

Biochemical Characterization of Temi Tea from Sikkim

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Received 26 May 2023, Accepted 31 August 2023, Published on 3 June 2024

ABSTRACT

Tea is the most popular non-alcoholic beverage in the world well known for its refreshing and stimulative properties. Temi tea is a top-notch organic tea brand being grown in the sole tea estate in the mountainous state of Sikkim which has high demand in the international market and has not been studied scientifically so far. Therefore, the present study was conducted to assess and compare the total polyphenol content, total flavonoid content, total tannin content and antioxidant activity in the processed Temi tea samples collected across different season (flush) which is expressed on dry weight basis. Total polyphenols, flavonoid, tannin and antioxidant activity were studied spectrophotometrically. Results of the analysis revealed that the highest total polyphenol content, highest flavonoid content and highest antioxidant activity was found in T₁ (Green tea sample of Spring flush) while the highest total tannin content was found in (T₂) Black tea (Spring flush).

Keywords Temi tea, Polyphenol, Antioxidant activity, Flavonoid, Tannin.

INTRODUCTION

Tea is the most popular non-alcoholic drink on earth after water consumed by almost people of all age group from child to older and all class of people from rich to poor. The principal tea-producing states in India are Assam, West Bengal, Tamil Nadu and Kerala. It is also being grown in small scale in few other states like, Karnataka, Himachal Pradesh, Tripura, Manipur, Arunachal Pradesh and Sikkim. Based on the period of fermentation of leaves, tea is classified as black tea, green tea and oolong tea. Approximately 76–78% of the tea produced and consumed in the world is black tea, 20–22% is green tea and <2% is oolong tea and other teas. The chemical composition differs among various types of tea. This difference is caused due to numerous factors such as agronomic practices like plucking standard, manure application, harvesting season, climate and processing method.

Tender shoots of *Camellia sinensis* are a rich source of secondary metabolites. Beside the attractive aroma and unique taste, the potential health-promoting properties of tea are gaining more attention and popularity in tea products all over the world. The secondary metabolites present in tea plants beside bestowing with unique quality and providing wider adaptability and normal growth also provide numerous pharmacological health benefits in human like antiobesity, prevention of cardiovascular diseases, suppression of tumor cell formation and lowering the

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risk of atherosclerosis (Shang *et al.* 2021).

Temi tea garden is the sole tea estate in the mountainous state of Sikkim producing top-notch quality of organic tea marketed under the name “Temi tea” that has a huge demand in the global market. So far Temi tea has not been studied properly, only few reports are available on this aromatic organic tea related to social aspects of it. Considering these facts, the present study was carried out to assess and compare the total polyphenol content (TPC), total flavonoid content, total tannin content and antioxidant activity in the processed Temi tea sample collected from different season (flush).

MATERIALS AND METHODS

The samples of processed tea leaf were collected season (flush) wise from March–November 2017 from Temi tea garden, South Sikkim with latitude and longitude of 27.2367°N and 88.4222°E. The research work was carried out in the laboratory, Department of Horticulture, Sikkim University, 6th mile, Samdur during the year 2017-2018. The experiment was a Completely Randomized Design with three replications from which the mean values and standard deviation were calculated. Significant difference was carried out by performing one way analysis of variance (ANOVA). Sample extract were prepared according to the specified assay for different parameter. For the estimation of total polyphenols and total antioxidant content, Methanolic extract were prepared according to the method described by Kaur *et al.* (2015) and Ethanolic extract were prepared for the estimation of total flavonoids and total tannin.

Treatment details

Season/Flush	Tea type
1 Spring flush (T ₁)	Green tea
2 Spring flush (T ₂)	Orthodox black tea
3 Summer flush (T ₃)	Orthodox black tea
4 Monsoon flush (T ₄)	Orthodox black tea
5 Autumn flush (T ₅)	Orthodox black tea

Total polyphenols estimation

Total polyphenol content in tea samples was deter-



Fig. 1. Total polyphenol content (TPC) analysis of Temi tea samples.

mined by spectrophotometer using gallic acid as standard (Fig. 1), according to the method described by Abdolmaleki (2016). Briefly, 1.0 ml of the diluted sample extract was transferred to separate tubes followed by addition of 5.0 ml of Folin-Ciocalteu’s reagent (1/10 dilution) and 4.0 ml of sodium carbonate solution (7.5% w/v) and the final volume was made upto 10 ml by addition of distilled water. The tubes were allowed to stand in the dark before the absorbance was, measured at 765 nm. The TPC was expressed as gallic acid equivalents (GAE) in mg/g of the sample.

