International Journal of Advance and Applied Research
www.ijaar.co.in
ISSN – 2347-7075 Impact Factor – 7.328
Peer Reviewed Bi-Monthly
Vol.11 No.3 January – February 2024

Chromatographic, Spectroscopic And Etectroanalytical Analysis Of Extracts Of Indian Tea Samples

Swati Satish Mane[1], Dr. Suprita[2] & Dr. Prakash Arun Bansode[3]

[1]*Research Scholar, Department of Chemistry,*
Shri Jagdishprasad Jhabarmal Tibrewala University, Rajasthan, India
[2]*Professor & Research Guide, Department Chemistry,*
Shri Jagdishprasad Jhabarmal Tibrewala University, Rajasthan, India
[3]*Research Co-guide, Department Chemistry,*
Sangola Mahavidyalaya Sangola, Solapur, Maharashtra, India
Corresponding Author - Swati Satish Mane
DOI - 10.5281/zenodo.11024661

Abstract:

India is one of the major tea producers among various other countries in the world t is a traditionally ancient popular and medicinal beverage in many parts of the world. Tea is Known to contain hundreds of the rapeutically privileged compounds pertaining to plethora of important medicinal properties like anti-carcinogenic, anti-islamic anti diabetic, anti-microbial, ,anti-inflammatory and anti-diuretic etc Hence it is important to determine the precise amounts of bioactive compounds present in India's samples the present investigation involves determination of presence and amounts of active tea compounds such as polyphenols flavonoids catechins phenolic acids using various instrumental techniques such as Fourier Transform infra-red (FTIR) spectroscopy gas chromatography mass spectroscopy (GCMS) highly performers liquid chromatography (HPLC) X-ray Photoelectron spectroscopy (XPS) and Cyclic Voltammetoly (CV) the an analysed tea samples including Indian black tea green tea or found to contain higher amounts of bioactive Compounds in order to justify their medicinal value.

Keywords: Black Tea, Green Tea Camellia Sinesis, Anticancer, Antihypertensive, Antidiabetic.

Introduction:

The quantification of the overall phenolic and flavonoid levels in commercial tea samples is necessary *via* the use of instrumental analysis. The total phenolic and flavonoid makeup of tea are significant markers of its potential health benefits and antioxidant qualities. Nevertheless, the existing techniques used to examine these elements require a significant amount of manual effort, consume a considerable amount of time, and may include the utilization of dangerous chemicals. Hence, to ascertain the overall phenolic and flavonoid levels in commercial tea samples, it is essential to use a quick, precise, and dependable instrumental analytical technique. The

suggested technique has the capacity to overcome the constraints of conventional procedures by generating dependable results at a faster pace and with less use of hazardous chemicals. This approach is capable of effectively assessing a diverse array of tea samples, including different brands and types that are commercially accessible. Tea has been a prominent focus of research in the realms of nutrition and medicine worldwide (1-3).

The reason for this is not only because tea is the second most drank beverage after water, but rather because tea contains bioavailable and more powerful therapeutic chemicals compared to other plants. These chemicals have a wide range of effects due to their widespread presence. Furthermore, tea has a distinctive quality resulting from the intrinsic amalgamation of fragrant and therapeutic constituents. This research is quite compelling and convincing. Tea is composed of a complex chemical structure that includes over 650 different components. The main constituents of tea leaves are mostly polyphenols, making up about 25-35% of the total weight of the dried leaves. The polyphenols found in tea consist of chemicals that may be classified into six distinct classes: The compounds present include anthocyanins, flavones, hydroxyl-4-flavanols, flavonols, and phenolic acids. The bitterness, astringency, and sweetness of the tea's aftertaste are all caused by polyphenolic chemicals. Tea includes flavonols, namely glycosides like quercetin, kaempferol, and myrecetin (4-6).

Black tea production involves the oxidation of polyphenols, resulting in the formation of proanthocyanidin polymers and complexes of gallic acid, such as asinensis and arubigins, as well as catechins and gallic acid complexes. Methylxanthines make up around 2-4% of the makeup, along with small amounts of caffeine, theophylline, and theobromine. The tea plant includes a variety of amino acids, with theanine being the most prevalent and comprising fifty percent of the total amino acid composition. Amino acids undergo degradation as a component of the biosynthesis process responsible for the production of the tea scent. Although not the main constituents of a tea blend, chlorophyll, volatile chemicals, lipids, and carotenoids all play a vital role in the creation of the scent. Tea consists of volatile components such as terpenoids, carotenoids, linoleic acid, and metabolites resulting from the breakdown of amino acids. In addition, tea includes small amounts of carbs and B vitamins such as C, E, K, and A. Furthermore, tea provides significant quantities of manganese, potassium, and fluoride ions to the diet. Tea polyphenols have strong antioxidant properties. Antioxidants shield the body from the harmful consequences of free radicals, which are endogenously generated by the body (7-9).

