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Assessment of Proximate Composition and Antioxidant Potential of Different Commercially Packaged Tea Samples in West Bengal

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ABSTRACT

Background: Worldwide tea consumption as beverage ranks next to water. The high consumption of tea is attributed to richness in flavor, aroma, antioxidant properties and health benefits. Tea has complex chemical composition having catechins, flavonoids, alkaloids, carotenoids, chlorophyll, amino acids, polysaccharides, lipids, vitamins, minerals and more than 600 different volatile compounds.

Methods: The present study focuses on determining the physicochemical parameters, phyto-chemical assay and microbiological qualities of commercially packaged green and black tea samples within West Bengal following standard methods.

Result: The values of proximate parameters like moisture, ash, acid insoluble ash and water extractive have complied with standard specifications. High pressure liquid chromatography (HPLC) analysis quantified significantly higher polyphenol content of green tea in compare to black tea samples respectively with 65.12 ppm and 18.58 ppm with gallic acid equivalence and green tea epigallocatechin gallate (EGCG) content was found 449.28 ppm with respect to pure EGCG standard. *In vitro* antioxidant activity of green tea and black tea extracts as reported by IC_{50} value were found to be 192.97-301.08 µg/ml and 342.54-481.91 µg/ml respectively.

Key words: Antioxidant, Catechins, Contamination, EGCG, ISO specification.

INTRODUCTION

Since ancient times tea had been consumed for its medicinal and functional properties, but recently tea as beverage gained importance mainly because of its high content of some important bio-stable and direct acting therapeutic compounds. There is great variation in the chemical composition of tea depending on climatic conditions, soil properties, growth altitude, plucking season, grading, processing conditions and storage (Jiang *et al*., 2019). Chemical analysis proves that tea is a complex of over 2000 components of which polyphenols, the main constituents of tea, make up 20-35% of tea's dry weight (Abdel-Rahman *et al*., 2011). Catechins belonging to the family of flavonoids account for 60-80% of tea polyphenols (Tong *et al*., 2019). Yashin *et al*. (2015) identified twelve catechins by chromatographic techniques out of which catechin, epicatechin, gallocatechin, epigallocatechin, catechingallate, epicatechingallate, gallocatechin gallate and epigallocatechin gallate are present in significant quantity. Green tea polyphenols exhibit significant antiviral properties, mediated through a series of mechanisms, which is extremely essential during a pandemic situation. It is suggested that, EGCG attaches to the viral hemagglutinin, preventing the virus from binding to the cell's target receptor, which eventually prevents the virus from spreading. EGCG also changes the virus's envelope, halting the virus from infecting other cells. L-theanine is found in green tea, which stimulates human gamma delta T lymphocytes to produce interferon-c (IFN-c), an antimicrobial cytokine (Tallei *et al.,* 2021). In addition, green tea EGCG demonstrates strong efficacy in inhibiting infection of live SARS-CoV-2 and human corona virus (HCoVOC43) (Liu *et al.,* 2021). Tea catechins and polyphenols have effective scavenging activity of free radicals or the reactive oxygen species that are generated during various oxidative processes (Zhao *et al*., 2019). The secondary metabolites particularly amino acids, catechins and alkaloids are mostly associated with tea quality characteristics including colour, aroma and taste as well as various physiological and functional properties (Gao *et al*., 2020; Qu *et al*., 2020). The content of healthy constituents of tea depends on the type of tea, its quantity, temperature and time of brewing (Jakubczyk *et al*., 2020).

Understanding of the chemical composition of tea is a key technological underpinning in the systematic study of the beverage for healthy addition to the diet. Different commercial brands of green and black tea are available in the market having variation in their constituents and quality which directly influence its sensory attributes and storage stability. Therefore, the present research work has focused

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on understanding different bioactives present in commercially packaged market tea samples along with its antioxidant potential.

MATERIALS AND METHODS

Commercially packaged green and black tea samples of five popular international and national brands are purchased from different shopping malls of Kolkata. Analytical grade chemicals are used for analysis and purchased from HIMEDIA®.

Brewing of tea

Green tea samples are brewed by adding 1 gram tea leaves in 50 ml hot distilled water for 10 minutes. However, black tea samples are brewed by boiling the same amount in distilled water for 5 minutes.

