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Separation of Tannins and Caffeine in Black Tea Using Modified Microwave-Assisted Extraction and High-Performance Liquid Chromatography

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Abstract. Black tea is known to contain condensed tannins including catechin (C), epicatechin (EC), and epigallocatechin gallate (EGCG), as well as caffeine (CAF). Some consumers of specific diets avoid consuming tannins or CAF. Therefore, this study aimed to explore the fast and simple development of a method for separating and quantifying tannins and CAF in black tea using microwave-assisted extraction (MAE) followed by high-performance liquid chromatography (HPLC) analysis. MAE, a type of solid-phase extraction recently developed, was used with a modified microwave that simultaneously accommodates up to 24 samples. The effects of various MAE parameters, including microwave irradiation power, temperature, and solvent, were studied, with concentrations measured in mg/kg. The results showed that optimal extraction conditions were achieved at a microwave irradiation power of 1500 W, a temperature of 80°C, and methanol as the extraction solvent. The extracted samples were analyzed using a C18-M-SE reverse phase column (150 × 4.6 mm) with a mobile phase consisting of acetonitrile, methanol, and H3PO4 (1:49:50), and NaH2PO4 (20 mM) at pH 2.5. The three tannin compounds and CAF were separated in less than 10 min. The method demonstrated excellent repeatability (RSD ≤ 2%, n = 3), accuracy (96.7-102.5%), and was successfully applied to determine tannin content in commercial black tea samples.

Keywords: Black tea; Caffeine; Catechin; Epicatechin; Epigallocatechin gallate

1. Introduction

Black tea is among the most favorite teas (Zhang, Qi, and Mine, 2019) and has many health benefits, including antioxidants, anti-inflammatory, anti-obesity, and anti-cancer effects as well as preventing high blood pressure (Durgawale, Durgawale, and Khanwelkar 2016). The benefits are due to the components, such as tannins, flavonoids, phenolic acids, caffeine (CAF), amino acids, and other compounds (Shaukat *et al.,* 2023). The most crucial component is tannins (Naveed *et al.,* 2018). Tannins are phenol derivatives soluble in water

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and have a molecular weight of 300-5000 Daltons. Plants synthesize tannins naturally as secondary metabolic products (Mohan, Mohan, and Savithramma, 2019). Furthermore, tannins are divided into two types, namely hydrolyzed and condensed. Hydrolyzed tannins are polyesters of sugar molecules and organic acids such as gallic and ellagic acids (Plaza *et al.,* 2016). Weak acids or bases can hydrolyze - tannins to produce carbohydrates and phenolic acids. Examples of hydrolyzed tannins are gallotanin and ellagitannin (Amarowicz and Janiak, 2018). Meanwhile, condensed tannins are polymers of 2 to 50 or more carbon-bonded flavonoid units and are not susceptible to hydrolysis. Examples of condensed tannins (Figure 1) are catechin (C), epicatechin (EC), and epigallocatechin gallate (EGCG) (Soldado, Bessa, and Jerónimo, 2021).

C has been studied for pharmacological properties, including antihypertensive, anti-oxidative, anti-carcinogenic, and preventing dental caries (Rana *et al.,* 2016). EGCG is reportedly capable of suppressing brain dysfunction (Pervin *et al.,* 2019), and also the component with the highest antioxidant and free-radical-scavenging properties. It has 25 times more antioxidant activity than vitamin E and 100 times more than vitamin C, hence, EGCG can be an anti-cancer agent (Chen *et al.,* 2020). EC (flavan-3-ols) is a potential antioxidant with significant biological, pharmacological, and medicinal properties (Shay *et al.,* 2015). CAF is the most significant component of tea, hence, when measuring tannin concentration, CAF is always detected (Zhang *et al.,* 2020). Appropriate consumption drives away drowsiness, relieves fatigue, and stimulates the nerves (Iswanto *et al.,* 2023). High intake of CAF also has many side effects that can damage human health because of stimulatory effects but it is an essential factor in the quality of tea (Saraiva *et al.,* 2023).

