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Comparative assessment of total polyphenols, antioxidant and antimicrobial activity of different tea varieties of Bangladesh

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ABSTRACT

Objective: To determine the total polyphenol content, antioxidant activity and antibacterial properties of the extracts of different Bangladeshi tea varieties such as flowery broken orange pekoe, broken orange pekoe, red dust and green tea.

Methods: Total phenolic and flavonoid contents were determined by Folin–Ciocalteu and aluminum chloride colorimetric assay, respectively. The antioxidant capacity was determined by ferric ion reducing antioxidant power and phosphomolybdenum method. Antibacterial activity was evaluated by disc diffusion method in agar plate and subsequently, the minimum inhibitory concentration was determined by broth dilution method.

Results: Total phenolic and flavonoid contents were significantly higher ($P < 0.05$) in green tea compared to other three black tea varieties. The green tea also showed a higher free radical scavenging and antioxidant activities than all the other tea varieties tested ($P < 0.05$). In addition, the extracts of all four tea varieties showed inhibitory activity against several pathogenic bacteria and also the same trend of higher antimicrobial activity of green tea than other tea varieties was observed.

Conclusions: Taken together, the results of this study demonstrated that Bangladeshi tea, especially the green tea, may act as a substitute for natural antioxidants and as a promising antibacterial agent for beneficial influence in human health.

1. Introduction

Tea, an aqueous extract derived from *Camellia sinensis* (*C. sinensis*) plant, is one of the most widely consumed beverages in the world. Based on the manufacturing process, teas are classified into several groups such as green tea (non-fermented), oolong tea (semi-fermented) and black tea (fully fermented) [1]. Among the various grades of black tea flowery broken orange pekoe (FBOP), broken orange pekoe (BOP) and red dust are widely known in South Asia [2]. Black tea is consumed mostly in North America, Europe and South Asia, whereas green and oolong teas are consumed mainly in East Asian countries [3–5]. Numerous studies have reported that drinking tea imparts various physiological and pharmacological benefits which include, antidiabetic, anti-inflammatory, antioxidant, anticholesterolemic, antimutagenic, anticarcinogenic and antibacterial activities [2–8].

The active components playing key roles in most of the biological activities of tea are known to be catechins (also known as polyphenols) [2,3]. Moreover, tea polyphenols are well-known for possessing antioxidant properties. The presence of different forms of catechins and their derivatives in both green and black teas made them capable of working as potential antioxidants [9]. Due to being non-fermented, green tea is thought to contain higher polyphenols and thereby has greater antioxidant potential than black tea [10,11]. Stronger antioxidant potential of green tea is mainly attributed to catechin derivatives such as epicatechin gallate and epigallocatechin gallate [4,5,10,12]. Numerous studies have indicated that tea catechins and polyphenols are effective scavengers of free radicals/reactive oxygen species generated due to various oxidative stress [1,13]. We have recently reported that tea extract prevents damage of cellular DNA *in vitro* caused by arsenic-mediated oxidative stress [13].

Tea polyphenols also exhibit remarkable antibacterial activity [1,3,14]. In general, an inverse relationship exists between the

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antibacterial activities of tea and the extent of tea fermentation, suggesting a stronger antibacterial activity of green tea than black tea [15,16]. Several studies have shown that both green and black tea have antibacterial activity against both Gram positive and Gram negative bacteria [3,17,18]. Although great deals of work have been performed, however, the precise antibacterial spectrum of tea extract is difficult to assess because there are many conflicting reports of presumptive antimicrobial activity. The main reason for the variability of tea's antibacterial activity is probably due to involvement of different research laboratories that generate the results with their optimized methods. It seems thus more reasonable to look at the results obtained from a single laboratory. Therefore, this study aims to compare the total antioxidant contents and antibacterial activities of four commercially available tea varieties of Bangladesh such as green tea, BOP, FBOP and red dust.

2. Materials and methods

2.1. Preparation of tea extract

Four different varieties of tea such as FBOP, BOP, red dust and green tea were collected from the local market as granular powder of a particular local brand. Tea extract was prepared according to the method described previously [3]. Briefly, tea powder (50 g) was mixed with 300 mL distilled water and boiled for 45 min. All the granular materials were removed by filtration with Whatman No. 1 filter paper. Filtered tea extract was then lyophilized in order to obtain dry fine powder. The yield of boiling water extracts for BOP, FBOP, red dust and green tea were 13.94%, 13.32%, 13.56% and 11.87%, respectively. Dry fine powder was stored at 4 °C and when necessary it was dissolved in phosphate buffered saline (PBS) to make 400 mg/mL working solution.

