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Original Research Article

Comparative Phytochemical and Biological Analyses of commercial Green Tea Products Marketed in Dhaka, Bangladesh

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ABSTRACT

Green tea (*Camellia sinensis*) is a "non-fermented" tea popular worldwide attributed to its pleasing flavor and beneficial impact on human health. Commercially available green tea products differ in their quality, constituents and health benefits which can be attributed to the variations in the processing methods, geographical factors, and plant parts used. This study aims to compare the phytochemical constituents and biological activity of four commercial green tea brands of Bangladesh (GTE1-4). Methanolic extracts of four marketed green tea products were employed in this study. The functional groups and characteristic peaks of the known chemical entities were determined by IR and 1D-NMR spectroscopy, respectively. Gas chromatography-mass spectroscopy (GC-MS) technique was utilized for the quantitative determination of caffeine and other volatile substances. Antioxidant and antimicrobial properties were assessed using the (2,2-Diphenyl-1-picrylhydrazyl) DPPH free radical scavenging assay and the disc diffusion method, respectively. Finally, the products were tested for their potential cytotoxic effects. Caffeine, gallic acid, (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate and quinic acid were identified as the major constituents in the qualitative phytochemical analyses. GTE-1 contained the highest concentration of 1,2,3-benzenetriol while caffeine was the most abundant in GTE-2 (96.16%). Majority of the samples exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria at 1000 µg/mL concentration. Sample GTE-1 exhibited a positive cytotoxic effect at 500 µg/mL concentration when tested on HeLa cells. Taken together, the results indicate that methanol extracts of green tea in different commercial products can differ considerably.

***Keywords*:** Green tea, Nuclear Magnetic Resonance, cytotoxic activity, antimicrobial activity, Gas Chromatography–Mass Spectrometry.

Introduction

Green tea refers to a product made from fresh tea leaves by steaming or pan-frying to deactivate the enzyme polyphenol oxidase, thereby preventing the oxidation of polyphenolic components such as catechins. *Camellia thea* is a synonym of the green tea plant.[1] *Camellia sinensis* sinensis and *Camellia sinensis* assamica are the two most common varieties of *C. sinensis*. Typically, *sinensis* variety produces green tea, while the assamica variety generates black tea.[2] Considering its health benefits, green tea has been recognized to be superior to black tea since it possesses more polyphenols. Flavonoids are the predominant polyphenols present in green tea. Catechins and theanine are the two primary constituents unique to green tea, and their antioxidant and other medicinal values are gaining a great deal of attention worldwide.

Green tea has been included into the category of beverages with functional qualities as a result of the growing interest in its therapeutic implications.

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Components of green tea exhibit a wide range of biological activities. The optimal consumption of green tea with antioxidants offers beneficial properties, including prevention of cancer and cardiovascular diseases. Researchers have been investigating the anti-inflammatory, antiarthritic, antibacterial, antiangiogenic, antioxidative, antimutagenic, antiviral, neuroprotective, and cholesterol-lowering activities of green tea and its isolated constituents (Figure 1). [2,3] Green tea's polyphenols, or catechins, may provide some protection against degenerative diseases, according to studies done on animal models.\[4]

The assessment of the physiological as well as pharmacological impacts of tea involves background information on manufacturing, leaf composition, the availability of various types of tea components, and, most importantly, the chemical alterations that occur throughout the production of various commercial products.[2] Because the complex biochemical composition of tea strains varies with leaf position in the shoot, season, climate, cultivation location, horticultural practices, technique of processing, storage conditions, shaping of tea leaves like rolling and drying, size of leaves (small pieces or whole leaf), chemical treatment or organically grown, not all green teas have the same flavor and taste.[5]

Different techniques of preparing tea are used in different nations, which may affect the amount of active chemicals in the final brew.[6] In simplest terms, phytochemical screening is a valid approach for assessing the phytochemical profile of herbs and medicinal plants like *C. sinensis* in order to assess their therapeutic potential. For phytochemical screening, plant extracts or herbal formulations, a variety of approaches have been explored. One of them is the gas chromatography-mass spectrometry (GC-MS) approach (effective separation method for quantitative analysis), which has been used to

