

FINLAY GREEN TEA POSSESSES THE HIGHEST IN VITRO ANTIOXIDANT ACTIVITY AMONG THE 20 COMMERCIALY AVAILABLE TEA BRANDS OF BANGLADESH

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In vitro antioxidant activities of water extract of 20 brands of tea of Bangladesh were assessed in the present investigation. Antioxidant activities were determined by estimating total antioxidant capacity (TAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) and ferrous ion chelating (FIC) ability. Finlay Green Tea had the highest polyphenol content [103.0±0.3 mg gallic acid equivalent (GAE)/g], concurrently with the highest DPPH-radical scavenging activity (IC₅₀) (19.0±3.0 µg/mL), FRAP (97.0±1.4 mg GAE/g) and TAC (325.0±0.6 mg GAE/g) but moderate FIC ability [1.22±0.09 mg disodium ethylenediaminetetracetate (EDTA)/g]. The level of antioxidant activity, without FIC ability, was strongly associated with the total phenolic content (TPC). Therefore, the teas of Bangladesh, especially the green tea (*Camellia sinensis* L.), may serve as a potential dietary source of natural phenolic antioxidants.

Keywords: Antioxidant property, Tea, Natural phenolic antioxidants, Polyphenols

INTRODUCTION

Tea is the most commonly drunk beverage on earth and is being consumed socially and habitually by people since 3000 BC. Tea (*Camellia sinensis* L.), a cultivated evergreen plant, is native to China, later spread to India and Japan, then to Europe and Russia, arriving in the New World in the late 17th century (Sharangi 2009). Antioxidants are capable of stabilising or deactivating reactive oxygen species (ROS) such as hydroxyl radical, ferryl ion, superoxide radical anion, peroxy radical and hydrogen peroxide, which are induced by oxidative stress, before they later attack cells and biological targets. Antioxidants are therefore believed to be crucial for maintaining optimal cellular and systemic health and well-being (Rahman 2007; Dufresne and Farnworth 2001). During the last decade, the effects of tea and tea polyphenols were extensively investigated and studies showed that tea is capable of lowering the risk of cardiovascular diseases and cancers (Chen *et al.* 2008; Mukamal *et al.* 2007), reducing body fat, systolic blood pressure (SBP), and low density lipoprotein (LDL) cholesterol (Nagao, Hase and Tokimitsu 2007). Among age-associated pathologies and neurodegenerative diseases, green tea was shown to confer significant

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protection against Parkinson's disease and Alzheimer's disease (Hu *et al.* 2007; Rezai-Zadeh *et al.* 2005). On the other hand, persistent tea consumption by mothers during pregnancy might be associated with an increased risk of preeclampsia, especially severe preeclampsia (Wei *et al.* 2009).

Little information is reported in the literature regarding the antioxidant activity and the total phenolics of different brands of teas in Bangladesh. Therefore, the present study was undertaken to investigate total phenolic content (TPC), total antioxidant capacity (TAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging activity, ferric reducing antioxidant power (FRAP) and the ferrous ion chelating (FIC) ability.

METHODS

Chemicals

Twenty different brands of teas were purchased from departmental stores of Dhaka, the capital of Bangladesh. The teas were used as sold by the vendor without further identification. A voucher specimen was retained in the Department of Biochemistry and Molecular Biology, University of Dhaka for further reference. Folin-Ciocalteu's reagent (2N) and DPPH was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, potassium ferricyanide, anhydrous ferric chloride, di-sodium hydrogen phosphate, potassium di-hydrogen phosphate, 1,10-phenanthroline monohydrate, tris-hydrochloric acid (Tris-HCl) and sulphuric acid were purchased from Merck (Darmstadt, Germany). Disodium ethylenediaminetetraacetate (EDTA) was purchased from Invitrogen (Carlsbad, California, USA). Trichloroacetic acid and ammonium molybdate were purchased from Merck (Mumbai). All chemicals and solvents used were of analytical grade.

