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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(8): 629-635

www.thepharmajournal.com
Received: 28-06-2022
Accepted: 30-07-2022

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Physical and antioxidant properties of selected tea varieties

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DOI: https://doi.org/10.22271/tpi.2022.v11.i8h.14745

Abstract
Tea (*Camellia sinensis* L.) is amongst the most popular beverages around the globe. Tea contains polyphenols and flavonoids, which have huge health significance. Hence, polyphenols and flavonoids can be considered as good indicator for tea quality. In the present investigation four different tea varieties i.e., Nilgiri, Darjeeling, Assam and Munnar were analyzed for their moisture content, water activity, total phenol content and total flavonoid content using UV-visible spectrophotometer. The total amounts of phenol content and total flavonoids were determined by Folin-Ciocalteu and Aluminium chloride method respectively. The total phenol was obtained as in the range of 20.76 ± 0.010 to 25.97 ± 0.027 mg gallic acid equivalent (GAE)/g of dry weight and flavonoids were expressed between 45±0.012 to 53.84±0.017 mg/g of catechin equivalents (mg/g of Catechin of extracted compound) for the sample taken. Nilgiri and Assam tea infusion were determined to have the highest phenol and total flavonoid contents i.e., 25.97 ppm and 53.84 ppm respectively.

Keywords: Flavonoids, folin-ciocalteu, phenol, UV spectroscopy, gallic acid, catechin

1. Introduction
Tea is amongst most popular beverages, consumed by people all over the world for its appealing flavor, stimulating effects and health benefits. It is prepared by boiling of processed tea leaves, *Camellia sinensis* in luke warm water to extract its beneficial compound. (Vinodh Kumar & Shruthi, 2014) [17]. Globally, tea ranks second after water in terms of consumption (Hayat *et al*., 2015) [9]. According to the varied degrees of fermentation, tea is generally classified into six categories: white tea i.e., mildly fermented, green tea i.e., unfermented, black tea i.e., fermented, oolong tea i.e., semi-fermented, and dark tea i.e., post-fermented, yellow tea ie., slightly fermented (Zhao *et al*., 2014). Among them, black, green, and oolong tea are primarily consumed.

Apart from providing relief from tiredness, tea has numerous health benefits. Tea has been shown to have anti-cancer properties in several laboratory models of malignancies generated by chemical carcinogens in the lung, intestine, pancreas, liver, breast, and skin. Additionally, it has been discovered to be helpful in the metabolism of xenobiotic substances, atherosclerosis, coronary heart disease, high blood pressure, and cholesterol. Tea has been proven to positively influence insulin sensitivity, glucose metabolism, fatty acid oxidation, and other physiological processes in addition to having good effects under physiologically changed situations. It also offers protection against neuro-damage and ageing. According to research, green tea polyphenols can prevent tissue damage to the heart during both oxidative damage and hypoxia/reoxygenation damage (Bordoni *et al*., 2002) [5]. Tea can shield humans from ultraviolet radiation-induced genotoxicity (DNA damage), even when only occasionally consumed (Malhomme de la Roche *et al*., 2010) [12].

Undoubtedly one of the most widely used techniques for phenolic analysis is the Folin-Ciocalteu assay (Singleton *et al*., 1999) [14]. The Folin-Ciocalteu reagent (FCR) is reduced in the presence of phenolics to produce molybdenum-tungsten blue, which can be spectrophotometrically measured at 760 nm and whose reading is directly interrelated with the phenols concentration in the reaction medium (Swain T & Hillis W E, 1959) [16]. After extraction of oil present in tea using solvent extraction method, total flavonoid content (TFC) can be often measured colorimetrically. One of the most utilized methods for flavonoid test for plant extract is the aluminium chloride colorimetric assay described by Christ and Müller, 1960 [7] in which Al (III) is used as a complexing agent. When $AlCl_3$ is introduced without the presence of $NaNO_2$, the development of yellow-colored Al (III)-flavonoid complexes is

observed experimentally.

