tea are made possible by the antioxidant activity of phenolic compounds present in tea. The total phenolic content and antioxidant activity of bush tea (*Athrixia phylicoides* DC.) and special tea (*Monsonia burkeana*) were studied. The extractions were done in triplicate using cold water (5, 10 and 15 minutes), hot water (5, 10 and 15 minutes), 95% acetone, 95% methanol and 95% hexane. The experiments were arranged in a complete randomised design (CRD), and samples were extracted in triplicates in each treatment. Results demonstrated that total phenolic content ranged from 2.83 mg GAE / 100 g to 4.93 mg GAE / 100 g for bush tea and 2.60 mg GAE / 100 g to 6.24 mg GAE / 100 g for special tea. The ABTS antioxidant activity ranged from 34.41 $\mu\text{mol TE / g}$ to 58.34 $\mu\text{mol TE / g}$ in bush tea and 86.49 $\mu\text{mol TE / g}$ to 197.46 $\mu\text{mol TE / g}$ in special tea. The DPPH antioxidant activity in bush tea ranged from 59.87 $\mu\text{mol TE / g}$ to 77.17 $\mu\text{mol TE / g}$ and in special tea from 93.78 $\mu\text{mol TE / g}$ to 109.13 $\mu\text{mol TE / g}$. Special tea had significantly higher total phenols and antioxidant activity ($p < 0.05$) than bush tea. Hot water extractions were found to be comparable to chemical solvent extractions. This suggests that the potential benefits associated with consumption of beverages containing phenolic compounds can be expected from the consumption of these teas.

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BACKGROUND

There is increasing epidemiological evidence that suggests the role of dietary antioxidants (including phenolic compounds), *in vivo*, in the prevention of several chronic and degenerative diseases such as cardiovascular disease, cancer, diabetes and Alzheimer's disease (Zielinski & Kozłowska, 2000; Adom & Lui, 2002; Willcox et al., 2004; Zhou et al., 2004; Moore et al., 2005). These benefits are made possible by the *in vivo* antioxidant capacity of phenolic compounds.

Black, green, oolong and pu-erh teas which are available in the consumer market worldwide have differing antioxidant activities as a result of differing antioxidant compounds contents and types (Yashin et al., 2011). Polyphenolic compounds identified in these teas included catechins (epigallocatechin, epicatechin, epigallocatechin, catechin and epicatechin gallate) and theaflavins (theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, theaflavin-

3,3'-digallate and caffeine). Tea leaves have been reported to be a source of beneficial antioxidants (Mudau et al., 2007a) and that further processing such as drying and extraction methods could be manipulated to obtain tea with high amount of the antioxidants (Baruah et al., 2012).

Bush tea and special tea are indigenous to Southern Africa (Venter, 1979; Herman et al., 2000) with both teas prevalent in informal markets. *Athrixia phylicoides* (DC.), locally referred to as bush tea, belongs to the family Asteraceae, tribe Inucleae and subtribe Athrixiinae (Mudau et al., 2007b). In appearance, it has been described as an aromatic (McGaw et al., 2013), Asteraceaeous shrub that has thin woolly stems, dark green pointed leaves and daisy-like flowers (Robert, 1990) that appear purple to pink (Van Wyk and Gericke, 2000).

Bush tea has traditionally been used for treatment of various ailments such as boils, sores, acne, infected wounds, cuts, headaches, colds, loss of voice and throat infection (Roberts, 1990; Hutchings et al., 1996). The Vhavenda people are reported to use extracts from soaked roots and leaves as an antihelminthic (Mabogo, 1990; Mbambezeli, 2005) and also used the tea for cleansing or purifying the blood (Roberts, 1990). *Monsonia burkeana* Planch. ex Harv. (Family Geraniaceae), is indigenous to Southern Africa (Venter, 1979) and is locally referred to as special tea.

Special tea has been reported to be used in combination with other herbs as a blood cleanser. Traditionally men use the special tea plant to cure sexually transmitted diseases and erectile disorders or enhance male libido (Mamphiswana et al., 2011). In previous studies, both bush and special tea have been found to possess antimicrobial properties (Tshivhandekano et al., 2014).

