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Comparative Studies on Green Tea and Black Tea of Various Brands Obtained From Commercial Market for its Gallic Acid Content by HPLC Technique

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ABSTRACT

Standardization of herbal drugs is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive quantitative and qualitative values that carry on assurance of quality, safety, efficacy and reproducibility. The objectives of present study is to carry out the estimation of gallic acid which is one of the polyphenolic compound responsible for antioxidant activity from green tea and black tea by High Performance Liquid Chromatographic Techniques (HPLC) to check the content uniformity. The collected tea samples were subjected to aqueous extraction and the extract was used for HPLC analysis using gallic acid as standard. The result reveals that there are lots of variations between the samples and the percentage of gallic acid is not uniform in all the collected samples. The results clearly reveal that the gallic acid content is high in green tea compared with black tea. The highest amount of gallic acid present in the green tea sample is 0.338%w/w and from black tea is 0.187%w/w. From this study it was concluded that the better antioxidant activity of green tea may be due to the presence of high content of polyphenols.

Keywords: HPLC analysis, *Camellia sinensis*, Tea samples, Gallic acid.

INTRODUCTION

According to world health organization (WHO), 70-95% of the world's population rely on traditional medicines for their primary health care and most of practices includes the use of plant extracts or their active components. Usually the natural product is extracted from the source, then concentrated, fractionated and purified, yielding essentially single biological active compounds. It is still routine practice for scientists to investigate medicinal plants just to find the single chemical substance responsible for the therapeutic effect. Considering that the biological activity may be the results of combination of several compounds. In fact, it is already well known that sometimes complex mixtures of compounds in herbal medicines have greater effects than isolated compounds due its synergistic activity[1].

Many phenolic compounds are known to show potent antioxidant activity and a number of naturally occurring melanogenic inhibitors contain a phenol structure. Gallic acid is a naturally occurring polyphenol antioxidant that was recently shown to have potential healthy effect[2]. Gallic acid and its structurally related compounds are found widely distributed in fruits and plants. Gallic acid and catechin derivatives are the important phenolic compounds of both black and green tea. Esters of gallic acid have a diverse range of industrial uses as antioxidants in food, in cosmetics and in pharmaceutical industry[3]. Derivatives of gallic acid have potential biological activities like antibacterial, antifungal, antimalarial, anticancer and neuroprotective activity[4].

Tea (*Camellia sinensis*) is reported to contain nearly 4000 bioactive compounds including polyphenols. Polyphenols are bonded benzene rings with multiple hydroxyl groups present in the form of either flavonoids or non-flavonoids but found in tea are mostly flavonoids. They are secondary plant metabolites derived from the condensation reaction of cinnamic acid with three malonyl-CoA groups[5]. The objective of the present work is to estimate the gallic acid content from green tea and black tea samples obtained from commercial market by HPLC as a part of standardization technique.

MATERIALS AND METHODS

Sample collection

The green and black tea samples were collected from the commercial market, which is marketed by various companies all over the India. The collected tea samples were uniformly crushed by using mortar and pestle and stored in air tight container for further analysis.

Standard preparation

Weigh accurately about 25mg of gallic acid standard and transferred into a 25ml volumetric flask. Added 10ml of 70% methanol and sonicated for 10minutes and then made up to the volume with mobile phase at a concentration of 1mg/ml.

Sample preparation

Weigh accurately about 1000mg of powdered sample into a 25ml volumetric flask and added 10ml of 70% methanol, sonicated for 10minutes and then made up to the volume with mobile phase at a concentration of 40mg/ml.