Material and Methods:

Material:

Reagents and Apparatus Used:

n-hexane, carbon tetra chloride (CCL4), chloroform (CHCl3), ethyl acetate, acetone, methanol, and ethanol

(from Sigma Aldrich, USA), phytochemical reagents, haematoxylin & eosin stains, 10 % formalin dissecting kits, liver function and lipid profiles kits, mindray BC-2800 auto haematology analyzer, Roche reflotron plus auto analyzer, compound microscope, etc.

Sample Collection and Preparation:

A diverse and inclusive array of commercial tea, including herbal, green, and black variations, from various brands were collected and selected pan to study. The samples were dehydrated in an oven at a temperature of 37°C for a duration of six days. After undergoing the processes of drying, weighing, and blending using a warring blender, the components were then submerged in methanol for a duration of two days before being filtered using Whatman No. 1 paper (10). The methanol was fully extracted by the use of a vacuum evaporator operating at a temperature of 50°C, leading to the creation of a thick and sticky material. The unrefined extracts were measured and kept at a temperature of 4°C before being examined. To ensure the integrity and preservation of the tea samples, it is essential to keep them in hermetically sealed containers, thereby preventing any kind of contamination (11).

Preparation of Tea Powder:

The tea samples were grounded into a fine powder using a grinder or mortar and pestle. The powdered samples were placed into appropriately labelled containers that are sealed tightly in order to facilitate subsequent examination (12).

Figure 1 Powdered samples

Methods:

Analytical instruments play a crucial role in the development and assessment of new goods, as well as in the preservation of the environment and public health. It is used for research and development, process optimization and control, quality assurance of end products, testing the quality of raw materials, including compounds used in integrated circuit chips, and detecting and estimating pollutants to assure the safety of foods, drugs, water, and air. A considerable number of contemporary devices are operated by computers or microprocessors and have user-friendly software for the collecting, modification, and display of data. This chapter presents a comprehensive review of the wide range of analytical instrumental methods that are used in different sectors. Methods such as molecular spectroscopy, sensors, X-ray diffraction, and scanning electron microscopy may be used for analytical purposes (13, 14).

FT-IR Analysis:

The analysis of tea sample was carried out using spectroscopy.Therefore, the expected ability of plant extracts to serve as antioxidants may be accurately

and quickly assessed by using FT-IR measurement (15).

GC-MS Analysis:

Each kind of tea samples, weighing ten grams each, were individually crushed in an electric blender until they reached a fine consistency. Subsequently, the pulverized samples were sieved through muslin cloth. After dissolving the fine powder in 100 milliliters of methanol, the extracts were produced using a maceration process that lasted for 48 hours. After the solvent was concentrated at a temperature of 40°C, the finely ground dry extracts produced were used in further experiments. The yield amounted to around 5-6% of the originally harvested tea stuff. The three GT samples examined in this article are appropriately identified and given names that correspond to the states from where they originate. To perform a first phytochemical screening, the plant extract underwent chemical tests using recognized techniques and the methodology of Sofowara (1993), Trease and Evans (1989), and Harborne (1984). The Shimadzu QP 2010 plus mass analyzer was used for the GC-MS analysis. The temperature of the column furnace is set at 100°C, while the injection temperature is set at 250°C. The injection is carried out in the standard manner. The relative percentage amount of each component may be determined by comparing the average peak area to the overall area. Through the use of the National Institute of Standards and Technology (NIST) database, which has more than 62,000 patterns, distinct components were found. The spectra of the known component contained in the NIST library were compared to the spectrum of the unknown component. The test samples were described based on their individual names, molecular weights, and structures (16).

XPS Analysis:

The X-ray Particle Analysis MKIII X-ray phosphoriming system is equipped with a 1253.6 eV Mg K2 X-ray radiation source and functions as an XPS instrument. The ion pump was used to keep the pressure inside the vacuum chamber at $1.33 \times 10{-}10$ kPa. Under these circumstances, a total of one hundred pictures were captured at a resolution of 0.1 eV for further analysis. By using the specified parameters and a pre-established scanning range, binding energy spectra were produced (17).