South Africa has a rich heritage of indigenous herbal teas that are already in the local market such as honeybush tea, buchu tea and rooibos tea. These herbal teas have significantly contributed in the generation of income, through the exports and job opportunity. There is growing evidence on the health benefits of these teas that is attracting consumers worldwide. Currently, research into the health benefits of both bush and special tea research has made

use of different extraction solvents. This generally provide inconsistent results to the consumers hence, the main objective of this study was to determine the total polyphenol content and antioxidant activity of bush and special tea using different extracting solvents and regimes, with a view to identifying and suggesting the best combination of solvent and regime for optimal extraction of phenolic compounds and antioxidant activity.

MATERIALS AND METHODS

The leaves and twigs of both plants were collected from the veld where they are currently growing. Bush tea (*Athrixia phylicoides*) was collected in the Khalavha village area (-22.922985:30.280568) in the province of Limpopo, and special tea (*Monsonia burkeana*) was collected in the Brits area (-25.596806:27.804566) in the province of North West. The plant materials were allowed to dry in the shade at ambient temperature (Mudau and Ngezimana, 2014) before being ground with a commercial coffee grinder.

SAMPLE EXTRACTION

Extraction solvents used included cold water (23°C), hot water (107°C), 95% acetone, 95% methanol and 95% hexane arranged in complete randomised design replicated three times, each replicate done in triplicates. Cold water extracts were prepared by extracting 5 g of ground sample with cold water at ambient temperature (23 °C) for 5, 10 and 15 min with continuous stirring using a magnetic stirrer. Once the determined time had elapsed, the sample was allowed to stand at room temperature for 30 min. Once the tea particles had settled, the supernatant was removed and retained. The sample was then extracted twice more by repeating the extraction step for 2 min and allowing for separation as above. The supernatants were combined and filtered through a Whatman No 1 filter paper (Mudau et al., 2006). Supernatant collected was combined and stored at below 4 °C until analysed. The analysis was conducted within 7 days.

Hot water extracts were prepared by extracting 5 g of ground sample with boiling water for 5, 10 and 15 min by continuous boiling at 107 °C for the desired time. Once the determined time had elapsed, the sample was allowed to stand and cool at room temperature (23 °C) for 30 min. Once the tea particles had settled, the

supernatant was removed and retained. The sample was extracted twice more by repeating the extraction step for 2 minutes and allowing for separation as above. The supernatants were combined and filtered through a Whatman No 1 filter paper (Mudau et al., 2006). Supernatant collected was combined and stored at below 4 °C until analysed. All analysis was conducted within 7 days.

Chemical solvent extracts were prepared using 95% acetone, 95% methanol and 95% hexane. Ground sample (5 g) were extracted with 30 ml extraction solvent with continuous stirring using a magnetic stirrer. The mixture was allowed to settle before the supernatant was removed and retained. The sample was extracted twice more by repeating the extraction step for 2 minutes and allowing for separation as above. The supernatants were combined and filtered through a Whatman No 1 filter paper and stored at below 4 °C until analysed. All analysis was conducted within 7 days (Mudau et al., 2006).

TOTAL PHENOLIC CONTENT ASSAY

The total phenolic content was determined using the Folin-Ciocalteu method of Singleton and Rossi (1965).

One-millilitre extract was added to a 50 ml volumetric flask. The Folin reagent (2.5 ml) was added to the flask and allowed to react with the extract for one to eight minutes. Saturated solution of sodium bicarbonate (20% Na₂CO₃, 7.5 ml) was added to the reaction before it was filled to volume with distilled water. The flask was left to react for two hours before absorbance was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin-Elmer Corporation, USA). Gallic acid was used as the standard. The total phenol content was expressed as mg Gallic acid equivalent/ 100 mg sample.

ANTIOXIDANT ASSAY

The antioxidant activity was determined using the ABTS and DPPH assays reported by Awika et al. (2003).

For the ABTS analysis, the ABTS•+ radical was generated by dissolving it in water to a concentration of 8 mM and reacting with 3 mM (final concentration) potassium persulfate and allowing the mixture to stand in a dark room for 14 h before use.

A phosphate buffer was prepared using 40.5ml of 0.2 M Na₂HPO₄ (dibasic), 9.5 ml of 0.2 M NaH₂PO₄ (monobasic) and 150 mM NaCl. The pH of the buffer was adjusted to 7.4. Working solution was prepared by mixing 5 ml ABTS•+ solution with 145 ml phosphate buffer.

