



Comparative Study of Antioxidant Capacity of Raw Powder and Waste Black Tea by FRAP Assay

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Abstract

Since many centuries tea has been used as important bioactive compounds like folk medicine and it is also an important source of polyphenol as a powerful antioxidant which has used in anticancer activity and protects cell damages from free radicals. This study aimed to compare antioxidant capacity of raw tea powder and tea waste sample by FRAP assay. Antioxidant activity of methanolic (50%) and distilled water extract of raw tea powder and tea waste sample were analyzed by FRAP assay. Total phenolic content was determined using Folin-Ciocalteu reagent and calculated as gallic acid equivalent per gram dry weight. Total antioxidant activity was found to be 0.930 mmol Fe^{II}/g of dry weight (waste tea) and 0.995 mmol Fe^{II}/g of dry weight (raw powder). Total phenolic content was 0.232 g gallic acid/g of dry weight (waste tea) and 0.162 g gallic acid/g of dry weight (raw powder). A correlation between total antioxidant activity, total phenolic content and gallic acid was tested between two samples. Because of contribution of phenolic compounds these samples possess higher antioxidant activity. It is strong radical scavenger and can consider as a good source of natural antioxidant for medicine purposes.

Keywords: Poly phenolic, FRAP, Antioxidant activity, Phenolic, Radical scavengers.

Introduction

The Worldwide 30 different countries are grown *Camellia sinensis* L. (tea) plant. For the best growth it requires adequate rainfall and slightly acidic soil in tropical and subtropical areas. Because its desirable flavor, taste and putative positive physiological functions it is most consumed beverage¹. High levels of flavonoids and polyphenols in green and black tea can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals after uptake including anticarcinogenic, antioxidative, and hypolipidemic activities². Studies have shown green tea possess cholesterol lowering³, anti-inflammatory⁴, antibacterial and antiviral properties^{5,6}.

In both black and oolong teas, Theaflavins (TF) Polyphenol Pigments found. During manufacturing of black or oolong Tea, at fermentation or semi fermentation stage Theaflavins are formed from polymerization of Catechins.

In ~2g/100g of dried water extract of black tea bright orange-red colour is formed because of presence of TF. In cancer and cardiovascular diseases, both catechins and TF have recently received much attention as protective agents. It is also believed that they both have other pharmaceutical benefits including antioxidative and antihypertensive activity.

Till now studies have been conducted to find out antioxidant activity and phenolic content in (Black and Green tea) tea leaves, tea infusion and waste from tea processing units. Our

aim of study was to evaluate that whether antioxidant activity in the (black tea) waste samples after boiling of tea (waste that we get after infusion) is found or not by FRAP assay (Ferric Reducing Ability of Plasma). Our study also indicates a possible connection between phenolic content and antioxidant activity between the samples of tea waste and raw tea powder.

Material and Methods

Tea Sample: Black tea powder ("Waghbakri" CTC Fanning Tea) was purchased from a local super market. Waste tea sample was collected from mess of Hostel B; Ganpat University; Ganpat vidyanagar -384 012.

Chemicals and Reagents: TPTZ (2, 4, 6-tri [2-pyridyl]-s-triazine), Vitamin-C (ascorbic acid) and Folin- Ciocalteu phenol reagent were used. Gallic acid (3, 4, 5-trihydroxybenzoic), FeCl₃.6H₂O, FeSO₄.7H₂O, Sodium acetate Trihydrate, Glacial acetic acid, Ethanol, Methanol and Sodium carbonate were obtained from Sd fine chemicals limited.

Antioxidant activity and phenolic content extraction method: Sample of waste (after boiling of tea) was dried in oven at 40°C for 16-18 hours then homogenized under blender immediately before extraction. The raw tea powder was used directly without drying and homogenized in the same as the sample of waste tea powder. Each ground sample (50g) separately weighed, 50% aqueous methanol (1:5W/V) (250ml) and 250ml of distilled water was mixed with both samples and

put in environmental shaker at 120 rpm for 6hr at 37^oC-40^oC. Then supernatant was obtained by centrifugation of filtrate at 2000 rpm for 20min at room temperature. The supernatant was directly used for estimation of total phenolic compound and FRAP.

FRAP assay used for measurement of total antioxidant activity: By Benzie and Strain, FRAP⁷ (Ferric Reducing antioxidant power assay) method was followed to check total antioxidant activity. Aliquots of 100µl sample were mixed with 3ml of FRAP reagent and incubated mixture at 37^oC for 10min and by using spectrophotometrically absorbance of reaction mixture was measured at 593nm Using five different concentration of FeSO₄.7H₂O (1000,750,500,250,125µmol/L) calibration curve was prepared. Sample solutions were used as a absorbencies. The values were expressed as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1mmol/L FeSO₄.

