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Simultaneous Extraction, Purification, and Quantification of Polyphenols, Caffeine, and Theophylline from BB35 Green Tea Leaves (Kenya cultivar) Using High-Performance Liquid Chromatography (HPLC) with Gradient Quantification

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

This study introduces a novel technique that uses a solvent mixture of 60% water and 40% ethanol to extract caffeine, theophylline, and polyphenols from green tea leaves (*Camellia sinensis*) all at once. On a dry basis, the Folin-Ciocalteu Reagent Method (ISO 14502-1) estimated the total polyphenol content of green tea leaves to be 25% (Gallic Acid Equivalent (GAE) in mg/g of dry sample). The extraction procedure showed remarkable effectiveness, recovering 94% of the caffeine, 92% of the theophylline, and 95% of the polyphenols. We verified each compound's stability and retention periods using HPLC-UV/PDA analysis at a fixed wavelength of 280 nm. The concentrations of the following compounds were noted: epicatechin at 19.2 ppm (RT 8.0 min), theophylline at 19.5 ppm (RT 3.2 min), catechin at 20.0 ppm (RT 7.2 min), and caffeine at 18.9 ppm (RT 4.0 min). These findings demonstrate the method's environmental advantages and scalability, suggesting that the nutraceutical industry may find use for it.

Keywords: Green tea extraction; gallic acid equivalent; nutraceuticals; agronomy; BB35 green tea variety; cultivar Kenya.

1. INTRODUCTION

In Kenya, the BB35 tea variety is one of several cultivars that are primarily grown in the high-altitude areas of the East and West Rift Valley. These highlands, which are between 1,500 and 2,700 meters above sea level, provide ideal growing conditions for tea because of their rich volcanic soils and consistent rainfall, which enable the production of premium varieties like BB35 [1,2]. In Kenya's central and western highlands, the counties of Kericho, Bomet, Nandi, Kisii, Nyamira, Kakamega, Vihiga, Bungoma, Nyeri, Kirinyaga, Murang'a, Kiambu, Embu, and Meru are notable producers of tea [1]. The year-round tea harvests made possible by this exceptional climate distinguish Kenyan tea production from that of other tea-growing regions across the world. High polyphenol and caffeine content are two important characteristics of the Kenyan tea variety BB35 that increase its market value and health benefits [3]. Research on Kenyan teas typically indicates that 20–30% of the dry weight of the tea is composed of polyphenols [4,5]. Catechins are among the polyphenols prized for their strong antioxidant qualities, which are advantageous to health. Kenyan tea varieties such as BB35 usually contain 2-3 percent caffeine in addition to polyphenols, though these amounts can vary depending on the conditions of cultivation and processing [5,6]. Conversely, tea's minor compound theophylline is present in much smaller amounts (approximately 0.1–1.2 percent) [3,4,5]. The rich composition of BB35 contributes to its commercial appeal by satisfying consumer demand for functional, health-promoting beverages. It is more desirable in wellness-focused markets because of the potential health benefits of the antioxidants found in its high polyphenol content. Additionally, the temperate climate of Kenya allows for consistent, year-round tea production, making BB35 a dependable choice for both domestic and foreign markets where supply stability and quality are highly valued. Theophylline, polyphenols, and caffeine are important bioactive substances that are highly prized in pharmaceuticals, functional foods, and nutraceuticals [6,7,8.9]. Effective techniques for extracting these substances are essential since they provide several therapeutic advantages. A bronchodilator, theophylline, and a stimulant of the central nervous system, caffeine [9,10].

2. EXTRACTION TECHNIQUES FOR BIOACTIVE COMPOUNDS FROM GREEN TEA

Among the many bioactive substances found in green tea leaves are theophylline, caffeine, and polyphenols such as epicatechins and catechins. Researchers have investigated a variety of extraction techniques to effectively separate and maintain the effectiveness of each compound because of their disparate polarities and stability

requirements. Selecting an extraction technique is crucial for pharmacological and nutraceutical applications since it affects yield, purity, and cost-effectiveness [10].

1. Solvent Extraction Methods

The solvent-based extraction technique is one of the most extensively researched ways to extract theophylline, caffeine, and polyphenols from green tea. Acetone, methanol, and ethanol are frequently used because of their strong ability to solubilize caffeine and polyphenols. Researchers have experimented with different temperatures and solvent ratios to maximize yield and prevent compound degradation [10,11].

