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Assessment of the Antioxidative Composition Present in Different Teas by Using Hplc Method

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KEYWORDS

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ABSTRACT:

Antioxidants are bioactive compounds present in various types of teas that are known for their potential health benefits. This study aimed to investigate the concentration of antioxidants in different tea varieties through the application of High-Performance Liquid Chromatography (HPLC) analysis. Samples of green tea and black tea were subjected to extraction and HPLC analysis to quantify the levels of key antioxidants, including catechins-Epicatechin (EC), Epigallocatechin (EGC), Epicatechin-3-gallate (ECG), Epigallocatechin-3-gallate (EGCG)). The teas were brewed under controlled conditions to simulate typical consumption practices. HPLC reveals the presence of vast varieties of antioxidants in them particularly catechins, theaflavins, thearubigins, flavonoids, and polyphenols etc. This study concluded that indian teas and herbal infusions have potential to provide several benefits for human health, due to polyphenolic compounds present in them.

Introduction

Tea is one of the most consumed beverages globally, renowned not only for its diverse flavors but also for itspotential health benefits. Among the many health-promoting compounds found in tea, antioxidants play a pivotal role in safeguarding the body against oxidative stress and associated diseases. The basis of different technological process, we perceive green and yellow teas as non-matured, oolong and white teas as semi-fermented, and black teas as completely aged (Zhang, et.al. 2018). During the oxidation interaction associated with the maturation of black tea, oxidation items, for example, theaflavins and thearubigins are created. These address the primary contrast among black and green tea. Research has shown that dark tea has around 20-30% *Camellia sinensis* polyphenolic content (Dong, et.al. 2009). Antioxidants, such as catechins, flavonoids, and polyphenols, are known for their ability to combat free radicals and reduce the risk of chronic illnesses, including cardiovascular diseases, cancer, and neuro degenerative disorders.(Yang & Koo 2000; Mandel & Youdim, 2004; Butt et al., 2015)

However, the fate of these antioxidants in different types of teas and their bio-availability upon consumption remains a topic of significant interest and research. While green, black, white, oolong, and herbal teas all originate from the Camellia sinensis plant or other botanical sources, their processing methods, growing conditions, and oxidation levels result in variations in antioxidant content and composition. Additionally, howthese antioxidants are absorbed, metabolized, and excreted by the human body can depend on factors such astea preparation methods and individual differences in metabolism.(Manzocco,et. al. 2012)

This study seeks to explore the fate of antioxidants present in different teas, including green tea, black tea, white tea, oolong tea, and various herbal infusions. To accomplish this, we employ the High-Performance Liquid Chromatography (HPLC) method, a powerful analytical technique widely used to separate, identify, and quantify compounds in complex mixtures. By utilizing HPLC, we can accurately measure the concentrations of specific antioxidants in tea samples, both before and after

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preparation, and assess how they change during steeping or brewing. (Hayat, et.al. 2015,Bobková, et.al.2021)

Green tea and black tea are the most consumed beverages worldwide, and their antioxidant properties are well investigated (Almajano, et.al. 2008, El-Shahawi, et.al. 2012, Namal Senanayake, et.al. 2013). On the other hand, some traditional or folk teas from various edible plant leaves are also popular in Asia (Hayat, et.al. 2015, Frei, et.al. 2018, Jin, et.al. 2016).Tea leaves comes from the *Camellia sinensis* plant and it is classifiedinto four major class i.e., white tea, black tea, green tea and oolong tea. The traditional manufacturing processof tea is similar to green tea, and people also usually drink tea by soaking it in boiling water as a health beverage. However, the growing environment of tea is different, and there is no standard for manufacturing process. Recently, it has been reported that the antioxidant composition of teas are affected by geographical location, plantation elevation, and leaf grades (Zhang, 2018). To clarify whether the antioxidant composition of tea are affected by the geographical locations , we chose tea samples from local markets and agencies in this study. First, we used different solvents to optimize an efficient extraction method to obtain the major compounds in tea were then determined by high-performance liquid chromatography (HPLC).

