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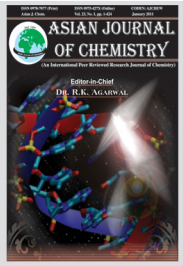

Determination of Catechins and Antioxidant Properties of Commercial Green and Black Teas

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Catechins and antioxidant activity of commercial green tea and black tea were determined and compared with each other. Catechins were measured by HPLC method. Antioxidant activity was assessed by measuring the ability to scavenge DPPH radical, reduction Fe(III) to Fe(II) and scavenging effect of hydrogen peroxide. The epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin and (+) catechin in green tea was found to be higher than black tea. The DPPH activity and reducing power of green tea were found to be higher when compared to black tea. But the scavenging effect of hydrogen peroxide on black tea showed higher than green tea. The results clearly indicated that green tea had higher amount of individual catechins and antioxidant activity than black tea sample.

Key Words: Green tea, Black tea, Catechins, Antioxidant activity, DPPH.

INTRODUCTION

Tea is one of the commonly consumed beverages in the world for its desirable aroma, taste and putative positive physiological functions[1]. Several epidemiological studies have suggested the importance of secondary plant products-polyphenol consumption in reducing the incidence of degenerative diseases[2]. Those substances possess strong antioxidative activity and are counterparts to oxidative stress[3].

The ability to scavenge the free radicals by tea polyphenols is due to the presence of hydroxyl group attached to the flavan-3-ol structure which has been associated with the therapeutic action against free radical medicated disease; thereby attracting tremendous research interest. It has become quite clear that, free radicals play an important role in the development of many human diseases. It is now thought that once free radicals are produced, they are the primary cause of certain human disease involving many organs[4,5].

Tea polyphenols, in particularly catechins have showed strong antioxidant activity, especially in free radical scavenger activities *viz.*, singlet oxygen, hydroxyl radical and metal chelating capacity[6]. Green tea polyphenol, (-)-epigallocatechin-3-gallate is a potential anticancer agent[7] and a meta-analysis of epidemiological studies indicates that consumption of green and black tea lowers the risk of breast cancer[8]. Thus regular green tea drinking might protect smokers from oxidative damages and could reduce cancer risk or other diseases caused by free radicals associated with smoking[9] and also enhance the insulin activity[10]. Tea flavonoids possessing antiallergic[11] and antibacterial, antitoxin, antiviral, antifungal activities[12] and anti-mutagenicity[13].

The main objective of the present study is to compare individual catechins, radical scavenging activities of green tea with black tea as potential source of natural antioxidants. That should be complement to their previously known therapeutic value and improve the popularization of green tea drinking in addition to that of black tea.

EXPERIMENTAL

2,2-Dihydryl-1-picryl radical (DPPH), (+)-catechins were purchased from Sigma Aldrich Chemicals Private Limited, Bangalore for the present study. Vitamin-C, methanol, glacial acetic acid, hydrogen peroxide, ferric chloride, trichloroacetic acid, potassium hexacyanoferrate, acetonitrile and other chemicals were purchased from S.D. Fine Chemicals, Mumbai, India.

Tea samples: Green tea and black tea were obtained from The United Nilgiris Tea Estate Co. Ltd, Coimbatore and Kannan Devan Tea Estate, Moonar, Kerala. The purchased tea samples were consequently subjected to analysis of various tea quality parameters.

Tea extracts preparation: About 2 g of powdered tea sample was boiled with 70 % aqueous ethanol for 0.5 h and extract was centrifuged and the supernatant was diluted with 100 mL distilled water and directly used for analysis.