Aside from the benefits, tannins also have adverse effects. People with iron deficiency should not consume excessive amounts of tannins because it potentially inhibits the reduction of iron from food (Delimont, Haub, and Lindshield, 2017). CAF is a stimulant that causes sleep disorders, anxiety, and increased heart rate (O'callaghan, Muurlink, and Reid, 2018). The daily standard for CAF consumption has been set by The Scientific Committee on Food of the European Commission at 150 mg L^{-1} (Turck *et al.,* 2022). Therefore, this study significantly contributes to the beverage industry by providing a method to separate and determine the concentrations of tannins and CAF in tea. The developed method also offers an alternative to existing methods. Tea producers can better tailor products to consumer preferences by separating CAF and tannins.

Tannins and CAF separation methods from the matrix are required for sample analysis. Naturally, the sample contains other interfering compounds that can interfere with the measurements. The analyte concentration is also usually on a micro or nano-scale, hence, a fast and simple separation method is needed before the sample is injected into high-performance liquid chromatography (HPLC). The separation methods include Soxhlet (Ruiz-Aquino *et al.,* 2023), liquid-liquid (Chaugule *et al.,* 2019), and solid-phase extraction (Martins *et al.,* 2020). A standard method for tannins and CAF separation is liquid-liquid extraction. This method requires large volumes of solvent and a long extraction time to produce large volumes of organic solvent for disposal (Wangkarn *et al.,* 2021; Das *et al.,* 2020) which is not in accordance with the principles of green chemistry. Molecular imprinting technology has been reported to separate CAF. However, this method does not determine the concentration of tannins and CAF in tea (Mehamod *et al.,* 2015). In this context, supercritical carbon dioxide fluid extraction (SFE) has also been applied (Serdar Demir, and Sökmen, 2019) but this method uses chlorinated solvents, which harm the environment. Microwave-assisted extraction (MAE) is a type of solid-phase extraction that has recently been developed due to the cheapness, specificity, fastness, accuracy, preciseness, and robustness of various molecules (Aparamarta *et al.,* 2019). MAE using non-

chlorinated solvents such as methanol and acetonitrile is a suitable method for extracting tannins from biomass in a short time and with excellent recovery (Ciuperca *et al.,* 2023; Brantsen *et al.,* 2021; Silva *et al.,* 2021). Following capillary electrophoresis, the method has been used to determine C and EC from green tea, producing extraction recoveries of more than 100% (Li *et al.,* 2010).

Several analytical methods have been used to determine tannins and CAF concentration. The simplest method has been reported using an ultraviolet-visible (UV-Vis) spectrophotometer (Loum, Byamukama, and Wanyama, 2020). Advanced methods, such as liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), are used to analyze tannins. These methods provide more reliable quantitative and qualitative data with improved analytical accuracy and precision but are expensive. HPLC (Rahim, Nofrizal, and Saad, 2014) is the primary method used to determine tea tannins and CAF. Fast analytical methods have been developed in the past few years. A simple tannins and CAF determination method is adapted from the initial screening chromatogram, then the mobile phase composition is varied, changing the flow rate, temperature, and other measurement conditions. Due to the demand for fast and accurate separations from chromatography, optimizing separation conditions is still an art. Different separation methods and equipment produce various separations.

This study presents the relationship between initial screening chromatograms and rapid separation of tannins (C, EC, EGCG) with optimized CAF (Figure 1) using MAE method on black tea samples to produce optimal separation rates. The microwave has been modified to accommodate the simultaneous extraction of up to 24 samples. The modification made was increasing the number of cells. A previous study used 6 sample cells for extraction (Rahim, Nofrizal, and Saad, 2014), while 24 cells were used in this study to make extraction more efficient (see Figure 2).

