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Determination of Total Phenolic Content and Antioxidant Activity of Epigallocatechin Gallate *Rich Fraction* from Tea Leaves (*Camellia sinensis* L.)

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ABSTRACT: The tea plant, *Camellia sinensis* L., is a well-known natural product with significant health advantages. The main catechin found in green tea leaves is epigallocatechin gallate (EGCG), which has potent antioxidant properties. EGCG is a common active ingredient in products from the medical and cosmetics industries. This study examines the antioxidant properties and total phenolic content (TPC) of an EGCG-rich fraction (ERF) that is extracted from green tea leaves. TPC was quantified using the Folin-Ciocalteu method, with absorbance measured via UV-VIS spectrophotometry at 760.5 nm. Antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, with absorbance recorded at 516 nm. The findings indicated that the TPC of ERF was 437.18 mg GAE/g ± 5.54, while the IC_{50} value for antioxidant activity was 4.47 mg/L, This value was lower than that of the positive control, Vitamin C, which exhibited an IC_{50} of 5.07 mg/L. These results suggest that ERF could be developed into a natural ingredient for cosmetics and dietary supplements.

Keywords: **antioxidant; DPPH; epigallocatechin gallate; folin-ciocalteu; green tea; total phenolic compound.**

Introduction

Tea (*Camellia sinensis* L.) is one of the most popular traditional beverages, especially in Indonesia. There are four types of tea: black, white, green, and oolong tea. Green tea is treated without fermentation, which entails the deactivation of oxidase and phenolase enzymes in fresh tea leaves. This processing method maintains high polyphenol content, which ranges between 30% and 35% [1]. Catechins, which constituting approximately 42% of the polyphenols in green tea, are classified into four major types: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [2]. The most abundant of them is EGCG which comprising approximately 13% of total polyphenols in green tea [3]. EGCG is recognized for its antioxidant capabilities [3,4], which are beneficial in cosmetic applications, especially for anti-aging effects. Therefore, further investigation into the total phenolic content of green tea leaves is essential to ensure the quality and efficacy of their applications in cosmetic formulations.

The phenolic content is closely related to antioxidant activity, with increased phenolic concentrations generally leading to improved antioxidantive potential [5]. The Folin-Ciocalteu method is commonly utilized for detection of Total Phenolic Compoun (TPC). This assay works by reducing the Folin-Ciocalteu reagent (FCR) in the presence of phenolics, which produces a molybdenum-tungsten blue complex that can be detected spectrophotometrically at 760 nm. The reaction intensity is related to the sample's phenolic concentration [6]. TPC levels are commonly given as gallic acid equivalents (GAE) per gram of extract [7].

While various studies have been conducted on the TPC of green tea extracts, research focused on specific chromatographic fractions, especially those high in EGCG, is very limited. The purpose of this work is to quantify the TPC in the EGCG-rich fraction of green tea extract obtained using chromatographic fractionation.

Methods

Material

Fresh green tea leaves, 50% ethanol, analytical-grade ethanol, *n*-hexane, ethyl acetate, silica

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gel 60 (0.063-0.2 mm) (Merck), TLC silica gel 60 F_{254} (Merck), chloroform (Merck), acetic acid (Merck), formic acid, isopropanol, 10% H_2SO_4 (Merck), EGCG standard (Sigma Aldrich), $FeCl_3$, gallic acid (Merck), Folin-Ciocalteu reagent, distilled water, and 7.5% Na_2CO_3 were used in this study.

Plant Collection and Determination

Green tea leaves were collected from Cileueur, Sukamanah, Bogor, West Java. The botanical determination was conducted at the Bogoriense Herbarium, National Research and Innovation Agency (BRIN), Cibinong, Bogor Regency, West Java.

Simplicia Preparation

Collected tea leaves were sorted and washed to remove foreign particles. The leaves were then cut into smaller pieces and air-dried at room temperature to prevent oxidative degradation [8].