Normally, consumers all over the world enjoy tea by brewing tea leaves in hot-boiled water for 2 to 3 min, and usually the time for brewing tea is not sufficient to extract the complete phenolic content from the tea leaves. Spent tea leaves or tea wastes are the tea leaves left after the preparation of the beverage.(Yang, et.al. 2009) Spent tea leaves could be a source of natural polyphenols, because it might contain residual phenolic compounds that have not been completely extracted from the tea leaves during the brewing process. Nevertheless, spent tea leaves have received little attention on their bio active compound and bio-activities. (Wang, et.al. 2016)

Understanding the fate of antioxidants in different teas is crucial for consumers seeking to maximize the health benefits of their tea consumption. It can also inform the tea industry about optimal processing techniques to retain antioxidant levels in tea products. Furthermore, this research may contribute to the development of personalized tea recommendations based on individual antioxidant needs and preferences, ultimately promoting healthier life styles and reducing the risk of chronic diseases.(Liu. et. al.(2009)

Chemical Compounds in teaPolyphenols

In steeped teas, polyphenols are largely responsible for astringency, a taste experience that causes a drying sensation on the tongue and bitterness. The term polyphenol simply refers to a categorization of compounds composed of many phenolic groups, hence the name poly-phenol. These compounds are plant metabolites produced as a defense against insects and other animals, and they are the most abundant compounds in tea. Polyphenols comprise as much as 30–40% of freshly plucked tea leaves and solids in tea liquor. (Chan, et.al. 2010) Polyphenolsare derived from amino acids via sunlight, and therefore tea grown in the shade has a smaller concentration of polyphenols and a higher concentration of amino acids. (Frei, et.al. 2018) The bud and first leaf have the highest concentrationof polyphenols, and polyphenol levels decrease in each leaf moving down the plant. There are an estimated 30,000 polyphenolic compounds in tea.There are several known categories within polyphenols. (Shahawi, et.al. 2012)

Flavanoids are arguably the most important category;they are the reason for many health claims surrounding tea because they contain antioxidants.

Within the flavonoid group are flavanols, flavonols, flavones, isoflavones, and anthocyanins. Flavanols (short for flavan-3-ols) are the most prevalent and thus the most studied. Flavanols are often referred to as tannins or catechins. The major flavanols in tea are: catechin (C), epicatechin (EC), epicatechin gallate (ECG), gallocatechin (GC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG)(Yashin et al., 2015). EGCG is the most active of the catechins, and this flavanol is often the subject of studies regarding tea antioxidants y (Zeeb et al., 2000). Flavanols are converted to theaflavins and thearubigins during oxidation. They are the compounds responsible for the darkcolor and robust flavors that are present in oxidized teas. Flavonols, flavones, isoflavones and anthocyanins are thought to contribute to the color of a tea's infusion and its taste.(Kartsova & Alekseeva, 2008)

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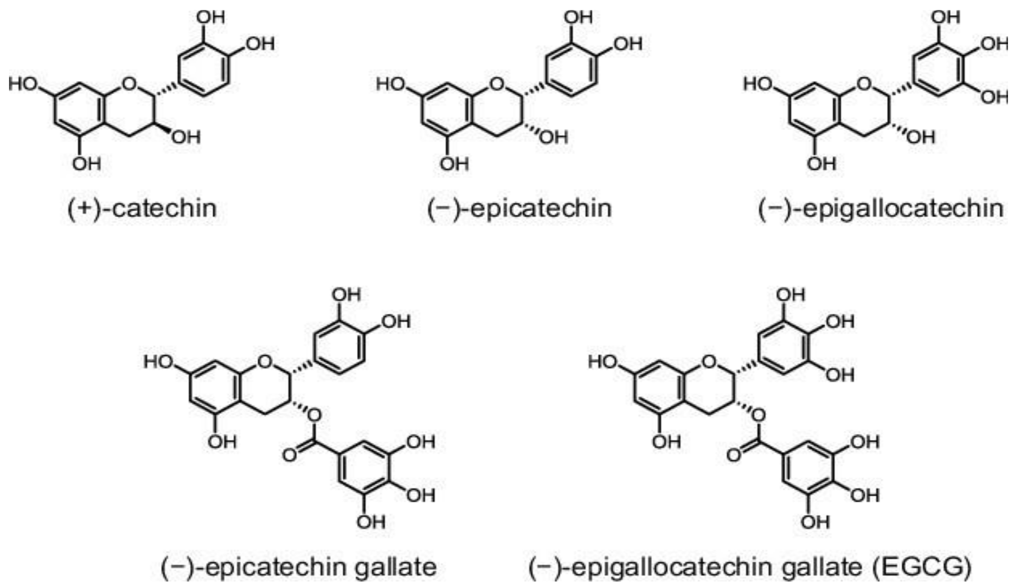