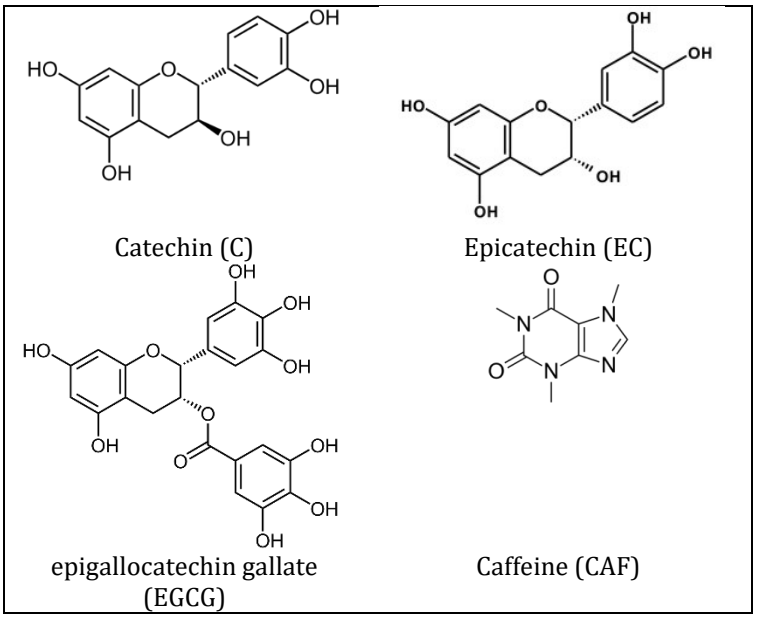

Figure 1 Molecular structure of C, EC, EGCG, and CAF

Therefore, the developed method can separate tannins and caffein compounds faster. Several extraction parameters, such as temperature, microwave irradiation power, and solvent composition, have been optimized to obtain separation conditions for tannins and caffein compounds at mg g^{-1} levels. A previous study used microwave irradiation power in the 300-600 W range and high temperatures. The result showed that Malaysian green tea tannins and CAF extraction have good separation (Rahim, Nofrizal, and Saad, 2014), but the

DBS Hypersil gold C-18 monolithic column used is expensive. Therefore, in this study, the extraction of tannins and CAF from Indonesian black tea was reported using a C18-M-SE reverse phase column (150 × 4.6 mm) that can be found in standard laboratories due to the lower price. MAE temperatures (60-90°C) and irradiation power (1200-1800 W) were optimized for highly efficient extraction. The optimized procedure was validated through recovery and comparison with previous results to determine tannins in black tea samples.

2. Methods

2.1. Chemical and Reagents

All chemicals used were of analytical reagent grade. The C, EC, EGCG, and CAF standards were purchased from Sigma (Germany). Standard stock solutions were prepared by weighing 0.01 mg of each and dissolving in 100 mL of acetonitrile and were stored in the dark at -20°C. The standard solution of C, EC, EGCG, and CAF for calibration or spiking purposes was prepared daily by diluting the stock solutions in acetonitrile (HPLC grade) supplied by Merck (Germany). Water was purified with Milli-Q IQ 7003 Water Purification System (Merck, Germany). The solution was filtered using a 0.45 µm nylon filter before HPLC injection. Black tea used for the recovery study was purchased from a local store (Jakarta, Indonesia).

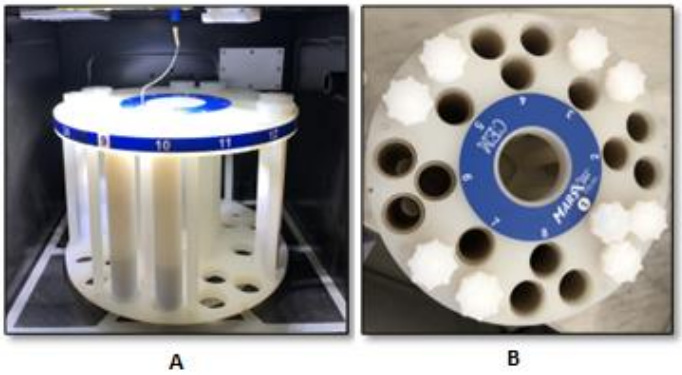

Figure 2 24 Microwave assisted extraction cells side view (A) and top view (B). This cell is placed in a microwave to extract tannins and CAF from black tea

2.2. Apparatus

Black tea sample extraction was conducted using a microwave (Mars 6, CEM Corporation, USA), as shown in Figure 2. Chromatographic analysis was carried out with an HPLC (Agilent 1260 Infinity Quaternary LC) equipped with diode array detector (DAD). A C18 reverse phase VDSpher PUR 100 C-18-M-SE (150 × 4.6 mm for length vs inner diameter) with particle size of 3 µm and pore size of 100 Å. The column was purchased from Germany and used as the analytical column.

2.3. *Preparation of Sample and Sample Extraction Method*

Black tea samples were ground with mortar and pestle then passed through a 20-mesh sieve (The Cary Company, USA). After grinding, 100 g of sample was dried in an oven at 40°C for 24 hours. A 2 g sub-sample was prepared and placed in the extraction vessel. For extraction using the spike method, standard solutions of C, EC, EGCG, and CAF in methanol (1 mg L^{-1}) were added to the sample. The recovery data was obtained from spiked samples by adding 1 mL of a 25 mg L^{-1} tannin standard solution and caffein mixture into a 25 mL volumetric flask.