2.2. Determination of total polyphenol content (TPC) of tea extract

TPC was determined by spectrophotometry against gallic acid (GA) as standard by the Folin–Ciocalteu method [19]. Aqueous solution of tea extract at a concentration of 500 μg/mL was used in this analysis. Briefly, 0.5 mL of the diluted aqueous extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent dissolved in water and then vortexed. Afterwards, 2.5 mL of 7.5% $NaHCO_3$ was added. The tubes were then allowed to stand at room temperature for 60 min. Absorbance was measured at 765 nm against water. Each sample was prepared in triplicate. The same procedures were repeated for standard solution. The TPC was expressed as gallic acid equivalents (GAE) in mg of GA/g of tea extract.

2.3. Determination of total flavonoid content (TFC) of tea extract

TFC content of tea extract was determined by spectrophotometry against catechin as standard using aluminum chloride colorimetric assay [20]. Briefly, 1 mL of aqueous tea extract at concentration of 30 mg/mL was dissolved in 10% 0.3 mL $AlCl_3$ solution and 5% 0.3 mL $NaNO_2$ followed by addition of 200 μL NaOH. The sample was incubated for 1 h at room temperature. Absorbance was measured at 510 nm against water. Each sample was prepared in triplicate. The same procedure was repeated for the standard solution. The TFC was expressed as catechin equivalents in mg of catechin/g of tea extract.

2.4. Reducing power assay (iron reducing activity)

The total reducing power of four varieties of *C. sinensis* extracts was determined according to previously described method [21]. Briefly, 2.5 mL of various concentrations of four varieties of tea extracts were mixed with 2.5 mL of 0.2 mol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Next, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 650 r/min for 10 min. The upper layer (5 mL) of the solution was gently mixed with 5 mL deionized water and 1 mL of 0.1% of ferric chloride. After that, the absorbance was measured at 700 nm. Each assay was performed in triplicate and the results are expressed as mean ± SD.

2.5. Determination of total antioxidant capacity using phosphomolybdenum method

The antioxidant activity of four varieties of tea extracts was determined by the phosphomolybdenum method according to the method described previously [22]. Briefly, 0.3 mL of the extract (200 μg/mL, 600 μg/mL, and 1 000 μg/mL) was mixed with 3 mL of reagent solution (0.6 mol/L sulfuric acid, 28.0 mmol/L sodium phosphate, 4.0 mmol/L ammonium molybdate). For blank, 0.3 mL of methanol was used instead of tea extract. The mixture was then incubated in water bath at 95 °C for 90 min. After the reaction became cooled, the absorbance was taken at 695 nm. Each sample was prepared in triplicate. The antioxidant capacity of each sample was expressed as ascorbic acid equivalent (AAE) using the following linear equation established using ascorbic acid as standard:

$$A = 0.003\,7C + 0.034\,3;\ R^2 = 0.991$$

where A is the absorbance at 695 nm and C is the concentration as AAE (μg/mL).

2.6. Bacterial strains and growth conditions

Bacterial strains were collected from the Department of Genetic Engineering and Biotechnology, University of Dhaka. *Shigella dysenteriae* (*S. dysenteriae*), *Shigella boydii* (*S. boydii*), *Vibrio cholerae* (*V. cholerae*), *Salmonella typhi* (*S. typhi*), *Salmonella paratyphi* (*S. paratyphi*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Escherichia coli* (*E. coli*) were routinely cultivated in either nutrient agar or broth medium (HiMedia, India) at 37 °C with shaking. *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*) were cultivated in trypticase soy medium (HiMedia, India) at 37 °C. *Streptococcus* spp. were cultivated in brain heart infusion medium (Scharlau Co., EU), if necessary supplemented with 5% sheep blood at 37 °C in microaerophilic condition.

2.7. Determination of antibacterial activity of tea extract

Antibacterial activity was measured using the standard agar well diffusion method described in earlier studies [3,18]. Briefly, overnight grown bacteria were adjusted to McFarland turbidity

standard (~1.0 × 10^8 CFU/mL) and then 0.1 mL of each culture of bacteria was spread on agar plate surfaces. Wells were made on the agar medium using a sterile borer and the test extracts or control (PBS) were then added into the wells at 100 mg/mL concentration and incubated for 24 h. The zones of inhibition around the wells were observed and measured as millimeter (mm) in diameter after incubation at 37 °C in microaerophilic condition. For antibacterial assay, all bacterial strains were grown in Mueller Hinton agar medium (Difco, USA). For *Streptococcus* spp., 5% sheep blood was added for antibacterial assay.

