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Research Article

Karakterisasi Metabolit Teh dan Tanah dari Berbagai Sumber di India Timur Laut

Characterization of Tea Metabolites and Soil of Different Origins in North Eastern India

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Abstract: *Tea harvested in North-Eastern parts of India is renowned for high quality tea including various therapeutic potentials like antioxidant and antimicrobial properties. Tea samples collected from Darjeeling, Assam and Jalpaiguri gardens are subjected for metabolite quality analysis processes. The geographic factors including soil characters can influence the qualities of tea according to their inherent secondary metabolites. One of the major class of tea secondary metabolite is tea flavonoids responsible for tea quality parameters including Rutin, Quercetin, Epigallocatechin gallate, Tannic acid, Gallic acid etc. Spectrophotometric, Spectrofluormetric scan analysis, HPLC, FTIR analysis characterize the differential contents of different tea flavonoids of different origin. The results suggest that tea extracts from Assam, Darjeeling, and Jalpaiguri possess valuable bioactive secondary metabolites to impart antimicrobial and antioxidant activities. The variations in these properties can be attributed to a combination of factors, including soil characteristics and cultivation practices of the different origin of North eastern Indian tea gardens.*

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Keywords: *tea metabolites; flavonoids; HPLC; FTIR; antioxidant; antimicrobial; geographical variation; Camellia sinensis*

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Abstrak: Teh yang dipanen di bagian Timur Laut India terkenal dengan kualitas teh yang tinggi termasuk berbagai potensi terapeutik seperti sifat antioksidan dan antimikroba. Sampel teh yang dikumpulkan dari kebun Darjeeling, Assam, dan Jalpaiguri dianalisis untuk proses analisis kualitas metabolit. Faktor geografis termasuk karakteristik tanah dapat mempengaruhi kualitas teh sesuai dengan metabolit sekunder yang melekat. Salah satu kelas utama metabolit sekunder teh adalah flavonoid teh yang bertanggung jawab atas parameter kualitas teh termasuk Rutin, Quercetin, Epigallocatechin gallate, Asam Tanat, Asam Galat, dll. Analisis spektrofotometri, pemindaian spektrofluorometri, HPLC, dan analisis FTIR mengkarakterisasi kandungan diferensial dari berbagai flavonoid teh dari asal yang berbeda. Hasil penelitian menunjukkan bahwa ekstrak teh dari Assam, Darjeeling, dan Jalpaiguri memiliki metabolit sekunder bioaktif yang berharga untuk memberikan aktivitas antimikroba dan antioksidan. Variasi dalam sifat-sifat ini dapat dikaitkan dengan kombinasi faktor-faktor, termasuk karakteristik tanah dan praktik budidaya dari asal yang berbeda di kebun teh India Timur Laut.

Kata Kunci: metabolit teh; flavonoid; HPLC; FTIR; antioksidan; antimikroba; variasi geografis; Camellia sinensis

1. Introduction

Tea is one of the most widely consumed beverages in the world. It is a rich source of bioactive compounds, including epigallocatechin gallate (EGCG), rutin, quercetin, gallic acid, and tannic acid, which have been extensively studied for their potential health benefits. Tea plant (*Camellia sinensis*) belongs to genus Camellia L. and family Theaceae. Compared with other plants, secondary metabolites of tea plants not only endow tea with unique therapeutic quality, but also benefit human health. As an important economic plant, tea has been studied in many fields, including health, food production, and culture. These metabolites possess antioxidant, antimicrobial and anti-inflammatory properties, which may contribute to reducing the risk of chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders. The tea plant is a perennial and economically significant crop, and boasts a productive lifespan of up to 100 years (Xia et al., 2017; Wang et al., 2016). Research has highlighted the critical factors influencing peak tea yields, including genotype, environment, management practices, and their interactions.

EGCG, in particular, has garnered significant attention for its potential anti-cancer effects (Wan and Xia, 2015; Zhang et al., 2020), with studies suggesting its ability to inhibit tumour growth and metastasis. Rutin and quercetin, flavonoids with antioxidant and anti-inflammatory properties, have been shown to improve blood circulation and reduce the risk of heart disease. Gallic acid and tannic acid, polyphenols known for their astringent taste, have demonstrated antioxidant and anti-inflammatory activities as well (Wei et al., 2018; Zhao et al., 2020).

From a human perspective, secondary metabolites act as important components for economic crop quality. Various secondary metabolites contribute to the quality and function of tea (Wan, 2003). During tea plant growth (preharvest stage) and the tea manufacturing process (postharvest stage), many stresses are used to modify tea metabolites (Zeng et al., 2019; Yu and Yang, 2020; Yang et al., 2013). At the preharvest stage, shading treatment (abiotic stress) has been shown to enhance free amino acids and aromatic aroma compounds, and reduce catechins (Chen et al., 2017; Yang et al., 2012; Zhang et al., 2006).

Furthermore, tea leaf attacked by tea green leafhoppers (biotic stress) increases honey-fruit aroma compounds (Zeng et al., 2019; Cho et al., 2007). Compared with other economic crops, the postharvest manufacturing process is an important stage for improving tea quality, especially regarding tea aroma. A comprehensive investigation of aroma compound formation at this stage showed that jasmonic acid (JA)-key genes-characteristic aroma compounds was the main regulatory route (Zhou et al., 2017; Zhou et al., 2020; Wang et al., 2020; Zeng et al., 2016; Zeng et al., 2019). To date, much research has been conducted to clarify the mechanism involved in improving tea quality with stresses.

This study aims to investigate the geographical variation and altitudinal influence on tea metabolite profiles and associated soil parameters. By employing a combination of analytical techniques, including Fourier-Transform Infrared Spectroscopy (FTIR), Ultraviolet-Visible (UV-Vis) Spectroscopy, High-Performance Liquid Chromatography (HPLC), Thin layer chromatography (TLC), and Fluorimetry, we will characterize the changes in metabolite composition and explore the underlying mechanisms influenced by environmental factors.

