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Study of Antioxidant Properties in Black Tea and Green Tea

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A B S T R A C T

The present study was conducted to assess and compare the total polyphenol content (TPC), antioxidant activity in black and green tea. The green tea showed a higher polyphenol content and antioxidant levels than black tea ($p < 0.05$). The variations in polyphenolic levels in black tea (2.11- 0.26 mg TAE/g) and green tea (5.20- 1.17 mg TAE/g) observed in our study. In black tea, the FRAP levels ranged from (5.58 mg AAE/g to 1.21 mg AAE/g) whereas in green tea has ranged (8.88 mg AAE/g to 3.26 mg AAE/g. The total antioxidant capacity in green tea (22.866±7.036 mg AAE/g) is significantly higher than in black tea (3.386±1.28 mg AAE/g). The activities of antioxidant were well correlated the total polyphenol content r2 = 0.9571 for FRAP method and r2 = 0.9287 for Polymolybdenum assay. The Assam and Darjeeling green tea (North East India) had higher TPC and antioxidant levels than Munnar, Coimbatore Nilgiri green tea (south India). However, the more studies on Indian tea to emphasize the health benefits of Indian tea especially green tea which will help in its increased consumption as a favorite beverage in India.

Introduction

Tea is one of the most widely consumed beverages in the world, next to water (Cheng, 2004 and Vinson, 2000) with a per capita consumption of ~120 mL/day (Mckay, *et al.,* 2002). This popularity is probably related with its sensorial properties, relatively low retail price, stimulating effects and potential health benefits (Baptista *et al.,* 1998 and Baptista, *et al.,* 1999). The biological properties of tea include effects on the Central System (CNS) and antioxidant effects, attributed to the presence of methylxanthines, such as caffeine and phenolic compounds, especially catechins (Shagana, *et al.,* 2017). Tea after processed, which is one of the most popular beverages, is manufactured from the young tender leaves of the plant *Camellia sinensis* (Cabrera *et al.,* 2003). Tea is derived from terminal leaves of shoots of tea plant *Camellia sinensis* family. *Sinensis* species is divided into two distinct varieties *sinensis* and *assamica*. *Camellia sinensis var. sinensis* is indigenous to South east China, Darjeeling, Japan. *Camellia sinensis var assamica* is indigenous to Assam,

Thailand, Sri Lanka (Macfarlane *et al.,* 2004). There are mainly three types of Camellia tea which are Green, Oolong and Black tea. The difference lies in the 'fermentation' which refers to oxidative and enzymatic changes within the tea leaves during processing (Hicks, 2001). Worldwide, the consumer's preference is approximately 76-78% of black tea followed by green tea (20-22%) and oolong tea (2%). The bulk of tea is produced in the Northeast (West Bengal and Assam), followed by the Northern area (Himachal Pradesh) and small quantities are produced in the Southern India (Tamilnadu) tea followed by green tea (20-22%) and oolong tea (2%). Tea from Darjeeling, Assam, and Nilgiri are world famous for their taste and flavor. In contrast, during black tea processing, tea shoots are macerated to initiate oxidation by polyphenol oxidase (PPO) before firing. TFs (Theaflavins) are also present in tea which gives a yellow-red coloration in fermented black tea and contributes to the briskness and brightness of tea liquor. The manufacturing method for semi-fermented oolong tea consists of solar withering, panning, rolling and drying. Throught this process, the characteristic floral aroma of oolong tea is produced. White tea may be a rare specialty tea that gets its name from a paricular tea plant variety selection as well as a selected post-harvest processing technique that raises small silvery hairs on the dried buds. The differencesbetween the various processes of manufacture result in differences in the polyphenol profile between green, black, oolong and white tea.