The absorbance of these complexes is then measured between 410 and 440 nm. The total flavonoid concentrations are calculated using calibration curves based on a reference flavonoid standard (catechin in current studies) obtained under identical experimental condition and wavelength.

Considering the health benefits, total polyphenol content and flavonoid content are important criteria for assessment of high-grade tea product. The objective of current study was to assess the physical properties (moisture content and water activity) as well as antioxidant characteristics by determining total phenolic content and the flavonoid content of tea powders. Tea sample of four different varieties Nilgiri, Darjeeling, Assam, Munnar, will be used for the assessment of the total phenol content by Folin-Ciocalteu method and the computation of the total flavonoid content by using aluminium chloride colorimetric assay.

2. Material & Methods

2.1 Raw materials

Nilgiri (A), Darjeeling (B), Assam (C), Munnar (D) tea varieties were procured from local market in Thanjavur district, Tamil Nadu, India.

2.2 Reagents

Folin-Ciocalteu reagent, $NaHCO_3$, Gallic acid, Catechin, $AlCl_3$, $NaNO_2$, NaOH. The reagents used for the experimental purpose were purchased from Sigma-Aldrich. All the reagents were of analytical grade.

2.3 Moisture content determination

The moisture content for tea powders were determined by hot air-drying method. The oven temperature is maintained at 105±5 °C. 5±0.1 g of tea powder sample from different brands were taken in metal dish and placed in hot air oven for 24 hours. Drying was carried further until no significant consecutive weight changes occurred (You *et al*., 2018). The formula for calculation of moisture content is given in eq. (1)

$$\text{Moisture content (\% wb)} = \frac{\text{Initial weight} - \text{Oven dry weight}}{\text{Oven dry weight}} \times 100 \quad \ldots\ldots(1)$$

2.4 Water activity determination

Tea powder weighs 0.5 g was placed into a sample holder provided with a Rh probe and water activity meter (Make: Testo 650 Water Activity System, Cole-Parmer, USA) was used at ambient temperature (around 25 °C) to conduct the test. The relative humidity equilibration was used to record water activity values (Topuz *et al*., 2014) [15].

2.5 Preparation of tea extract

Tea in the granular powder form was purchased from the local market under the following four varieties: A, B, C, and D. Tea extract was prepared by using the technique outlined by Hossain MS *et al*., (2014) [10] given in Figure 1. 50 g of tea powder was infused distilled water (300 mL) and subjected to boil for 45 minutes. Whatman No. 1 filter paper was used to filter out the impurities.

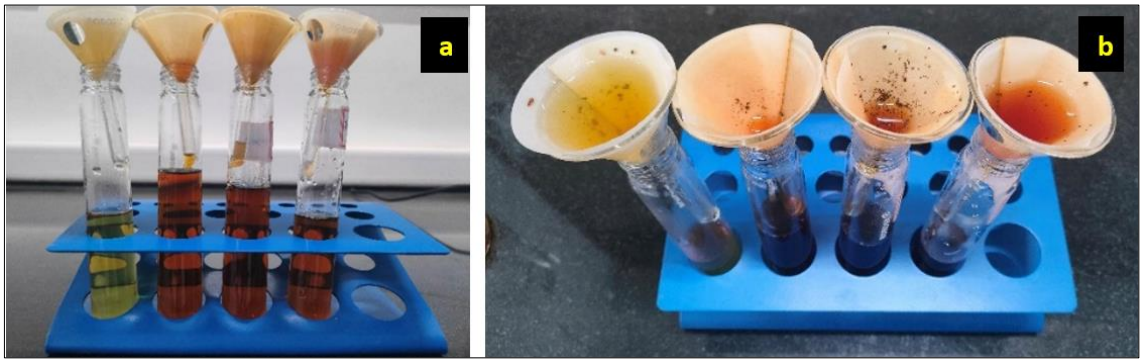

Fig 1: Extraction of different tea samples

2.6 Preparation of test standards

Standard gallic acid and catechin solutions were made by mixing homogenously 10 mg of both the compounds in 10 mL of methanol (1 mg/mL). Different concentrations of working standard of gallic acid solutions (5, 10, 20, 30, 40, 50 ppm) and catechin solutions (10, 20, 40, 60, 80, 100 ppm) were prepared from the stock solution.

