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Optimization Method of Caffeine Isolation of Merapi Green Coffee Beans

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Abstract : Merapi Green coffee bean is a green and immature bean, that is originated from coffee plantations nearby volcano located in the province of Daerah Istimewa Yogyakarta, Indonesia. They are derived from waste product during harvesting. Alkaloid is one natural ingredient compounds contained in coffee bean and has a basic structure of nitrogen with a bitter taste. The purpose of this study was to optimize method of caffeine isolation ofMerapi green coffee bean. Green coffee bean was macerated using chloroform and then partitioned with 80% of methanol. The results of partitioning were isolated by preparative thin layer chromatography (PTLC) method using a mobile phase of chloroform: ethyl acetate (1: 3) v/v. The active compounds were tested by TLC densitometry method. The results showed levels of caffeine in isolate was 67.27%.
Key words : green coffee bean, volcano, isolation, TLC, densitometry.

Introduction

Merapi Green coffee bean is a green and immature bean, that is originated from coffee plantations nearby volcano located in the province of Daerah Istimewa Yogyakarta, Indonesia. They are derived from waste product during harvesting. Alkaloid is one natural ingredient compounds contained in coffee bean and has a basic structure of nitrogen with a bitter taste. Coffee contents main active ingredients such as caffeine, sitosterin, choline, and terpenoids. Robusta and Arabica are widely consumed coffee species in Indonesia[9]. Green coffee bean contains caffein, phenolic compounds, and chlorogenic acid[2]. Robusta coffee has a higher caffeine content than Arabica coffee[12]. Caffeine content in green coffee *(C.arabica* and *C.canephora)*1.45% and 2.38%,respectively[1]. Coffee beans quality depends on moisture content, defects, bean size, some chemical compounds and preparation of a sample to perform cup tasting[8].

Caffeine or 1,3,7–trimetilxantin is a purine alkaloid compoundwith molecular formula $C_8H_{10}N_4O_2$.Caffeine is one of many constituents in foods that can exert physiological effects. Scientific and historical evidence shows that among the healthy adult population, moderate caffeine consumption (e.g., (400 mg/day) is not associated with adverse health effects. Improvements inmental alertness, concentration, fatigue, and athletic performance are well documented benefits[4,10].

Thin-layer chromatography (TLC)-densitometry is analytical method used for the determination of levels of caffeine in green coffee bean. This method is relatively simple, inexpensive, and rapid. Densitometry is intended for the quantitative analysis of analytes at low concentrations, which previously carried out the separation by Thin Layer Chromatography (TLC). Green coffee beans used in study was waste products from

mature coffee harvest. The purpose of this study was to determine the levels of caffeine in green coffee bean using TCL-densitometry method and optimize the caffein isolation method.

Experimental

Materials

The main material used in this study was green coffee bean obtained from coffee plantations in Petung subvillage, Kepuharjo village, Cangkringan Sleman District, Yogyakarta province. The standard of caffeine (Sigma), chloroform (Merck), methanol (Merck), ethyl acetate (Merck), distilled water, silica gel F_{254}(Merck).

Procedures

1. Preparation of the main ingredients

Merapi coffee beans obtained from wasted green coffee, which were harvested in March 2015 from Petung subvillage, Kepuharjo village, Cangkringan Sleman District, Yogyakarta province.

2. Preparation of extract

Merapi green coffee beans were macerated with chloroform in the vessel for 3 x 24 hours, and every 1x24 hours the solvent was replaced and stirred frequently. Extract was filtered and the filtrate was concentrated with a rotary evaporator in water bath to obtain chloroform extract.

3. Preparation of a 80% methanol-soluble partition

Chloroform extract was dissolved in 80% methanol. Extract was vortexed and sonicated. The filtrate was dried with a water bathto obtain a 80% methanol-soluble partitions.

4. Making fraction

The results ofa 80% methanol-soluble partition was vacuum in liquid colom (VLC) system with a gradient mobile phase of chloroform (F1), chloroform: ethyl acetate (1: 1) (F2), chloroform: ethyl acetate (1: 4) (F3), and ethyl acetate (F4). VLC F1 was chosen for the isolation.

5. Separation of isolates

Results of VLC F1 was then chromatographed in preparative thin layer using a mobile phase of chloroform: ethyl acetate (1: 3). The preparative result was dried to produce isolates.

6. Identification of co-chromatographic isolates and caffeine standard

Isolate solution and caffeine standard are mixed and spotted on a TLC plate. The plate was eluted in mobile phase of chloroform: ethyl acetate (1: 3). Spots were observed and calculated as R_f value.

7. Determination of the active compound using TLC densitometry method

Stock solution of isolates and caffein standard were prepared with concentration of each 1 mg / 1 mL in chloroform. Prepared standard solution with concentration of 1µg/mL,3 µg/mL, 5µg/mL, 7µg/mL, and 9 µg/mL and active compound of1µg / mL, 5 mg / mL, and 9 mg / mL.5µl of standard solution and isolated were spotted on a TLC plate using linomat 5 (Camag). TLC plates was placed into the vessel that has been saturated with a mixture of Clororform: ethyl acetate (1: 3). The vessel was closed and allowed to mix the with mobile phase until reaching to the upper limit. TLC plate was then scanned with a densitometer (TLC Scanner 3 Camag) at a wavelength of 254 nm to determine the value of AUC (area under curve). The concentration of the active compound in green coffee bean was calculated using linearity equations.

