# COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITY OF FOUR POPULAR GREEN TEA BRANDS AVAILABLE IN INDIA

AUTHOR DETAILS
Daniel Getachew Tikuye
School of Pharmacy, Sharda University, Greater Noida, India
E-mail: danielgt143@gmail.com

**ABSTRACT**

The study was designed compare the antioxidant potential of the various brands of green tea available in India. The main objective of this study is to compare the hot water extracts antioxidant properties at different concentration of four green tea brands. Four commercial green tea brands (Lipton, Typhoo, Tetley, Tulsi) were extracted in hot water (90-100$^{o}$C). UV-vis Spectrophotometer is used to measure the absorbance and determine total polyphenol concentration (TPC) by Folin-Ciocallteu's method. Percentage inhibition of DPPH radical, Nitric Oxide, Superoxide anion radical scavenging activities and reducing power capacity were calculated from the absorbance measurement by UV-Vis Spectrophotometer in 50-250µg/ml concentrations for each brand. The TPC of the four green tea brands in decreasing order are Tetley (708.2±2077) > Lipton (572.13±3.3) > Typhoo (430±2.55) > Tulsi (390.73±2.44) µg GAE/gm per sample. All samples showed an increase in antioxidant activity with increasing of extract concentrations.

**Keywords:** *Green tea, Antioxidant scavenging activity, Radicals, Hot water extraction, Polyphenols.*

## I. INTRODUCTION

Since the accidental discovery of tea by a Chinese emperor, Shen Nung, in 2737 BC, while boiling water and some leaves and fell into it producing an apparent aroma and taste, it is the nature's wealth to human in the world and is the second most consumed drink next to water [1]. Tea is prepared by steeping fermented leaves, twigs and buds of a plant, Camellia sinensis in water or other solvents.[2] Tea can be black (78%), green (20%) or oolong tea (2%) which varies in aroma, color, name and flavor depending upon how the tea leaves are manufactured [3,4]. Black tea and oolong tea are produced by full fermentation and semi-fermentation respectively and have less antioxidant potential than green tea which is produced by steaming fresh leaves to prevent catechin oxidation by polyphenol oxidase. With no fermentation green tea leaves preserve their colors and almost all of their catechin content and therefore have a greater antioxidant potential than black tea and oolong tea [5,6].

Green tea is rich in the flavonol group of polyphenol called catechin, which act as antioxidant by sequestering metal ions and by scavenging free radicals that can damage DNA, proteins and lipids thereby contribute to cancer, metabolic dysfunctions, coagulations and atherosclerosis [7,8].

The antioxidant properties of green tea and its components catechins were detected in many diseases associated with reactive oxygen species (ROS) such as cancer and neurodegenerative diseases. Many epidemiological studies have been conducted to show that green tea can provide protection against several cancers such as breast cancer, prostate, bladder, ovarian, colorectal, esophageal, lung, pancreatic and skin cancers [5].

The free radicals may be oxygen derived, reactive oxygen species (ROS) which includes superoxide anion ($O_2$), hydrogen peroxide ($H_2O_2$), peroxyl radicals (ROO) and hydroxyl radicals (OH); or nitrogen derived free radicals, reactive nitrogen species (RNS), including nitric oxide (NO), peroxyl nitrite anion (ONOO), nitrogen dioxide ($NO_2$) and dinitrogen trioxide ($N_2O_3$) [13]. To combat this, now a days people throughout the world use antioxidant supplements or natural foods and drinks like green tea which are rich in antioxidant activity. Hence, the present study was designed to compare the various brands of green tea in the Indian market.

## II. MATERIAL AND METHODS

### *A. Chemicals*

Chemicals used were as follows: Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl, L-Ascorbic acid, and phosphate buffered, Trichloroacetic Acid, Nitro Blue Tetrazolium Chloride, β-Nicotinamide Adenine Dinucleotide hydrogen (β-NADH), Phenazine Methosulphate, Gallic acid, Griess's reagent, Potassium ferric cyanide, Ferric Chloride Anhydrous, Sodium Nitroprusside, Sodium Carbonate Anhydrous, and Methanol. most of these chemicals and reagents were purchased from Sisco research laboratories Pvt. Ltd

### *B. Sample Preparation*

Hot tea extracts were prepared by adding 200 mL of water at hot temperature (90 -100°C) to a teabag that was previously weighed (net weight of 10 gm); the infusions remained at 90 -100°C temperature for 5 minutes and were agitated manually every minute for 15 seconds, and the temperature were controlled every 30 seconds for each sample. Initially all samples were diluted at 1000 ppm (=1000µg/ml) in distilled water as stock solution and stored at 4°C for subsequent analysis. From 1000µg/ml concentration 0.5ml, 1ml, 1.5ml, 2ml and 2.5ml were taken and added to 10 ml volumetric flasks and then filled to the mark with distilled water to prepare 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml concentrations respectively.

