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Anti-diabetic and anti-proliferative activities of herbal teas, *Athrixia phylicoides* DC and *Monsonia burkeana* Planch. ex Harv, indigenous to South Africa

Lutendo Patricia Mathivha, Vuyisile Samuel Thibane and Fhatuwani Nixwell Mudau
Department of Agriculture and Animal Health, University of South Africa, Florida, South Africa

Abstract

Purpose – The purpose of this paper is to investigate the health and medicinal importance of bush tea (*Athrixia phylicoides* DC) and special tea (*Monsonia burkeana* Planch. ex Harv), two of Southern African indigenous herbal teas.

Design/methodology/approach – The two herbal teas, *A. phylicoides* and *M. burkeana* were extracted individually and in combined ratios for analysis. The phenolic content was determined and the different phenolic compounds were identified using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The anti-diabetic activity of the teas was determined by evaluating the inhibition of both α-amylase and α-glucosidase *in vitro*. The anti-proliferative activity was measured on human cervical cancer (HeLa) cell line using the MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium) assay.

Findings – Gallic acid, chlorogenic acid and quercetin were identified to be present in significant quantities by TLC. The HPLC quantified the presence of catechin (1.567 mg/g) and chlorogenic acid (1.862 mg/g) in special tea while chlorogenic acid (1.288 mg/g) was present in bush tea. Bush tea and special tea expressed significant levels of phenolic content and high antioxidant activities. Special tea (S100) expressed high inhibition of α-amylase, α-glucosidase and HeLa cell line proliferation when compared to bush tea (B100).

Originality/value – Both bush tea and special tea could provide an alternative for treatment and management of both diabetes and cervical cancer. However, future studies are needed to investigate their synergistic effect with a wide range of other commercial herbal teas.

Keywords α-Amylase, α-Glucosidase, Herbal teas, Anti-diabetic, Anti-proliferative

Paper type Research paper

1. Introduction

The prevalence of both diabetes and cancer has been noted as a global health burden in developed and developing countries. The biggest increase in the number of diabetes death-related cases were observed more in developing countries such as South Africa (Shaw *et al.*, 2010). Diabetes has been estimated to cause approximately 3.6 per cent of South Africa's population deaths, making it one of the top 10 leading cause of death (Pillay-van Wyk *et al.*, 2016). Lung, liver, stomach, breast and cervical cancers are the leading estimated cause of death

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in the Southern African region. The increase in new cancer cases and deaths has been largely attributed to the aging population, increasing prevalence of lifestyle risk factors such as smoking, and the less physical activities associated with urbanisation (Torre *et al.*, 2015). There is increasing epidemiological evidence that suggests the role of dietary antioxidants, including phenolic compounds, in the prevention of several chronic and degenerative diseases which includes diabetes and cancer (Zielinski and Kozlowska, 2000; Willcox *et al.*, 2004). These benefits are made possible by the *in vivo* antioxidant capacity of phenolic compounds which are reported to assist with maintaining the balance between oxidants and antioxidants (Adom and Lui, 2002). Plants are known rich sources of phenolic compounds and have an extensive reported usage for medicinal purposes (Sapkota *et al.*, 2012). The South African plant flora has a rich source of indigenous herbal teas with some teas still unexplored even though they have diverse compounds with pharmacological properties.

Bush tea (*Athrixia phylicoides* DC) is a Southern African indigenous plant (Herman *et al.*, 2000) currently being used for treatment of boils, sores, acne, infected wounds, cuts, headaches, colds, loss of voice and throat infection as a gargle (Mabogo, 1990; Roberts, 1990; Hutchings *et al.*, 1996). The Vhavenda people use extracts from soaked roots and leaves as an anthelmintic (Mbambezeli, 2005). It is also used for cleansing or purifying the blood (Roberts, 1990; Joubert *et al.*, 2008). Special tea (*Monsonia burkeana* Planch. ex Harv) is indigenous to the Southern African region (Venter, 1979) and is used in combination with other herbs as a blood cleanser, cure for sexually transmitted diseases, to restore erectile dysfunction disorders and to enhance male libido (Mamphiswana *et al.*, 2011). The phenolic compounds and resultant antioxidant activity have been reported to be significant in both special tea (Mamphiswana *et al.*, 2010) and bush tea (Mudau *et al.*, 2006), which could attribute to their observed activities. Studies have further demonstrated that extracts from both teas to have antimicrobial properties against known pathogens (Tshivhandekano *et al.*, 2014).