Chromatographic conditions

Solvent

Water	:	Acetonitrile	:	Trifluroacetic acid
950ml		50ml		0.5ml

Instrument set up

Column	:	Inertsil ODS C18 250cm × 4.6mm, 5µm
Flow	:	1.0ml/min
Detector	:	272nm
Injection volume	:	20µl
Diluent	:	70% methanol and mobile phase

RESULTS AND DISCUSSION

The HPLC analysis of tea samples were carried out obtained from commercial market which is marketed by various companies all over India. We were selected 10 samples out of which five samples are green tea and another five are black tea. Gallic acid is one of the chemical constituent of the *Camellia sinensis* and used as analytical marker for this study. The results of estimation of gallic acid are tabulated in Table No.1 to 3 and Fig 1 to 13.

Table 1: Results of HPLC analysis with respect to retention time of green teasamples.

Name of the Marker	Standard Retention Time	Sample No. allotted	Retention Time of Samples
Gallic Acid	11.178	G1	10.971
		G2	10.947
		G3	11.280
		G4	10.796
		G5	11.016

Table 2: Results of HPLC analysis with respect to retention time of black teasamples.

Name of the Marker	Standard Retention Time	Sample No. Alloted	Retention Time of Sample
Gallic Acid	11.178	B1	11.047
		B2	10.768
		B3	11.012
		B4	10.845
		B5	10.863

Table 3: Results of HPLC analysis with respect to percentage of gallic acid.

S.No	Sample No	Content of Gallic Acid (in %w/w)
1	G1	0.213
2	G2	0.219
3	G3	0.288
4	G4	0.338
5	G5	0.172
6	B1	0.155
7	B2	0.187
8	B3	0.053
9	B4	0.130

10	B5	0.162

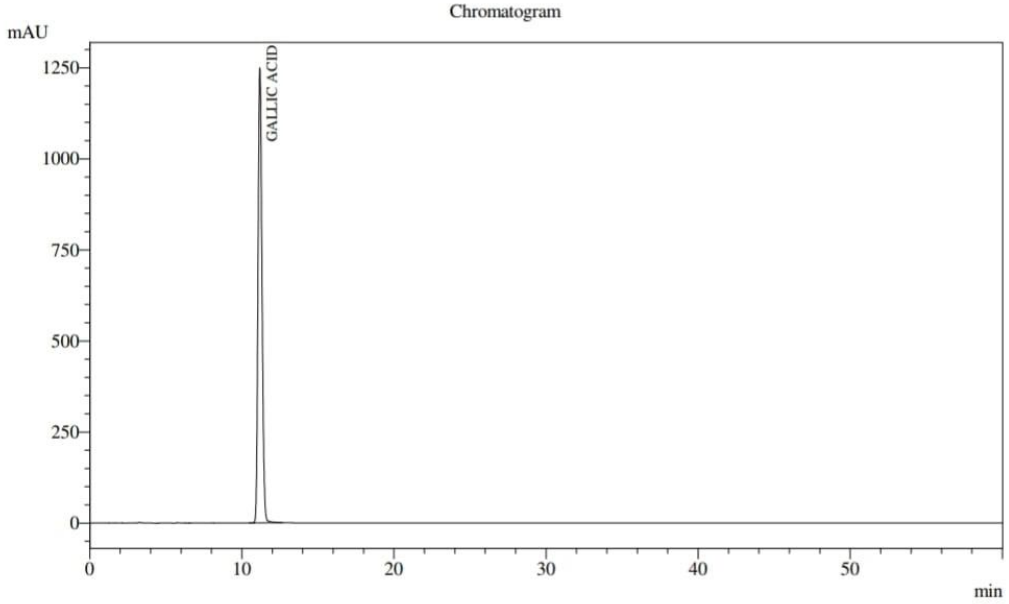

Fig 1: The HPLC Chromatogram of Standard Gallic Acid.