The chemical composition of tea is complex and includes alkaloids (caffeine, theophylline, and theobromine), polyphenols, carbohydrates, amino acids, proteins, volatile compounds, minerals, chlorophyll, trace elements and other unidentified compounds. Among these, polyphenols constitute the most interesting group and are the main bioactive molecules in tea (Cabrera *et al.*, 2003). The major polyphenolic compounds in tea are the flavan-3-ols called catechine (Peterson *et al.,* 2005). Catechins account for 6-16% of the dry green tea leaves. When the black tea is oxidized, the catechins are converted into theaflavins and thearubigins which still act as antioxidants (Ho *et al.,* 1994). The theaflavins and thearubigins are sometimes called tannins and are responsible for the darker color of black tea and more heavily-oxidized oolong teas (Wang *et al.,* 2001). Green tea is rich in chemicals called catechins which are a form of flavanol monomers, a type of flavonoid. The catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin-3- gallate (ECG), and epigallocatechin-3-gallate (EGCG). Green tea also contains a little amount of an antioxidant, Vitamin C, which is also an essential nutrient (Farhoosh *et al.,* 2007). Polyphenols found in black tea are very strong antioxidants. The tannins in tea have a therapeutic effect on gastric and intestinal illnesses (Siemann *et al.,* 1992).

Among tea producing countries the principal producers are China, India, Sri Lanka, Kenya and Indonesia which account for 80% of global production. India is a major producer, consumer, and exporter of tea accounting for 31% of the total global production of tea. In India, there are three different geographical areas of cultivation and production of tea. However, in India green tea accounts for only 1% of total production of tea. Since there are very few studies which document the antioxidant potential of Indian tea, an attempt is made to study total polyphenol content and antioxidant activity in different brands of tea and also the possible correlation between TPC and antioxidant activity. A very few peoples are known for their better antioxidants potential in black tea and green tea**.** My present study was done to estimate the total phenolic content (TPC), FRAP activity or antioxidant power and total antioxidant capacity in both black and green teas and

investigate the better one in both and was also done the relationship between the phenolic content with the antioxidant activity of the tea samples.

Materials and Methods

We had used six different green tea samples (labeled as AF) and ten different black tea (labeled G-P) available in the local departmental stores of different brand name of the particular area of cultivation, India. Each tea sample was studied in triplicate.

Preparation of extract

The preparation of extract was done in the dept. of biochemistry laboratory, Dr. R.P.C.A.U, Samastipur, Pusa, Bihar 200 mg of each sample was weighed, and to it, 5 ml of 70% methanol at 70 °C was added. The extract was mixed properly and heated at 70 °C on a vortex for 10 min. After cooling at room temperature, the extract was centrifuged at 2000 g for 10 min. The supernatant was collected in a centrifuge tube. The extraction step was repeated twice. Both extracts were pooled, and the volume was adjusted to 10 ml with cold 70% methanol. One milliliter of the extract was diluted with water to 100 ml. Analysis of the tea extract was done in triplicate.

Determination of Total Polyphenol Content (TPC)

The total polyphenol content of the water extract was determined by the Folin-Ciocallteu method (s. Toda, 2005). 0.5 mL tested sample was mixed with 0.5 mL of Folin-Ciocallteu reagent and 0.5 mL of 10% sodium carbonate solution. After the mixture was incubated at room temperature for 1 h, the absorbance was measured at 760 nm. The total polyphenol was expressed as mg/g tannic acid equivalent.

Determination of ferric ion reducing antioxidant power assay (FRAP)

The total antioxidant power was determined by spectrophotometry, using ascorbic acid as standard, modified FRAP assay. 0.1 ml of 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 ferricyanide, 0.5 ml of 1% SDS and 0.5 ml of 0.2% of ferric chloride was added. This mixture was boiled in a water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at 700 nm to measure the reducing power of the tea extract. The antioxidants in samples were derived from a standard curve of ascorbic acid ranging from 10 to100 µg/mL. The total antioxidant power was expressed as mg ascorbic acid equivalent (AAE)/ g

Determination of Total antioxidant

For the determination of total antioxidant capacity by using phosphomolybdenum assay, the tubes contained 0.2 ml of extract is mixed with 1.8 ml of distilled water, 2 ml of phosphomolybdenum reagent (2.8 mM (40 mg) of sodium phosphate and 4 mM ammonium molybdate (49 mg) in 10 ml of 0.6 M sulphuric acid). The tubes were incubated at 95°C for 90 minutes. The mixtures were cooled to room temperature, and absorbance was measured at 695 nm. The antioxidant capacity was expressed as mg Ascorbic Acid Equivalent (AAE) /g.