Combinational studies can greatly enhance the activity of the individual herbal teas when a synergistic interaction is achieved (Rakholiya and Chanda, 2012). Tea polyphenols and bleomycin hydrochloride were reported to express an antioxidant-based synergistic interaction and enhanced the inhibition of uterine cervical cancer cells (Alshwati *et al.*, 2016). The intracellular bioactivity and synergy of botanical extracts was further reported to be stable when determined using human red blood cells and therefore encouraging their study (Zhou *et al.*, 2000; Blasa *et al.*, 2011). However, one should be cognisant to the fact that the interaction between botanical extracts is never synergistic as an additive or antagonistic interaction can be expressed (Eja *et al.*, 2011; Tshivhandekano *et al.*, 2014). Therefore, with the increased interest on using more natural products it is vital to understand the health and medical benefits of bush tea or *A. phylicoides* and special tea or *M. burkeana* and their possible synergistic interaction by evaluating their anti-diabetic and anti-proliferative activity.

2. Materials and methods

2.1 Plant materials

Bush tea (*A. phylicoides*) was collected in the Khalavha village area (−22.922985:30.280568) of the Limpopo Province (South Africa) and special tea (*M. burkeana*) was collected in the Brits area (−25.596806:27.804566) of North West Province (South Africa). The stem and leaves were collected as these are used during the preparation of traditional teas. Both bush tea and special tea were formally identified by FN Mudau, Professor in the Agriculture and Animal Health Department at the University of South Africa. The plants material were allowed to dry under shade, at ambient temperature (approximately 25 °C) for 14 days or until constant dryness was achieved and ground into powders using a commercial grinder. The ground plant material was stored in an air-tight container at room temperature in the dark before extraction. Voucher specimens of bush tea (*Athrixia phylicoides*-9055000) and special tea (*Monsonia burkeana*-3925000) are kept at the South African National Biodiversity Institute, National Herbarium.

2.2 Plant material preparations

In total, 5 g of ground plant material was prepared as indicated in Table I and extracted with 30 mL of 90 per cent aqueous methanol (v/v) by continuously stirring using a magnetic stirrer. The supernatant was removed and retained. The sample was rinsed twice with the extraction solvent by repeating the extraction step for 2 min and allowing to separate as above. The supernatants were combined and filtered through a Whatman No 1 filter paper and stored at 4 °C until ready for assay analysis. Treatments were arranged as per Table I in a complete randomized design and replicated three times.

2.3 Total phenolic content (TPC) determination

The Folin-Ciocalteu reagent was used to determine the TPC of the plant extracts (Singleton and Rossi, 1965). Briefly, 1 mL extract and 2.5 mL Folin C reagent (1 N) were added into a 50 mL volumetric flask and allowed to stand for 8 min. A saturated solution of sodium bicarbonate (20 per cent Na_2CO_3 (w/v), 7.5 mL) was added to the reaction mixture and filled to volume with distilled water. The solution was incubated at room temperature for 2 h and the absorbance measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin-Elmer Corporation, USA). Gallic acid was used as the standard. The assay was performed in triplicate. The TPC was expressed as mg gallic acid equivalent/g dried sample (mgGAE/g). ($R^2 = 0.9998$).

