Antioxidant Activity of Different Forms of Green Tea: Loose Leaf, Bagged and Matcha

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Abstract
Green tea is commercially available in three forms: loose leaf, bagged and powdered. The objective of this study was to compare the radical scavenging capacity of different forms of green tea like loose leaf (3), bagged (2) and powdered matcha (2) of various brands. The green tea forms were prepared at 95-100 °C for 5 min., to mimic conditions usually used for tea preparations at home. The comparison of combined IC\textsubscript{50} values of different green tea forms (loose leaf, bagged and matcha) showed no significant difference in their radical scavenging activity except bagged tea that exhibited slightly more DPPH radical scavenging potential as compared to matcha. Individually, the Bud white loose leaf demonstrated highest antioxidant activity followed by Laplant bag, Lipton bag, Laplant loose, Gourmet matcha, Wow matcha and Lipton loose. These findings revealed that on the basis of form, it may not be possible to generalize which form of tea whether loose leaf, bagged or matcha, is more effective in scavenging free radicals.

Introduction
Green tea is obtained from the dried leaves of tea plant *Camellia sinensis* and is one of the drinks gaining popularity throughout the world\textsuperscript{1}. It is manufactured from newly picked leaves that are exposed to heat, and then pan fried or steamed prior to rolling or shaping and drying\textsuperscript{2}. It is widely consumed, due to its refreshing taste, aroma and various other properties like antioxidant, anticarcinogenic, antimutagenic and antihypertensive\textsuperscript{3-10}. All the above mentioned properties are mainly attributed to high polyphenolic content present in the green tea\textsuperscript{11}. The catechins constitute the major part of the polyphenolic content in green tea, main catechins and its derivatives present are: (-) – epigallocatechin (EGC), (-) - epigallocatechin 3- gallate (EGCG), (-) - epicatechin gallate (ECG), (-) - epicatechin gallate (ECG), (-) - gallolatechin gallate (GCG) and (-) – epicatechin (EC)\textsuperscript{12-15}. It is reported that EGCG is one of the major and biologically effective catechin of green tea\textsuperscript{16}.
Green tea is commercially available in the market in three forms: Loose leaf, bagged and powdered (matcha). There have been few comparative studies conducted on different forms of tea and their association with antioxidant potential. It was demonstrated that antioxidant capability of different forms of tea (bagged and loose leaf) was similar at 80 °C/5'17 but another study showed that maximum extraction efficiency for green tea bioactive compounds also depends on steeping time at constant temperature for different tea types i.e. 5' (powder), 15' (bagged) and 30' (loose leaf) at 80 °C18. The powdered form of green tea revealed greater scavenging effect on the production of reactive oxygen species (ROS) in vitro compared with the same amount of loose leaf tea19. Matcha is a powdered form of green tea that is produced from shade grown tea leaves that are briefly steamed after harvesting and then grinded in a stone mill19,20. It is found to be effective in quenching reactive oxygen species, inhibition of blood glucose accumulation and stimulation of lipid metabolism21. The aim of this study was to assess and compare the effect of different forms of green tea such as loose leaf (Bud white, Lipton and Laplant loose), bagged (Lipton and Laplant bag) and powdered (Gourmet and Wow matcha) on their antioxidant potential at conditions usually used for tea preparations at home.

Materials and Methods

Chemicals and Reagents
Methanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). All these chemical are of analytical grade and procured from Himedia (Mumbai, India).

Sample Preparation
Different forms of green tea such as loose [Bud white (BW), Lipton loose (LL), and Laplant loose (LAL)], bagged [Lipton bag (LB) and Laplant bag (LB)] and powdered (Gourmet matcha (GM) and Wow matcha (WM)] of various brands were evaluated in this study. Various tea samples were prepared by adding 0.2 g of tea in 10 ml of distilled water and steeped for 5 minutes at 95-100 °C. The hot water samples were filtered using Whatman’s filter paper and the filtrate was used for further investigations22.