The specific objectives of this research are to identify and quantify key tea metabolites, such as EGCG, rutin, quercetin, gallic acid, and tannic acid, in tea samples collected from different geographical locations and altitudes; investigate the effects of varying soil properties on tea metabolite production; explore the interplay between geographical factors, soil characteristics, and tea plant physiology in determining metabolite profiles; and provide insights into the potential implications of geographical variations in tea metabolites for the quality and health benefits of tea. The amount of these compounds in tea can vary depending on factors such as the type of tea, growing conditions, and processing methods (Zhao et al., 2020; Wang et al., 2016; Yang et al., 2019; Zhu et al., 2019; Wan, 2003). Therefore, consuming a variety of teas can help maximize the potential health benefits associated with these bioactive compounds.

By addressing these objectives, this research will contribute to a deeper understanding of tea plant biology and provide valuable information for tea production and quality control, particularly in identifying regions with optimal conditions for cultivating tea with desired metabolite profiles. Furthermore, this study may have broader implications for the development of functional foods and nutraceuticals derived from tea. By understanding the factors that influence the production of bioactive compounds in tea, researchers can explore strategies to enhance their levels and develop products with targeted health benefits. Additionally, this research may contribute to the development of sustainable tea production practices, as it can help identify regions with optimal growing conditions and inform the development of management strategies that minimize environmental impact.

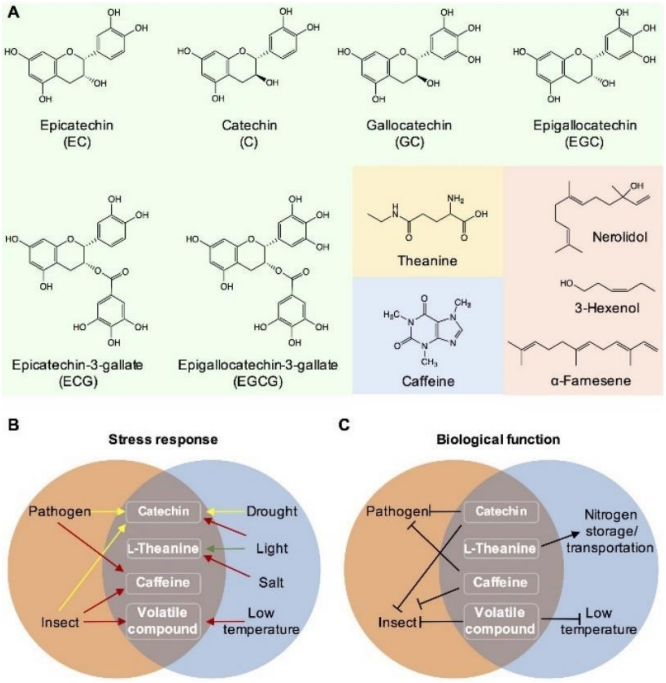

Figure 1. An overview of the various metabolites found in tea plants and their possible biological roles. Panel A shows the chemical structures of several metabolites that are classified as volatile chemicals (pink background) and catechins (green background). Panel B illustrates how these metabolites react to various stressors, including pathogen infection, insect attack, and drought, by showing whether their levels rise (red arrow), fall (green arrow), or show a range of responses (yellow arrow). Panel C illustrates the possible biological roles of these specific metabolites in tea plants.

2. Materials and Methods

2.1. Origin of Tea Samples

Tea plants (*Camellia sinensis*) from three different places were analyzed in this study: located Dibrugarh (27.4705° N, 94.9125° E), Assam; Balasun (26.8606°N 88.2357°E), Darjeeling; and VVtea garden (26.5683° N, 88.5396° E) Fatapukur, Jalpaiguri, India. Fresh tea leaves TV 25 were plucked at these places between June 20, 2024, and June 26, 2024, immediately kept in dry ice, sent to St. Xavier's College (Autonomous), Kolkata, and stored at −80 °C until further analysis.

2.2. Extraction of Tea Leaf Samples

Tea leaf samples from Assam, Jalpaiguri, and Darjeeling were extracted by maceration technique in dimethyl sulfoxide (DMSO) solvent. Leaf samples were dried, ground, and homogenized to ensure uniform particle size. 50 grams of each sample were weighed and placed in separate centrifuge tubes. 10 millilitres of DMSO were added to each tube, followed by vigorous homogenization using a mortar and pestle. The homogenized mixtures were then centrifuged at 5°C for 5 minutes at 10,000 rpm to separate the solid residue from the extracted compounds. The supernatant, containing the extracted tea leaf compounds, was carefully transferred to clean Eppendorf tubes for further analysis. This modified maceration technique offers several advantages over traditional extraction methods (Zhao et al., 2020; Xia et al., 2017; Zhou et al., 2020). The direct homogenization and centrifugation steps streamline the process, reducing the risk of sample degradation and minimizing the time required for extraction. By using a small volume of DMSO, the technique is suitable for extracting bioactive compounds from limited quantities of tea leaf samples.

2.3. UV-Visible Spectrophotometric Analysis of Tea Polyphenols and Flavonols

To characterize the polyphenolic compounds present in the extracted tea leaf samples, UV-visible spectrophotometric scanning was performed. This technique allows for the qualitative and quantitative analysis of these compounds based on their absorption of light in the ultraviolet and visible regions of the spectrum. Standard solutions of tannic acid, gallic acid, quercetin, epigallocatechin gallate (EGCG), and rutin were prepared at known concentrations of 1 mg/ml and used as reference standards. The extracted tea leaf samples were diluted with DMSO to a suitable concentration and scanned in a UV-visible spectrophotometer over a wavelength range of 200-400 nm. The absorbance spectra of the samples were compared to the spectra of the standard solutions to identify and quantify the specific polyphenols present. Tannins and flavonoids typically exhibit absorption maxima between 260 and 280 nm (Koornneef

and Pieterse, 2008; Langenheim, 1994), while flavanols like EGCG often show absorption peaks around 270-280 nm and 360-370 nm.