Theophylline: Usually extracted in ethanol-water mixtures at high temperatures (60–80°C), which enables solvent partitioning to separate the substances from caffeine and polyphenols [12,13].

Polyphenols: Tea leaves' cellular structures are disrupted by ultrasonication, which increases yield. Ethanol or methanol mixtures are particularly effective at extracting catechins [10,11,12,13].

Caffeine: Caffeine is frequently separated from the aqueous phase after polyphenol extraction by adding a secondary solvent because it is more soluble in chloroform or ethyl acetate in alkaline conditions [10,11,12, 13,14,15].

2. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction, notably with CO_2 as a solvent, has gained traction due to its selective extraction capabilities, minimal solvent residue, and environmental benefits. By adjusting the temperature and pressure parameters, SFE has shown effectiveness in [16]:

Polyphenol Extraction: Supercritical CO_2 with ethanol as a co-solvent has been shown to extract catechins efficiently without significant degradation [16].

Caffeine Isolation: CO_2 extracts caffeine selectively from tea, especially at higher pressures, while leaving behind polyphenols due to their lower solubility in nonpolar CO_2 [16].

3. Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction uses microwave energy to heat the solvent and sample, which can accelerate the extraction of polar compounds like catechins and caffeine [17].

Catechins and Epicatechins: Are highly responsive to MAE, with extraction times significantly reduced compared to conventional heating, preserving more of the bioactive components [17].

Theophylline: Although less common, MAE has shown promise in simultaneously extracting theophylline and polyphenols in optimized solvent mixtures, enhancing recovery through rapid heating [17].

4. Ultrasound-Assisted Extraction (UAE)

Ultrasonic waves create cavitation bubbles in liquid media, disrupting plant cell walls and enhancing solvent penetration. UAE has been found effective for [18]:

Catechin and Epicatechin Extraction: UAE, especially when combined with ethanol, promotes rapid extraction of these polyphenols due to enhanced diffusion and improved solvent interactions [18].

Simultaneous Extraction: Studies have shown that UAE can be coupled with a water-ethanol mixture to extract caffeine, theophylline, and catechins together, optimizing energy use and reducing processing time [18].

5. Chromatographic Techniques for Purification

Following initial extraction, chromatographic techniques such as high-performance liquid chromatography (HPLC) are often employed to purify and separate caffeine, theophylline, and polyphenols [19]. Some researchers have explored:

Caffeine and Theophylline Separation: HPLC with specific mobile phases and gradient elution techniques offers high selectivity, often targeting caffeine at pH 9 to optimize its distinct retention from theophylline [19].

Polyphenol Purification: Techniques such as reverse-phase HPLC isolate catechins and epicatechins based on hydrophobic interactions, facilitating the analysis and preparation of pure compounds for further research or formulation [19].

6. Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction uses specific enzymes to degrade cell walls, enhancing the

release of bioactive compounds. This method has been less commonly used for theophylline but is explored for polyphenol and caffeine extraction [20].

Polyphenol Enhancement: Enzymes like cellulase and pectinase can improve catechin extraction by breaking down the tea leaf cell matrix, especially in water-based extractions [20].

Potential for Simultaneous Extraction: Enzymatic methods are increasingly being studied for simultaneous extraction, as they offer mild processing conditions that preserve the natural structure of catechins and other polyphenols [20].

Each extraction method presents unique advantages and trade-offs, influencing the choice of technique based on specific compound requirements and industrial constraints [21]. This review highlights that solvent extraction remains widely adopted, but advances in supercritical fluid, microwave-assisted, and ultrasound-assisted methods offer promising alternatives for green, efficient extraction of caffeine, theophylline, and polyphenols from tea leaves.

3. MATERIALS AND METHODS

3.1 Raw Material Preparation

High-quality green tea leaves rich in polyphenols, caffeine, and theophylline were selected for extraction. The Leaves are procured from local tea gardens in Kiambu Central Kenya. The leaves were dried at 50°C until they reached a moisture content of about 5-10%. Once dried, the leaves were ground into a fine powder (100-200 microns) to maximize the surface area for optimal extraction.

