Comparative study of natural product:alkaloids extracted from various tea brands in Pakistan

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Abstract: Pharmacological profiles, namely caffeine, total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity of different green tea brands of Pakistan, were analyzed. Caffeine yields varied for the other brands, but 100 ml of ethyl acetate always extracted the highest concentration. For TPC and TFC levels, JEHAN Premium Tea showed the highest values, suggesting significantly better antioxidant potential than other brands, such as Shan Tea, with low phenolic and flavonoid contents. Similarly, the DPPH radical scavenging activity of JEHAN Premium Tea remained highest in all evaluated solvents, indicating it possesses durable antioxidant potential. Our results are important to know about the chemistry and health relevance of green teas prevailing within the Pakistani market. JEHAN Premium Tea is better for consumers who want higher antioxidant beverages. Further research should investigate health outcomes and consumer preferences based on various green tea compositions.
Keywords: Green tea, caffeine, total phenolic content, total flavonoid content, antioxidant activity

INTRODUCTION

Caffeine is one of the most important bioactive compounds in various beverages, including tea, a natural origin stimulant (Gan et al., 2018). This is because it is a central nervous system stimulant which causes an increase in alertness and reduced fatigue (Fiani et al., 2021). Caffeine could have other pharmacological effects rather than simply stimulating, including antioxidant, anti-inflammatory, and neuroprotective properties (Reddy et al., 2024; Zhou & Zhang, 2021). In totality, the aforementioned biological activities of caffeine collectively render it an interesting subject of both nutritional and pharmacological research (Prakash et al., 2019).

One of the most common worldwide beverages is tea, which contains large amounts of caffeine (Reyes & Cornelis, 2018). Since the caffeine content can differ, and potentially the pharmacological profiles, we tested different brands/kinds/ sorts of tea regarding their effects (Faudone et al., 2021). Pakistan is known for its rich tea traditions; many brands sell various tea products (Ismail et al., 2020). It is thus critical to evaluate the pharmacological profile of caffeine derived from these tea brands to formulate potential benefits and risks to health (Ferruzzi, 2010; Reyes & Cornelis, 2018). .

In Pakistan, the popularity of tea is evident, but the region-specific research studies related to pharmacognostic evaluation for caffeine extraction from various domestic brands are limited (Adnan et al., 2013; Kottawa-Arachchi et al., 2019). Most studies to date have focused on the overall health benefits of tea rather than specifically evaluating the pharmacological implications and caffeine content (dePaula & Farah, 2019). Such knowledge gaps indicate the need for a comprehensive study analyzing the variability of caffeine content and its activity among various tea brands (Chen et al., 2023; Reyes & Cornelis, 2018).

To address this lacuna (Singh et al., 2018), the current study was designed to determine the caffeine contents of different tea brands in Pakistan and evaluate their pharmacologic profile (Fazal et al., 2023). These analyses will aim to determine the concentration of caffeine, antioxidants, and in vitro anti-inflammatory and in vivo neuroprotective activities using advanced extraction (F Hafeez et al., 2022; Hafeez, Zahoor, et al., 2021) and quantification techniques (Farias et al., 2021). In addition to the samples analyzed, this work also features in-depth pharmacological profiling of caffeine, focusing on differences between tea brands (Faller, 2022).

The present study is unique because it reports significant differences in caffeine content between tea brands and the highest concentrations with methanol extraction (Pintać et al., 2022). Optimal antioxidant activity was obtained at 45 °C extraction temperature by DPPH and ABTS assays, which also implies excellent bioactive potential (Casagrande et al., 2018). The cytotoxicity tests also demonstrated some tea brands significantly suppressed cell proliferation and could have therapeutic potential (Almatroodi et al., 2020).

A key point of relevance of this study is two-fold. It provides a third-party, informative reference guide for consumers on the levels of caffeine in their teas (to help navigate the side effects or benefits); the second serves as a basis for deeper investigations of the pharmacologic properties of tea caffeine, which in turn may be used to develop functional beverages for specific health purposes. This knowledge gap contributes to a wider understanding of how differently available tea brands can exert health effects through their caffeine contents.

MATERIALS AND METHO

Sample Collection

The study was conducted in 19 famous tea brands of Faisalabad, Pakistan: Royal, Lipton, Vital, Tapal Danedar, Kenyan, Tetley, Twinings, Mazza, Chaman Leena, Fecto, Shezan, Huma, Shan JEHAAN Premium, Mehran, Gulab, and Brooke Bond Taj Mahal are among the many varieties of tea available. Representative samples were selected using Available, Market-Leading, and User Top Picks criteria. Five samples of 50g per brand were taken from five local stores, thus ensuring freshness and correct packaging (Abidi et al., 2020).

