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Research article

ARE COMMERCIALLY PACKED GREEN TEA SAMPLES OF HIGHER QUALITY THAN UNPACKED ONES? A COMPARATIVE PHYTOCHEMICAL, BIOACTIVITY, AND CHEMOMETRIC ANALYSIS

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ABSTRACT

Green tea, derived from the unfermented leaves of *C. sinensis* var. *sinensis,* is a widely consumed beverage known for its therapeutic benefits. But unfortunately, tea is among the top ten easily adulterated foods. The current study conducted for the first time in Pakistan aimed to compare the adulteration level, phytochemical composition and therapeutic benefits of commercially available packed and unpacked green tea samples. The results showed no adulteration with sand or leather flakes in any of the samples, but colour adulteration was more prominent in packed samples. There was no significant difference in the sugar content between unpacked and packed samples. Quantitative phytochemical analysis revealed no significant difference in TPC and TFC; however, unpacked samples had significantly higher TAC compared to packed samples. Antioxidant activity was moderate for unpacked samples and weak for packed ones. Mineral analysis showed no presence of toxic heavy metals (Pb and Cd) and no significant difference in mineral content (Zn, Fe, and Cu) between the two sample types. Both sample types showed weak antibacterial activity against *Klebsiella pneumoniae* at 500µg/mL but no activity against *Staphylococcus aureus.* Chemometric analysis indicated that unpacked samples exhibited greater overall variance. In conclusion, unpacked green tea samples demonstrated better quality, with higher antioxidant potential and less colour adulteration.

1. Introduction

Green tea is a widely consumed, unfermented herbal beverage obtained from the infusion of *Camellia sinensis var. sinensis* with water (Ahmed & Stepp, 2013; Anand *et al*., 2014). It belongs to family *Theaceae* (Zakir *et al*., 2015) and is indigenous to Southeast Asia, with China historically introducing it to the world thousands

of years ago (Perva-Uzunalic *et al*., 2006). Globally, approximately three billion kilograms of dried tea leaves are produced each year (Abualhasan *et al*., 2020), with green tea accounting for 20% of total production (Ligor *et al*., 2008). China is recognized as the leading producer and consumer of tea, followed by Turkey, Indonesia, India, Kenya, and Sri Lanka (Yang *et al*., 2023). In Pakistan, the production of green and black tea is limited to a small area in district Mansehra, Khyber Pakhtunkhwa, resulting in the country being one of the world's largest tea importers (Rameeza & Eun, 2022).

In recent years, green tea has gained a lot of attention due to its wide range of therapeutic effects when consumed unadulterated (Shraim *et al*., 2021). Numerous bioactive constituents found in green tea have been evaluated to have anti-arteriosclerotic (Wang *et al*., 2001), anti-fibrotic (Cabrera *et al*., 2006), cardioprotective (Babu & Liu, 2008), anti-inflammatory (Chacko *et al*., 2010), osteogenic (Shen *et al*., 2010), anti-aging (Y. Li *et al*., 2015), anti-mutagenic (Bushman, 1998), antimicrobial ((Jigisha *et al*., 2012), antioxidant (Oliveira *et al*., 2023), antiplatelet (Shahbazi *et al*., 2019), hypoglycemic (Chung *et al*., 2019), anti-metastatic (Kochman *et al*., 2020), neuroprotective (Malar *et al*., 2020), antiviral (Wang *et al*., 2021; Z. Zhang *et al*., 2021), antiallergic (Li *et al*., 2021), antidiabetic (Oliveira *et al*., 2023), anti-obesogenic (Abiri *et al*., 2023), and anticancerous potential (Oh *et al*., 2023).

Generally, it is estimated that over 4,000 bioactive compounds are present in green tea (Anand *et al*., 2014). Among these include the polyphenols mainly catechins and flavonoids, alkaloids majorly caffeine, theobromine and theophylline, carbohydrates mainly polysaccharides, amino acids mainly theanine, pigments such as chlorophyll and carotenoids, sterols, vitamins such as B, C and E, lipids, volatile acids and inorganic elements (Adnan *et al*., 2013; Xu *et al*., 2021). The levels of these bioactive components and contaminants can vary across different tea samples depending upon the growing conditions (climate, geographical location, horticultural practices, and leaf age), processing methods, storage conditions, and brewing practices (Nafisah *et al*., 2024). The main steps involved in processing of green tea include harvesting, spreading, fixing, rolling, drying and packaging (Yin *et al*., 2022). These help to remove moisture, maintain green colour, dry and shape the leaves into desired form to be sold in the market (Jakubczyk *et al*., 2020).

The main component of green tea leaves are polyphenols that make up about more than 30% of the fresh leaves by dry weight (Perva-Uzunalić *et al.,* 2005; Subhashini *et al*., 2010). Protein content in green tea is about 15 to 20 percent (Musial *et al*., 2020). Mineral elements found in green tea, known as ash, includes a complex mineral makeup containing elements that are both bioavailable like P, K, Zn, Cu, F, Ca, Mg, Fe, Mn, Al, S, Si (Zhao *et al*., 2022) and harmful like Pb, Cd and Ni (Koch *et al*., 2018). The therapeutic properties of green tea are majorly because catechin (known for their antioxidant properties) (Braschi *et al*., 2022) and caffeine (which has anti-obesity effects) (Nishitani & Sagesaka, 2004). The amino acids found in tea play a significant role in producing biological effects, such as sedation and relaxation (Bagheri *et al*., 2021).

In current era, where both intentional and unintentional adulteration is prevalent in other foods, it is also common in the tea (Pal & Das, 2018). In tea, adulteration is done in order to improve its flavor, appearance, bulk or quality (Jayawardhane *et al*., 2016; L. Li *et al*., 2021). Currently, in Europe, tea is one of the top 10 easily adulterated foods (Yang *et al*., 2023). Major adulterants added to the tea include caffeine, sand, leather flakes, legume husks, colouring agents, cereal starch, chicory, used tea leaves, sugar, starch, and China clay (Sagaya *et al*., 2023; Romers *et al*., 2023). The presence of these adulterants not only makes the tea sub-standard but may also pose potential health risks (Bhatt *et al*., 2013) such as digestive system disorders, stomach cancer, stomach infections, carcinogenesis, liver disorders, diarrhea, epidemic dropsy, blood disorders, glaucoma, lathyrism, cardiac arrest etc. (Mishra & Mishra, 2017; Amsaraj & Mutturi, 2024). Safety and quality concerns arise due to regulatory gaps in evaluation and authentication processes.

Therefore, reliable and precise analytical techniques are essential to identify and authenticate the quality of different types of green tea (Zou *et al*., 2023).

The current study, conducted for the first time in Pakistan, assesses adulteration levels, evaluates the quality through the quantitative analysis of phytochemical composition, and compares the potential health benefits of various packed and unpacked green tea samples available in the market using various biochemical and chemometric analysis of the obtained data.

2. Materials and methods

2.1. Sample collection and labelling

A total of 20 green tea samples, including 10 commercially packed and 10 unpacked (sold locally in open form), were collected from Rawalpindi, Pakistan, with unpacked samples labelled as "S1-S10" and packed samples labelled as "S11-S20".

2.2. Sample preparation

1 g of the green tea leaves were boiled for 2-3 minutes in 20ml distilled water for the preparation of green tea infusions (Barreira *et al.,* 2020)

2.3. Detection of adulteration

2.3.1. Test for colour and sugar adulteration

The Colour Assessment Scale method, as described by Gunathilaka, & Warnasooriya, 2021, was used to detect water-soluble colours in green tea samples. 1g of leaves were placed in 20ml distilled water for 60 seconds and assigned a scale from 1-5 based on the intensity of colour observed.

Sugar content in green tea infusions was detected using modified method proposed by Oti *et al,* 2016. For this purpose, 2-3 drops of the brewed green tea samples were added to completely cover the prism of brix refractometer and brix value was noted. Mass of sugar was calculated by following method:

$$\text{Mass of sugar} = \text{Mass of tea sample (a)} \times \%\ \text{of sugar brix value (z)} / 100 \quad (1)$$

2.3.2. Test for sand and leather flakes adulteration

Sedimentation test and Burning test described by Gunathilaka, & Warnasooriya, 2021 were used to detect the sand and leather flakes adulteration in green tea samples respectively. Sediments were detected by placing 1g of tea in 20ml distilled water, while leather flakes were detected by burning 1g of tea for 2-3 minutes on flame.

2.4. Quantitative Phytochemical Analysis

The photochemical analysis of green tea samples was carried out by quantitative estimation of total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC).