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profile the phytochemical contents of medicinal plants, including volatile chemicals. When analyzing tea extract qualitatively, many techniques such as TLC, UV-Visible spectrometry, NMR, and IR spectroscopy can be employed to identify the phytochemicals.[7] In addition to phytochemical analysis, green tea's biological properties, which also rely on its phytochemical composition, play a significant role in determining its quality. Biological activity of the green tea products can be examined through various assays including antioxidant test, antimicrobial assays and cytotoxic activity tests. However, concentrations of the major phenolic catechins in green tea products and hence the biological properties may differ due to the climate changes, time of collection and method of preparation.[8-10]
Several investigations associated with the phytochemical analyses, antioxidant activity and antimicrobial effects of black and green tea compounds in Bangladesh have been conducted. [11-14] However, no cytotoxic assay has been used previously to evaluate the anticancer efficacy of any of the marketed green tea products. Therefore, the objective of this study is to compare the phytochemical constituents and biological activities including anticancer potential of multiple green tea brands marketed in Bangladesh. This comparative study intends to determine the phytochemical constituents of the methanolic extracts of the products first and then to contrast their biological properties utilizing several biological assays. To the best of our knowledge this is the first study in Bangladesh demonstrating differential cytotoxic effect of the methanolic extracts of the marketed green tea products in Bangladesh. Since people take green tea not only as a beverage drink but also for enormous therapeutic and health benefits, it is imperative to maximize its health benefits by obtaining comprehensive knowledge on the contents and quality of green tea products.

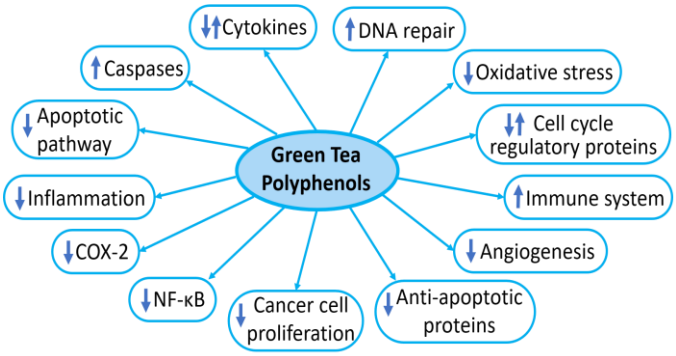

Figure 1: Molecular target of green tea polyphenols.[4]

Method and Materials

Sample collection and extraction
Four commercial green tea products (Kazi &Kazi, Finlays, Summing Genial and Lipton) were procured from a department store in the month of July 2022 in Dhaka, Bangladesh and labelled as GTE-1, GTE-2, GTE-3 and GTE-4, respectively. The selection of these brands was based on their popularity in the market. Among them, GTE-4 was imported from other countries. About 25 grams extract of green tea samples of each variety were weighed and taken in a beaker. Then 200 mL methanol (Sigma-Aldrich-, HPLC grade, 99.99% pure) was added and the extracts were obtained by maceration (48 hours). The Whatman filter paper grade no. 1 (Merck, Germany) was used to filter the solvent extracts. The filtrates were subsequently concentrated by using rotary evaporator (EYELA N-1100) at temperature 40°C. Finally, freeze-drying method (Labconco, Model: Freezone 2.5, USA) was used to obtain fine powder form, which were used for further testing and studies. The yield fraction was approximately 5-6% of the GT content extracted initially.[5]

Phytochemical analysis
Equipment and Reagents: Shimadzu portable IR Spirit FTIR (IRSPIRIT-T) spectrometer (Japan) was used to conduct FT-IR analysis to determine the functional groups in the methanolic crude extracts. Fine powdered sample was placed in the sample holder within the path of the IR source and scanned from 650 to 4000 cm^{-1} frequency to collect spectra. NMR spectra (both ^{1}H and ^{13}C) were measured on a BRUKER AvanceCore (Germany) spectrometer operating at a probe temperature of 294.1 K using a dual $^{1}H/^{13}C$ 5 mm probe. Methanol-d_4 (CD_3OD) was used as the solvent, and spectra were referenced relative to residual non-deuterated solvent peak of methanol. The operating NMR frequency was 400.17 MHz for proton and 100.25 MHz for carbon NMR. 20 mg powdered methanolic extract was dissolved in 3 mL 100% methanol-d_4 (CD_3OD) without any internal standard, in a vial. The supernatant was centrifuged and transferred to a 5-mm NMR tube. Tube was placed in the sample compartment for data acquisition.[15] Characteristic peaks of the known compounds were compared with published values to identify their presence in the crude extracts.

Quantitative analysis using GC-MS
GC-MS test was performed in the Institute of National Analytical Research and Service (INARS) of Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka. The first step involved dissolving 0.1 g of each sample in 1 mL of methanol, filtering the mixture, and using 1 µL of the filtrate for the extract's phytochemical analysis using GC-MS. Using the Shimadzu GCMS-QP2010 ultra, GC-MS analysis was carried out. The instrument was equipped with Shimadzu AOC-20i autosampler and split/splitless injector. A fused silica Rxi-624Sil MS Column with 30 m length × 0.32 mm i.d. × 1.80 µm film thickness was used. The analytes were thermally desorbed in the splitless mode at 250°C injection temperature. Oven temperature program was 40°C for 1 min then raised to 300°C with total program time of 37 minutes. The pressure and average velocity were 9.7 psi and 42.7 cm/sec respectively, carrier gas was Helium (99.99%) with 1.40 mL/min flow rate. The MS interface was heated to 250 °C, and the ion source was heated to 200°C. MS was conducted in electron impact ionization (EI) mode using an acceleration voltage of 70 eV and detector voltage 0.4 kV. The detector was operated in the scan mode (from 50 to 1000 m/z), scan speed 3333 u/sec and solvent delay time was 6.0 min. Comparison of mass-spectrum with reference spectra from the National Institute of Standards and Technology (NIST) library database was used to interpret mass-spectrum for detection and quantification of phytochemical substances contained in samples. The relative proportion of each element was computed through a comparison of the mean peak area to the overall area. The test materials' component names, molecular weights, and structures were identified.[5]