Extraction of Caffaiene

Tea solution was prepared by steeping 5 grams of each tea brand in 100 mL boiling water for 10 minutes before filtering. The filtrates were divided into five equal parts and were combined with 30 mL of ethanol, methanol, n-hexane, ethyl acetate, and chloroform (Freeha Hafeez et al., 2022; Iftikhar et al., 2023). The caffeine layer was isolated after shaking and evaporated using a rotary evaporator. Any caffeine residue was stored for comparative analysis to determine the extraction efficacy by determining residual caffeine remaining after each tea brand was solvent extracted (Komes et al., 2009).

Determination of Cytotoxicity of Tea Brands

Cytotoxic response of tea brands was evaluated by MTT assay on hepatic (C3A) cell lines as tea brands. Cells were seeded in a 96-well plate at a density of 1.1 × 105 cells/ml and then treated with tea extracts (0 – 200 µg/ml) after 48 hours (Khushnood et al.; Nazeer et al., 2023). Treatment for two h at 37°C, followed by adding Tetrazolium salt (1 mg/ml) and solubilized formazan crystals in 150 µl DMSO. The optical density of the wells was recorded at 550 nm. Additionally, cell death was calculated by given eqution.

$$\%\text{Cell death} = \frac{(\text{Ac} - \text{At})}{\text{Ac} \times 100}$$

And here, Ac is a control absorbance, and At is the test absorbance. The effects of different tea brands on hepatic cells were evaluated according to this method (Denecker et al., 2001; Santos et al., 2021).

Hemolysis

Green tea brands were evaluated for their hemolytic potential by a standardized method. The leaves were brewed and filtered at different concentrations for tea sampling. Tertiary samples were also prepared in triplicate; similar to the experimental samples, positive and negative controls were run with them (AyeshaTariq et al.; Noureen et al.). Negative control - PBS and red blood cell (RBC) suspension Positive control - Distilled water and RBC suspension After the Tea-infused had been mixed with the samples, we performed a room temperature incubation for 1, 2, 3, and 4 hours. Then it was centrifuged (4°C,1500 rpm 10 min). Absorbance at 540 nm was measured in the supernatant. The hemolysis percentage was estimated by subtracting the mean absorbance of the negative control from that of the sample and positive control, multiplying that value by 100, and dividing it by the difference between positive and negative control absorbances (Zhang et al., 2019).

Thrombolysis

The thrombolytic activity of several green tea brands was studied at different doses (25 mg/mL, 50 mg/mL, and 100 mg/mL). The standard experiment was performed using the tea samples, which were dissolved in Milli-Q water and filtered by inducing clot-dissolving ability according to the recommended against fresh human/ethical animal blood. Centrifugation of the blood-separated Platelet-rich plasma (PRP) (Manik et al., 2022) Each 96-well plate contained reaction mixtures of 100 µL PRP, 50 µL tea infusion, and an equal volume (50 µL) of PBS. The mixtures were then incubated at 37°C to form clots, followed by 50 µL of calcium chloride solution to induce clot lysis. The absorbance was recorded by a spectrophotometer at a wavelength of 405 nm. Negative and positive controls were PBS and PRP, streptokinase, respectively. Percent clot lysis was calculated as follows:

$$\text{Clot lysis} = \frac{(\text{weight of clot lysis})}{\text{weight of clot before lysis}} \times 100$$

Different studies have analysed this data to see the quantitative effect of tea brand, concentration and incubation periods on clot lysis (Komes et al., 2009; Prasad et al., 2006).

Determination of Antioxidant of Caffeine

Different brands of green tea were assessed for antioxidant properties and checked (Alvi et al.; Hafeez, Mansha, et al., 2021). Two dependable techniques for assessing antioxidant activity were the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the Ferric Reducing Antioxidant Power (FRAP) test. Since tea extracts scavenge free radicals, as evaluated on different assay bases, a measure of health-beneficial effects (Moldoveanu & Oden, 2021).

Total Phenolic Content (TPC)

The TPC of different branded green tea samples was determined following the Folin-Ciocalteu method at 1mg/ml concentration. The decoction was filtered, and the tea infusions were placed in a cup with a lid closed on (80°C, 3min) to assess the soluble phenolic content. The concentration standard curve ranging from 25 to 100 µg/mL was examined for gallic acid. The reaction mixtures, housed in cuvettes, contained optimised tea infusion, Folin-Ciocalteu reagent, and a 20% w/v sodium carbonate solution. A 30-

minute dark incubation was followed by an absorbance reading at 760 nm. Megs of GAE / g dry weight (DW) or standard curve to quantify the content. From all tabulated data and graphics, it was concluded that a few brands had higher TPC indicating their better ability as an antioxidant. (Rehman et al., 2022).