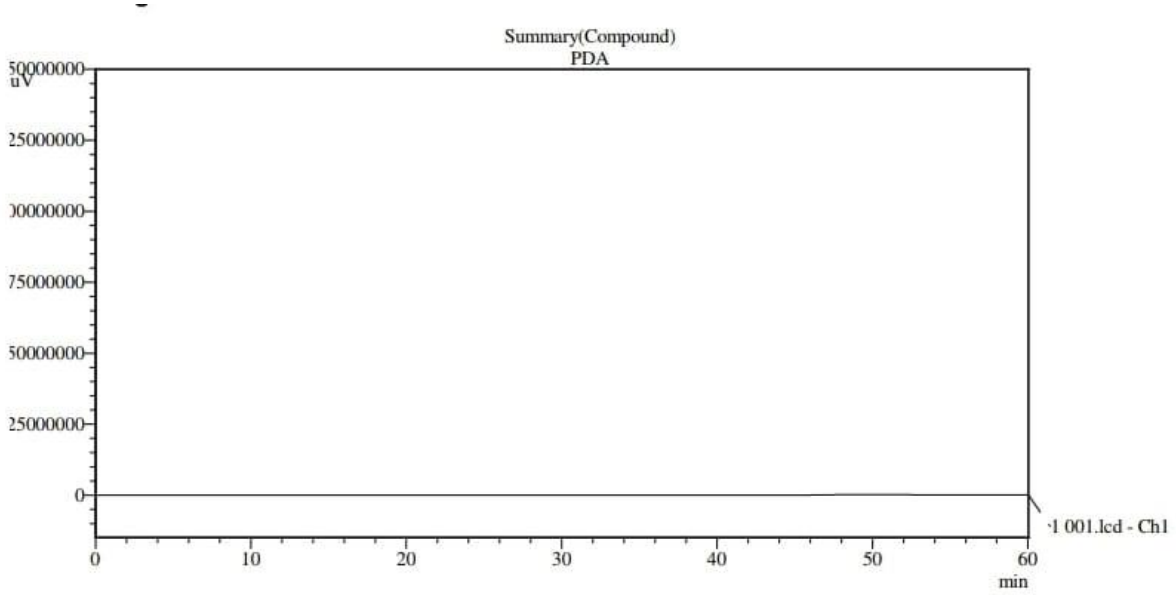

Fig 2: The HPLC Chromatogram of Blank.