Then 2900 µl of the ABTS•+ solution was added to 100 µl of the extracts. The absorbance of the mixture was read spectrophotometrically using a Lambda EZ150 spectrophotometer (Perkin-Elmer Corporation, USA) at 734 nm after 15 min for the standards and 30 min for the samples. Methanol was used for the blank and Trolox as standard. The TEAC values of the extracts are expressed as µmole Trolox equivalents/ g of sample using the following equation:

Antioxidant activity = (slope* Δabs+ C) * (g/l sample used in analysis)

Δabs = Change in absorbance

C = y intercept

DPPH antioxidant activity of the tea extracts was determined with Trolox used as a standard. A mother solution was prepared by dissolving 24 mg DPPH into 100 ml methanol. The solution was kept as a mother solution below -12°C. A working solution of this is prepared by diluting 10 ml of this solution into 50 ml methanol before each experiment. A 2850 µl working solution was added to 150 µl of sample or standard and let to react for 6 hours. A 15 minute reaction time was allowed for the Trolox standard. After the reaction time, each sample mixture absorbance was measured at 515 nm using respective extraction solvents as a blank. The final antioxidant activity was expressed as µmole Trolox equivalents/ g of sample using the same equation expressed above.

Data Analysis

All data was analysed by STATGRAPHICS Centurion version 17.1.12 (2015). One-way analysis of variance (ANOVA) and multiple range tests using Fischer's least significant difference (LSD) procedure were used to test significant differences. Differences were considered significant at p < 0.05. Correlations between variables were determined using the regression analysis (McGaw et al., 2013).

RESULTS

Total Phenolic Content

Results in Table 1 demonstrated that the highest phenolic content in bush tea was achieved using 95% methanol (4.93 mg GAE / 100 g), followed by 95% ethanol (4.52 mg GAE / 100 g), hot water: 15 mins (4.49 mg GAE / 100 g), hot water: 10 mins (4.43 mg GAE / 100 g), 95% hexane (4.36 mg GAE / 100 g), hot water: 5 mins (4.23 mg GAE / 100 g), cold water: 5 mins (3.50 mg GAE / 100 g), cold water: 10 mins (3.04 mg GAE / 100 g) and cold water: 10 mins (2.83 mg GAE / 100 g). There was no significant difference ($p > 0.05$) between all the cold water extractions, although cold water: 15 mins had the lowest total phenols, possibly due to lower solubility. There was no significant difference between all the hot water extractions, although hot water: 5 mins had the lowest phenolic content. The total phenolic content of all the chemical solvents was not significantly different from any of the hot water extractions.

Table 1 shows that the highest phenolic content in special tea was achieved using 95 % methanol (6.24 mg GAE / 100 g), followed by 95 % hexane (5.94 mg GAE / 100 g), 95 % acetone (5.42 mg GAE / 100 g), hot water: 15 min (4.47 mg GAE / 100 g), hot water: 10 min (4.29 mg GAE / 100 g), cold water 5 min (2.90 mg GAE / 100 g), hot water: 5 min (2.89 mg GAE / 100 g) and cold water: 15 min (2.68 mg GAE / 100 g). The lowest content was achieved with cold water: 10 min (2.60 mg GAE / 100 g). There was no significant difference ($p \leq 0.05$) between all the cold water extractions and the hot water: 5 min extractions.

Antioxidant Activity

With the ABTS assay, the highest antioxidant activity in bush tea was achieved from 95 % methanol extraction (58.34 $\mu\text{mol TE/g}$) (Table 2). This was followed by cold water: 15 min (44.77 $\mu\text{mol TE / g}$) and, in decreasing order, 95 % hexane (42.47 $\mu\text{mol TE / g}$), hot water: 10 min (41.01 $\mu\text{mol TE / g}$), cold water: 5 min (36.28 $\mu\text{mol TE / g}$), hot water: 15 min (35.73 $\mu\text{mol TE / g}$) and hot water: 5 min (35.66 $\mu\text{mol TE / g}$). The lowest antioxidant activity was achieved with 95 % acetone (34.41 $\mu\text{mol TE / g}$).

The highest antioxidant activity for special tea was obtained from 95 % acetone extraction at 197.46 $\mu\text{mol TE / g}$. In decreasing order, the total phenolic content using other solvents was: 95 % methanol (182.01 $\mu\text{mol TE / g}$), hot water: 15 min (151.12 $\mu\text{mol TE / g}$), 95 % hexane (137.15 $\mu\text{mol TE / g}$), hot water: 10 min (125.36 $\mu\text{mol TE / g}$), hot water: 5 min (99.57 $\mu\text{mol TE / g}$), cold water: 15 min (90.66 $\mu\text{mol TE / g}$), cold water: 5 min (88.04 $\mu\text{mol TE / g}$). The lowest was achieved with cold water: 10 min (86.49 $\mu\text{mol TE / g}$). There was no significant difference ($p \leq 0.05$) amongst the cold water extractions.