Fig 1: Chemical Compounds of Catechin in teas

MATERIALS AND METHODS

All samples including Green and Black teas were bought from the local markets and agencies. Ultrasonic apparatus were used for the sake of filtration for the HPLC grade solvents through a 0.22 µm hydrophilic membrane. Total Green tea extract (GTE) obtained containing known amounts of epicatechin (EC) 10.1%, epicatechin gallate (ECG) 18.2, epigallocatechin (EGC) 4.2%, and epigallocatechin gallate (EGCG) 45.5% was used as a standard.

ANALYSIS AND EXTRACTION:

Preparation of Standard Solution:

Catechins stock solution of 1000 ppm was prepared by accurately weighing 100.00 mg of pure each catechinsseparately and quantitatively transferring it into 100ml volumetric flask and making it to the mark with the mobile phase. Mix Working standards of 100 ppm were prepared by serial dilution of the stock solution with the mobile phase.

Sample Preparation:

For extraction,0.50g of each tea samples named GT_1, GT_2, BT_1, BT_2 were weighed separately into 250ml beakers,120ml of distilled water was added and extracted by ultrasonic apparatus at room temperature for 1 hr, the solution was filtered with a hydrophilic membrane of 0.22 um.The sample was then stirred slowly with a magnetic stirrer for 5 min, cooled, and centrifuged at 12000 × *g* for 10 min at 1 °C. The supernatant was filtered on a 0.22 µm millipore nylon filter before analysis. The standards and the samples were run in the HPLC system. The following were the HPLC Conditions.

HPLC was performed out on a agilent quaternary HPLC system with 1260 quat pump equipped with an auto sampler 1260 ALS and the stainless-steel Column Type and Size: C18 (4.8X150 mm inner dia) .The gradient system consisted of a mixture of Dimethyl Formamide (HPLC) and Methanol (HPLC) . The flow rate was 1 ml/min at a column temperature of 30 °C. A Variable wavelength detector 1260 VWDVL was set having the flow rate: 1 ml/min ,run time: 25 minutes, sample injection volume: 20µl and detector: setat 280 nm. The tea extract was injected directly into the column. Analyses, each in triplicate, were carried out with 3 extracts prepared from 3 different tea bags for each sample.

Mobile Phase: Initially it contains Distill Water (57%), Dimethyl formamide (40%), Methanol (2%), Acetic acid(1%).Assessment and Analysis was maintained by the comparison of the respective chromatographic peak areas of tea samples to peak area of the known amounts of standards.Single peak was analysed by contrasting the time of retention and absorption spectra of the tea samples toward the standards.

This is because, cooling the infusion during centrifugation allows the precipitate of some matter and what is being measured is the compensation of

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compounds during the earlier process and filtration polished the sample for the required analysis.

RESULTS AND DISCUSSION :

Green tea is also known as unfermented tea where it does not undergo fermentation or oxidation process dueto polyphenol oxidase (PPO), which is an enzyme that exist in tea leaves, and can be deactivated by steaming. (M Skerget, et.al.2005)Therefore, most of the catechins are preserved and green tea is believed to have the greatest number of total phenolic and flavanoid contents. Furthermore, previous study unveiled that the chemical composition of green tea differs very little from that of the fresh leaves because of being unfermented. (Lee, et.al.2004).

In contrast, black tea or fermented tea contains a mixture of catechins, theaflavins, and thearubigins, and produced when the tea leaves undergo a complete fermentation process. As a result, PPO enhance the conversion of the catechins into theaflavins, which is a soluble oxidation products. Fermentation of black tea is accelerated when the PPO enzyme is released during rolling step (Wheeler, et.al.2004).

HPLC analytical aspects

The HPLC in house method was adapted after various earlier described procedures for the quantitave analysis of catechins. (Verma et. al.) Analysis of the vulnerability of retention times of blank and various tea samples column temperature in the range of 25℃ to 35℃ reveals that for suitable chromatographic peak 30℃ is optimum for the separation .