2.4 Phenolic compound identification

2.4.1 Thin-layer chromatography (TLC). Phenolic compounds were separated by adding 10 μL of the extract on a TLC F_{254} analytical plate using EMW (ethyl acetate/methanol/water 40:5.4:4) as the eluent (Wagner and Bladt, 1996). The plates were sprayed with vanillin-sulphuric acid reagent and heated for 5 min at 100 °C for colour development. Separated compounds were visualised under visible light.

2.4.2 High-performance liquid chromatography (HPLC). The quantitative analysis of selected phenolic compounds was determined, with slight modifications, using a quadra pump HPLC instrument fitted with a RP-C18 column and a C18 guard column (Fernendez *et al.*, 2000).

Chromatographic conditions: a two-solvent gradient elution was performed at a flow rate of 1 mL/min. The solvents A (water:acetonitrile:formic acid – 94.7:4.3:1 v/v) and B (water: acetonitrile:formic acid – 49.5:49.5:1 v/v). Solvent A was run at 90 per cent for the initial 10 min after which it was decreased to run at 70 per cent for 20 min. The solvent was further decreased to 20 per cent and left to run for 10 min. The total running time was recorded at 40 min. Detection was done using an ultraviolet (UV) detector at 280 nm. The assay was performed in triplicate. The results obtained were expressed as mg/g of dried plant material.

2.5 Antioxidant activity assays

2.5.1 ABTS antioxidant activity. The antioxidant activity of the extracts was measured using the Trolox Equivalent Antioxidant Capacity (TEAC) assay with Trolox used as a standard (Awika *et al.*, 2003). The $ABTS^{\bullet+}$ radical was generated by dissolving ABTS in

Sample code	Bush tea (w/w) (%)	Special tea (w/w) (%)
B100	100	0
BS75:25	75	25
BS50:50	50	50
BS25:50	25	75
S100	0	100

Notes: B, bush tea; BS, bush tea and special tea ratio; S, special tea

Table I. The sample codes according to bush tea – special tea combinations used

water to a concentration of 8 mM and reacting with 3 mM potassium persulfate (final concentration). The mixture was allowed to stand in a dark room for 14 h prior to usage. A working solution was prepared by mixing 5 mL $ABTS^{\bullet+}$ with 145 mL phosphate buffer (40.5 mL of 0.2 M Na_2HPO_4, 9.5 mL of 0.2 M NaH_2PO_4, 150 mM NaCl, pH 7.4). A volume of 2.9 mL $ABTS^{\bullet+}$ solution was added to 0.1 mL of the extracts and the reaction mixture incubated for 15–30 min at room temperature. Absorbance was measured at 734 nm using a Lambda EZ150 spectrophotometer (Perkin-Elmer Corporation, USA). Methanol served as a blank and Troxol as the standard. The assay was performed in triplicate. The TEAC values of the extracts were expressed as μmole Trolox equivalents/g of dried sample (μmolTE/g) using the following antioxidant activity equation:

$$\text{Antioxidant activity} = (\text{slope} \times \Delta\text{abs} + C) \times (g/L \text{ sample}),$$

where Δabs is the change in absorbance; C the y intercept ($R^2 = 0.9996$).

2.5.2 DPPH radical scavenging activity. The antioxidant activity of the extracts was determined using the DPPH radical with Trolox used as a standard (Awika *et al.*, 2003). A stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and stored below −10 °C. A working solution was prepared by diluting 10 mL of the stock solution with 50 mL methanol. A volume of 2.85 mL working solution was added to 0.15 mL sample or standard and incubated for 15–30 min at room temperature for 6 h. Absorbance was measured at 515 nm using a Lambda EZ150 spectrophotometer (Perkin-Elmer Corporation, USA). Methanol served as a blank and Troxol as the standard. The assay was performed in triplicate. The antioxidant activity was expressed as μmole Trolox equivalents/g of dried sample (μmolTE/g) using the antioxidant activity equation.