Antioxidant Potential

DPPH Free Radical Scavenging Assay
The scavenging activity of different tea extracts against stable DPPH free radical was examined spectrophotometrically. DPPH solution (0.3 mM) was prepared by dissolving DPPH in methanol. The optical density (OD) of DPPH solution was set between 0.8-1 by diluting it with 50% methanol. Different concentrations of tea extracts were added separately to 2 ml of DPPH solution23. After 30 minutes of incubation, the discoloration of the purple to yellow color was observed at 520 nm. Methanol was taken as blank and 2 ml of DPPH solution was taken as control. The test was carried out in triplicates. Radical scavenging potential was determined applying the following relationship:

\[
\text{% Scavenging activity} = \frac{A_{520}(c) - A_{520}(s)}{A_{520}(c)} \times 100
\]

Where, \(A(c)\) = absorbance of control and \(A(s)\) = absorbance of sample.

ABTS Free Radical Scavenging Activity
This assay depends on the ability of different substances to scavenge ABTS [2, 2'- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)]. The radical cation was generated by reacting ABTS stock solution (7 mM) with potassium persulfate (2.4 mM) in 1: 1 ratio. The reaction mixture was kept in dark for 16 hours at room temperature. The optical density (OD) of ABTS solution was set between 0.8-1 by diluting it with 50% methanol. Different concentrations of tea extracts were added to every 2 ml of ABTS solution24. After 30 minutes of incubation, absorbance of respective samples was taken at 745 nm. The scavenging potential of the test samples was determined by following equation:

\[
\text{% Scavenging activity} = \frac{A_{745}(c) - A_{745}(s)}{A_{745}(c)} \times 100
\]

Where, \(A(c)\) = absorbance of control and \(A(s)\) = absorbance of sample.

Statistical Analysis
The data was expressed as mean ± S.D for triplicate readings. The inter group comparisons were performed by one way analysis of variance (ANOVA) succeeded by Tukey’s honestly significant difference test using SPSS software (version 18).
The observations were considered statistically significant when the p-values are 0.05 or less.

**Results and Discussion**

Antioxidant activity of different forms of green tea was determined using DPPH and ABTS tests as shown in Figure 1 and 2.

### DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of different forms of green tea were ranked upon IC$_{50}$ values as followed: Lipton loose, LL (30.48 µg/ml) > Wow matcha, WM (29.93 µg/ml) > Gourmet matcha, GM (27.55 µg/ml) > Laplant loose, LAL (26.26 µg/ml) > Lipton bag, LB (24.42 µg/ml) > Laplant bag, LAB (20.38 µg/ml) > Bud white loose leaf, BW (18.93 µg/ml) as depicted in Figure 1. Lesser the IC$_{50}$ value, greater is the antioxidant potential. It was observed that BW and LAB are significantly more effective in scavenging DPPH radical followed by LB, LAL, GM, WM and LL.

![Fig. 1: DPPH radical scavenging activity of different brands and forms of green tea (mean ± sd, n=3). Different alphabets symbolize significant variation between different tea brands. Bud white (BW), Gourmet matcha (GM), Laplant bag (LAB), Laplant loose (LAL), Lipton bag (LB), Lipton loose (LL) and Wow matcha (WM) are various brands of different forms of green tea](image)

### ABTS Radical Scavenging Activity

The results obtained from the ABTS test shown in Figure 2. The IC50 values of different forms of green tea based upon their scavenging activity are as followed: LL (13.62 µg/ml) > WM (11.95 µg/ml) > GM (11.44 µg/ml) > LAL (11.16 µg/ml) > LB (10.89 µg/ml) > LAB (9.75 µg/ml) > BW loose leaf (8.84 µg/ml). It was demonstrated that BW was significantly more effective in radical scavenging activity followed by LAB, LB, LAL, GM, WM and LL.