Mobile phase consists Distill **Water** (57%), **Dimethyl formamide** (40%), **Methanol** (2%), **Acetic acid** (1%) and prepared in linear isocractic modes fortunately separated the described four catechins.The method reported linearly over the retention time, area, percentage area and height of all four samples are given .

Blank

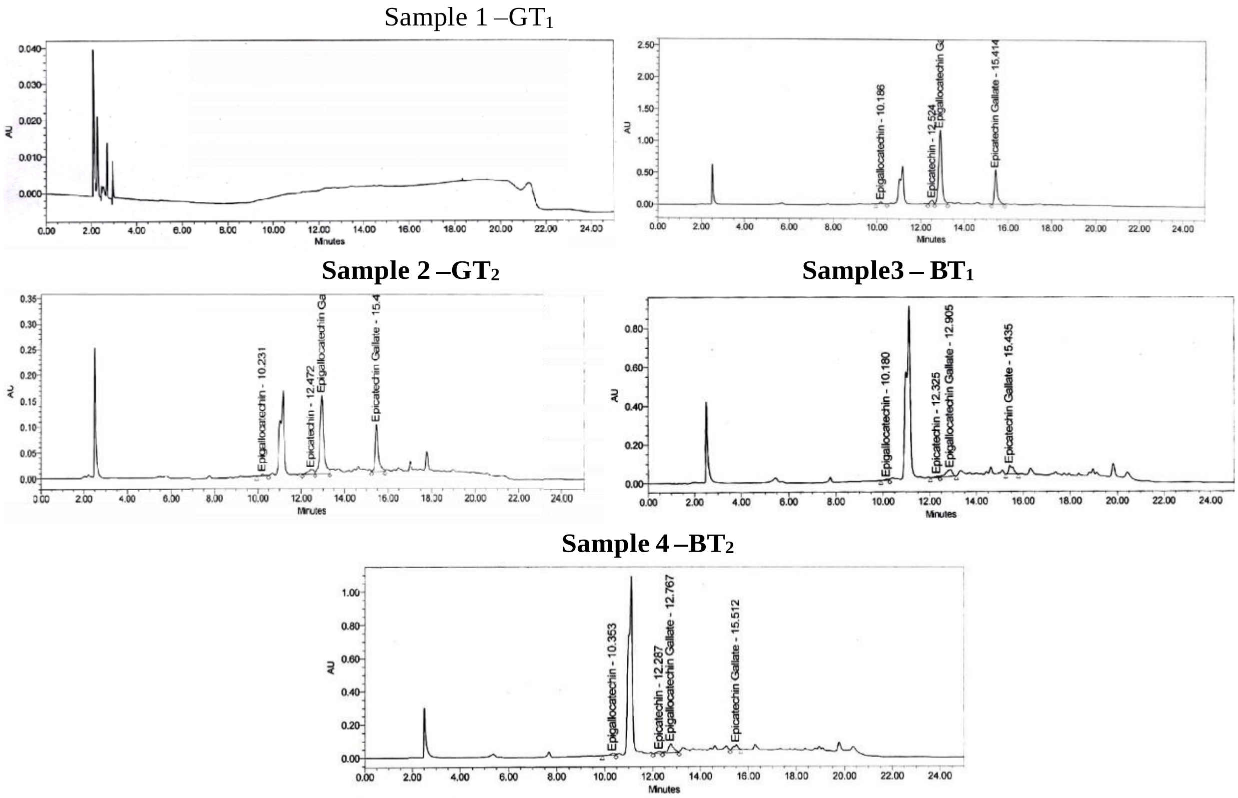

Fig. 2 High performance liquid chromatography (HPLC) at 280 nm of blank, Green tea and Black tea samples

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(6), 2333-2339 | ISSN:2251-6727

	RT					Area				% Area				RT			
	Blank	**GT-1**	**GT-2**	**BT-1**	**BT-2**	**GT-1**	**GT-2**	**BT-1**	**BT-2**	**GT-1**	**GT-2**	**BT-1**	**BT-2**	**GT-1**	**GT-2**	**BT-1**	**BT-2**
Epigallocatechin (EGC)	10.127	10.019	10.231	10.180	10.535	252503	42231	70233	163961	1.40	10.44	5.04	11.50	34060	3759	5306	11368
Epicatechin (EC)	12.462	12.524	12.472	12.325	12.287	650646	152346	77377	125721	3.60	5.18	5.56	8.82	67117	8121	5873	9248
Epigallocatechin-3-gallate (EGCG)	12.852	12.878	12.972	12.905	12.767	12281867	1869590	636394	797803	167.99	63.55	45.69	55.95	1161790	151376	33859	53299
Epicatechin-3 -gallate (ECG)	15.350	15.414	15.477	15.435	15.512	4878786	877757	608802	338459	27.01	29.84	43.71	23.74	543648	92033	44649	29183

Table – 1 HPLC blank, Green tea and Black tea samples listed in order of retention time, Area,

% Area and Height.

