QUANTITATIVE ANALYSIS OF GREEN TEA POLYPHENOLS IN INDIAN CULTIVARS

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Abstract

Green tea catechins (GTCs), namely (-)-epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), were analysed in 25 prominent Indian tea cultivars cultivated in North, South and North-east states of India using an optimized procedure of extraction and HPLC determination. Kangra Jawala grown in Palampur (North India) showed the highest content of EGCG (6.88%), followed by TRI-2026 (6.83%) grown in Coonoor (South India) and lowest content were recorded in P-126 (1.2%) grown in Darjeeling. The TV-1 cultivars from Palampur showed the highest content of ECG (2.74%) among all the samples analysed.

Key words: *Camellia sinensis*; green tea catechins (GTCs); epigallocatechin gallate (EGCG); epicatechin gallate (ECG)

INTRODUCTION

Tea is derived from terminal three leaves of shoots of tea plant *Camellia sinensis* (L.) O. Kuntze (syn. *Thea sinensis* L.) family Theaceae. It is the most popular non-alcoholic beverage in the world. A number of dissimilar chemical reactions initiated by an enzyme, polyphenol oxidase during fermentation, are of practical value in commercial manufacture of black tea. The primary polyphenols are oxidised during the fermentation process and are transformed to compounds with tanning properties. The oxidized polyphenols in black tea are responsible for briskness, strength, colour, taste and pungency of the black tea infusion. These polyphenols remain intact in green tea as steaming of leaves following plucking in green tea manufacture inactivates the enzyme polyphenol oxidase. The natural polyphenols in green tea include (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3- gallate (ECG), and epicatechin (EC). The highest concentration is of EGCG followed by EGC, ECG and EC in decreasing order [Nakabayashi, 1991; Oshima, 1936; Bradfield, et al., 1947 & 1948]. The pharmacological importance of these catechins decreases in the order of EGCG, EGC, ECG and EC. Other minor catechins, (+)-gallocatechin (GC), (-)-gallocatechin gallate (GCG), (-)-catechin gallate (CG) and (+)-catechin (C) are also present in tea [Yamamoto, et al., 1997]. The green tea contains 30 to 42% polyphenols on the dry weight basis and a cup of green tea contains about 300 to 400 mg of polyphenols [Balentine, et al., 1997].

The public awareness of the health giving properties of tea has increased in the recent past. The majority of beneficial effects of tea have been attributed to primary polyphenolic constituents of green tea. Strong antioxidant potential of these polyphenols is thought to mediate most of the beneficial effects of tea [Balentine, et al., 1997]. The health benefits in cancer [Suganuma, et al., 2000], arthritis [Haqqi, et al., 1999], cardiovascular diseases [Muramatsu, et al., 1986], diabetes [Deng and Tao, 1998] and