2.5.3 Ferric reducing power (FRP) activity. The FRP of the extracts was determined according to the method described by (Yildirim *et al.*, 2001). Briefly, 1 mL of the extract was mixed with 2.5 mL phosphate buffer (0.2 M, pH 7.4) and 2.5 mL potassium ferricyanide solution (1 per cent, w/v). The reaction mixture was incubated in a water bath at 50 °C for 20 min. After incubation, 2.5 mL trichloroacetic acid (10 per cent in phosphate buffer w/v), 1.25 mL distilled water and 1.25 mL ferric chloride ($FeCl_3$) (0.1 per cent in phosphate buffer, w/v) were added to the reaction mixture and further incubated at room temperature for 10 min. Absorbance was measured at 700 nm using a Lambda EZ150 spectrophotometer (Perkin-Elmer Corporation, USA). Ascorbic acid was used as a standard. The assay was performed in triplicate. The FRP was expressed as gram ascorbic acid equivalent/100 g dried plant material (gAAE/100 g). ($R^2 = 0.9977$).

2.6 Anti-diabetic activity assay

2.6.1 α-Glucosidase inhibition assay. The anti-diabetic activity of the tea extracts was evaluated by examining the inhibition of the α-glucosidase as described by Mahendran *et al.* (2015). Briefly, α-glucosidase solution was prepared by diluting 10 units/mL (0.1 M potassium phosphate buffer v/v, pH 6.8). A volume of 5 μL of the α-glucosidase solution was mixed with 250 μL of the different extracts and incubated in a water bath at 37 °C for 20 min. After incubation, 10 μL (10 mM p-NPG) was added to the reaction mixture and further incubated at 37 °C for 30 min. The reaction was terminated by adding 650 μL of Na_2CO_3 solution (1 M). A 0.3 mL aliquot of the reaction mixture was added to 4.7 mL distilled water. A buffer solution without the extract was used as a blank. The absorbance of the reaction mixture was measured at a wavelength of 410 nm using a spectrophotometer. The assay was performed in triplicate. The enzyme activity was measured using the following equation:

$$\alpha\text{−Glucosidase \% inhibition} = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}} \times 100\right].$$

2.6.2 α-Amylase inhibition assay. The anti-diabetic activity of the tea extracts was evaluated by examining the inhibition of the α-amylase as described by (Mahendran *et al.*, 2015). Briefly, soluble starch solution (1 per cent w/v in 0.05 M sodium phosphate buffer, 1 mM $CaCl_2$, pH 6.8) was prepared and used as a substrate. A reaction mixture was initially prepared by mixing a volume of 400 μL of the α-amylase solution (20 units/mL), 1 mL sodium phosphate buffer (pH 6.8) and 200 μL of tea extracts and incubated in a water bath at 37°C for 10 min. After incubation, 300 μL of the starch solution to reaction mixture and further incubated at 37°C for 20 min. The total volume was made up to 8.0 ml with distilled water. A buffer solution without the extract was used as a blank. Acarbose was used as a positive control. The absorbance of the reaction mixture was measured at a wavelength of 540 nm using a spectrophotometer. The assay was performed in triplicate. The enzyme activity was measured using the following equation:

$$\alpha\text{-Amylase}\ \%\ \text{inhibition} = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}} \times 100\right].$$

2.7 Anti-proliferative activity assay

The potential of the tea extracts in cancer treatment was evaluated by determining their anti-proliferative activity of the human cervical cancer (HeLa) cell line as described by Mahendran *et al.* (2015). The HeLa cell line was maintained in DMEM supplemented with 10 per cent FBS in a 5 per cent CO_2 incubator. The extracts and positive control were dissolved in DMSO and diluted in serum free medium. A volume of 100 μL cells was seeded into a 96-well microtiter plates at a plating density of 10,000 cells/well and incubated at 37 °C, 5 per cent CO_2, 95 per cent air and 100 per cent relative humidity for 24 h. After incubation, the cells were treated by adding 100 μL of the medium containing extracts and further incubated at 37 °C, 5 per cent CO_2 for 48 h. After treatment, 15 μL of the MTT (5 mg/mL in PBS) was added to each well and incubated at 37 °C, 5 per cent CO_2 for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were stabilized by adding 100 μL of DMSO. The absorbance of the stabilized reaction mixture was measured at 570 nm using a microplate reader. Camptothecin was used at various concentrations as a standard and the medium without extracts was used as a negative control. The assay was performed in duplicates and repeated three times. The lethal dose (LD_{50}) was determined as the concentration required to reduce the activity by more than 50 per cent when compared to untreated cells.