3.2 Extraction Process

A 60:40 water-ethanol [21,22] mixture was selected for its effectiveness in extracting caffeine, theophylline, and polyphenols without compound degradation. The ground tea powder was added to a 5:1 solvent ratio (by weight) and heated in a glass-lined reactor to 60°C, where it was stirred continuously for 2 hours [23,24]. The mixture was then filtered to remove plant material, yielding a filtrate containing the target compounds.

3.3 Filtration and Concentration

Following the initial extraction, the filtrate underwent a secondary filtration to remove fine particulates. A vacuum evaporator reduced the volume by 60%, enabling more efficient separation in subsequent stages.

3.4 Separation of Compounds

Polyphenols were separated using a solvent partitioning method. Equal parts of ethyl acetate were added to the concentrated extract in a liquid-liquid extraction setup, where polyphenols were partitioned into the ethyl acetate layer while caffeine and theophylline remained in the aqueous layer [21,22]. The ethyl acetate layer was evaporated to yield a polyphenol-rich residue.

The aqueous layer, which contained caffeine and theophylline, was adjusted to a pH of 9 to increase the solubility of theophylline and decrease the solubility of caffeine. A 1:1 chloroform mixture was then used to extract the caffeine, which was later evaporated under reduced pressure to obtain caffeine crystals [21,22,23]. Theophylline was crystallized by neutralizing the pH to 6.5 and cooling the solution to 4°C.

3.5 HPLC Method Validation

HPLC-UV/PDA analysis was employed for the simultaneous detection and quantification of caffeine, theophylline, catechin, and epicatechin [25,26]. The procedure included the preparation of standard solutions, calibration, and optimized chromatographic conditions using a C18 column at a detection wavelength of 280 nm. Retention times were established for each compound, ensuring accurate quantification in the samples.

3.6 Principle

Separation and quantification of caffeine, theophylline, catechin, and epicatechin are achieved through reversed-phase HPLC with UV detection [25,26]. The method leverages differences in retention times and absorbance at 280 nm, allowing accurate identification and quantification of each compound.

3.7 Reagents and Materials

- Solvents: HPLC-grade water, methanol, acetonitrile, and formic acid (or acetic acid).
- Standards: Certified standards of theophylline, caffeine, catechin, and epicatechin from Xianji Biotech, China.

- Mobile Phase: Gradient mixture of water and acetonitrile.
- Column: C18, 250 mm × 4.6 mm, 5 µm (Thermo Fischer)

3.8 Chromatographic Conditions

- HPLC System: UV or PDA detector
- Column Temperature: 25°C
- Flow Rate: 1 mL/min
- Injection Volume: 10 µL
- Detection Wavelength: 280 nm
- Run Time: 10 minutes
- Gradient Program: Initial composition 90% water and 10% acetonitrile (Table 1).

3.9 Preparation of Standard Solutions

1. Prepare each standard solution at 100 ppm in methanol as stock solutions.
2. Dilute the stock solutions to create working concentrations ranging from 5 to 100 ppm for calibration.

3.10 Sample Preparation

1. Extract target compounds from herbal samples using a suitable solvent, such as methanol.
2. Filter and dilute to match the calibration range (5–100 ppm).

3.11 Method Validation Parameters

a) Calibration Curve and Linearity: Standard solutions were prepared at concentrations of 5, 10, 20, 50, and 100 ppm, and injected to generate calibration curves by plotting peak area versus concentration. The linear regression equation ($y = mx + c$) was used to calculate the correlation coefficient (R^2) for each compound. Table 2.

b) Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were calculated using the formula: $LOD = 3.3 \times (\sigma / S)$ and $LOQ = 10 \times (\sigma / S)$, where σ is the standard deviation of the response, and S is the slope of the calibration curve Table 3.

c) System Suitability Test: A standard mixture solution was injected to confirm peak resolution, retention time consistency, and peak symmetry. Table 4.

d) Robustness: The robustness of the method was tested by varying flow rate, column temperature, and acetonitrile percentage. Table 5.

Procedure:

1. System Suitability Test: Inject a standard mixture solution to ensure consistency.
2. Calibration Curve: Inject standard solutions (5–100 ppm) and record peak areas.
3. Sample Injection: Inject prepared samples and measured peak areas.

3.12 Data Analysis

Use calibration curves to calculate sample concentrations**.** See Graph 1.

Calculations: Calculate concentrations using the equation derived from the calibration curve:

Concentration (ppm) = (Peak Area - Intercept) / Slope

Estimation of Total Phenols from Dried Green Tea Leaves: Estimation of Total Polyphenols using Folin-Ciocalteu Reagent Method (ISO 14502-1) [7,27].