Cyclic voltammetry (CV):

Cyclic voltammetry (CV) is a technique used to measure the current response of an electrochemical system as a function of an applied voltage that is varied in a cyclic manner. For the experiment, a standardized amount of tea leaves weighing five grams was produced (18). The samples underwent the following preprocessing steps: initially, each sample was infused in 100 mL of boiling distilled water for a duration of 20 minutes; subsequently, a sieve was employed to eliminate the tea leaves; finally, the resulting liquid was cooled to a temperature of 25°C using a water bath, which took approximately 15 minutes. This process was followed before obtaining data from the taste sensors. After adding a 2 mol L^{-1} NaOH solution,

voltage measurements were conducted on the tea samples, resulting in a final concentration of 1 mol L^{-1}.

HPLC Methods for Identification of Tea Extract:

The procedure involves a 50 g sample of fresh commercially available tea by immersing it in 500 mL of water at a temperature of 80C for a duration of one hour. After the filtering procedure, the resulting liquid was concentrated to a volume of 100 mL using rotary evaporation. In order to remove triglycerides, caffeine, and chlorophyll, the concentrated solution was mixed with 50 mL of chloroform. A 100 mL amount of 2 ethyl acetate was used to extract the collected aqueous phase. The tea extracts were obtained by evaporating ethyl acetate at 40 degrees Celsius under decreased pressure. Dehydrated tea extracts were stored in a refrigerated facility. To conduct HPLC and spectrophotometric studies, the tea extracts and particular catechins were diluted in ethanol/water solutions (1:1, v/v) after accurate weighing. Various quantities of working solutions were created by diluting and filtering them through a 0.45 mm Millipore filter before being fed into the HPLC (19).

Result And Discussion:

FT-IR Analysis:

FTIR spectra at 3273 and 3271 an-1 for black and green tea resp indicated presence of hydroxyl group due to flavonoid and polyphenol content. the bands at 1629 and 1637 cm -1 for black and green tea resp indicated the presence of oleifinic double bond present in tea compounds. the bands at 1239-1148 an-1 indicated c-o streching vibration of tea polyphenols.