2.4. Characterization of Tea Polyphenols Including Flavonoids Using FTIR Spectrometry

Fourier-Transform Infrared (FTIR) spectroscopy was employed to characterize the functional groups present in the extracted tea leaf samples. The tea leaf extracts and standard compounds were prepared in DMSO solvent and scanned in the wavenumber range of 4000 cm^{-1} to 1000 cm^{-1}. Key regions of interest in the FTIR spectra include: 3400-3200 cm^{-1} (O-H stretching vibrations of phenolic hydroxyl groups); 1700-1600 cm^{-1} (carbonyl C=O stretching vibrations in flavonoids and tannins); 1600-1500 cm^{-1} (aromatic C=C stretching vibrations characteristic of polyphenols); and 1200-1000 cm^{-1} (C-O stretching vibrations in phenolic compounds). By comparing the FTIR spectra of the tea leaf extracts to the spectra of the standard compounds, it was possible to identify the presence and relative abundance of specific polyphenols and flavonols in the samples.

2.5. Spectrofluorimetric Analysis of Tea Polyphenols and Flavonols

To further characterize the polyphenolic compounds in the extracted tea leaf samples, spectrofluorimetric analysis was conducted (Yang et al., 2012; Yang et al., 2013). Standard solutions of tannic acid, gallic acid, quercetin, epigallocatechin gallate (EGCG), and rutin were prepared at known concentrations of 1 mg/ml. The excitation wavelength was set to 270 nm for all standards except for tannic acid (excited at 275 nm) and rutin (excited at 256 nm). The emission wavelength was scanned from 270 nm to 620 nm. The fluorescence spectra of the samples and standards were compared to identify the presence of specific polyphenols and to estimate their relative concentrations.

2.6. HPLC Analysis of Tea Polyphenols and Flavonols

To comprehensively characterize the polyphenolic compounds present in the extracted tea leaf samples, high-performance liquid chromatography (HPLC) analysis was conducted. A reverse-phase C18 column was employed for the separation of polyphenols. The mobile phase consisted of a gradient of acetonitrile and milli-Q water, with increasing concentrations of acetonitrile over time to elute compounds of varying polarity. The eluted compounds were detected using a UV-visible detector set at 285 nm, a wavelength at which many polyphenols exhibit strong absorbance. The retention times of the peaks in the chromatograms were compared to those of the standards to identify the individual polyphenolic compounds (Koornneef and Pieterse, 2008; Langenheim, 1994).

2.7. Total Free Amino Acids Assay

To determine the total free amino acids content in the extracted tea samples, a spectrophotometric method using ninhydrin was employed. Ninhydrin reacts with primary and secondary amino acids to produce a purple-colored complex, measured at 570 nm. For the assay, 1 ml of ninhydrin solution (3.5 mg/ml in ethanol) was added to 5 ml of the extracted tea sample. The mixture was incubated at 100°C for 5-7 minutes. After cooling to room temperature, the absorbance of the resulting solution was measured at 570 nm using a spectrophotometer. Alanine was taken as standard (1 mg/ml).

2.8. Antimicrobial Activity of Tea Metabolites

To assess the antimicrobial activity of the extracted tea leaf samples, a well diffusion assay was employed. The bacterial strains used in this study were *Escherichia coli* and Bacillus species, representing Gram-negative and Gram-positive bacteria, respectively. Ampicillin, a well-established antibiotic, was used as a positive control. The extracted tea leaf samples were dissolved in DMSO. Agar plates were prepared containing Mueller-Hinton broth. After incubation at 37°C for 24 hours, the zones of inhibition around the wells were measured and compared.

2.9. Characterization of Antioxidant Properties Using DPPH Assay

To evaluate the antioxidant properties of the extracted tea leaf samples, a spectrophotometric assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was employed. For the assay, 1 mL of each extracted tea sample in DMSO was mixed with 1 mL of a 0.04 mg/mL DPPH solution in DMSO. The mixture was incubated in the dark at room temperature for 30 minutes. Subsequently, the absorbance of the mixture was measured at 517 nm using a spectrophotometer. The decrease in absorbance at 517 nm compared to the positive control indicates the scavenging of DPPH radicals by the antioxidant compounds in the tea samples.

2.10. Colony Forming Unit Assay for Soil Samples

To determine the microbial activity within the soil samples associated with tea cultivation at varying altitudes, a colony-forming unit (CFU) assay was conducted (Wei et al., 2018). Soil samples were initially prepared by suspending 250 mg of each sample in 10 mL of distilled water. Serial dilutions (1:10, 1:100, and 1:1000) were then prepared and plated onto separate autoclaved nutrient agar plates. The plates were incubated at 37°C for 24-48 hours. Following

incubation, visible colonies were counted on each plate and multiplied by the corresponding dilution factor to determine the total CFUs per mL.

2.11. Nutrient Estimation of Soil Samples

To assess the micronutrient and macronutrient content of soil samples collected from tea-cultivating regions in Assam, Jalpaiguri, and Darjeeling, a total of 12.5 grams of soil from each location was mixed with 25 millilitres of water and filtered. The resulting filtrate was analyzed using the Soil Saathi app, which employs radioimmunoassay (RIA) technology. Dedicated liquid reagents were used to determine the concentrations of magnesium, calcium, sulfur, zinc, iron, and manganese. For nitrogen, phosphorus, potassium, and organic carbon, capsules containing reagent powder were used.