***1: Total Polyphenol Content (TPC) Determination***

The total polyphenol content of the water extracts was determined by the Folin-Ciocallteu method [11]. Folin-Ciocallteu phenol reagent were prepared (10%). To prepare standard Gallic acid initially 100mg of Gallic acid was weighed and dissolved in 100ml of distilled water which is equivalent to 1000µg/ml. then to prepare 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml concentration 0.5ml, 1ml, 1.5ml, 2ml and 2.5ml respectively were taken and added to 10 ml volumetric flasks and then filled with distilled water.

Total Polyphenol content were quantified using calibration curve equation of Gallic acid, [$y = 0.0122x - 0.061$, $R^2 = 0.9903$], (Figure 4.2) and were expressed as µg Gallic acid equivalent/gm per sample (µg GAE/gm per sample). The tests were performed in triplicate and the results are expressed as the mean ± standard deviation.

The TPC in all samples was calculated using the formula: [C = c V/m], where, C = total phenolic content mg GAE/g dry extract, c = concentration of Gallic acid obtained from calibration curve in µg/mL, V = volume of extract in ml, m = mass of extract in gram [12].

***2: DPPH Radical Scavenging Activity***

The free radical scavenging activity of the extracts was examined in vitro using DPPH radical as described by Shimada K. *et al* [13]. DPPH solution (0.1mM in methanol) were prepared first by weighing accurately 39.432 mg and dissolving it in 100ml methanol. Then 10ml was taken and added to 100ml volumetric flask and filled with methanol to the mark. Then it was stored in the dark by covering with aluminum foil. Negative control solution was prepared by adding extracting solvent (distilled water) instead of sample extracts.

Standard Ascorbic acid solution was prepared by dissolving 500mg of Ascorbic acid in 500ml distilled water and 0.5ml, 1ml, 1.5ml, 2ml and 2.5ml volumes were add to 10ml volumetric flask and filled to the mark by distilled water to prepare 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml respectively.

Absorbance was measured at 517 nm (Figure 3.4). L-ascorbic acid and DPPH solution with distilled water without extracts were used as a positive control and negative control respectively. The percentage DPPH radical scavenging activities were calculated by comparing the absorbance values of control and samples and calculated using the following formula: [%DPPH scavenging activity = ($A^o_{con}$ - $A^o_{sam}$/ $A^o_{con}$) x100%], where $A^o_{sam}$ = absorbance of test sample and $A^o_{con}$ = absorbance of control. The results are expressed as the mean ± standard deviation.

***3: Nitric Oxide Radical Scavenging Activity***

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes. Nitric Oxide (NO) and reactive nitrogen species (RNS) are free radicals that are derived from the interaction of NO with oxygen or reactive oxygen species.[101] Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals. This forms the basis of this experiment. Sodium nitroprusside solution at physiological pH generates Nitric oxide (NO) radicals.

Percentage Nitric Oxide scavenging activities of each brands in different concentration were calculated by using the formula: [%Nitric Oxide scavenging activity = ($A^o_{con}$ - $A^o_{sam}$/ $A^o_{con}$) x100%], where $A^o_{sam}$ = absorbance of test sample and $A^o_{con}$ = absorbance of control. This process was repeated in triplicate and mean ± standard deviation was taken to compare among different concentrations and $IC_{50}$ values were used to compare different brands.

***4: Reducing Power Capacity***

The reducing power were determined according to the method of Oyaizu [15]. The aim of this assay is to measure the reduction of ferric (Fe3) to ferrous (Fe2) ion.[16] The absorbance (OD) were measured at 700nm (Figure 3.6). These processes were repeated in triplicate and mean ± standard deviation were taken to compare among different concentrations and $IC_{50}$ values were used to compare different brands. Increased absorbance of the reaction mixture indicates increase in reducing power capacity. The % reducing power was calculated by using the formula: [$A^o_{sam}$- $A^o_{con}$/$A^o_{sam}$) x100%], where $A^o_{sam}$ = absorbance of test sample and $A^o_{con}$ = absorbance of control [18].

***5: Superoxide Anion Scavenging Activity***

Measurement of superoxide anion scavenging activity of extracts in different concentrations (50-250µg/ml) were performed by using the method explained by Nishimiki (Nishimiki et al., 1972) and modified by Ilhami et al.[19] The reaction mixtures were incubated at 25°C for 5 minutes, and the absorbance were measured at 560 nm against blank (Figure 3.7). The control blank

solution was prepared by adding all solutions except the sample extracts. L- Ascorbic acid was used as positive control standard.

Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. Percentage Superoxide scavenging activities were calculated by using the formula: [%Superoxide scavenging activity = ($A^o_{con}$ - $A^o_{sam}$/ $A^o_{con}$) x100%], where $A^o_{sam}$ = absorbance of test sample and $A^o_{con}$ = absorbance of control. This process was repeated in triplicate and mean ± standard deviation was taken to compare among different concentrations and $IC_{50}$ values were used to compare different brands.