Analysis of physicochemical properties of tea samples

The moisture content is determined by using digital moisture meter (Model No: MLS 50-3 HA 250, KERN, Germany) following the method I.S 3077: 1972. od No. 925.19. For estimation of crude protein content, the nitrogen content is determined using micro Kjeldahl method followed by multiplication with the factor 6.25, fat percentages are examined using Soxhlet apparatus using petroleum ether as solvent, total and acid insoluble ash in muffle furnace (AOAC, 2000). Total carbohydrate is determined by Anthrone method. The water extract of different samples are determined following ISO 9768 method.

Estimation of total phenolic content

The amount of total tea polyphenols are estimated as described by the ISO 14502-1 in spectrophotometer (UV-1800 ENG 240V, Shimadzu) at 738 nm taking gallic acid as a working standard.

DPPH* radical scavenging assay

The DPPH* (1, 1,-Diphenyl-2-picrylhydrazyl) assay is performed using a spectrophotometer (UV-1800 ENG 240V, Shimadzu) at 517 nm taking gallic acid as standard (Shekhar *et al.*, 2014). The results are reported as the IC_{50}, which is the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

HPLC assay

Pure green and black tea polyphenol is quantified using reverse phase high performance liquid chromatography in isocratic mode HPLC system (Shimadzu, UPLC load; SPD 20A) equipped with absorbance detectors set to 278 nm. C18 column (3.9 × 300 mm) is used to carry out the analysis. Elution is conducted at ambient temperature between 24 to 28°C using water: methanol (80: 20: 0.5 v/v) with 0.5% glacial acetic acid as a mobile phase at a flow rate 1.0 mL/min. Triplicate sample extracts are prepared and each extract is analyzed thrice taking Gallic acid as an external standard. Further with slight modification from Saito *et al.* (2006), same chromatographic condition is kept with changing mobile phase combination water, acetonitrile, methanol, ethyl acetate, glacial acetic acid (89: 6: 1: 3: 1 v/v/v/v/v) and same absorbance at 278 nm, EGCG content of pure green and black tea is quantified taking pure EGCG as an external standard.

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean±standard deviation. Differences between group means were estimated using a one way ANOVA followed by Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Determination of physico-chemical properties of tea samples

The various physico-chemical properties of different brands of green and black tea samples collected from the markets of Kolkata are represented in the Table 1 and 2 respectively. The results indicated the variations in average content of different components such as moisture 7.61-8.46%, total fat 1.62-2.26%, total protein 1.58-1.95%, total carbohydrate 12.48-15.33%, total ash 5.35-6.09%, acid insoluble ash 0.79-1.07%, water extract 35.43-43.02% and alcoholic extract 33.81-37.14% for commercially packaged green tea samples. For black tea samples the moisture content ranged between 6.13-7.18%, total fat 0.98-1.12%, total protein 0.94 -1.06%, total carbohydrate 11.32-13.88%, total ash 5.315-6.24%, acid insoluble ash 0.91-1.08%, water extract 36.18 - 41.86% and alcoholic extract 14.37-22.78%.

Determination of total polyphenol content

The total phenolic content of the commercially packaged green and black tea samples reported as mg/gm of sample in terms of gallic acid equivalence are represented in the Table 3. Among different green tea brands S_3 has shown highest amount of phenolic contents (248.06 mg/gm) and for black tea samples S_2 is found with highest polyphenols (80.44 mg/gm).

Results of DPPH* radical scavenging assay

The 1, 1,-Diphenyl-2-picrylhydrazyl radical scavenging assay represented as IC_{50} value of different commercially packaged green and black tea samples are depicted in Table 4. The table clearly revealed that both green and black tea samples showed different degree of antioxidant potential. The IC_{50} value of green tea samples are ranging from 192.97-301.08 µg/ml as compared to 16.34 µg/ml for pure gallic acid as antioxidant. However, the IC_{50} values of different black tea samples are higher with the values ranging from 342.54- 481.91 µg/ml. The IC_{50} value between different green and black tea brands is found to be significant at 1% ($p<0.01$) level.