2.12. Statistical Analysis

All the results and the correlations were tested (regression analysis) with ANOVA by SPSS analysis software version 20.0.

3. Results and Discussion

3.1. Analysis of Tea Metabolites by UV-Vis Spectrophotometer

The absorption spectra of tea extracts and the standards in dimethyl sulfoxide (DMSO) are recorded at room temperature using a UV-VIS spectrophotometer (Figure 2). Figure 3 demonstrates how the absorption peaks that describe the tea from each of the three places are not exact, but rather exhibit subtle variations. Another prominent difference was that the peak observed in DMSO solvent was quite broad, as DMSO—a highly polar solvent with strong hydrogen bonding capabilities and high viscosity—can lead to broader UV-Vis peaks compared to other solvents, resulting in broader spectral lines.

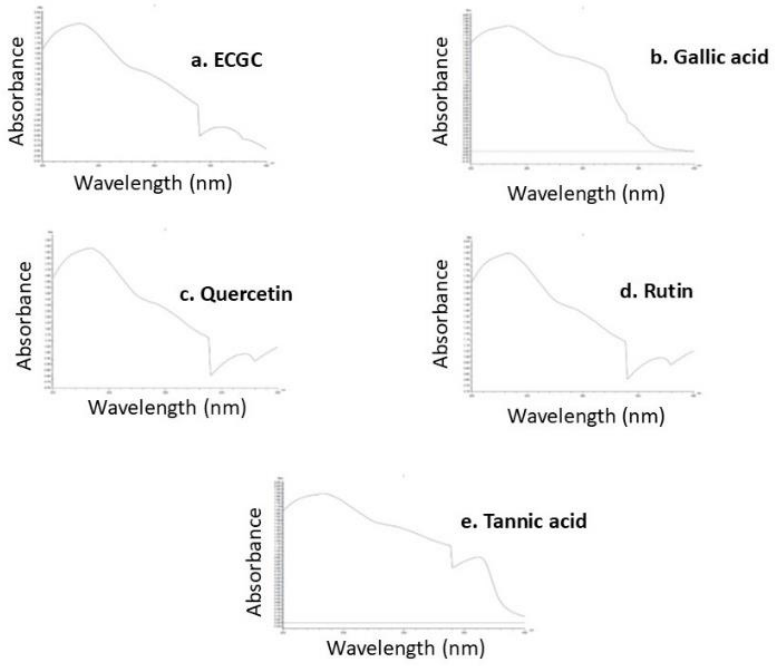

Figure 2. Absorption spectra recorded at room temperature using UV-VIS Spectrophotometer for different standards.

The characteristics of tea extract from Dibrugarh, Assam (grown at 358 to 423 ft height) can be identified by two main absorption peaks at 233 and 370 nm in DMSO. As the sample shows peaks at 370 and 233 nm, it suggests similarity to quercetin and rutin's carbonyl group (peaking at 371 nm), contains aromatic rings (233 nm), and has a chromophore that absorbs at longer wavelengths (370 nm). This likely indicates the presence of rutin or quercetin.

Two primary absorption peaks, at 229.5 nm and 370.5 nm, are characteristic of Balasun tea extract (grown at an elevation of around 365 to 1,375 meters). The signal at 229.5 nm is frequently linked to proteins and aromatic amino acids, which may indicate the presence of theanine. The peak at 370.5 nm is often associated with flavonoids, possibly quercetin or rutin. Two primary absorption peaks, at 234.0 nm and 371.5 nm, are present in the DMSO tea extract of Fatapukur VVtea (grown at around 328.08 feet). The peak at 234.05 nm is compatible with the distinctive absorption of EGCG, and the peak at 371.5 nm suggests the probable presence of rutin.

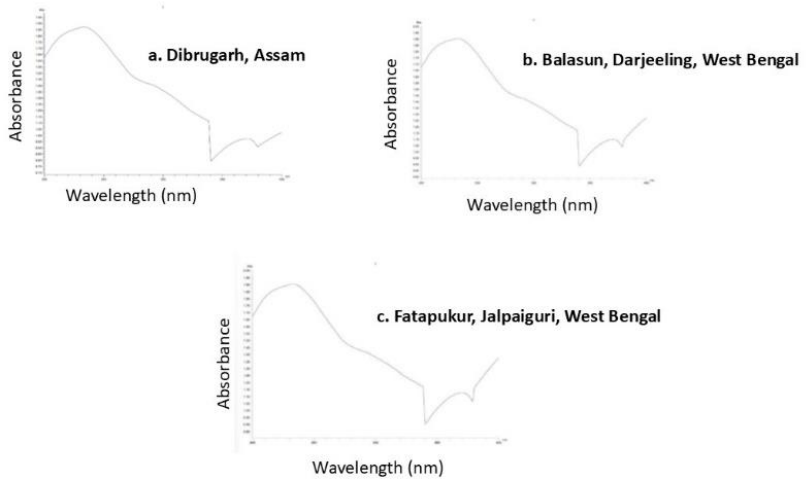

Figure 3. Absorption spectra recorded at room temperature using UV-VIS Spectrophotometer for tea extract samples from three different geographical locations.

3.2. Fourier Transform Infrared (FTIR) Analysis of Tea Samples

Fourier-Transform Infrared Spectroscopy (FTIR) analysis of the tea extracts revealed distinctive spectral patterns consistent with the presence of bioactive compounds. The observed spectral features were compared to reference spectra of rutin and tannic acid (Figure 4).

