Short Communication:

Determination of Caffeic Acid in Ethanolic Extract of Spent Coffee Grounds by High-Performance Liquid Chromatography with UV Detection

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Abstract: *We developed a method for determining the caffeic acid in spent coffee grounds. The spent coffee ground solution was prepared by blending 3 g spent coffee grounds with 60 mL ethanol/water (40/60 v/v) for 2 h on a hot plate magnetic stirrer (60 °C, 350 rpm). The mixture was filtered and the filtrate was concentrated under vacuum (60 °C) to 5 mL. The method employed a reversed-phase high-performance liquid chromatography with a UV detector. We used a Phenomenex Luna column (250 × 4.6 mm; i.d., 5 µm) under isocratic elution, and the mobile phase was acetonitrile-methanol-aqueous formic acid (10:10:80 v/v), with a flow rate of 0.9 mL/min. Analysis was performed at 324 nm. The column temperature was set at 27 °C temperature. The results showed that this method was selective for quantifying the caffeic acid in spent coffee grounds with good linearity in the range of 1.31–17.07 µg/mL. The detection and quantitation limits were 0.28 and 0.84 µg/mL, respectively. The mean intraday and interday recoveries were 83.80–95.17% and 82.16–97.40%, respectively. Intraday and interday precision expressed as the relative standard deviation (RSD) were below 7.3%. There was 0.17% ± 0.006 w/w caffeic acid in the spent coffee grounds (RSD = 3.63%, n = 3).*

Keywords: *caffeic acid; reversed phase high performance liquid chromatography; spent coffee grounds*

■ INTRODUCTION

Indonesia is the third-largest coffee producer and exporter in the world after Brazil and Vietnam [1]. Based on USDA Foreign Agricultural Service annual report data, Indonesian coffee production in 2019/2020 was forecast to reach 10.7 million bags: up to 9.45 and 1.25 million 60-kg bags of Robusta and Arabica, respectively. Consumption for 2019/2020 was forecast at 4.9 million bags based on continued strong consumer demand, and Robusta coffee has become one of the favorite drinks of Indonesians [2].

Spent coffee grounds (SCG) are the insoluble organic residue after coffee beans have been dehydrated, milled, and brewed [3-4]. In January 2020, the International Coffee Organization (ICO) estimated that coffee consumption would increase from 1.24 million bags to 169.34 million bags by 2019/2020 [5]. According to these data, a high quantity of SCG will be produced from coffee beverage preparation, discarded as domestic or industrial trash, and cause environmental problems [6]. It has been estimated that 1-ton green coffee beans can generate 650 kg SCG, and 1 kg soluble coffee produced results in 2 kg wet SCG [7]. Therefore, a good waste management plan is required to reduce SCG waste.

One means of reducing SCG waste is to recycle and utilize it. SCG from the brewed coffee process has been used as a renewable fuel resource because it contains lignocellulose biomass [8]. Unfortunately, society has not utilized SCG to its maximum potential [9] even though SCG contains phenolic compounds with the potential to be useful for medical uses, such as caffeic acid [10-11]. Caffeic acid is a hydroxycinnamate and a phenylpropanoid metabolite which are more widely distributed in plant tissues. It has been reported that the biological activities of caffeic acid include carcinogenic

inhibition, antioxidant, antibacterial, and preventing atherosclerosis and other cardiovascular diseases. It can be used as a potent human matrix metalloproteinase-9 (hMMP-9) inhibitor also [12-15]. Therefore, SCG could be explored as a source of caffeic acid for medical purposes [16].

Caffeic acid in SCG can be detected and quantified by high-performance liquid chromatography with diode array detection (HPLC-DAD) and HPLC–tandem mass spectrometry (MS/MS) [17-18]. Unfortunately, both samples in previous research were SCG from *Coffea arabica*. *Coffea robusta* contains more chlorogenic acid (a caffeic acid ester-linked to quinic acid) than *C. arabica* [19]. Besides that, that previous study performed gradient elution, which requires re-equilibration with the initial mobile phase composition, requiring more time than isocratic elution [20].