Quantitative determination of tea polyphenols by reverse phase HPLC analysis

Fig 1 and Fig 2 clearly quantified and represented much higher concentration of pure green tea catechin in comparison with pure black tea standard when eluted

Table 1: Physico-chemical analysis of different green tea samples.

Green tea samples	Chemical composition (%)							
	Moisture	Fat	Protein	Total carbohydrate	Total ash	Acid insoluble ash	Water extract	Alcoholic extract
Brand 1	7.63±0.82	1.74±0.07^{a}	1.60±0.03^{a}	14.16±0.51^{b,c}	5.35±0.18^{a}	0.98±0.04^{c}	43.02±0.75^{d}	37.14±1.01^{b}
Brand 2	8.12±0.52	2.01±0.13^{b}	1.82±0.06^{b}	15.33±0.47^{d}	6.09±0.17^{c}	1.07±0.01^{d}	38.94±0.77^{b}	33.81±0.83^{a}
Brand 3	7.33±0.35	2.26±0.09^{c}	1.95±0.06^{c}	15.04±0.67^{c,d}	5.38±0.17^{a}	0.86±0.04^{b}	42.65±0.99^{d}	34.17±0.82^{a}
Brand 4	8.46±0.42	1.78±0.06^{a}	1.64±0.08^{a}	12.48±0.56^{a}	5.81±0.08^{b}	0.79±0.03^{a}	41.06±0.77^{c}	35.22±0.79^{a}
Brand 5	7.86±0.41	1.62±0.1^{a}	1.58±0.03^{a}	13.51±0.27^{b}	5.41±0.09^{a}	1.02±0.05^{c,d}	35.43±0.87^{a}	35.11±0.86^{a}
F value (ANOVA)	2.029	22.316	23.534	15.37	15.056	34.033	42.086	6.699
Sig. (P-value)	0.166 (NS)	<0.01* (S)	<0.01* (S)	<0.01* (S)	<0.01* (S)	<0.01* (S)	<0.01* (S)	<0.01* (S)

[x]Means of three trials and standard deviation for groups in homogeneous subsets (a, b, c, d) are displayed.

[y]NS: Not Significant; S: Significant.

[z]*1% level of significance.

Table 2: Physico-chemical analysis of different black tea samples.

Black tea samples	Chemical composition							
	Moisture	Fat	Protein	Total carbohydrate	Total ash	Acid insoluble ash	Water extract	Alcoholic extract
Brand 1	6.72±0.26^{b}	1.04±0.07^{a,b,c}	0.95±0.01^{a}	11.32±0.53^{a}	5.42±0.35^{a}	1.08±0.02^{c}	36.53±0.97^{a,b}	14.37±0.76^{a}
Brand 2	6.36±0.13^{a}	1.12±0.05^{c}	0.98±0.03^{a,b}	12.95±0.9^{b}	5.73±0.41^{a,b}	0.91±0.02^{a}	39.87±1.29^{c}	16.92±0.9^{b}
Brand 3	6.13±0.12^{a}	1.1±0.02^{b,c}	1.02±0.05^{b,c}	13.88±0.58^{b}	5.15±0.15^{a}	0.98±0.01^{b}	41.86±0.96^{d}	22.78±0.5^{d}
Brand 4	7.03±0.13^{c}	0.98±0.03^{a}	1.06±0.04^{c}	13.48±0.7^{b}	5.73±0.4^{a,b}	1.05±0.06^{c}	38.44±1.16^{b,c}	20.27±0.95^{c}
Brand 5	7.18±0.15^{c}	1.01±0.07^{a,b}	0.94±0.03^{a}	12.62±0.68^{b}	6.24±0.3^{b}	1.03±0.02^{c}	36.18±0.8^{a}	21.46±0.39^{c,d}
F value (ANOVA)	21.119	3.909	6.231	6.066	4.435	15.025	15.172	66.433
Sig. (P-value)	<0.01* (S)	0.037**(S)	0.009* (S)	0.010** (S)	0.026** (S)	<0.01* (S)	<0.01* (S)	<0.01* (S)

[x]Means of three trials and standard deviation for groups in homogeneous subsets (a, b, c, d) are displayed.

[y]NS: Not Significant; S: Significant.