2.7 Total phenolic content (TPC) determination

The calibration curve was created by plotting 0.5 ml of solutions containing 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm Gallic acid with tenfold-diluted Folin Ciocalteau reagent (0.5 ml) and 0.4 ml of sodium carbonate solution. At 765 nm, the absorbance was measured after 30 minutes. In order to create the calibration curve, 1 ml of each of the aqueous and ethanolic extracts (1 gm/100 ml) was combined separately with the same reagents. Using the procedure, the absorbance was measured after one hour to determine the total phenolic content for each individual standard.

TPC was measured using the Folin-Ciocalteu method in spectrophotometry using gallic acid (GA) as the standard (Singleton *et al*., 1999) [14]. This analysis made use of tea extract dissolved in water at a 500 mg/mL concentration. Diluted aqueous extract (0.5 mL) was mixed with water-dissolved 10% Folin-Ciocalteu reagent (2.5 mL). After that, the mixture was vortexed. Then, 2.5 mL of $NaHCO_3$ at 7.5 percent was mixed together. The tubes were then remained at ambient temperature for an additional 60 minutes. At a wavelength of 765 nm, absorbance was calculated in relation to distilled water. Each sample was made into three copies. The processes were repeated for the usual sample. The TPC were calculated with respect to mg of Gallic acid/g of dry tea weight, the gallic acid equivalents (GAE).

2.8 Total flavonoid content (TFC) determination

10mg of catechin was mixed homogenously in 100ml methanol and then diluted to 10 ppm, 20 ppm, 40 ppm, 60

ppm and 100 ppm using methanol. Using distilled water, 10 percent aluminium chloride and 1M potassium acetate were formed. Separate 0.5ml samples of the standard catechin dilution and each extract stock solution were taken in test tubes. aluminium chloride solution (0.1 ml), methanol (1.5 ml), potassium acetate solution (0.1 ml), and distilled water (2.8 ml) were put into each test tube. By replacing distilled water with an aluminium chloride solution instead of water, every dilution of standard catechin was made in the same way. The solutions were prepared, passed through Whatmann filter paper, and then the absorbance was determined. The standards' absorbance at 450 nm was taken against distilled water (Akbay *et al*., 2003) [2].

Utilizing a colorimetric test with aluminium chloride, the total flavonoid content of tea extract was measured with spectrophotometer using catechin as the reference (Kumar *et al*., 2008) [11]. 10% $AlCl_3$ solution (0.3 mL) and 5% $NaNO_2$ (0.3 mL) were combined with tea extract (1 mL) at a concentration of 30 mg/mL before 200 mL of NaOH was added. At room temperature, the sample was incubated for one hour and absorbance at 450 nm was measured. Three duplicates of each sample were created. The TFC was expressed as mg of catechin/g of tea extract, which represents comparable amount of catechin.

2.9 Statistics

Statistical analysis was carried for the test results by MiniTab 20 software. All the experiments were conducted in triplicate runs to find the variability in data collection and measuring the standard deviation. Using ANOVA and Tukey's test, the statistically significant variation (P<0.05) was found in the moisture content, water activity, total phenol content and total flavonoid contents.

3. Result & Discussion

3.1 Moisture content

Moisture contents of tea powders were found to be in between 3.41±0.49 to 5.58±0.16% wb. Variety A tea contains higher moisture content 5.58±0.16 followed by C, B and D. There was no significant (P<0.05) variation between varieties A & C and B & D in terms of moisture content. The results were shown in Figure 2. The higher moisture content in variety A may be related to the fermentation process' exclusion because throughout the tea-processing procedure, many of the polyphenols that help retain moisture content are destroyed. Utilizing packing materials to maintain a steady moisture level when storing samples of commercial tea is another crucial component, making moisture content in commercial tea a crucial indicator of quality (Adnan *et al*., 2013) [1].