![Fig. 2: ABTS radical scavenging activity of different brands and forms of green tea (mean ± sd, n=3). Different alphabets symbolize significant variation between different tea brands. Bud white (BW), Gourmet matcha (GM), Laplant bag (LAB), Laplant loose (LAL), Lipton bag (LB), Lipton loose (LL) and Wow matcha (WM) are various brands of different forms of green tea](image)
The fluctuations in antioxidant activity of green tea from various brands were reported. This is in agreement with previous studies, which concluded that phytochemical content of tea is affected by the cultivation conditions, horticultural practices, cultivar, age of leaf, grade, geographical area, storing and type of processing\(^{25-27}\).

The combined IC\(_{50}\) values of different forms of green tea such as loose (Bud white, Lipton and Laplant loose), bagged (Lipton and Laplant bag) and matcha (Wow and Gourmet matcha) showed no statistical significant difference in both the antioxidant assays except in case of DPPH radical scavenging, bagged tea demonstrated a small but significant difference in radical quenching ability as compared to matcha as depicted in Table 1.

**Table 1: Average values of combined IC\(_{50}\) (µg/ml) of loose, bagged and powdered form of green tea (mean ± sd, n=3)**

<table>
<thead>
<tr>
<th>Green tea form</th>
<th>DPPH</th>
<th>ABTS</th>
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<tbody>
<tr>
<td>Bag tea (Lipton and Laplant bag)</td>
<td>22.40 ± 2.26</td>
<td>10.32 ± 0.77</td>
</tr>
<tr>
<td>Loose tea (Bud white green tea, Lipton and Laplant loose)</td>
<td>25.45 ± 5.12</td>
<td>11.21 ± 2.10</td>
</tr>
<tr>
<td>Matcha tea (Wow and Gourmet matcha)</td>
<td>28.74 ± 1.57 *</td>
<td>11.70 ± 0.65</td>
</tr>
</tbody>
</table>

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2’ azino- bis (3- ethylbenzothiazoline-6-sulfonic acid) (ABTS). * Represents a significant difference as compared to bagged tea at \(p \leq 0.05\).

A previous study on different forms of green tea such as powdered (Matcha), loose leaf (Kukicha, Gyokuro, Longjing, Sencha J, Bancha, Yunnan, Sencha CH, Gunpowder and Rose of the Orient) and bagged (Twinings of London, Taylors of Harrogate and Franck) demonstrated that antioxidant potential of different forms of tea varies with extraction time and temperature. The same study also showed that antioxidant potential and total phenolic content was found to be maximum for all green tea forms at 100 °C/3 ′, the bagged tea possess highest scavenging activity followed by powdered and then loose leaf tea\(^{16}\). In vivo studies demonstrated that regular consumption of green tea exerts protection against benzo(a)pyrene mediated toxicity in mice model\(^{28,29}\). In the present work, no significant difference was found between the radical quenching ability of different forms of tea except bagged tea, which exhibited slightly more scavenging potential in case of DPPH radical. Interestingly, earlier studies also revealed that antioxidant capability of different forms of green tea were similar for steeping time of 5′ and 10′ at 80 °C\(^{17,18}\). It was also suggested that high water temperature and short steeping time is best for extraction of tea bioactive compounds\(^{18,30,31}\). Our investigation was concentrated on short steeping time (5′) and high water temperature (95-100 °C) as these conditions mimic the household method of tea preparation.

**Conclusion**

The study on the antioxidant activity of different forms of commercially available green tea like loose (Bud white, Lipton loose and Laplant loose), bag (Lipton and Laplant bag) and matcha (Gourmet matcha and Wow matcha) prepared at 95-100 °C and steeped for 5 min, showed no significant difference in the IC\(_{50}\) value against DPPH and ABTS radical except bagged tea that displayed slightly more DPPH radical scavenging potential in comparison to matcha. But individually, BW (loose leaf) showed the highest antioxidant activity followed by LAB (bagged), LB (bagged), LAL (loose leaf), GM (powder), WM (powder) and LL (loose leaf). Moreover, this study reported it might not be possible to rank the radical scavenging potential on the basis of green tea form.
Acknowledgments
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Conflict of Interest
The authors declare no conflict of interest, financial or otherwise.

References


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