2.4.1. Estimation of Total Phenolic Content (TPC)

The Folin-Ciocalteau method proposed by Fatima *et al.,* 2024 was used to quantitatively evaluate the TPC in green tea samples. For calibration curve, 0.5 mL aliquots of methanolic gallic acid solutions were mixed with 2.5 mL of 10% Folin-Ciocalteau reagent. After 5 minutes, 2 mL of 7% Na_2CO_3 was added, and incubated for 40 minutes. Absorbance was recorded at 760 nm. The total phenolic content (TPC) was then determined using the same reagents and procedure with 0.5 mL methanolic green tea infusion. TPC was expressed as µg gallic acid equivalents (GAE) per mg of methanolic green tea infusion.

2.4.2. Estimation of Total flavonoid Content (TFC)

Ferric chloride test ($AlCl_3$) described by Essien *et al.,*2020 was modified for quantitative determination of the TFC. Methanolic green tea infusions (1 µL/mL) were prepared and diluted with 200 µL of distilled water. To each sample, 150 µL of 5% sodium nitrate ($NaNO_2$) solution was added and incubated for 5 minutes, followed by the addition of 150 µL of 10% $AlCl_3$ and further incubation for 6 minutes. Subsequently, 2 mL of 4% sodium hydroxide (NaOH) was added, and the final volume was adjusted to 5 mL with distilled water. After a 15-minute incubation at room temperature, absorbance was measured at 570 nm. A standard calibration curve was

generated using different concentrations of quercetin for TFC quantification. TFC of green tea infusions was expressed as µg quercetin equivalent (QE) per mg of sample.

2.4.3 Estimation of Total Alkaloid Content (TAC)

Bromocresol Green (BCG) Method described by Ambre & Hase, 2020 was modified for the quantitative evaluation of the TAC. A 0.25 mL aliquot of green tea infusion was placed in a separating funnel, to which 1.25 mL of BCG solution and 1.25 mL of phosphate buffer were added. Then gradually chloroform was added to each funnel in volumes of 0.25ml, 0.5ml, 0.75ml and 1ml and shaken. After separation, the chloroform extract was collected, and its absorbance was measured at 470nm. Different concentrations of atropine were used to generate the standard calibration curve for the quantification of TAC. TAC of the green tea samples was expressed as µg atropine equivalent (AE) per mg of sample.

2.5. Determination of antioxidant activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay described by Safdar *et al.,* 2016 was modified for the estimation of antioxidant activity. Four concentrations (50, 250, 500, and 1000 µg/mL) of the methanolic green tea infusions were mixed with 1.5 mL of 1 mM methanolic solution of DPPH. The tubes were incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 517 nm. Percentage Radical scavenging activity (RSA) was calculated using the following formula:

$$\%RSA = \frac{\text{Absorbance of the control } (Ac) - \text{Absorbance in the presence of the green tea infusions} (As)}{Ac} \times 100 \quad (2)$$

In order to find the strength of antioxidant activity, half maximal Inhibitory concentration (IC50) value of each sample and ascorbic acid was also calculated.

2.6. Elemental analysis

Elemental analysis was performed using the method proposed by Abualhasan *et al.,* 2020. 0.2 g of powdered green tea leaves were oxidized using 10 mL digestion mixture of nitric acid and perchloric acid in a 2:1 ratio, and left overnight at room temperature to ensure complete oxidation. The samples were then heated to 120.5°C until white fumes appeared. After cooling, the mixture was filtered, and 0.5 mL of each sample was diluted to a final volume of 12.5 mL with distilled water. Each sample was prepared in triplicate. These prepared samples were subsequently analyzed for microelements (Fe, Cu, and Zn) and toxic heavy metals (Pb and Cd) using a flame atomic absorption spectrophotometer, with concentrations reported in parts per million (ppm).

2.7 Antibacterial activity

The antibacterial activity of the green tea samples was evaluated against *Staphylococcus aureus* and *Klebsiella pneumoniae,* collected from Microbiology and Biotechnology Research Lab, Fatima Jinnah Women University, Rawalpindi, using the agar well diffusion method described by Nadeem *et al.,* 2022. Bacterial strain cultures were prepared to a final concentration of 10^8 CFU/mL using a 0.5 McFarland standard and 20µl of the prepared inoculum was evenly spread onto Mueller Hinton agar. 40 µL volume of each 500 mg/mL green tea extract was applied to the sealed wells and petri plates were incubated at 37°C for 24 hours. Mean zones of inhibition were measured in millimeters, and activity index was determined.

$$\text{Activity Index (AI)} = \frac{\text{Zone of inhibition (mm) of the sample}}{\text{Zone of inhibition (mm) of the standard}} \quad (3)$$

For the negative control, distilled water was added to the sealed wells and as a positive control the antibiotic discs, 30 µg of Chloramphenicol (C) for *Staphylococcus aureus*, and 25 µg of Trimethoprim-sulfamethoxazole (SXT) for *Klebsiella pneumoniae* were used.

2.8 Chemometric analysis

MATLAB software was used for chemometric analysis of green tea samples. PCA score, loading, biplots, and scree plots were made to compare similarity and variability among the different green tea samples.

3. Results and discussions

3.1. Results

3.1.1. Detection of adulteration

The colour intensity was observed more in packed samples as compared to unpacked samples as shown in table 1. 60% of the unpacked whereas 70% of the packed green tea samples showed colour in water after 60 secs. Therefore, colour adulteration was found more in packed samples as compared to unpacked samples. Figure 1 shows the pictorial representation of colour changes of green tea samples with water.

The sugar content analysis shown in table 1 revealed a maximum of 0.02 g per gram in 4 unpacked (S5, S6, S8, S9) and 7 packed (S11–S17) green tea samples. The average sugar content was higher in packed samples compared to unpacked ones but none of the green tea sample was adulterated with sugar (Figure 2).

Table 1. Adulterants in unpacked and packed green tea samples

Tea Samples	Colour adulteration		Sugar Content (g/g of tea sample)	Tea Samples	Colour adulteration		Sugar Content (g/g of tea sample)
	Result	Scale assigned			Result	Scale assigned	
S1	Negative	1	0.01	**S11**	Positive	3	0.02
S2	Positive	4	0.01	**S12**	Negative	1	0.02
S3	Positive	2	0.01	**S13**	Positive	5	0.02
S4	Positive	2	0.01	**S14**	Positive	4	0.02
S5	Positive	2	0.02	**S15**	Positive	4	0.02
S6	Positive	3	0.02	**S16**	Positive	4	0.02
S7	Negative	1	0.01	**S17**	Positive	4	0.02
S8	Positive	2	0.02	**S18**	Negative	1	0.01
S9	Negative	1	0.02	**S19**	Negative	1	0.01
S10	Negative	1	0.01	**S20**	Positive	2	0.01

The sedimentation and burning test revealed that none of the tested green tea sample was adulterated with sand and leather flakes respectively.

3.1.2. Phytochemical screening

The results of total phenolic, flavonoid and alkaloid content found in the unpacked and packed green tea samples are shown in Figure 3. The average phenolic, flavonoid and alkaloid content in ten unpacked and packed samples of green tea was calculated (Figure 4) and it was found that more phenolic and alkaloid content was present in unpacked as compared to packed samples but more flavonoid content was found in packed as compared to unpacked samples. On applying T-test, it was concluded that there is no significant difference in average phenolic and flavonoid content whereas significant difference in average alkaloid content of unpacked and packed samples was found.

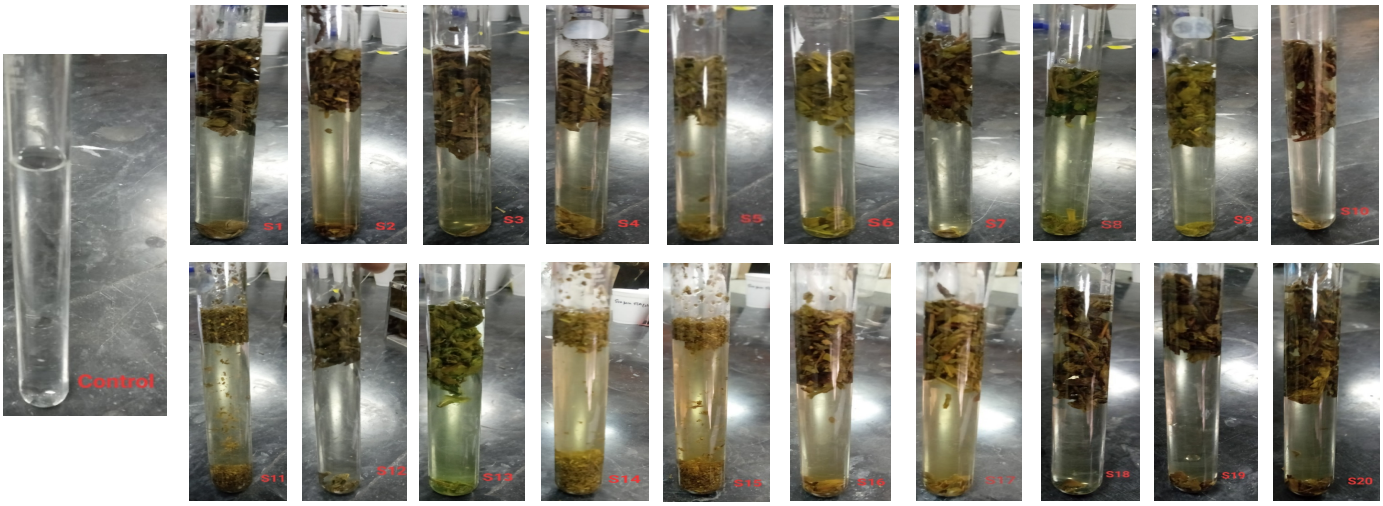


Figure 1. Colour changes of green tea samples with water at room temperature (25°C) after 60 seconds.

