# A Comparative Study of the Phenolic Content and In-vitro Antioxidant Activity in Different Varieties of Tea

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## ABSTRACT

Tea is the most commonly consumed beverage. The annual utilisation of tea raises upto 6.3 billion kilograms globally and 1.2 billion kilograms in India. There are different varieties of tea such as most commonly recognised varieties *Camellia sinensis var. sinensis* and *Camellia sinensis var. assamica*.

Tea has medical applications in prevention and treatment of cardiovascular, neurodegenerative diseases, cancer and delayed ageing as it is rich in certain phenolic compounds such asflavan-3-ols, flavonols and glycosides (kaempferol, quercetin, myricitin), phenolic acids , tannins, theaflavins and thearubigins along with certain alkaloids (caffeine, theophylline, and theobromine), the principle compound being Catechins.

Tea is classified on the basis of the oxidation of polyphenols and other processing conditions. In this paper, the amount of polyphenols and anti-oxidant activity has been determined and compared in different varieties in samples of tea used commercially by determining the scavenging activity of DPPH (2,2-Diphenyl-1-picrylhydrazyl) which is a stable free radical that can be used to measure the radical scavenging activity of antioxidants, on tea samples.


## 1. Introduction

Tea is one of the oldest and most popular beverages in the world. Its usage dates back to the ancient times as a medicine to cure various illnesses. Later it gained popularity as a beverage when it was first introduced in Europe in 1559 and was later commercialised by 1657 (Wickremasinghe, 1978). With commercialisation, the trade of tea became an economic mainstay and expanded beyond China to other tropical and sub- tropical regions. Today major exporters of tea include China, India, Kenya, Sri Lanka and Argentina. The beverage enjoys an annual consumption of 6.3 billion kilograms globally in various forms of preparations. India contributes a major fraction to the global tea drinking population with a consumption volume of 1.2 billion kilograms while also being its second largest producer in the world, after China. The cultivation of tea in India is heterogenous and is grown in different regions such as Tamil Nadu, West Bengal and Assam. The most commonly recognised varieties hail from China (*Camellia sinensis var. sinensis)* and from the region of Assam in North-Eastern India (*Camellia sinensis var. assamica.* The tea bushes are periodically pruned and new buds are harvested manually or mechanically, and are then subjected to a variety of treatment steps which determine the type of tea produced. A traditional *Camellia sinensis* (tea) infusion is characterised by the presence of alkaloids (caffeine, theophylline, and theobromine), and several polyphenols such as flavan-3- ols, flavonols and glycosides (kaempferol, quercetin, myricitin), phenolic acids , tannins, theaflavins and thearubigins ((Zhang et al., 2019). Principal among these are monomeric flavonols called catechins such as (-)-epicatechin (EC)[2], (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) (Graham, 1992). Catechins constitute about up to 36% of the total dry leaf weight of which 59% is EGCG and appears to be the most bioactive polyphenol with the highest amount of oxidative resistance. (Khan & Mukhtar, 2007; Lorenz, 2013). There are numerous classifications of tea that can be non-fermented, partially fermented or fully fermented. This is done on the bases of their processing conditions like degree of oxidation of polyphenols, temperature and humidity (Zhang et al., 2019). The leaves contain the polyphenol oxidase enzyme which comes in contact with tea catechins upon rolling and maceration of the leaves and oxidises them to form theaflavin and thearubigins. Black tea is manufactured by the complete enzymatic fermentation of rolled tea leaves and hence contains fewer catechins in contrast to green tea. The polyphenols in green tea are hardly oxidised due to the inactivation of polyphenol oxidase through steam and drying. White tea is prepared from dried unopened tea buds keeping rolling

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and crushing to a minimum, whereas oolong tea is allowed to undergo partial fermentation by limiting the degree of oxidation(Truong & Jeong, 2021). Black teas are fully fermented to allow oxidation of catechins into oxidation products. Due to production differences, there is a variation in their tastes ranging from plain, mellow tastes to brisk, astringent flavour.