2.8 Statistical analysis

All data are expressed as means ± standard deviation of triplicate measurements and analysed by STATGRAPHICS Centurion version 17.1.12 (32 bits, 2015). A one-way analysis of variance and multiple range tests using Fischer's least significant difference procedure was used to test for any significant differences between results. Differences were considered significant at $p < 0.05$. Correlations between variables were determined using the regression analysis.

3. Results

3.1 Total phenolic content determination and identification

The results for the TPC for bush tea (B100), special tea (S100) and their combinations (BS75:25, BS50:50, BS25:75) are represented by Table II. The TPC for S100 was significantly high when compared to B100 with values of 66.1 and 48.0 mgGAE/g, respectively. This was further established by the observed increase of the TPC with increased ratio of special tea. There was no significant difference between the tea combinations for BS75:25, BS50:50 and BS25:75 with values of 51.8, 54.9, 56.9 mgGAE/g, respectively. The difference between the TPC of S100, 100 per cent special tea, and B100, 100 per cent bush tea, was 18.1 mgGAE/g.

The B100 sample had more visible lines on the TLC plate (Figure 1). Gallic acid, chlorogenic acid and quercetin were the only ones identified from the standard compounds included in the assay for both B100 and S100.

The three phenolic acids were also identified and quantified in the HPLC analysis of B100 and S100 samples (Figure 2). In addition to the three identified phenolic compounds, catechin was also identified in the HPLC assay of the special tea only. Table III indicates the retention times and concentrations interpreted from the HPLC chromatographs.

3.2 Antioxidant activity assays

The antioxidant activity of bush tea (B100), special tea (S100) and their combinations (BS75:25, BS50:50, BS25:75) are represented by Table II. The overall antioxidant activity of special tea was significantly higher than that of bush tea. Increasing the special tea ratio in the tea combinations expressed a slight increase in the antioxidant activity from BS75:25 to BS25:75. The antioxidant activity values for S100 were recorded at 170.67 μmolTE/g, 105.94 μmolTE/g and 4.62 gAAE/100 g for the ABTS, DPPH and FRP assays, respectively. There was no significant difference in the DPPH radical scavenging activity of S100 and BS25:75 with values of 105.94 and 99.12 μmolTE/g, respectively. The BS25:75 combinations expressed notable radical scavenging and metal chelating activity when compared to the other tea combination.

Tea combination	TPC mgGAE/g		ABTS μmolTE/g		DPPH μmolTE/g		FRP gAAE/100 g	
B100	48.0[b]	±0.56	36.58[d]	±3.65	74.53[b]	±3.81	2.69[c]	±0.04
BS75:25	51.8[b]	±0.26	37.01[d]	±3.67	75.11[b]	±3.70	2.32[c]	±0.05
BS50:50	54.9[a,b]	±0.64	47.00[c]	±4.75	81.04[b]	±0.58	2.67[c]	±0.03
BS25:75	56.9[a,b]	±0.54	83.42[b]	±3.90	99.12[a]	±1.94	3.94[b]	±0.03
S100	66.1[a]	±1.04	170.67[a]	±6.08	105.94[a]	±7.01	4.62[a]	±0.53

Notes: Values are expressed as means ±SD ($n = 3$). Values marked with the different lower cases (a–d) are significantly different ($p < 0.05$)

Table II. The TPC, ABTS, DPPH and FRP of bush tea, special tea and tea combinations