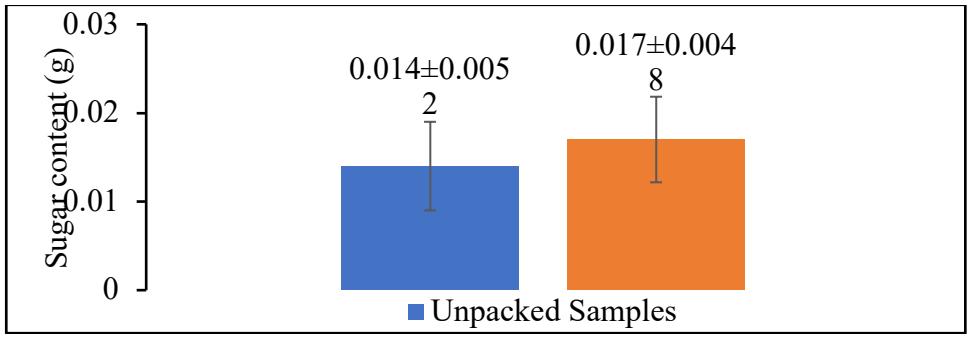


Figure 2. Average sugar adulteration in ten unpacked and packed samples

No significant difference in sugar content between packed and unpacked samples ($p > 0.05$) was observed when analysed through t-test.

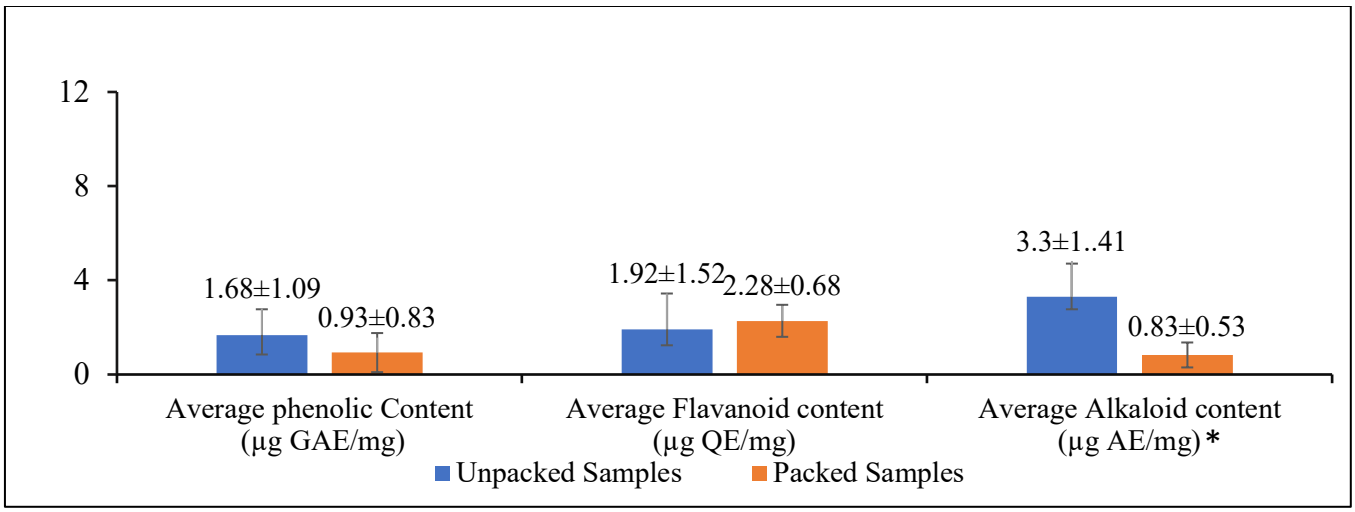


Figure 4. Average phenolic, flavonoid, and alkaloid content in ten unpacked and packed green tea.

Asterisk represents significant difference ($p < 0.05$) observed between the alkaloid content of testing samples.

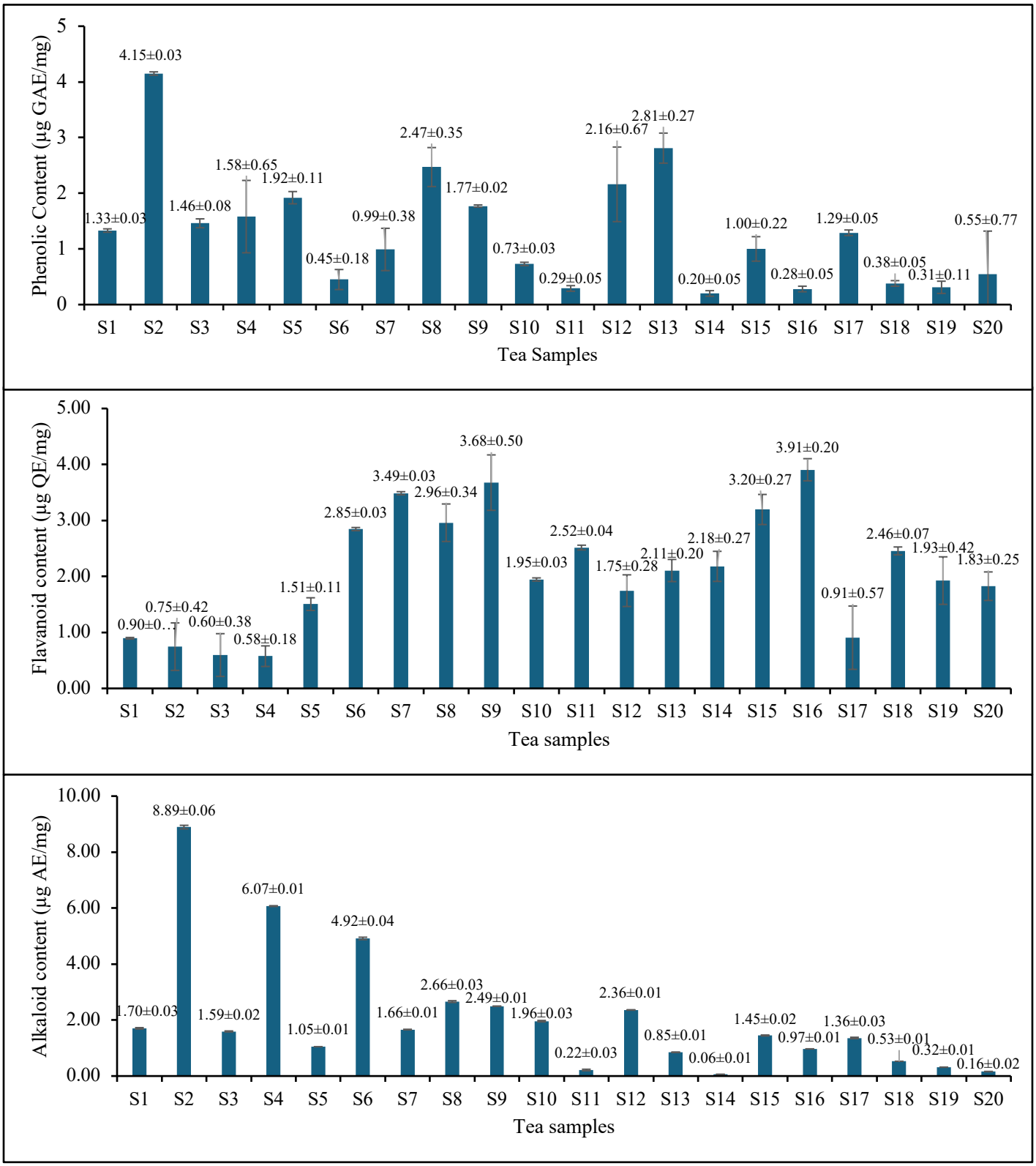


Figure 3. Total phenolic content (TPC), Total flavonoid content (TFC), and Total alkaloid content (TAC) in unpacked and packed green tea samples. The data represents the average of three replicates

3.1.3. Analysis of Antioxidant Potential

The results of the percentage radical scavenging activity of green tea samples at four different concentrations, and their IC50 values are shown in the figure 5 and 6a respectively. Samples S6, S7, and S8 exhibited the lowest IC50 values, indicating strong antioxidant activity. On contrary, S14 had the highest IC50 value, signifying weak antioxidant activity. When comparing the average IC50 values of unpacked and packed green tea samples, it was observed that the unpacked samples displayed moderate antioxidant activity, while the packed samples showed weaker antioxidant potential (Figure 6b).