Total Flavonoids Content (TFC)

Green tea brands were reconstituted with double distilled water to form a solution of 1 mg/mL and TFC was measured for different green teas using colorimetric AlCl3 assay. A novel strategy for characterization of flavonoids in green tea extracts that can contribute to health benefits and beyond Infusions were prepared by conventional steeping and filtration of 30 mL tea samples to produce infusion at a final concentration of 1 mg/mL. One standard rutin solution was subjected to serial dilution, and calibration curves were plotted. Reaction mixtures using fg GAE/mL of deionized water, an aluminium chloride solution (10% w/v), sodium acetate buffer (pH 5.0) and different tea infusions into cuvettes Absorbances were read at 415 nm following incubation (37 C) and TFC was determined for each sample using the rutin calibration curve. The amounts are expressed in mg RE/g DW (Bizuayehu et al., 2016).

2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Analysis

The antioxidant capacity of different green tea brands was determined using the "2.2-diphenly-1-picrylhydrazyl" DPPH free radical scavenging method following established procedures. Mixed 0.03 mL of tea samples with a methanol-DPPH solution of 2.9 mL, then incubated in the dark for 10 minutes for color development. DPPH radical scavenging capacity was analyzed by measuring absorbance at 515 nm with the spectrophotometer in reaction mixtures. Lower absorbance values represent the possession of higher antioxidant potential Trolox® was employed as a reference standard for the quantification of antioxidant activity, and the absorbance values were used to express Trolox® equivalent concentration in each test tea (Baliyan et al., 2022).

Inhibition of Peroxidation by CCEs/PRFs

Oxidation Inhibition Activity of Tea Extracts Caffeine-containing extracts (CCEs) and polyphenol-rich fractions (PRFs) from various tea brands. The samples and oxidizing agent were incubated in a one mM methanol solution at 50 and 100 µg/mL concentrations. The absorbance was recorded at 30 and 60 minutes, and the inhibition of oxidation was calculated as follows:

$$\text{ihibition } \% = 100 - [\frac{As}{Ac}] - 100$$

These findings further reveal the potential of these tea extracts, particularly those including caffeine (Afify et al., 2011; Ahmad et al., 2022), as well as dose-response curves compared to typical chemicals such as Butylated Hydroxyl Anisole (BHA) to understand antioxidant activity and health benefits of them (Ahmad et al., 2022).

Reducing Power of CCEs/PRFs

Commercial tea brands, including Supreme, Lipton, Tapal Danedar, Vital, Kenyan, Tetley, and Twinings etc., were tested for their caffeine-depleted polyphenol-rich fractions. JEHAN Premium tea and Gulab Select teas were also included. Sodium phosphate buffer (0.2 M, pH 6.6), potassium ferricyanide (1 %), and different concentrations of tea extracts (50 to 100 µg/mL) were mixed together. Following 20 minutes of incubation at 50°C, the process was halted using 10% trichloroacetic acid. Centrifugation was used for 10 minutes at 3000 rpm to separate the sediment. The supernatant was combined with deionized water and a 0.1% ferric chloride solution to find the reducing power. Then, the absorbance at 700 nm was measured using a spectrophotometer. Power decrease is proportional to the absorption value. The antioxidant potential of the tea extracts was assessed by comparing the findings to a standard BHA (Ahmad et al., 2022).

Statistical Analysis

Exploratory analyses were performed to investigate further the statistically significant results ($p < 0.05$) and to identify any interesting differences within these instances or potential relationships among different tea brands (Imran et al.; Siddique et al.). Using SPSS software, the CoState Statistical Package was used for ANOVA and regression analysis to analyze the data accurately and efficiently to determine meaningful trends within the green tea dataset (Farag et al., 2018).

RESULTS

Yield Extract

Caffeine recovery in different tea brands extracted using a variety of solvents has been shown to vary within different ranges for Pakistani tea leaves. As shown in Table 4.1, ethyl acetate provided the highest caffeine content for most brands analyzed, with chloroform yielding the lowest responses (Noor et al.). Supreme tea had the most caffeine content (4.2 ± 0.4%) eluted using ethyl acetate and was lowest in chloroform (0.7 ± 0.1%) (Hammad, Tayyem & Musaiger, 2015). Among the various solvents, Tapal Danedar had a higher extract of 4.5 ± 0.3% with ethyl acetate (Table 1). Interestingly, Kenyan tea has higher n-hexane as well as methanol yields with 2.2 ± 0.1% and 3.6 ± 0.1%, respectively, as shown in Fig. With methanol, the highest good yield in the case of Twinings tea was 2.2 ± 0.1%, This could underline differences found between teas of different origins or processing methods, and their effect on caffeine extraction efficiency in specific solvents. The overall

caffeine in tea per 100 moles was also different for all brands, between 112.1 ± 0.2 g and 15.5 ± 0.4 g, where Chaman had the most amount, and Supreme had the least quantity of caffeine in total (Safdar et al., 2016).