MAE extraction stages include lean (temperature increase), holding (temperature maintenance), and cooling. The extraction time selected referred to previously reported studies (Ahmad *et al.,* 2023). The parameters to be studied include microwave irradiation

power (1200, 1500, and 1800 W), the extraction temperature (60°C, 70°C, 80°C, and 90°C), and the extraction solvent (acetone, methanol, water, and ethanol). The extract was diluted with extraction solvent to 30 mL and then the solvent was evaporated at 90°C. The dried extract was added with 1 mL of methanol and ready to be injected into HPLC.

2.4. *The Analysis of* high-performance liquid chromatography (*HPLC)*-diode array detector *(DAD)*

The mobile phase composition used was acetonitrile/methanol/0.05 % H_3PO_4 1/49/50 (v/v/v) and 20 mM NaH_2PO_4 with pH 2.5 at a flow rate of 0.8 mL min^{-1} and injection volume of 8 µL. NaH_2PO_4 was weighed as 3.1202 grams, then dissolved in aqua millipore and adjusted to pH 2.5 using H_3PO_4 85%. The choice of mobile phase was based on the validated C separation method using a C18 HPLC reverse phase VDSpher PUR 100-C-18-E (150 x 4.6 mm for length vs inner diameter) with a particle size of 3.5 µm (Andreas *et al.,* 2019). The column was maintained at ambient temperature and DAD was set at a wavelength of 210 nm. All the measurements were carried out in triplicate. Reverse phase chromatography is a separation method where the stationary phase is a nonpolar compound (C18) and the mobile/solvent phase is a polar compound.

Figure 3 shows HPLC instrumentation where a peristaltic pump sets the solvent (mobile phase) flow rate in mL/min. The analyte was injected, solvents produced tannins and CAF, then the separation occurred in the column (stationary phase). The injector used is the autosampler.

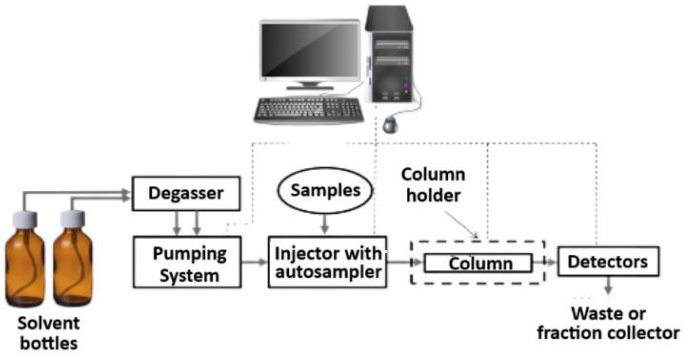

Figure 3 HPLC instrumentation with autosampler

3. Results and Discussion

3.1. HPLC Method Optimization

Separation of C, EC, EGCG, and CAF was carried out using a C18 column. Good separation resolution was obtained using a mobile phase composition of acetonitrile/methanol/0.05% H_3PO_4 1/49/50 (v/v/v) and 20 mM NaH_2PO_4 with pH 2.5, flow rate of 0.8 mL min^{-1} and a total analysis time of 10 min (Table 1). The average retention time (min ± SD., n = 10) under the condition was 6.000 ± 0.3 (C), 7.912 ± 0.3 (EGCG), 8.901 ± 0.3 (CAF), and 9.991 min ± 0.3 (EC) (Supplementary file). Previous studies have reported separating C, EGCG, and EC using a VDSpher Pur 100 C18-E column with the same mobile phase but the retention times obtained were longer. C, EGCG, and EC had retention times of 12.09, 12.98, and 13.75 min, respectively (Andreas *et al.,* 2019). The difference in retention time from the previous study is because the larger the particle column size, the slower the separation. The column particle size of the previous study was 3.5 µm compared to 3.0 µm used in this experiment. Compared to this study, all the compounds can be determined in less than 10 min using an isocratic mode of the mobile phase, decreasing the analysis time per sample. Four compounds were separated with a resolution above 1.5, indicating that all the compounds are well-separated. Table 1 shows the retention time of black tea sample,

with CAF having the highest peak height. This indicates that the concentration of CAF in black tea is the greatest.