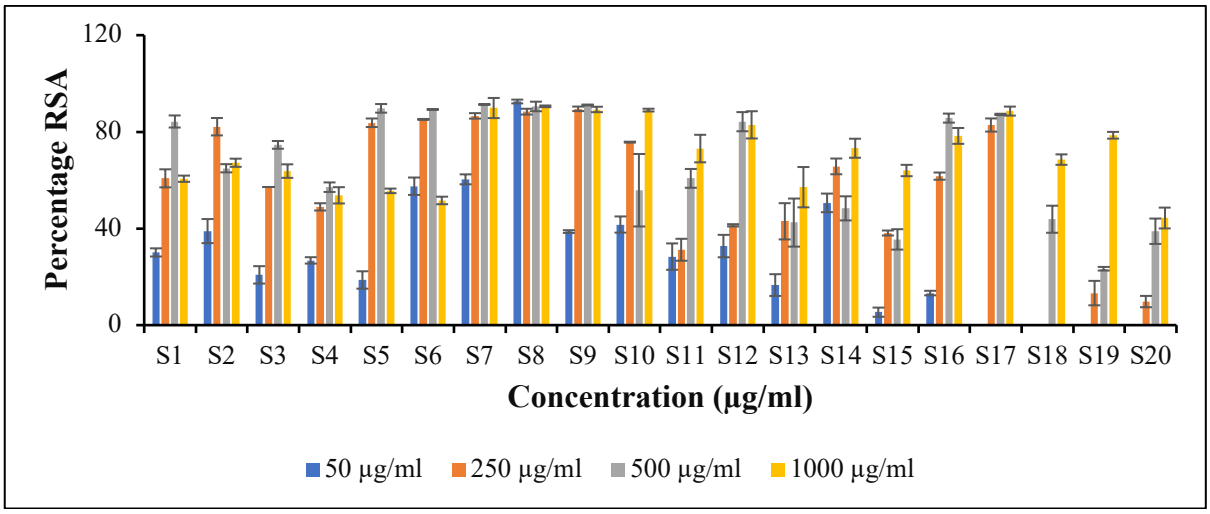


Figure 5. Percentage Radical Scavenging activity of the green tea samples at four different concentrations. Data represents the average of three replicates per sample with error bars.

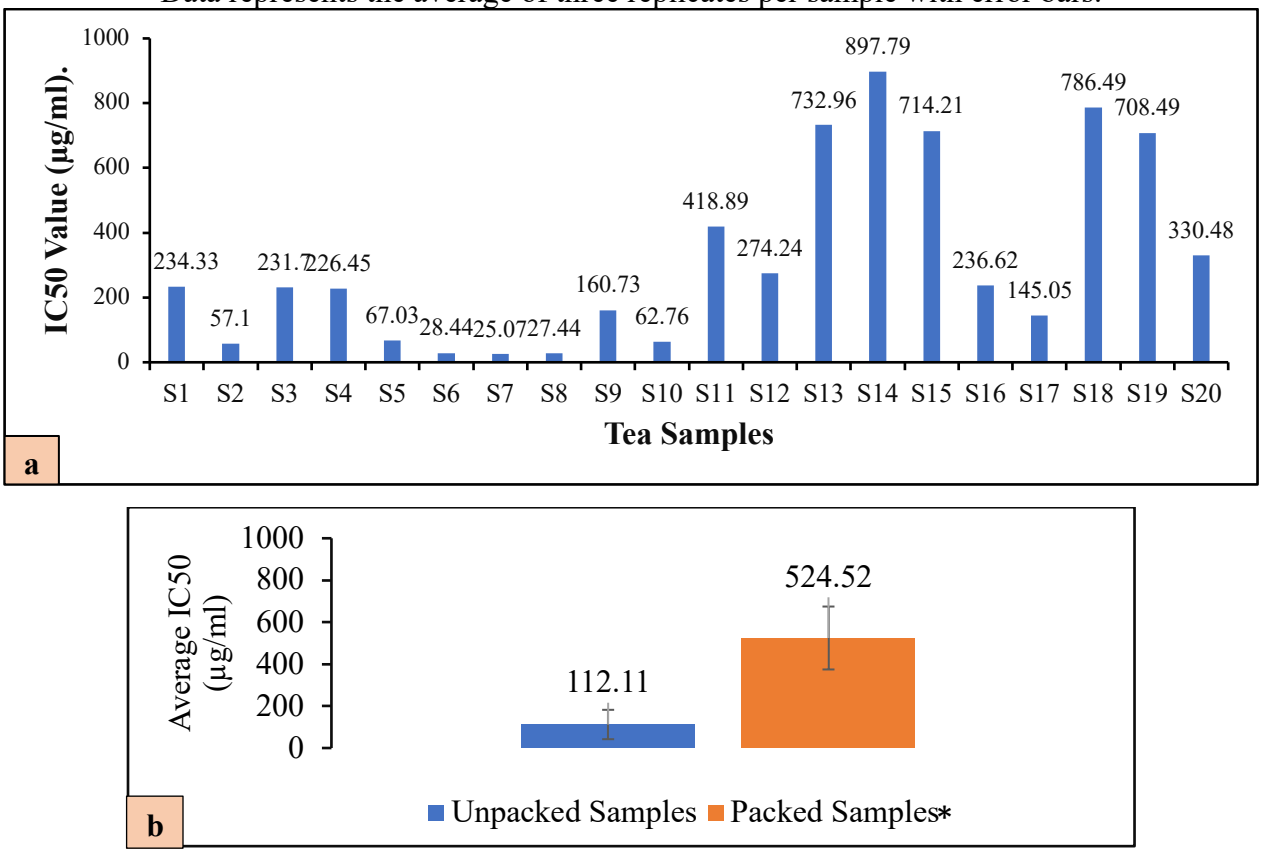


Figure 6. Individual (a) and Average (b) IC50 values of ten unpacked and packed samples of green tea
Asterisk represents significant difference ($p < 0.05$) observed between the IC50 values of testing samples.

3.1.4. Pearson's Correlation Coefficient (r)

A negative correlation was observed between IC50 values and the content of phenolics, alkaloids, and flavonoids in case of both packed and unpacked samples. However, a weak positive correlation was found between IC50 values and flavonoid content specifically in the packed samples (Table 2). This suggests that as the levels of phytochemical constituents (such as phenolics, alkaloids, and flavonoids) increase, the antioxidant activity of the samples also increases.

Table 2. Correlation analysis of IC50 of green tea samples with their bioactive constituents

		TPC (µg GAE/mg)	TFC (µg QE/mg)	TAC (µg AE/mg)
UNPACKED SAMPLES	**IC50 (µg/ml)**	-0.094	-0.569	-0.098
PACKED SAMPLES	**IC50 (µg/ml)**	-0.124	0.164	-0.432

3.1.5. Linear regression analysis

The analysis revealed a very weak negative linear relationship between IC50 values and phenolic content for both unpacked ($R^2 = 0.0088$) and packed ($R^2 = 0.0153$) green tea samples, suggesting that higher phenolic content corresponds to lower IC50 values. Similarly, for flavonoid content, a weak negative relationship is observed in unpacked samples ($R^2 = 0.3241$), while a very weak positive relationship is seen in packed samples ($R^2 = 0.027$), suggesting that additional factors influence the observed variability. For alkaloid content, a weak negative linear relationship is found for both unpacked ($R^2 = 0.0095$) and packed ($R^2 = 0.1867$) samples, with low R^2 values, implying that other variables significantly affect IC50 (Figure 7). These results highlight the complexity of factors influencing phenolic, flavonoid, and alkaloid content in both unpacked and packed green tea samples.