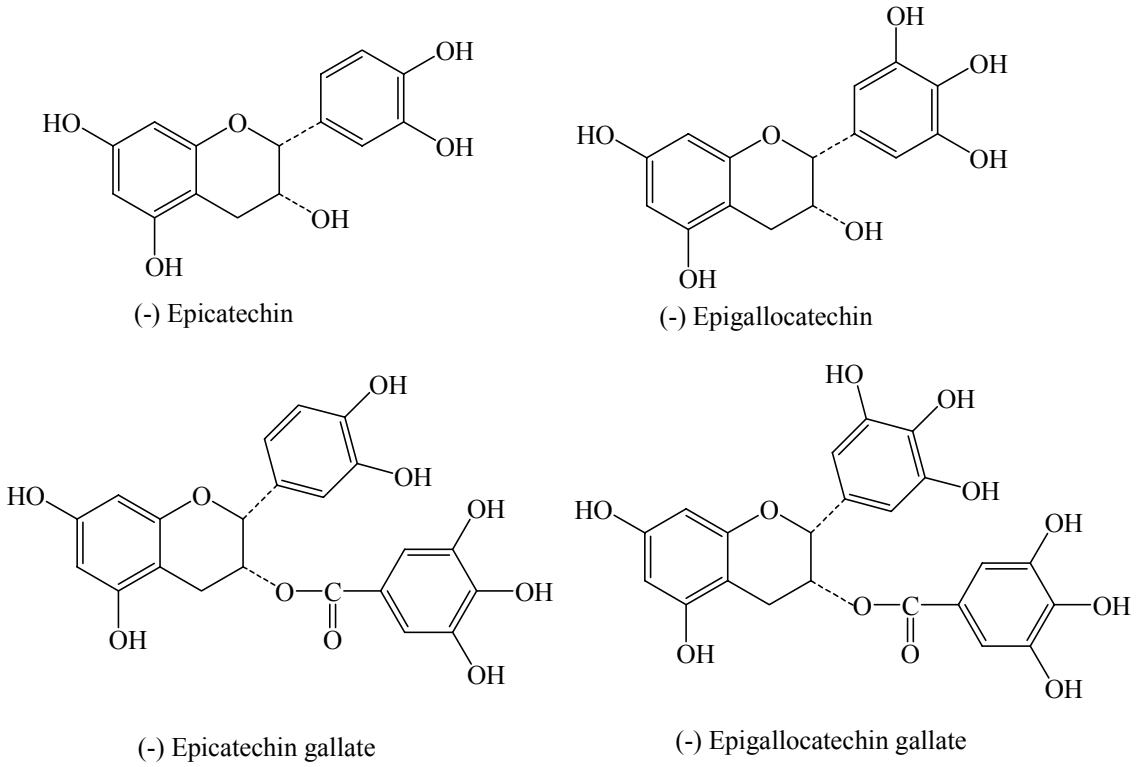

Fig. 1. Tea polyphenols

obesity [Kwanashie, et al., 1989] have been focused for scientific investigations in the recent past [Weisburger, 1999]. The results of these investigations have indicated that green tea catechins have a great potential to be developed as therapeutic agents [Kohri, et al., 2001].

The estimation of polyphenols has been done by various techniques viz. nuclear magnetic resonance [Schulz, et al., 1999], near-infrared reflectance spectroscopy [Zhu and Xiao, 1991], high performance thin layer chromatography (HPTLC) [Lee and Ong, 2000], liquid chromatography coupled with mass spectroscopy (LC-MS) [Ding, et al., 1999], high performance capillary electrophoresis (HPCE) [Goto, et al., 1996] and high performance liquid chromatography (HPLC) [Beecher, et al., 1999; Larger, et al., 1998]. The common and most frequently used technique for estimating tea polyphenols is reversed phase HPLC with UV-absorbance detection. The presence of an acid in the mobile phase seems essential for complete and efficient resolution of catechins, specifically for elimination of peak tailing and its detection at shorter wavelengths [Beecher, et al., 1999; Poon, 1998].

India is the largest producer of tea and it chiefly manufactures black tea. The country has ignored the potential to produce and propagate green tea. However, with increasing interest in green tea polyphenols, there is a potential for the Indian tea industry to enhance green tea production and develop innovative products based on natural tea polyphenols. To achieve this objective, it is important to identify and promote the Indian tea cultivars with optimal content of tea polyphenols for industrial exploitation. The objective of the present study was to identify the Indian tea cultivars containing highest content of EGCG, pharmacologically the most active polyphenol of tea. The results of the present study will help the Indian tea industry to focus attention on the production of green tea and develop non-beveragic innovative products based on green tea polyphenols.

EXPERIMENTAL

Material and Methods

Tea samples

Samples of different tea cultivars were collected from three major tea-growing areas in India including Palampur (North India), Guwahati and Darjeeling (Northeast India), Coonoor and Ooty (South India) in the year 2001-2002. In one case, collection was repeated at the beginning (April), in the middle (September) and at the end (October-November) of the plucking season. The hand picked tea leaves were processed for sample preparation following the usual manufacturing procedure of green tea. For this, the leaves were steamed for 5 min immediately after plucking and then dried in an oven at a temperature not exceeding 65°C. The sun-dried samples were prepared by drying the leaves directly in the sun immediately after plucking for comparison.

Chemicals

All chemicals and reagents were of AR or HPLC grade (E. Merck or S. D. Fine Chemicals, India). (-) EGCG (Catalogue No. E4143) and (-) ECG (Catalogue No. E3893) were obtained from Sigma-Aldrich Chemicals Ltd. Distilled water was used wherever water is mentioned.