[z]*1% level of significance; **5% level of significance.

against gallic acid and EGCG standard. The retention time against gallic acid and EGCG is found to be 3.78 min and 7.66 min respectively. The polyphenol content of green tea and black tea was found 65.12 ppm and 18.58 ppm, where as EGCG content in pure green tea is quantified with 449.28 ppm.

Table 3: Total phenolic content (mg/gm in terms of gallic acid equivalent) of different green and black tea samples.

Brands	Green tea	Black tea
Brand 1	152.75±8.44^{b,c}	55.21±0.99^{a}
Brand 2	183.41±32.93^{c}	80.44±3.85^{d}
Brand 3	248.06±34.47^{d}	78.35±2.55^{d}
Brand 4	128.67±14.94^{a,b}	61.03±1.03^{b}
Brand 5	92.94±5.35^{a}	69.98±1.62^{c}
F value (ANOVA)	19.978	68.205
Sig. (P-value)	<.01*(S)	<.01*(S)

[x]Means of three trials and standard deviation for groups in homogeneous subsets (a, b, c, d) are displayed.

[y]S: Significant.

[z]*1% level of significance.

There was no significant difference in the moisture content among different green tea brands. However, other physicochemical parameters were highly significant at 1% ($p<0.01$) level. Table 2 clearly depicted that moisture, crude protein, acid insoluble ash, water extract and alcoholic extract of black tea samples were significant at 1% ($p<0.01$) level whereas other parameters are significant at 5% ($p<0.05$) level. The moisture, fat and protein content of green tea samples showed slight higher values as compared to black tea samples. Similar trends are also noticed by and Adnan *et al.* (2013) and Rehman *et al.* (2002). As per IS specification the total ash and acid insoluble ash content of both green and black tea samples should be 4-8% by mass and maximum 1% respectively. The tea water extract is a complex mixture of polyphenols, alkaloids, amino acids, sugars and many minor soluble substances like minerals and pigments which largely depend on the ratio of tea and water, infusion temperature, type of tea, particle size and

Table 4: DPPH radical scavenging assay (µg/ml in terms of gallic acid equivalent) of different green and black tea samples.

Samples	Green tea		Black tea	
	IC_{50} value	R^2 value	IC_{50} value	R^2 value
Control	16.34	0.981	16.34	0.981
Brand 1	238.26±16.14^{b}	0.986	481.91±20.43^{c}	0.974
Brand 2	195.2±8.19^{a}	0.997	342.54±21.39^{a}	0.991
Brand 3	192.97±19.59^{a}	0.987	370.12±11.74^{a}	0.982
Brand 4	282.11±16.99^{c}	0.981	471.83±23.51^{b,c}	0.985
Brand 5	301.08±24.03^{c}	0.975	440.79±18.08^{b}	0.979
F value (ANOVA)	23.07		30.529	
Sig. (P-value)	<.01*(S)		<.01*(S)	

[x]Mean of three trials and Standard Deviation for groups in homogeneous subsets (a, b, c) are displayed.

[y]S: Significant.

[z]*1% level of significance.

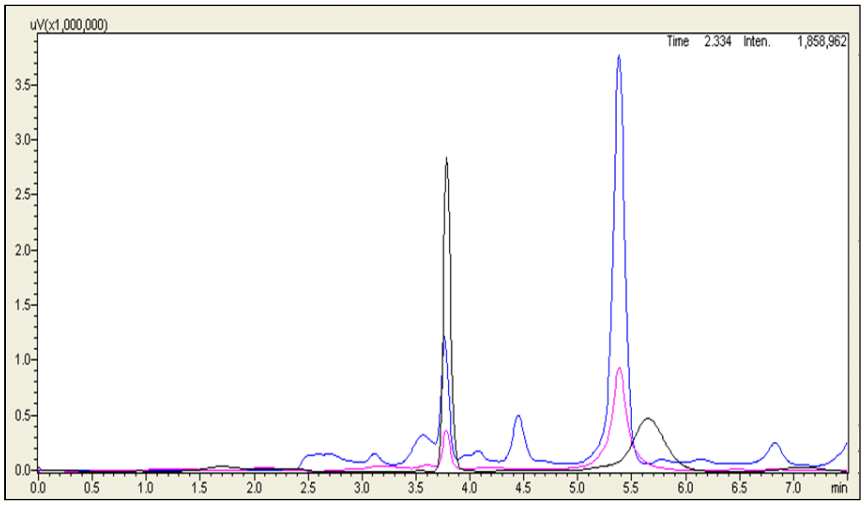

Fig 1: Comparison of pure green tea and black tea polyphenol content with respect to pure gallic acid standard.