3.1.6. Mineral analysis

Atomic absorption spectroscopy analysis of green tea samples revealed the concentrations of Fe, Zn, and Cu in green tea samples in ppm (figure 8) with Pb and Cd not detected in any sample. The average Fe, Zn and Cu concentration in ten unpacked and packed samples of green tea was calculated (Figure 9) Comparison between unpacked and packed samples showed that unpacked samples had higher Fe and Cu levels, while packed samples had higher Zn concentrations. On applying T-test, it was concluded that there is no significant difference in average mineral content of testing samples.

3.1.7. Antibacterial activity

The results of disc diffusion assay indicated that none of the green tea samples exhibited any zones of inhibition against *Staphylococcus aureus*, suggesting no antibacterial activity of the tested samples against this strain. On the other hand, antibacterial activity against *Klebsiella pneumoniae* was observed in 15 out of 20 green tea samples, with zones of inhibition ranging from 0 to 20 mm. The highest inhibition was found in packed sample 20 (20±0.5mm). Five samples (S9, S10, S12, S13, and S15) exhibited no antibacterial activity against the *K. pneumonia* strain. The activity index (AI) of samples was calculated and is shown in figure 10. Calculating the average of ten samples, it was found that packed green tea samples showed slightly higher average antibacterial activity than unpacked samples, as illustrated in Figure 11. On applying t-test, it was concluded that the difference in antibacterial activity of unpacked and packed green tea samples against the *K. pneumonia* strain was not statistically significant.

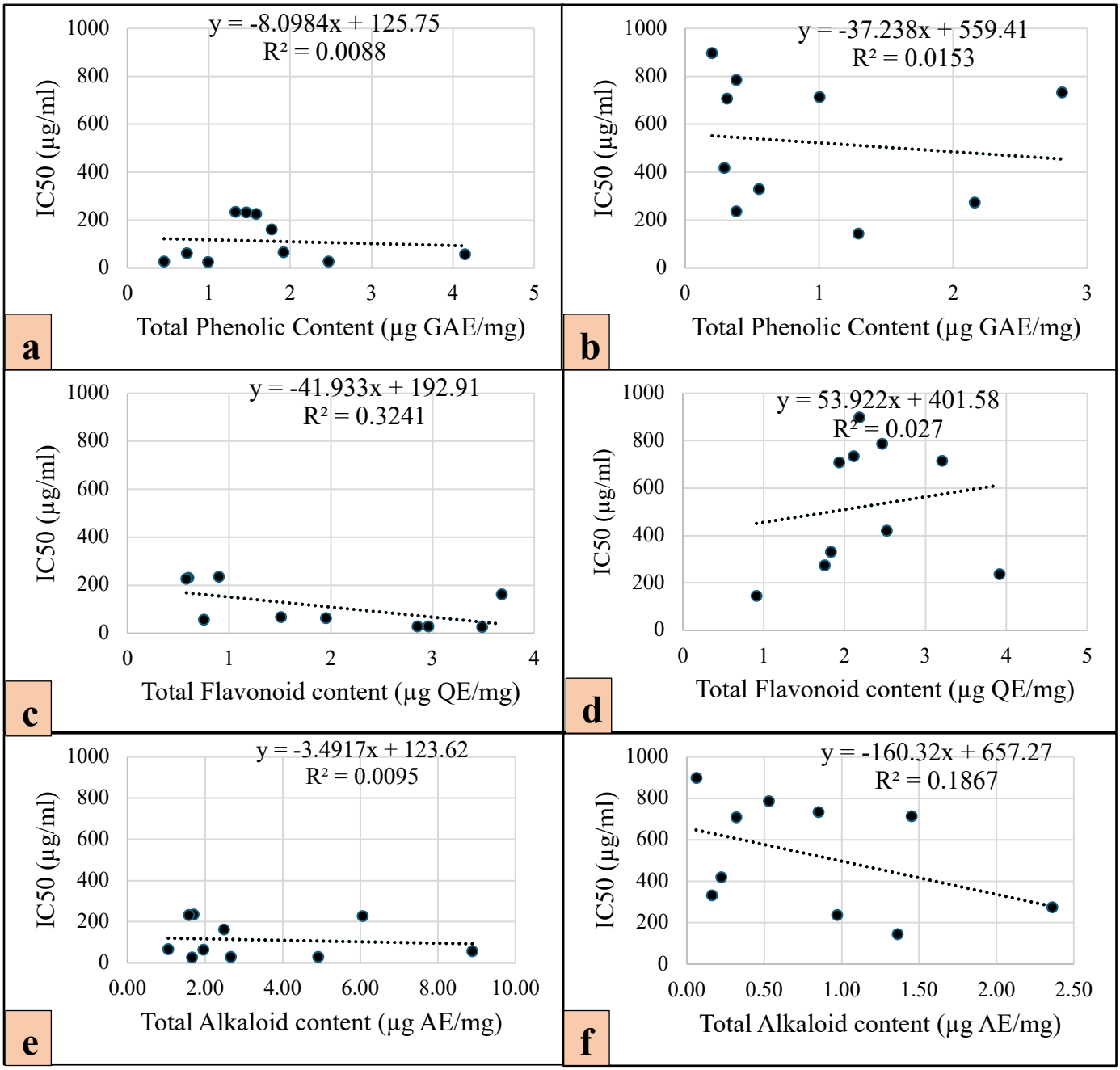
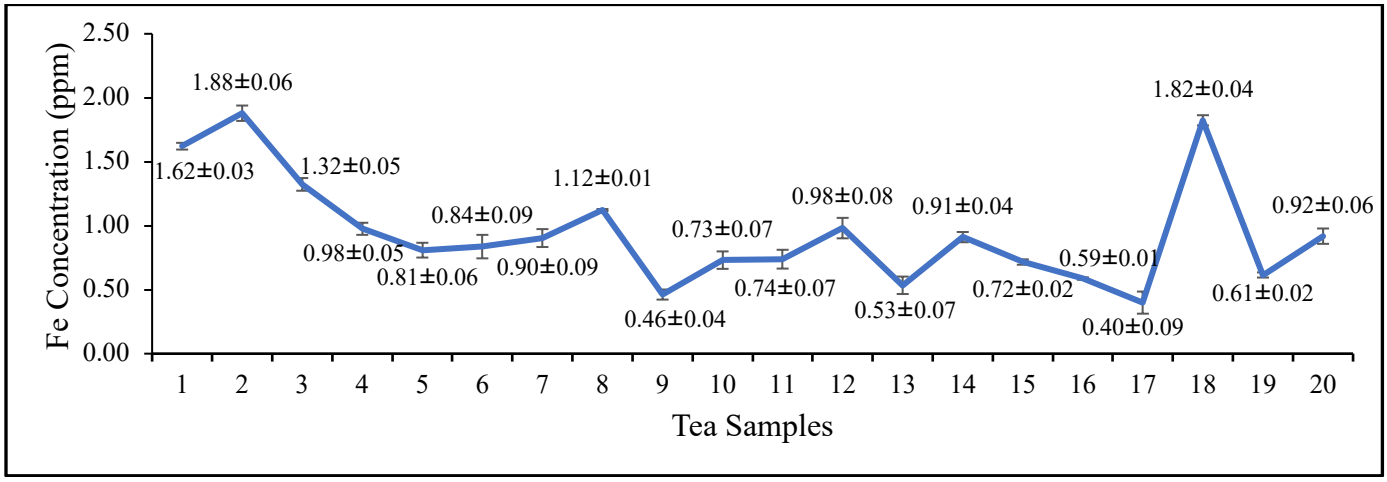
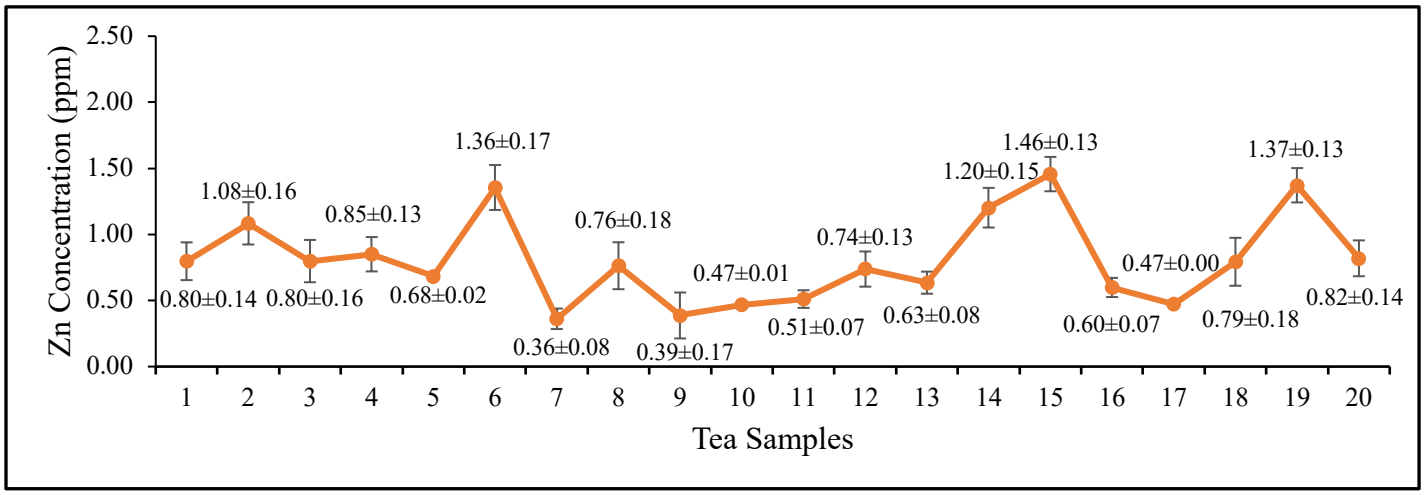