### *C. Statistical Analysis*

Data are expressed as the mean ± standard deviation of the mean of five independent experiments performed in triplicate. Multivariate ANOVA's Tukey multiple comparison test was used to compare the variance in the dependent variables of four different green tea brands, and also amongst five different extract concentrations. The statistical hypotheses used are Ho: μ1 = μ2 = μ3 = μ4....μn, which refer to the regression variable response of each of the four brands in five different concentrations for each analytical test. The Alternative hypothesis is: H1: At least one mean is different.

The statistical significance was based on the total error criteria with a confidence level of 95.0%, for this reason P-values less than 0.05 were considered statistically significant. The data obtained from the study were entered in to MS excel 2016 spread sheet and statistically analyzed using 'SPSS version 21' software to determine the mean value, standard deviation and analysis of variance ANOVA's Tukey test (significant $p < 0.05$). The figures were made using MS excel 2016 program.

## III. RESULTS

TABLE 3.1: MEAN ±SD SUMMARY OF TPC(μG/GM GAE), %DPPH RADICAL SCAVENGING ACTIVITIES, %NITRIC OXIDE RADICAL SCAVENGING ACTIVITIES, %SUPEROXIDE RADICAL SCAVENGING, %REDUCING POWER CAPACITIES OF FOUR DIFFERENT GREEN TEA BRAND IN DIFFERENT CONCENTRATION (50-250(μG/ML)

**TABLE 3.1**

| **Brands** | **Concentration (μg/ml)** | **Lipton** | **Typhoo** | **Tetley** | **Organic India Tulsi** | **p-value** |
|---|---|---|---|---|---|---|
| TPC(μg/gm GAE) | 150 | 572.13±3.30 | 430±2.55 | 708.2±2.77 | 390.73±2.44 | 0.000 |
| DPPH scavenging(%) | 50 | 16.44±0.39 | 11.94±1.03 | 14.41±0.78 | 7.88±0.39 | 0.000 |
| | 100 | 24.77±1.03 | 22.75±1.03 | 29.28±1.03 | 22.07±1.03 | |
| | 150 | 34.68±0.39 | 34.23±0.78 | 40.31±1.03 | 29.28±0.78 | |
| | 200 | 46.87±0.80 | 45.24±0.68 | 50.45±0.39 | 44.59±1.35 | |
| | 250 | 62.61±0.39 | 59.23±0.78 | 66.88±0.68 | 57.43±0.68 | |
| | IC50 (μg/ml) | 206.46 | 215.48 | 188.61 | 223.01 | |
| Nitric oxide scavenging (%) | 50 | 7.88±0.17 | 5.52±0.39 | 7.46±0.34 | 4.14±0.30 | 0.000 |
| | 100 | 13.29±0.23 | 11.87±0.22 | 12.84±0.23 | 11.16±0.28 | |
| | 150 | 29.90±0.34 | 28.22±0.34 | 29.19±0.28 | 24.71±0.47 | |
| | 200 | 43.37±0.47 | 41.77±0.34 | 42.40±0.17 | 40.13±0.47 | |
| | 250 | 56.59±0.39 | 54.16±0.28 | 55.43±0.22 | 49.76±0.28 | |
| | IC50 (μg/ml) | 227.62 | 235.24 | 231.83 | 249.93 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Superoxide scavenging (%) | 50 | 6.70±0.10 | 6.0±0.06 | 6.48±0.13 | 5.97±0.88 | 0.000 |
| | 100 | 19.30±0.11 | 22.20±0.06 | 20.24±0.13 | 17.99±0.15 | |
| | 150 | 41.25±0.09 | 38.54±0.08 | 40.96±0.06 | 32.93±0.10 | |
| | 200 | 62.17±0.08 | 53.58±0.10 | 61.51±0.08 | 53.19±0.13 | |
| | 250 | 73.95±0.08 | 67.93±0.13 | 73.41±0.13 | 67.66±0.08 | |
| | IC50 (µg/ml) | 176.31 | 189.76 | 177..05 | 195.54 | |
| Reducing power (%) | 50 | 20.08±0.78 | 25.73±0.67 | 26.74±0.73 | 8.25±0.38 | 0.000 |
| | 100 | 32.98±0.36 | 33.56±0.81 | 31.77±0.42 | 19.06±0.3 | |
| | 150 | 38.13±0.17 | 41.75±0.41 | 43.07±0.39 | 28.0±0.41 | |
| | 200 | 58.73±0.08 | 47.28±0.22 | 48.71±0.24 | 37.73±0.18 | |
| | 250 | 63.3±0.16 | 53.79±0.09 | 56.25±0.15 | 48.78±0.21 | |
| | IC50 (µg/ml) | 182.77 | 218.54 | 207.24 | 258.42 | |

### *A. Total Polyphenol Content (TPC) Determination*

Total polyphenol concentration for hot water extracts (90-100ºC) of four different green tea brands which are quantified using calibration curve of Gallic acid in different concentrations (50µg/ml-250µg/ml) are shown in (figure 3.1). The calibration curve equation of Gallic acid is y = 0.0122x - 0.061, $R^2$ = 0.9903 (Figure 3.2).