Statistical analysis

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences did by Student's t-test ($p < 0.05$). Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations using Microsoft office Excel 2007 and SPSS, version 16.

Results and Discussion

The total polyphenol content of 10 samples of black tea and six samples of green tea were estimated. The variations in polyphenol levels in black tea (2.11- 0.26 mg TAE/g) and green tea (5.20- 1.17 mg TAE/g) had observed in our study (Fig. 1) could be due to variations in the climate and agronomic practices and other factors such as tea types, commercial brands of tea, tea plantation area etc. The other results also supported to our study that the total polyphenol content in green tea (3.066±1.911 mg TAE/g) was significantly higher than in black tea (0.72±0.55 mg TAE/g) ($p<0.05$) (Suteerapataranon *et al.,* 2008). The higher levels of polyphenols in green tea (unfermented tea) than black tea could be due to the conversion of the tea polyphenols into thearubigins and theaflavin during the fermentation process (Jain, 1999). Various other studies have also reported higher total polyphenol content in green tea (Anesini *et al.,* 2008; Shrestha *et al.,* 2010, Nor Ohairul Izzreen and Mohd Fadzelly, 2013). In our study, the Kangra tea had higher polyphenol content than Darjeeling tea as reported by Vashisht *et al.,* (2007) as the Kangra tea has higher amounts of epigallocatechin-3-gallate as compared to North-East Darjeeling tea. Polyphenols in tea have been reported to have a strong antioxidant property which could be attributed to various types of the flavan-3-ols present in tea. These flavan-3-cells have been reported to prevent oxidative stress by chelating free ferrous ions which are responsible for the formation of reactive oxygen species by various metabolic processes (Tsai *et al.*, 2007).

Total Antioxidant power of 10 samples of black tea and six samples of green tea were estimated by using modified ferric ion reducing antioxidant power (FRAP) method. The results showed that the FRAP levels were significantly higher in green tea than ten samples of black tea. In black tea, the FRAP levels ranged from (5.58 mg AAE/g to 1.21 mg AAE/g) whereas in green tea had ranged (8.88 mg AAE/g to 3.26 mg AAE/g) (Fig. 2). Our results also supported from other studies have also shown that the FRAP levels were significantly higher in green tea (5.866±2.73 mg AAE/g) as compared to black tea (2.034±1.39 mg AAE/g) ($p<0.05$) (Su *et al.,* 2007, Dutta *et al.,* 2013). The higher the absorbance at 700 nm indicates the more significant the reducing power of the extract. The reducing power is associated with the presence of reductones (Duh, 1998). Reductones react with certain precursors of peroxides and prevent peroxides formation. Being good electron donor, phenolic compounds show the reducing power and ability to convert the ferric ion Fe^{+3} to Fe^{+2} ion by donating an electron. Gordon (1990) reported that the antioxidant action of reductones is based on the breaking of the free-radical chain by donating a hydrogen atom.

Total Antioxidant Capacity of 10 samples of black tea and six samples of green tea was estimated by using phosphomolybdenum assay. This assay usually detects total antioxidants such as ascorbic acid, some phenolics, α- tocopherol and carotenoids. In the present study, the total antioxidant capacity in green tea (22.866±7.036 mg AAE/g) is significantly higher than in black tea (3.386±1.28 mg AAE/g) ($p<0.05$) (Fig. 3).