High performance liquid chromatography

The analytical determinations of EGCG and ECG were carried out using reverse phase-high performance liquid chromatography in isocratic mode. The Waters HPLC system equipped with automated gradient controller, 510 pumps, U6K injector, 481 detector, 746

data module and Waters μ-bondapak C18 column (3.9 x 300 mm), was used for the analysis. Elution was carried out at ambient temperature between 24 to 28°C using water : methanol : acetic acid (70: 30: 0.5) as a mobile phase at a flow rate 1.0 mL/min. All extracts were prepared in triplicate and each extract was analysed in triplicate. The UV detection was carried out at 280 nm.

Standard stock solutions

A standard solution of EGCG was prepared by dissolving 4.72 mg of EGCG in 50 ml methanol. Standard plot for HPLC analysis was prepared by injecting in triplicate a constant volume of 5 μl of serially diluted concentrations containing 9.85, 19.70, 39.35, 78.50, 157.50 and 283.20 ng/5 μl of EGCG and noting AUC corresponding to each concentration.

The standard solution of ECG was prepared by dissolving 3.02 mg of ECG in 10 mL methanol. The stock solution was diluted to make 9.40, 18.85, 37.50, 75.50, 151.0 and 302 ng/5 μl dilutions of ECG. A constant volume of 5 μl of each concentration was injected in triplicate. The standard plot was prepared as described for EGCG and same conditions of analysis were used for the two catechins.

Estimation of polyphenols in tea samples

The samples for analysis were prepared following the conditions developed for optimized extraction of tea polyphenols in our laboratory [Vasisht, et al., 2003]. Accurately weighed, about 2 g of moderately fine powder of tea sample was taken in a vacuum flask and 100 mL of boiling water was added to it. The flask was stoppered and kept on a

rotary shaker for 5 min. The contents were filtered quickly while still hot using vacuum and the marc was washed with 10 mL of boiling water. The volume of the extract was adjusted to 100 mL with cold water and 1mL of this extract was diluted to 25 mL of mobile phase (water: methanol: acetic acid :: 70: 30: 0.5). The diluted extract was filtered through a 0.45 µm membrane filter and a constant volume of 5 µl was injected for each analysis. The amount of EGCG and ECG in the extract was calculated from the area under the curve corresponding to the respective peaks of two polyphenols and their standard plots.

Linearity of HPLC system and its sensitivity

Linear regression was obtained by plotting the peak area versus concentration of a series of dilutions for each phenolic compound. The regression lines, expressed as correlation coefficients, were linear (r^2 =1 and 0.9999 for EGCG and ECG respectively) in the experimental range.

Sensitivity (defined as the lowest measurable concentration of a compound in the sample) was determined as that concentration which generated a peak at least three times higher than the baseline noise range (Table 1).

Table 1: Linearity and sensitivity of the detection method for EGCG and ECG

Compound	Range studied (ng/5 µl)	Correlation coefficient (r^2)	Sensitivity (ng/5 µl)
Epigallocatechin gallate	9.85-283.2	1.0	9.85
Epicatechin gallate	9.4-302	0.9999	9.4

Recovery and reproducibility

The percent recovery of analyte in the method was established by spiking the sample at 100 % level. The per cent recovery of EGCG from triplicate set of analysis was 95.92 ± 0.58. Reproducibility was found to be with in limits by repeating the analysis at different times.

