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Original Paper

Total Phenolic Contents and Antioxidant Activities of Selenium-Rich Black Tea Versus Regular Black Tea

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ABSTRACT
Two black aqueous tea extracts of Camellia sinensis, (selenium-rich black tea (Se-BTE) and regular black tea (R-BTE)) were assessed for their total phenolic content (TPC) and antioxidant properties. Aqueous tea extracts were prepared using different extraction temperature and time. TPC was measured using the Folin-Ciocalteu method, while antioxidant activity was measured by ferric-reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The R-BTE showed significantly higher TPC ($P < 0.05$) and antioxidant activity ($P < 0.05$) than the Se-BTE, which may be due to these being different cultivars, or differences in growing conditions. Our findings demonstrate that both extraction temperature and time are important determinants of extracted TPC and antioxidant activities of these teas. In addition and in contrary to our expectations, the high level of organic selenium in black tea did not enhance the antioxidant activity when compared with black tea containing very low level of organic selenium. Other elements and minerals may contribute to the high antioxidant activity detected in the regular black tea.

Keywords: selenium-rich black tea; regular black tea; total phenolic contents; ferric-reducing/antioxidant power; DPPH-scavenging activity

INTRODUCTION

Tea is an infusion beverage made from the leaves of the plant *Camellia sinensis* and has been consumed worldwide for centuries. Tea is served in a diverse range of varieties, with the most common forms being black, green, oolong and white teas, all categorized according to their method of production [1]. Tea has a high polyphenol content, with

catechins and their polymerized forms such as theaflavins and thearubigins being responsible for most of the health-giving properties of tea. The major tea catechins are (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin (EC) and (-)-epicatechin gallate (ECG), which are found abundantly in green and white teas. The theaflavins and thearubigins are complex phenolic compounds derived from the enzymatic oxidation of catechins during the fermentation stage of oolong and black tea production [2] and are only present in these teas.

The four major theaflavins identified in black and oolong teas, include theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-gallate. However, the chemical structure of thearubigins is not well-defined and still remains unclear [3].

The most widely recognized property of tea polyphenols is their potent antioxidant activity, as evidenced to have antioxidative roles by *in vitro* and *in vivo* studies [4, 5]. Tea polyphenols act as antioxidants by scavenging reactive oxygen and nitrogen species free radicals, as well as chelating redox-active transition metal ions [4]. Epidemiological and experimental studies have demonstrated that tea polyphenols may reduce the risk of oxidative-related diseases such as cancers [6], chronic degenerative diseases [7] and obesity [8]. Due to their strong antioxidant activities, tea catechins are utilised in the food industry as a potential source of natural antioxidants to improve food quality and prolong the shelf-life of food products [9].

There has been increased interest in the antioxidant properties of Selenium-containing teas, particularly in response to their purported health claims surrounding their use, and also the potential use of tea extracts as nutraceuticals. Selenium (Se) is an important trace mineral that is found in meats and seafood, with Se levels in agricultural products solely dependent on soil selenium concentrations and the bioavailability to plants [10]. Importantly, Se is an essential component of selenoenzymes, which are selenium-dependent antioxidant enzymes, that play an antioxidant role in cellular membrane protection. Tea plants grown in Se-rich soils are high in Se, the tea brewed from these plants is suggested to be more antioxidant rich when compared to regular tea, possibly due to a higher Se content, which may act with other polyphenolic compounds synergistically [5]. Previous research has shown that a Se enrichment in tea can significantly enhance the antioxidant activity of tea [11].

To the best of our knowledge, there has been no investigation of the antioxidant properties of selenium-rich black tea. Promising reports on the antioxidant effects of tea, as well as Se have led us to hypothesize that selenium-rich black tea might be more effective as an antioxidant compared to regular black tea. Therefore, studies on selenium-rich black tea for its total phenolic content and antioxidant capacity must first be performed to confirm this hypothesis. Accordingly, the aim of the present study is to assess the phenolic content and antioxidant capacity of Se-rich black tea in comparison with regular tea. In addition, we also studied the effect of different tea extraction conditions (water temperature and extraction time) on polyphenolic content and antioxidant properties. The correlation between the total phenolic content (TPC) and antioxidant properties of the tea extracts was also investigated.