Total flavonoids estimation

Total flavonoids content in tea samples were determined by spectrophotometer using quercetin as standard (Fig. 2), according to the method described by Mohammed and Manan (2015). Briefly, 200 μ l ethanolic sample extract was taken and to it 150 μ l of



Fig. 2. Total flavonoids content (TFC) analysis in Temi tea samples.



Fig. 3. Antioxidant activity (AOA) analysis of Temi tea samples.

sodium nitrite (NaNO_2) (5% w/v), was first incubated for 6 minutes at room temperature. After this, 150 μl of aluminium chloride hexahydrate $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (10% w/v) was added and incubated for 6 minute at room temperature followed by addition of 800 μl solution of NaOH (10% w/v) and incubated at room temperature for 15 minute and the final volume was made up to 5 ml. For blank, extract was replaced with distilled water. Absorbance was measured at 510 nm to measure total flavonoids content in the samples. Total flavonoids content was expressed as mg Quercetin equivalent (QE)/g.

Antioxidant activity estimation

Antioxidant activity in tea samples was determined by spectrophotometry, using ascorbic acid as standard (Fig. 3), according to the method described by Kaur *et al.* (2015). 0.1 ml of methanolic sample extract was taken and to it 0.9 ml of ethanol, 5 ml of distilled water, 1.5 ml of HCl, 1.5 ml of potassium fericyanide, 0.5 ml of 1% SDS and 0.5 ml of 0.2% ferric chloride was added and the final volume was made upto 10 ml. This mixture was boiled in water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at 750 nm to measure the reducing power of the tea extract. The antioxidants in samples were derived from a standard curve of ascorbic acid. The total antioxidant power was expressed as mg ascorbic acid equivalent (AAE)/g.

Total tannin estimation

Total tannin content in tea samples was determined by



Fig. 4. Total tannin content (TTC) analysis in Temi tea samples.

spectrophotometry, using tannic acid as standard (Fig. 4), according to the method described by Thimmaiah (2004). For the preparation of Folin-Denis Reagent, 100 g of sodium tungstate was dissolved in 20 g phosphomolybdic acid and 750 ml of distilled water was added in a suitable flask with the addition of 50 ml phosphoric acid. This mixture was reflux for 2 hour and the final volume was made up to 1 liter with water. The prepared reagent should be protected from exposure to light.

Sodium carbonate solution : 175 g of sodium carbonate was dissolved in 500 ml of water at 70–80°C. The mixture was filtered through glass wool after allowing it to stand overnight.

Standard tannic acid solution : 100 mg of tannic acid was dissolved in 100 ml of distilled water.

Procedure : 0.1 ml of ethanolic sample extract was taken and to it 0.5 ml of FDR solution, 1ml of sodium carbonate solution was added and the final volume was made up to 5 ml. Absorbance was measured at 700 nm to measure total tannin content in the samples. Total tannin content was expressed as mg tannic acid equivalent (TAE)/g.

RESULTS AND DISCUSSION

Total polyphenols (TPC) in Temi tea samples

The content of total polyphenols in tea samples were derived from a standard curve of gallic acid. The TPC was expressed as mg gallic acid equivalents

Table 1. Total polyphenol content (TPC), total flavonoids content (TFC), antioxidant activity (AOA) and total tannin content (TTC) in Temi tea samples (mg g⁻¹).