Extraction of Teas

Tea powder (1 g) was extracted with 50 mL boiling water and allowed to steep for 1 h with continuous swirling. Extracts were filtered through Whatman No. 1 filter paper, aliquoted and stored at 4°C for immediate analyses and/or stored at -20°C if analyses were performed later. Analyses of aqueous tea extracts were done in triplicate.

Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu assay (Turkmen *et al.* 2007). Samples (1.0 mL, in triplicate) were introduced into test tubes followed by 1.0 mL of Folin-Ciocalteu's reagent (diluted 3 times with deionised water) and 2.0 mL of sodium carbonate (35%, w/v). The mixture was shaken thoroughly and diluted to 6 mL with deionised water. The tubes were allowed to stand for 30 min before the measurement of absorbance at 700 nm (Shimadzu UV-VIS mini 1240, Shimadzu Corporation, Kyoto). TPC was expressed as gallic acid equivalent (GAE) in mg per g extract.

Ferric Reducing Antioxidant Power (FRAP)

FRAP of tea extracts was determined using the procedure of Turkmen *et al.* (2007). Different dilutions of extracts (0.5 mL) were added to 1.25 mL phosphate buffer (0.2 M,

pH 6.6) and 1.25 mL of potassium ferricyanide (1%, w/v). The mixture was incubated at 50°C for 20 min. After trichloroacetic acid solution (1.25 mL, 10%, w/v) was added, the mixture was separated into aliquots of 1.25 mL and diluted with 1.25 mL of water. To each diluted aliquot, 0.250 mL of ferric chloride solution (0.1%, w/v) was added. After 10 min, absorbance was measured at 700 nm. FRAP of extracts was expressed as mg GAE/g.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was measured by measuring the decrease in absorbance of methanolic DPPH solution at 517 nm in the presence of tea extract (Turkmen *et al.* 2007). The hot water extract was diluted with methanol and 100 µL of diluted extracts (with different concentrations) were added to 100 µL with methanolic DPPH solution (final concentration of 0.4 mM). The reaction mixture was vortex mixed and allowed to stand for 30 min at room temperature in the dark before the absorbance at 517 nm was measured using methanol as blank. Antioxidant activity was expressed as percentage inhibition (% I) of the DPPH radical and was determined by the following equation: % I = [1 - Absorbance (Abs) of the sample/Abs of the control] x 100. Data of % I were subjected to nonlinear regression analysis using dose-response curve for inhibition. The unit for the concentration of the extracts required to decrease the absorbance of the DPPH by 50% (IC₅₀) was µg of tea (powder)/mL hot water extract.

Total Antioxidant Capacity (TAC)

The assay is based on the reduction of molybdenum (VI)-molybdenum (V) [Mo (VI)-Mo (V)] by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Banerjee, Dasgupta and De 2005). Different dilutions of extracts (0.1 mL) were combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance of the samples was measured at 695 nm. Results of the TAC were expressed as mg GAE/g (mg of gallic acid/gm of solid tea).

Ferrous Ion Chelating (FIC) Ability

FIC ability of the tea extracts was determined according to the method of Olabinri *et al.* (2010). In this assay, the tea extract binds with Fe²⁺ ion generated in vitro using 500 µM iron (II) sulphate as ion donor. Different dilutions of tea extracts (0.2 mL) were added to Tris-HCl (0.336 mL, 0.1 M, pH 7.4) and 0.436 mL of saline (0.9%, w/v). After 5 minutes, 0.26 mL of 1, 10-phenanthroline (0.25%, w/v) was added to the reaction mixture. The absorbance of the samples was measured at 510 nm against control which consists of Tris-HCl, saline and phenanthroline without the tea extract. The ability of extracts to chelate ferrous ions was calculated as mg EDTA/g of solid tea.

Statistical Analysis

The experimental results obtained were expressed as mean±SEM. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 16 (Chicago, IL, USA).