Analysis of total phenolic content Folin-Ciocalteu reagent
Spectrophotometric technique was employed to determine the total phenolic contents of the plant extract which was previously described using Folin-ciocalteu reagent as oxidizing agent.[16] Gallic acid (Merck, Germany) was used as the standard to prepare the curve with positive slope to identify the concentration of the unknown crude extracts.[16]
Sample preparation: At first, a suitable volume of methanol was used to dissolve the crude extract in order to achieve a stock concentration of 1 mg/mL. To make the volume 2 mL, 0.2 mL of this stock sample solution was diluted with distilled water. Then 0.1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) was added, shaken well and after 5 minutes 2 mL of saturated sodium carbonate (Merck, Germany) solution was added to the mixture. The ultimate volume was 10 mL after adding distilled water. The absorbance readings were taken at 765 nm using a UV-vis spectrophotometer (UV-1800, Shimadzu Corporation, Japan) after incubation at room temperature for 1.5 hour. By comparing the samples to the gallic acid standard curve, the total phenolic content of the samples was calculated and expressed as mg/g Gallic acid equivalent (GAE). (Supplementary Table 2). Each of the experiments was performed in triplicate.

Antioxidant activity by DPPH free radical scavenging assay
Stable free radical DPPH (Sigma-Aldrich, USA) was used to determine the antioxidant activity of several green tea extracts using with a slight modification of the published method.[17] The assay is

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based on measuring the ability of antioxidants to scavenge it. Initially, ethanol (Merck, Germany) was used to make a stock solution of the crude extracts at a concentration of 1 mg/mL. From the stock solution, a series of distinct concentrations (0, 5, 20, 50, 100, 250, 500 µL) of these crude extract solutions were created by diluting with appropriate quantities of ethanol until the final volume was 2 mL.Then 1 mL of 0.04% (w/v) freshly prepared DPPH (Sigma-Aldrich, USA) was added, shaken well and incubated in a dark place for 60 minutes. After an incubation period at room temperature, absorbance measurements were obtained at 517 nm. The crude extracts' DPPH free radical inhibition ability was determined as follows:

$(I\%) = (1 - A_{sample} / A_{blank}) \times 100$

Here, Blank represents the absorbance of the control reaction (which contains all reagents except the test substance). The proportion of DPPH inhibition vs. extract concentration was plotted on a graph to express the antioxidant activity of the extracts as IC_{50}, which was then compared to the IC_{50} values of ascorbic acid (Merck, Germany).

Antimicrobial activity by disc diffusion method

The disc diffusion method was used to screen for antimicrobial susceptibility using strains from the American Type Culture Collection (ATCC) complying with the published methodology.[18] For antibacterial activity assessment, Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria were chosen. The test samples were dissolved in suitable amount of distilled water to prepare solutions of 1 mg/mL or 1000 µg/mL concentration in an aseptic condition. Subsequent dilutions were also performed to prepare solutions of 500 µg/mL with distilled water. About 20 µL of test samples were applied to the sterilized filter paper discs (about 8 mm diameter) and dried. Using sterile forceps, the sample discs, standard antibiotic (Chloramphenicol- as positive control, 30µg/disc, HiMedia, India) and blank discs (as negative control) were placed gently on the previously marked zones at equal distances in the agar plates. All culture plates were incubated in an inverted position for 24 hours at 37°C. For antifungal activity determination, one fungus strain *Aspergillus niger* (collected from soil sample) was cultured in Potato Dextrose Agar (PDA) media using Amphotericin B as positive control (10µg/disc, HiMedia, India, ZOE 18cmm). Lastly, the diameter of the zone of inhibition was measured in millimeters and was used to evaluate the test extracts' antimicrobial potential.

Cytotoxic activity against HeLa cells

Using commercial services, cytotoxic activity was studied at the Centre for Advanced Research in Sciences (CARS) via utilizing their established protocol.[19] Briefly, each of the sample was prepared by mixing 10 mg of dried extracts in 20 mL of distilled water, which yielded a 500 µg/mL concentration. HeLa, a human cervical carcinoma cell line was kindly provided by Professor H. Hosino (Gunma University Graduate School of Medicine, Gunma, Japan) and maintained in DMEM (Dulbecco's Modified Eagles' medium, Gibco, USA) containing 1% penicillin- streptomycin (1:1) and 0.2% gentamycin and 10% fetal bovine Serum (FBS) (all from Sigma-Aldrich, USA). Cells (2.0×10^4/100 µL) were seeded onto 96-well plate and incubated at 37°C+5% CO_2. Next day, 25 µL sample (filtered by 0.45µm pore size filter) was added each well. After 48 hours of incubation, cytotoxicity was assessed using an inverted light microscope. For each sample, duplicate wells were used.