Table 1 Retention time of tannins and CAF in standard and sample solution

Retention time		Compound
Standard solution	Sample solution	
6.0	5.9	catechin (C)
7.9	8.2	epigallocatechin gallate (EGCG)
8.9	9.0	caffeine (CAF)
9.9	9.8	epicatechin (EC)

3.2. Optimization of MAE for C, EGCG, CAF, and EC Extraction in Black Tea Method

3.1.1. MAE Irradiation Power

Microwaves produced electromagnetic waves that interacted with water molecules, polar solvents, and other polar substances in the sample, causing rapid heating. Therefore, thermal energy increased, as well as the process of dissolving tannins and CAF. Extraction without the use of microwave irradiation takes a longer time. Optimization of irradiation power is needed to obtain optimum conditions while also avoiding tannins and CAF degradation. Figure 4 shows a series of samples to study the effect of microwave irradiation on the % extraction recovery. There was no significant color change when the power was increased from 1200, 1500, and 1800 W. However, the % recovery was optimum at 1500 W, and the calculation was carried out using the results of HPLC measurements.

This study examined the effect of 1200 W, 1500 W, and 1800 W power on extraction efficiency. Based on the C, EGCG, CAF, and EC recovery %, the recovery data was obtained from spiked black tea samples by adding 1 mL of a 25 mg L^{-1} tannin standard solution and CAF mixture into a 25 mL volumetric flask. The recovery % was calculated based on Equation 1.

$$\text{Recovery } \% = \frac{[\text{spike}]_{\text{measured}}}{[\text{spike}]_{\text{calculated}}} \times 100\% \quad (1)$$

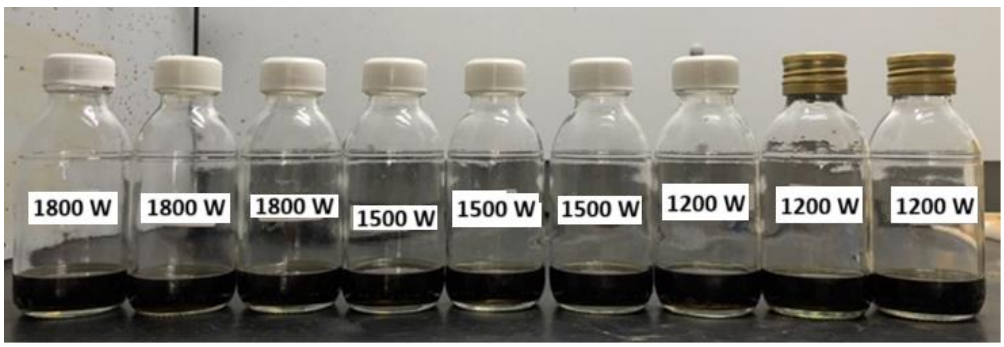

Figure 4 Black tea samples after extraction with MAE using variations in irradiation power of 1200, 1500, and 1800 W

The recovery % for C, EGCG, CAF, and EC with microwave power irradiation of 1200 W ranged from 75-80%. When the power was increased to 1500 W, the tannins recovery % started to increase to 96%. According to a previous study (Ismail-Suhaimy *et al.,* 2021), increasing microwave power can increase the extracted analyte concentration. When the power was increased to 1800 W, there was a decrease in tannins and CAF recovery up to 77-80% (Figure 5). Generally, increasing power will enhance the extraction recovery. However, extremely high microwave power can also reduce the extraction results due to the degradation of compounds. The results are consistent with a previous study (Ciuperca *et al.,* 2023), stating that tannin concentrations decrease with increasing microwave power.