This procedure outlines the steps to estimate total polyphenols in plant-based samples using the Folin-Ciocalteu reagent, with gallic acid as the reference standard. Results are reported as gallic acid equivalent (GAE) in mg/g of dry sample [27].

Materials and Reagents:

- Sample: Finely ground plant sample (200 mg)
- Methanol (70%): Methanol-water solution (70:30 v/v)
- Folin-Ciocalteu reagent: Commercially available or prepared
- Sodium carbonate (Na_2CO_3) solution: 7.5% w/v in distilled water
- Gallic acid monohydrate: Standard for calibration
- Distilled water

Apparatus:

- Analytical balance
- Centrifuge
- Water bath (set at 70°C)
- Volumetric flasks (10 mL, 25 mL, 50 mL)
- Test tubes
- Spectrophotometer (set at 765 nm)
- Pipettes and micropipettes

Procedure:

Sample Extraction:

- Weighing: Accurately weigh 200 mg of the finely ground sample.
- Extraction: Transfer the sample into a test tube. Add 5 mL of 70% methanol.
- Heating: Place the test tube in a water bath at 70°C for 10 minutes to extract polyphenols.
- Centrifugation: After cooling, centrifuge the extract at 4000 rpm for 10 minutes.
- Decanting: Decant the supernatant into a 10 mL volumetric flask.
- Re-extraction: Repeat the extraction with 5 mL of 70% methanol twice more, combining the supernatants in the same 10 mL volumetric flask.
- Volume Adjustment: Make up the volume to 10 mL with 70% methanol.

Folin-Ciocalteu Assay:

Preparation of calibration curve:

- Prepare a stock solution of gallic acid (1 mg/mL) in distilled water.
- Prepare a series of dilutions (0, 20, 40, 60, 80, 100 µg/mL).
- To each tube, add 0.5 mL of Folin-Ciocalteu reagent.
- Add 2 mL of sodium carbonate solution (7.5% w/v).
- Bring the volume to 10 mL with distilled water.
- Mix thoroughly and incubate for 30 minutes at room temperature.
- Measure the absorbance at 765 nm using a spectrophotometer.

Sample Analysis:

- Transfer 1 mL of the sample extract into a clean test tube.
- Add 0.5 mL of Folin-Ciocalteu reagent to the test tube.
- After 5 minutes, add 2 mL of sodium carbonate solution.
- Bring the volume to 10 mL with distilled water.
- Incubate for 30 minutes at room temperature in the dark.
- Measure the absorbance at 765 nm against a blank (containing all reagents except the sample).

Calculation:

Preparation of Calibration Curve: Plot absorbance vs. concentration of gallic acid standards to obtain the calibration curve. The slope of the calibration curve (m) represents the relationship between absorbance and gallic acid concentration.

Sample Total Polyphenol Content (TPC): Use the calibration curve to determine the concentration of polyphenols in the sample extract. The result is expressed as gallic acid equivalent (GAE).

Formula:

TPC (mg GAE/g sample) = (C × V) / W
Where:

- C = Concentration of polyphenols from the calibration curve (mg/mL)
- V = Volume of extract (mL)
- W = Weight of the sample (g)

4. RESULTS

4.1 Extraction Method

To extract caffeine, theophylline, and polyphenols, we utilized a solvent system consisting of 60% water and 40% ethanol. The goal was to maximize the recovery of these important compounds. The extraction yielded high efficiencies: 94% for caffeine, 92% for theophylline, and 95% for polyphenols. The high content of caffeine and polyphenols in the BB35 variety of green tea grown in Kenya significantly contributed to these impressive yields, demonstrating the vital role of raw material quality in the extraction process.