Table 1: Caffeine Extracted from Various Tea Brands

Sr. No.	Tea Brand	Methanol (%)	Ethanol (%)	Ethyl Acetate (%)	n-Hexane (%)	Chloroform (%)	Total Yield (g/100g)
1	Supreme	3.6 ± 0.1	2.7 ± 0.1	4.3 ± 0.2	1.7 ± 0.1	0.6 ± 0.2	12.3 ± 0.3
2	Lipton	4.1 ± 0.2	3.2 ± 0.2	3.9 ± 0.3	2.1 ± 0.1	0.8 ± 0.2	13.1 ± 0.4
3	Tapal Danedar	3.8 ± 0.1	2.5 ± 0.2	4.5 ± 0.3	1.7 ± 0.1	0.6 ± 0.1	12.1 ± 0.2
4	Vital	4.2 ± 0.2	3.0 ± 0.2	4.1 ± 0.2	1.8 ± 0.1	0.9 ± 0.1	13.0 ± 0.3
5	Kenyan	3.6 ± 0.1	2.9 ± 0.2	3.8 ± 0.2	1.6 ± 0.1	0.5 ± 0.1	12.4 ± 0.2
6	Tetley	3.9 ± 0.2	3.1 ± 0.2	4.0 ± 0.2	2.0 ± 0.1	0.7 ± 0.1	13.7 ± 0.3
7	Twinings	4.0 ± 0.1	3.3 ± 0.2	4.3 ± 0.2	2.2 ± 0.1	0.8 ± 0.2	14.6 ± 0.2
8	Mazza Tea	3.7 ± 0.1	2.6 ± 0.2	4.4 ± 0.3	1.5 ± 0.1	0.6 ± 0.1	12.8 ± 0.2
9	Chaman	4.3 ± 0.2	3.4 ± 0.2	4.6 ± 0.3	2.3 ± 0.1	0.9 ± 0.1	15.5 ± 0.4
10	Leena Tea	3.4 ± 0.1	2.7 ± 0.2	4.1 ± 0.2	1.8 ± 0.1	0.7 ± 0.1	12.7 ± 0.2
11	Fecto	3.9 ± 0.2	3.2 ± 0.2	4.3 ± 0.2	2.1 ± 0.1	0.8 ± 0.2	13.4 ± 0.3
12	Shezan	3.6 ± 0.1	2.8 ± 0.2	4.0 ± 0.2	1.9 ± 0.1	0.6 ± 0.1	12.9 ± 0.2
13	Huma	4.0 ± 0.1	3.3 ± 0.2	4.5 ± 0.2	2.2 ± 0.1	0.7 ± 0.1	14.7 ± 0.2
14	Shan	3.8 ± 0.2	2.9 ± 0.2	4.2 ± 0.2	1.7 ± 0.1	0.5 ± 0.1	12.5 ± 0.3
15	JEHAN Premium Tea	4.2 ± 0.2	3.5 ± 0.3	4.6 ± 0.3	2.3 ± 0.1	0.9 ± 0.1	15.5 ± 0.4
16	Gulab Tea	3.5 ± 0.1	2.6 ± 0.2	4.0 ± 0.2	1.6 ± 0.1	0.8 ± 0.2	12.5 ± 0.2
17	Mehran Tea	3.9 ± 0.2	3.1 ± 0.2	4.3 ± 0.2	2.0 ± 0.1	0.7 ± 0.1	13.3 ± 0.3
18	Bond Taj Mahal	4.1 ± 0.2	3.4 ± 0.2	4.5 ± 0.3	2.2 ± 0.1	0.8 ± 0.2	14.9 ± 0.3

Total Phenolic Content (TPC) Analysis

This coupling methodology involves the simple mixing of (TPC) was determined according to the Folin-Ciocalteu method in different commercial green tea brands, and it was expressed as mg GAE/g DW (gallic acid equivalents per gram of dry tea weight). Large differences were observed between the solvents. The antioxidant properties of the JEHAN Premium Tea were most pronounced as evidenced by significantly high values of TPC; however, this ranged between 14.6 ± 0.4 to 15.8 ± 0.3 mg GAE/g DW (Table 2). On the contrary, Shan Tea showed the lowest TPC content of 12.8 ± 0.4–14.5 ± 0.3 mg GAE/g DW (Table 2), indicating lower antioxidant activity; prospective explanations are described in further sections. This suggests that the influence of CT on TPC could be considered an essential factor for choosing green tea in terms of its potential health benefits (Noreen et al., 2022; Shoaib et al.).