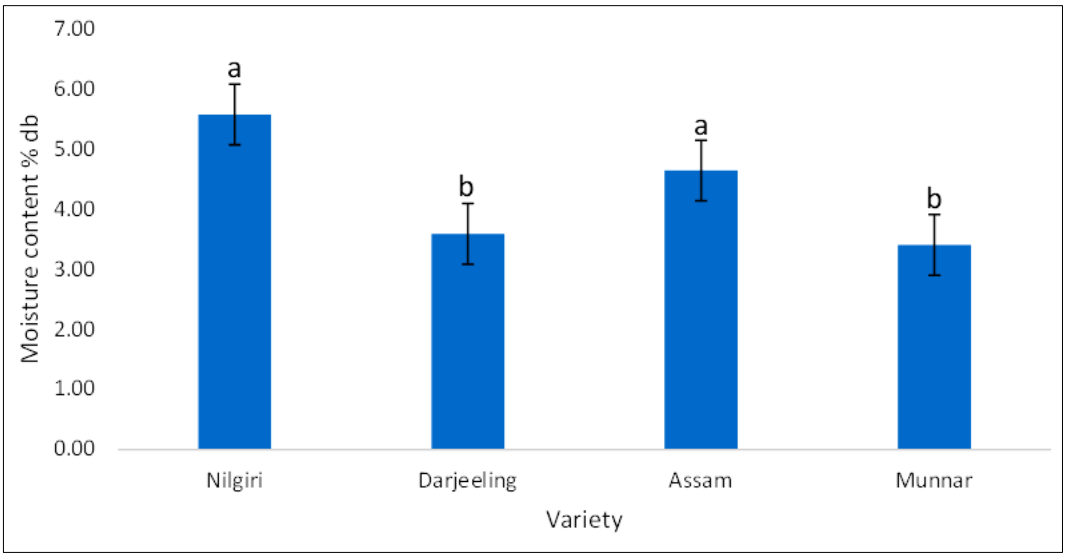

Fig 2: Moisture content of tea samples from different brands

3.2 Water activity

Water activity results were observed from the conducted experiment in between 0.319±0.003–0.348±0.009. The highest water activity content was found in variety B tea sample. There was no significant (P<0.05) variation between varieties A & D, however B & C shown differences in terms of water activity content. The results were shown in Figure 3. Higher water activity signifies availability of greater degree of free water. Consequently, the product will be more prone to microbial decomposition and does not meet high shelf-life criteria.