Treatment	TPC mg GAE/g DW	TFC mg QE/g DW	AOA mg AAE/g DW	TTC mg TAE /g DW
T ₁	10.56±0.80	5.45±0.91	6.00±0.50	6.00±2.30
T ₂	7.68±0.51	4.80±0.65	5.93±4.23	17.2±4.03
T ₃	7.56±0.31	4.55±0.44	3.56±2.34	12.0±3.26
T ₄	7.37±0.25	3.90±0.38	1.87±0.82	10.0±2.30
T ₅	6.43±0.55	3.72±0.30	1.43±0.87	8.00±4.61

per gram of sample (mg GAE/g) which ranged from 6.43±0.55 to 10.56±0.80 mg GAE/g DW (Table 1). Among all the treatments, highest value of total polyphenols was observed in T₁ (Green tea sample of Spring flush) with TPC of 10.56±0.80 mg GAE/g DW while the lowest total phenolic content was observed in T₅ (Black tea sample of Autumn flush) with TPC of 6.43±0.55 mg GAE/g DW. Spring flush green and black tea has been found to contain more polyphenols as compared with other flush sample. This is because Spring flush samples are being harvested after the rest/dormancy period of the plant. During this phase the plant produces more secondary metabolites to cope up with the stress condition and as a result TPC has been found more in the Spring flush. Similar trends in the phenolic content of black and green tea have been noted in a number of other investigations. Bizuayehu *et al.* (2016) have reported the TPC of commercially available Ethiopian tea which ranged from 21.3±0.24 to 31.6±0.31 mg of GAE/ g of dry matter. Likewise, Ghabru and Sud (2017), have reported that the rainy and summer flush season's samples of fresh tea shoots of Kangra local tea had higher levels of total polyphenols content in comparison to first and winter flush seasons.

Total flavonoids (TFC) in Temi tea samples

The total flavonoid content in Temi tea samples were estimated from a standard curve of Quercetin and expressed as mg Quercetin equivalents per gram of sample (mg QE/g) which ranged from 3.72±0.30 to 5.45±0.91 mg QE/g DW (Table 1). Among the studied treatment, (T₁) green tea (Spring flush) had the highest flavonoid content (5.45±0.91 mg QE/g DW) while (T₅) black tea (Autumn flush) had the lowest flavonoid content (3.72±0.30 mg QE/g DW). The most possible reason that led to differ the flavonoid content among

Temi tea samples of different flush/season might be due to the degree of fermentation. Since green tea is made without fermentation, the majority of its polyphenols are left intact, while in the case of black tea, during the fermentation process, the polyphenols oxidise to become theaflavins (TF) and thearubigins (TR) or degrade to other forms. Likewise, Pereira *et al.* (2014) have reported the TFC of green, black and white tea bags in which the TFC ranged from (6.35 to 8.92 mg of quercetin/g) and Gonbad *et al.* (2015) have reported the total flavonoid content of tea clone which ranged from 3.45 ± 1.02 to 5.40 ± 0.72 mg rutin equivalents per gram dry weight which is lower than the current findings.

Antioxidant activity (AOA) in Temi tea samples

The level of antioxidant activity of Temi tea samples was estimated from a standard curve of ascorbic acid and expressed as mg ascorbic acid equivalents (AAE)/g DW. The antioxidant activity in the studied Temi tea samples of different flush ranged from 1.43±0.87 to 6.00±0.50 mg AAE/g DW (Table 1). (T₁) Green tea (Spring flush) had the AOA (6.00±0.50 mg AAE/g DW) while (T₅) black tea (Autumn flush) had the lowest AOA (1.43±0.87 mg AAE/g DW). The most probable reason for the higher antioxidant activity is due to potent antioxidant activities of catechins in green tea due to their three adjacent hydroxyl (OH) groups on the β-ring as in EGCG, GCG, EGC, ECG and GC which are more effective in scavenging free radicals than the two adjacent OH groups as in ECG, CG and EC (Kaur *et al.* 2015). The content of EGCG and EGC in green tea is much higher than in black tea (Musial *et al.* 2020). Similarly, Bizuayehu *et al.* (2016) have reported the antioxidant content of commercial Ethiopian tea which ranged from 28.8± 1.86 to 80.0 ±0.63 mg/g which is higher than the

current findings.