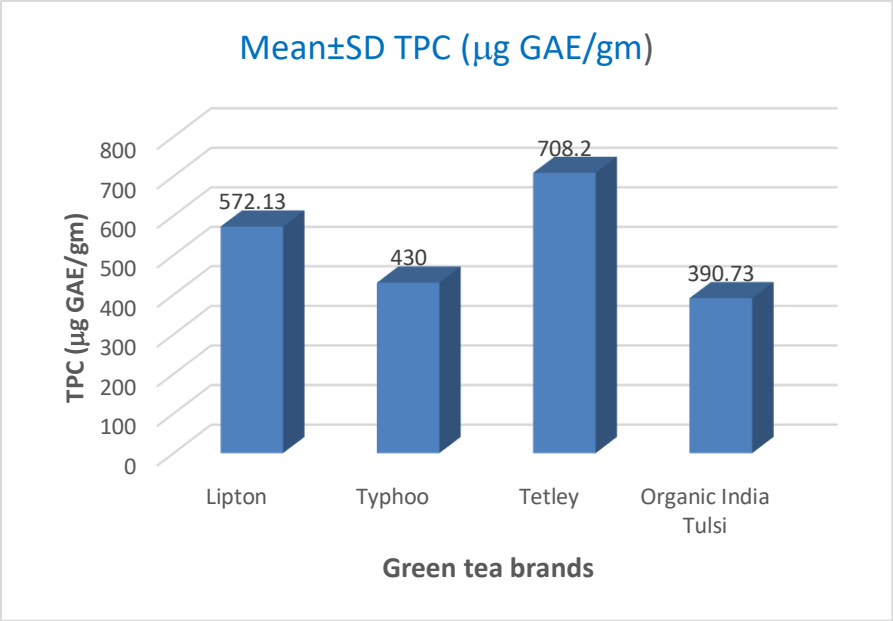


**Figure 3.1.**: Total polyphenol concentrations of the four green tea brands

The TPC of the four green tea brands (Lipton, Typhoo, Tetley, Organic India Tulsi) are 572.13±3.30, 430±2.55, 708.2±2.77, 390.73±2.44 µg GAE/gm per sample respectively. The TPC in decreasing order is Tetley > Lipton >Typhoo >Organic India Tulsi. The analysis of variance by comparison of means showed that there is significant difference in the Gallic acid equivalence of TPC of the hot water extracts of the four green tea brands (P< 0.05).

The difference in TPC can be due to maturity of the leaves during harvesting time, climate, season of harvesting, altitude(topography), and processes of harvesting and steaming.

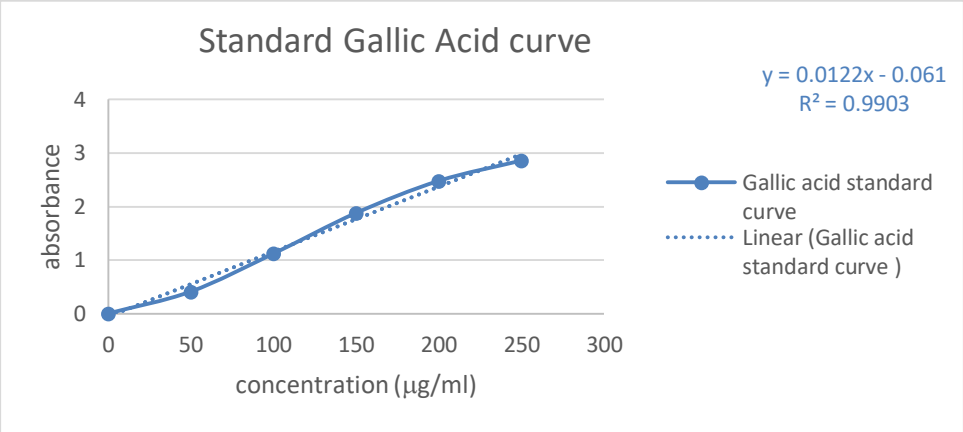


Figure 3.2: Standard curve of Gallic acid (50-250µg/ml) concentration at 760nm.

### B. *DPPH Radical Scavenging Activity*

The antioxidant capacities of four green tea brands in different concentration (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml) were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The absorbance of test green tea samples in five different concentrations for each brand were measured by using UV-visible spectrophotometer. Then percent DPPH free radical scavenging activity were calculated by the equation, [%DPPH scavenging activity = ($A^{o}_{con}$ - $A^{o}_{sam}$/ $A^{o}_{con}$) x100%].

The absorbance decreases with increasing of the concentrations of antioxidants (extracts) for each brands, which indicates the amount of DPPH free radical scavenged (reduced) increases when the amount (concentration) of the antioxidant increases. Comparison of the capacity of DPPH free radical scavenging activities were performed among different concentrations with in one brand and among different brands. Figure 3.3 shows % DPPH free radical scavenging activity curves at different concentration of the four brands and standard.