Results and Discussion

This study demonstrates qualitative and quantitative evaluation of four marketed green tea products of Bangladesh by utilizing their methanolic extracts. Qualitative analysis of the experimental green tea samples by IR and NMR spectroscopy revealed the presence of several catechins, phenolic compounds, flavonoids, carboxylic acids, amines, esters and caffeine. In addition, various biological tests including DPPH assay, TPC assay, antimicrobial test and cytotoxicity test were conducted to evaluate the biological properties of the products. This is the very first study demonstrating the anticancer potential of the studied products of Bangladesh.

Table 1: IR absorption spectra of green tea samples

Reference Wavenumber (cm^{-1})	Vibration band/group	Wavenumber (cm^{-1})				Possible chemical compounds
		GTE-1	GTE-2	GTE-3	GTE-4	
3550 - 3200	O–H stretching	3243.24	3210.33	3220.35	3214.62	Phenols, alcohols (e.g. catechins)
3000-2925	C–H stretch (asym.)	-	-	2922.78	2924.21	Alkanes
	O–H stretch					Carboxylic acid
2925-2850	C–H stretch (sym.)	-	-	2854.11	2854.11	Alkanes
2375-2250	N=C=O Stretch	2360.54	2360.54	2359.11	2363.40	Isocyanate
		2331.93	2341.94	2337.65		
1710 -1685	C=O stretch (carbonyls)	1691.00	1689.57	1695.30	1695.30	Flavonoids Polyphenols, catechins
1650-1600	C=C stretch	1605.17	1603.74	1605.17	1606.60	Aromatics
1447	C–C stretch (in ring)	1447.80	1447.80	1447.80	1447.80	Aromatics
1342-1225	C–N stretch	1336.21	1339.07	1339.07	1337.64	Aliphatic amines, Aromatic amine, caffeine
		1234.63	1233.20	1231.77	1236.06	
		1188.85	1188.85	1190.28	1190.28	
1210-1000	C–O stretch	1141.61	1141.64	1143.07	1143.07	Alcohols, esters, carboxylic acids
		1031.48	1093.00	1031.48	1031.48	
		1014.32	1030.05	1014.32	1015.75	
830-790	C-H Bending	819.75	819.75	819.75	819.75	

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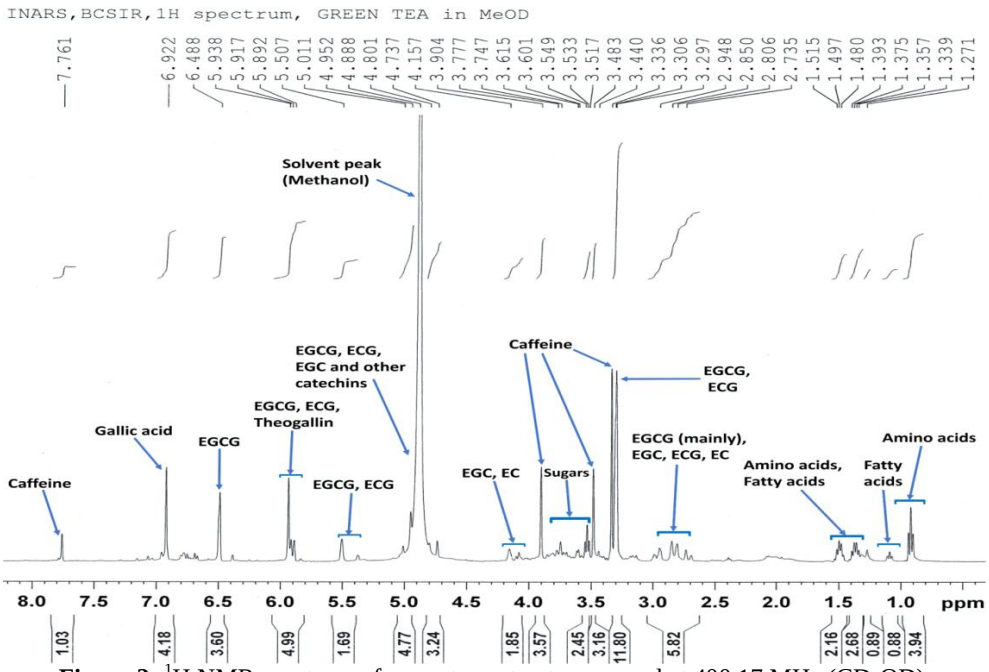

Figure 2: ^{1}H NMR spectrum of green tea extracts appeared at 400.17 MHz (CD_3OD).