Results and Discussion

The yields of extract, partition, fraction, and isolates were 1.53%, 27%, 51.12%, and 41.77%, respectively. Co-chromatography of isolates and caffein standard eluted with chloroform : ethyl acetate (1: 3) v/v identified in UV_{254}resulted the same R_f value of 0.33.TLC profile can be seen in Figure 1.

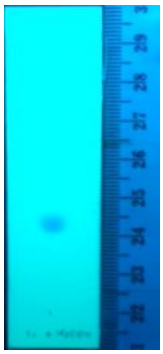
Determination by using a combination of TLC and densitometry methods was quite economic because it uses less mobile phase, consumes a relatively short time and can be done multiple assay simultaneously[13]. The solution of isolates and caffeine standard were spotted 5 µL, respectively on the TLC plate with various concentrations. TLC plates were inserted into the vessel that has been saturated with a mixture chlororform: ethyl acetate (1: 3) v/v. The vessel was closed and the mixture allowed to rise to the limit. The results of elution TLC can be seen in the Figure 2. TLC was scanned with a densitometer at maximum wavelength 254 nm. The results of this study were shown in Figure 3. The calculation showed that the equations resulted r value of 0.9122.thethree-dimensional densitogram profile seen in figure 4 showed that isolate detected at a wavelength of 254 nm has a R_f value of 0.33.

Based on the data from densitometry, the concentration isolates were detected adalah3.542 µg/mL and 5.733 µg/mL, while the isolates concentration were at 5 mg / mL and 9 mg / mL. The different concentration of isolates caused by several factors such as isolates are not pure enough, the measurement process and the lack of proper spotting. The level of caffeine concentrations in both isolates were 70.84% and 63.7%, respectively, with the average concentration of caffein was 67.21% The concentration of caffein is presented in Table 1.Differences in chemical content and the caffeine content of coffee beans depending on the type of coffee, geography, and the roasting process[3,7].

Conclusion

Itcan be concluded that the average concentration of caffein in Merapi green coffee bean obtained from coffee plantations in Petung subvillage, Kepuharjo village, Cangkringan Sleman District, Yogyakarta province using TLC densitometry method with a mobile phase mixture of chloroform: ethyl acetate (1: 3) on silica gel F_{254} was 67.27%.

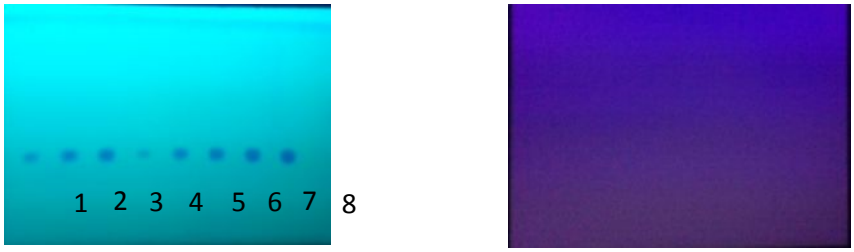
Figures and Tables



A

b

Figure 1.Elusion of isolate and caffein standard co-chromatography using chloroform : ethyl acetate (1: 3) v/v on silica gel F_{254} (a) identification by UV_{254} (b) identification by UV_{366}



A b

Figure 2. Elution of caffein standards and isolates using usingchloroform : ethyl acetate (1: 3) v/v on silica gel F_{254} (a) identification by UV_{254} (b) identification by UV_{366}

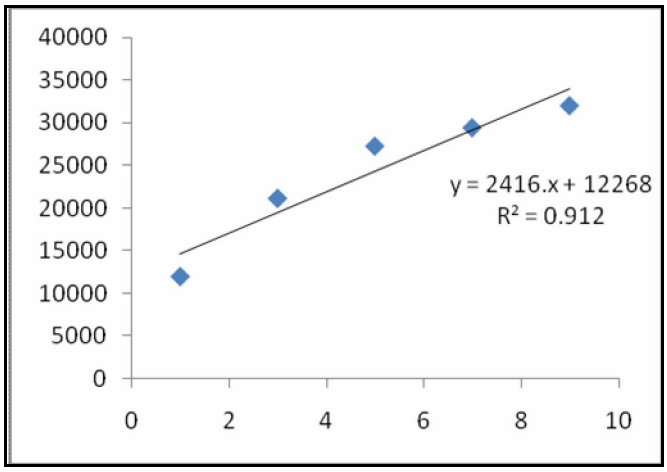


Figure 3. Equation of calibration curve of caffein standard using TLC-densitometry method

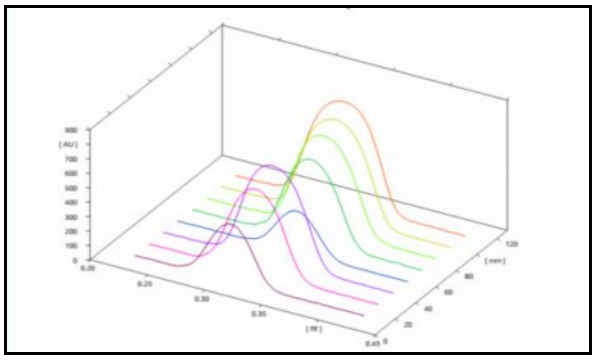


Figure 4. Three-dimensional densitogram profile derived from elusion of mobile phase chloroform : ethyl acetate (1:3) v/v

Table 1.the concentration of caffein in Merapi green coffee beans

No.	Concentration (µg/mL)		Caffein in isolates (%)	Average caffein in isolates (%)
	Calculation	Densitometer		
1	5	3.542	70.84	67.21
2	9	5.733	67.21	

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