## 2. Significance of Tea Polyphenols in Health and Disease

The popularity of tea is attributed to a number of epidemiological studies that link tea consumption to health benefits. This is mostly due to two main groups- the xanthine bases like caffeine and theophylline and tea catechins. (Del Rio et al., 2013) Caffeine is largely known for its stimulatory potential while catechins are advocated to be helpful in the prevention and treatment of cardiovascular, neurodegenerative diseases, cancer and delayed ageing (Yan et al., 2020). The well-known anti-oxidant activity of tea catechins is due to their ability to scavenge free-radicals such as superoxide radical, hydroxyl radical and hydrogen peroxide. Although necessary for signalling pathways, an imbalance in the accumulation of ROS and endogenous antioxidant

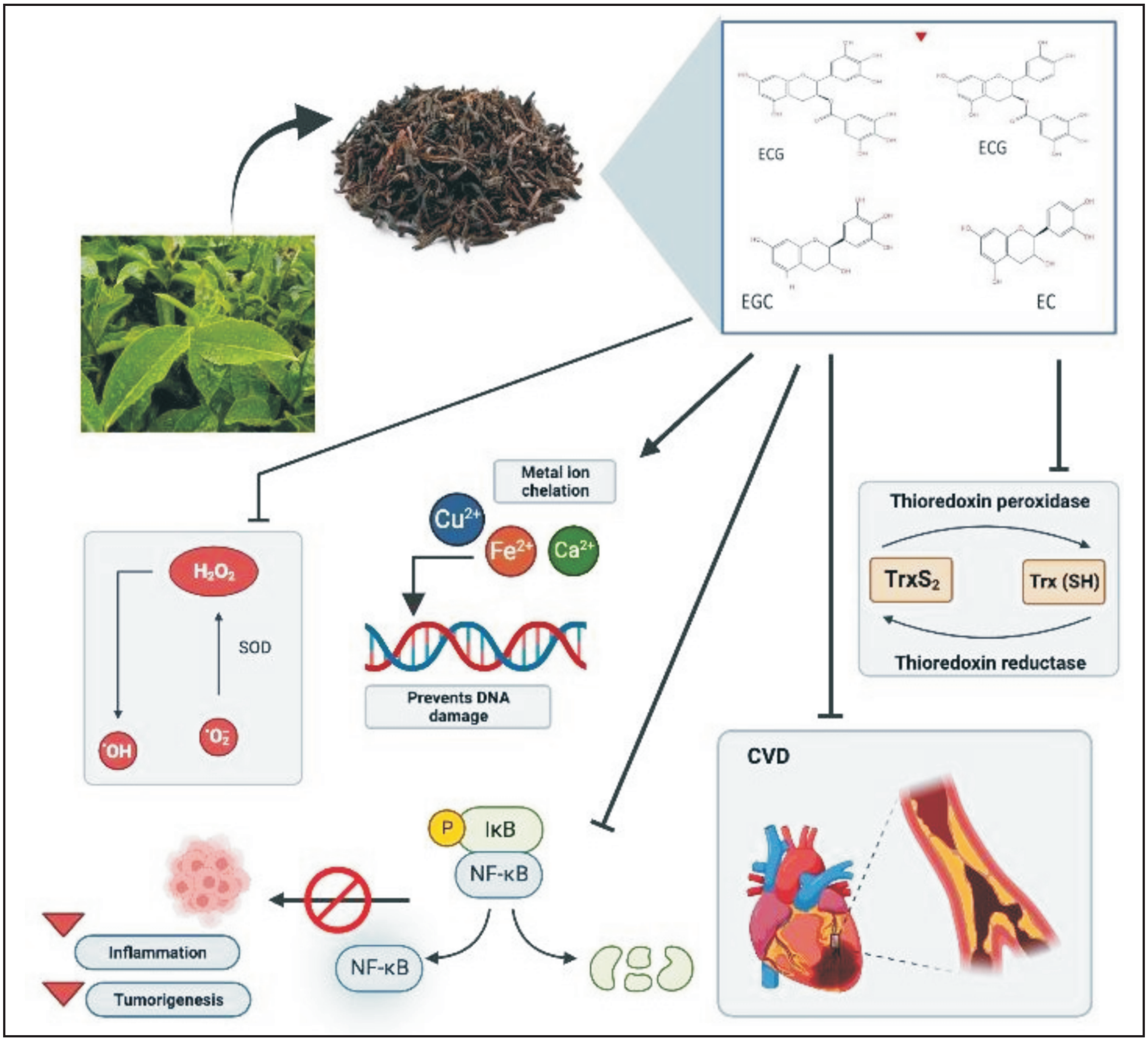


***Figure1:*** *Multifarious benefits of tea polyphenols in the biological system. Tea polyphenols such as ECG, EGC, EGCG, EC show anti-oxidant behaviour by redox-active metal ion chelation to protect against oxidative DNA damage, impede pro-oxidant enzymes such as xanthine oxidase, cyclooxygenase; inactive NF-KB pathway to reduce tumorigenesis and inflammation; increase the oxidation of thioredoxin and glutathione.*

pathways results in oxidative stress that is largely implicated in atherosclerosis and cancer(Du et al., 2012; Hayashi & Iguchi, 2010) They can directly act on ROS by forming stable phenolic oxygen radicals (Nakagawa & Yokozawa, 2002) or indirectly, by increasing the levels of antioxidant enzymes like SOD, GSH-Px and impeding pro-oxidant enzymes like xanthine oxidase via Ca2+ chelation, and NAPDH oxidase. (Guo et al., 2005; Yang et al., 2011a) This has been exemplified by In vivo experiments wherein tea polyphenols were found to be protective against hepatic injury and bacterial