RESULTS

Total Phenolic Content (TPC)

TPC of 20 tea samples are shown in the Figure 1. Finlay Green Tea showed significantly ($p < 0.05$) higher TPC (103.0 ± 0.3 mg GAE/g) than those of other brands of tea. The second highest TPC was found in the Ispahani Zareen Tea, while the third highest content was found in the Kazi and Kazi (Black) brand. The HRC Clone Tea, Lipton Taza, Kazi Tea State and Ispahani Mirzapur Tea Premium brands had TPC in the range of 69~74 mg GAE/gm. All other brands contained total polyphenol in the range of 57~65 mg GAE/gm.

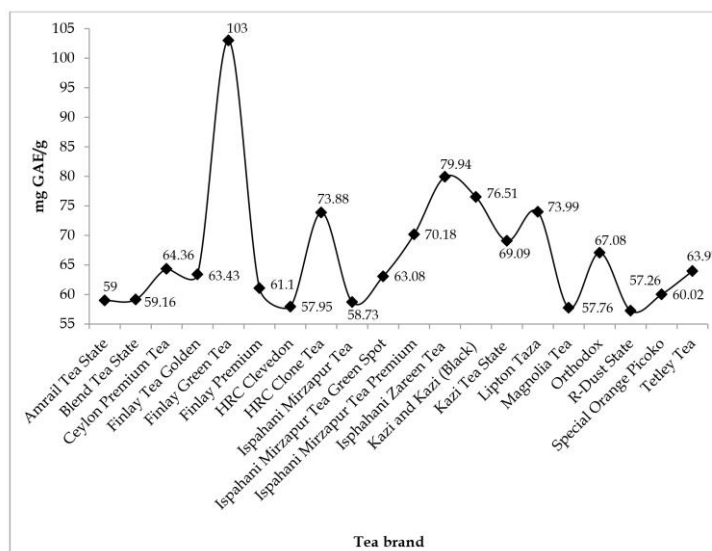


Fig. 1: TPC of different tea brands. TPC was determined against GAE.

DPPH Radical Scavenging Activity

DPPH-radical scavenging activities of teas are shown in the Table 1, in term of IC_{50} . Finlay Green Tea showed the highest DPPH-radical scavenging activity (19.0 ± 3.0 μ g tea/mL hot water). The next one was Tetley Tea, which contained 27.0 ± 2.0 μ g tea/mL. For the others, IC_{50} values ranged 31–104 μ g/mL. DPPH activity also showed significant correlations with TPC and TAC ($R^2 = 0.568$, $p < 0.05$ and $R^2 = 0.491$, $p < 0.05$, respectively).

Total Antioxidant Capacity (TAC)

The phosphomolybdenum assay usually detects antioxidants such as ascorbic acid, some phenolics, α -tocopherol and carotenoids. A higher absorbance indicates a higher antioxidative activity. TAC of the Finlay Green Tea (324.9 ± 0.6 mg GAE/g) was considered as 100%, while those of the others were calculated against the TAC of the Finlay Green Tea brand (Fig. 2). Total antioxidant capacities of the teas of other brands were one third to one fourth (22%~33%) of that of the Finlay Green Tea brand.

Table 1: Antioxidant potentials of different tea brands of Bangladesh.