Total tannin content (TTC) in Temi tea samples

The concentration of total tannin in Temi tea samples was derived from a standard curve of tannic acid and TTC in the tea samples was expressed as mg tannic acid equivalents (TAE)/g DW which ranged from 6.00 ± 2.30 to 17.2 ± 4.03 mg TAE/g DW (Table 1). (T₂) Black tea (Spring flush) had the total tannin content (17.2 ± 4.03 mg TAE/g DW) while (T₁) green tea (Spring flush) had the lowest total tannin content (6.00 ± 2.30 mg TAE/g DW). The significant differences in tannin concentration between tea samples may be attributed to differences in the manufacturing method, the ageing process of tea leaves, and variations in climate. According to the report of Khasnabis *et al.* (2015), tannin content in black tea ranged from 11.76 to 15.14% with an average of 13.36% while in green tea it was 3.11% with an average of 2.65%. Similarly, Bizuayehu *et al.* (2016) have reported TTC of commercially available Ethiopian tea which ranged from 5.64 ± 0.39 to 7.45 ± 0.27 mg tannic acid equivalent/g of dry weight basis which are lower than the present data.

CONCLUSION

Significant seasonal variations in secondary metabolite content were observed across the different flush tea sample. T₁ (Green tea of Spring flush) had the highest TPC, TFC and AOA while T₂ (Black tea of Spring flush) had highest tannin content. The most probable reason that induced seasonal variations in the level of secondary metabolites of tea samples may include the weather parameter variation across different harvesting season.

REFERENCES

- Abdolmaleki F (2016) Chemical analysis and characteristics of black tea produced in North of Iran. *Journal of Food Biosciences and Technology* 6 (1) : 23—32.
- Bizuayehu D, Atlabachew M, Ali MT (2016) Determination of some selected secondary metabolites and their *in vitro* antioxidant activity in commercially available Ethiopian tea (*Camellia sinensis*). *Springer Plus* 5 : 1—9. DOI 10.1186/s40064-016-2056-1
- Ghabru A, Sud RG (2017) Variations in phenolic constituents of green tea (*Camellia sinensis* (L.) O Kuntze) due to changes in weather conditions. *Journal of Pharmacognosy and Phytochemistry* 6 (5) : 1553—1557.
- Gonbad RA, Afzan A, Karimi E, Sinniah UR, Swamy MK (2015) Phytoconstituents and antioxidant properties among commercial tea (*Camellia sinensis* L.) clones of Iran. *Electronic Journal of Biotechnology* 18 (6) : 433—438. <https://doi.org/10.1016/j.ejbt.2015.08.007>.
- Kaur A, Kaur M, Kaur P, Kaur H, Kaur S, Kaur K (2015) Estimation and comparison of total phenolic and total antioxidants in green tea and black tea. *Global Journal of Bio-Science and Biotechnology* 4 (1) : 116—120.
- Khasnabis J, Rai C, Roy A (2015) Determination of tannin content by titrimetric method from different types of tea. *Journal of Chemical and Pharmaceutical Research* 7 (6) : 238—241.
- Mohammed S, Manan FA (2015) Analysis of total phenolics, tannins and flavonoids from *Moringa oleifera* seed extract. *Journal of Chemical and Pharmaceutical Research* 7 (1) : 132—135.
- Musial C, Jankowska AK, Ponikowska MG (2020) Beneficial properties of green tea catechins. *International Journal of Molecular Sciences* 21 (5) : 1744. <http://dx.doi.org/10.3390/ijms21051744>.
- Pereira VP, Knor FJ, Velloso JCR, Beltrame FL (2014) Determination of phenolic compounds and antioxidant activity of green, black and white teas of *Camellia sinensis* (L.) Kuntze, Theaceae. *Revista Brasileira de Plantas Mediciniais* 16 (3) : 490—498. http://dx.doi.org/10.1590/1983-084X/13_061
- Shang A, Li J, Zhou DD, Gan RY, Li HB (2021) Molecular mechanisms underlying health benefits of tea compounds. *Free Radical Biology and Medicine* 172 : 181—200. <https://doi.org/10.1016/j.freeradbiomed.2021.06.006>.
- Thimmaiah SR (2004) In: Standards methods of biochemical analysis, Kalyani Publishers, New Delhi 65 : 296—298.