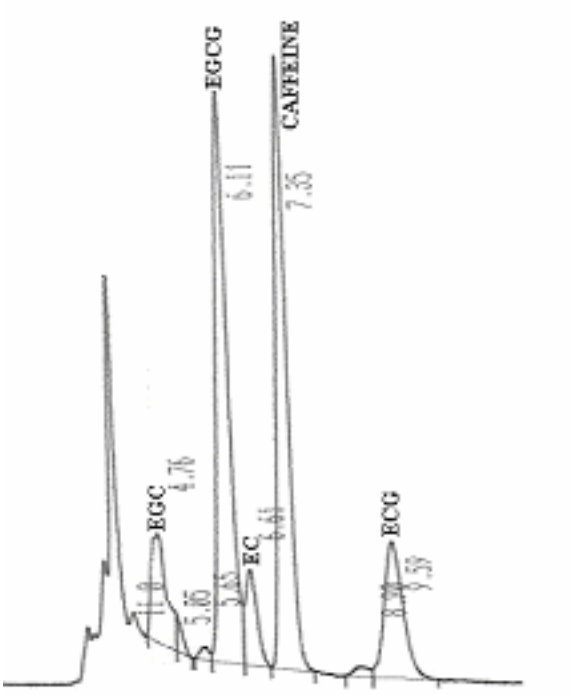

Fig. 2. A typical HPLC chromatogram of green tea extract

Table 2: Polyphenol content in green tea samples of different Indian cultivars

Region/place of collection	Tea cultivar	Period of collection	EGCG (mg/g)	EGCG (%)	ECG (mg/g)	ECG (%)
North/ Palampur	TV-23*	Sep. 01	2.70 ± 0.5	0.2	N.D.	N.D
	TV-23	Sep. 01	24.45 ± 2.48	2.4	6.36 ± 0.50	0.63
	Kangra Asha	Nov. 01	32.00 ± 2.93	3.2	7.04 ± 0.51	0.70
	TV-1	Nov. 01	42.54 ± 0.43	4.2	25.36 ± 0.07	2.5
	TV-1*	Nov. 01	29.61 ± 2.74	2.9	20.63 ± 2.63	2.0

	TV-23	Nov. 01	43.60 ± 0.25	4.3	16.40 ± 0.30	1.6
	TV-23*	Nov. 01	19.82 ± 0.20	1.9	9.58 ± 0.11	0.95
	Kangra Asha	Apr. 02	45.61 ± 1.67	4.5	9.56 ± 0.32	0.95
	Kangra Jawala*	Apr. 02	26.64 ±2.52	2.6	6.37 ± 0.58	0.63
	Kangra Jawala	Apr. 02	68.89 ± 2.77	6.8	19.04 ± 0.35	1.9
	TV-1*	Apr. 02	22.16 ± 0.52	2.2	15.56 ± 0.67	1.5
	TV-1	Apr. 02	43.22 ± 2.80	4.3	27.39 ± 2.20	2.7
	TV-23*	Apr. 02	31.87 ± 1.63	3.1	12.18 ± 0.22	1.2
	TV-23	Apr. 02	58.52 ± 1.34	5.8	18.95 ± 0.33	1.8
	Kangra Asha*	Sep. 02	23.50 ± 0.85	2.3	11.39 ± 0.85	1.1
	Kangra Asha	Sep. 02	64.38 ± 1.52	6.4	14.04 ± 0.90	1.4
	Kangra Jawala*	Sep. 02	27.14 ± 2.07	2.7	10.88 ± 0.38	1.0
	Kangra Jawala	Sep. 02	62.01 ± 4.52	6.2	14.30 ± 1.15	1.4
Northeast/ Guwahati & Darjeeling	P-126	Jun. 02	12.89 ± 1.24	1.2	7.16 ± 0.71	0.71
	Takda-7, 8	Jun. 02	19.48 ± 0.59	1.9	6.45 ± 0.30	0.64
	Tenali-17	Jun. 02	17.62 ± 1.48	1.7	5.63 ± 0.69	0.56
	TV-1	Jun. 02	27.48 ± 2.32	2.7	19.64 ± 1.71	1.9
	TV-9	Jun. 02	33.91 ± 3.67	3.3	10.72 ± 1.18	1.0
	TV-18	Jun. 02	50.18 ± 1.61	5.0	16.22 ± 1.52	1.6
	TV-20	Jun. 02	19.18 ± 0.82	1.9	6.60 ± 0.86	0.66
	TV-23	Jun. 02	23.52 ± 0.61	2.3	13.71 ± 1.49	1.3
	TV-26	Jun. 02	18.46 ± 1.40	1.8	7.33 ± 0.53	0.7
	China Bush + Clones	Jun. 02	30.36 ± 3.49	3.0	8.50 ± 0.85	0.8
South/ Ooty & Coonoor	China Variety*	Oct. 02	1.65 ± 0.07	0.16	1.37 ± 0.04	0.1
	UPASI-3*	Oct. 02	1.36 ± 0.22	0.13	0.32 ± 0.00	0.03
	VP-Clones*	Oct. 02	2.14 ± 0.50	0.21	0.71 ± 0.16	0.07
	BSS-1	Oct. 02	24.32 ± 0.86	2.4	8.04 ± 0.56	0.8
	C-1	Oct. 02	30.87 ± 0.89	3.0	10.54 ± 0.55	1.0
	China Variety	Oct. 02	38.12 ± 2.35	3.8	14.24 ± 1.08	1.4
	CR-6017	Oct. 02	44.18 ± 3.58	4.4	11.60 ± 1.20	1.1
	TRI-2024	Oct. 02	33.43 ± 3.60	3.3	12.76 ± 0.89	1.2
	TRI-2025	Oct. 02	42.31 ± 5.09	4.2	10.29 ± 0.82	1.0
	TRI-2026	Oct. 02	68.35 ± 5.21	6.8	22.20 ± 1.91	2.2
	UPASI-2	Oct. 02	51.47 ± 0.74	5.1	12.15 ± 0.33	1.2
	UPASI-3	Oct. 02	19.75 ± 6.99	1.9	5.53 ± 2.02	0.5
	UPASI-8	Oct. 02	45.44 ± 3.09	4.5	14.68 ± 1.26	1.4
	UPASI-9	Oct. 02	57.82 ± 1.62	5.7	14.24 ± 0.98	1.4
	UPASI-10	Oct. 02	21.64 ± 2.24	2.1	8.33 ± 0.76	0.8