infections acting via anti- inflammatory antioxidant, antiapoptotic mechanisms in azathioprine induced rats and *S. typhimurium* infected mice. (El- Beshbishy et al., 2011; Zhang et al., 2019)) by increasing rat serum catalase, SOD, GSH-Px levels (Ahmed et al., 2017; Negishi et al., 2004). Besides this, catechin esters have been reported to be more effective antioxidants than vitamin C (Rice-Evans, 1999). Another study demonstrated that the antioxidant effect of tea polyphenols was not only better than that of vitamin E or Vitamin C but also showed enhanced efficacy in synergy with vitamin E and vitamin C. (Dai et al., 2008)

Catechins also exhibit positive cardiovascular effects which is attributed to its ability to increase endothelial nitric oxide synthase activity(Hertog et al., 1993). In chronic hyperglycaemia, increased glucose metabolism is conducive to glucose oxidation and oxidative stress that reduces the compensative efficiency of endogenous antioxidative mechanisms. Under such conditions, I-κB is rapidly phosphorylated and this releases NF-kB into the nucleus. NF-κB is a transcription factor that regulates the expression of anti- apoptotic genes and activates proinflammatory cytokines. (Iliopoulos et al., 2009)Hence its constant activation will exacerbate inflammation. The inhibition of NF-KB by tea polyphenol was evaluated and it was found that they could induce a decrease in NF-κB pathway, and EGCG had the best inhibitory effect which could improve the body's antioxidant capacity (Yang et al., 2011b) Activation of NF-KB negatively regulates the Nrf2 pathway responsible for redox homeostasis via the interaction of P65 protein with Keap1 inhibitory protein. (Jaramillo & Zhang, 2013) A pre-treatment of tea polyphenol in rats was found to reduce P65 levels which in turn inhibits the activation of NF-κB (Li et al., 2014).