2.8. Determination of minimum inhibitory concentration (MIC)

To determine the MIC of the tea extracts, a stock solution of 10 mg/mL was prepared in PBS. The broth dilution method was used to determine the MIC as described for antimicrobial susceptibility testing by the Clinical and Laboratory Standards Institute with little modification [18,23]. In brief, overnight grown bacterial culture was adjusted to 0.5 McFarland standards (~1.0 × 10^8 CFU/mL) for each bacterium and inoculated (1:100 dilution of each culture) into serially diluted aqueous extract of tea in 96-well round-bottomed micro-titer plates in Mueller Hinton broth. After 18 h incubation, 5 µL of serially diluted cultures were plated onto tryptic soy agar as drop plate method and incubated for 24–48 h. Different dilutions were performed into PBS. MIC was determined as the minimum concentration of tea extract which inhibited the apparent growth on the agar plate.

2.9. Statistical analysis

All experiments were done in triplicate (n = 3) and results were expressed as mean ± SD. Significance differences for multiple comparisons were determined using One-way ANOVA using SPSS for Windows, version 22.0 (SPSS, Chicago, IL). The significant difference was based on $P < 0.05$.

3. Results

3.1. Higher total phenolic and flavonoid compounds in green tea than the other three varieties of tea

Aqueous extracts of all four varieties of *C. sinensis* (BOP, FBOP, red dust and green tea) were used for the determination of their total phenolic and flavonoid contents. The TPCs in tea extracts were expressed in terms of GAE (mg of GA/g of extract). On the other hand, TFCs in tea extracts were expressed in terms of catechin equivalent (mg of catechin/g of extract). Among the four varieties of tea, green tea contained significantly ($P < 0.05$) higher amount of total phenolic and flavonoid compounds compared to other tea varieties (Table 1).

Table 1
Comparison of total phenolic and flavonoid contents among four varieties of tea extracts.

Tea variety	Total phenolic content (mg GA/g extract)	Total flavonoid contents (mg catechin/g extract)
BOP	8.84 ± 0.50	17.7 ± 0.82
FBOP	6.78 ± 0.55	13.93 ± 1.08
Red dust	8.20 ± 0.49	19.12 ± 0.33
Green tea	26.33 ± 1.73*	50.12 ± 0.60*

*: $P < 0.05$ compared to other tea varieties.

3.2. Higher antioxidant activity of green tea compared to other varieties

We next evaluated whether higher contents of total phenolic and flavonoid compounds in green tea was correlated with its higher total antioxidant activity. For this reason, the antioxidant activity of all four varieties of tea extracts was determined. The total antioxidant capacity in four varieties of tea extracts was expressed in terms of ascorbic acid equivalent (AAE µg/mL) with the standard curve equation: y = 0.2046x. Green tea showed higher antioxidant activity at all the concentrations tested compared to other tea varieties (Figure 1). Total antioxidant capacity of the aqueous extract of green tea was found 10.19 ± 0.54, 14.08 ± 0.78 and 21.33 ± 0.59 µg/mL AAE at 200 µg/µL, 600 µg/mL and 1 000 µg/µL extract concentrations, respectively (Figure 1). Total antioxidant capacity was found to be the lowest for red dust followed by BOP and FBOP.

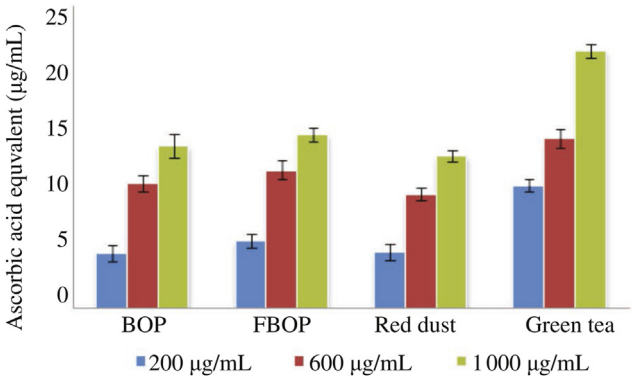

Figure 1. Total antioxidant ability of four varieties of tea.
Total antioxidant ability was expressed in terms of ascorbic acid equivalent (µg/mL). Each assay was performed in triplicate and the results are expressed as mean ± SD.

3.3. Green tea showed significantly higher ferric reducing antioxidant power (FRAP) activity compared to BOP, FBOP and red dust

FRAP activity of four tea varieties were expressed in terms of GAE (mg of GAE/g of extract). Among four varieties of tea extracts, green tea exhibited the highest FRAP activity (102.33 ± 1.02 mg GAE/g) as shown in Figure 2. The difference in FRAP activity was also statistically significant ($P < 0.05$) when compared between green tea and all other tea varieties.