Estimation of catechins by HPLC method: About 0.2 g of black tea and green tea powder were extracted using 10 mL

of 70 % (v/v) methanol maintained at 70 ºC. One mL of the extract was diluted to 5 mL with stabilizing solution. Stabilizing solution was prepared by mixing 0.025 % (w/v) each of EDTA, ascorbic acid and acetonitrile (10 %). It was prepared freshly on the day of use and filtered through 0.45 µ nylon membrane filter and then used for HPLC analysis. The mobile phase A was 2 % acetic acid in 9 % acetonitrile in water and mobile phase B was 80 % acetonitrile in water. The gradient program was, flow rate of mobile phase, 1 mL binary gradient conditions were, 100 % mobile phase A for 10 min then over 15 min. A linear gradient mobile phase A 32 % mobile phase B and hold at this composition for 10 min. Then the rest to 100 % mobile phase A and allowed to equilibrium for 10 min before next injection. A phenomenex Lana 5 µm phenyl-hexyl column (250 mm length × 4.6 mm internal diameter) fitted with a phenomenex security guard (4 mm × 3 mm) phenyl-hexyl bonded cartridge was used for the analysis. The absorbance of compounds measured at the wavelength of 278 nm using UV- detector.

DPPH radical scavenging activity: Free radical scavenging activity was estimated according to Zhu *et al.*[1] 1 mM solution of DPPH radical solution in ethanol was prepared and then 1 mL of this solution was mixed with 3 mL of extract of various concentrations. The mixture was then vortexed vigorously and left for 0.5 h at room temperature in the dark and the absorbance was measured at 517 nm. The activity was calculated as DPPH scavenging activity = (control absorbance - extract absorbance) × 100/control absorbance).

Scavenging of hydrogen peroxide: The ability of tea extracts to scavenge hydrogen peroxide was determined using a method described in literature[14]. Hydrogen peroxide solution (2 mM/L) was prepared and added to 0.6 mL of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Free radical scavenging activity of H_2O_2 was expressed in percentage.

Ferric reducing power: Ferric reducing power was carried out using assay described in literature[15]. One mL of the extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferric cyanide (1 %) solution. The mixture was incubated at 50 ºC for 0.5 h. Afterwards 2.5 mL of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer was pipetted out and mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1 %) was added and the absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

Catechins: Chromatograms of green tea and black tea are shown in Figs. 1 and 2. The catechins fractions such as catechin (C), epicatechin (EC), epigallocatechin (EGC), (+)-catechin [(+)-C], epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) of green tea and black tea are given in Table-1 and determined by HPLC method. The result indicated that the catechins present in green tea were found to be higher than black tea. The percentage of individual catechins in both teas were found to be in the following order of EGCG > EGC > ECG > EC > (+) -C. Among the catechins, the EGCG content of green tea was the highest. This result was similar to others results. They reported on the Nilgiris tea and which varied from the results of the Darjeeling tea[16,17]. It has been reported that trihydroxy flavan-3-ols are oxidized faster during the fermentation of black tea processing thereby resulting in the lower levels of EGCG and EGC and subsequent increase in green tea[18].

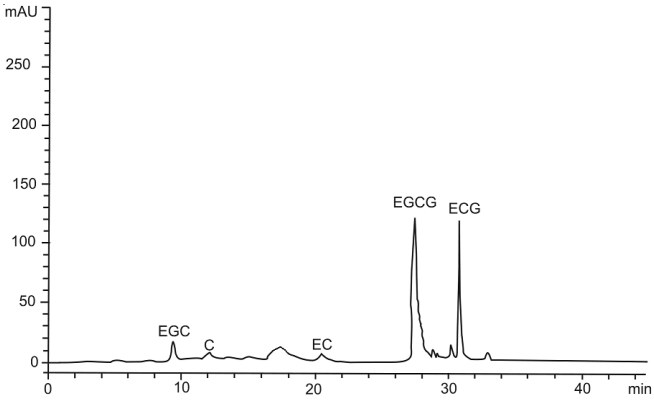

Fig. 1. HPLC chromatogram of green tea at 280 nm. Peaks: epigallocatechin (EGC); (+) -catechin; EC, -epicatechin (EC) EGCG, epigallocatechin gallate (EGCG); ECG, epicatechin gallate (ECG).