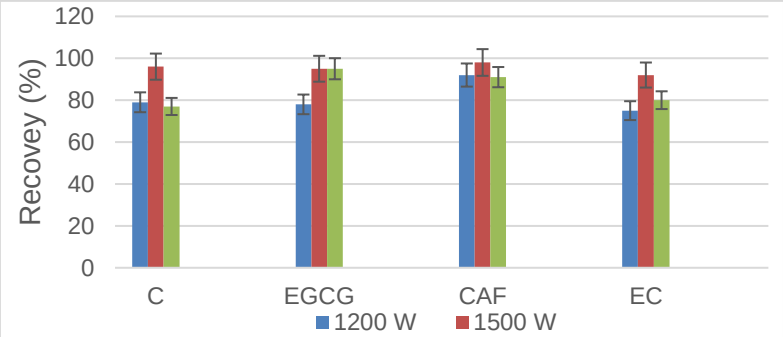

Figure 5 Extraction recoveries of C, EGCG, CAF, and EC from black tea sample spiked at 1 mgL^{-1} using MAE and HPLC analysis (n = 3) with various microwave irradiation power

MAE method in this study has several advantages, including short extraction time, where it can simultaneously extract samples in large quantities. Furthermore, the solvent used in MAE is only a small amount (30 to 50 mL) compared to other extraction methods. Previous study proved that extraction using MAE method produced the highest tannins concentration, compared to maceration, reflux, and ultrasonic-assisted extraction (UAE) methods with concentrations of 0.41% each, 0.45%, 0.65% respectively (Zhang Lin, and Ye, 2018). Therefore, MAE has advantages over other extraction methods.

3.1.2. Extraction temperature

C, EGCG, CAF, and EC extraction using MAE were strongly influenced by temperature. Generally, temperature affects the ability of the solvent to absorb microwave power. The temperatures optimized in this study were 60°C, 70°C, 80°C, and 90°C. The extraction temperature used was only up to 90°C because, according to a previous study (de Hoyos-Martínez *et al.,* 2019), higher temperatures cause lower tannins due to stability. Moreover, selectivity was reduced in extraction, co-extracting other substances in the sample.

Based on Figure 6, C, EGCG, CAF, and EC recovery % increased from 60°C to 80°C, ranging from 76-97%. The highest recovery % was at 80°C and decreased tannins levels at 90°C. Generally, high temperatures can increase tannins and CAF concentration. An increase in intermolecular interactions in the solvent causes a rise in the solubility, leading to enhanced final recovery. Higher temperatures also affect pressure, which causes bond rupture and increases the components to be extracted (Chaves *et al.,* 2020).

Tannin levels will generally increase until optimum temperature conditions are reached and then followed by a decrease due to degradation. In this study, tannins and CAF were degraded at an extremely high temperature (90°C).

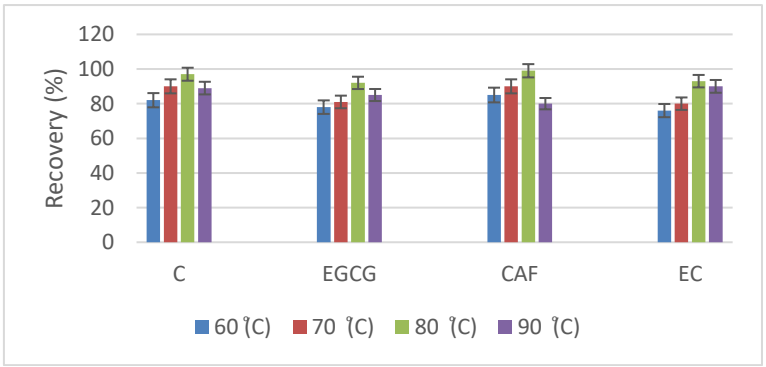

Figure 6 Extraction recoveries of C, EGCG, CAF, and EC from black tea sample spiked at 1 mgL^{-1} using MAE and HPLC analysis (n = 3) at different temperatures (60-90°C)

The optimum temperature for tannins at 80°C and degradation at 90°C is consistent with previous study. The results showed that the highest tannins were at an optimum temperature of 80°C and decreased at 90°C (Handayani *et al.,* 2019).

3.1.3. Extraction Solvent

Methanol, ethanol, water, and acetone were applied for MAE extraction solvent. These solvents were selected according to the solubility of C, EGCG, CAF, and EC in the sample without co-extracting other matrix components. Moreover, the polar solvents were selected due to the ability to easily absorb microwave energy. Polar solvents have molecules with permanent dipole moments that can interact with microwaves efficiently. The solvent molecules interact with the electric field of microwaves. This polarity causes the molecular dipoles to vibrate rapidly, producing heat when exposed to microwave radiation. Solvents with high dielectric constants, such as methanol (ε': 23.9) and ethanol (ε': 24.3), can absorb microwave energy more efficiently than non-polar ones. Although less polar than methanol and ethanol, acetone has a sufficient dielectric constant (ε': 21.1) to absorb microwave heat.