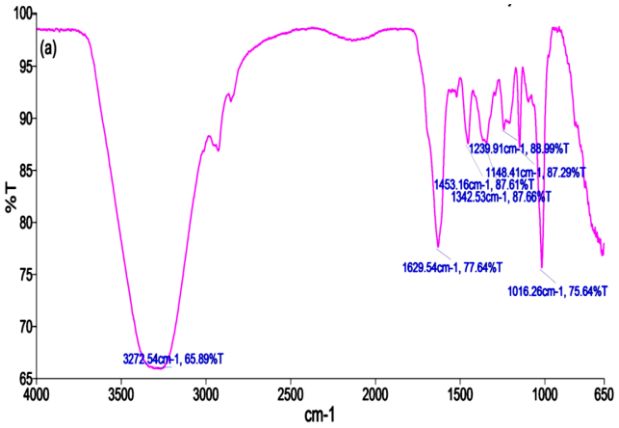

Figure 2 FT-IR spectra of black tea samples

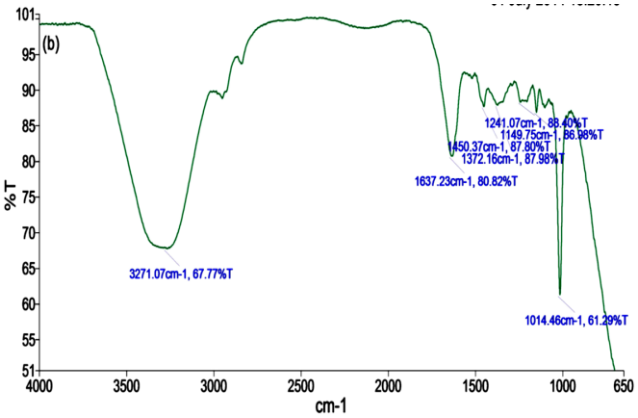

Figure 3 FT-IR spectra of green tea samples

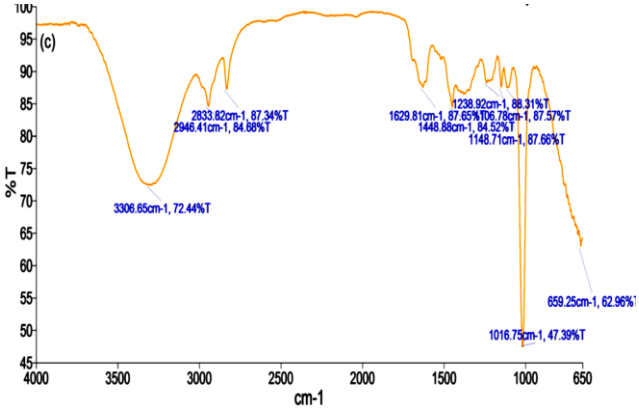

Figure 4 FT-IR spectra of herbal tea samples

Table 1: Infrared vibrational bands of three tea samples

Wave number (cm–1)	**Vibration band/group**	**Chemical compound**
3270 ~ 3320	O–H stretch, H–Bonded	Phenols, alcohols
2946	C–H stretch (Asym.)	Alkanes
	O–H stretch	Carboxylic acid
2833	C–H stretch (sym.)	Alkanes
1629 ~ 1663	C=O stretch (carbonyls)	Flavonoids
		Polyphenols, catechins
	C=C stretch	Aromatics
1449	C–C stretch (in ring)	Aromatics
1239	C–N stretch	Aliphatic amines
1113	C–O stretch	Alcohols, esters, carboxylic acids
1014 ~ 1019	C–O stretch	Alcohols, esters, carboxylic acids
	C–N stretch	Aliphatic amines
	C–OH stretch	Secondary alcohols

GC-MS Analysis:

The qualitative detection of phytochemicals in tea extracts is reported in Table 2, utilizing conventional screening techniques.

Table 2 Phytochemicals screening results of Tea extracts

S. No.	**Phytochemical**	**Result (qualitative)**
1	Tannin	−−−
2	Saponin	−−−
3	Steroids	+
4	Terpenoids	+++
5	Alkaloids	++
6	Amino acids	−−−
7	Glycoside	++
8	Polyphenols	+++
9	Protein	+++
10	Flavonoids	+++
−: Absence, +: Presence, ++, +++: Intensity of color, GT: Green tea		

The GC-MS analysis of the GT samples identified many major phytochemicals that had antibacterial, antioxidant, and other health-promoting properties. These phytochemicals are summarized in Table 3. Table 3 demonstrates that a significant proportion of phytochemicals, many of which have a diverse range of beneficial qualities, were found in all of the commercial GT

samples. GT tea has the greatest concentration of content, with roughly 96%, while ASS tea has the lowest concentration, with approximately 83%. The significant presence of biologically active compounds highlights the crucial role of GT as the ultimate superfood.

Table 3 Phytochemicals in the Tea samples

Compounds	**Peak area (%) of Tea samples**		
	Black tea	**Green tea**	**Herbal tea**
Pyrogallol	18.40	16.28	28.36
Quinic acid	13.96	9.64	1.67
Caffeine (alkaloid)	46.25	57.17	56.48
Xanthine (alkaloid)	3.00	3.25	4.33
Palmitic acid	2.90	1.53	2.11
Palmitic acid	-	1.19	1.03
Phytol ethyl ester	1.34	-	-
α-linolenic acid	5.39	-	2.07
Total	91.24	89.06	96.05
-: Not detected, GC-MS: Gas chromatography-mass spectroscopy			

Six commercially available teas from India were analyzed by GC-MS in an effort to identify the primary phytochemicals that might provide health benefits. The results of the study revealed that the tea samples contained a significantly high concentration of various phytochemicals, each of which possessed numerous health-promoting properties.

XPS Analysis:

X-ray photoelectron spectroscopy (XPS) was used to examine the surface chemical composition and structure of both the leaf and root of the tea plant. The analysis revealed that the surface mostly consisted of four elements: carbon (C), oxygen (O), nitrogen (N), and aluminum (Al). Additionally, the underside of the leaf included small amounts of phosphorus (P) and fluorine (F). To classify the binding energy of C(1s) on the leaf surface, we used the online standard spectrum data library, findings from a wood XPS study, and a combination of line Gaussian and Lorentzian methods. These were used in conjunction with the structure and molecular composition of the botanic epidermis. The earliest variant was denoted as C1, which refers to lipid molecules like wax and cutin. Its electron binding energy is 285 eV. C2 is mostly linked to cellulose and is formed by a single carbon-oxygen bond (C-O). It exhibits adaxial binding energies of 286.35 eV and abaxially 286.61 eV.

