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HPLC Analysis of Polyphenols in Green Tea Extracts

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HPLC is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. By virtue of its ability to simultaneously separate, identify and analyze the complex mixture of organic substances, HPLC will be highly useful for the analysis of herbal drugs and formulations. Green tea contains polyphenols which constitute one of the most numerous and ubiquitous groups of plant metabolites and are an integral part of both human and animal diets. In the present communication we report the HPLC analysis of the green tea extract.

Key Words: HPLC, Polyphenols, Green tea, Epicatechin, Epicatechingallate, Epigallocatechin, Epigallocatechin-gallate.

INTRODCUTION

Polyphenols constitute one of the most numerous and ubiquitous groups of plant metabolites and are an integral part of both human and animal diets. Ranging from simple phenolic molecules to highly polymerized compounds with molecular weights of greater than 30,000 Da, the occurrence of this complex group of substances in plant foods is extremely variable[1, 2].

Green tea leaves are obtained from dried leaves of *Camellia sinensis*, belonging to the family theaceae. Steaming or drying fresh tea leaves at elevated temperatures makes commercial green tea. Its chemical composition is similar to that of fresh tea leaves. Green tea contains polyphenols, which include flavanols, flavandiols, flavonoids and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the green tea polyphenols are flavonols, commonly known as catechins[3]. Some major green tea catechins are (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC) (+)-gallocatechin and (+)-catechin[4]. The chemical structures of some of these compounds are shown in Fig. 1.

Caffeine, theobromine and theophylline, the principal alkaloids, account for about 4% of the dry weight. In addition, there are phenolic acids such as gallic acids and characteristic amino acids such as theanine[5]. HPLC is an efficient method to ensure the identity and purity of the herbal[6, 7].

In the present communication we report a simple, gradient method using acetic acid and acetonitrile as mobile phase, which gives distinct separation of polyphenolic units in green tea extract.

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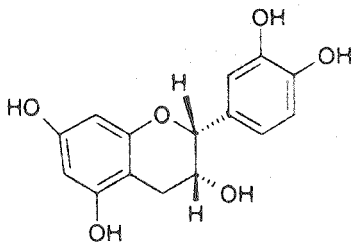
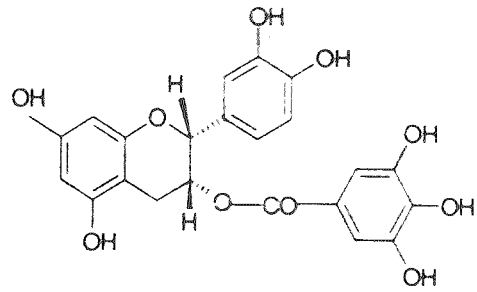
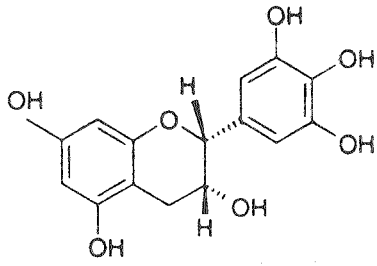


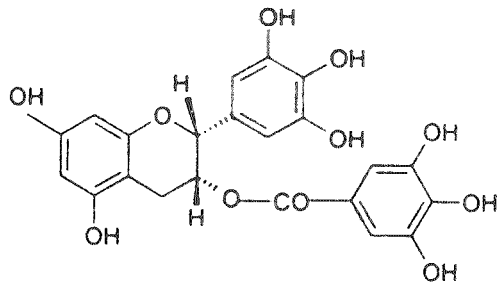
Epicatechin (EC)



Epicatechingallate (ECG)



Epigallocatechin (EGC)



Epigallocatechingallate (EGCG)

Fig. 1. Structures of polyphenols

EXPERIMENTAL

HPLC: Shimadzu-Class VP Software; Column: C_{18} Column; Detector: UV 280 nm Mobile phase: Binary gradient, A: 0.05% acetic acid and B: 100% acetonitrile; Flow rate: 1 mL/min; Injection volume: 20 µL

Acetic acid, acetonitrile and methanol of HPLC grade were used. Double distilled water (all glass assembly) was used throughout the work.