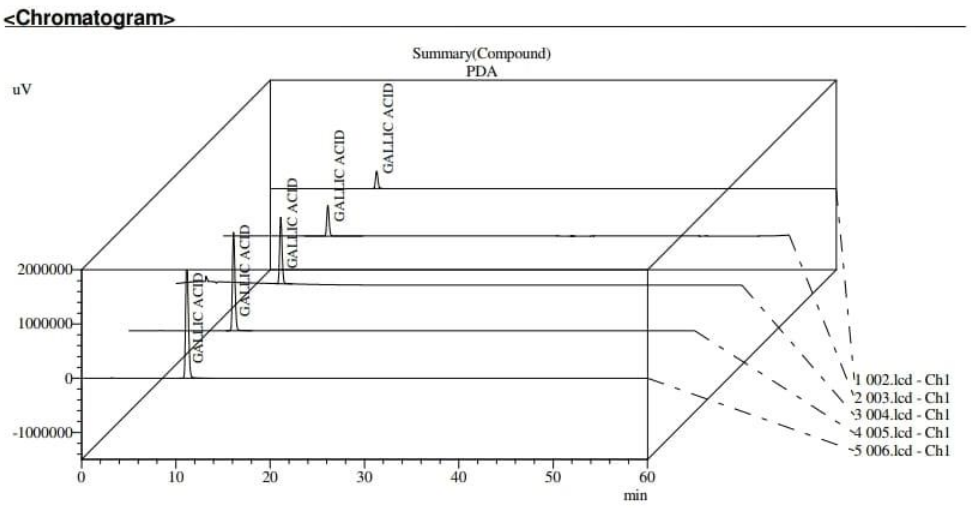

Fig 3: The HPLC Chromatogram of Linearity

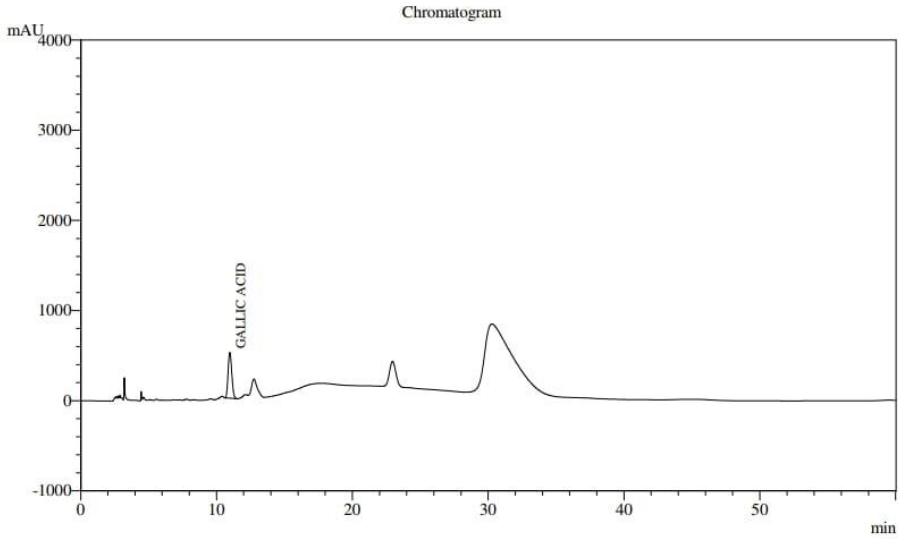

Fig 4: The HPLC Chromatogram of Tea Sample G1.

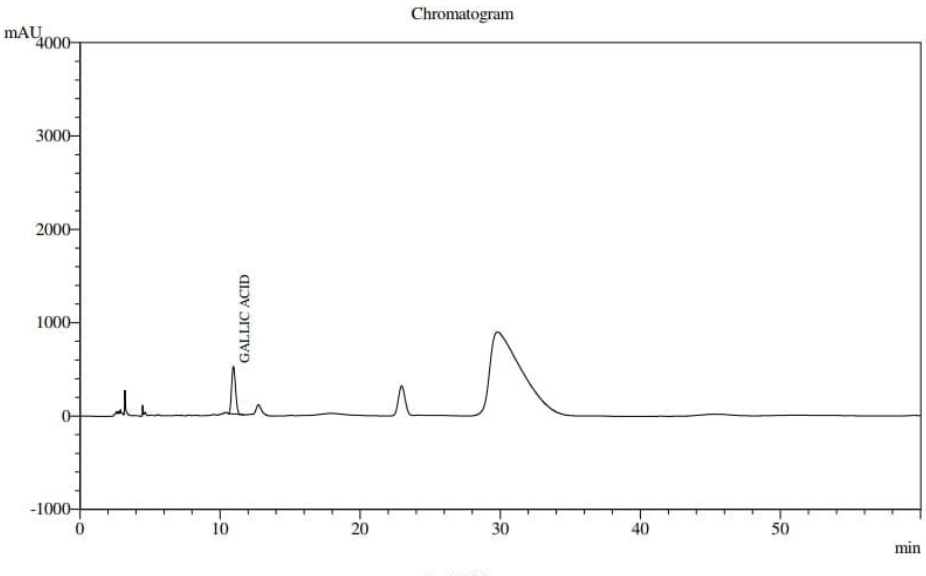

Fig 5: The HPLC Chromatogram of Tea Sample G2.