Various other studies have reported higher antioxidant activity in green tea than in black tea (Yen *et al.,* 1995; Chan *et al.,* 2010). The higher antioxidant activity is due to potent antioxidant activities of catechins in green tea are due to their three adjacent hydroxyl (OH) groups on the β-ring as in EGCG, GCG, EGC and GC which are more effective in scavenging free radicals than the two adjacent OH groups as in ECG, CG, and EC.

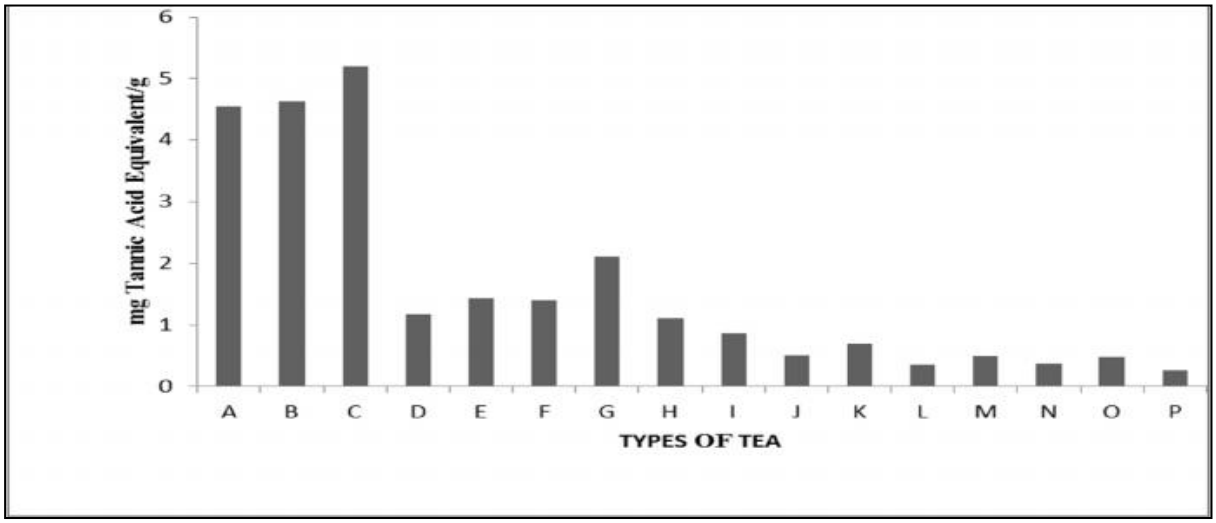

Fig.1 Total Phenolic Content of Green Tea (A to F) & black tea (G to P)