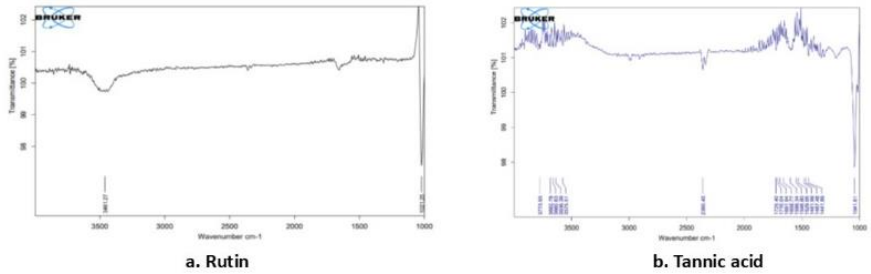

Figure 4. Fourier Transform Infrared (FTIR) spectra of the standards. The x-axis represents wavenumber (cm^{-1}), and the y-axis represents absorbance intensity.

FTIR analysis of the tea extract from Dibrugarh, Assam (Figure 5a) revealed distinctive spectral patterns indicative of the presence of specific functional groups. Prominent peaks were observed at 3426.66 cm^{-1}, 1659.35 cm^{-1}, and 1315.20 cm^{-1}. The broad peak at 3426.66 cm^{-1} is characteristic of O-H stretching vibrations, suggesting the presence of hydroxyl groups, consistent with the structure of both rutin and tannic acid. The peak at 1659.35 cm^{-1} is indicative of C=O stretching vibrations, attributable to conjugated C=O stretching in aromatic rings or C=O stretching in ester linkages.

FTIR analysis of the tea extract from Balasun and Jalpaiguri (Figure 5b) revealed prominent peaks at 3428.31 cm^{-1}, 1658.78 cm^{-1}, and 1014.40 cm^{-1}. The broad peak at 3428.31 cm^{-1} is characteristic of O-H stretching vibrations, consistent with the structure of several tea metabolites, including rutin, tannic acid, and gallic acid. The FTIR spectrum of the tea sample from Fatapukur, Jalpaiguri (Figure 5c) exhibited prominent peaks at 3432.51 cm^{-1}, 1658.83 cm^{-1}, 1315.34 cm^{-1}, and 1016.07 cm^{-1}. Based on these findings, the potential compounds in the tea sample also probably contain both rutin and tannic acid.

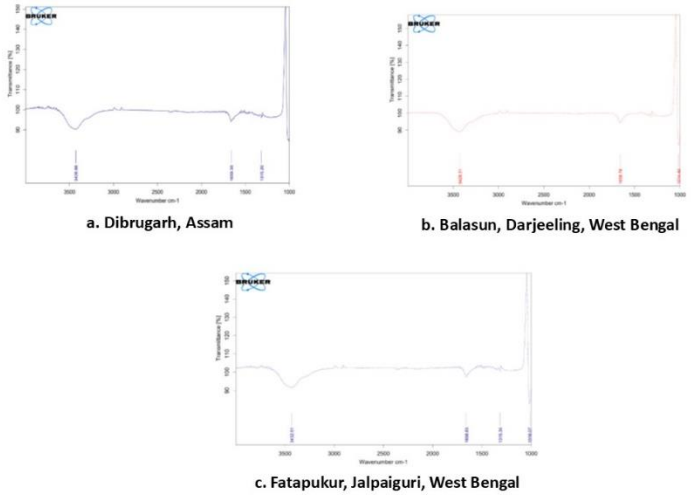

Figure 5. Fourier Transform Infrared (FTIR) spectra of the tea extract from different locations. The x-axis represents wavenumber (cm^{-1}), and the y-axis represents absorbance intensity.

3.3. Spectrofluorimetric Analysis of Tea Extracts

Fluorometric analysis of the tea extracts revealed distinct fluorescence emission spectra, indicative of the presence of specific bioactive compounds. Spectrofluorimetric analysis of the tea extract from Dibrugarh, Assam, revealed a prominent emission peak at 540.6 nm and 512 nm. This emission peak is consistent with the fluorescence characteristics of EGCG, rutin, and gallic acid, all of which exhibit emission maxima in the 540-550 nm range. The observed emission peak at 540.6 nm is most likely due to a combination of rutin and gallic acid. Rutin has been reported to exhibit emission peaks around 513 nm and 551 nm, while gallic acid has been shown to emit fluorescence around 540 nm.

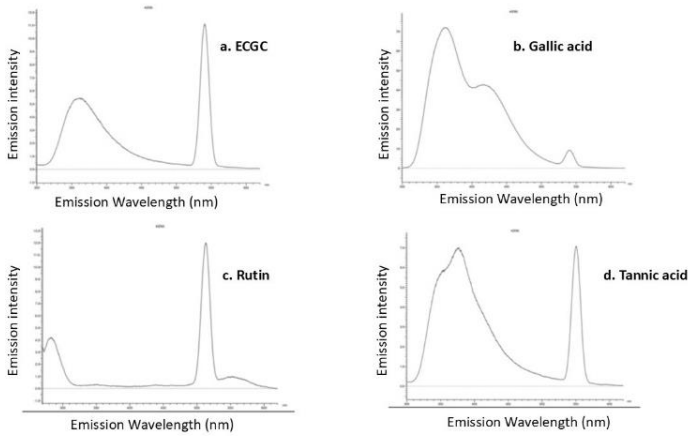

Figure 6. Fluorescence emission spectra of standards. The x-axis represents the emission wavelength (nm), and the y-axis represents the emission intensity.

Spectrofluorimetric analysis of the Balasun, Darjeeling tea extract revealed an emission maximum at 500.6 nm. This emission peak is most consistent with the emission spectra of rutin and tannic acid, both of which exhibit emission maxima around 550 nm. The Fatapukur VVtea tea extract also exhibited an emission maximum at 500.4 nm, consistent with the emission spectra of rutin and tannic acid. The observed shifts in emission maxima could be attributed to factors such as matrix effects, concentration differences, or environmental conditions.