Material and Methods

Chemicals

All chemicals used in this study were of analytical grade purchased from Sigma-Aldrich Chemical Co. (Auckland, New Zealand). Ultrapure Milli-Q (MQ) water was used throughout the study which was obtained from a Milli-Q system (Millipore, Milford, MA).

Preparation of tea extracts

Dry leaves of regular black tea powder (R-BTE) were purchased from a local retail shop in New Zealand, while dry leaves of selenium-containing black tea (Se-BTE) were obtained from a tea plantation in China which is only 19 km away from a Se mine. 1% aqueous extracts were prepared using a modified infusion method of Molan et al. [5]. Prior to use, dry tea leaves were ground into fine powders. Then, 1 g of each of the powdered tea leaves were infused with 100 mL of MQ water at different brewing temperatures (50, 70, 90 and 100ºC) and allowed to brew for different lengths of time (2, 5 and 10 min) with continuous stirring. The infusions were decanted and filtered through Whatman No. 4 filter paper (0.45 mm) to remove insoluble solid matter.

Mineral analysis of dry tea material

Dry leaves of both SGT and CGT were ground into fine powders and sent to the Hill Laboratories (www.hill-labs.co.nz), Ruakura Research Centre, Hamilton, New Zealand for mineral analysis. The Ca, Fe, K, Na and P were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES), while Cu, Mn, Se and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS).

Determination of total phenolic content

The total phenolic content (TPC) of each tea sample was quantified using Folin-Ciocalteu's reagent, as described previously by Molan et al. [5]. The total polyphenol content of the tea extracts was calculated using a calibration curve for gallic acid and expressed as mg gallic acid equivalents per gram of tea leaves on a dry basis (mg GAE/g DW). Results are also expressed as mg of TPC estimated to be held in a typical serving of a common tea strength consumed (1 %, w/v).

Ferric-Reducing/Antioxidant Power (FRAP) assay

Antioxidant activity (AOA) of the tea extracts was determined using a modified FRAP assay [5]. The FRAP values were expressed as mg $FeSO_4$ equivalents per g tea leaves on a dry basis (mg $FeSO_4$ E/g DW), where the calculations were based on the standard curve of $FeSO_4 \cdot 7H_2O$. Results are also expressed as mg of FRAP estimated to be held in a typical serving of a common tea strength consumed (1 %, w/v).

Diphenyl-picrylhydrazyl (DPPH) radical scavenging activity assay

The antiradical activity of tea extracts was assessed on the basis of the ability to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical [5] with modification. The DPPH-scavenging activity was expressed as mg ascorbic acid (Vitamin C) equivalent antioxidant capacity per gram dry weight of tea leaves (mg VCEAC/g DW), calculated from the calibration curve of ascorbic acid standard solutions (17.6 – 176 µg/mL). Results are also expressed as mg of DPPH estimated to be held in a typical serving of a common tea strength consumed (1 %, w/v).

Statistica analysis

Absorbance readings of tea samples and standard were corrected for the blank and dilution factor before TPC, FRAP and DPPH values were determined. All measurements were carried out in triplicate across three independent experiments, and results statistically analyzed using SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA). Simple linear regression analysis was performed to calculate the dose-response relationship of standard solutions used for calibration; Fe(II), gallic acid and ascorbic acid. Pearson correlation coefficients (*R*) between assays were performed using the PROC CORR procedure to

achieve the correlation between the phenolic contents and antioxidant activity assays. One-way analysis of variance (ANOVA) was used to test for significance between means, while factorial ANOVA was performed to analyze the effect of tea extract, extraction temperature and time on the total phenolic contents. Both ANOVA tests employed Tukey's *posthoc* analysis. A difference was considered to be statistically significant when $p < 0.05$.