The investigations on N (1s) (399-401 eV) and O(1s) provided confirmation about the nature of the C=O group in proteins, namely that it was an acyl group located at position C3. The binding energy of this group was found to be around 288 eV (288.04 eV on the adaxial side and 288.09 eV on the abaxial side). The root surface had a C5 type with a binding energy of 283.32 eV, similar to the substances wax and cutin (C1, 285 eV), cellulose (C2, 286.49 eV), and protein (C3, 288.78 eV) that were also identified on the leaf. It was hypothesized that carbon was building a chemical bond with

metal owing to its reduced electronegativity compared to C1. This indicates that the organic acid released by the root is found on the surface of the root without being chemically bound to any surface components. In addition, both the leaf and root surfaces did not include C4, a common form of O-C=O found on wood surfaces, known for its high oxidized carbon binding energy ranging from 289 to 289.5 eV. The findings from the isolated spectra of O (1s) confirmed the previously described findings from C (1s). The higher concentration of oxygen groups in the abaxial surface, as seen by the ratio of each kind of carbon, implies that the abaxial surface exhibited stronger chemical reactivity. The root had a higher abundance of oxygen groups and a lower amount of cutin and wax compared to the leaf. This indicates that the root surface had more chemical activity and allowed for a larger movement of solute and water molecules. The hydration values for the protein contents in the root, abaxial, and adaxial areas were the same. A higher level of oxidized aluminum in the root indicates improved capacity to be absorbed, and an Al binding energy over 73.50 eV suggests the existence of oxidized aluminum on the tea plant's surface, which might possibly promote absorption.

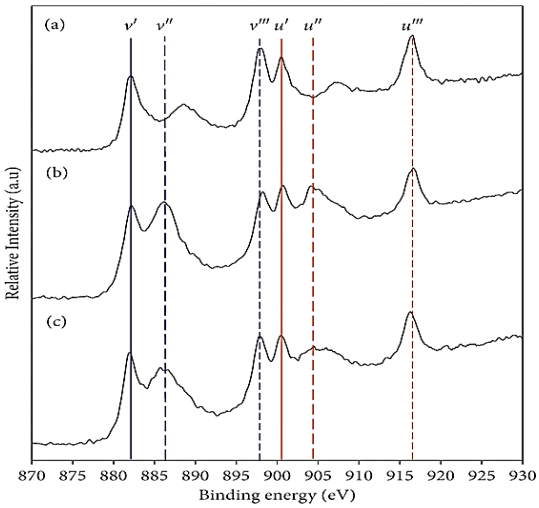

Figure 5 XPS spectra of tea samples

Cyclic Voltammetry (CV):

Electrochemical Characterization of Tea Samples:

The various tea varieties are produced by distinct processing methods. Green tea is produced with unfermented leaves and is rich in monomer flavanols. In contrast, black tea undergoes full fermentation, resulting in the production of Theaflavins and Thearubigins. Through several tests, these phenolic compounds have shown their ability to serve as antioxidants by effectively neutralizing reactive oxygen species and binding to metal ions. Electrochemical analysis has been used to evaluate polyphenols and may provide valuable insights into the physicochemical characteristics of substances. Hence, the first step in our endeavour was to analyze the electrochemical characteristics of the teas. Figure 6 displays several cyclic voltamogram obtained from four electrodes that were modified with metal oxides. These electrodes were used to evaluate two tea samples, which were part of a total of 16 samples tested.