Studies on epigallocatechin gallate in particular have demonstrated its anti-tumour activity via the downregulation of LPS-induced NF- κB activity and also selectively induced apoptosis in cancer cell lines.(Chen et, al 2001) . In vitro studies show that tea polyphenols also have the ability to chelate redox-active transition metal ions, such as iron and copper(Perron & Brumaghim, 2009) These form covalent complexes with DNA bases and react with $H_2O_2$ under oxidative stress to form $OH^-$ free radicals that can result in the DNA strand cleavage or formation of damaged bases such as 8-oxo-guanine (Henle & Linn, 1997; Imlay & Linn, 1988) to cause cell death. Studies on green tea polyphenols (Anghileri & Thouvenot, 2000) have proved to be protective against iron-induced lipid peroxidation in mouse liver tissue suspensions. Furthermore, polyphenols also aid in the prevention of neurodegenerative diseases like Alzheimer's (Markesbery & Lovell, 2006).

Green tea catechins were especially reported to be protective against iron-induced neurodegeneration in mice as measured by their down-regulation of the amyloid precursor protein (S. Mandel et al., n.d.; S. A. Mandel et al., 2005).

While existent, endogenous antioxidant mechanisms are not always sufficient in the counteraction of ROS production. Hence diet-derived antioxidant are pertinent in protection against oxidative. There are a large variety of tea available in India. Many teas are marketed with the addition of various herbs and additives for better health effects and flavour. In the present study, different commercially available tea samples were studied for a comparative assessment of their anti-oxidant activity and polyphenol content. The effect of additives like ginger, cardamom on the free radical scavenging activity, if any, was also studied and compared.

## 3. Materials and Methods

### 3.1 Tea Samples

A total of 20 commercial samples of CTC (curl, tear, crush) and orthodox green and black were were procured. CTC Green tea leaves with additives were also procured. For the determination of total phenolic content (TPC), Folin phenol (SRL Chem), gallic acid (SRL Chem), and anhydrous sodium carbonate (99% purity) were used. 1,1-diphenyl-2-picrylhydrazyl (DPPH; SRL Chem) was used for the determination of the free-radical scavenging activity.

### 3.2 Total Polyphenol Content

All decoctions were freshly prepared for the assay at a stock concentration of 10 mg/mL by boiling 100 mg of *Camellia sinensis L.* leaves in 10 ml distilled water, followed by filtration with layers of filter paper and diluted to 20x working concentration. 1 ml each of (10x) Folin-Ciocalteu reagent and 10% (w/v) sodium carbonate solution were added to each filtrate and incubated for 2 hours in the dark. Absorbance was spectrophotometrically measured at 760 nm against an appropriate blank. All samples were prepared in triplicates. A five-point 2-fold dilution standard curve of gallic acid was prepared and TPC was calculated as mg GAE/ 100 mg of tea sample.

### 3.3 DPPH Assay

A decoction was prepared by boiling 100 mg of Camellia sinensis L. leaves in water, filtered and diluted (20x). A 20x

DPPH stock solution was obtained and then stored at -20℃ till further use. In the dark 0.5 mL tea filtrate was mixed with 0.5 mL prepared methanolic DPPH solution. The samples were incubated and then their absorbance was recorded at 516 nm spectrophotometrically. The scavenging activity was determined based on the following equation :

$$\% \textbf{ DPPH scavenging activity} = \frac{A(Blank) - A(Sample)}{A(Blank)} \times 100$$

## 4. Statistical analysis

The results obtained for Total Polyphenol Content and DPPH scavenging activity in CTC and orthodox, green and black tea samples were tested for significant differences using two-way student's T-test on Microsoft Excel. Data obtained by experimental results were expressed as the means of three independent experiments.