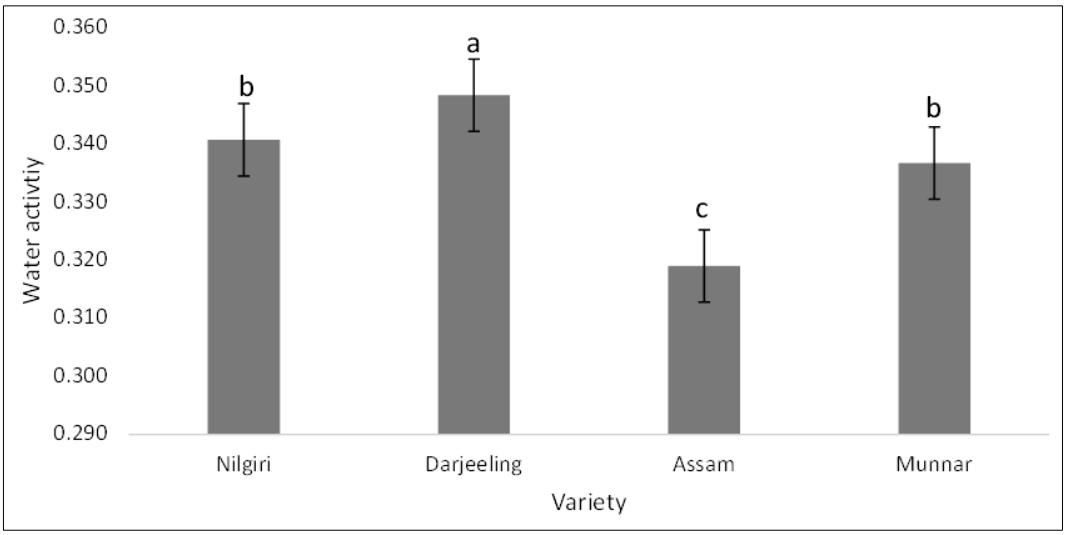

Fig 3: Water activity of tea samples from different brands

3.3 Total phenolic content (TPC)

Aluminium chloride colorimetric method was employed for total phenolic content assay. Gallic acid was the standard compound for UV spectroscopy at a wavelength of 765 nm. The proportion of phenols present in aqueous tea extracts was quantified. Gallic acid concentrations of 5, 10, 20, 30, 40, and 50 ppm were formed in methanol. Three copies of each reading were recorded. Total phenol concentrations were represented as gallic acid equivalents (GAE/g) of dry weight with good correlation, i.e., R^2=0.993, as shown in Figure 4, and the results were calculated using the standard calibration curve of gallic acid.

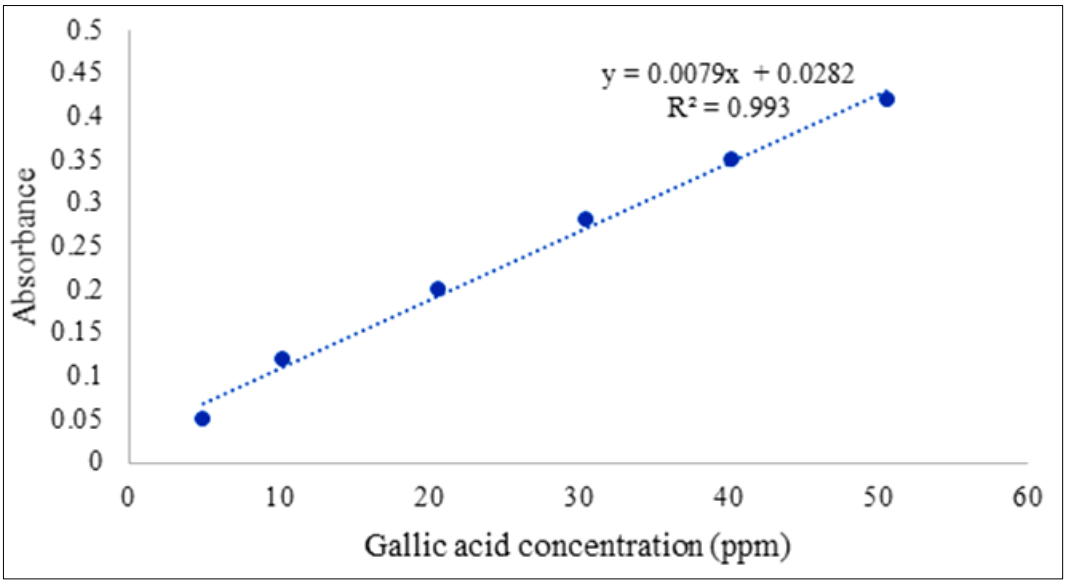

Fig 4: Calibration curve for standard gallic acid

Then after, the total phenolic contents (TPC) were found by performing Folin-Ciocalteu method for the tea infusion from different brands. As seen from Figure 5, it was found to be in the range of 20.76 ± 0.010 to 25.97 ± 0.027 mg gallic acid equivalent (GAE)/g of dry weight. There is no significant difference between Darjeeling & Munnar tea varieties whereas Nilgiri & Assam tea resembles variation in total phenol content. The total phenolic content was found to be higher in young tea leaves than in older ones at the start of the fermentation. This demonstrated that variety A had a greater amount of young tea leaves (De Filippis *et al*., 2018) [8].