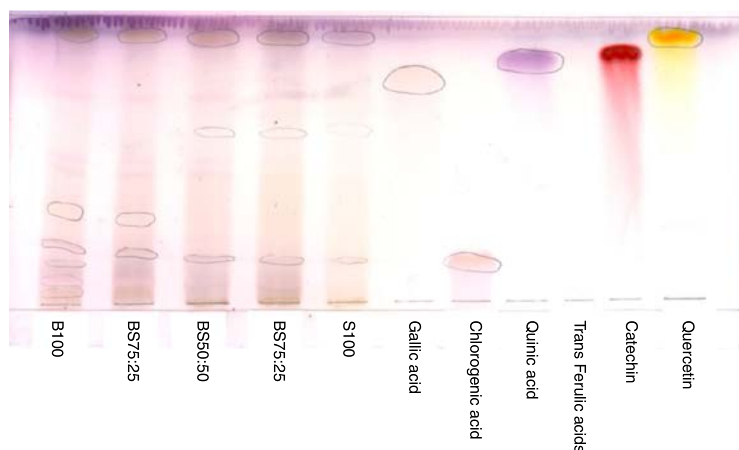
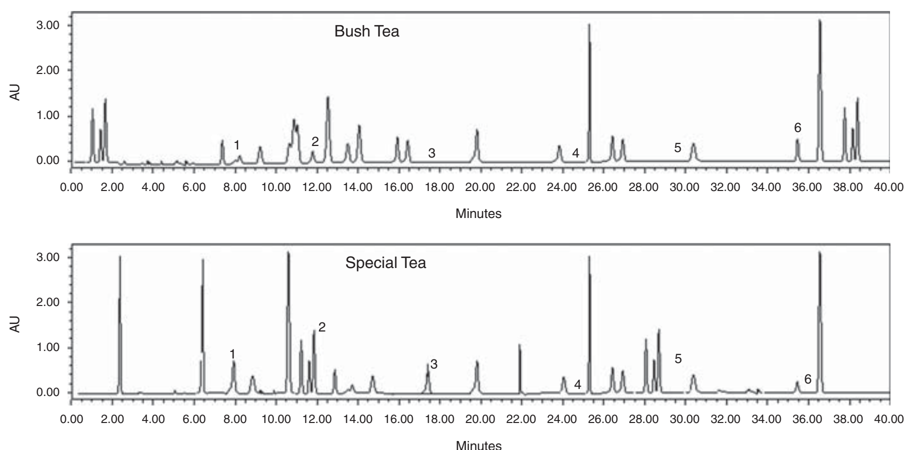


Figure 1. TLC chromatogram developed in an EMW system



Notes: 1 – gallic acid, 2 – Chlorogenic acid, 3 – Catechin, 4 – trans Ferulic acid, 5 – Quinic acid, 6 – Quercetin

Figure 2.
HPLC chromatograms of bush tea and special tea

Table III.
The retention times and concentrations of phenolic compounds identified

Phenolic compounds	Retention time (min)	B100 mg/g	S100 mg/g
Gallic acid	7.10	0.291±0.11	0.369±0.03
Chlorogenic acid	11.9	1.288±0.02	1.862±0.05
Catechin	17.3	nd	1.567±0.23
Trans ferulic acid	24.4	nd	nd
Quinic acid	30.2	nd	nd
Quercetin	35.5	0.297±0.03	0.096±0.01

Notes: nd, not detected. Values are expressed as means ±SD ($n = 3$)

3.3 Anti-diabetic activity assay

The anti-diabetic activity results for the tea combinations represented by the per cent inhibition of α-amylase and α-glucosidase are expressed in Table IV. The S100 extract inhibited 100 per cent activity of both the α-amylase and α-glucosidase. However, the inhibitory effect was lower for B100 extract against both the α-amylase and α-glucosidase with values of 26.67 and 36.67 per cent, respectively. There was a notable increase in the inhibitory effect of bush tea when the ratio of special tea was increased. The inhibitory activity of BS50:50 was recorded at 65.0 and 73.33 per cent, this was further increased to 80.0 and 86.67 per cent by increasing the