## 5. Results and Discussion

***Table 1:*** *Total Polyphenol Content (mg GAE/100 g tea) and %DPPH Scavenging Activity of green/black tea preparations.*

| Sample | | | Total Polyphenol Content (mg GAE/100 g tea) | % DPPH scavenging activity |
|---|---|---|---|---|
| GreenTea | CTC | 1 | 57.93 | 70.35 |
| | | 2 | 72.85 | 77.28 |
| | | 3 | 106.54 | 74.24 |
| | | 4 | 76.00 | 71.36 |
| | Orthodox | 1 | 36.73 | 85.01 |
| | | 2 | 76.49 | 87.57 |
| BlackTea | CTC | 1 | 51.42 | 69.23 |
| | | 2 | 52.74 | 75.31 |
| | | 3 | 56.15 | 36.64 |
| | | 4 | 64.02 | 75.73 |
| | Orthodox | 1 | 36.34 | 71.36 |
| | | 2 | 70.10 | 78.24 |

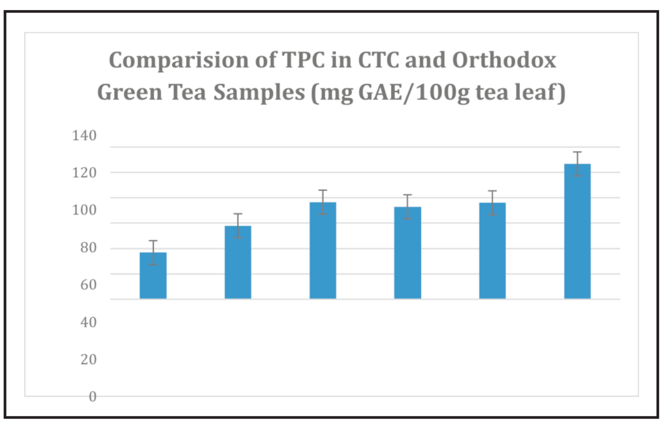


***Figure 2:*** *Shows the total polyphenol content in different samples of green tea prepared by CTC & Orthodox techniques.*

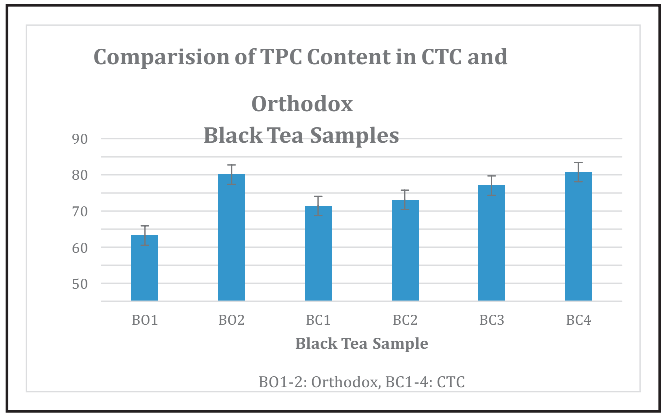


***Figure 3:*** *Shows the total polyphenol content in different samples of black tea prepared by CTC & Orthodox techniques.*

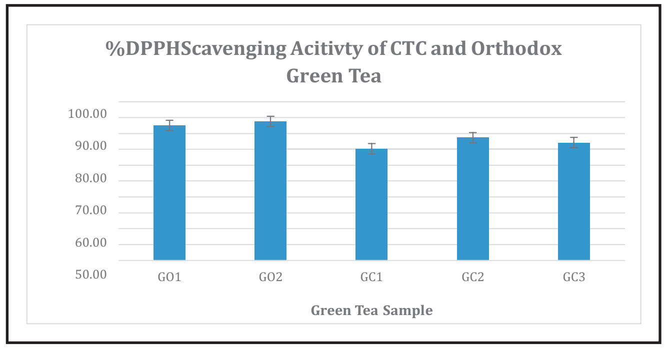


***Figure 4:*** *Shows % DPPH scavenging activity in different samples of green tea prepared by CTC & Orthodox techniques.*