Phytochemical analysis of the green tea samples

Using IR and NMR spectroscopy, qualitative phytochemical investigations of the marketed experimental green tea items were conducted to determine the functional groups and structural attributes of the main ingredients, respectively. The vibrational band assignment between 4000-650 cm^{-1} frequency for the prominent peaks and the chemical compounds in the FT-IR spectra of the four green tea samples are provided in Table 1 and the spectral data have been shown in Supplementary Figure 1(i-iv). Identification of functional groups has been done considering (Senthilkumar *et al*., 2017)[20, 21] and Sigma-Aldrich [22] as reference. Broad OH stretching with rounded tip between 3550-3200 cm^{-1} frequency indicates the presence of polyphenolic components (e.g. catechins, flavonoids, 1,2,3-benzenetriol).[23] Weak band with sharp spike C=O stretch (carbonyls) between 1710-1685 cm^{-1} and medium sharp band C=C stretch in the range of 1650-1600 cm^{-1} frequency is also due to catechins. Weak overtone and combination tone bands found from 2000 to 1600 cm^{-1} confirms aromatic compounds. IR spectra depicted that all the marketed products are rich in polyphenolic compounds. The region between 1700 - 1600 cm^{-1} corresponds to C=C and C=N stretching vibrations, as well as those of the carbonyl groups (1710 -1685 cm^{-1}) and sharp 1342-1225 cm^{-1} peaks split into a doublet area for C–N stretch that indicates caffeine (Supplementary Figure 1(i-iv)).

Green tea is an excellent source of retaining the original components of tea which possess beneficial antioxidant, anticancer, antidiabetic, neuroprotective and antimicrobial properties. Many investigations have reported the presence of polyphenols, flavonoids, catechins, alkaloids, amino acids and aromatic compounds in it.[24-26] Qualitative analyses of the green tea samples by IR spectra depicted that all the samples contained the characteristic functional groups and structures of polyphenolic compounds, aromatic amines, carboxylic acids, flavonoids and aliphatic compounds which is in accordance with previous studies. Interpreting the ^{1}H and ^{13}C NMR spectra of the methanolic extracts of sample green tea products provides additional evidence for the presence of these compounds. NMR interpretation plays a pivotal role in molecular identifications. Several factors associated with NMR spectroscopy such as chemical shift, spin multiplicity, coupling constants, and integration can be exploited to assign the structures as well as dynamics, reaction state, and chemical environment of molecules in addition to their identification of chemical compounds. In this study, ^{1}H and ^{13}C NMR spectra have been employed to identify the common structures of green tea components (Figure 2). Chemical shift values from ^{1}H-NMR analysis (δ 6.922, 6.488, 5.50, 4.888, 2.948 ppm) are indicative of epigallocatechin-3-gallate (EGCG) at 400.17 MHz. Presence of other catechins such as epicatechin-3-gallate (ECG) (δ 6.922, 6.488, 5.938, 5.011, 2.850 ppm), epigallocatechin (EGC) (δ 6.488, 5.938, 4.157, 2.948, 2.806 ppm) and epicatechin (EC) (δ 6.922, 6.488, 5.938, 5.507, 2.850 ppm) have also been confirmed when compared with the published values.[27] The presence of caffeine can be verified by looking at the ^{1}H-NMR data peaks at δ 3.336, 3.483, 3.904, and 7.761 ppm. ^{13}C-NMR spectrum data are also recorded at 100.2 MHz that confirms the structures of EGCG, EGC, ECG, EC (Data not shown) in all the commercial green tea products.[28] Comparative results from NMR analysis of 4 types of green tea samples is presented in Table 2.

Quantitative analysis of the tested samples

The volatile components in the commercial green tea products have been identified and quantized using GC-MS technique and the data are compared with the standard mass spectra from NIST Mass spectral library. The peak area detected in the spectra in relation to overall area is used to determine the concentration of the chemicals. Data have been shown in Table 3 and the spectral data have been displayed in Supplementary Figure 2 (i-iv). Major aromatic compounds that are found in these samples include alkaloids, alcohols, lipids, carboxylic acids, esters and indoles supporting the findings of a previous study.[29] Caffeine and 1,2,3-benzenetriol are present in the highest concentrations among the four samples that were analyzed, which is consistent with previous investigations.[30-33] Nonetheless, notable variations in the levels of caffeine content in the examined specimens have been noted. While GTE-2 contains the highest amount of caffeine (96.16%), GTE-1 is superlative with respect to the concentration of 1,2,3-benzenetriol among all the green tea extracts (Table 3). Studies suggest that, various factors, e.g., plant species, plant part used, environmental conditions as well as manufacturing process impact the range and quantity of green tea constituents.

