Journal of
Food and Pharmaceutical Sciences

Original Article

The Effect of Variation in Solvent Concentration on Caffeine Content in Green Arabica Coffee Bean Extract (*Coffea arabica*) using UV-Vis Spectrophotometry

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Received: 30 November 2024; Revised: 20 December 2024; Accepted: 25 December 2024; Published: 31 December 2024

Abstract: Coffee is one type of beverage that is widely favoured by the community, Arabica coffee plants are a variant that grows a lot in Indonesia. Coffee beans contain various chemical compounds, one of which is caffeine. This study aims to determine the caffeine content with different solvent concentrations. Green Arabica coffee beans (*Coffea arabica*) were extracted using the maceration method. Ethanol extract 70% and ethanol extract 96% of green Arabica coffee beans were then fractionated in liquid-liquid extraction of chloroform and water phases. Then the analysis and determination of caffeine content were carried out with a UV-Vis Spectrophotometer. Qualitative tests were carried out using the Parry reagent color test showing positive results with a change in color to green. The results of the study in the form of a % caffeine content value to the weight of the extract sample, showed that in the 70% ethanol extract of green Arabica coffee beans contained a caffeine content of 10.115 ± 0.06% and the 96% ethanol extract of green Arabica coffee beans was 12.760 ± 0.580%. The results of the analysis showed that the amount of caffeine content had a significant difference with a p-value <0.05. So it can be concluded that the optimum solvent for extracting caffeine from green Arabica coffee bean extract is 96% solvent.

Keywords: Arabica Green Coffee Beans; Caffeine; Maseration; UV-Vis Spectrophotometer.

1. INTRODUCTION

Arabica coffee (*Coffea arabica*) is a type of coffee that is often planted and popular in global trade. The characteristics of Arabica coffee are small leaves, thin crowns, few ketai, and small flowers. Arabica coffee beans are different from other coffee beans. The shape is rather long with a convex surface but not too high. The color of the beans is lighter and the tips are bright. The center of the beans has a flat and bent groove [1]. Coffee contains high phenolic compounds such as caffeine, chlorogenic acid, caffeic acid, ferulic acid, pyrogallic acid, and trigonelline which are often used in diet programs. In addition, there are also other bioactive compounds such as polyphenols, flavonoids, melanoidins, trigonelline, various minerals, and carbohydrates that function as antiproliferatives, antimicrobials, anti-inflammatories and antioxidants [2]. Caffeine has clinically useful pharmacological effects, such as stimulating the central nervous system by relieving feelings of fatigue, hunger, and drowsiness[3]. Green Arabica coffee beans are chosen because they have a better caffeine and chlorogenic acid content than roasted Arabica coffee. Caffeine and chlorogenic acid can act as photoprotectors so they can protect against UV-B radiation [4].

The amount of compounds contained in coffee beans depends on the solvent used[5]. Caffeine solubility is very low in water, slightly in ethyl acetate, pyrimidine, pyrrole, acetone, and very high in petroleum ether, ether, benzene and chloroform. In FI Edition V, 2015 it is stated that caffeine is soluble in 1:1 parts of hot water, 1:7 in chloroform and 1:130 in ethanol. The analytical method used in determining caffeine levels is UV Spectrophotometry. Caffeine is a compound that has chromophore and auxochrome groups, so it can be analyzed using UV Spectrophotometry [6]. The purpose of this study was to determine the acquisition of caffeine compounds in 96% ethanol extract of green Arabica coffee beans and 70% ethanol extract of green Arabica coffee beans using UV Spectrophotometry.

2. MATERIALS AND METHODS

2.1. Materials and tools

The tools used in this study were analytical scales, porcelain cups, funnels, Erlenmeyer flasks, beakers, measuring cups, sieves, grinders, measuring flasks, micropipettes, sonicators, hotplate stirrers, UV-Vis Spectrophotometry. The samples used were green Arabica coffee beans (*Coffea arabica*) taken from coffee plantations in the Genting Village area, Semarang, Central Java. The materials used in this study were 70% ethanol, 96% ethanol, and chloroform (Merck). caffeine standard, filter paper, $CaCO_3$.

2.2. Method

2.2.1. Sample Preparation

Arabica green coffee beans were taken from Genting Village, Semarang, Central Java. The clean samples were dried again using an oven at a temperature of 50^0C then ground and sieved with a sieve with a mesh number of 30.