Extraction and Fractination

Powdered green tea leaves (400 g) were subjected to maceration flasks with 5 L of 50% ethanol at room temperature for 14 days. The macerate was filtered and concentrated using a rotary evaporator at 45°C. The resulting extract (95.86 g) was subjected to liquid-liquid extraction with *n*-hexane and ethyl acetate, followed by fractionation via column chromatography using silica gel 60 as the stationary phase and an ethyl acetate-ethanol as mobile phase. The resulting fractions were analyzed via thin-layer chromatography (TLC) with a chloroform:acetic acid:formic acid:isopropanol solvent system [9-13].

Determination of Total Phenol Content

The TPC was determined by the Folin-Ciocalteu method. A gallic acid standard calibration curve was prepared using concentrations ranging from 60 to 100 mg/L. Fraction samples (200 µL) were mixed with 2 mL of Folin-Ciocalteu reagent (1:10), followed by the addition of 1.6 mL of 7.5% Na_2CO_3. A sample's absorbance was measured at 760.5 nm using a UV-Vis spectrophotometer. The mixture is then mixed with a vortex for 15 seconds and then heated for 15 minutes at 45°C [14].

Antioxidant Activity Assay

DPPH radical scavenging activity was determined by mixing 1 mL of sample solution with 2 mL of 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark for 30 minutes, and absorbance was measured at 525 nm. Vitamin C was used as a positive control. The IC_{50} value was calculated from the inhibition curve [14].

Result and Discussion

The botanical identification confirmed that the collected plant material belonged to *Camellia sinensis* L. of the Theaceae family. The extraction method rodiced 95.86 g of concentrated extract, which corresponds to a 23.55% yield and meets the standard minimum limit (7.8%) for green tea extract [5]. Liquid-liquid extraction effectively separated non-polar compounds into the *n*-hexane phase, while phenolic compounds remained in the ethyl acetate phase [4]. Following column chromatography, five fractions (F1-F5) were obtained, with F1 identified as the EGCG-rich fraction (ERF) based on TLC analysis , with

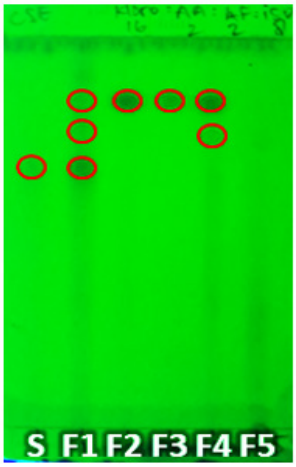

Figure 1. TLC profile of EGCG standard and column chromatography fraction (visualization by UV 245 nm)
S = Standard EGCG, F1= Fraction 1, F2= Fraction 2, F3= Fraction 3, F4= Fraction 4, F5= Fraction 5.

Table 1. Column chromatography fraction and Rf values.

Fraction	Vial number	Rf Value(s)
F1	1-10	0.6 0.75 0.8
F2	11'-18', 11"-20"	0.8
F3	11-18, 12"'-15"'	0.8
F4	19-50	0.8
F5	51-128	-

Table 2. Absorbance of gallic acid standards at 760.5 nm.

Concentration (mg/L)	Absorbances (λ) 760,5 nm			
	Replicate 1	Replicate 2	Replicate 3	Average
60	0.41	0.42	0.42	0.42
70	0.48	0.48	0.48	0.48
80	0.53	0.54	0.55	0.54
90	0.59	0.60	0.60	0.60
100	0.65	0.64	0.65	0.65

Rf value of 0.6 corresponding to the EGCG standard (Figure.1) (Table1).

Each fraction of the column chromatography results was measured with a UV-VIS spectrophotometer at a wavelength of 760.5 nm for three replicates. The TPC analysis revealed that F1 (ERF) had the highest phenolic content (437.18 mg GAE/g ± 5.54) followed by F4 (160.23 mg GAE/ g ± 3.63), F2 (144.42 mg GAE/ g ± 2.04), F3 (86.65 mg GAE/ g ± 4.99), and F5 (46.36 mg GAE/ g ± 0.79).