3.4. Antibacterial activity of tea extract

We next investigated the antibacterial activity of the four varieties of tea against several pathogenic bacteria. We determined the antibacterial activity by agar well diffusion method as described in methods section. The mean ± SD of the zones of bacterial growth inhibition by tea extract is shown in Table 2. Our results revealed that all of the tested bacteria were susceptible to the exposure of all four kinds of tea extracts (Figure 3). Among the varieties of tea extract tested, green tea exhibited greater antimicrobial activity followed by red dust. The least activity was found in the remaining cases of FBOP and BOP. All

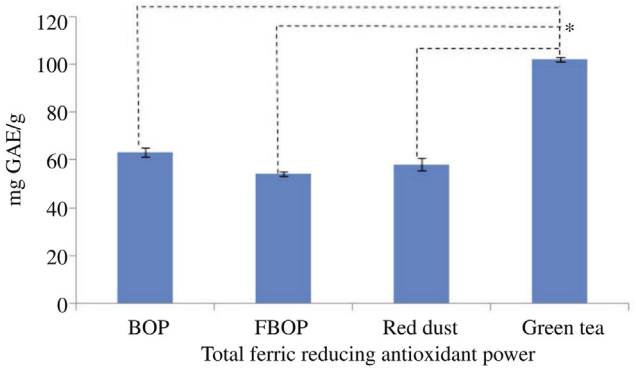

Figure 2. Total ferric reducing antioxidant power (FRAP) activity of four varieties of tea.
Total FRAP activity was expressed in terms of gallic acid equivalent (mg of GAE/g of extract). Asterisk indicate the significant of difference or *P* values that are <0.005. Data represented as mean ± SD.

varieties of tea extract showed maximum activity against *S. boydii* and minimum against *E. coli*. The minimum inhibitory concentration was also lower for green tea extract compared to other varieties of tea (Table 3).

Table 2
Mean area of the zones of bacterial inhibition by the aqueous extract of Bangladeshi tea.

Bacteria	Zone of inhibition (mm)			
	BOP	FBOP	Red dust	Green tea
S. dysenteriae	15 ± 1	13 ± 1	20 ± 1	23 ± 1
S. boydii	21 ± 1	26 ± 1	28 ± 1	35 ± 1
S. typhi	8 ± 1	9 ± 1	11 ± 1	15 ± 1
E. coli	7 ± 1	9 ± 1	12 ± 1	14 ± 1
K. pneumoniae	9 ± 1	10 ± 1	10 ± 1	11 ± 1
S. paratyphi	9 ± 1	9 ± 1	7 ± 1	11 ± 1
E. faecalis	10 ± 1	11 ± 1	12 ± 1	14 ± 1
V. cholerae	21 ± 1	23 ± 1	21 ± 1	16 ± 1
S. aureus	15 ± 1	17 ± 1	18 ± 1	19 ± 1

Values are means ± SD resulting from at least three independent experiments performed in duplicates.

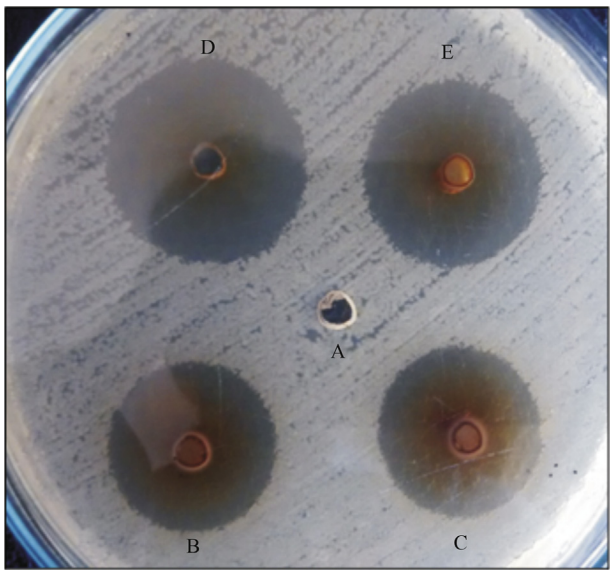

Figure 3. Antibacterial activities of four different varieties of tea extracts against various pathogenic bacteria.
A: PBS control; B: Black tea (FBOP); C: Black tea (BOP); D: Green tea; E: Red dust. A representative plate from at least three independent experiments is shown here.

Table 3
MICs of the four varieties of Bangladeshi tea against various pathogenic bacteria (μg/mL).