3.4. HPLC Analysis of Tea Metabolites

High-Performance Liquid Chromatography (HPLC) was employed to analyze the bioactive compounds present in tea extracts from various regions. The retention times of the analytes were compared to those of reference standards: gallic acid (3.042 min), tannic acid (3.742 and 6.828 min), EGCG (3.820 min), quercetin (1.290, 1.734, 1.907, 2.235, and 3.456 min), and rutin (2.380, 6.500, and 6.850 min).

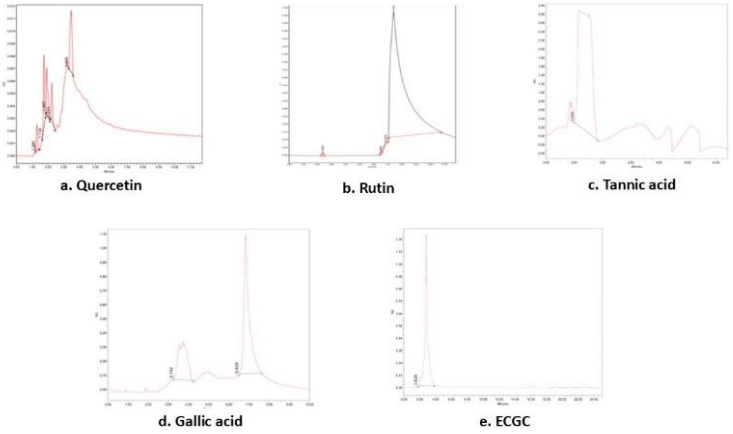

Figure 7. HPLC chromatogram of tea standards. The x-axis represents the retention time (minutes), and the y-axis represents the absorbance intensity at 285 nm.

HPLC analysis of the Fatapukur tea extract revealed prominent peaks at 1.435, 2.433, and 7.255 minutes, which align with the retention times of gallic acid, rutin, and tannic acid, respectively. HPLC analysis of the Balasun tea extract revealed prominent peaks at 2.174 and 7.237 minutes, suggesting the presence of gallic acid and tannic acid. HPLC analysis of the Assam tea extract revealed prominent peaks at 1.959, 2.749, 3.571, 5.343, 5.874, 6.070, and 6.503 minutes, suggesting the presence of gallic acid, rutin, tannic acid, and potentially EGCG and quercetin.

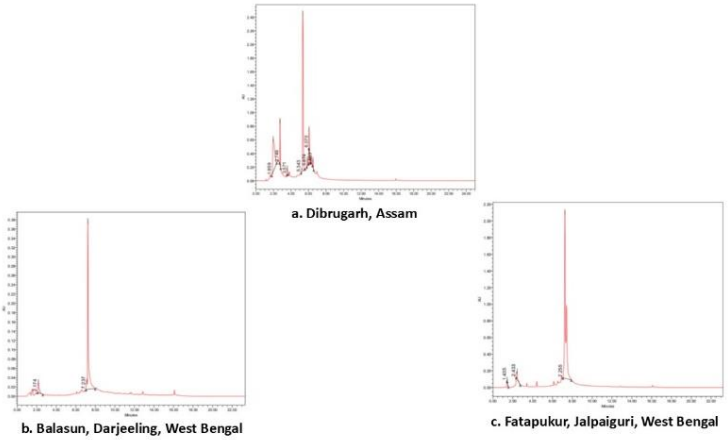

Figure 8. HPLC chromatogram of tea samples. The x-axis represents the retention time (minutes), and the y-axis represents the absorbance intensity at 285 nm.

3.5. Antioxidant Assay of Tea Extracts

The antioxidant potential of various tea extracts was evaluated using the DPPH assay. All tea extracts exhibited significant antioxidant activity, with Fatapukur Untreated demonstrating the highest potential (81.152±0.01%). Balasun and Assam tea extracts also showed notable antioxidant activity, with values of 77.161±0.02% and 69.711±0.01%, respectively. The average antioxidant potential of the tea samples was calculated to be 82.006±0.02 V%. These findings highlight the potent antioxidant properties of tea-based compounds and their potential contribution to overall health benefits.

3.6. Antimicrobial Assay of Tea Extracts on Escherichia coli and Bacillus subtilis

The well diffusion method was employed to evaluate the antimicrobial activity of tea extracts against both *Escherichia coli* and *Bacillus subtilis*. A clear zone of inhibition was observed around the Fatapukur tea extract well against both bacterial strains (Figure 9b and 10b), indicating the presence of antimicrobial compounds. The zone of inhibition measured 1.4±0.001 cm for *Escherichia coli* and 1.3±0.001 cm for *Bacillus subtilis*. DMSO did not exhibit any antimicrobial activity, while ampicillin displayed potent inhibition.

The well diffusion method was also employed to evaluate the antimicrobial activity of Assam tea extract. A clear zone of inhibition was observed against both bacterial strains (Figure 9a and 10a). The zone of inhibition was 1.3 cm for

Bacillus subtilis and 1.2 cm for *Escherichia coli*. Balasun tea extract exhibited antimicrobial activity (Figure 9c and 10c) with a zone of inhibition of 1.3±0.01 cm against *Bacillus subtilis* and 1.5±0.01 cm against *Escherichia coli*.