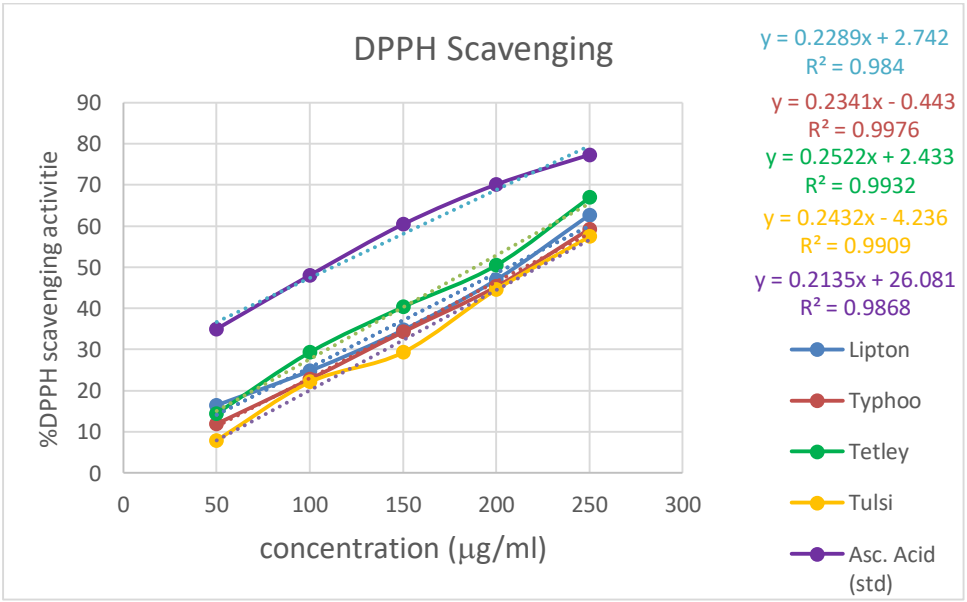


Figure 3.3: Percentage DPPH scavenging activity of the study green tea brands and their curve equations

The capacity of DPPH free radical scavenging activity in decreasing order is Tetley > Lipton > Typhoo > Organic India Tulsi. The $IC_{50}$ Organic India Tulsi is highest and Tetley is lowest, which implies Organic India Tulsi has the least efficient antioxidant and Tetley has most efficient. The analysis of variance by two-way ANOVA multivariate comparison showed that concentrations have high significant effect on percentage DPPH radical scavenging activities of all four green tea brands ($P<0.05$). In addition, brands of green tea also have a high significance effect on percentage DPPH scavenging activities ($P<0.05$).

### *C. Nitric Oxide Radical Scavenging Activities*

Nitric Oxide radical scavenging activity was performed according to Awah and Verla [30 The percentage nitric oxide free radical scavenging activities of hot water extracts of four different green tea brands in increasing concentrations are shown in (Figure 3.4). All four green tea brands exhibited an increasing in %nitrite free radical scavenging activities with increasing of concentrations ($P < 0.05$).

Comparing $IC_{50}$ values of the four green the brands give a decreasing order in their percentage Nitric Oxide radical scavenging activities as Lipton > Tetley > Typhoo > Organic India Tulsi, which indicates Lipton is most efficient and Organic India Tulsi is least efficient, but all are less effective than the standard Ascorbic acid. The analysis of variance by two-way ANOVA multivariate comparison of means showed that there are high significant differences in percentage nitrite free radical scavenging activities with different concentrations in all green tea brands ($P <0.05$). In addition, brands of green tea also have a high significance effect on percentage Nitric Oxide scavenging activities ($P<0.05$).