Two samples of green tea extract named GT-I and GT-II were procured from two different local herbal extract agencies. Total green tea extract (GTE) obtained from Sami Labs, Bangalore, containing known amounts of EGC (4.2%), EC (10.1%), EGCG (45.5%), GCG (2.97%), ECG (18.2%), catechins (1.6%) and caffeine (2.5%) was used as a standard.

Standard preparation: 50 µg of the standard green tea extract was dissolved in 50 mL of methanol, 2 mL of acetonitrile was added to it and the volume was made up to 100 mL with distilled water.

Sample preparation: 5 g each of GT-I and GT-II were soaked separately in 50 mL of distilled water by keeping overnight. These were then centrifuged and supernatant was collected separately and made up to 100 mL with distilled water. The aqueous solution was extracted with chloroform (25 mL × 3 times) to remove caffeine. Aqueous layer was extracted with ethyl acetate (25 mL × 3 times). The combined ethyl acetate fractions were concentrated under vacuum at 50°C to dryness[8]. Residue was dissolved in 25 mL of methanol. 0.10 mL of this methanolic solution was diluted to 10 mL for HPLC analysis.

RESULTS AND DISCUSSION

In the present work we have used standard green tea extract containing known amount of polyphenolic units to standardize two samples of green tea extracts obtained from two different local herbal extract agencies.

Green tea extract GT-I yielded maximum amount (74.75%) of total constituents and accounted for highest EGCG content (48.54%). GT-II yielded 63.07% of total constituents with 37.76% of EGCG and relatively higher ECG (19.57%) content compared to GT-I. A typical chromatogram of standard Green tea extract is shown in Fig. 2.