In this experiment, total phenol content was determined using the Folin-Ciocalteu technique and a UV-Vis spectrophotometer. Gallic acid was chosen as a standard because of its structural simplicity, chemical stability, and relevance as a common phenolic constituent. Gallic acid reacts with the Folin-Ciocalteu reagent, producing a yellow hue that changes to blue with the addition of sodium carbonate (Na_2CO_3). The alkaline conditions of Na_2CO_3 promote the redox reaction between phenolic chemicals and the Folin-Ciocalteu reagent, resulting in the creation of a blue-colored complex. The intensity of the blue coloring corresponds to the concentration of phenolic chemicals present [7].

The total phenolic content is an important parameter in determining antioxidant capacity because phenolic compounds are well known for their ability to donate

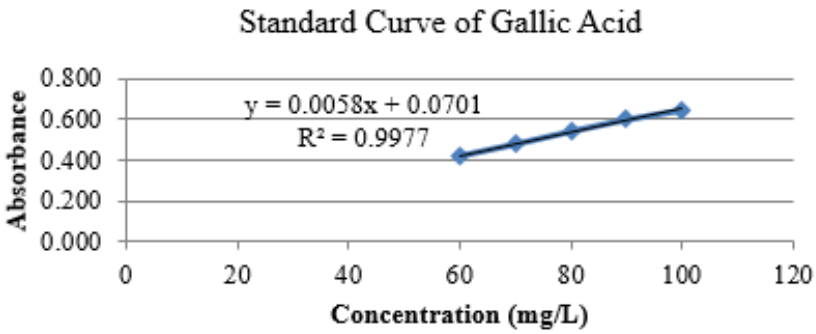

Figure 2. Standard curve of gallic acid.

Table 3. Total phenol content from the fraction of column chromatography fractions.

Fraction	Concentration (mg/L)	Sample absorbance	Total Phenolic Content (mgGAE/g)	Average (mgGAE/g)	SD
		0.33	441.21		
1	100	0.32	430.86	437.18	5.54
		0.32	439.48		
		0.56	142.21		
2	600	0.57	144.80	144.42	2.04
		0.58	146.24		
		0.60	92.22		
3	1000	0.56	85.15	86.65	4.99
		0.55	82.57		
		0.73	163.44		
4	700	0.70	156.29	160.23	3.63
		0.72	160.97		
		0.34	47.05		
5	1000	0.33	45.50	46.36	0.79
		0.34	46.53		

electrons or hydrogen atoms to neutralize free radicals, thereby inhibiting oxidative stress and related cellular damage. Thus, a higher absorbance value indicates a greater total phenolic content, which in turn suggests a stronger antioxidant potential of the tested sample [3].

The antioxidant activity assay of the F1 (ERF) obtained from column chromatography of ethanol extract of green tea leaves revealed an IC_{50} value of 4.47 mg/L, indicating its capacity to scavenge 50% of DPPH radicals at this concentration. This value was lower than that of the positive control, Vitamin C, which exhibited an IC_{50} of 5.07 mg/L. Furthermore, the Antioxidant Activity Index (AAI) values of the F1 (ERF) and Vitamin C were 8.95 mg/L and 7.89 mg/L, respectively, classifying both as very strong antioxidants. These findings demonstrate that the ERF possesses superior antioxidant potential compared to Vitamin C.

Conclusion

This study demonstrated that the EGCG-rich fraction (ERF) of green tea extract exhibited a high total phenolic content (437.18 mg GAE/g ± 5.54) and strong antioxidant activity (IC_{50} = 4.47 mg/L). The promising antioxidant activity of the EGCG-rich fraction support its potential utility in the development of natural antioxidant agents for pharmaceutical and cosmetic formulations. Nevertheless, it is important to acknowledge the study's limitation as it primarily focused on in vitro analyses, excluding factors such as drug stability, permeability, metabolic transformation, and systemic bioavailability. Additionally, interactions with other phytoconstituents were not explored. Consequently, further research involving *in- vivo* models, stability testing, and formulation evaluations is necessary to validate the therapeutic potential and commercial viability of this fraction.

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