Results and Discussion

Mineral profile of teas

The Se-BTE and R-BTE powdered leaves were analyzed for mineral content (Fig. 1). Elemental analysis indicated that the Se-BTE leaves had a 23.7-fold higher total selenium concentration (1.42 versus 0.06 mg of Se/kg). Moreover, Se-BTE contains higher concentrations of phosphorus, zinc and copper than R-BTE (Fig. 1), but R-BTE contains more iron, boron, and manganese.

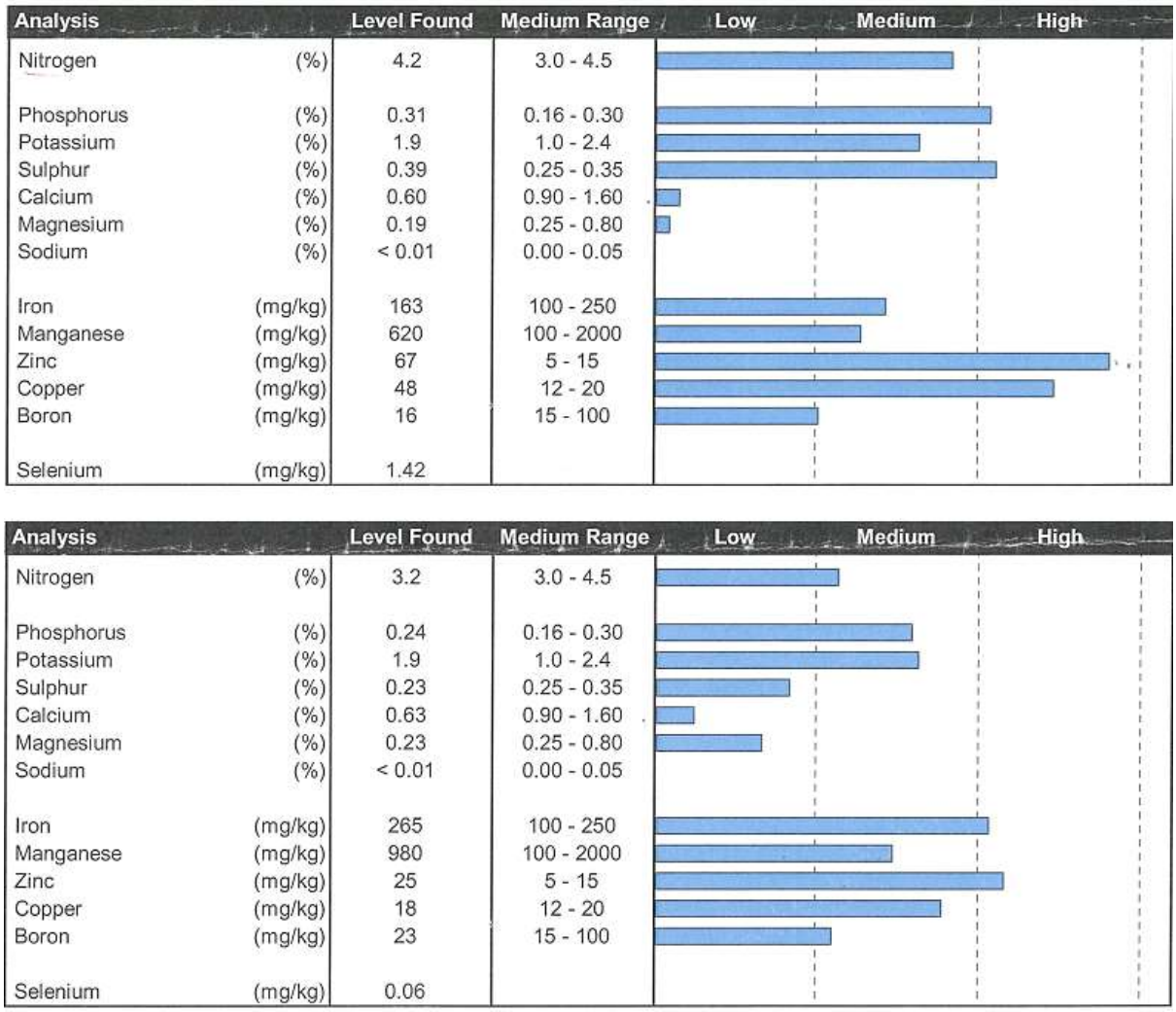

Fig. 1. Mineral profile of selenium-rich black tea (upper panel) and regular black tea (bottom panel). This Figure was provided by the Hill Laboratories (www.hilllabs.co.nz), Ruakura Research Centre, Hamilton, New Zealand.

Total phenolic content (TPC)

Phenolic compounds have drawn attention because of their significant antioxidant activities [5]. They have an important role in stabilizing lipid oxidation, scavenging reactive oxygen species and free radicals, breaking radical chain reaction and chelating metals [12]. The TPC values, FRAP values and DPPH-scavenging properties of the tea extracts obtained under different extraction conditions, as well as their respective estimated amounts in one cup servings are presented in Tables 1 and 2. The R-BTE showed significantly ($p < 0.05$) higher TPC values (57.6 ± 0.3 to 82.2 ± 0.4 mg GAE/g DW) than Se-BTE (34.7 ± 0.6 to 44.1 ± 0.2 mg GAE/g DW). The significant variation in TPC between the tested teas might be influenced by several factors such as different tea variety, geographical area, environmental conditions, as well as agricultural practices of the two teas [13].

All teas are derived from the Camellia plant (*Camellia sinensis*), which has two sub-varieties: *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*. The *sinensis* variety originates from China, while *assamica* originates mainly from India [14]. Fresh tea leaves from the *assamica* variety are generally higher in polyphenols than the *sinensis* variety [1, 15]. Thus, the locality effect of each tea and its variety might be another reason for the differences in TPC found in both black teas. Other contributing factors behind the TPC variations of different tea samples might be due to particle size [15], climate and region of production [16], genetic make-up of different tea variety [17], fermentation conditions of tea processing [18], as well as the plantation and harvesting season [13].