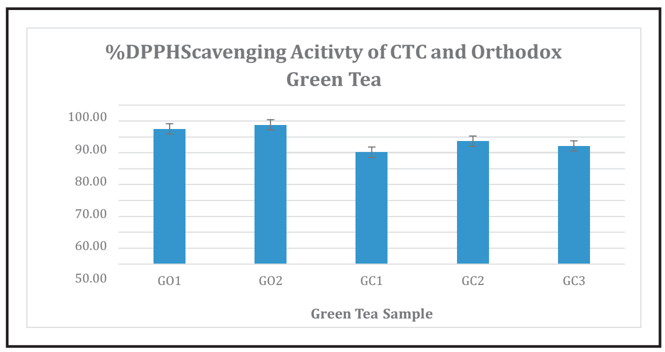


***Figure 5:*** *Shows % DPPH scavenging activity in different samples of black tea prepared by CTC & Orthodox techniques.*

## 6. Discussion

The estimation of Total Polyphenol Content of black and green tea showed that showed significant variation in the phenol content among CTC preparations of green and black tea ($p<0.1$) while no such difference was seen in the orthodox preparations. It was observed that there was a highly significant difference in the % DPPH scavenging activity among orthodox and CTC green tea samples ($p<0.001$) whereas no significant difference was observed in the % DPPH scavenging activity of CTC and Orthodox black tea samples ($p>0.1$). Furthermore, it was also found that the % DPPH scavenging activity in the CTC preparations of green and black tea, and orthodox preparations of green and black also showed no significant difference ($p>0.1$). Hence from the results it can be understood that although there might not be a variation in the overall phenolic content in the tea samples, however there can be variations among the constituent polyphenols. As previous research suggests that polyphenols display a wide range of oxidation resistance among themselves i.e. EGCG ≈ ECG > EGC > GA > EC with Epigallocatechin gallate (EGCG) being the most potent, it is plausible that some samples are abundant in highly scavenging polyphenols than others. (Ravindranath et al., n.d.; Rice- Evans, 1999) This is due to the difference in the number and position of the hydroxyl groups of the polyphenols. (Namal Senanayake, 2013) This is justified by the highly significant variance ($p=0.004$) in the % DPPH scavenging activity among preparations of green tea. Furthermore, it was observed that both DPPH scavenging activity and TPC content was the greatest in freshly procured samples than those in tea bags. In this context, a previous temporal assessment of tea anti-oxidant activity observed that initial activity was maintained for 90-120 days post which rapid declination by 60-75% was seen. Furthermore, the $Fe^{2+}$ ion chelating activity of both black and green tea decreased after only 30 days. Similar experiments showed that green tea bags lost 30% of their EGCG and 50% of ECG content in a span of six months when kept in the dark. (Friedman et al., 2009) In black tea, too, antioxidants like theaflavins decreased by 37% in storage. (Hazra et al., 2020)Thus shelf life and processing could be a potential factor in the non-Significant variation in the antioxidant capacity of the tea samples used in the study that could have led to a decline in the amount of polyphenols.

## 7. Conclusion

There are many purported benefits of tea that include delayed ageing, a reduced risk of cancer, improved lipid profile and better cardiometabolic health. For this, various preparations of tea are commercially available to the end-user. However right from the moment of harvesting till brewing, there are multiple numbers of steps that give rise to varied tastes, aroma and degrees of anti-oxidant activities. This is because the contents of polyphenols vary in different types of tea depending on the manufacturing procedure. Green tea, because of being enzyme-inactivated, undergoes the least amount of fermentation and hence retains most polyphenols. Also, orthodox preparations where the leaf's structural integrity is maintained is more antioxidative potent than CTC preparations which was demonstrated by this study. Besides manufacturing procedures, storage conditions and shelf life are also important determiners. In conclusion, it was seen that upon comparing different commercial samples of tea, freshly procure green tea leaves prepared by the orthodox technique showed high antioxidant and polyphenol content than others.

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