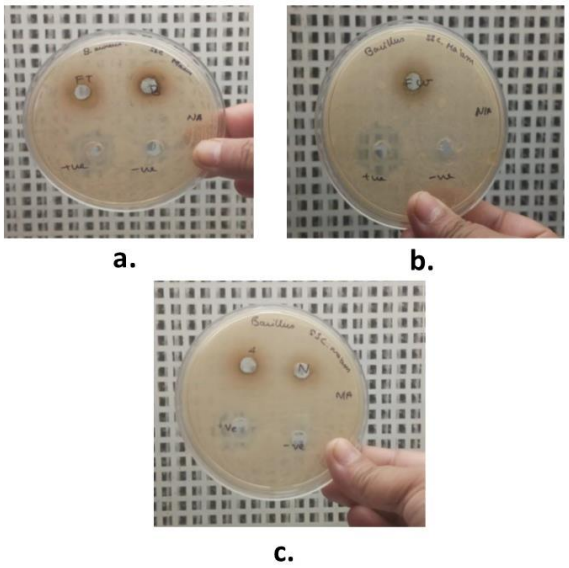

Figure 9. Agar plates showing the antimicrobial activity of (a) Dibrugarh, (b) Fatapukur, and (c) Balasun tea extracts against *Escherichia coli*. Clear zones of inhibition indicate the presence of antimicrobial compounds.

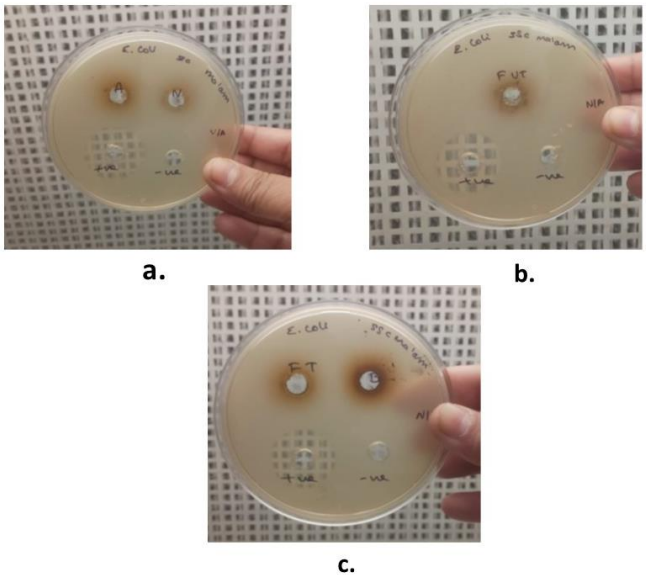

Figure 10. Agar plates showing the antimicrobial activity of (a) Balasun, (b) Fatapukur, and (c) Dibrugarh tea extracts against *Bacillus subtilis*. Clear zones of inhibition indicate the presence of antimicrobial compounds.

3.7. Analysis of Free Amino Acid Content in Tea Samples

The analysis of free amino acid content in tea samples provides valuable insights into the nutritional composition and potential health benefits of tea (Zeng et al., 2019). Balasun exhibited the highest content (0.38±0.001 mg/ml), suggesting the highest concentration of free amino acids. Fatapukur and Assam tea samples showed lower values (0.25±0.002 mg/ml and 0.28±0.002 mg/ml, respectively). These findings suggest that the amino acid composition of tea can vary depending on factors such as soil conditions, climate, and cultivar.

3.8. Macronutrient and Micronutrient Content of Soil from Tea Gardens

The evaluation of macronutrient and micronutrient content in tea samples from Assam, Darjeeling, and Jalpaiguri is crucial for understanding the factors influencing tea quality and composition. The following tables show the amount of macro and micronutrients found in the soil of Assam, Darjeeling, and Jalpaiguri.

Table 1. Macronutrient content of soil from tea gardens in Dibrugarh (Assam), Balasun (Darjeeling), and Fatapukur (Jalpaiguri).

Macronutrients	Assam soil (Dibrugarh)	Darjeeling soil (Balasun)	Jalpaiguri soil (Fatapukur)
Nitrogen (N)	252 kg/ha	296 kg/ha	305 kg/ha
Organic Carbon (OC)	0.45%	0.53%	0.59%
Phosphorous (P)	158 kg/ha	136 kg/ha	92.7 kg/ha
Potassium (K)	469 kg/ha	464 kg/ha	495 kg/ha

Table 2. Micronutrient content of soil from tea gardens in Dibrugarh (Assam), Balasun (Darjeeling), and Fatapukur (Jalpaiguri).

Micronutrients	Assam soil (Dibrugarh)	Darjeeling soil (Balasun)	Jalpaiguri soil (Fatapukur)
Magnesium (Mg)	1.74 mg/kg	1.57 mg/kg	1.03 mg/kg
Calcium (Ca)	0.68%	<0.01%	<0.01%
Sulphur (S)	6.93 mg/kg	6.70 mg/kg	6.66 mg/kg
Iron (Fe)	10.6 mg/kg	5.31 mg/kg	4.46 mg/kg
Manganese (Mn)	3.93 mg/kg	3.99 mg/kg	3.98 mg/kg
Copper (Cu)	<=0.4 mg/kg (deficient)	<=0.4 mg/kg (deficient)	<=0.4 mg/kg (deficient)

3.9. Colony-Forming Unit (CFU) Counts of Soils from Various Tea Gardens

The bacterial colony counts obtained from soil samples collected from Assam, Darjeeling (Balasun subsoil), and Jalpaiguri (Fatapukur) demonstrate variations in microbial populations.

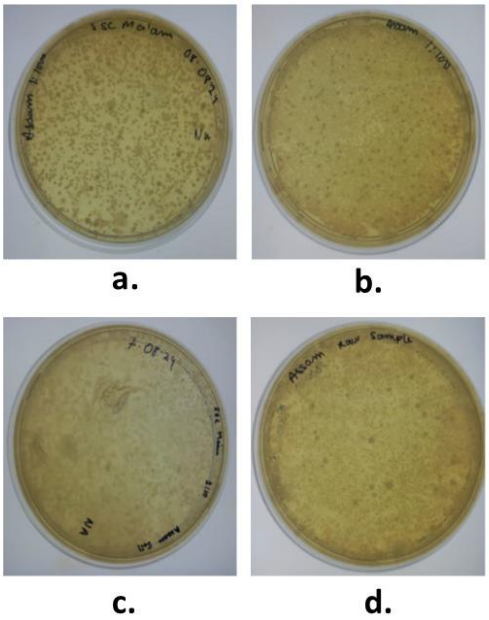

Figure 11. CFU counts of Assam soil samples at different dilutions (1:10, 1:100, and 1:1000).