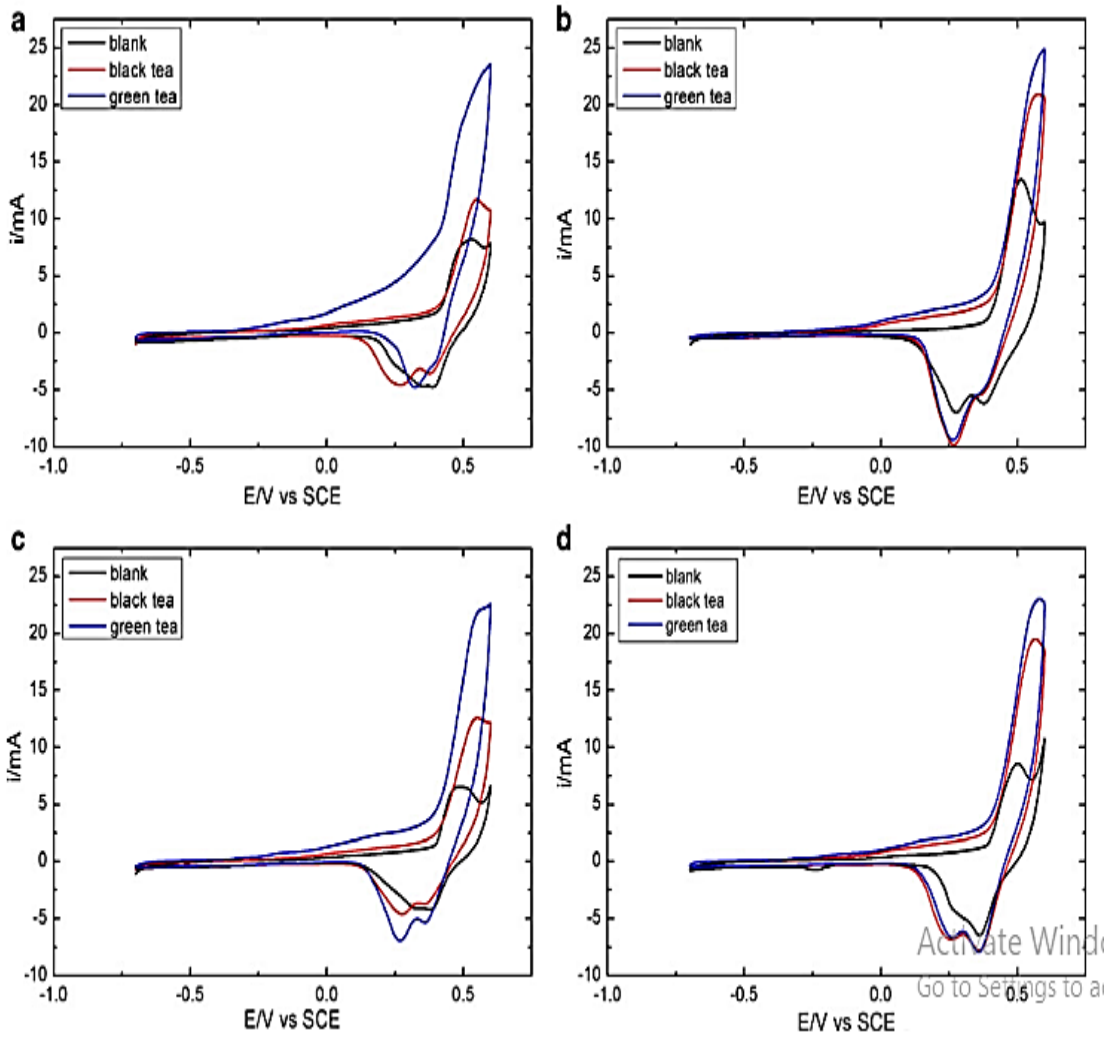

Figure 6 CV scans recorded on different kinds of teas

Comparable are the responses of the four electrodes to both brews. To illustrate, two oxidation-reduction reactions take place at approximately 0.05 and 0.52 V in the anodic region of the majority of cyclic voltammetry (CV) diagrams, with respect to the standard calomel electrode (SCE). Furthermore, a discernible pattern can be observed in the signal at 0.52 V, wherein the peak current exhibits an upward trend as the various teas are scrutinized. To provide greater precision, the maximal current observed in green tea surpasses that of black tea. Conversely, the minimum current is generated by a blank NaOH solution containing 1 mol L^{-1}. However, prior research has demonstrated a distinct correlation between these maximal concentrations and the polyphenolic content of the samples. Variations in phenolic content are observed among distinct varieties of tea. The predominant constituents of black tea are Theaflavins, while green tea is distinguished by the abundance of epigallocatechin gallate (EGCG). Cyclic voltammetry experiments have demonstrated that EGCG demonstrates greater intensities in comparison to Theaflavins. Furthermore,

the overall effect would be attributed to the synergy of all the concurrent constituents, with green tea containing a greater abundance of flavonoids in comparison to black tea. The presence of redox reactions can be observed in the reverse scan of the catholic wave, which exhibits a range of 0.32 to 0.40 V. While there are similarities in the redox processes of the electrodes under investigation, their responses vary in terms of peak intensities and diagram shapes. These differences arise from the interactions that occur between the electrode and the molecules dissolved in the solution under scrutiny. The disparities in peak currents are more conspicuous in Figures 6a and c in comparison to those in Figures 6b and d.

HPLC Method:

Following the completion of first testing, which included the examination of a number of different mixtures of acetonitrile, methanol, and water, the parameters of the HPLC were adjusted to perfection. In accordance with the explanation provided in Section 2.3, the gradient elution method that used the methanol-0.2% acetonitrile system demonstrated exceptional resolution. Figure 8 is a representation of the chromatography of the tea extracts, which reveals the presence of gallic acid and caffeine that are still present. A successful separation of each catechin was achieved within a time window of fifteen minutes, with the exception of C and EGC, which were not separated. Although catechin C is found naturally in tea and tea extracts, its prevalence is often substantially lower when compared to the categories of catechins that are found in other beverages. It is advised that, in order to obtain accurate EGC quantification, the error be minimized by making certain that the peaks of C and EGC coincide to the maximum degree feasible within the range of possibility. Therefore, it is possible to determine the total quantity of catechins that are contained in tea extracts by using the HPLC method that has been specified for this purpose. Several different standard catechin solutions were investigated in order to determine whether or not the HPLC test was linear. The formulation of calibration curves was accomplished by using peak areas as a function of concentration. Presented in Table 4 are the results of the regression analysis that was performed. In the HPLC readings of the catechins that were tested, a significant linear connection was seen. The correlation values for all of the standards were more than 0.9899. This linearity was seen over the whole concentration range, which was from 3 to 300 mg L^{-1}.