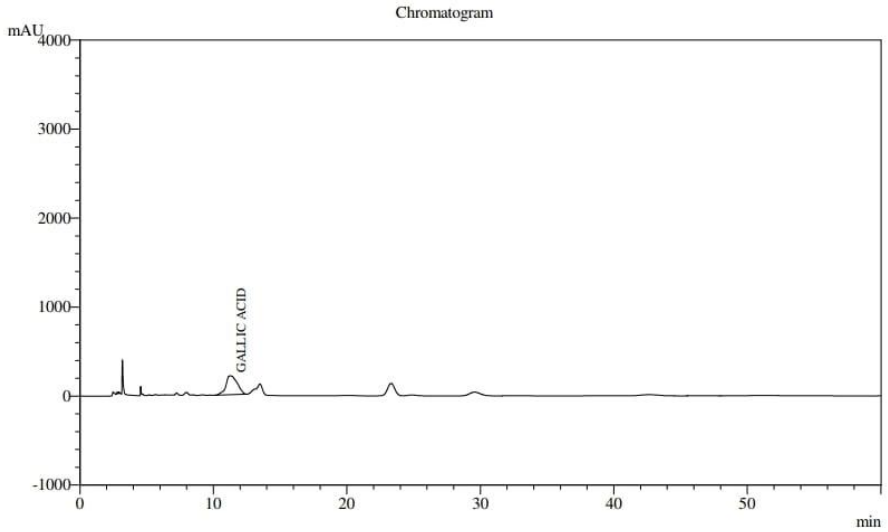

Fig 6: The HPLC Chromatogram of Tea Sample G3.

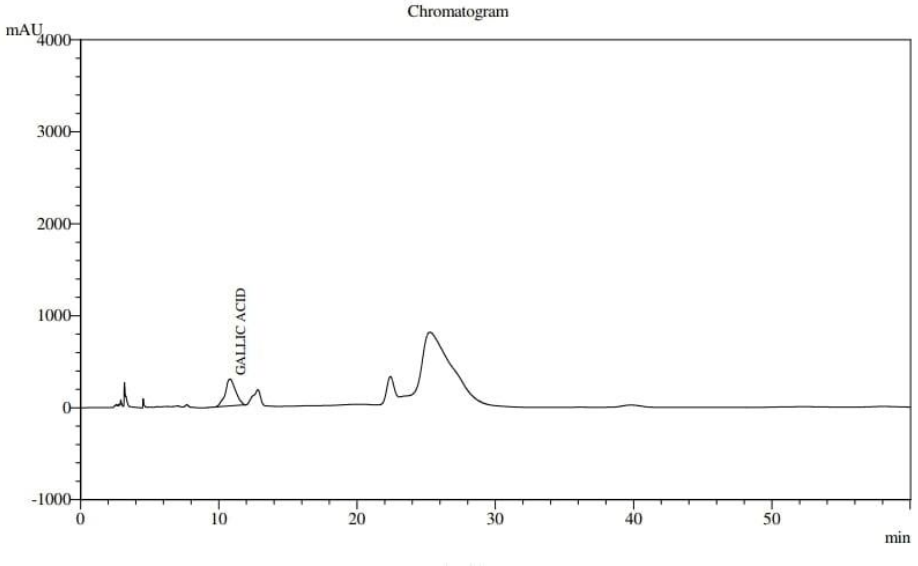

Fig 7: The HPLC Chromatogram of Tea Sample G4.