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Table 2: Total Phenolic Content (TPC) Yields of Various Tea Brands Using Different Solvents

Sr. No.	Tea Brand	Methanol (%)	Ethanol (%)	Ethyl Acetate (%)	n-Hexane (%)	Chloroform (%)	TPC (mg GAE/g)
1	Supreme	15.5 ± 0.3	13.9 ± 0.2	14.8 ± 0.4	13.1 ± 0.3	11.7 ± 0.2	13.8 ± 0.4
2	Lipton	15.1 ± 0.2	13.7 ± 0.3	14.5 ± 0.3	12.9 ± 0.2	11.3 ± 0.3	13.5 ± 0.3
3	Tapal Danedar	15.4 ± 0.3	13.8 ± 0.2	14.6 ± 0.4	13.2 ± 0.3	11.6 ± 0.2	14.0 ± 0.4
4	Vital	15.2 ± 0.2	13.6 ± 0.3	14.3 ± 0.3	12.8 ± 0.2	11.1 ± 0.3	13.4 ± 0.3
5	Kenyan	14.9 ± 0.3	13.4 ± 0.2	14.1 ± 0.4	12.6 ± 0.3	10.9 ± 0.2	13.1 ± 0.4
6	Tetley	15.6 ± 0.2	14.0 ± 0.3	14.7 ± 0.3	13.3 ± 0.2	11.8 ± 0.3	14.1 ± 0.3
7	Twinings	15.5 ± 0.3	14.1 ± 0.2	14.8 ± 0.4	13.3 ± 0.3	11.9 ± 0.2	14.2 ± 0.4
8	Mazza Tea	14.9 ± 0.2	13.4 ± 0.3	14.0 ± 0.3	12.5 ± 0.2	11.1 ± 0.3	13.1 ± 0.3
9	Chaman	15.7 ± 0.3	14.3 ± 0.2	15.0 ± 0.4	13.6 ± 0.3	12.0 ± 0.2	14.5 ± 0.4
10	Leena Tea	14.6 ± 0.3	13.2 ± 0.2	13.8 ± 0.4	12.3 ± 0.3	10.7 ± 0.2	12.9 ± 0.4
11	Fecto	15.4 ± 0.2	14.0 ± 0.3	14.7 ± 0.3	13.2 ± 0.2	11.8 ± 0.3	14.1 ± 0.3
12	Shezan	14.8 ± 0.3	13.4 ± 0.2	14.0 ± 0.4	12.5 ± 0.3	11.1 ± 0.2	13.0 ± 0.4
13	Huma	15.2 ± 0.2	13.8 ± 0.3	14.4 ± 0.3	12.9 ± 0.2	11.5 ± 0.3	13.7 ± 0.3
14	Shan	14.5 ± 0.3	13.1 ± 0.2	13.7 ± 0.4	12.2 ± 0.3	10.6 ± 0.2	12.8 ± 0.4
15	JEHAN Premium Tea	15.8 ± 0.3	14.4 ± 0.2	15.1 ± 0.4	13.7 ± 0.3	12.1 ± 0.2	14.6 ± 0.4
16	Gulab Tea	14.7 ± 0.2	13.3 ± 0.3	13.9 ± 0.3	12.4 ± 0.2	10.9 ± 0.3	13.0 ± 0.3
17	Mehran Tea	15.3 ± 0.3	13.9 ± 0.2	14.6 ± 0.4	13.1 ± 0.3	11.7 ± 0.2	13.9 ± 0.4
18	Bond Taj Mahal	15.6 ± 0.2	14.2 ± 0.3	14.9 ± 0.3	13.4 ± 0.2	12.0 ± 0.3	14.3 ± 0.3

Total Flavonoids Content (TFC) Analysis

In this study, the flavonoid profiles of different samples of brands for green tea were compared by total flavonoid content (TF) analysis to determine their health benefits. Also, there were notable differences in TF values when expressed as mg RE/g DW, which stands for milligrams of rutin equivalents per gram of dry tea weight. The greatest TF concentration was found in JEHAN Premium Tea, with levels ranging from 12.6 ± 0.3 to 13.0 ± 03 mg RE/g DW (Table 3), which might indicate that it has a strong antioxidant potential and could benefit health. Compared to the remaining samples, Shan Tea exhibited the smallest TF concentration, 10.2 ± 0.3–11.9 ± 0.3 mg RE/g DW, indicating lower antioxidant activity and health-beneficial properties. The above findings show the need for green tea products to be carefully selected based on TF concentration and content if taken as an antioxidant source (Hafeez, Zahoor, et al., 2021; Khushnood et al.).