Name of tea brand	*IC ₅₀ value (µg/mL)	FRAP (mg GAE/g)	FIC ability (mg EDTA/g)
Finlay Green Tea	19.0±3.0 ^a	97.0±1.4 ^d	1.02±0.07 ^f
Tetley Tea	27.0±2.0 ^b	60.0±0.2	1.59±0.05 ^{ij}
Ispahani Mirzapur Tea Green Spot	31.0±4.0 ^{bc}	47.0±0.4 ^j	1.88±0.02 ^k
Finlay Premium	32.0±1.0 ^{bc}	55.0±0.9 ^{ef}	1.73±0.05 ^{gh}
Ceylon Premium Tea	34.0±2.0 ^{bc,d}	58.0±0.5 ^a	1.41±0.05 ^{de}
Kazi and Kazi (Black)	35.0±3.0 ^{cd}	63.0±0.2 ^m	1.49±0.03 ^{ej}
HRC Clone Tea	39.0±1.0 ^{d,e}	67.5±0.3 ^h	1.33±0.11 ^{bd}
Magnolia Tea	39.0±4.0 ^{d,e}	56.0 ±0.1 ^f	1.78±0.07 ^{kg}
Special Orange Picoko	40.0±3.0 ^{d,e}	55.0±0.3 ^{ef}	1.42±0.07 ^{de}
Blend Tea State	43.0±5.0 ^{ef}	58.0±0.3 ^{ab}	1.24±0.03 ^{bc}
HRC Clevedon	43.0±3.0 ^{ef}	59.0±0.6 ^g	1.36±0.05 ^d
Finlay Tea Golden	44.0±1.0 ^{ef,g}	53.0±0.2 ^c	1.22±0.09 ^{bc}
Kazi Tea State	46.0±5.0 ^{ef,g}	68.0±0.5 ^h	1.54±0.09 ^{ij}
Amrail Tea State	50.0±5.0 ^{g,h}	58.0±0.8 ^{ab}	0.75 ±0.05 ^{ab}
Ispahani Mirzapur Tea Premium	51.0±5.0 ^{g,h}	58.0±0.3 ^{ab}	1.24±0.03 ^{bc}
Lipton Taza	55.0±8.0 ^{h,i}	57.0±0.2 ^b	1.55±0.05 ^{ij}
Orthodox	57.0±3.0 ^{h,i}	66.20±0.0 ⁿ	0.97±0.05 ^f
Ispahani Zareen Tea	73.0±2.0 ^j	64.0±0.5	2.06±0.05 ^l
Ispahani Mirzapur Tea	75.0±1.0 ^j	55.0±0.5 ^{ei}	1.64±0.03 ^{ih}
R-Dust State	104.0±5.0 ^k	54.0±0.3 ⁱ	1.58±0.05 ^{ij}

Notes: Results have been expressed as mean ± Standard Error Mean (SEM). Values that do not share a common superscript are significantly different at $p < 0.05$. *Data of percentage of inhibition were subjected to nonlinear regression analysis using dose-response curve for inhibition. DPPH concentration used was 0.4 mM in ethanol. Unit of IC₅₀ value was µg of tea (powder)/mL. Except for the Finlay Green Tea, all other teas are black tea as they are confirmed by the manufacturer on the packet.

Ferric Reducing Antioxidant Power (FRAP)

Finlay Green Tea exhibited the highest (97.0±1.4 mg GAE/g) FRAP. The FRAP of other brands ranged from 47.0±0.4 (Ispahani Mirzapur Tea) to 71.0±0.3 (Ispahani Mirzapur Tea Premium) (Table 1).

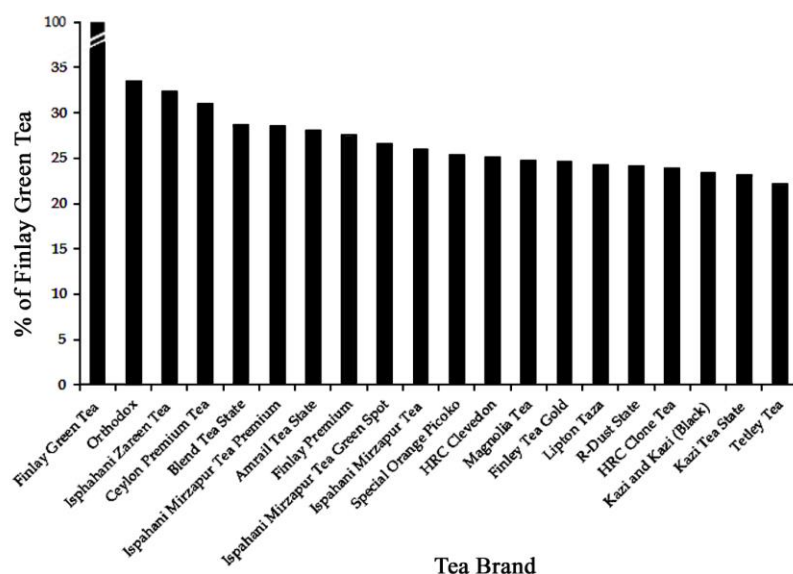


Fig. 2: TAC of different tea brands. TAC was determined against GAE.