Our results show that TPC varies depending on the temperature of the water and infusion time, where TPC was found to increase with increasing extraction temperature and time, as previously encountered by Jayasekera et al. [13]. TPC increased significantly ($p < 0.05$) as extraction time increased from 2 to 5 min at all temperatures. However, after 10 min of infusion with extraction temperatures higher than 50°C, TPC decreased slightly but not significant. Dai and Mumper [19] explained that the use of high water temperatures for tea infusion increases TPC extraction, as well as reducing viscosity and surface tension. However, TPC extraction using high temperatures at a longer time may lead to the loss of unstable polyphenolic components in the tea extracts, as the result of degradation and oxidation of phenolics [19]. Therefore, the extraction of total phenolics, total flavonoids, catechins, and theaflavins from tea using water at the boiling point are relatively inefficient at infusion times less than 2 min [20]. This finding supports the results of previous studies conducted by Su et al. [21] which demonstrated a loss of phenolic contents when teas were extracted at 100°C for 10 min. There is further evidence that catechins, the major polyphenolic compounds in tea, tend to degrade when extracted in water at high temperatures [22] and for prolonged extraction times [23].

The initial extraction of TPC at 50°C for 2 to 5 min were lower than those obtained with higher temperatures (70 to 100°C). The TPC extracted at 50°C gradually increased after 5 to 10 min but was not significant, and this increase was not observed at other temperatures. The increase in TPC extraction at 50°C for 10 min might be due to low saturation of phenolics in the tea extracts, which allows a maximum solubility of the compounds. In addition, low extraction temperature could prevent phenolic compound degradation as previously explained by Dai and Mumper [19]. An appropriate infusion time and temperature for tea brewing has been generally considered as one of the critical factors for polyphenols extraction from tea leaves, such as catechins and theaflavins [15].