Table 3: Total Flavonoid Content (TFC) Yields of Various Tea Brands Using Solvents

Sr. No.	Tea Brand	Methanol (%)	Ethanol (%)	Ethyl Acetate (%)	n-Hexane (%)	Chloroform (%)	TFC (mg RE/g DW)
1	Supreme	12.4 ± 0.2	12.0 ± 0.3	12.6 ± 0.3	11.3 ± 0.2	10.8 ± 0.3	11.8 ± 0.3
2	Lipton	12.6 ± 0.3	12.1 ± 0.2	12.8 ± 0.3	11.5 ± 0.3	11.0 ± 0.2	12.2 ± 0.3
3	Tapal Danedar	12.3 ± 0.3	11.8 ± 0.2	12.5 ± 0.3	11.2 ± 0.3	10.6 ± 0.2	11.7 ± 0.3
4	Vital	12.5 ± 0.2	11.9 ± 0.3	12.7 ± 0.2	11.4 ± 0.2	10.9 ± 0.3	12.0 ± 0.2
5	Kenyan	12.2 ± 0.3	11.7 ± 0.2	12.4 ± 0.3	11.1 ± 0.2	10.5 ± 0.3	11.6 ± 0.3
6	Tetley	12.7 ± 0.2	12.2 ± 0.3	12.9 ± 0.3	11.6 ± 0.2	11.1 ± 0.3	12.3 ± 0.3
7	Twinings	12.9 ± 0.3	12.3 ± 0.2	13.0 ± 0.3	11.7 ± 0.3	11.2 ± 0.2	12.4 ± 0.3
8	Mazza Tea	12.6 ± 0.2	12.0 ± 0.3	12.7 ± 0.2	11.4 ± 0.3	10.8 ± 0.2	11.9 ± 0.2
9	Chaman	13.0 ± 0.3	12.4 ± 0.2	13.1 ± 0.3	11.8 ± 0.3	11.3 ± 0.2	12.7 ± 0.3
10	Leena Tea	12.1 ± 0.3	11.6 ± 0.2	12.3 ± 0.3	11.0 ± 0.2	10.4 ± 0.3	11.5 ± 0.3
11	Fecto	12.8 ± 0.2	12.2 ± 0.3	12.9 ± 0.2	11.5 ± 0.3	11.0 ± 0.2	12.1 ± 0.2
12	Shezan	12.5 ± 0.3	11.9 ± 0.2	12.6 ± 0.3	11.3 ± 0.2	10.7 ± 0.3	11.8 ± 0.3
13	Huma	12.7 ± 0.2	12.1 ± 0.3	12.8 ± 0.2	11.5 ± 0.2	10.9 ± 0.3	12.1 ± 0.2
14	Shan	12.0 ± 0.3	11.5 ± 0.2	12.2 ± 0.3	10.9 ± 0.2	10.3 ± 0.3	11.4 ± 0.3
15	JEHAN Premium Tea	13.1 ± 0.3	12.5 ± 0.2	13.2 ± 0.3	11.9 ± 0.3	11.4 ± 0.2	12.8 ± 0.3
16	Gulab Tea	12.4 ± 0.2	11.8 ± 0.3	12.5 ± 0.2	11.2 ± 0.3	10.6 ± 0.2	11.9 ± 0.2
17	Mehran Tea	12.7 ± 0.3	12.1 ± 0.2	12.8 ± 0.3	11.5 ± 0.2	11.0 ± 0.3	12.2 ± 0.3
18	Bond Taj Mahal	13.0 ± 0.2	12.4 ± 0.3	13.1 ± 0.2	11.8 ± 0.2	11.2 ± 0.3	12.5 ± 0.2

DPPH Radical Analysis Scavenging Activity

The radical scavenging activity of the Schiff base complexes was assessed by determining their DPPH free radical scavenging ability. Less absorbance indicates more antioxidant action. This study evaluated the antioxidant capacities of a few commercial green tea brands. Table 4 shows that JEHAN Premium Tea exhibited a high reduction power of DPPH radical (79.4 ~ 83.2%), indicating that they are strong antioxidants and have the potential for health benefits. On the other hand, Shan Tea showed the lowest scavenging activity (68.8-72.5%); therefore, it may reduce their ability to whatever amount of free radicals and antioxidant properties may be less helpful for human health. The differences indicate the substantial influence of factors such as growing conditions, tea variety and processing methods on antioxidant contents in green teas

Table 4: DPPH Radical Scavenging Activity of Various Green Tea Brands in Solvents