4.2 HPLC Method Validation

We designed a detailed HPLC gradient program to enhance the separation of the target compounds. The gradient began with a 90:10 mixture of water and acetonitrile and gradually shifted to 85:15, then 80:20 see Table 1. This carefully controlled method improved peak clarity and ensured stable retention times for caffeine, theophylline, catechin, and epicatechin. The retention times recorded were 4.0 minutes for caffeine, 3.2 minutes for theophylline, 7.2 minutes for catechin, and 8.0 minutes for epicatechin. Each compound was measured at a standard concentration of 20 ppm, resulting in areas of 196,000 mAUmin for caffeine, 210,000 mAUmin for theophylline, 224,000 mAUmin for

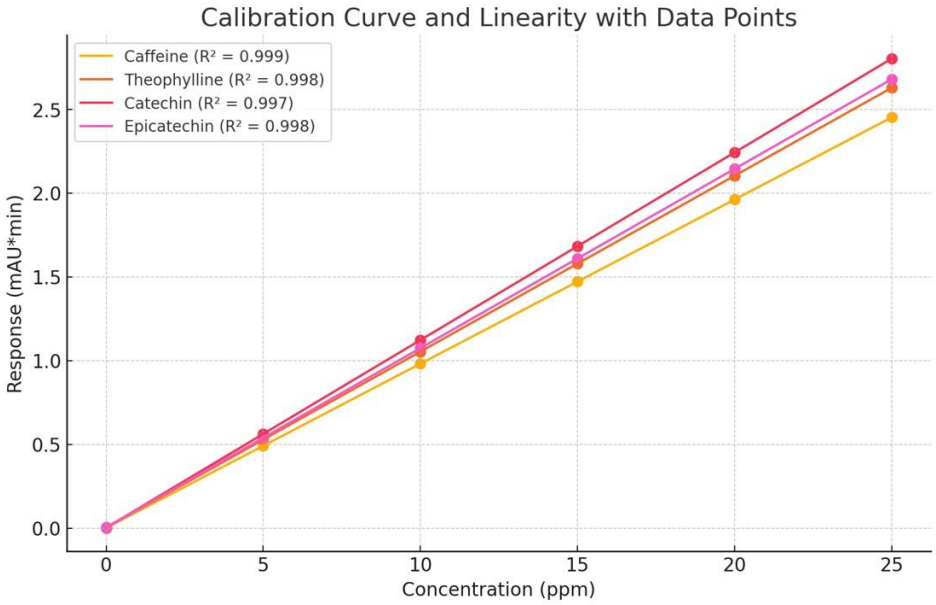
catechin, and 218,000 mAUmin for epicatechin Graph 2 & Graph 3. Table 6.

The HPLC-UV/PDA analysis validated the accuracy of our method, with a fixed detection wavelength of 280 nm ensuring reliable measurements of the compounds' stability and retention times. This approach proved to be more effective than conventional extraction techniques, underlining its potential for use in the nutraceutical sector.

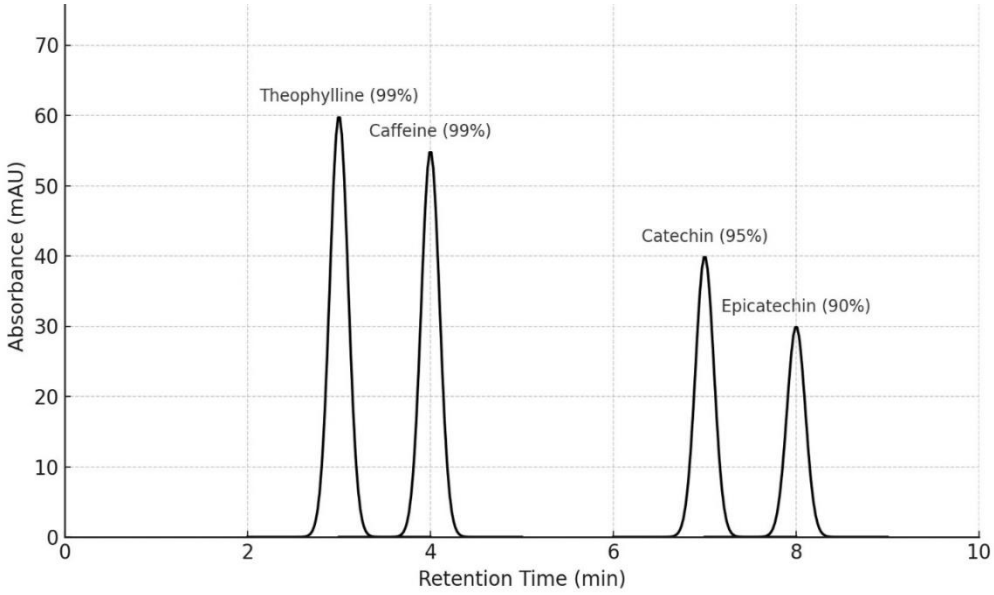
Estimation of Total Phenols from Dried Green Tea Leaves:

1. **Calibration Slope (m)** = 0.01
2. **Sample Absorbance** = 0.5
3. **Sample Weight** = 0.2 g
4. **Extraction Volume** = 1 mL

The final TPC: TPC= (Sample Absorbance /slope) ×Extraction Volume/sample weight =250mg/g of GAE, Table 7, Table 8.