For all these reasons, this research was aimed at developing a simple, selective, and sensitive method for quantifying the caffeic acid in SCG by HPLC. The SCG from the roasted bean of *C. robusta* was selected for analysis in this research. The results indicate that the method is valid and can be readily for quantifying caffeic acid in SCG.

■ EXPERIMENTAL SECTION

Materials

Caffeic acid (98%) was purchased from Sigma-Aldrich (Missouri, USA). Methanol (LC grade) was purchased from Supelco (Pennsylvania, USA). Formic acid (98–100%), ethyl acetate (99.5%), and acetonitrile (LC grade) were from Merck Millipore (Darmstadt, Germany). Ethanol (96%) was purchased from Brataco Chemica (Jakarta, Indonesia). Double-distilled water was obtained from the Analytical Instrument Laboratory, Faculty of Pharmacy, Universitas Sanata Dharma, Yogyakarta, Indonesia. Samples of coffee (Excelso Robusta Gold, Batch No. 8991002102132, Santos Jaya Abadi, Sidoarjo, Indonesia) were purchased from a local market.

Instrumentation

A Shimadzu® (Kyoto, Japan) LC-2010HT system (No. C21255111004 LP with UV/Vis detector) was used. T460 ultrasonicator, analytical balance (Scaltec® SBC 22, max, 210 g; min, 0.01 mg), rotary evaporator (Buchi®), oven (UN 45), membrane filter holder (Whatman®, 300-mL capacity, Cat. No. 1960-004) were used. Inorganic solvent membrane filter (Whatman®, 0.45-µm pore size, 47-mm diameter), syringe filter (Millipore®, 0.20-µm pore size, 25-mm diameter), and a set of micropipettes (Socorex®) were used in this study.

Chromatographic Conditions

The Shimadzu LC-2010HT system with Lab Solution software and UV-Vis detector was developed and modified based on Doncea et al. [21], with the mobile phase composition and stop time modification. A C18 column (Luna Phenomenex®, 250 × 4.6 mm, 5 µm) was used. The mobile phase composition was acetonitrile-methanol-aqueous formic acid (10:10:80 v/v/v) with an isocratic elution system at a flow rate of 0.9 mL/min and detection at 324 nm. The injection volume was 10 µL; the column temperature and stopped time were set at 27 °C temperature and 50 min, respectively.

Procedure

Preparation of stock solution and calibration solution

The stock solution of caffeic acid at 1,313 mg/L was prepared with methanol. A series of calibration solutions were prepared by diluting adequate caffeic acid stock solution volumes with methanol.

Preparation of SCG

The Robusta coffee grounds (5 g) were accurately weighed and placed in a beaker glass. About 200 mL of hot water (60 °C) was added to the beaker glass and stirred four times, then left to brew for 4–5 min. The coffee was filtered, and the filtrate was removed and dried in the oven for 24 h at 60 °C.

Sample preparation

The extraction conditions were a combination and modification by Andrade et al. [22] and Juliantari et al. [23]. SCG sample (3 g) was accurately weighed and blended with 60 mL ethanol-water (40:60 v/v) for 2 h on a hot plate magnetic stirrer (60 °C, 350 rpm). The mixture was filtered, and the filtrate was concentrated

under vacuum (60 °C) to 5 mL. The caffeic acid was extracted by liquid/liquid extraction with ethyl acetate (20 mL × 3). The extracts were then combined, and the ethyl acetate was evaporated. The residue was dissolved in 7 mL methanol. The dissolved residue (1 mL) was transferred to a 5.0-mL volumetric flask, then 500 µL of this solution was transferred into a microtube and diluted to 1.0 mL with methanol. All diluted samples and stock solution series were sonicated for 10 min and filtered using a Millipore® syringe filter before injection.