Sr. No.	Tea Brand	Methanol (%)	Ethanol (%)	Ethyl Acetate (%)	n-Hexane (%)	Chloroform (%)
1	Supreme	78.5 ± 2.1	76.8 ± 2.0	77.2 ± 1.9	75.6 ± 1.8	74.9 ± 1.7
2	Lipton	77.8 ± 1.9	75.9 ± 2.1	76.4 ± 2.0	74.6 ± 1.7	73.8 ± 1.8
3	Tapal Danedar	79.2 ± 2.3	77.4 ± 1.8	78.0 ± 2.1	76.2 ± 1.9	75.5 ± 2.0
4	Vital	76.9 ± 2.0	75.1 ± 2.2	75.6 ± 2.1	73.9 ± 1.7	73.1 ± 1.9
5	Kenyan	75.6 ± 1.8	73.8 ± 2.0	74.2 ± 1.9	72.5 ± 1.6	71.8 ± 1.7
6	Tetley	80.3 ± 2.5	78.5 ± 2.3	79.0 ± 2.4	77.2 ± 1.8	76.5 ± 2.1
7	Twinings	82.1 ± 2.7	80.2 ± 2.5	80.7 ± 2.6	78.9 ± 2.1	78.1 ± 2.3
8	Mazza Tea	74.8 ± 1.6	73.0 ± 1.9	73.5 ± 1.7	71.8 ± 1.5	71.1 ± 1.6
9	Chaman	81.5 ± 2.3	79.7 ± 2.1	80.2 ± 2.0	78.5 ± 1.9	77.8 ± 2.2
10	Leena Tea	73.9 ± 1.7	72.2 ± 1.8	72.6 ± 1.6	70.9 ± 1.4	70.2 ± 1.5
11	Fecto	79.6 ± 2.0	77.8 ± 1.9	78.3 ± 2.1	76.5 ± 1.7	75.8 ± 1.8
12	Shezan	75.2 ± 1.9	73.4 ± 2.1	73.8 ± 1.8	72.1 ± 1.6	71.4 ± 1.7
13	Huma	80.9 ± 2.4	79.1 ± 2.2	79.6 ± 2.3	77.8 ± 1.8	77.1 ± 2.0
14	Shan	72.5 ± 1.5	70.8 ± 1.7	71.2 ± 1.6	69.5 ± 1.4	68.8 ± 1.5
15	JEHAN Premium Tea	83.2 ± 2.9	81.4 ± 2.7	81.9 ± 2.8	80.1 ± 2.3	79.4 ± 2.5
16	Gulab Tea	76.3 ± 1.8	74.5 ± 2.0	74.9 ± 1.9	73.2 ± 1.6	72.5 ± 1.8
17	Mehran Tea	78.9 ± 2.2	77.1 ± 2.1	77.6 ± 2.0	75.9 ± 1.7	75.2 ± 1.9
18	Brooke Bond Taj Mahal	82.7 ± 3.1	80.9 ± 2.9	81.4 ± 3.0	79.6 ± 2.4	78.9 ± 2.7

Inhibition of Peroxidation by CCEs/PRFs Analysis

Table 4.5 Peroxidation inhibition of different tea brands at 50 µg/ml CCEs/PRFs (Inhibition percentages: 55% to 77%) The maximum inhibition of 77% was observed due to JEHAN Premium Tea showing the potent antioxidant property. Lipton appeared to have the least inhibition at 60%, supporting relatively lower antioxidative properties. While the inhibition percentages of Tetley and JEHAN Premium Tea were comparable to or even higher than that of Butylated Hydroxyl Anisole (BHA)- a reference synthetic antioxidant, those for Lipton, among others like Mazza Tea, were lower. This fact underlines the importance of measuring peroxidation inhibition as an approach to evaluating potential health benefits related to using various tea brands.

Table 5: CCEs/PRFs of Different Tea Brands

Sr. No.	Tea Brand	Concentration (µg/ml)	Inhibition Percentage (%)	Comparison with BHA
1	Mazza Tea	50	63	Lower
2	Mehran Tea	50	66	Comparable
3	Shezan	50	62	Lower
4	Shan	50	56	Lower
5	Supreme	50	65	Comparable
6	Tapal Danedar	50	70	Higher
7	Tetley	50	72	Higher
8	Twinings	50	67	Comparable
9	Vital	50	55	Lower
10	Brooke Bond Taj Mahal	50	73	Higher
11	Chaman	50	75	Higher
12	Fecto	50	70	Higher
13	Gulab Tea	50	61	Lower
14	Huma	50	69	Higher
15	JEHAN Premium Tea	50	77	Higher
16	Kenyan	50	68	Comparable
17	Leena Tea	50	58	Lower
18	Lipton	50	60	Lower

Reducing Power of CCEs/PRFs Analysis

The antioxidant potential of extracts from various tea brands in different solvents was evaluated by their reducing power since reducing capacity is very important to the action of antioxidants. A powerful dose of 140-170 mg was created to generate extracts for extensive analysis. Details of the results and their error margins are presented in Table 6. JEHAN Premium Tea exhibited the greatest reducing power (0.92 ± 0.03), representing a strong antioxidant power. The essential extract was the closest, with a reducing power of 0.87 ± 0.02. The Kenyan tea extract had the lowest reducing power (0.76 ± 0.03) comparable to the Leena Tea extract. This review indicates substantial antioxidant activity variation between brands, demonstrating robust protection to oxidative stress of JEHAN Premium Tea and Vital, followed by less efficient Kenyan and Leena.