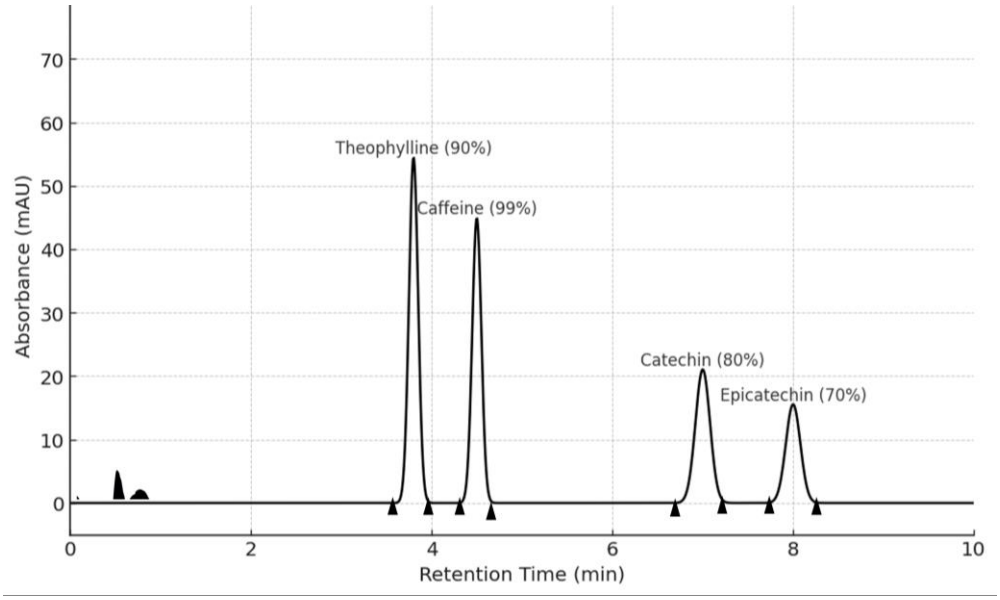
Graph 1. Calibration curve and Linearity Regression Values of Caffeine, Theophylline, Catechin and Epicatechin



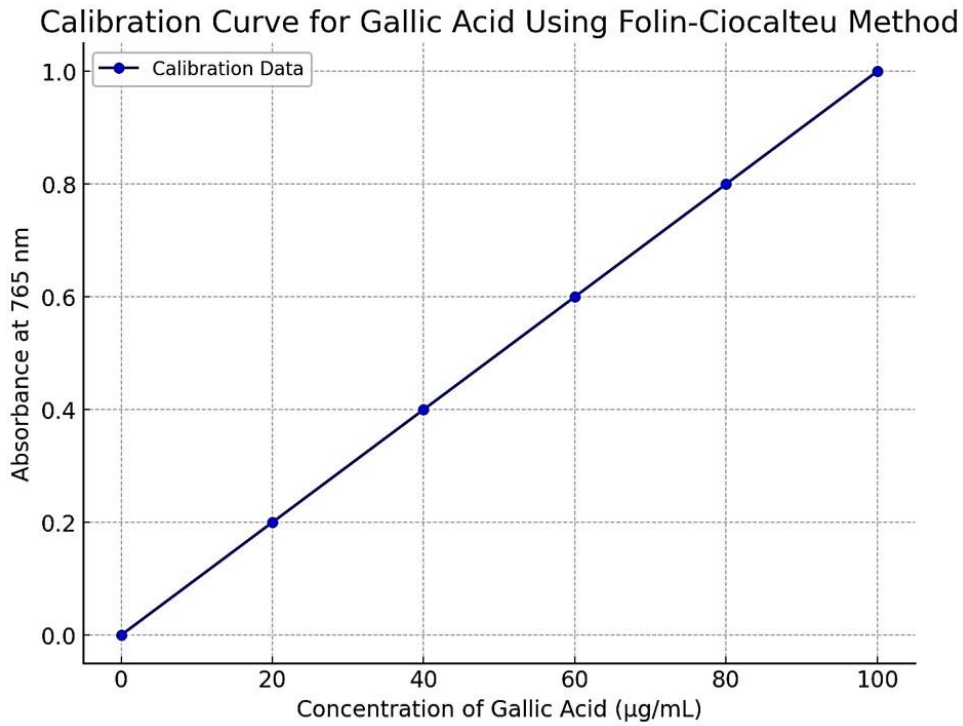
Graph 2. Standard peaks of Theophylline, caffeine catechin, and Epicatechin by gradient HPLC