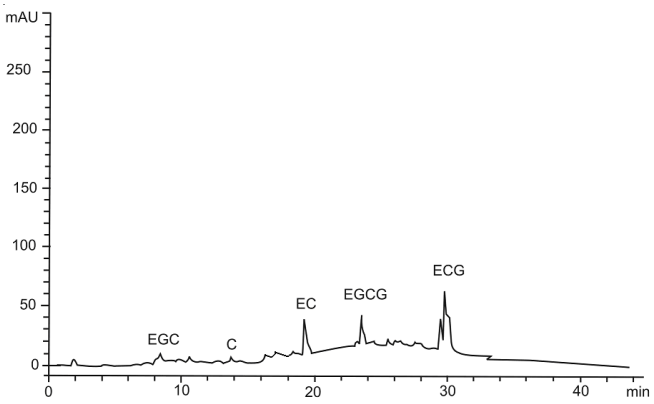

Fig. 2. HPLC chromatogram of black tea at 280 nm. Peaks: epigallocatechin (EGC); C-catechin; epicatechin (EC); epigallocatechin gallate (EGCG); epicatechin gallate (ECG).

TABLE-1
CATECHIN OF GREEN AND BLACK TEA BY HPLC METHOD

Catechins	Green tea (%)	Black tea (%)
Epigallocatechin	2.94 ± 0.05	1.77 ± 0.12
(+)-Catechin	0.18 ± 0.05	0.13 ± 0.06
Epicatechin	1.19 ± 0.08	0.77 ± 0.12
Epigallocatechin gallate	7.09 ± 0.13	5.27 ± 0.64
Epicatechin gallate	1.74 ± 0.03	1.73 ± 0.06

Note: The results were expressed in percentage ± SD.

DPPH radical scavenging activity, ferric reducing power and scavenging effects of hydrogen peroxide: The antioxidant capacities of the tea samples were analyzed using the free radical scavenging (DPPH), ferric reducing power and hydrogen peroxide scavenging activities. DPPH radical scavenging effects on green and black tea extracts were found to be

56.5-59.9 and 33.2-35.5 %, respectively. The scavenging effect of green tea and black tea extracts on the DPPH radical was found to be in the order of green tea > black tea. Lowest antioxidant capacity in green tea due to fast fermentation process and the highest DPPH free radical scavenging ability of green tea followed by black tea extracts also have been reported by other investigators[19,20]. The results of investigations have permitted us to rank extracts according to their decreasing antiradical properties in the order of green tea > black tea. This may be due to presence of catechins and followed by polyphenols. High antiradical potential in scavenging of DPPH radical was affirmed for EGCG[21]. The result coincided with the report of Yen and Chen[13]. The catechins present in tea, donate hydrogen atom to DPPH free radical and neutralize it hence it become a stable radical.

Ferric reducing power is used to study the ability of the antioxidants in reducing iron (III) to iron (II) in a redox-linked reaction, involving a single electron transfer mechanism[22]. The reducing powers of green and black teas were 0.524-0.549 % and 0.400-0.460 %. The greater reducing power was observed more in green tea than in black tea. Tea extracts are electron donors and can react with free radicals to convert them to more stable products and terminate radical chain reaction[13].

Conclusion

It is concluded that green tea extract possess significant antioxidant activities, their overall strength being in the order of green tea>black tea when compared to black tea. Highest DPPH radical scavenging activity, ferric reducing power observed in green may be due to presence of EGC, (+) -C, EC, EGCG and ECG. But black tea showed the highest inhibitory effect on hydrogen peroxide than green tea. The results suggest that catechins are the major contributors to the antioxidant activities of green and black tea. Thus drinking of green tea in addition to black tea could be additionally ascribed to the confirmed here strong antioxidant properties and rich content of catechins.

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