Bacteria	BOP	FBOP	Red dust	Green tea
S. dysenteriae	172	200	167	142
S. boydii	180	200	170	140
S. typhi	242	250	218	200
E. coli	690	750	625	575
K. pneumoniae	350	375	310	290
S. paratyphi	235	250	210	200
E. faecalis	475	500	435	392
V. cholerae	250	285	200	175
S. aureus	482	500	460	445

The results presented here are representative of at least three independent experiments performed in duplicates.

4. Discussion

Aqueous infusion of tea is one of the most popular beverages in the world. Tea extract is enriched with numerous secondary compounds which have immense health benefits [24]. The important beneficial components of tea are the polyphenols, most importantly the flavonoids. The scarcity of reports about chemical composition and bioactivity of different tea varieties of Bangladesh kept its consumers less concern about the potential health benefits of tea constituents. We, therefore, performed this study and demonstrated the phenolic composition, *in vitro* antioxidant activity and antimicrobial activity of locally available four tea varieties such as FBOP, BOP, red dust and green tea. Our study revealed that the total polyphenolic content was significantly higher in green tea compared to other varieties (Table 1). Relatively lower levels of polyphenols in black tea (FBOP, BOP and red dust) can be attributed to the conversion of tea polyphenols into theaflavin and thearubigins during fermentation process [25]. Our results are in accordance with several previous studies that demonstrated the higher levels of polyphenols in green tea compared to black tea [26–28].

Tea polyphenols have been reported to have strong antioxidant property and free radical scavenging activity due to possession of a phenolic hydroxyl group attached to the flavan-3-ol structure [28,29]. Free radicals are generated constantly due to metabolism of food ingredients, physical stress, and oxidative stress mediated by various environmental pollutants/chemicals/toxins, radiation *etc*. These free radicals are implicated in numerous disorders in human such as cancer, angina pectoris, neurodegenerative diseases and atherosclerosis [30]. Due to free radical scavenging activity, antioxidants are beneficial for reducing or preventing the progression of these diseases. Antioxidant activity measured by FRAP and phosphomolybdenum assay also supported the significantly stronger antioxidant activity of green tea than other varieties of tea (Figures 2 and 3). The ferric reducing antioxidant activity of green tea was found significantly higher compared to other black tea varieties tested (Figure 2). Our results are in agreement with several previous reports where they reported the higher FRAP levels in green tea compared to black tea [29,31,32]. A change in tea composition may occur while fermenting tea leaves to produce black tea, and this change might be the main cause of relative lower antioxidant potential of black tea than green tea found in our study [29]. In corroboration with previous study [32], our study also indicated a strong correlation between total polyphenolic content and antioxidant

activity, which implies that polyphenols have antioxidant property to protect against oxidative damage. This view is supported by our recent report in which we demonstrated that tea extract prevents apoptotic cell death and damage of cellular DNA caused by arsenic-mediated oxidative stress [13].

Our study also demonstrated that all four varieties of tea are able to inhibit various bacterial pathogens (Figure 3 and Tables 2 and 3). However, the green tea extract was much more effective to inhibit bacterial pathogens than all other black tea extracts. The decreased antibacterial activity of black tea is probably due to fermentation. Gram negative bacteria such as *Vibrio*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Pseudomonas* and Gram positive opportunistic pathogen, *S. aureus* were more sensitive; however, *E. coli* was found to be the least sensitive to all four types of tea extracts tested. Our results comply with the findings of several earlier studies that have demonstrated antibacterial effects of both green and black tea extracts [15,17]. The antimicrobial activity of tea extracts, however, is not always consistent; a previous report demonstrated no activity of the extracts against Gram-negative *E. coli*, *S. typhi*, and *P. aeruginosae* [1]. The disparity in findings could be due to differences in bacterial strains used, and to the differences in concentrations/types of extracts used by various research laboratories. Antibacterial activity of green and black tea extracts has been attributed to the presence of catechins (importantly EGCG and ECG) [1,3,14]. Although the exact mode of action is still unclear, however, damage of bacterial cell membrane, and inhibition of crucial enzyme activity, fatty acid synthesis and various other physiological activities may contribute collectively or individually to the overall antimicrobial activity of the tea extracts.

In this study, extracts of four different brands of Bangladeshi tea (FBOP, BOP, red dust and green tea) were analyzed for their polyphenol content, antioxidant property and antibacterial activity. Total polyphenol content, antioxidant property and antibacterial activity were higher in green tea extract compared to other tea varieties. We conclude that Bangladeshi tea especially green tea may act as a promising antibacterial agent as well as a natural antioxidant substitute and thereby contribute to human health.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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