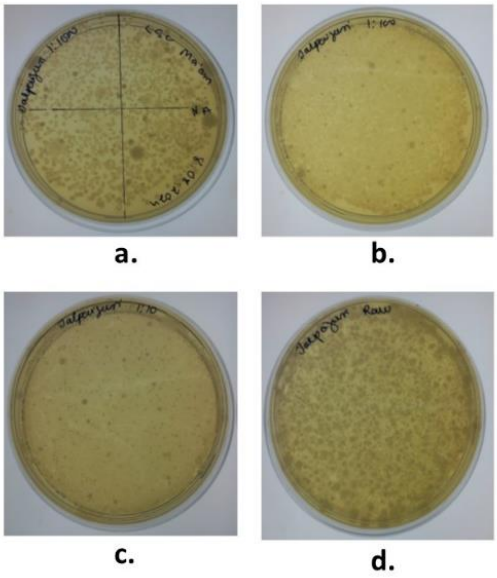

Figure 12. CFU counts of Fatapukur, Jalpaiguri soil samples at different dilutions (1:10, 1:100, and 1:1000).

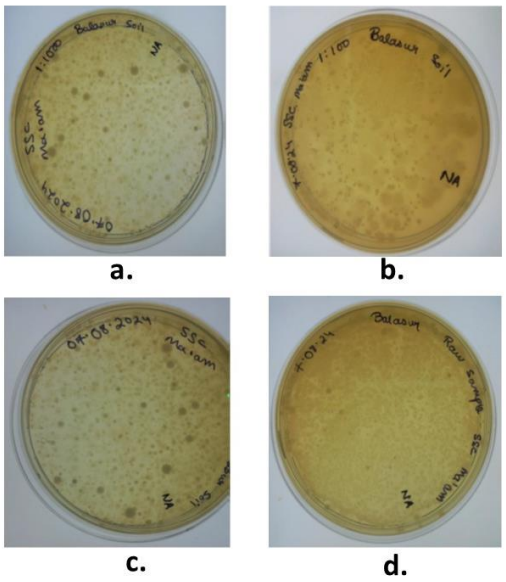

Figure 13. CFU counts of Balasun, Darjeeling soil samples at different dilutions (1:10, 1:100, and 1:1000).

Assam (Dibrugarh): Colony count (1:1000) dilution = 284 x 4 = 1134 CFU/mL (approximately). Jalpaiguri (Fatapukur): Colony count (1:1000) dilutions = 297 x 4 = 1188 CFU/mL (approximately). Darjeeling (Balasun): Colony count (1:1000) dilutions = 318 x 4 = 1272 CFU/mL (approximately). Darjeeling (Balasun) soil exhibited the highest bacterial colony count, suggesting a relatively higher microbial diversity or abundance. Assam and Jalpaiguri soils displayed comparable bacterial counts, indicating similar levels of microbial activity.

4. Conclusions

The present study investigated the influence of geographical factors and soil characteristics on the metabolite composition and biological activities of tea extracts from Assam, Jalpaiguri, and Balasun. The results obtained from UV-Vis spectroscopy, FTIR analysis, spectrofluorimetric analysis, antimicrobial assays, HPLC analysis, and total free amino acid (TFAA) determination provide valuable insights into the interrelationships between these factors.

The UV-Vis and FTIR analyses revealed distinct spectral patterns in the tea extracts, suggesting the presence of specific functional groups and compounds consistent with gallic acid, rutin, tannic acid, and potentially EGCG and quercetin. HPLC analysis confirmed the presence of several bioactive compounds in the tea extracts. The relative

abundance of these compounds varies among the different regions due to variations in soil characteristics, climate, and cultivation practices.

Based on the HPLC analysis, gallic acid was detected in all three tea extracts, with Balasun and Assam showing higher levels compared to Fatapukur. All three tea extracts contained tannic acid with similar levels in Balasun and Fatapukur. EGCG was detected in all three extracts, with the highest levels observed in Fatapukur VVtea extract. Quercetin was detected in all three extracts, with similar levels in Balasun and Assam. Rutin may be present in varying concentrations in all three samples.

The antimicrobial assays demonstrated that tea extracts from all three regions exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria. The correlation between total metabolite content and antimicrobial activity was found to be 0.77, indicating a strong positive correlation. The correlation between total metabolite content and antioxidant potential was found to be 0.52, indicating a moderate positive correlation.

The analysis reveals that Fatapukur tea extract, with its higher total metabolite content, exhibits the most pronounced antimicrobial and antioxidant activities. Balasun tea extract, despite having a lower total metabolite content, still demonstrates significant bioactivity. Assam tea extract maintains moderate antimicrobial and antioxidant properties. The soil macronutrients, particularly nitrogen and organic carbon, are positively correlated with the antimicrobial and antioxidant properties of tea extracts, highlighting the importance of soil health and nutrient management in tea cultivation.

Region	Nitrogen (kg/ha)	Organic Carbon (%)	Phosphorous (kg/ha)	Potassium (kg/ha)	Antimicrobial activity	Antioxidant potential	Free amino acids
Assam	252	0.45	158	469	Moderate	High	Low
Darjeeling (Balasun)	296	0.53	136	464	High	Medium	High
Jalpaiguri (Fatapukur)	305	0.59	92.7	495	Moderate	Low	Medium

Table 3. Data analysing the macronutrients found in the soil and the antioxidant, antimicrobial activity of the tea leaves extract from that region. P<0.05.

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