Ferrous Ion Chelating (FIC) Ability

Metal chelating activity is also shown in the Table 1. FIC ability ranged from 0.75 ± 0.05 (Amrail Tea State) to 2.06 ± 0.05 mg EDTA/g (Ispahani Zareen Tea) ($p < 0.05$) (Table 1). FIC ability showed significant correlations with FRAP, DPPH and TAC ($R^2 = 0.167$, $p < 0.05$; $R^2 = 0.226$, $p < 0.05$; $R^2 = 0.107$, $p < 0.05$), respectively. On the other hand the correlation with TPC was poor and insignificant ($R^2 = 0.018$, $p > 0.05$).

DISCUSSION

Tea is the chief non-alcoholic beverage drinks in Bangladesh and increasingly being recognised as one of the important 'health drinks' in view of its significant medicinal value. Bangladesh produces about 56 million kg of tea per year. The domestic markets consume about 41 million kg, though the local consumers are mostly unaware of the beneficial effects particularly of the tea's impact on oxidative defence in maintaining the good health. Therefore, the intention of this investigation was to evaluate the TPC of different tea brands and whether the hot water extract could act as antioxidant. The antioxidant potentials were evaluated by determining TAC, ferric reducing ability, ferrous chelating ability and DPPH-free radical scavenging activity. Analyses of TPC of different tea revealed that Finlay Green Tea brand had the highest TPC ($p < 0.05$), and other brands of the tea varied and ranged 50–80 mg of GAE per gram of tea (Fig. 1). Finlay Green Tea showed significantly higher TPC value than those of other teas, including Kazi and Kazi (Black) tea. Higher values for TPC in Argentinian green and black tea have been reported (Anesini, Ferraro and Filip 2008). These values are, however, not comparable to ours, since Anesini, Ferraro and Filip (2008) used methanol as the extractant. Chan, Lim and Chew

(2007) found, however, a value of 191 mg GAE/g in an aqueous extract of Malaysian green tea, compared to 103 mg GAE/g for Finlay Green Tea by us. The higher content of total phenolics in green tea than in black tea reported by Anesini, Ferraro and Filip (2008) and by Chan *et al.* (2010), who used water as extractant, is in accordance with our results. After having the TPC data of the teas of our local market, the antioxidant power were evaluated by using a variety of methods, such as TAC, DPPH-free radical scavenging activity, FRAP and FIC ability. The phosphomolybdate assay usually detects total antioxidants such as ascorbic acid, some phenolics, α -tocopherol and carotenoids. A higher absorbance indicates a higher antioxidative activity. Because of its simplicity, it was decided to extend its application to tea extracts. Except Finlay Green Tea (325 mg GAE/g), the TAC in tea was found to vary from 72 (Tetley Tea) to 109 mg GAE/g (Orthodox) (Fig. 2). Finlay Green contained about 3 to 5 times higher level of TAC than those of other teas. There was no report available on the TAC of tea extracts by using this method. DPPH, a nitrogen centred free radical, is one of the most commonly used substrates for fast evaluation of antioxidant activity because of its stability (in radical form) and simplicity of the assay (Bozin *et al.* 2008). The amount of tea required to scavenge 50% of DPPH, IC_{50} , is summarized in Table 1. The lower the IC_{50} , the higher is the antioxidant activity (Brand-Williams, Cuvelier and Berset 1995). Finlay Green Tea had the lowest IC_{50} value ($19.0 \pm 3.0 \mu\text{g/mL}$) of the teas tested (Table 1). Chan, Lim and Chew (2007) reported similar values to ours for DPPH scavenging activity for different types of tea and different procedures. The antioxidant properties of different compounds within a group can vary remarkably so that the same levels of phenolics do not necessarily correspond to the same antioxidant responses (Parejo *et al.* 2002; Zheng and Wang 2001). Moreover, the response of phenolics in the Folin-Ciocalteu assay also depends on their chemical structure, and the radical-scavenging capacity of an extract cannot be predicted on the basis of its TPC content alone (Parejo *et al.* 2000).