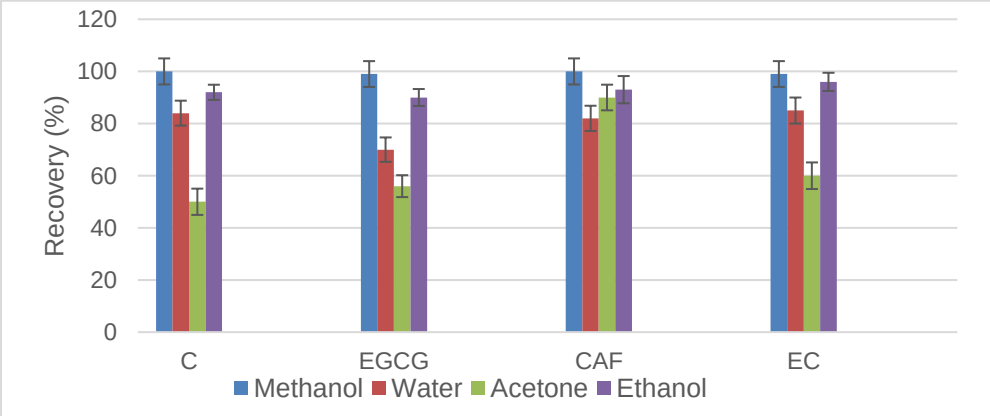

Figure 7 Extraction recoveries of C, EGCG, CAF, and EC from black tea sample spiked at 1 mgL^{-1} using MAE and HPLC analysis (n = 3) with various solvent extraction.

The extraction recoveries of C, EGCG, CAF, and EC from black tea samples spiked at 1 mgL^{-1} using MAE and HPLC analysis (n = 3) with various extraction solvents are shown in Figure 7. Excellent recoveries were obtained for C, EGCG, and CAF in methanol, ranging from 97% to 100%. The extraction recoveries were slightly reduced when ethanol and water were used as solvents. Acetone as an extraction solvent showed a high reduction in recovery. Tannins are considered polyphenolic compounds with polarity properties due to the presence of many hydroxyl groups (-OH), which increase solubility. Methanol has very high polarity compared to ethanol and acetone, hence, it forms stronger hydrogen interactions with the hydroxyl groups of tannins.

Methanol smaller molecular size than ethanol and acetone allows faster diffusion into the material matrix during extraction, leading to the selection as the optimum solvent. Water can also dissolve tannins due to the polarity but precipitates in large aggregates, making it less effective for pure extraction. Methanol is more effective in dissolving tannins than ethanol, acetone, and water due to the high polarity, ability to form strong hydrogen bonds with hydroxyl groups, small molecular size, and better interaction with functional groups. Although other solvents, such as water and ethanol, can be used, methanol provides more efficient extraction results due to the more suitable physicochemical properties.

A previous study (Bhebhe *et al.,* 2016) stated that in black tea, water was found as the most optimal solvent with the highest total phenol concentration, and the lowest was

achieved with acetone. Another study (Anand *et al.,* 2015) proved that extraction using methanol for green tea samples produced the highest total phenol concentration while the lowest was recorded with ethanol.

3.1.4. Performance of the Analytical Method

HPLC method developed has been validated for the linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy. A linear calibration curve was obtained by plotting the peak area vs standard concentration, ranging from 0.5-20 mg L^{-1}. The coefficient of determination for C, EGCG, CAF, and EC ranged from 0.9926 to 0.9999. In conclusion, all the analytes showed good linearity.

LOD and LOQ were calculated based on a signal-to-noise ratio of 3:1 and 10:1 (Shrivastava and Gupta, 2011). The calculations were based on measurements of blank samples spiked with 1 mg L^{-1} of each standard (Table 2). LOD for the four compounds ranged from 0.1-0.2 mg L^{-1} range, while LOQ was between 0.35-0.59 mg L^{-1}. A previous study also found the range of LOD and LOQ at the mg L^{-1} level (Rahim, Nofrizal, and Saad, 2014). Data observations for the repeatability and reproducibility of the peak areas were obtained by injecting each standard mixture (1, 5, and 10 mg L^{-1}) on the same day (intra-day) and for three consecutive days (inter-day). The repeatability was obtained from relative standard deviation (RSD) value for n = 3. RSD value < 2.5% was found for all experiments, as shown in Table 2.