Total phenolic compound analysis: Total amount of phenolic contents in the extracts was estimation calorimetrically by using Folin-Ciocalteu (FC) reagent⁸. 100 µl of undiluted (FC) reagent mixed 20 µl of extract and incubated at 40 °C in the dark for 30 min. after incubation colour development was determined (absorbance) at 765 nm by UV-1650PC visible spectrophotometer. By using standard curve of gallic acid, phenolic contents of the samples were calculated and expressed as Gallic acid equivalents (GAE)/g FW or DW of the plant materials.

Table-1
Total Antioxidant activity and total phenol content of samples.

Sample	Total Antioxidant activity (mMF ^{II} /g Dry weight)	Total Phenol content (g GA/g sample)
Raw tea powder (D.W)	0.990	0.155
Raw tea powder (50% Methanol)	1.000	0.170
Tea waste (D.W)	0.910	0.230
Tea waste (50% Methanol)	0.950	0.235

Results and Discussion

Estimation of total antioxidant: Results of the FRAP assay with distilled water and 50% diluted methanol are shown in table-1. Antioxidant activity was found from 0.990 to 1.000 mMF^{II}/g Dry weight in raw tea powder and 0.910 to 0.950 mMF^{II}/g Dry weight in tea waste powder sample.

Estimation of total phenol: The results of Folin-Ciocalteu assay with distilled water and 50% methanol are shown in table-1. Phenolic content was found from 0.155 to 0.170 g GA/g

sample in raw tea powder and 0.230 to 0.235 g GA/g sample in tea waste sample.

Analysis of data by statistical method: Correlation analyses of antioxidant activity versus the total phenolic content was carried out using the correlation and regression program of SPSS16.0 and the results are shown in figure 1.

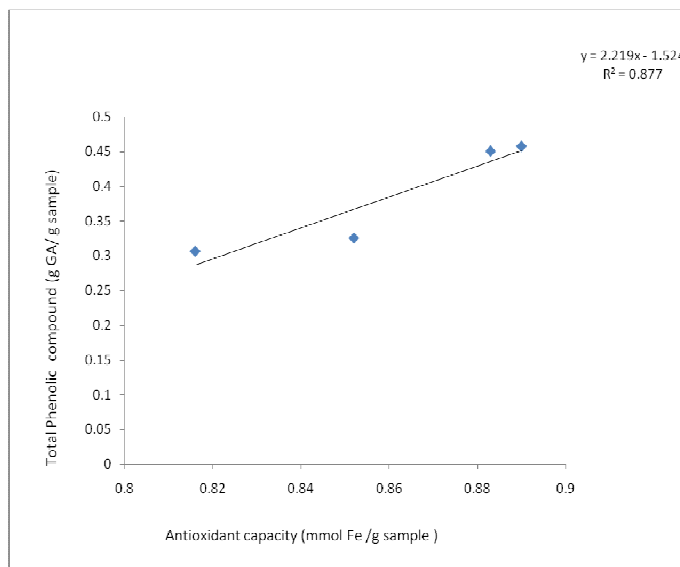


Figure-1
Correlation between Fe^{II} equivalent antioxidant activity and total phenolic content in black tea samples.

Discussion: In present study it was observed that total antioxidant activity was found nearer in both raw tea powder and tea waste sample ranging from 0.930 to 0.995 mMF^{II}/g Dry weight. The phenolic content was found little higher in tea waste sample i.e. 0.232 g GA/g sample than that of raw tea powder i.e. 0.162 g GA/g sample. The antioxidant effect of black tea was estimated using in vitro FRAP method to identifying that samples possessing high antioxidant power for further studies. The study was also attempted to quantify the total phenolic content present in black tea (table-1). Black tea is rich in a range of compounds such as catechins, flavonoids, theaflavins, thearubigins, and phenolic acids that could potentially have health-promoting properties. Many studies have confirmed that black tea possesses considerable antioxidant capacity. Antioxidants are known to protect tissues against damage caused by oxygen free radicals and lipid peroxidation, and it is proposed that the protective effect of tea against cardiovascular disease may be attributed to its antioxidant components¹⁴. Black tea is widely consumed by Indian people and mostly it is prepared by boiling in water. After boiling of tea the infusion is used as beverage while the remaining tea waste powder is discarded. The present research work was carried out for comparative estimation of antioxidant capacity in tea waste (after boiling) and raw tea (before boiling).

Conclusion

From Present study it is concluded that tea waste sample has high antioxidant power So it can be used as a favorite beverage.

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