The reducing power of a compound serves as a significant indicator of its antioxidant activity (Joshi, Verma and Mathela 2010). Assay of reducing activity is based on the reduction of Fe^{3+} / ferricyanide complex to the ferrous form in the presence of reductants (antioxidants) in the tested samples. The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu 1986). Except very high value for Finlay Green Tea ($97 \pm 1.4 \text{ mg GAE/g}$), FRAP ranged from 47 ± 0.4 (Ispahani Mirzapur Tea) to 71 ± 0.2 (Ispahani Mirzapur Tea Premium) (Table 1). The highest FRAP was found in Finlay Green Tea, and this value is similar to those reported for different brands of commercially available green teas of Malaysia (Chan *et al.* 2010; Chan, Lim and Chew 2007). Values for black teas in our investigation are also in good accordance with values given by Chan *et al.* (2010) and Chan, Lim and Chew (2007). In this study, correlation between FRAP and TPC was significantly strong ($R^2 = 0.732$, $p < 0.05$). Turkmen *et al.* (2007) also reported strong correlation between TPC and FRAP of different tea extracts.

Finally, the antioxidant potentials of the tea extracts were evaluated by determining their FIC ability (Table 1). Metal chelating activity is one of the better understood antioxidant mechanisms because of its ability to reduce the concentration of the catalysing transition metals in lipid peroxidation. Among the transition metals, iron is the most effective lipid oxidation pro-oxidant due to its high reactivity (Qiao *et al.* 2009). FIC ability measures the ability of secondary antioxidants to chelate metal ions. Primary antioxidants prevent oxidative damage by directly scavenging free radicals, while secondary antioxidants act indirectly by preventing the formation of free radicals through

Fenton's reaction (Chan *et al.* 2010). FIC ability ranged from 0.75 ± 0.05 (Amrail Tea State) to 2.06 ± 0.05 mg EDTA/g (Ispahani Zareen Tea) ($p < 0.05$) (Table 1). The high FIC ability of Ispahani Zareen Tea, Ispahani Mirzapur Tea, Magnolia Tea and Finlay Premium suggests that they contain greater amounts of ligands that react very well to chelate ferrous metal ions (Chan, Lim and Chew 2007). Although Finlay Green Tea showed high level of TPC and strong antioxidant activity as compared to that of the Kazi and Kazi Black tea, it had poorer FIC ability than that of the Kazi and Kazi (Black) tea. Previous studies also reported that green teas contained higher TPC, FRAP and DPPH radical scavenging activity but poorer FIC ability than black teas (Chan, Lim and Chew 2007; Chan and Lim 2006). We also examined the correlation between TPC and the antioxidant potential. Significantly strong correlation was found between TAC with TPC and FRAP ($R^2 = 0.607$, $p < 0.05$ and $R^2 = 0.684$, $p < 0.05$ respectively). In contrary to our results, Marwah *et al.* (2007) reported a poor correlation between TPC and antioxidant activity of some wound healing plants of Oman by using this method. FIC ability showed significant correlations with FRAP and TAC ($R^2 = 0.167$, $p < 0.05$; $R^2 = 0.10$, $p < 0.05$), respectively.

CONCLUSION

This study is the first report on the antioxidant activities and the phenolic contents of the different brands of teas available in Bangladesh. Most of the methods used for antioxidant activity showed strong correlation with TPC. Regarding the putative importance of antioxidant in various degenerative diseases, it can be concluded that teas from Bangladesh can be an important dietary source of antioxidants and could act as one of the important health drinks.

ACKNOWLEDGEMENT

The authors are thankful to Bangladesh Rice Research Institute (BRRI) for providing some chemicals and reagents used in the study.

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