Figure 7. Linear regression analysis between IC50 and bioactive compounds of unpacked (a,c and e) and packed green tea samples (b,d and f)





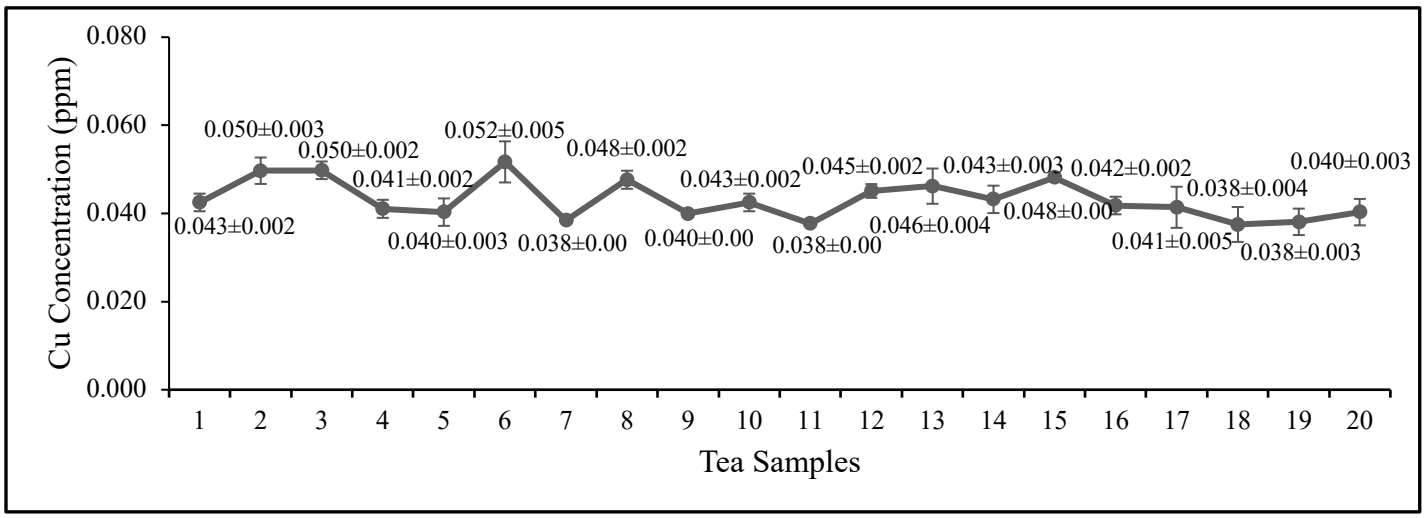


Figure 8. Concentrations (ppm) of the three microelements in different green tea samples.
Data represents the average of three replicates per sample with error bars.

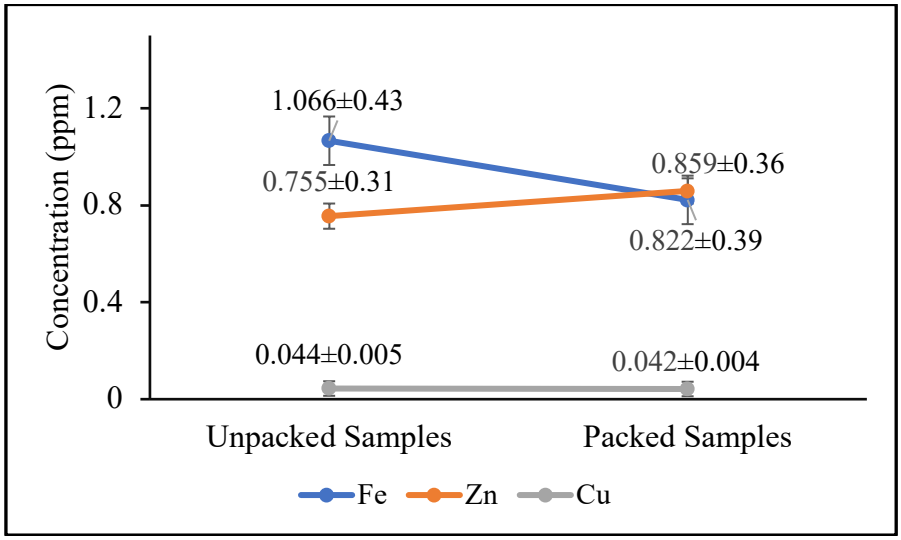


Figure 9. Average mineral content of ten unpacked and packed samples of green tea,
No significant difference ($p > 0.05$) observed between the mineral content of testing samples.

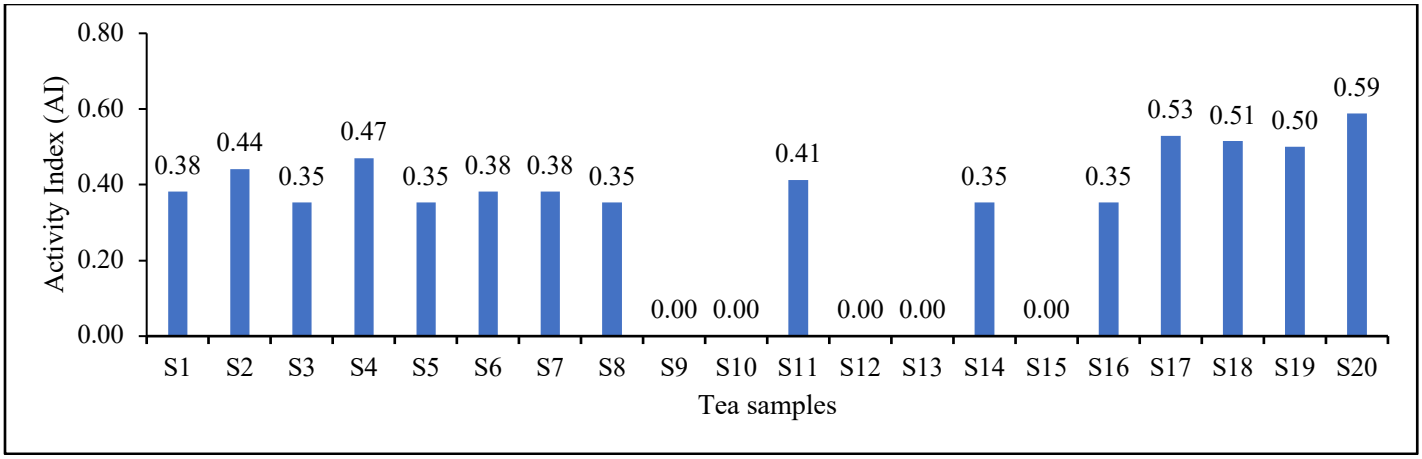


Figure 10. Activity index of green tea samples against *K. Pneumoniae*

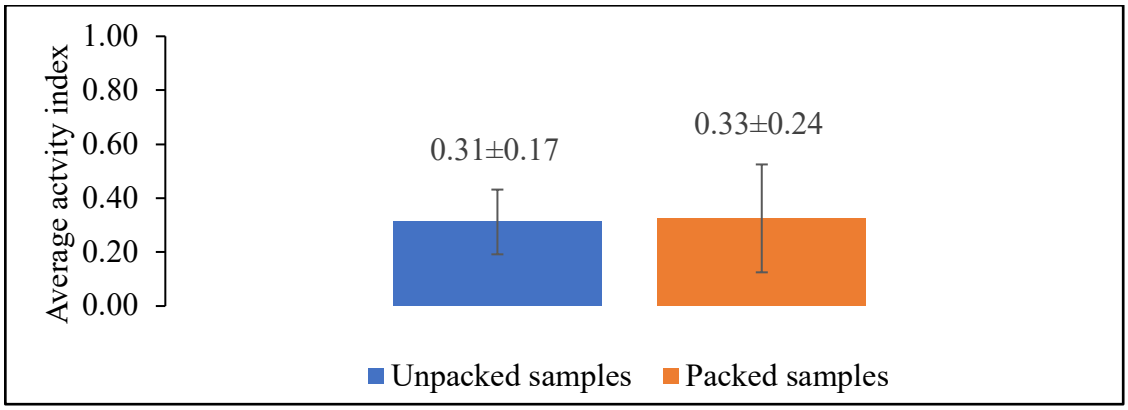


Figure 11. Average activity index of ten unpacked and packed samples against *K. pneumoniae.*
No significant difference ($p > 0.05$) observed between the antibacterial activity of testing samples.