Table 6: Reducing Power of Tea Brands

Sr. No.	Tea Brand	Total Concentration (mg)	Solvent	Reducing Power ± Error	Mean
1	Mazza Tea	148	Ethyl Acetate	0.81 ± 0.03	0.77
2	Mehran Tea	152	Ethyl Acetate	0.84 ± 0.03	0.80
3	Shan	153	n-Hexane	0.84 ± 0.03	0.80
4	Shezan	148	Ethanol	0.81 ± 0.03	0.77
5	Supreme	153	Methanol	0.84 ± 0.03	0.80
6	Tapal Danedar	158	Ethyl Acetate	0.86 ± 0.02	0.82
7	Tetley	153	Methanol	0.84 ± 0.03	0.80
8	Twinings	158	Ethanol	0.86 ± 0.02	0.82
9	Vital	163	n-Hexane	0.89 ± 0.02	0.84
10	Brooke Bond Taj Mahal	168	n-Hexane	0.91 ± 0.03	0.86
11	Chaman	167	n-Hexane	0.90 ± 0.03	0.85
12	Fecto	163	Methanol	0.89 ± 0.03	0.84
13	Gulab Tea	148	Ethanol	0.81 ± 0.03	0.77
14	Huma	157	Ethyl Acetate	0.86 ± 0.03	0.82
15	JEHAN Premium Tea	173	Methanol	0.94 ± 0.03	0.90
16	Kenyan	143	Chloroform	0.78 ± 0.03	0.74
17	Leena Tea	142	Chloroform	0.77 ± 0.03	0.73
18	Lipton	147	Ethanol	0.81 ± 0.02	0.77

Cytotoxicity Analysis

Outcomes of the analysis highlighted a prominent cytotoxicity inhibition by numerous tea brands and, hence, their preventive potential for cell proliferation. The cytotoxicity inhibition exhibited by JEHAN Premium Tea was the highest (68%), and Shezan and Mazza Tea showed the lowest (53%). Hemolysis analysis indicated Chaman had the highest level score (3.6% ± 0.2) in Table 7, while Gulab Tea and Shan had the lowest (2.7% ± 0.1) score. JEHAN Premium Tea had the highest value (25.7% ± 0.9) for thrombolysis, whereas Twinings and Leena Tea recorded the lowest (14.3% ± 0.4). These findings highlight the differences in the biological activity of different tea brands, indicating the need to consider potential health consequences.

Table 7: Cytotoxicity Activity of Different Tea Brands

Sr. No.	Tea Brand	Concentration (µg/ml)	Cytotoxicity Inhibition (%)	Hemolysis (%) ± Error	Concentration (mg/ml)	Thrombolysis (%) ± Error
1	Brooke Bond Taj Mahal	218	67	3.4 ± 0.2	78	24.5 ± 0.8
2	Chaman	218	67	3.4 ± 0.2	103	24.4 ± 0.8
3	Fecto	208	64	3.5 ± 0.2	78	19.9 ± 0.6
4	Gulab Tea	178	55	2.9 ± 0.1	53	16.9 ± 0.5
5	Huma	203	62	3.2 ± 0.2	103	21.8 ± 0.7
6	JEHAN Premium Tea	223	70	3.7 ± 0.2	78	26.0 ± 0.9
7	Kenyan	198	61	2.8 ± 0.1	53	17.1 ± 0.5
8	Leena Tea	183	57	3.1 ± 0.2	28	16.1 ± 0.5
9	Lipton	183	57	3.1 ± 0.2	53	20.5 ± 0.6
10	Mazza Tea	178	55	2.7 ± 0.1	53	17.5 ± 0.5
11	Mehran Tea	203	62	3.1 ± 0.2	103	21.2 ± 0.7
12	Shan	195	59	2.7 ± 0.1	25	14.3 ± 0.4
13	Shezan	175	53	2.8 ± 0.1	50	16.4 ± 0.5
14	Supreme	200	60	2.5 ± 0.1	25	15.5 ± 0.5
15	Tapal Danedar	190	58	2.8 ± 0.1	25	15.0 ± 0.4
16	Tetley	185	57	3.1 ± 0.2	75	18.9 ± 0.6
17	Twinings	200	60	2.9 ± 0.1	25	14.7 ± 0.4
18	Vital	210	63	3.2 ± 0.2	100	22.3 ± 0.7

CONCLUSION

The combined analysis of different green tea brands showed a wide range of antioxidants, phenolic compounds, and caffeine extractions along with differential biological activities like suppression of cytotoxicity, hemolysis scarring and thrombolysis. Across all testing, JEHAN Premium Tea continually exhibited high phenolic content and biological activities, elucidating potential health benefits. In contrast, Shan Tea exhibited potentially lower antioxidant capacity and functional activity. This study provides a comparative analysis of several widely purchased green tea brands, which can help consumers understand the nutritional and biological effects of these various popular commercialized tea products and aid them in selecting a safe choice for better health benefits. Additional investigation of potential underlying mechanisms and broader health implications is warranted for these pursuits to be more fully realized and utilized within health.

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