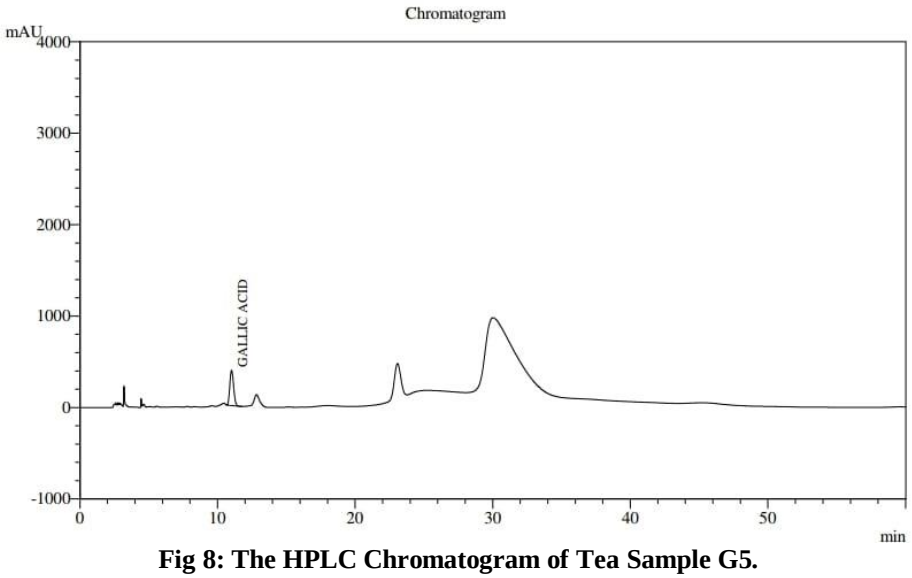

Fig 8: The HPLC Chromatogram of Tea Sample G5.

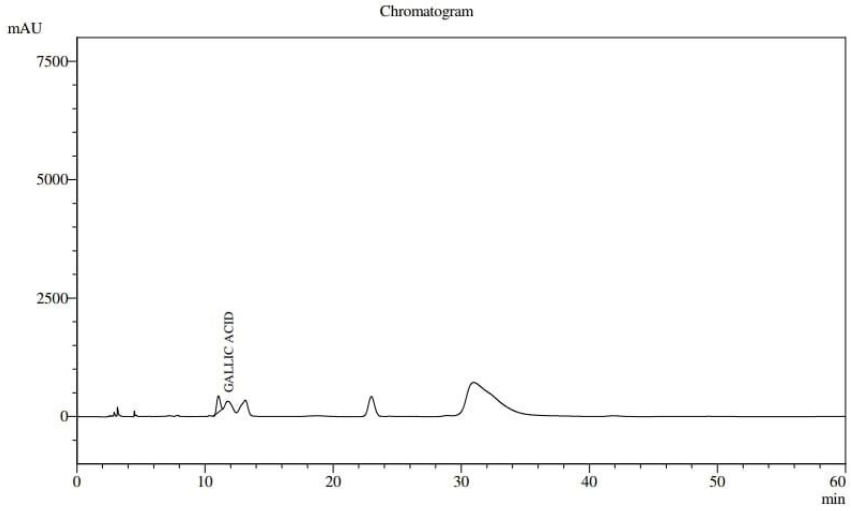

Fig 9: The HPLC Chromatogram of Tea Sample B1.

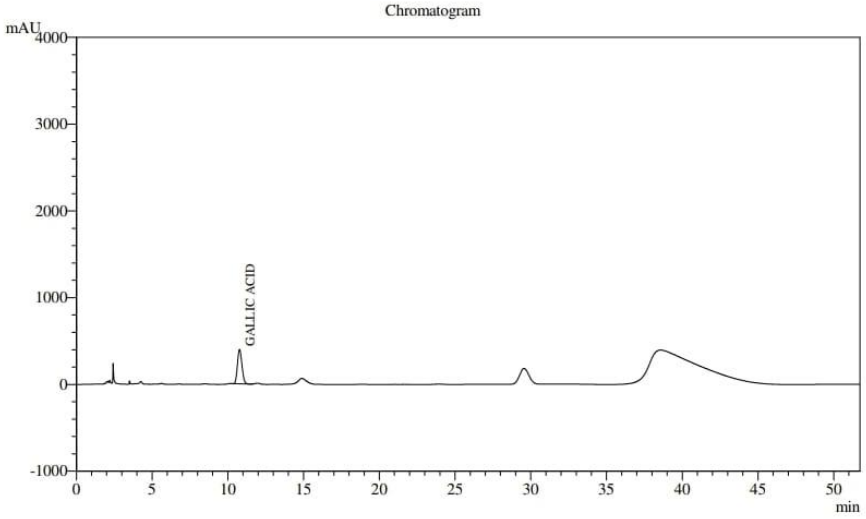

Fig 10: The HPLC Chromatogram of Tea Sample B2.