Table 2 Limit of detection, limit of quantification, and precision using MAE and HPLC

Analyte	Linear range (mgL^{-1})	R^2	LOD (mgL^{-1})	LOQ (mgL^{-1})	Intra-day (%RSD), n = 3			Inter-day (%RSD), n = 3		
					Fortified concentration level (mg L^{-1})					
					1	5	10	1	5	10
C	0.5-20	0.996	0.12	0.41	1.12	1.35	1.24	1.40	1.30	1.82
EGCG	0.5-20	0.993	0.11	0.35	1.29	2.28	1.86	1.90	1.43	2.46
CAF	0.5-20	0.998	0.20	0.59	1.48	1.35	1.61	1.10	1.15	2.35
EC	0.5-20	0.996	0.17	0.59	2.06	1.41	1.74	1.85	2.05	1.70

The optimized MAE and HPLC methods were applied to determine tannins and CAF in one commercial black tea sample. C, EGCG, CAF, and EC concentrations were determined using the external standard calibration curve method, and analysis was carried out in triplicate. Based on the calculated data, the concentrations obtained were 61.91, 393.1, 582.9, and 159.8 mg kg^{-1}, respectively (Table 3).

C concentration in black tea sample was the lowest. This is due to maximum oxidation and changes in C, which turns into the aflavin and the arubigin during fermentation (Abudureheman *et al.,* 2022). The highest concentration in black tea sample was CAF. Generally, CAF concentration depends on the origin of the tea and the processing process (Deka *et al.,* 2021). A comparative extraction study with previous investigations is shown in Table 4. This study showed a better efficiency in terms of recoveries and consumed time per sample, than other previous studies.

Table 3 The concentration of C, EGCG, CAF, EC in black tea sample

	Fortified concentration (mg kg^{-1})	Found concentration (mg kg^{-1})
C	61	62 ± 1.0
EGCG	391	393 ± 2.3
CAF	578	583 ± 3.0
EC	161	160 ± 2.2

Table 4 Previous extraction method for the analysis of tannins in tea

Extraction Method	Sample	Retention time (min)	Extraction time (min)	Extraction solvent	Literature
Maceration	Green tea leaves	C: 13.78; EC: 16.49; EGCG: 17.68	60	100 mL of 70 % ethanol	(Maslov *et al.*, 2021)
Liquid-liquid extraction	Turkish green tea	C: 13; EC: 16.5; EGCG: 19	40	Citric acid water extraction	(Demir, *Serdar, and Sökmen,* 2016)
Supercritical carbon dioxide fluid extraction	Green tea	CAF: 11.18; C: 16.44; EC: 20.60; EGCG: 22.03	4	ethanol: water mixture	(Ruiz-Aquino *et al.,* 2023)
Solid phase extraction using N-vinylpirolidon-divinylbenzen copolymer	Wine and apple cider	C: 15.9; EC: 19.4	20	10 mL of 70 % aqueous ethanol containing 1 % formic acid for o	(Tomaz and Maslov, 2016)
Microwave assisted-extraction	Black tea	C: 5.991; EGCG: 8.201; CAF: 9.000; EC: 9.880	15	30 mL of methanol	this study

Conventional liquid-liquid extraction including maceration, and separating funnel takes longer because heat transfer occurs by conduction. Polar molecules absorb microwaves, leading to vibration and generation of internal heat in the sample. The temperature in the microwave can also be adjusted, in contrast to liquid-liquid extraction, which depends on room temperature while maceration uses slow conventional heating. In MAE, temperature can be precisely controlled during analyte extraction, allowing users to achieve optimal temperatures in a shorter time.

4. Conclusions

In conclusion, the determination of C, EGCG, CAF, and EC in black tea samples using MAE and HPLC offers several advantages over other HPLC methods with shorter retention times. Compared to other methods reported in the literature, this process renders extraction times in 15 min with a fast separation chromatography in 10 min. MAE extraction procedure significantly reduced the sample preparation. It also showed excellent characteristics in terms of precision and accuracy. The combination of MAE and HPLC in this study can be used as an alternative method for determining tannins and CAF content. Based on the result, measuring actual black tea samples showed that CAF concentration was the highest while C was the lowest.

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Conflict of Interest

The authors declare no conflicts of interest.

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