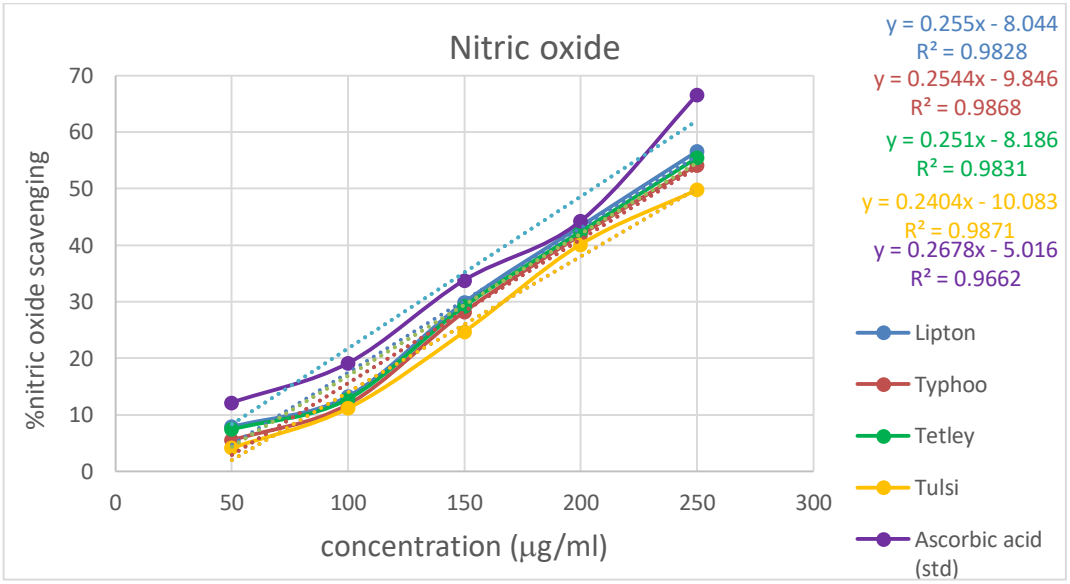


Figure 3.4: Percentage Nitric Oxide scavenging activities of all four green tea brands and standard with their curve equation.

### *D. Reducing Power Capacity*

Reducing power assay of four different green tea brands of hot water extracts were carried out in different concentrations and the reducing power of all the extracts increased with increase in concentration as shown in (Figure 3.5). The percent reducing power capacity was calculated by using the formula: $[A^{o}_{sam}- A^{o}_{con}/A^{o}_{sam}) \times 100\%]$, where $A^{o}_{sam}$ = absorbance of test sample and $A^{o}_{con}$ = absorbance of control.

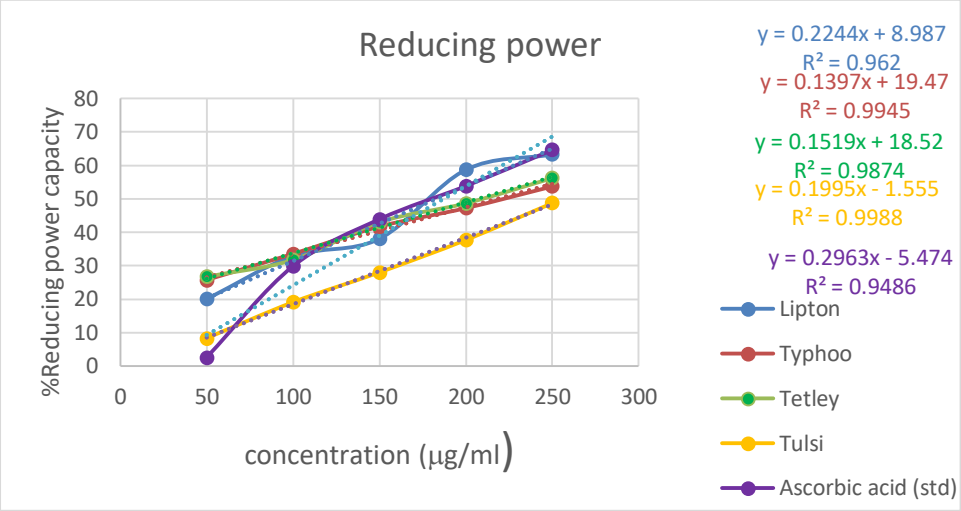


Figure 3.5: Percentage reducing power capacities of the studied green tea brands and standard with their curve equation.

By comparing $IC_{50}$ values of the studied green tea brands the reducing power capacity of Lipton green tea was highest and Organic Indian Tulsi green tea is lowest. The second and third highest were Tetley and Typhoo respectively. As compared to the standard Ascorbic acid Lipton showed better reducing power efficiency than the standard, ($P < 0.05$) but the other green tea brands studied showed lower reducing power capability than standard, ($P < 0.05$). The analysis of variance by two-way ANOVA multivariate comparison of means showed that there are high significant differences in percentage reducing power capacities with different concentrations in all green tea brands, ($P < 0.05$). In addition, brands of green tea also have a high significance effect on percentage reducing power capacities, ($P<0.05$).

### *E. Superoxide Anion Free Radical Scavenging Activity*

The percent superoxide anion radical scavenging activities of hot water extracts of four green tea brands in 50µg/ml - 250µg/ml concentrations are shown in (Figure 3.6).

Percentage Superoxide anion radical scavenging activities of these green tea brands in decreasing order are Lipton > Tetley > Typhoo > Organic India Tulsi, by comparing their $IC_{50}$ values. The analysis of variance by two-way ANOVA multivariate comparison of means showed that there are high significant differences in percentage Superoxide radical scavenging activities with different concentrations in all green tea brands ($P < 0.05$). In addition, brands of green tea also have a high significance effect on percentage Superoxide scavenging activities ($P<0.05$).