Table 1. Total phenolic content (TPC) of selenium-rich black tea (Se-BTE) and regular black tea (R-BTE), and their respective estimated amounts per cup serving (2g/serving).

Tea	Temperature (˚C)	Time (min)	TPC[a] (mg GAE/g DW)	TPC estimation in one cup of tea[b]
Se-BTE	100	2	37.2 ± 0.2	74.4
		5	39.7 ± 0.4	79.4
		10	38.9 ± 0.3	77.8
	90	2	40.2 ± 0.6	80.4
		5	44.1 ± 0.2	88.2
		10	42.4 ± 0.5	84.8
	70	2	35.8 ± 0.4	71.6
		5	40.8 ± 0.6	81.6
		10	39.4 ± 0.8	78.8
	50	2	34.7 ± 0.6	69.4
		5	36.4 ± 0.2	72.8
		10	38.3 ± 0.2	76.6
R-BTE	100	2	78.6 ± 0.3	157.2
		5	81.8 ± 0.5	163.6
		10	80.5 ± 0.5	161.0
	90	2	80.0 ± 0.3	160.0
		5	82.2 ± 0.4	164.4
		10	81.4 ± 0.2	162.8
	70	2	71.6 ± 0.3	143.2
		5	75.9 ± 0.3	151.8
		10	75.9 ± 0.3	151.8
	50	2	57.6 ± 0.3	115.2
		5	61.3 ± 0.3	122.6
		10	64.5 ± 0.3	129.0

*Values are expressed as mean ± SEM. [a]Total phenolic content (mg gallic acid equivalent (GAE)/g dry weight). [b]One serving is 200 mL of a 1% (w/v) tea infusion.

Antioxidant activities (AOA) of the tea extracts

FRAP values of the tea extracts and an estimated level in a one cup serving are shown in Table 2. The R-BTE showed significantly higher ($P < 0.05$) antioxidant power (86.8 ± 0.2 to 139.7 ± 0.3 mg $FeSO_4$ E/g DW) than Se-BTE (44.1 ± 0.2 to 67.0 ± 0.4 mg $FeSO_4$ E/g DW), a similar pattern as that shown by the TPC values of the tea extracts. As shown in Table 3, DPPH-scavenging activity is expressed as an ascorbic acid equivalent to represent the antioxidant capacity of tea through a commonly accepted measure [24], since ascorbic acid is an antioxidant compound usually found in nutritional labelling on food products. The R-BTE showed DPPH-scavenging values ranging from 129.8 ± 2.4 to 192.0 ± 4.2 mg VCEAC/g DW), which is significantly higher ($p < 0.0001$) than the Se-BTE (88.1 ± 1.2 to 113.0 ± 1.7 mg VCEAC/g DW).

In general, AOA was significantly increased in both teas extracted for 2 to 5 min as measured by FRAP and DPPH assay ($P < 0.05$). In line with results of the TPC, teas extracted for 10 min with temperatures higher than 50°C had slightly decreased AOA, but did not differ significantly. In contrast, AOA increased in both teas when extracted at 50˚C temperature for 5 to 10 min, but again this was not significant. The present study shows that both teas have similar and high DPPH-radical scavenger activity even after a 10-fold dilution of the tea, which indicates a promising potential for use as nutraceutical agents. Overall, using both antioxidant methods, the highest AOA was exhibited by the R-BTE

when extracted at 90°C for 5 min, whereas the Se-BTE extracted at 50°C for 2 min had the lowest AOA ($P < 0.05$).

Table 2. Ferric-reducing antioxidant power (FRAP) of selenium-rich black tea (Se-BTE) and regular black tea (R-BTE), and their respective estimated amounts per cup serving (2g/serving).