3.1.8. Chemometric Elaboration of the data

3.1.8.1. PCA scatter and loading plots

Figure 12 (a) and (b) present the PCA scatter plots for unpacked and packed green tea samples, respectively. For unpacked samples, Principal Component 1 (PC1) accounted for 46.78% of the variance, while PC2 explained an additional 22.48%. Sample 2 exhibited the highest variance, being farthest from the origin, indicating significant deviation from the mean. Conversely, samples 10 and 5 showed minimal variance, positioned closest to the origin. In packed samples, PC1 accounted for 36.55% of the variance, and PC2 explained 25.64%.

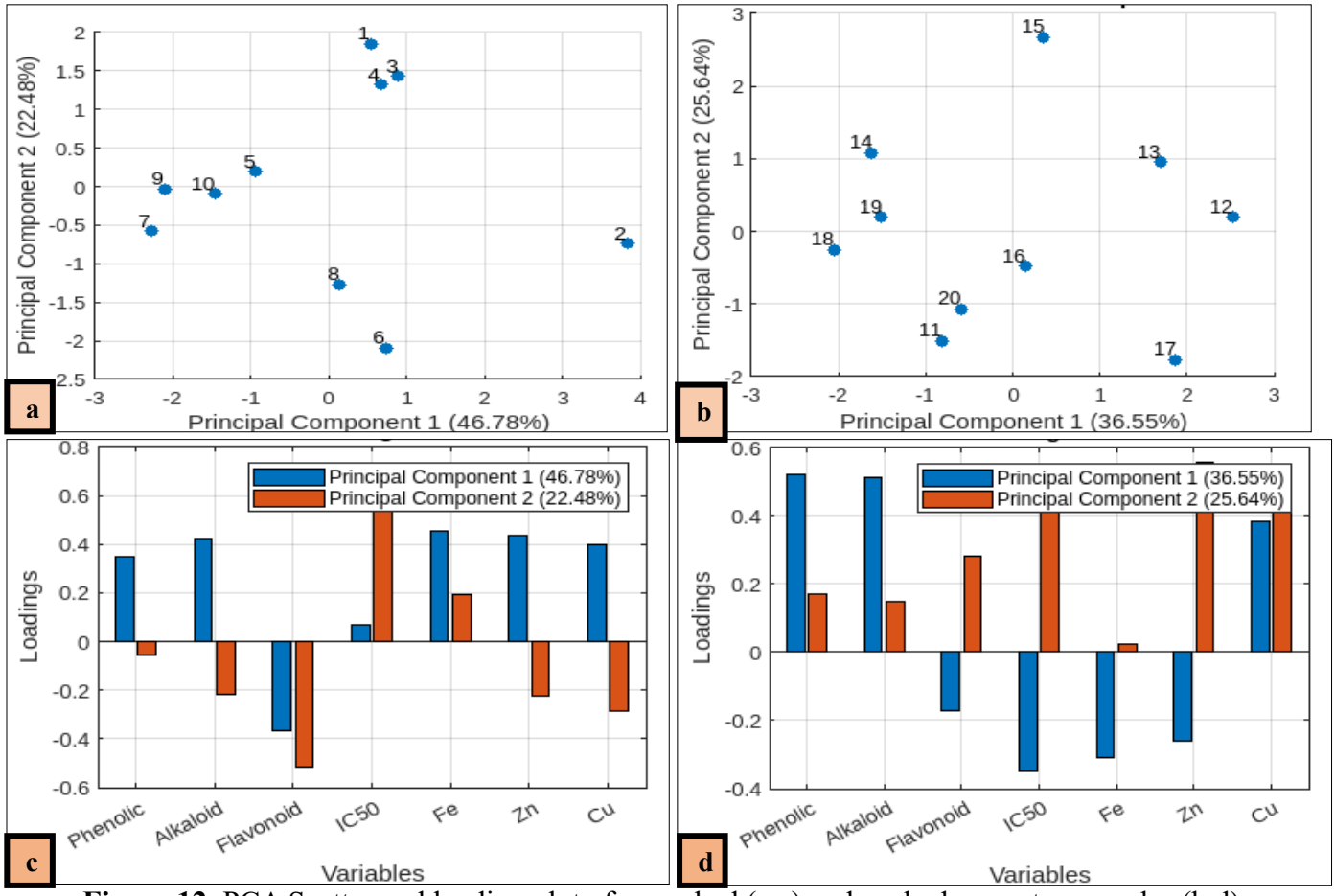


Figure 12. PCA Scatter and loading plot of unpacked (a,c) and packed green tea samples (b,d)

Figure 12 (c) and (d) present the PCA loading plots for unpacked and packed green tea samples, respectively. In unpacked samples, phenolic compounds, alkaloids, iron, zinc, and copper had positive loadings on PC1, while flavonoids exhibited negative loadings on both PC1 and PC2. IC50 values showed a small positive loading on PC1 and a significant positive loading on PC2, emphasizing bioactivity's role in PC2. In packed samples, phenolic compounds, alkaloids, and copper contributed positively to both PC1 and PC2, while flavonoids, IC50 values, iron, and zinc showed inverse relationships with PC1 and positive loadings on PC2. These differences highlight the impact of packaging on the biochemical composition and interactions within green tea, altering the contributions of certain variables to the principal components.

3.1.8.2. PCA Biplots and scree plots

Figure 13 (a) and (b) present the PCA biplots for unpacked and packed green tea samples, respectively, highlighting the relationships between sample scores and variable loadings. In unpacked samples, the first two principal components explained 69.26% of the variance, with IC50 and Fe having the strongest influence, as seen in Sample 1.