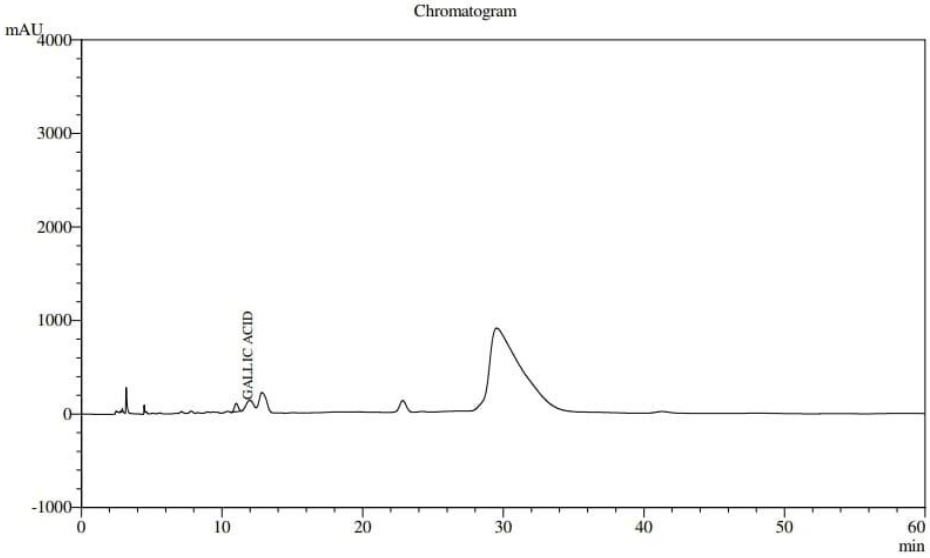

Fig 11: The HPLC Chromatogram of Tea Sample B3.

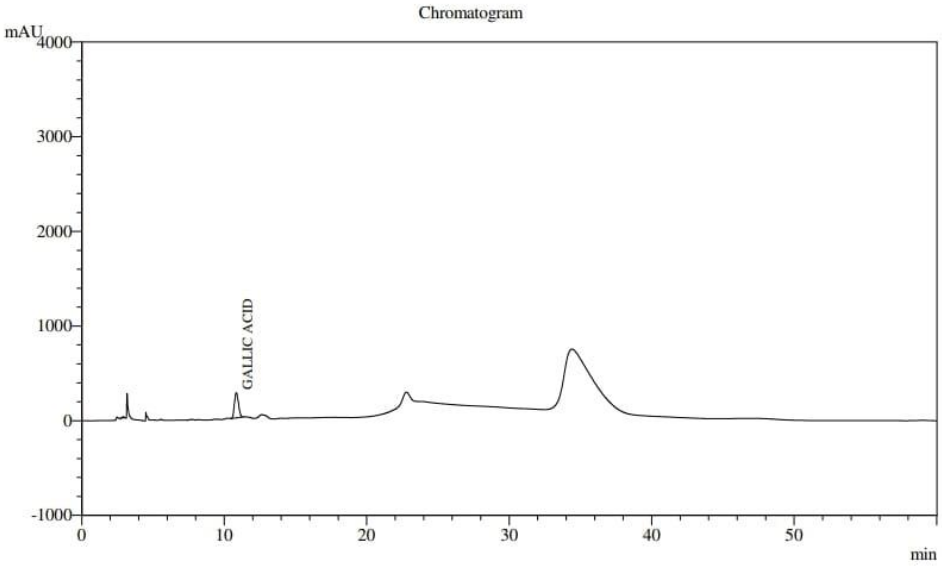

Fig 12: The HPLC Chromatogram of Tea Sample B4.