Tea	Temperature (˚C)	Time (min)	FRAP[a] (mg $FeSO_4$E/g DW)	FRAP estimation in one cup of tea[b]
Se-BTE	100	2	60.2 ± 0.3	120.4
		5	67.0 ± 0.4	134.0
		10	63.3 ± 0.9	126.6
	90	2	62.8 ± 0.4	125.6
		5	66.9 ± 0.7	133.8
		10	65.5 ± 0.7	131.0
	70	2	55.8 ± 0.3	111.6
		5	60.8 ± 0.6	121.6
		10	59.9 ± 0.9	119.8
	50	2	44.1 ± 0.2	88.2
		5	51.1 ± 0.4	102.2
		10	53.6 ± 0.3	107.2
R-BTE	100	2	132.2 ± 0.3	264.4
		5	135.0 ± 0.2	270.0
		10	133.6 ± 0.2	267.2
	90	2	137.1 ± 0.2	274.2
		5	139.7 ± 0.3	279.4
		10	139.1 ± 0.4	278.2
	70	2	125.8 ± 0.2	251.6
		5	128.0 ± 0.5	256.0
		10	127.1 ± 0.8	254.2
	50	2	86.8 ± 0.2	173.6
		5	98.8 ± 0.6	197.6
		10	101.9 ± 0.6	203.8

*Values are expressed as mean ± SEM. [a] Ferric-reducing antioxidant power (mg $FeSO_4$ equivalent/g dry weight). [b]One serving is 200 mL of a 1% (w/v) tea infusion.

Table 3. DPPH-scavenging activity of selenium-rich black tea (Se-BTE) and regular black tea (R-BTE), and their respective estimated amounts per cup serving (2g/serving).

Tea	Temperature (˚C)	Time (min)	DPPH[a] (mg VCEAC/g DW)	DPPH estimation in one cup of tea[b]
Se-BTE	100	2	97.7 ± 2.8	195.4
		5	105.8 ± 2.8	211.6
		10	100.2 ± 2.7	200.4
	90	2	103.5 ± 2.9	207.0
		5	113.0 ± 1.7	226.0
		10	111.6 ± 1.4	223.2
	70	2	90.8 ± 1.2	181.6
		5	105.1 ± 3.5	210.2
		10	106.2 ± 1.4	212.4
	50	2	88.1 ± 1.2	176.2
		5	94.4 ± 1.6	188.8
		10	99.1 ± 2.3	198.2
R-BTE	100	2	179.3 ± 2.2	358.6
		5	184.5 ± 2.6	369.0
		10	181.3 ± 3.3	362.6

	90	2	186.2 ± 3.8	372.4	
		5	192.0 ± 4.2	384.0	
		10	189.1 ± 3.9	378.2	
	70	2	177.7 ± 1.6	355.4	
		5	187.0 ± 1.7	374.0	
		10	184.1 ± 2.8	368.2	
	50	2	129.8 ± 2.4	259.6	
		5	150.9 ± 3.0	301.8	
		10	155.0 ± 2.4	310.0	

*Values are expressed as mean ± SEM. [a]Diphenyl-1-picrylhydrazyl radical scavenging activity (mg vitamin C equivalent antioxidant capacity/g dry weight). [b]One serving is 200 mL of a 1% (w/v) tea infusion.

Effect of extraction temperature and time on TPC of the tea extracts

As shown in Table 4, it was confirmed that the extraction efficiency of polyphenols was significantly affected by the type of tea ($F_{1,192}$ = 43915.00, p < 0.0001), extraction temperature ($F_{3,192}$ = 1115.72, p < 0.0001) and extraction time ($F_{2,192}$ = 168.00, p < 0.0001). The interaction effects of tea x temperature ($F_{3,192}$ = 492.92, p < 0.0001), temperature x time ($F_{6,192}$ = 13.47, p < 0.0001) and tea x temperature x time ($F_{6,192}$ = 3.23, p = 0.0048), were also significant, indicating that the effect of extraction temperature on TPC strongly depends on the extraction time (and *vice versa*). The optimal extraction condition was found to be 90°C at 5 min, as the tea extracts prepared under these conditions showed higher TPC than other extraction temperatures and times.