Table IV.
The anti-diabetic and anti-proliferative activity of bush tea, special tea and tea combinations

Tea combinations	Anti-diabetic assay α-amylase % inhibition		Anti-diabetic assay α-glucosidase % inhibition		Anti-proliferative assay HeLa % inhibition	
B100	26.67^{c}	±11.55	36.67^{d}	±5.77	54.82^{c}	±0.57
BS75:25	55.00^{b}	±25.98	54.00^{c}	±12.17	60.21^{b,c}	±25.12
BS50:50	65.00^{b}	±8.66	73.33^{b}	±11.55	86.92^{a,b}	±5.81
BS25:75	80.00^{a,b}	±10.00	86.67^{a,b}	±5.77	58.21^{c}	±22.52
S100	100.00^{a}	±0	100.00^{a}	±0	90.14^{a}	±0.58

Notes: Values are expressed as means ±SD ($n = 3$). Values marked with the different lower cases (a–d) are significantly different ($p < 0.05$)

special tea content to 75 per cent for both the α-amylase and α-glucosidase, respectively. The results for the anti-diabetic activity assay where consistent with the antioxidant activity assays results, where higher levels of special tea increased the activity.

3.4 Anti-proliferative activity assay

The anti-proliferative activity results for the tea combinations represented by the per cent inhibition of the HeLa cell line are expressed in Table IV. The S100 extracts had significantly higher anti-proliferative activity than B100 with an inhibitory value of 90.14 per cent. The BS75:25 expressed an improved inhibitory effect of HeLa cell line with a value of 60.21 per cent. Reduction of the special tea content in BS25:75 expressed reduced inhibitory activity against the HeLa cell line. Interestingly, BS50:50 (50 per cent of bush tea and 50 per cent of special tea) improved the inhibitory effect of the tea combination with a value of 86.92 per cent. The observed improvement in the inhibitory activity of BS50:50 will result in reduced levels of bush tea and special tea being used to achieve higher activities.

4. Discussion

The efforts to develop a phytochemical fingerprint for both bush tea and special tea were undertaken to identify and quantify some of the phenolic compounds that could justify the potential health benefits of these teas. The HPLC chromatogram of special tea expressed more peaks when compared to the bush tea chromatogram. The HPLC results for bush tea confirmed the TLC findings and only gallic acid, chlorogenic acid and quercetin were identified and quantified. However, catechin was also identified in the HPLC analysis of special tea. There is no data currently available on the phenolic content of both bush tea and special tea. Similarly, Qasim *et al.* (2017) identified chlorogenic acid, gallic acid, catechin and quercetin as abundant phenolic compounds in five South African medicinal plants. Caffeic acid, syringic acid, coumarin, narigenin and kaempferol were also identified.

There was a notable upward trend between the phenolic content and all antioxidant assays examined. This positive trend was further observed in the anti-diabetic assays, signalled by an observed high anti-diabetic activity with high phenolic compound content. There was no notable trend between the anti-proliferative assay and TPC, ABTS and both the anti-diabetic assays. Furthermore, a similar trend was observed between the anti-proliferative assay and DPPH and FRP assay.