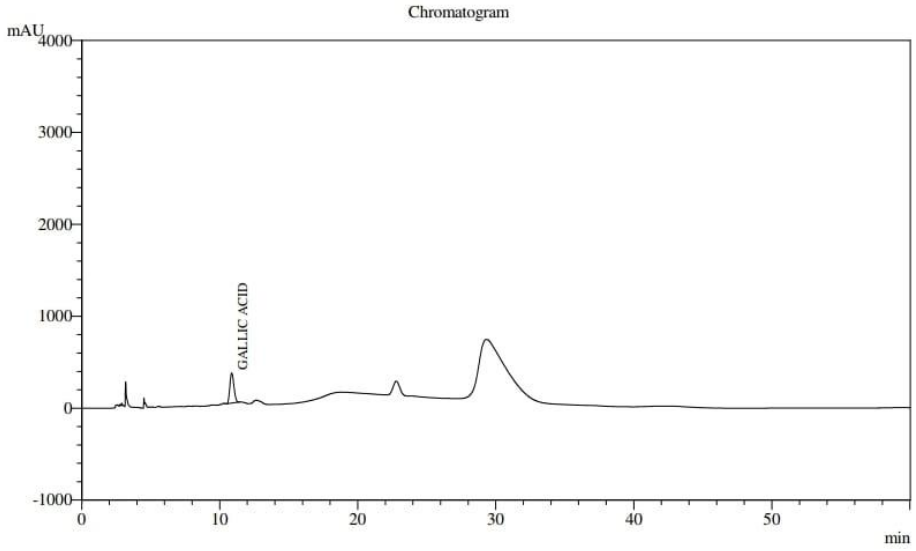

Fig 13: The HPLC Chromatogram of Tea Sample B5.

The retention time of the standard gallic acid was found to be 11.178 and the retention time of gallic acid present in the various collected green tea samples were found to be 10.971, 10.947, 11.280, 10.796 and 11.016 for samples G1, G2, G3, G4 and G5 respectively and confirmed the presence of gallic acid in all the samples. The retention time of gallic acid present in the various collected black tea samples were found to be 11.047, 10.768, 11.012, 10.845 and 10.863 for samples B1, B2, B3, B4 and B5 respectively and confirmed the presence of gallic acid in all the samples. The amount of gallic acid was found to be 0.213%w/w, 0.219%w/w, 0.288%w/w, 0.338%w/w and 0.172%w/w from the green tea samples G1, G2, G3, G4 and G5 respectively. The amount of gallic acid was found to be 0.155%w/w, 0.187%w/w, 0.053%w/w, 0.13%w/w and 0.162%w/w from the black tea samples B1, B2, B3, B4 and B5 respectively.

From the results it was clearly reveals that the gallic acid content is high in green tea samples compared with black tea. The highest amount of gallic acid present in the green tea sample was G4 with 0.338%w/w and the lowest amount present was in G5 with 0.172%w/w. The highest amount of gallic acid present in the black tea sample was B2 with 0.187%w/w and the lowest amount present was in B3 with 0.053%w/w.

SUMMARY AND CONCLUSION

Currently, nutraceuticals are becoming a part of the daily diet, because the current lifestyle could generate many diseases which lead to the scientific community to the search for natural sources of compounds that help to maintain a balance in the consumer health. At present, the consumption of tea increased due to studies reported the number health benefits associated with consumption reducing cardiovascular diseases, action against some cancers, inflammatory diseases, diabetes and weight loss. The present study was carried out to determinate how antioxidant activity varies from the different

brands of green tea and black tea in order to associated the antioxidant activity with the content of flavonoids and phenolic compounds to establish a relationship between the structure and the ability to remove the free radicals. Based on the above facts that we are selected the research work to carry out the comparative studies of black tea and green tea. We were selected five samples of green tea and black tea for the estimation of gallic acid by HPLC analysis. In this study reveals that the gallic acid content is high in green tea compared with black tea. It was concluded that the higher percentage of gallic acid in green tea may be responsible for potent antioxidant activity. The content variation may be due to different climatic conditions and soil variations.

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