Table 4. Analysis of variance for the effect of tea variety, extraction temperature, extraction time and their interactions on total polyphenolic content by Folin – Ciocalteu's phenol reagents.

Source of variance	DF[a]	Total polyphenol	
		MS[b]	F value
Tea	1	67267.68	43915.00***
Temperature	3	1709.03	1115.72***
Time	2	257.34	168.00***
Tea x Temperature	3	755.04	492.92***
Tea x Time	2	3.89	2.54
Temperature x Time	6	20.64	13.47***
Tea x Temperature x Time	6	4.94	3.23**
Error	192	1.53	

[a] Degree of freedom; [b] Means squares; * p < 0.05, ** p < 0.01, ***p < 0.001

Correlation between TPC and AOA of the tea extracts

The AOA of all teas correlated well with their TPC (Table 5). A moderate to strong relationship was found between the TPC and AOA (FRAP and DPPH), which implies that TPC are the major contributors to the antioxidant activity of the tea extracts. Katalinic et al. [25] reported that polyphenol compounds are important ingredients that denote the antioxidant ability in teas. Most of antioxidant properties are well correlated with the corresponding polyphenolic compounds rather than ascorbic acid [26], and that the relationship is highly dependent on the extraction conditions used [23].

In conclusion, R-BTE contained significantly higher TPC, and exhibited significantly higher ferric-reducing/antioxidant power and DPPH-scavenging activities than Se-BTE. These differences may be due to tea varieties, environmental conditions and agricultural practices from which the tea plants were grown and processed, but not necessarily on the level of selenium the tea contains.

Table 5. Correlation coefficients between total phenolic content (TPC) and antioxidant properties of water extract from Se-rich black tea and regular black tea. The antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays.

		Correlation coefficients (*R*)	
Sample	TPC *vs* FRAP	TPC *vs* DPPH	FRAP *vs* DPPH
Se-BTE	0.7173***	0.6984***	0.5761***
R-BTE	0.9772***	0.8705***	0.8877***

* $p < 0.05$, ** $p < 0.01$, ***$p < 0.0001$

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Karori, S.M., Wachira, F.N., Wanyoko, J.K. and Ngure, R.M. (2007). Antioxidant capacity of different types of tea products. Afr. J. Biotech., 6: 2287-2296.
2. Ostadalova, M., Pazout, V., Straka, I., Tauferoya, A., Bartl, P. and Pokorna, J. (2011). Monitoring tea pigments theaflavins and thearubigins in dependence on the method and duration of storage. J. Food Technol., 9: 50-56.
3. Bandyopadhyay, P., Ghosh, A.K. and Ghosh, C. (2012). Recent developments on polyphenol-protein interactions: Effect on tea and coffee taste, antioxidant properties and the digestive system. Food Funct., 3: 592-605.
4. Das, A.S., Mukherjee, M., Das, D. and Mitra, C. (2009). Protective action of aqueous black tea (*Camellia sinensis*) extract (BTE) against ovariectomy-induced oxidative stress of mononuclear cells and its associated progression of bone loss. Phytother. Res., 23: 1287-1294.
5. Molan, A.L., Flanagan, J., Wei, W. and Moughan, P.J. (2009). Selenium-containing green tea has higher antioxidant and prebiotic activities than regular green tea. Food Chem., 114: 829-835.
6. Yuan, J-M., Sun, C. and Butler, L.M. (2011). Tea and cancer prevention: Epidemiological studies. Pharmacol. Res., 64: 123-135.
7. Jose, P.A. and Marco, A. (2012). Protective effects of chronic green tea consumption on age-related neurodegeneration. Curr. Pharm. Design, 18: 4-14.
8. Grove, K.A., Sae-tan, S., Kennett, M.J. and Lambert, J.D. (2011). (-)-Epigallocatechin-3-gallate inhibits pancreatic lipase and reduces body weight gain in high fat-fed obese mice. Obesity, 20: 2311-2313.
9. Vuong, Q.V., Stathopoulos, C.E., Nguyen, M.H., Golding, J.B. and Roach, P.D. (2011). Isolation of green tea catechins and their utilization in the food industry. Food Rev. Int., 27: 227-247
10. Hu, Q., Xu, J. and Pan, G. (2001). Effect of selenium spraying on green tea quality. J. Sci. Food Agric., 81: 1387-1390.