Analytical method validation

The selectivity, linearity, range, detection limit, quantitation limit, accuracy, and precision of the analytical method for the determination of caffeic acid was validated according to the International Conference on Harmonization (ICH) and the Association of Official Analytical Chemists (AOAC) guidelines [24-25].

■ RESULTS AND DISCUSSION

Selectivity

The selectivity test was performed on both standard and sample solutions containing caffeic acid. It was set by determining the resolution value, which indicates the separation of each compound's peak. Fig. 1(a) and 1(b) depict a representative chromatogram of the caffeic acid standard and SCG, respectively. The resolution of caffeic acid in the sample was 3.178.

Linearity and Range

Caffeic acid was quantified using the standard external method. The slope, intercept, and coefficient of determination (R^2) for the calibration plot was calculated using linear regression analysis. The linearity was determined with various concentrations of the standard solution. The calibration curve, obtained by plotting the

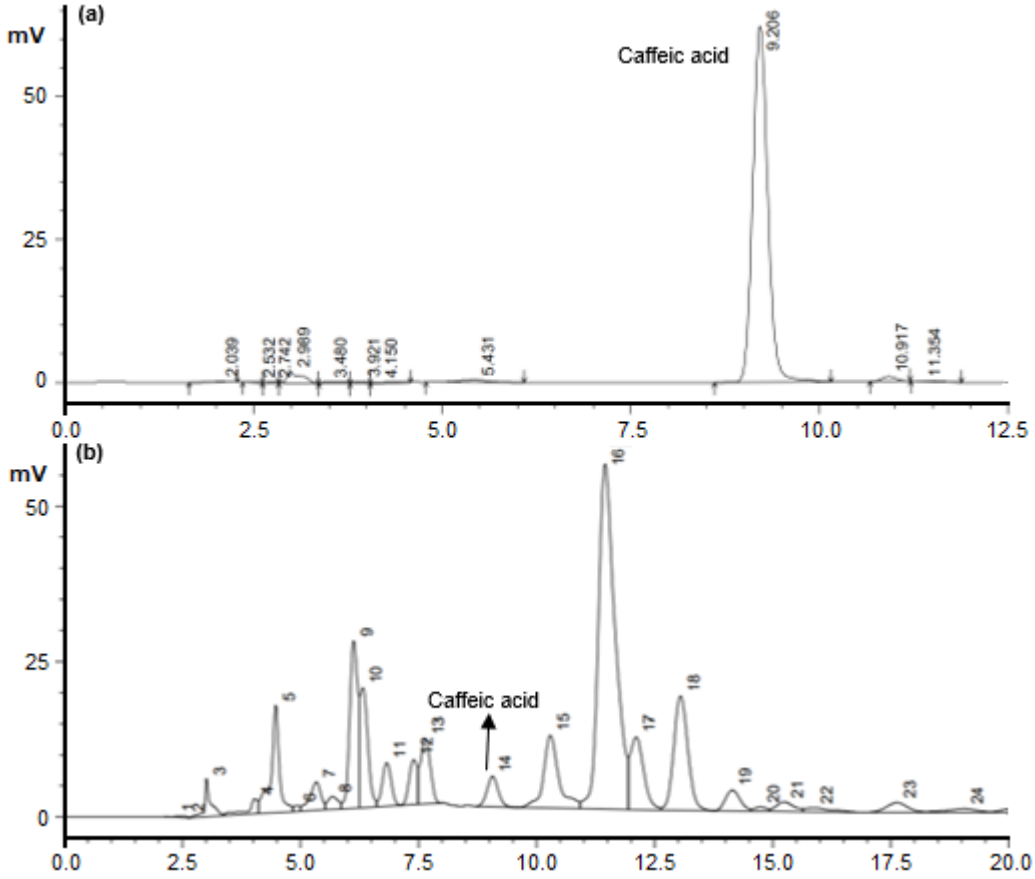

Fig 1. (a) Chromatogram of caffeic acid standard. (b) A close-up of the SCG chromatogram. All chromatograms were run using: a Phenomenex® C18 column (250 × 4.6 mm, 5 µm). Mobile phase: methanol-acetonitrile-aqueous formic acid (10:10:80 v/v). The flow rate was set at 0.9 mL/min and detection was at 324 nm