2.2.2. Extract Preparation

The sieved powder was weighed as much as 300 g to then be extracted by maceration using 70% ethanol and 96% ethanol as much as 1200 ml each. The macerate was left for 24 hours then the macerate was re-macerated twice using 70% ethanol and 96% ethanol each as much as 600 ml for 24 hours with occasional stirring. The total macerate was filtered with a Buchner funnel to separate it from impurities or other solids, then evaporated with a rotary evaporator at a temperature of 50°C to remove the solvent. The extract results after being thickened are evaporated again with a water bath to produce a thick extract that can be weighed and its yield value calculated [7].

$$\textbf{\% Yield}= \frac{\text{Weight of thick extract}}{\text{Weight of Simplisia}} \times 100\%$$

2.2.3. Liquid-Liquid Extraction

The separation of caffeine from Arabica green coffee extract was carried out using the extraction method. Green coffee extract was dissolved in 100 ml of distilled water and then stirred using a magnetic stirrer for 1 hour accompanied by heating to a temperature of 70°C. Then 250 mg of $CaCO_3$ was added and the solution was filtered to separate the solids from the extract powder. A total of 25 mL of chloroform was put into a separating funnel, then shaken, and two layers were formed, take the chloroform layer and evaporate to dryness. After the chloroform was dry, the caffeine was dissolved in distilled water up to 100 mL to be used for identification and determination of caffeine levels .

2.2.4. Qualitative Caffeine Test

Parry's reagent was made by reacting $Co(NO_3)_2$ with methanol p.a. The Parry test was carried out by taking 1 ml of the preparation sample and then adding Parry's reagent and dilute ammonia. A positive solution contains caffeine if the solution changes color to dark blue/green [8].

2.2.5. Quantitative Caffeine Test

a. Making Caffeine Standard Solution

Caffeine standards were weighed as much as 10.0 mg each then dissolved with distilled water up to 10.0 ml at a temperature of ±70^{0}C to obtain a stock solution with a concentration of 1 mg/ml and sonicated to dissolve completely [7].

b. Making Standard Curve

Stock solutions were taken as much as 60, 80, 100, 120, 140, 160 µl then dissolved with distilled water up to 10.0 ml to obtain a series of standard solutions with concentrations of 6, 8, 10, 12, 14, 16 µg/ml. Then the absorbance value was read according to the maximum lambda of each standard and the linear regression equation was calculated [1],[8].

c. Determination of Maximum Wavelength

Each standard solution of caffeine and chlorogenic acid was taken as much as 80 µl, put into a 10.0 mL measuring flask (concentration 8 µg/ml), then added with distilled water to the boundary line and homogenized. The solution was measured for maximum absorbance in the wavelength range of 200-300 nm [11].

d. Determination of Content

The caffeine sample that had been separated using liquid-liquid extraction was read for its absorbance using UV-Vis spectrophotometry at the maximum wavelength of caffeine and chlorogenic acid obtained in the previous process. Then the absorbance results and spectral diagram images were obtained for each fraction. And then the results obtained were analyzed using SPSS.

3. RESULTS AND DISCUSSION

3.1. Extraction

The initial step of this research is that Arabica green coffee beans obtained from Genting Village, Central Java are re-dried to reduce the water content so that it can prevent rotting, and growth of fungi and mold in the sample. The filtering stage with a mesh fineness of 30 is intended so that the powder size is uniform and the extraction process takes place optimally. This is because the smaller the powder size, the wider the contact surface of the sample with the solvent so that the compounds contained in the simplicial will be more extracted. Maceration is the simplest extraction method that is most widely used by soaking the powder in a suitable solvent in a closed container at room temperature. The choice of this method is based on the presence of compounds that are not heat-resistant or thermolabile [12]. Ethanol is a universal solvent that can dissolve various compounds, mold and germs are difficult to grow in ethanol 20% and above, and to evaporate the solvent requires a relatively faster time. The yield percentage is calculated with the aim of knowing the compound content contained in the extract and that the influence of the concentration of the solvent used will affect the yield% results [13]. The yield obtained from the study results can be seen in Table 1. The yield of 96% ethanol extract is greater than 70% ethanol extract.

Table 1. Results of Arabica Green Coffee Bean Extraction Yield

Sample	% Yield
Ethanol Extract 70 %	11.15
Ethanol Extract 96 %	24.48

The thick extract obtained in this study was then re-extracted using the liquid-liquid method, namely the separation of compound components by dissolving the sample with another solvent. This extraction produces two immiscible phases, namely the water phase and the organic phase [14]. Some components will dissolve in the water phase and some will dissolve in the organic phase, the separation is according to the level of polarity with a fixed concentration ratio [15]. The organic solvent used is chloroform because caffeine has high solubility in chloroform [16]. The separation process occurs according to the level of polarity with a fixed concentration ratio [15]. So that later some of the compound components in the sample will dissolve in the water phase and some will dissolve in the chloroform phase. The function of adding $CaCO_3$ powder here is to break the bond between caffeine and other compounds in the sample, so that caffeine will be in a free base state. Caffeine in the form of a free base will be bound by chloroform. The chloroform layer is at the bottom because the specific gravity of chloroform is greater than water. Liquid-liquid extraction is repeated with the addition of new chloroform solution which aims to maximize the caffeine that can be extracted in chloroform.