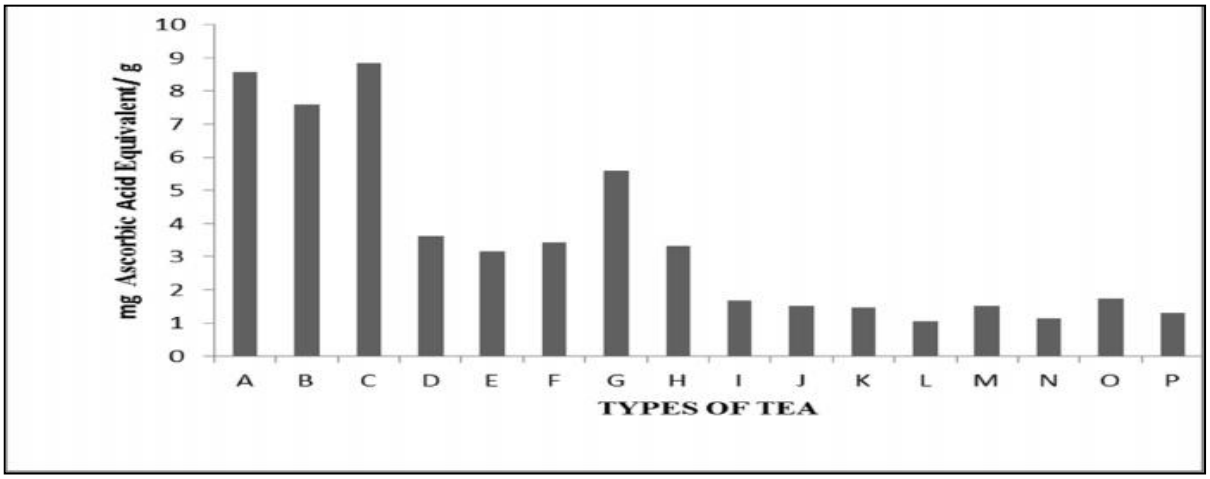

Fig.2 Total Antioxidant power of Green Tea (A to F) & black tea (G to P) FRAP method

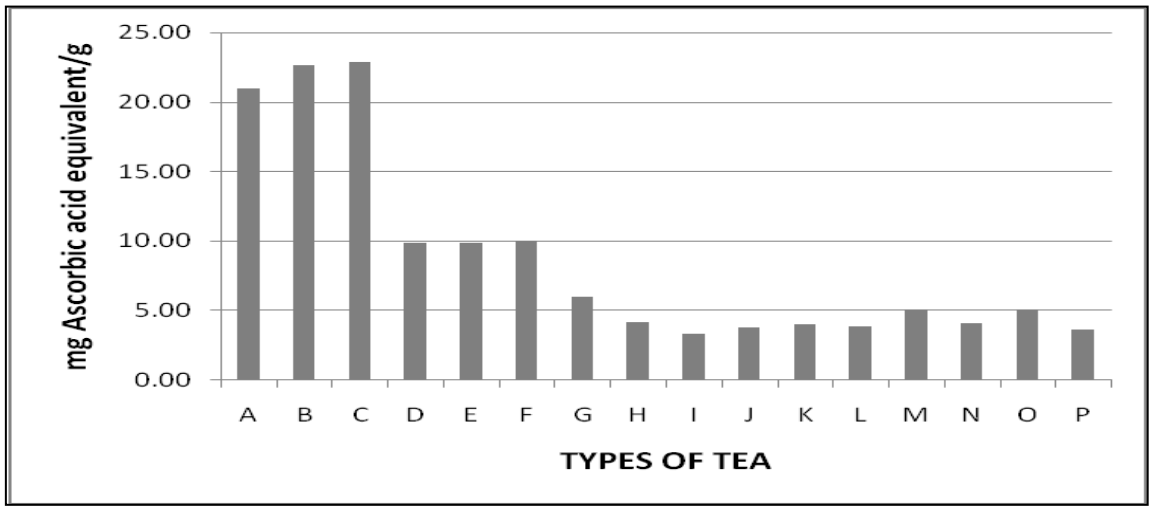

Fig.3 Total Antioxidant Capacity of Green Tea (A to F) & black tea (G to P)

The content of EGCG and EGC in green tea is much higher than in black tea (Almajano *et al.,* 2008). In case of black tea, the antioxidant properties have been attributed to its chemical components of thearubigins, phenolic acids, catechins, and theaflavins. Theaflavins which impart color, brightness, and astringency to black tea infusion also possess potent antioxidant properties (Shivaki *et al.,* 1994; Miller *et al.,* 1996). The total antioxidant capacity showed a strong correlation with TPC (and r_2 = 0.9287) as reported earlier by Dutta *et al.,* (2013) implying that polyphenol possesses antioxidant property that protects against oxidative stress. The evaluation of antioxidant activity and the total polyphenol contents of the different brands of black tea and green tea available in Indian market. The antioxidant activity in Indian tea showed a strong correlation with TPC. It can be concluded that Indian tea especially green tea may act as a natural antioxidant substitute. However, furthermore studies on Indian green tea have to be done to make it a popular beverage among Indians.

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Conflict of Interest Disclosure

"Hereby we are declaring that there is no conflict of interest regarding the publication of this paper."

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