There are currently very few scientific publications available on the phenolic composition and antioxidant activities of bush tea and special tea. Similar levels of TPC and antioxidant activity, using the ABTS method, in special tea have been previously reported (Mudau *et al.*, 2006). Another study by Tshivhandekano *et al.* (2014) also reported similar results when analysing the TPC of bush tea and special tea. However, there was little antioxidant activity reported from the same study on both bush tea and special tea. The differences in results between the study by Tshivhandekano *et al.* (2014) and this study can be as a result of the different extraction methods used (Tabart *et al.*, 2009). A recent study by Malongane *et al.* (2018) identified phenolic compounds, phenyl pyruvate and gallate, in special tea whereas only gallate was detected in bush tea. The same study also identified myricitin, hesperidin, orientin, luteolin and quercetin in both herbal teas. There is no published research report that suggests the anti-diabetic and anti-proliferative properties of the two herbal teas. The results of this study have suggested that bush tea and special tea do contain anti-diabetic and anti-proliferative activity similar to those reported for rooibos (Joubert and de Beer, 2011) green tea (Miao *et al.*, 2015) and honeybush tea (Joubert *et al.*, 2008). The α-glucosidase inhibition activity from both bush tea and special tea are higher than what has been reported for *Centella asiatica* (Pegagan) ranging from 5.39 to 13.30 per cent (Dewi and Maryani, 2015). Special tea and bush tea noted in this study were also considerably higher when compared to results reported on *Ligustrum robustum*

extracts on both α-amylase (70.61 per cent) and α-glucosidase (84.65 per cent) (Yu *et al.*, 2015). However, similar levels of inhibition were reported on α-glucosidase inhibition assay involving two Japanese herbal plants of *Mallotus japonicus* (96.54 per cent) and *Quercus phillyraeoides* (98.57 per cent) (Indrianingsih *et al.*, 2015).

This study has shown that special tea has significantly higher TPC and antioxidant activity than bush tea. An increase in special tea ratio also resulted in an increase in the TPC, DPPH, per cent α-amylase inhibition and per cent α-glucosidase inhibition. The results obtained from this study did not suggest any synergistic effect when bush tea and special tea were combined. These results were supported by combination studies conducted on bush tea and special tea that reported that there was no synergistic effect in terms of TPC, total flavonoids, total tanning and antimicrobial activity (Tshivhandekano *et al.*, 2014). They also demonstrated that there was no significant difference between bush tea and the combination sample.

There was no direct increase in the anti-proliferative activity with the increase in special tea ratio, although special tea expressed higher activity than bush tea. An equal ratio of both bush tea and special tea yielded a significant anti-proliferative activity with a value of 86.92 per cent, comparative to 100 per cent special tea. In another study by Carvalho *et al.* (2010) similar findings were reported, with a maximum of 97 per cent anti-proliferative activity against renal carcinoma cells. A ten-fold synergistic effect was noted when green tea was infused with grape phenolics against human cervical carcinoma (Morré and Morré, 2006).

5. Conclusions

Special tea contains higher TPC of 6.6 mgGAE/g than that of bush tea of 4.8 mgGAE/g and therefore that gives special tea more in terms of the antioxidant and health benefits tested. The highest ABTS and DPPH antioxidant values were 170.67 μmolTE/g and 105.94 μmolTE/g, respectively, for special tea. Special tea, S100, achieved the maximum anti-diabetic properties for both α-amylase and α-glucosidase inhibition of 100 per cent whereas bush tea, B100, had a higher α-glucosidase inhibition (36.67 per cent) than α-amylase (26.67 per cent). Special tea, S100, also had a higher anti-proliferate activity of 90.14 per cent than bush tea, B100, which had an anti-proliferative activity of 54.82 per cent. There was no synergistic effect in combining the two herbal teas on any of the tests conducted in any of the different combination ratios prepared. The reported anti-diabetic and anti-proliferative activities of bush tea and special tea are of great significance in combating diabetes and cervical cancer. South Africa is one of the developing countries burdened by the prevalence of diabetes and cancer deaths. The consumption of bush tea and special tea in the more urbanized areas of South Africa can aid in reducing the prevalence of diabetes and cancer as consumption is still more popular in the rural parts of the country. Future studies will be to determine the synergistic effects with a wide array of commercial teas on the anti-diabetic and anti-cancer properties of these herbal teas. Another important aspect to be investigated is the isolation and quantification of the different phenolic compounds present in the teas and their contribution to the antioxidant and health benefits of these teas.

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Corresponding author
Fhatuwani Nixwell Mudau can be contacted at: mudaufn@unisa.ac.za

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