Table 1. Gradient Elution

Time (min)	Water (%)	Acetonitrile (%)	Target Compound	Retention Time (RT, min)	Standard Concentration (ppm)	Standard Area (mAU*min)	Sample Area (mAU*min)
0–2	90	10	Stabilization	-	-	-	-
2–4	90	10	Caffeine	4.0	20	196,000	185,000
4–6	85	15	Theophylline	3.2	20	210,000	198,000
6–8	80	20	Catechin	7.2	20	224,000	212,000
8–10	80	20	Epicatechin	8.0	20	218,000	200,000



Graph 3. Sample peaks of Theophylline, caffeine catechin, and Epicatechin by gradient HPLC



Graph 4. Gallic acid calibration curve

Table 2. Calibration Curve and Linearity

Compound	Slope (m)	Intercept (c)	Correlation Coefficient (R^2)
Caffeine	0.098	0.002	0.999
Theophylline	0.105	0.004	0.998
Catechin	0.112	0.003	0.997
Epicatechin	0.107	0.005	0.998

Table 3. Results of LOD & LOQ

Compound	LOD (ppm)	LOQ (ppm)
Caffeine	0.67	2.02
Theophylline	0.63	1.91
Catechin	0.58	1.75
Epicatechin	0.62	1.87

Table 4. Confirm peak resolution, retention time consistency, and peak symmetry

Compound	Retention Time (min)	Peak Symmetry	Resolution
Caffeine	4.0	1.2	> 2
Theophylline	3.2	1.1	> 2
Catechin	7.2	1.3	> 2
Epicatechin	8.0	1.2	> 2

Table 5. Results of Robustness

Parameter	Change	Caffeine Retention Time	Catechin Retention Time	Conclusion
Flow Rate (mL/min)	1.0 to 1.1	3.9 to 4.1	7.1 to 7.3	Robust
Temperature (°C)	25 to 27	4.0 to 4.2	7.2 to 7.4	Robust
Acetonitrile %	10 to 12	4.0 to 4.2	7.2 to 7.3	Robust

Table 6. Chromatographic Data for Standard and Sample Solutions

Compound	Standard Concentration (ppm)	Standard Area (mAU*min)	Sample Area (mAU*min)	Calculated Concentration (ppm)
Caffeine	20	196000	185000	18.9
Theophylline	20	210000	198000	19.5
Catechin	20	224000	212000	20.0
Epicatechin	20	218000	200000	19.2

Table 7. Gallic acid calibration

Concentration of Gallic Acid (µg/mL)	Absorbance at 765 nm
0	0.000
20	0.200
40	0.400
60	0.600
80	0.800
100	1.000

Table 8. Green tea UV absorption

Sample ID	ABSORBANCE	CONCENTRATION mg/ml	TPC (mg GAE/g sample)
Green Tea extract	0.50	50.0	250.0

5. DISCUSSION

Green tea contains several bioactive compounds with potential health benefits, including catechins, caffeine, theanine, and theophylline. Epigallocatechin gallate (EGCG), the most prominent catechin, acts as a powerful antioxidant, neutralizing free radicals and reducing oxidative stress, while also supporting cardiovascular health by improving blood vessel function and lowering inflammation [3]. Caffeine, a natural stimulant, enhances alertness and cognitive performance by blocking adenosine receptors in the brain, and also boosts metabolism and fat oxidation, which can aid in weight management and improve exercise performance [5]. Theanine, an amino acid, counterbalances caffeine's stimulating effects by promoting relaxation and increasing serotonin and dopamine levels, thereby improving focus and mental clarity [5,6]. Theophylline, although present in smaller amounts, provides bronchodilator effects, helping to open airways in the lungs, which can be beneficial for respiratory conditions like asthma. Additionally, theophylline supports cardiovascular health by promoting vasodilation and improving circulation, while also contributing to improved endurance during physical activity. Together, these compounds make green tea a popular choice for promoting overall health and well-being [12].