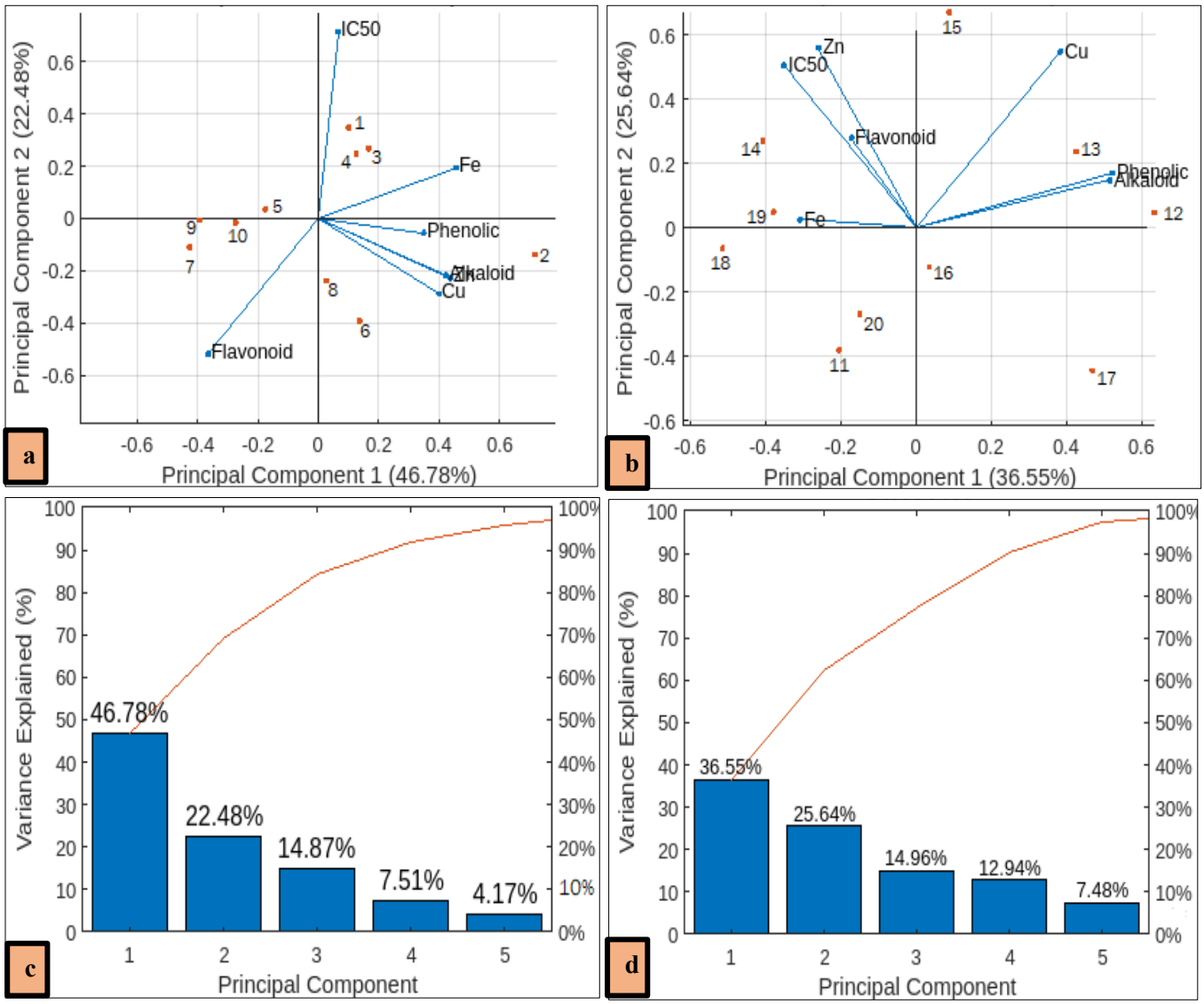


Figure 13. PCA biplot and scree plot of unpacked (a,c) and packed green tea samples (b,d)

Sample 2, which exhibited the highest variance, is positively correlated with Fe, Phenolic, Alkaloid, and Cu, indicating higher concentrations of these components. In packed samples, which explained 62.19% of the variance, Phenolic content and Zn are key variables. Sample 12 and 13 showed higher values for Phenolic content, while Sample 11 exhibited the lowest variance. These biplots demonstrated that packaging alters the chemical composition and antioxidant properties of green tea, with different variables influencing unpacked and packed samples.

Figure 13 (c) and (d) present the scree plots for unpacked and packed green tea samples, respectively, illustrating the variance explained by each principal component. In unpacked samples, the first two principal components (PC1 and PC2) explained 69.26% of the variance, with PC1 contributing 46.78% and PC2 an additional 22.48%. Subsequent components contributed progressively less, summing up to 100%. In packed samples, PC1 explained 36.55% and PC2 25.64%, together accounting for 62.19% of the variance, with the remaining components contributing less. This analysis showed that the first few components capture most of the data's

variability, suggesting dimensionality reduction to the first two or three components is sufficient for further analysis. Unpacked samples exhibited greater overall variance, with a faster cumulative explanation, indicating a wider range of factors influencing their properties. In contrast, packed samples showed a more even variance distribution, implying the packaging process introduces greater uniformity among samples.

3.2. Discussion

Extensive scientific research has unveiled the immense therapeutic potential of green tea, leading to its increased global consumption, production, and trade, while simultaneously raising concerns regarding its safety, quality, and authenticity. Adulteration being a significant problem compromises the safety, quality and authenticity of green tea. Therefore, in order to assess the adulteration in various unpacked and packed green tea samples, four types of adulteration tests were performed. Among these, sand and leather flakes adulteration was not found in any sample. However, colour adulteration was present in both unpacked and packed samples, with a higher occurrence in packed samples (70%) compared to unpacked samples (60%). Sugar content was also higher in packed samples, but the difference was not statistically significant and the sugar content found was within the safe consumption level in all the tested samples.

Phytochemical screening revealed variations in the total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) among samples. The highest phenolic content was observed in the unpacked sample S2 (4.15±0.03 µg GAE/mg), whereas the lowest was in the packed sample S14 (0.20±0.05 µg GAE/mg). Flavonoid content was highest in the packed sample S16 (3.91±0.20 µg QE/mg), while the lowest was found in S4 (0.58±0.18 µg QE/mg). Alkaloid content was significantly higher in unpacked samples, particularly in S2 (8.89±0.064 µg AE/mg), compared to packed samples, with S14 exhibiting the lowest (0.06±0.004 µg AE/mg). Statistical analysis confirmed that unpacked samples exhibited significantly higher alkaloid content, whereas phenolic and flavonoid content differences were not statistically significant. The observed variations in results can be attributed to the sensitivity of phytochemical composition to multiple factors, including environmental conditions during cultivation, leaf maturity, processing techniques, and storage conditions (Jakubczyk *et al*., 2020).

Antioxidant activity of the samples was evaluated by DPPH assay which is cost- effective and sensitive enough to detect active compounds at low concentrations (Neglo *et al.,* 2021). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a violet coloured, stable radical. It gets stabilized after reacting with antioxidant by electron donate mechanism. This was observed in the samples (green tea infusions) and control by decreased intensity of the violet colour of DPPH and gradually turning into yellow during incubation time (Indradi *et al.,* 2017)

Overall, the unpacked samples exhibited stronger radical scavenging activity than packed samples. IC50 values were inversely correlated with antioxidant potential (Alara *et al.,* 2018), with the lowest values in S6, S7, and S8, indicating high antioxidant activity. Packed samples exhibited weaker antioxidant activity, aligning with findings from previous studies (Anand *et al.,* 2014). Pearson correlation and regression analysis further confirmed a negative correlation between IC50 values and phytochemical content, reinforcing the relationship between phytochemical composition and antioxidant potential (Jumina *et al.,* 2019).

Mineral analysis using flame atomic absorption spectroscopy (FAAS) revealed no detectable levels of toxic heavy metals (Pb, Cd) in any sample, ensuring safety. Among microelements, Fe content was highest in S2 (1.879±0.19 ppm) and lowest in packed sample S17 (0.399±0.09 ppm). Zn concentration was highest in S15 (1.457±0.33 ppm) and lowest in packed sample S9 (0.387±0.17 ppm). Cu was highest in S6 (0.052±0.005 ppm) and lowest in four samples (0.038 ppm). No statistically significant differences were observed between packed and unpacked samples for mineral content, and all values were within the permissible limits set up by FAO/WHO limits for

these toxic metals and minerals in the edible plants (Kohzadi *et al.,* 2019).

Antibacterial activity was assessed against *Staphylococcus aureus* (Gram-positive) and *Klebsiella pneumoniae* (Gram-negative) strains. No inhibition zones were observed for *S. aureus*, contradicting previous findings (Zakir *et al.,* 2015), potentially due to differences in sample composition and test conditions. However, 15 out of 20 samples exhibited antibacterial activity against *K. pneumoniae*, with packed sample S20 showing the highest inhibition zone (20 mm). Despite these findings, the overall antibacterial activity was weak, as indicated by the low Activity Index (AI) values (0.31±0.17 for unpacked, 0.33±0.24 for packed), suggesting significantly lower efficacy, only 30% of that compared to standard antibiotics. Overall, this study presents novel findings on the antibacterial activity of green tea samples against *Klebsiella pneumoniae*, which has not been previously documented in the literature

Principal Component Analysis (PCA) indicated greater variance in unpacked samples, suggesting a broader range of quality variations due to environmental and processing factors. Packed samples showed more uniformity, likely due to standardized processing and packaging techniques, which may influence phytochemical stability.

4. Conclusions

The current study compared unpacked and packed green tea samples using PCA analysis and found notable differences. Packed samples showed higher colour adulteration, potentially misleading consumers about quality and safety. While phenolic and flavonoid content showed no significant variation, alkaloid content differed, leading to unpacked samples exhibiting significantly stronger antioxidant activity. Mineral content was within safe limits, with no detectable Pb or Cd in any sample. Both types showed weak antibacterial activity against *Klebsiella pneumoniae* with no significant difference. Overall, unpacked green tea demonstrated better quality due to higher antioxidant potential and richer phytochemical composition.

5. Future Prospects

Future research should focus on human clinical studies to validate green tea's health benefits, determine optimal consumption levels, and understand its mechanisms of action. Advanced analytical methods and predictive biomarkers are needed to study its interactions with the body. Nanotechnology-based delivery systems should be optimized to enhance its therapeutic potential. The development and evaluation of green tea-based functional foods and supplements for safety and efficacy are essential. Additionally, its effects on gut microbiota and potential prebiotic properties should be explored. Miniaturized analytical tools should be developed for efficient and cost-effective adulteration detection.

6. References

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