The quality of green tea products can be ensured by its phytochemical analyses to assess its polyphenolic constituents and also by biological assays to ascertain the numerous reported useful pharmacological activities.[9, 24] High-quality green tea often contains EGCG levels above 100 mg/g, caffeine content can range from 15 to 30 mg per 8 oz cup, total polyphenol content 150-300 mg/g, flavonoid content above 10 mg/g.[35, 36] Bioactive phytochemical analysis of commercial green teas of India shower caffeine ranging between 46-59%.[3] In this study

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the sample green tea products possess diverse chemical compounds in varying concentrations. However, previous investigations involving green tea have reported a wide collection of components, our study has detected fewer compounds possibly due to the limitation of the detection instrument or degradation of the components during their manufacturing process or methanolic extract preparation.

Evaluation of antioxidant activity and phenolic content of the samples

Antioxidant activity was evaluated by the free radical scavenging potentials using DPPH test. Higher antioxidant activity indicates better anticancer and anti-aging effects.[37] In DPPH test, lower value of IC_{50} indicates greater antioxidant potentials. Accordingly, GTE-1 has been found to have lower IC_{50} value (50.73 µg/mL) compared to other three samples (Table 4) and comparable to the reference standard ascorbic acid in it as antioxidant property which can be attributed to its higher amount of polyphenolic content (Table 4). In comparison to the reference standard ascorbic acid (45.03 µg/mL), all of the samples possess free radical (DPPH) scavenging potential as reflected by their IC_{50} values (Supplementary Figure 3(i-v)) indicating their possession of high levels of polyphenols, mostly catechins and hence prominent antioxidant functions as mentioned in another study.[38]

Total phenolic content (TPC) test indicates important plant constituents with hydroxyl groups having redox properties and responsible for antioxidant activity facilitating free radical scavenging activity[39]. TPC is evaluated relative to a reference concentration of gallic acid (Supplementary Figure 4). The findings of TPC test involving green tea samples in this study are presented in Table 4 in the form of mg Gallic Acid Equivalent (GAE) per gram. GTE-4 (245.93 mg of GAE) has been found to possess the highest phenolic content among the four green tea samples signifying its remarkable antioxidant activity. Furthermore, the TPC assay indicates that GTE-1 has a significant quantity of phenolic content, which supports its high antioxidant activity (Table 4).

Determination of antimicrobial activities of the products

Therapeutic and prophylactic battles against infectious diseases are becoming challenging now-a-days because of the rapid emergence of resistance to antibiotics of the microbes. In this context plant derived ingredients continue to reveal enormous possibilities in developing safer antimicrobial agents. Several studies have reported the antimicrobial functions of green tea against various microorganisms attributable to the presence of polyphenol catechins (mainly EGCG and ECG).[40-42] In the present study, Kirby-Bauer disk diffusion method has been performed to determine the antimicrobial activities of the marketed green tea products. At 1000 µg/mL concentration, all four extracts demonstrated mild antimicrobial activities against Gram-negative *Escherichia coli* (Table 5). At highest, i.e., 1000 µg/mL concentration, all four samples except GTE-3 showed antibacterial activity against Gram-positive *Staphylococcus aureus*. (Table 5).

Table 2: Assignment of proton signals in the representative ^{1}H-NMR spectrum (CD_3OD, δ in ppm, *J* in Hz)

Analyte	Obtained proton signals (samples)	Test Samples				Reference signals
		GTE-1	GTE-2	GTE-3	GTE-4	
Caffeine	3.336 (s, N_1-CH_3); 3.483 (s, N_7-CH_3); 3.904 (s, N_3-CH_3); 7.761(s, H-8)	+	+	+	+	3.23 (s, N_1-CH_3); 3.42 (s, N_7-CH_3); 3.79 (s, N_3-CH_3); 7.65 (s, H-8) (21)
Gallic acid	6.922 (s, H-2,6)	+	+	+	+	7.16 (s, H-2,6) (Yuan *et al*., 2014)
Epigallocatechin (EGC)	2.806 (1H, m, H-4); 2.948 (1H, m, H-2); 4.157 (1H, m, H-6); 4.888 (1H, m, H-3);5.938(1H, m, H-8); 6.488 (s, H-2′,6′)	+	+	+	+	2.81 (1H, m, H-4); 2.93 (1H, m, H-2); 4.32 (1H, m, H-3); 4.92 (1H, m, H-3); 6.59 (s, H-2′,6′) (Yuan *et al*., 2014)
Epigallocatechin-3-gallate (EGCG)	2.948 (m, H-4a); 3.297 (m, H-4b); 3.306 (m, H-2); 4.888 (m, H-3); 5.507 (s, H-8); 5.938 (s, H-6); 6.488 (s, H-2′,6′); 6.922 (s, H-2″,6″)	+	+	+	+	2.93 (m, H-4a); 3.08 (m, H-4b); 5.03 (m, H-3); 5.50 (s, H-8); 6.09 (s, H-6); 6.62 (s, H-2′,6′); 6.87 (s, H-2″,6″) (Yuan *et al*., 2014)
Epicatechin (EC)	2.735, 2.948, 3.297, 4.157, 4.888, 5.917, 6.922	+	+	+	+	2.77, 2.95, 4.27, 4.81, 5.90, 6.83, 6.92, 7.02 (Yuan *et al*., 2014)
Epicatechin-3-gallate (ECG)	2.850, 3.306, 5.011, 5.507, 5.938, 6.488, 6.922	+	+	+	+	2.89, 3.03, 5.09, 5.50, 6.03, 6.78, 6.88, 6.99 (Le Gall *et al*., 2004)
Sucrose	3.747, 3.615	+	-	+	+	3.43, 3.53, 3.76, 3.80, 3.84, 4.05, 4.19, 5.42 (Yuan *et al*., 2014)
Fructose	3.777	+	+	-	-	3.56, 3.70, 3.79, 3.88, 4.00, 4.10 (Yuan *et al*., 2014)
2-O-Arabinopyranosyl-myo-inositol	3.601	-	+	-	+	3.26, 3.61, 3.68, 3.89, 3.97, 4.18, 5.14 (Yuan *et al*., 2014)
Theogallin	1.515, 1.497, 1.480, 4.801, 5.892	+	+	+	-	2.02, 2.15, 2.20 (Le Gall *et al*., 2004)
Quinic acid	3.440, 3.549	+	+	-	+	3.98, 4.05, 3.55 (Yuan *et al*., 2014)
Solvent Methanol	4.952	+	+	+	+	4.87