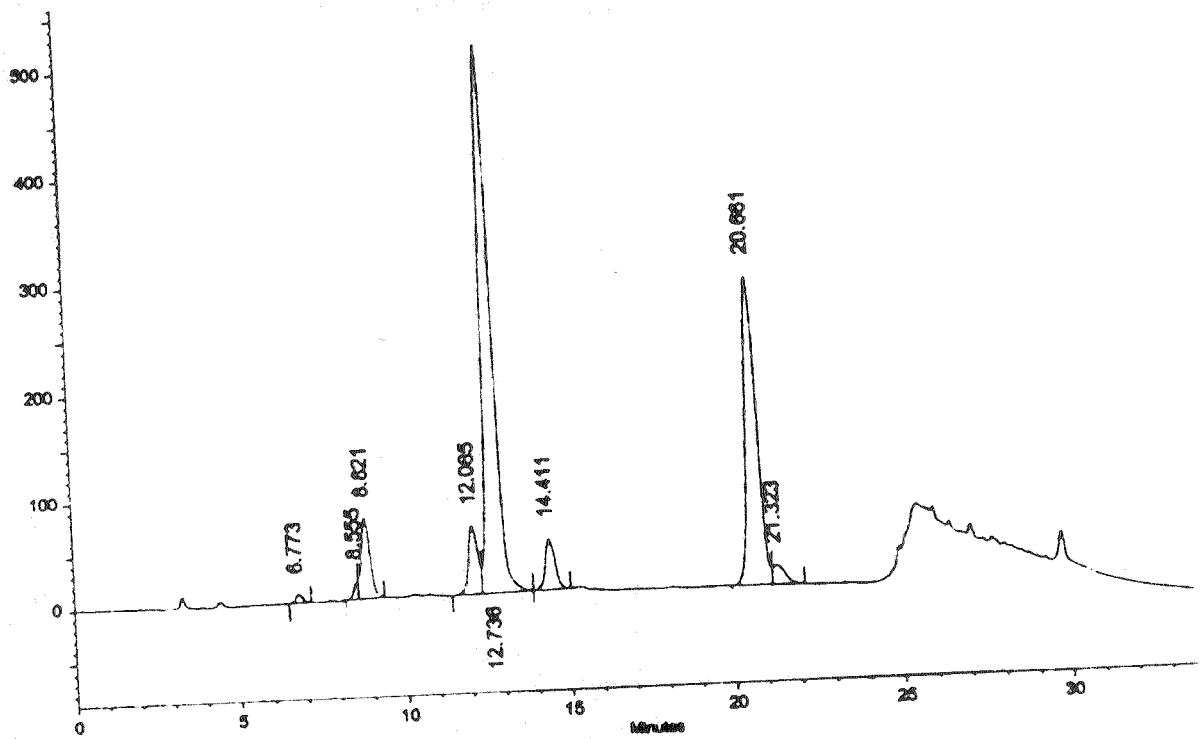


Fig. 2. Chromatogram of standard green tea extract

TABLE-1
PERCENTAGE OF POLYPHENOLIC UNITS IN STANDARD GREEN TEA EXTRACT [GTS]

Contents	Percentage (%)	Retention time [R_t] (min)
EGC	4.2	6.773
EC	10.1	12.085
EGCG	45.5	12.736
ECG	18.2	20.661
Total	78.0	—

A typical chromatogram of green tea extract [GT-I] is shown in Fig. 3. The result revealed that GT-I shows highest content of EGCG compared to GT-II.

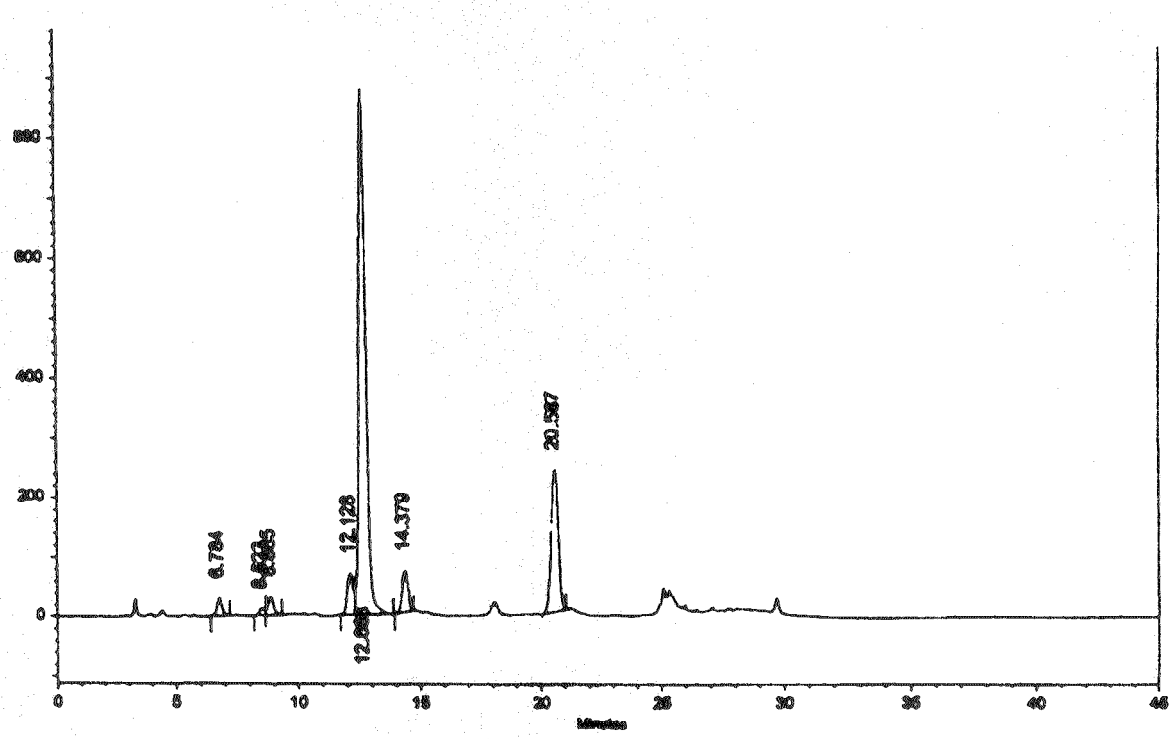


Fig. 3. Chromatogram of sample green tea extract [GT-I]