11. Li, F., Wang, F., Yu, F., Fang, Y., Xin, Z., Yang, F., Xu, J., Zhao, L. and Hu, Q. (2008). *In vitro* antioxidant and anticancer activities of ethanolic extract of selenium-enriched green tea. Food Chem., 111: 165-170.
12. Jayathilakan, K., Sharma, G.K., Radhakrishna, K. and Bawa, A.S. (2007). Antioxidant potential of synthetic and natural antioxidants and its effect on warmed over-flavour in different species of meat. Food Chem., 105: 908-916.
13. Jayasekera, S., Molan, A.L., Garg, M. and Moughan, P.J. (2011). Variation in antioxidant potential and total polyphenol content of fresh and fully-fermented Sri Lankan tea. Food Chem., 125: 536-541.
14. De la Paix, M.J., Ge, J., Habiyaremye, G. and Nadege, T.L. (2010). Materials on rivers around tea growing areas of Rwanda. International Conference on Environmental Science and Information Application Technology *(ESIAT)*, 3: 406-411.
15. Astill, C., Birch, M.R., Dacombe, C., Humphrey, P.G. and Martin, P. (2001). Factors affecting the caffeine and polyphenol contents of black and green tea infusions. J. Agric. Food Chem., 49: 5340-5347.
16. Bhattacharya, S. and Sen-Mandi, S. (2011). Variation in antioxidant and aroma compounds at different altitude: A study on tea (*Camellia sinensis* L. Kuntze) clones of Darjeeling and Assam, India. Afr. J. Biochem. Res., 5: 148-159.
17. Magoma, G.N., Wachira, F.N., Obanda, M., Imbuga, M. and Agong, S.G. (2000). The use of catechins as biochemical markers in diversity studies of tea (*Camellia sinensis*). Genet. Resour. Crop Ev., 47, 107-114.
18. Obanda, M., Owuor, P.O. and Mang'oka, R. (2001). Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. Food Chem., 75: 395-404.
19. Dai, J. and Mumper, R.J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules, 15: 7313-7352.
20. Lakenbrink, C., Lapczynski, S., Maiwald, B. and Engelhardt, U.H. (2000). Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. J. Agric. Food Chem., 48: 2848-2852.
21. Su, X., Duan, J., Jiang, Y., Duan, X. and Chen, F. (2007) Polyphenolic profile and antioxidant activities of oolong tea infusion under various steeping conditions. Int. J. Mol. Sci., 8: 1196-1205.
22. Robinson, E.E., Maxwell, S.R.J. and Thorpe, G.H.G. (1997). An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. Free Rad. Res., 26: 291-302.
23. Komes, D., Horžić, D., Belščak, A., Ganić, K.K. and Vulić, I. (2010). Green tea preparation and its influence on the content of bioactive compounds. Food Res. Int., 43: 167-176.
24. Kim, D.O., Lee, K.W., Lee, H.J. and Lee, C.Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. J. Agric. Food Chem., 50: 3713-3717.
25. Katalinic, V., Milos, M., Kulisic, T. and Jukic, M. (2006). Screening of 70 medicinal plants extracts for antioxidant capacity and total phenolics. Food Chem., 94: 550-557.

26. Vamanu, E. and Nita, S. (2013). Antioxidant capacity and the correlation with major phenolic compounds, anthocyanin, and tocopherol content in various extracts from the wild edible Boletus edulis mushroom. Biomed. Res. Int., 2013: 1-11.