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Table 3: GC-MS analysis of the green tea samples

Name	Retention Time	m/z	Area	Conc.	% Similarity
GTE-1					
1,2,3-Benzenetriol	14.989	52.00	15172892	14.57 %	99
Caffeine	21.330	67.00	88967335	85.43 %	99
GTE-2					
1,2,3-Benzenetriol	14.994	52.00	11876774	3.11 %	99
Caffeine	21.382	67.00	122280939	96.16 %	98
Theobromine	21.538	67.00	1152862	0.30 %	90
erythro-(Z) (1,4), (E) (1',4')-4,4'-Dihydroxybicyclooctyl	20.510	67.00	187869	0.04 %	81
Cyclooctene,1-(diethylboryl)oxy-	20.751	67.00	275252	0.07 %	90
3-Tridecene	21.538	67.00	1152862	0.3 %	89
GTE-3					
1,2,3-Benzenetriol	14.992	52.00	1201818	1.517 %	83
Caffeine	21.211	67.00	39711817	50.124 %	99
1,2,3,5-Cyclohexanetetriol	-	60.00	---	N.D.(Ref) %	---
Cyclooctane, ethenyl-	21.211	67.00	38035764	48.008 %	76
Pentadecanoic acid	21.877	55.00	48027	0.061 %	58
Phytol	23.485	71.00	229779	0.290 %	75
GTE-4					
1,2,3-Benzenetriol	15.005	52.00	2635092	4.545 %	94
Caffeine	21.253	67.00	54788485	94.509 %	99
Cyclopentaneundecanoic acid, methyl ester	-	74.00	-	-	-
n-Hexadecanoic acid	21.885	55.00	221710	0.382 %	79
Oxirane, octyl-	23.472	71.00	326203	0.563 %	83

Table 4: Free radical scavenging activity by DPPH test and total phenolic content of the methanolic extracts of green tea samples

Sample Code	IC_{50} in DPPH test (µg/mL)	Total Phenolic Content (mg GAE/g of DW)
GTE-1	50.73	242.39
GTE-2	62.81	156.32
GTE-3	63.37	172.89
GTE-4	60.60	245.93
Ascorbic Acid	45.03	-

Additionally, GTE-1 (1000 µg/mL) displays highest zone of inhibition (16 mm) against *E. coli*. No zone of inhibition was observed at the lower, i.e., 500 µg/mL concentration of all four samples against both Gram-positive and Gram-negative bacteria. Moreover, none of the four test samples show antifungal activity against *Aspergillus niger*, as shown by the experimental data (Table 5). The discrepancy in findings from the previous investigations may be due to the differences in the bacterial strains employed or concentrations or types of extracts used.

Assessment of cytotoxic activities of the samples

Reportedly, green tea possesses cytotoxic properties against a variety of cancer cell lines, including human colon adenocarcinoma, human hepatocellular carcinoma, and human cervical cancer cells, due to its higher content of polyphenolic and flavonoid components (ECG, EGC, EGCG).[43-45] Hence, to determine and compare the potential cytotoxic effects of the experimental green tea samples, percentage of survival and morphology of HeLa cells have been determined under an inverted light microscope (shown in Figure 3). It is observed that, at 500 µg/mL concentration, GTE-1 shows strong cytotoxic activity leading to less than 5% survival of the cells (Figure 3 (iii)) which can be attributed to its greater polyphenolic component and hence antioxidant property stated before. Interestingly, other three samples fail to elicit such activity at 500 µg/mL concentration (cell survival >95%). This deviation in cytotoxic effects can be because of their cell specific activity, containing less quantity of polyphenols in comparison to GTE-1 (found in GC-MS analysis) or error in the sampling procedure.