peak area from the HPLC chromatogram, was plotted against the known concentrations of stock solutions. The calibration curve equation of caffeic acid was y = 31,320x – 29,990 (R^2 = 0.9998). This method was linear in the range of 1.31–17.07 µg/mL.

Limit of Detection and Limit of Quantitation

Different dilutions of the caffeic acid were injected into the HPLC. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the standard deviation (SD) approach. The LOD and LOQ were 0.28 and 0.84 µg/mL, respectively.

Accuracy and Precision

The accuracy and precision were calculated using the standard addition method. Accuracy was expressed as recovery, and precision was expressed as the relative SD (RSD). They were determined over 3 consecutive days by spiking three caffeic acid concentrations into the SCG. In the present study, the recovery at a medium and high level was comparable with that of Angeloni et al. [18], who obtained 94.9% recovery with a spiking level of 2.5 mg/kg. However, at a low level, the recovery was lower than that of Angeloni et al. [18], where they obtained 93.0% recovery with a spiking level of 0.25 mg/kg. Here, the RSD at each level was comparable with that of Angeloni et al. [18] as they reported an RSD of < 10.7% for each level. Both accuracy and precision fulfilled the AOAC requirement [25] because they were 80–110% for recovery and < 7.3% for RSD. The results show that this method produces high precision and accuracy for determining caffeic acid at all concentration levels, not only for intraday evaluation but also for interday evaluation (Table 1).

Assay

We used an optimized and validated method for analyzing the caffeic acid in SCG. The determination of the samples of caffeic acid was based on the standard external method. Here, the SCG contained 0.17% ± 0.006 w/w caffeic acid, with RSD of 3.63% (n = 3). This result was less than that of the results by Angeloni et al. [18], as they obtained 5.826 mg caffeic acid per kg SCG. This discrepancy was caused by the SCG preparation and extraction conditions, which were sub-optimum for extracting all of the caffeic acids from the sample. The different methods of SCG preparation also affected the results. However, we could not compare our results with that of Ramón-Gonçalves et al. [17] because they did not report the caffeic acid content in their research.

■ CONCLUSION

We successfully developed a reversed-phase (RP)-HPLC for determining the caffeic acid in SCG. The proposed method showed good resolution, linearity, sensitivity, accuracy, and precision. The Robusta SCG examined contained 0.17% ± 0.006 w/w caffeic acid [RSD = 3.63% (n = 3)].

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Table 1. Evaluation of intraday and interday accuracy and precision

	Concentration spiking levels	Recovery (%) [a]	RSD (%)
Intraday	Low (1.31 µg/mL)	83.80 ± 2.82 [b]	3.37
	Medium (2.63 µg/mL)	97.17 ± 5.58 [b]	5.74
	High (3.94 µg/mL)	95.15 ± 5.81 [b]	6.12
Interday	Low (1.31 µg/mL)	82.16 ± 1.36 [c]	1.65
	Medium (2.63 µg/mL)	96.61 ± 6.73 [c]	6.97
	High (3.94 µg/mL)	97.40 ± 2.79 [c]	2.86

[a] Recovery expressed as mean ± SD; [b] Average from three replicates; [c] Average from three replicates on three consecutive days

■ AUTHOR CONTRIBUTIONS

EPI initiated the research project. MRG conducted the experiments and the analysis, MRG wrote the initial draft. MRG and EPI revised the manuscript. All authors agreed to the final version of this manuscript.

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