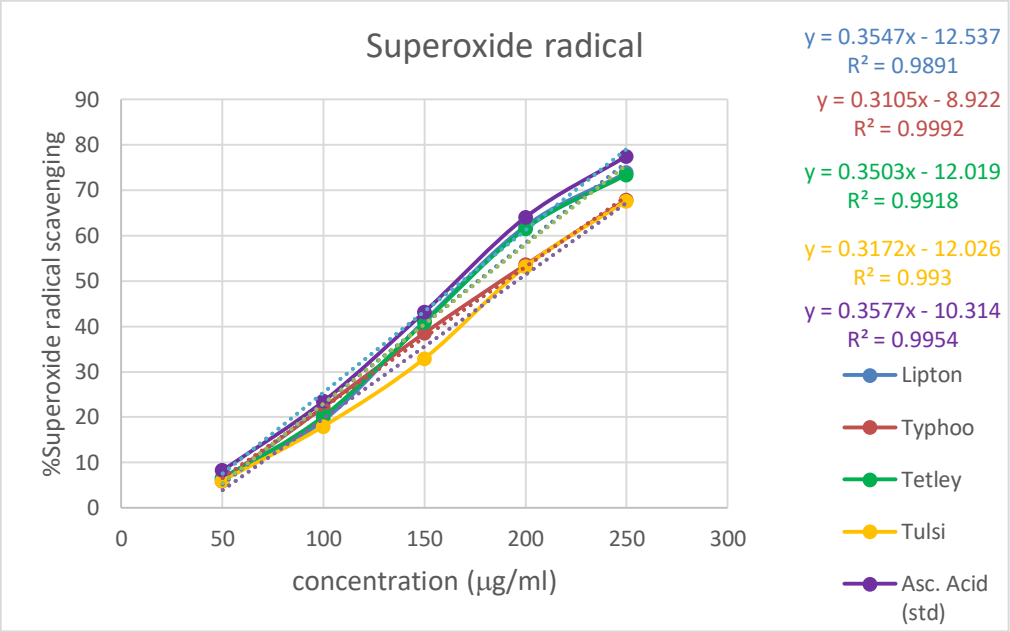


Figure 3.6: Percentage Superoxide radical scavenging activities of studied green tea brands and standard with their curve equations.

## IV. DISCUSSION

In this study four different green tea brands sold in local markets of India were compared for their total polyphenol content (TPC) and antioxidant free radical scavenging activities by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay, Nitric Oxide Radical Scavenging Assay, reducing power capacity and Superoxide anion scavenging activity. Hot water extracts (90-100$^{o}$C) was used. Each brand is compared for its antioxidant scavenging activities in five different extract concentrations (50-250µg/ml). $IC_{50}$ values are calculated from the curve equations of each brand for different assay models and used to compare TPC and every antioxidant scavenging activity assay models among different brands. The four different brands of green tea for this study are Lipton, Typhoo, Tetley and Organic India Tulsi. L-Ascorbic acid is used as standard (positive control) for antioxidant scavenging activity assays. Gallic acid is also used as a standard curve equation determination to calculate total polyphenol concentration of Gallic acid equivalent. Amongst the four brands Lipton is studied in previous studies for its antioxidant scavenging activities and TPC, but the other three brands were not studied before.

TPC obtained for hot water green tea extracts is presented as mean ± SD of µg GAE/gm per sample. The TPC in decreasing order is Tetley > Lipton > Typhoo > Organic India Tulsi with values 708.2±2.77, 572.13±3.30, 430±2.55, 390.73±2.44 µg GAE/gm per sample respectively. The analysis variance by multivariate two-way ANOVA showed there is high significant difference in Gallic acid equivalent values of the studied green tea brands ($P < 0.05$). A study by Ramírez-A. L.S. *et al* showed that there is significant difference in TPC of hot water extracts of Lipton and other three studied green tea brands,[20] but the TPC values are different with this study. The variation in TPC can be due to maturity of the leaves during harvesting time, climate, season of harvesting, altitude(topography), processes of harvesting and steaming, and the techniques used for analysis. A study conducted by Cleverdon R, *et al* indicated that steeping time has effect on the TPC of the extracts. Rate of polyphenol appearance may differ by time range of steeping.[21] Another study of antioxidant properties of green teas showed that TPC ranges from 1.17-5.58 mg TAE/gm[1]

The antioxidant scavenging activity obtained for hot water extracts (90-100$^{o}$C) of four green tea brands by DPPH assay is presented (Table 3.1). The analysis of variance by multivariate two-way ANOVA showed that there is high significant difference among various concentrations of DPPH radical scavenging activities of all brands ($P < 0.05$). This study shows the more the concentration of the extract the higher will be the percentage DPPH radical scavenging activity. DPPH possesses a proton free radical having characteristic absorption, which decreases on exposure to radical scavengers [23]. The method is widely used to predict the ability of flavonoids to transfer H atoms to radicals is based on the free radical, 1, 1-diphenyl-2-picrylhydrazyl in the DPPH assay.

DPPH radical scavenging activity among different brands were compared by its $IC_{50}$ values and it showed that Tetley > Lipton > Typhoo > Organic India Tulsi, with respective values of 188.61, 206.46, 215.48 and 223.01 μg/ml. Lower $IC_{50}$ values indicates higher antioxidant activity and higher $IC_{50}$ values indicates lower antioxidant scavenging activity [22].