Conclusion

- Qualitative IR and 1D-NMR spectroscopy of the extracts of four marketed green tea products in Bangaldesh identified caffeine, gallic acid, (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate and quinic acid as the major constituents. Quantitative analysis by gas chromatography-mass spectroscopy (GC-MS) technique has determined that caffeine is the most abundant in GTE-2 while1,2,3-benzenetriol is present in the highest concentration in GTE-1. Moreover, major aromatic compounds that are found in these samples include alkaloids, alcohols, lipids, carboxylic acids,

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esters and indoles. The products have also been tested for their potential antioxidant and antimicrobial activities. At the highest concentration, the majority of the samples exhibited antibacterial action against Gram-positive and Gram-negative bacteria, but no detectable antifungal impact. Finally, study of cytotoxic effects on HeLa cells demonstrates that GTE-1 possesses a positive cytotoxic effect at 500 µg/mL concentration. Taken together, the results indicate that methanol extracts of green tea in different commercial products can differ considerably, which, in turn, is attributed to the differences in the diverse sourcing and processing methods by manufacturers.
In future, this comparative study can be extended to test both black tea and green tea brands available in Bangladeshi market. Moreover, the quality of the marketed green tea products may be evaluated for their plausible anti-diabetic, antiviral and neuroprotective effects. More bacterial strains and cancer cell lines can also be included in the antimicrobial and cytotoxicity tests respectively to determine the extent of their biological properties.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 5: Result of antimicrobial activities of methanolic extracts of green tea samples.

Test Organisms	GTE-1		GTE-2		GTE-3		GTE-4	
	500 µg/mL	1000 µg/mL	500 µg/mL	1000 µg/mL	500 µg/mL	1000 µg/mL	500 µg/mL	1000 µg/mL
Gram-positive bacteria								
Staphylococcus aureus	-	13	-	9	-	-	-	11
Gram-negative bacteria								
Escherichia coli	-	16	-	11.5	-	13.5	-	12
Fungi								
Aspergillus niger	-	-	-	-	-	-	-	-

*The values are expressed as diameter of zone of inhibition (mm).

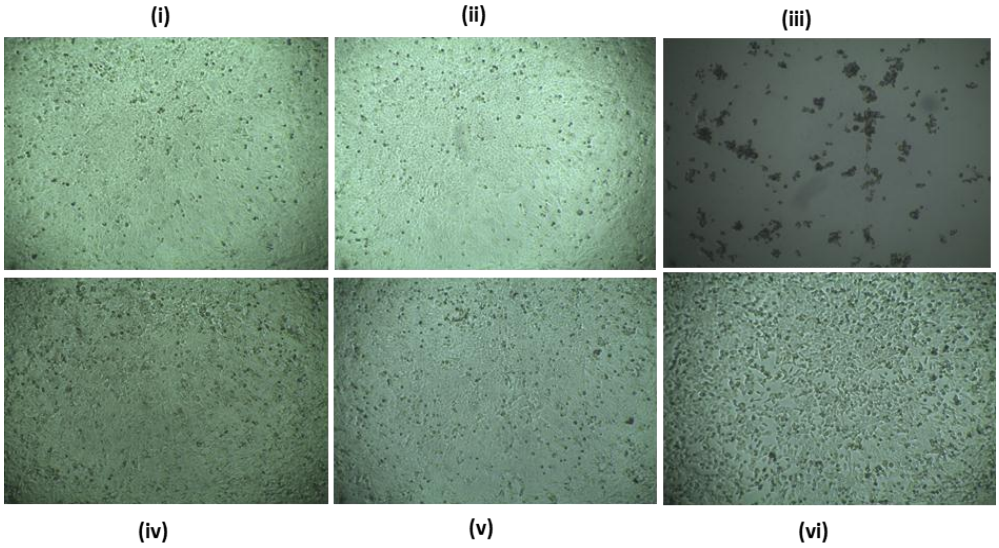

Figure 3: Determination of cytotoxic activity of green tea samples in HeLa cells. Cells were grown and 2.0×10^4/100 µL cells were seeded in 96 wells plate. After 24 hours incubation, test samples at 500µg/mL concentration were added to each well and incubated for another 48 hours. The percentage of cells survival were measured using an inverted light microscope. Duplicate wells were used for each sample (n=3). (i) Cells only (positive control), (ii) Cells with solvent (distilled water) (negative control), (iii) GTE-1, (iv) GTE-2, (v) GTE-3 and (vi) GTE-4.

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