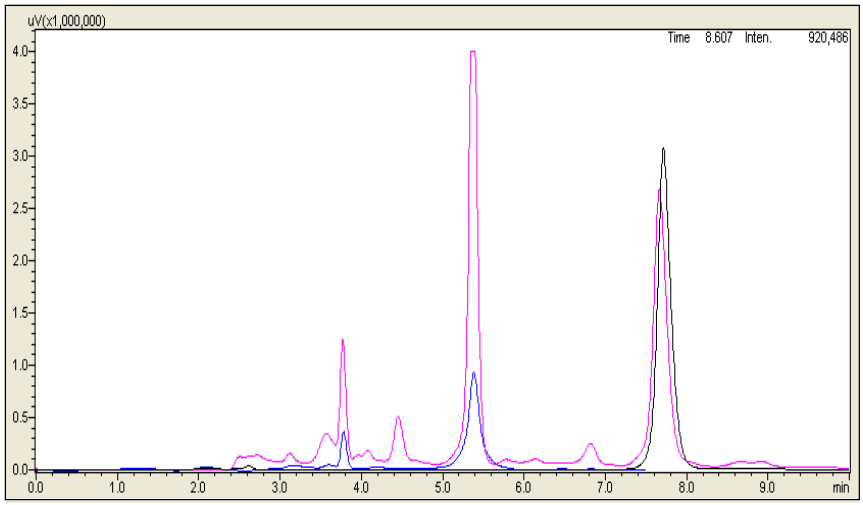

Fig 2: Comparison of pure green tea and black tea EGCG content with respect to pure EGCG standard.

proximate composition. According to international standards it should be more than 32% of the dry matter basis. In all the samples studied the water extract values follow the prescribed standard.

From the result of total phenolic content it is clear that green tea samples have higher amount of polyphenols than black tea samples. This might be due to processing conditions of black tea leaves as it undergoes complete fermentation process to oxidize catechin derivatives which results in the formation of the polymeric compounds, thearubigins and theaflavins. On the other hand, fresh tea leaves rich in polyphenols are not subjected to fermentation and steaming prevents the oxidation of catechin by polyphenol oxidase resulting in maximum amount of polyphenols in green tea. The results are varying from the observation of Abdolmaleki (2016) who reported the amount of total polyphenols in black tea samples in Iran varied from 17.10 to 12.20 g/100 g. The variations might be due to different regions of tea cultivation, soil property, processing conditions, storage conditions, extraction procedure *etc*.

From the DPPH* assay it can be concluded that brand 3 of both green and black tea samples are the best among analyzed tea samples as it showed much antioxidant potential. Bartoszek *et al.* (2018) also reported that among all tea varieties green tea is the best DPPH free radical scavengers.

In HPLC analysis, no elution for black tea sample is observed when pure black injected against EGCG standard at the particular retention time reported, which indicates green tea holds strong antioxidative properties with respect to black tea (Tallei *et al*., 2021; Lee *et al*., 2014; Sano *et al.,* 2001). The result also confirms the fact that tannin constituents such as thearubigins and theaflavins present in black tea are formed by the enzymatic oxidation of EC, EGC and EGCG followed by condensation (Sang *et al.,* 2016).

CONCLUSION

The study highlights greater percentage of moisture, fat, protein and carbohydrates in green tea samples and almost all the quality parameter of different samples follow the ISO specification. The total polyphenol content was also higher in green tea which confirms potential health benefits of the beverage as compared to black tea. The study enriches the knowledge about quality of commercial packaged tea available in the state of West Bengal which may directly affect the preference of consumers over brands and help in designing quality standards by the competent authorities. Thus, with the growing popularity of tea as healthy beverage, especially during the post COVID scenario the present study is certainly going to hold greater consumer acceptance to safely enjoy a cup of tea as a preferred beverage.

Conflict of interest: None.

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