This study showed that the percentage nitric oxide free radical scavenging activities of hot water extracts of all four different green tea brands increase with increasing concentration. Comparing $IC_{50}$ values of the four green the brands give a decreasing order in their percentage nitrite free radical scavenging activities as Lipton > Tetley > Typhoo > Organic India Tulsi, with respective values of 227.62μg/ml, 231.83μg/ml, 235.24μg/ml and 249.93μg/ml, which indicates Lipton is most efficient and Organic India Tulsi is least efficient, but all are less effective than the standard Ascorbic acid ($IC_{50}$ =205.44μg/ml). Lower $IC_{50}$ values indicates higher antioxidant activity and higher $IC_{50}$ values indicates lower antioxidant scavenging activity.[22] Nitric oxide radical scavenging activities of green teas were not studied before this study.

Reducing power capacity in this study is carried out for four various types of green tea brands in different concentrations (50μg/ml -250μg/ml). Different studies have indicated that the electron donation capacity, reflecting the reducing power, of bioactive compounds is associated with antioxidant activity [24]. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe3+/ ferricyanide complex to the ferrous form. Therefore, Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.[25,26] There are a number of assays designed to measure overall antioxidant activity or reducing potential, as an indication of host total capacity to withstand free radical stress [27].

By comparing $IC_{50}$ values of the studied green tea brands the reducing power capacity of Lipton green tea was highest (182.77 μg/ml) and Organic Indian Tulsi green tea is lowest (258.42μg/ml). The second and third highest were Tetley (207.24μg/ml) and Typhoo (218.54μg/ml) respectively. As compared to the standard Ascorbic acid (187.22μg/ml) Lipton showed better reducing power efficiency than the standard but the other green tea brands studied showed lower reducing power capability than standard. Lower $IC_{50}$ values indicates higher antioxidant activity and higher $IC_{50}$ values indicates lower antioxidant scavenging activity [22].

A study by Shaukath Ara Khanum *et al* indicated that the reducing power capacities of the green tea extracts increase with increasing in concentration [94]. He also presented that the reducing power capacities of five different flavor green tea brands by its $IC_{50}$ values (115.85±15 to 230±21) [22]. Safdar and colleagues by their study '*ten different brewing methods of green tea: comparative antioxidant study*' presented the reducing power capacity of different green tea brands of hot cocktail and compared the absorbance, which ranges from 3.725±0.016 to 3.789±0.07 at 700nm [2]. The percentage reducing power capacity, concentrations of the extracts and name of the green tea brands were not mentioned that makes comparison with this study impossible.

Oxygen is essential for the survival of aerobic cells, but it has long been known to be toxic to them when supplied at concentration greater than those in normal air. The biochemical mechanisms responsible for oxygen toxicity include lipid

peroxidation and the generation of $H_2O_2^+$, the superoxide radical, $O_2^-$. This superoxide radical can inhibit or propagate the process of lipid peroxidation. Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT) [28]. Assay for superoxide radical scavenging activity was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generated in NADH-NBT system [29]. Superoxide anion radical scavenging activities of hot water extracts of four green tea brands in 50µg/ml - 250µg/ml concentrations were calculated in this study. All brands exhibited an increasing percentage of superoxide anion scavenging activities with increase in concentration of extracts. The more antioxidants present the more free radicals will be scavenged or reduced.

Percentage Superoxide anion radical scavenging activities of these green tea brands by comparing their $IC_{50}$ values in decreasing order are Lipton > Tetley > Typhoo > Organic India Tulsi with $IC_{50}$ values 176.31, 177..05, 189.76 and 195.54µg/ml. Almost all brands show similar superoxide anion radical scavenging activities, but all shows lower scavenging activity than the standard L-Ascorbic acid (168.62µg/ml) in equivalent concentrations. Lower $IC_{50}$ values indicates higher antioxidant activity and higher $IC_{50}$ values indicates lower antioxidant scavenging activity [22]. Superoxide anion radical scavenging activities of green tea was conducted before this study.

## V. CONCLUSION

It is well known that hot water extraction of green tea is the most practiced and best yielding of antioxidants. The higher the antioxidant yield the higher will be the TPC, DPPH radical scavenging activity, Nitric Oxide scavenging activity, reducing power capacity and Superoxide anion radical scavenging activity. Individuals who consume green tea for its multiple benefits can be benefited by increasing the concentration of green tea per volume or by increasing daily consumption volume. Since the harvesting and preparation process, time range of steaming, harvesting stages of bud leaves of green tea, environment and season of collection, as well as geographical location where tea plant grow may vary in different green tea manufacturers. Due to this the choice of brand of green tea may have great influence in the antioxidant activity. Therefore, the daily intake amount or the amount of green tea per volume and choice of brand have great effect on antioxidant activity for beneficiaries. Further studies should be conducted on comparing all commercially available green tea brands, to determine the maximum extract concentration that gives best effect and isolation of the particular constituents responsible for the antioxidant activity.

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