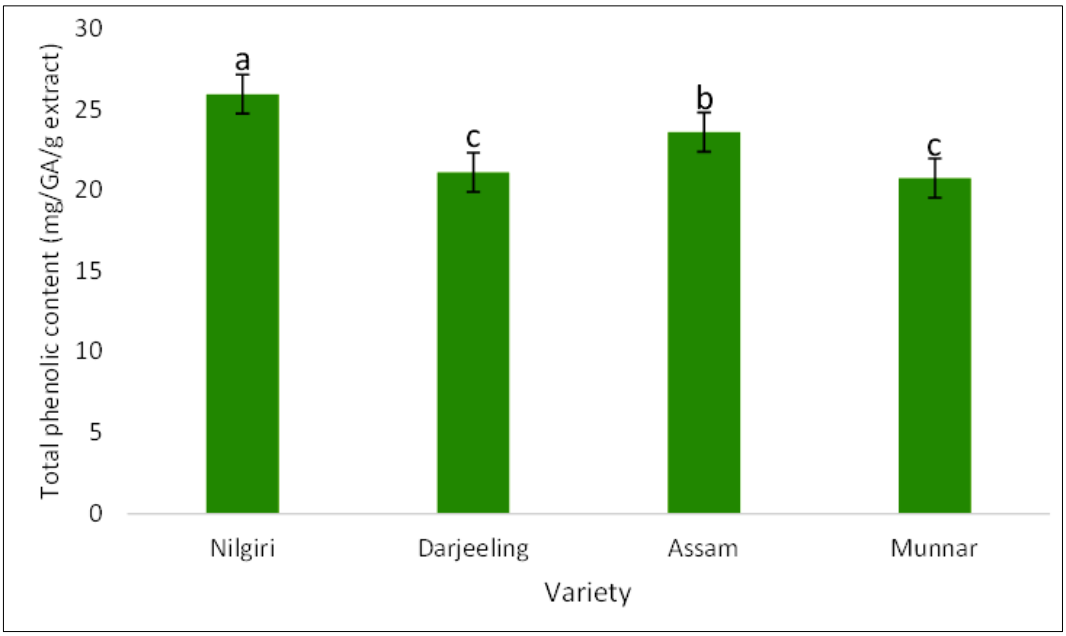

Fig 5: Total phenol contents of tea samples

3.4 Total Flavonoid content (TFC)

The flavonoid content in aqueous tea extracts was quantified by using the aluminium chloride colorimetric assay. Catechin concentrations of 10, 20, 40, 60, 80, and 100 ppm were made in methanol. Three duplicate copies of each reading were considered valid. Results from the catechin calibration standard are shown in Figure 6, and total flavonoid amounts are reported as catechin equivalents (mg/g of catechin of extracted compound), with $R^2 = 0.9918$.