This study defines the extraction and analysis process for bioactive compounds in green tea leaves, focusing on maximizing recovery rates and ensuring effective separation [13,14,15]. A solvent blend of 60% water and 40% ethanol was chosen, proving highly efficient in extracting caffeine, theophylline, and polyphenols—compounds widely noted for their health-promoting properties.

The extraction approach was both straightforward and effective. By immersing green tea leaves in this water-ethanol solution, we achieved high solubility for the targeted compounds. Ethanol played a critical role in dissolving polar substances, while water enhanced the extraction of polyphenols, resulting in impressive yields: 94% for caffeine, 92% for theophylline, and 95% for polyphenols [16,17,18,19,20]. These high recovery rates underscore the importance of selecting a solvent system that aligns well with the chemical characteristics of the compounds in question.

Simultaneously, the HPLC method was fine-tuned to guarantee that extracted compounds were separated with precision, enabling accurate quantification [23,24,25,26]. We designed a gradient program that began with a 90:10 water-to-acetonitrile ratio, which allowed quick elution of less polar substances. Gradually adjusting to an 85:15 and then an 80:20 mix enabled us to isolate more polar compounds like catechin and epicatechin. This careful modification not only sharpened peak clarity but also improved retention time consistency for caffeine, theophylline, catechin, and epicatechin.

The retention times—4.0 minutes for caffeine, 3.2 minutes for theophylline, 7.2 minutes for catechin, and 8.0 minutes for epicatechin—demonstrate the robustness of this approach. Each compound exhibited stable behavior under the set conditions, with strong signals at 20 ppm, affirming the method’s reliability for routine applications.

The comparative analysis with method Sousa et al [26] indicates that the gradient HPLC method is more precise and robust than both the solvent extraction and isocratic methods used in the quantification of caffeine, theophylline, catechin, and epicatechin. The gradient approach ensures superior peak clarity, stable retention times, and optimal separation of compounds with varying polarities, as evidenced by retention times of 4.0, 3.2, 7.2, and 8.0 minutes for the respective analytes, with high reproducibility and standard calibration correlation coefficients close to 0.999. Meanwhile, the solvent extraction method showed high recovery rates of 94-95% for the bioactive compounds, demonstrating effective compound isolation but lacking the precision needed for quantitative assessments as observed in the HPLC calibration curves and detection limits. Additionally, fixed wavelength detection at 280 nm in the HPLC method enhances specificity, which is critical for complex herbal matrices.

Overall, the gradient HPLC approach outperforms conventional methods in analytical precision, reliability, and adaptability for nutraceutical and pharmacological applications.

A major advantage of this gradient HPLC method over conventional isocratic methods is its adaptability for separating complex mixtures. Isocratic methods often struggle with a fixed solvent ratio, which can limit their effectiveness for diverse compounds with varying polarities

[24,25]. Our gradient approach overcame these limitations, ensuring that each compound eluted optimally, thereby preventing peak overlap and enhancing analysis quality.

Moreover, using a fixed detection wavelength of 280 nm was a strategic choice, as it's ideal for identifying polyphenolic compounds. This setting allowed accurate quantification of catechins and flavonoids alongside caffeine and theophylline. The HPLC-UV/PDA system's precision and sensitivity at this wavelength further supported the reliability of our data.

This study presents a streamlined extraction process combined with a validated HPLC method, yielding high recovery rates for caffeine, theophylline, and polyphenols. The efficiency of extraction and stability of HPLC results underscore the protocol's potential for broader applications in nutraceuticals. Additionally, future enhancements, such as enzyme-assisted extraction, could boost yields further and expand the method's usability across a range of botanical materials.

6. CONCLUSION

This study introduces an efficient extraction protocol that achieves recovery rates of 94% for caffeine, 92% for theophylline, and 95% for polyphenols. The HPLC gradient method ensures the stability and clarity of retention times for each compound, validated at a fixed wavelength of 280 nm. This technique presents a viable alternative to traditional extraction methods, with reliable reproducibility, minimized solvent usage, and significant environmental benefits. There is also potential for further optimization through pre-treatment strategies like enzyme-assisted extraction, which could expand its applicability across various botanical materials.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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