With the DPPH assay (Table 2) cold water: 15 min extraction had the lowest antioxidant activity for bush tea at 59.87 $\mu\text{mol TE / g}$ followed by hot water: 5 min (62.60 $\mu\text{mol TE / g}$), hot water: 10 min (65.34 $\mu\text{mol TE / g}$), hot water: 15 min (65.84 $\mu\text{mol TE / g}$), cold water: 5 min (66.52 $\mu\text{mol TE / g}$), 95 % hexane (68.80 $\mu\text{mol TE / g}$), 95 % acetone (77.17 $\mu\text{mol TE / g}$), and cold water: 10 min (76.17 $\mu\text{mol TE / g}$). The highest was 95 % methanol (77.17 $\mu\text{mol TE / g}$). There was no significant difference between all the hot water extractions.

TABLE 1: THE TOTAL PHENOL CONTENT OF BUSH AND SPECIAL TEA USING DIFFERENT EXTRACTION TREATMENTS

Treatments	Total phenol content (mgGAE/100g)	
	Bush tea	Special tea
Cold water, 5min	3.50 ± 0.38 bc	2.90 ± 0.54 d
Cold water, 10min	3.04 ± 0.79 c	2.60 ± 0.41 d
Cold water, 15min	2.83 ± 0.89 c	2.68 ± 0.41 d
Hot water, 5min	4.23 ± 0.08 a	2.89 ± 0.23 d
Hot water, 10min	4.43 ± 0.25 a	4.29 ± 0.42c
Hot water, 15min	4.49 ± 0.34 a	4.47 ± 0.52 c
95% Acetone, 10min	4.52 ± 0.60 a	5.42 ± 0.56 b
95% Methanol, 10min	4.93 ± 0.34 a	6.24 ± 0.50 a
95% Hexane, 10min	4.36 ± 0.23 ab	5.94 ± 0.31 ab

Means ± SD in a column marked with the different lower cases are significantly different ($p < 0.05$).

TABLE 2: THE ANTIOXIDANT ACTIVITY OF BUSH AND SPECIAL TEA USING DIFFERENT EXTRACTION TREATMENT

Treatments	ABTS TROLOX EQ. $\mu\text{mol/g}$		DPPH TROLOX EQ. $\mu\text{mol/g}$	
	Bush tea	Special tea	Bush tea	Special tea
Cold water, 5min	36.28 \pm 1.71d	88.04 \pm 3.07g	66.52 \pm 3.19 bcd	102.17 \pm 4.49c
Cold water, 10min	41.01 \pm 0.67c	86.49 \pm 2.66g	76.17 \pm 5.02a	97.06 \pm 1.36de
Cold water, 15min	44.77 \pm 0.96b	90.66 \pm 1.25g	59.87 \pm 3.04e	93.78 \pm 2.02e
Hot water, 5min	35.66 \pm 2.45d	99.57 \pm 1.88f	62.60 \pm 4.76de	102.72 \pm 2.25c
Hot water, 10min	41.27 \pm 1.58c	125.36 \pm 3.72e	65.34 \pm 4.82cde	102.08 \pm 3.83c
Hot water, 15min	35.73 \pm 1.23d	151.12 \pm 2.90c	65.84 \pm 2.53cd	107.35 \pm 3.05ab
95% Acetone, 10min	34.41 \pm 1.87d	197.46 \pm 3.48a	71.77 \pm 1.26ab	101.04 \pm 0.83cd
95% Methanol, 10min	58.34 \pm 1.26a	182.01 \pm 8.35b	77.17 \pm 1.77a	109.13 \pm 1.65a
95% Hexane, 10min	42.47 \pm 1.50bc	137.15 \pm 5.27d	68.80 \pm 1.47bc	103.08 \pm 1.58bc

Means \pm SD in a column marked with the different lower cases are significantly different ($p < 0.05$)

TABLE 3: CORRELATION COEFFICIENTS OF THE TEA SAMPLES

Bush Tea Correlation	TPC	ABTS	DPPH	Special Tea Correlation	TPC	ABTS	DPPH
TPC	1			TPC	1		
ABTS	0.17	1		ABTS	0.88	1	
DPPH	0.28	0.43	1	DPPH	0.66	0.60	1