Table 4 Calibration curves of catechins

Compound	Calibration curve[a]	Concentration range (mg L¯1)	Correlation coefficient (R)
EGC	Y = 8.1066X þ 23.866	3–300	0.9998
EGCG	Y = 11.603-330.990	3–300	0.9996
EC	Y = 8.6739X + 13.361	3–300	0.9997
GCG	Y = 14.402+ þ 9.1619	3–300	0.9915
ECG	Y =15.083+ þ 14.155	3–300	0.9899

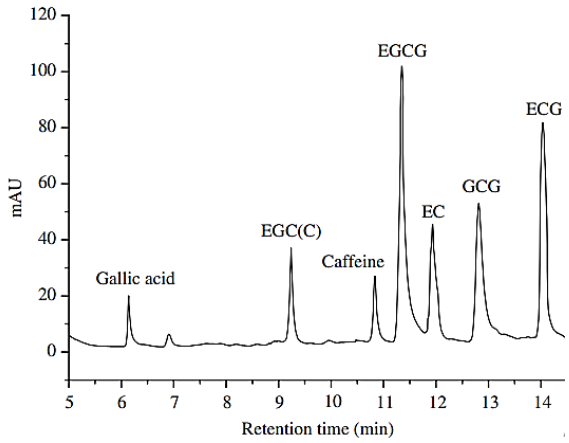

Figure 7 Chromatography of prepared tea extracts

The work focuses on the development and validation of HPLC and spectrophotometric techniques for accurately measuring the amount of total catechins. The HPLC approach demonstrated excellent separation of individual catechins and was determined to be accurate for quantifying catechins.

Table 5 Determination of total catechins in tea extracts.

Content (w/w, %)	93.60
RSD (n = 3, %)	3.29

The HPLC test, using an EGCG calibration curve, provided a straightforward and efficient alternative to the suggested HPLC approach for quantifying the overall content of catechins in tea extracts. Both approaches may be used to regularly measure the total amount of catechins in items that contain catechins.

Summary And Conclusion:

Indian teas are famous all over the world. The unique Flavors, aromas, are due to presence of bioactive components that these teas possess have earned them a well-deserved reputation. This move to a healthier lifestyle is now receiving a lot of attention, and nutraceuticals play a vital role in making this transformation easier to achieve. Scientists are increasingly considering tea as a superior alternative for controlling various common disorders and restoring its historical relevance (20). This is due to substantial research that has been conducted on the subject of harnessing the nutraceutical characteristics of tea over the years. In this study, a comparative analysis of a number of notable teas that may be found in India is carried out, with the main emphasis being on the investigation of the key bioactive components of these teas. In addition, the paper offers a full examination of the medicinal characteristics of these teas, which are used in the efficient treatment of a variety of ailments. In the future, research should place a higher priority on the exploration of additional bioactive compounds, as well as the analysis of the minerals and

vitamins that are present. Furthermore, it is necessary for research to emphasize the exploration of various health benefits linked with Indian teas. This is also a crucial aspect of research. From a nutraceutical point of view, this will give a full grasp of the nutritional qualities of this beverage that is often drunk (21, 22).

References:

1. Deshpande, S., Singh, S., Panneerselvam, A. and Rajeswari, V.D, "Nutrients in Caffeinated Beverages an Overview," Caffeinated and Cocoa Based Beverages, Wood head Publishing, 2019, 367-389.
2. Deb, S. and Pou, K.J., "A review of withering in the processing of black tea." Journal of Biosystems Engineering 41(4), 2016, 365-372.
3. Yang, X. and Kong, F., "Effects of tea polyphenols and different teas on pancreatic α-amylase activity in vitro." LWT-Food Science and Technology 66, 2016, 232-238.
4. Chandrasekara, A. and Shahidi, F., "Herbal beverages: Bioactive compounds and their role in disease risk reduction A review." Journal of traditional and complementary medicine 8(4), 2018, 451-458.
5. Lagad, R.A., Alamelu, D., Laskar, A.H., Rai, V.K., Singh, S.K. and Aggarwal, S.K., "Isotope signature study of the tea samples produced at four different regions in India." Analytical Methods 5(6), 2013, 1604-1611.
6. Gohain, B., Borchetia, S., Bhorali, P., Agarwal, N., Bhuyan, L.P., Rahman, A., Sakata, K., Mizutani, M., Shimizu, B., Gurusubramaniam, G. and Ravindranath, R., "Understanding Darjeeling tea flavour on a molecular basis." Plant molecular biology 78(6), 2012, 577-597.
7. Misra, U., "Assam tea: the bitter brew." Economic and Political Weekly, 2003, 3029-3032.
8. Pilgrim, T.S., Watling, R.J. and Grice, K., "Application of trace element and stable isotope signatures to determine the provenance of tea (Camellia sinensis) samples." Food Chemistry 118(4), 2010, 921-926.
9. Sourabh, A., Kanwar, S.S., Sud, R.G., Ghabru, A. and Sharma, O.P., "Influence of phenolic compounds of Kangra tea [Camellia sinensis (L) O Kuntze] on bacterial pathogens and indigenous bacterial probiotics of Western Himalayas." Brazilian Journal of Microbiology 44(3), 2013, 709-715.
10. Kumar, D., Gulati, A. and Sharma, U., "Determination of theanine and catechin in Camellia sinensis (Kangra Tea) leaves by HPTLC and NMR techniques." Food Analytical Methods 9(6), 2016, 1666-1674.
11. Khokhar, S. and Magnusdottir, S.G.M., "Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom." Journal of agricultural and food chemistry 50, 2002, 565-570.
12. Sharma, A., Bhondekar, A.P., Bari, S.S., Gulati, A., Kapur, P. and Singla, M.L., "Development and optimization of an HPLC method for the routine analysis of catechins, caffeine, and

gallic acid in tea (Camellia sinensis)." Journal of liquid chromatography & related technologies 34(19), 2011, 2243- 2255.

13. Sabhapondit, S., Karak, T., Bhuyan, L.P., Goswami, B.C. and Hazarika, M., "Diversity of catechin in northeast Indian tea cultivars." The Scientific World Journal 2012, 2012.
14. Chatterjee, S., Chatterjee, A. and Bandyopadhyay, S.K., "Seasonal variation of L-Theanine content in tea: A study on Darjeeling Black Tea." International Journal of Scientific Research in Science, Engineering and Technology 2(3), 2016, 522-4.
15. Deka, H., Barman, T., Sarmah, P.P., Devi, A., Tamuly, P., Paul, R.K. and Karak, T., "Quality characteristics of infusion and health consequences: a comparative study between orthodox and CTC green teas." RSC Advances 10(54), 2020, 32833- 32842.
16. Bhattacharya, U., Adak, S., Majumder, N.S., Bera, B. and Giri, A.K., "Antimutagenic and anticancer activity of Darjeeling tea in multiple test systems." BMC complementary and alternative medicine 14(1), 2014, 327.
17. Joshi, R., Sood, S., Dogra, P., Mahendru, M., Kumar, D., Bhangalia, S., Pal, H.C., Kumar, N., Bhushan, S., Gulati, A. and Saxena, A.K., "In vitro cytotoxicity, antimicrobial, and metal chelating activity of triterpene saponins from tea seed grown in Kangra valley, India." Medicinal Chemistry Research 22(8), 2013, 4030-4038.
18. Joshi, R., Rana, A., Kumar, V., Kumar, D., Padwad, Y.S., Yadav, S.K. and Gulati, A., "Anthocyanins enriched purple tea exhibits antioxidant, immunostimulatory and anticancer activities." Journal of food science and technology 54(7), 2017, 1953-1963.
19. Zhang, P.Y., Xu, X. and Li, X.C., "Cardiovascular diseases: oxidative damage and antioxidant protection." Eur Rev Med Pharmacol Sci 18(20), 2014, 3091-6.
20. Yanagimoto, K., Ochi, H., Lee, K.G. and Shibamoto, T., "Antioxidative activities of volatile extracts from green tea, oolong tea, and black tea." Journal of agricultural and food chemistry 51(25), 2003, 7396-7401.
21. Babu, P.V.A., Sabitha, K.E. and Shyamaladevi, C.S., "Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats." Chemico Biological Interactions 162(2), 2006, 114-120.
22. Kumaran, V.S., Arulmathi, K. and Kalaiselvi, P., "Senescence mediated redox imbalance in cardiac tissue: Antioxidant rejuvenating potential of green tea extract." Nutrition 25(7-8), 2009, 847-854.