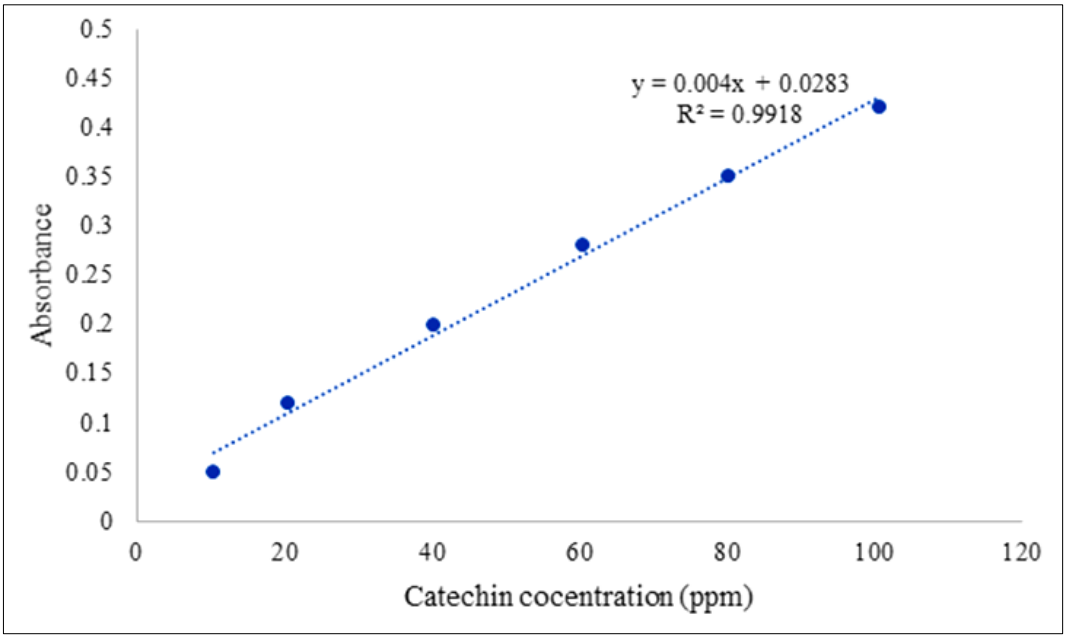

Fig 6: Calibration curve for standard catechins

The aluminium chloride colorimetric method was used to evaluate the total flavonoid content of several tea infusions. Total flavonoid concentrations were reported as catechin equivalents (mg/g of Catechin of extracted compound), using catechin as the standard ingredient. The range of total flavonoid was 45±0.012 to 53.84±0.017 (mg/g of Catechin of extracted compound). The Assam tea infusion contained the most flavonoid compounds (53.84 ppm). Figure 7 shows the results of all tea samples examined were rich in total flavonoids, which supports their use for enhancing human health, as presented by Bansode *et al*. (2015) [4].

The amount of catechins in tea is a crucial quality indicator in context to tea grade. Since most catechin components may be lost during the enzymatic oxidation process, the high catechin content in tea samples can be attributed to the usage of fresh leaves without undergoing oxidation as opposed to black tea (Muhammad Adnan *et al*., 2013) [1]. There is a significant different ($P<0.05$) in regards to the total flavonoid content of tea varieties.

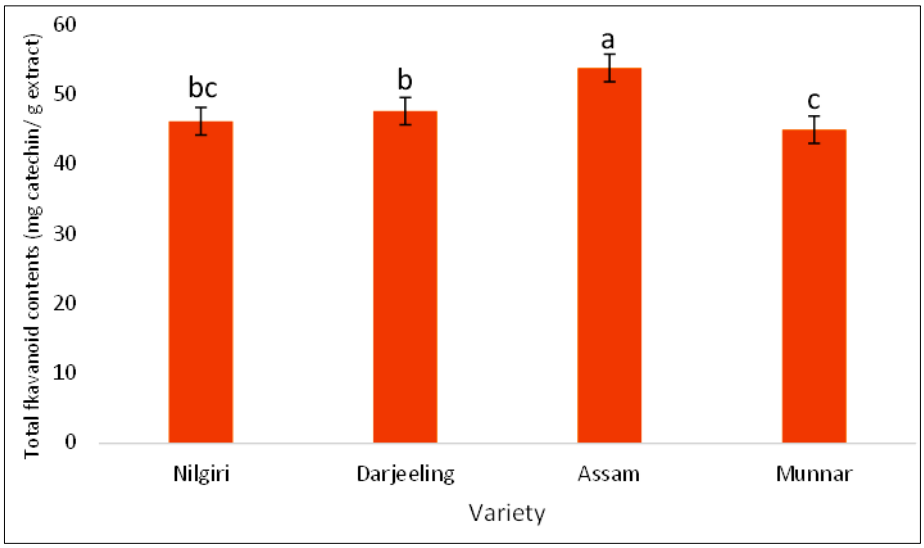

Fig 7: Total Flavonoid contents of tea samples

4. Conclusion

Moisture content and water activity are determining factor in imparting shelf stability to tea powder. Nilgiri variety tea infusion (25.97 ppm) and Assam variety tea infusion were determined to have the highest phenol and total flavonoid contents (53.84 ppm). In regards to total flavonoid compound determination, black tea infusions (Assam tea) contain higher levels of flavonoids content than green tea infusion (Nilgiri tea). The findings showed that all tea samples examined had comparable high levels of total phenols and flavonoids. All the selected varieties of tea have abundant sources of bioactive chemicals, which supports their health significance and consumption for healthy well-being.

5. Acknowledgement

The National Institute of Food Technology, Entrepreneurship and Management-Thanjavur (NIFTEM-T) is gratefully acknowledged by the authors for its support of this study.

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