3.2. Identification of Caffeine in Arabica Green Coffee Bean Extract

The qualitative identification results of Arabica coffee bean extract showed positive caffeine content as indicated by a significant color change using the Parry method. In this method, if several substances are dissolved in alcohol, then Parry's reagent and dilute ammonia are added, a positive result for caffeine content in the sample will show a change in the color of the solution to dark blue/green. The mechanism of the formation green and moss green colors produced come from the reaction between Parry's Reagent (which contains cobalt ions (Co) with a charge of +2) which binds the nitrogen group in the caffeine compound. Parry's Reagent is made by reacting Cobalt nitrate [$Co(NO_3)_2$] with methanol. This reaction forms a green complex compound [17]. The more caffeine content, the more concentrated the green color like moss green [1]. The results of the identification test can be seen in Table 2.

Table 2. Results of Caffeine Identification using the Parry Method

Sample	Change		Result
	Before	After	
Ethanol Extract 70 %	Clear	Green	+
Ethanol Extract 96 %	Clear	Moss Green	+

Description: (+) = Contains Caffeine, (-) = Does Not Contains Caffeine

3.3. Caffeine Content in Arabica Green Coffee Beans

Determination of caffeine levels in green Arabica coffee beans was carried out using the UV spectrophotometry method. The UV spectrophotometry method was chosen because this method is a relatively fast, inexpensive, and easy to perform method. Determination of the maximum absorbance wavelength of caffeine with a wavelength range (λ) of 200-300 nm, the results of the measurements obtained a wavelength of 273 nm which is not much different from the theoretical wavelength, namely the caffeine wavelength theory ranges from 272-276 nm [18]. The results of measuring the standard caffeine absorbance value can be seen in Figure 1.

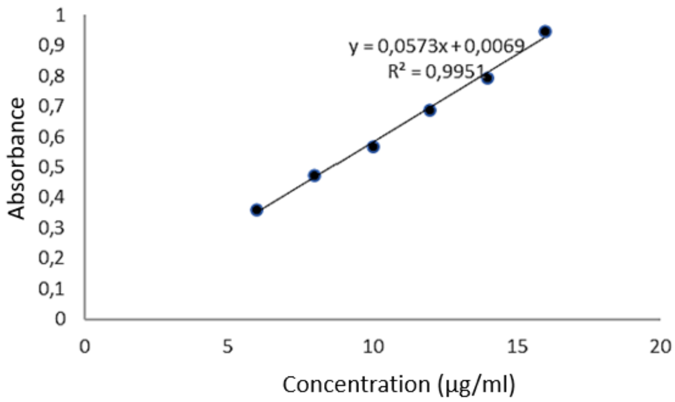

Figure 1. Caffeine standard curve

Caffeine is an alkaloid compound that is semi-polar or weakly polar, where there are two carbonyl groups together with a lone pair of electrons on the nitrogen atom increasing the polarity of the molecule. So caffeine will dissolve in more polar organic solvents. Caffeine which is polar will dissolve in solvents that are also polar or semi-polar according to the solubility principle "like dissolves like" that a substance will dissolve in a similar solvent [19]. Caffeine levels can be influenced by several factors, namely, the high concentration of solvents in dissolving caffeine components and the extraction temperature used. In this study, caffeine was highly soluble in ethanol solvent concentrations of 96% compared to ethanol solvent concentrations of 70%. Caffeine is an alkaloid that is non-polar so it tends to soluble in semipolar and non-polar solvents [20]. The results of determining caffeine levels can be seen in Table 3.

Table 3. Results of Caffeine Content of Arabica Green Coffee Beans

Sample	Result (Average± SD)
Ethanol Extract 70 %	10.115 ± 0.06 %
Ethanol Extract 96 %	12.760 ± 0.580 %

Further data were analyzed using SPSS showing that the significance value of $p<0.05$, which means that there is a significant difference in caffeine levels in 70% ethanol extract and 96% ethanol extract of green Arabica coffee beans.

4. CONCLUSION

Based on the research results obtained to determine the caffeine content in Arabica Green Coffee Beans (*Coffea arabica*), it can be concluded that the solvent that produces the highest caffeine content is 96% ethanol with a yield of 24.48% and a caffeine content of 12.760 ± 0.580%.

Acknowledgments: The author would like to thank Jenderal Achmad Yani University Yogyakarta for the support of facilities and infrastructure so that this research can run smoothly.

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