TABLE-2
PERCENTAGE OF POLYPHENOLIC UNITS IN SAMPLE GREEN TEA EXTRACT-I [GT-I]

Contents	Percentage (%)	Retention time [R_t] (min)
EGC	10.6	6.784
EC	6.59	12.128
EGCG	48.54	12.683
ECG	9.02	20.587
Total	74.75	—

TABLE-3
PERCENTAGE OF POLYPHENOLIC UNITS IN SAMPLE GREEN TEA EXTRACT-I [GT-II]

Contents	Percentage (%)	Retention time [R_t] (min)
EGC	2.30	6.731
EC	3.44	12.085
EGCG	37.76	12.597
ECG	19.57	20.512
Total	63.07	—

This HPLC technique was used to achieve distinct separation of all the polyphenolic units in green tea extract. A review of all the pharmacopoeias including phytotherapy monographs, WHO monographs show that all recommend

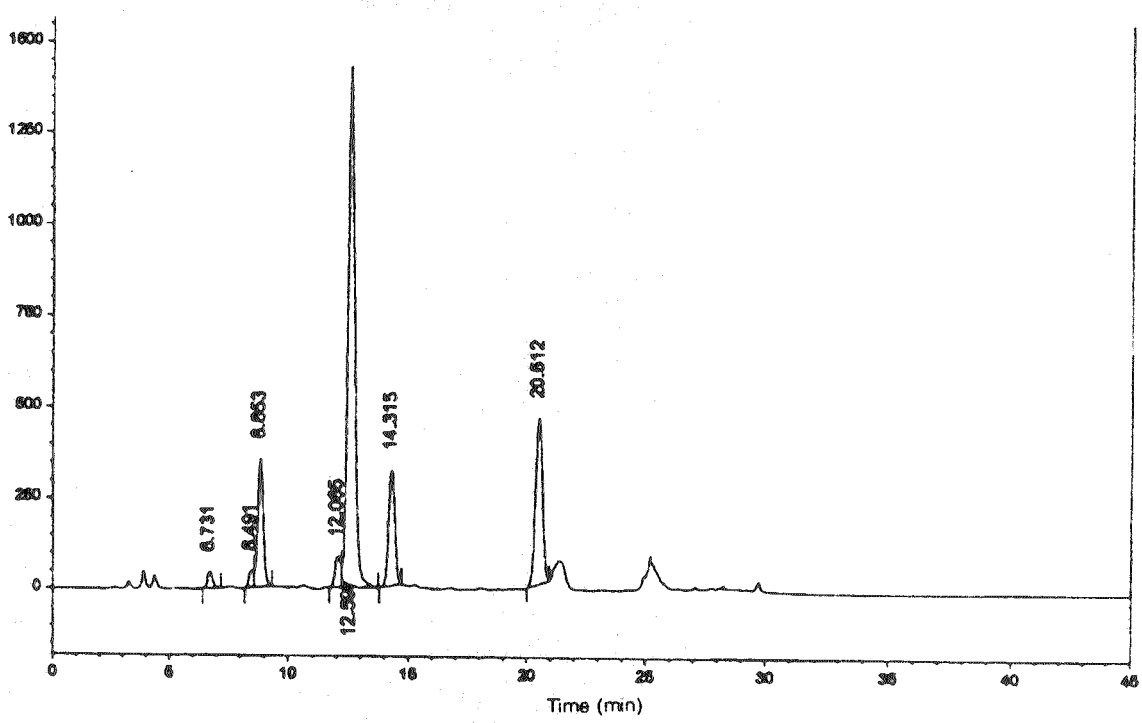


Fig. 4. Chromatogram of sample green tea extract [GT-II]

marker compound testing. Our pharmacopoeias, however, do not recommend the same as chromatographic techniques, as yet not used in our pharmacopoeia[9]. Standardization using marker compound is one of the best methods of standardizing herbs and herbal preparations[10]. Hence, the proposed HPLC method can be used to standardize green tea extracts for polyphenolic contents.

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