* =Sun dried; N.D. =Not detected

RESULTS & DISCUSSION

There are three different geographical areas of cultivation and production of tea in India. The bulk of green 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). In the year 2000, black tea constituted 99.07% of the total production of tea in India and the production of green tea during 1998 to 2000 decreased from 8,616 to 7,822 tonnes [Anonymous, 2000; Suchanti, 2001].

A large number of cultivars of tea have been developed in India of which 25 prominent cultivars were analysed in the present study.

Forty three samples of different cultivars were collected from various tea growing areas of the country. In one case (Kangra Asha), collection was repeated at different plucking seasons (April, September and November, year 2001-2002). The hand picked tea leaves in each case were processed for sample preparation following the usual manufacturing procedure of green tea at the collecting sites.

EGCG and ECG content of different cultivars were determined using optimised conditions of extraction and analysis developed in our laboratory. The content of EGCG and ECG in analysed samples varied over a wide range (0.14 to 6.88% for EGCG and 0.03 to 2.74% for ECG). The inactivation of enzymes quickly following plucking, preferably through steaming, is essential as observed from the very low content of EGCG in sun-dried samples. The EGCG content varied from 0.27 to 6.88% in North Indian sample, 1.28 to 5.01% in Northeast and 0.14 to 6.88% in South Indian samples. The

percentage of ECG varied from 0.64 to 2.73% (North), 0.56 to 1.96% (Northeast) and 0.03 to 2.22% (South India).

Among different cultivars, steamed samples of Kangra Jawala, collected during first flush of the crop (a cultivar developed at the Department of Tea husbandry and Technology, HPKV, Palampur North India) and TRI-2026 developed at the United Planters Association of Southern India (UPASI), showed the highest content of EGCG (6.88 and 6.83% respectively). Kangra Jawala sample collected during late flush showed a low content (6.2%) of EGCG. The TV1 cultivars from Palampur showed the highest content of ECG (2.74%) among all the samples analysed. The samples of two cultivars, TV-1 and TV-23 were collected from two regions, North (Palampur) and Northeast (Darjeeling) but the content of EGCG was lower in Northeast samples probably due to agroclimate conditions. Such variations are also noted in the literature [Bhatia and Ullah, 1968; Singh, et al., 1999].

In conclusion, the content of EGCG and ECG showed wide fluctuations in different Indian cultivars. These cultivars are primarily used for the production of black tea in India. Some promising cultivars with comparatively high content of EGCG and ECG have been identified in the present study. Kangra Jawala and TRI-2026 are particularly noteworthy for their high content of catechins and possible exploitation in the production of value added green tea and green tea based products.

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