The results from HPLC analysis showed that the value of catechin in black tea is lower than Green tea. The value of retention time ranges from 10.019 to 15.512 and area covers the value from 42231 to 12281867 while % area ranges from 1.40 to 167.99 and height have the covered values from 3759 to 1161790.

% of dry weight of various illustrated catechins of the taken samples of Green and Black tea with standards are q uantitative and ranged from 0.006 % to 7.68 % for epicatechin (EC) , epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) are given in Table 2.

CATECHINS	Sample -1	Sample -2	Sample -3	Sample -4
Epicatechin (EC)	0.75	0.18	0.09	0.06
Epigallocatechin (EGC)	3.14	0.54	0.87	0.93
Epicatechin-3 -gallate (ECG)	2.37	0.43	0.29	0.18
Epigallocatechin-3-gallate (EGCG)	7.68	1.19	0.40	0.42

Table – 2 The % dry weight of few catechin of various tea samples analysed by High performance liquid chromatography (HPLC)

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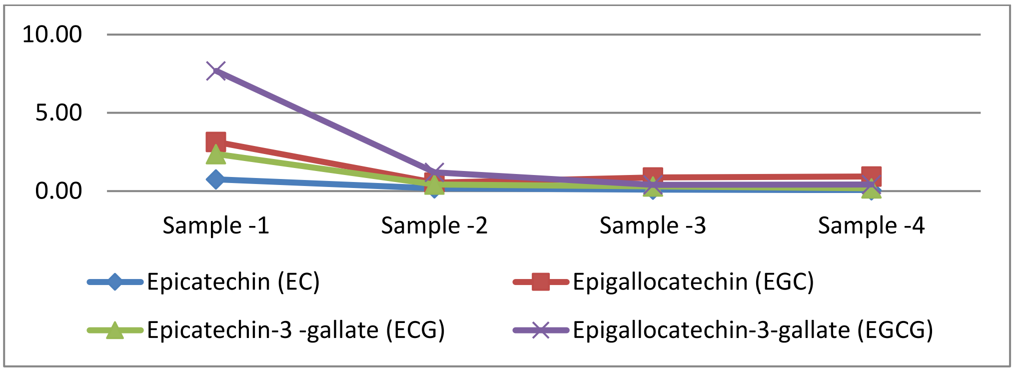

Fig. 3 Quantitative analyses of taken four catechins wrt % dry weight in Green and Black tea samples by High performance liquid chromatography (HPLC)

All four catechins are detected in GT and BT and the highest among all the four types of catechin content present in the taken samples are Epigallocatechin-3-gallate EGCG 7.68 and 1.19 (**Fig. 3**) for green tea (sample 1 and 2) while for black tea epigallocatechin (EGC) concentration proves its high value among all i.e. from 0.87 and 0.93 (**Fig. 3**) (sample 3 and 4)

Conclusion:

In the present investigation, HPLC analysis was used to monitor changes in antioxidant levels throughout the brewingprocess. Results indicated that steeping temperature and time significantly influenced antioxidant extraction and degradation kinetics. In conclusion, this study utilized HPLC analysis to assess the fate of antioxidants in different teas during the brewing and storage processes. The results highlighted the dynamics of antioxidants in teas contributes to optimizing brewing practices to maximize their potential health benefits and underscores the importance of selecting appropriate storage conditions to maintain antioxidant stability in tea products. In contrary, this study extend our knowledge about the composition of the sampled teas and provides a better understanding for the selection of teas by sake of the highest content of beneficial compounds. As all of such properties directly correlates to the geographics,post harvesting methods, brewing and fermentation process affects the health benefits of tea.

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JCHR (2023) 13(6), 2333-2339 | ISSN:2251-6727

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