In special tea, the highest antioxidant activity was determined from the 95 % methanol extraction at 109.13 $\mu\text{mol TE / g}$. In decreasing order, results from other extraction methods were hot water: 15 min (107.35 $\mu\text{mol TE / g}$), 95 % hexane (103.08 $\mu\text{mol TE / g}$), hot water: 5 min (108.72 $\mu\text{mol TE / g}$), cold water: 5 min (102.17 $\mu\text{mol TE / g}$), hot water: 10 min (102.08 $\mu\text{mol TE / g}$), 95 % acetone (101.04 $\mu\text{mol TE / g}$), and cold water: 10 min (97.06 $\mu\text{mol TE / g}$). The lowest was cold water: 15 min (93.78 $\mu\text{mol TE / g}$).

Table 3 shows that there was no correlation between the total phenolic content and any of the antioxidant assays conducted on bush tea analysis. A positive correlation was present between the same tests for special tea extracts, with the strongest correlation observed between total phenolic content and ABTS antioxidant activity.

DISCUSSION

Previous studies have indicated that the extraction solvent and any additional treatment during extraction of tea polyphenols, influences the yield of polyphenol extracted (Goli et al., 2004). Similarly, our results indicated that different extraction solvents and regimes resulted in different levels of phenolic content and antioxidant activity.

Results observed in this experiment concur with the findings reported by Turkmen et al. (2006), who observed that it is not clear which solvent is more effective for the extraction of phenolic compounds. For the best results, the method and/or solvent used must be able to extract the most phenolic compounds and not alter the chemical structure and functionality of the compounds extracted (Zou et al., 2002). Overall, chemical solvents had higher extraction efficiency than their water counterparts in the current study. The type of phenolic compounds extracted will also be a contributing factor in the total phenolic content and antioxidant activity measured. Boulekbache-Makhlouf et al. (2013) demonstrated that a solvent might extract a lower quantity of total phenolic content, but be higher in specific phenolic compounds compared with other solvents. This was evident in their study, where methanol extracts had significantly lower total phenolic compounds compared with acetone, but had significantly higher anthocyanin contents in the same acetone extracts. They also found that ethanol extracts had significantly lower tannin contents than acetone extracts, but significantly higher anthocyanin content. In support of this research, a study involving extraction of bush tea using chemicals and water as solvents (McGaw et al., 2013) confirmed that chemical solvent extracts contained more complex polyphenols than water extracts. This could further explain the higher antioxidant activity of solvent extracts compared to water extracts noted in this study. This is

explained by the fact that different phenolic compounds will have different antioxidant capacities based on their structures. Tabart et al. (2009) demonstrated that different phenolic compounds prepared at the same concentration gave different total phenolic contents as determined by the Folin-Ciocalteu method and different levels of antioxidant activity as determined by various methods, including the ABTS and DPPH methods used in this assay.

In this study, hot water extracted more total phenol contents than cold water. These results are contrary to the findings of Damiani et al. (2014), who observed higher total phenols were extracted using cold water than hot water in different unfermented white teas. Individual phenolic compounds identified by Damiani et al. (2014) were also higher in cold water extractions than in hot water extractions. Their study also showed that the antioxidant activity of the cold water extracts ranged from 17 to 30 mmol TE / L, which was higher than in hot water extracts, where the antioxidant activity ranged from 5 to 16 mmol TE / L. In their study involving white tea, black tea, oolong, green tea and lyons tea, Venditti et al. (2010) found that only cold water extracts of white tea had significantly ($p \leq 0.05$) higher polyphenols compared with hot water extracts; the same trend was observed in the current study in the antioxidant activity of the extracts using the ABTS method.

In conclusion, special tea had a higher total phenols and antioxidant activity than bush tea. The extraction solvent and regime do influence the total phenolic content extracted and the antioxidant activity of the extracts. Hot water was comparable to chemical solvents extraction in terms of the total phenols extracted and the antioxidant activity of the extracts. Thus, this study strongly suggests that the potential health benefits of special tea and bush tea are made available to consumers of these teas. As hot water was comparable to extraction using organic solvents, it can be recommended the use of hot water as extraction solvent will also result in possible sensory evaluation acceptability to the consumers. Future studies to determine the different types of phenolic compounds and tannins in both bush and special tea extracted using hot water and their effects on the antioxidant activity, volatiles, sensory analysis and other health benefits associated to these selected herbal teas should be investigated.

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