PROCEEDING UNICOPPS

UNJAYA International Conference on Pharmacy and Pharmaceutical Science

Vol. 1, November, 2025, page 54-60

Determination of Chlorogenic Acid Content in Arabica Green Coffee Beans (Coffea arabica) Using UV-Spectrophotometry

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ABSTRACT

Background: Coffee is a leading commodity in Indonesia, Arabica coffee is widely favored and grows well in Indonesia. Arabica coffee has many chemical compounds in it, one of which is chlorogenic acid. Chlorogenic acid (CGA) is a chemical compound of the polyphenol group that has antioxidant activity.

Objective: This study aims to determine the difference in CGA levels contained in green Arabica coffee bean extract using the maceration method with 70% ethanol and 96% ethanol solvents.

Research methods: Chlorogenic acid levels were measured using a UV-Vis spectrophotometer. Before determining the content of green coffee beans, microscopic identification was carried out to determine the fragment components in the powdered simplicia and to ensure the truth of the sample identity.

Results: The results showed that the CGA content in Arabica coffee beans extracted using 70% and 96% ethanol solvents had differences. The CGA content in 70% ethanol extract of green Arabica coffee beans showed results of 7,99 ± 0,153 while the 96% ethanol extract of green Arabica coffee beans was 6,26 ± 0,206. The results of the analysis showed that the caffeine content had a significant difference with a p value <0.05.

Conclusion: Based on the results of the study, it was concluded that the appropriate solvent for extracting chlorogenic acid in green Arabica coffee bean extract is a 70% solvent based on its polarity level**.**

Keywords: chlorogenic acid; arabica green coffee bean; UV-Spectrofomtometri; maceration

INTRODUCTION

Coffee plants are widely cultivated in Indonesia, in general two types of coffee are cultivated, namely Robusta coffee and Arabica coffee. Unroasted Arabica coffee beans are called green coffee beans. These green coffee beans have different chemical compounds and physical characteristics than roasted coffee beans (Handayani & Muchlis, 2021).
One of the chemical compounds that is abundant in green coffee beans is chlorogenic acid. The amount of chlorogenic acid contained in coffee beans can change due to the roasting process. During the roasting stage, chlorogenic acid will break down into volatile compounds and melanoidins, so that the amount will decrease (Furqan & Nurman, 2020). Chlorogenic acid is a phenolic acid compound that acts as an antioxidant. The antioxidant properties of flavonoid compounds come from their ability to transfer electrons to free radical compounds and can be understood as the ability to protect body cells from oxidative damage.(Hilda et al., 2021). The high chlorogenic acid content in green coffee beans is widely used as a treatment, including as an antioxidant, antiviral, hepatoprotective, and an antispasmodic. (Farhaty, 2016).

However, the amount of compounds contained in green coffee bean extract depends on the solvent used. The content of chlorogenic acid compounds has different levels of solubility in each type of solvent. Chlorogenic acid is polar so it can dissolve in water but is not soluble in nonpolar solvents. Chlorogenic acid can dissolve in organic solvents, ethanol, DMSO, and dimethyl formaldehyde. Because Chlorogenic acid has five active hydroxyl groups and one carboxyl hydroxyl group so it is more polar and soluble in ethanol (Wang et al., 2022). So, from this background, the aim was to determine the levels of chlorogenic acid in 70% and 96% ethanol extracts of green Arabica coffee beans (Coffea arabica) using the maceration extraction process and determination of levels using the UV Spectrophotometer method.

RESEARCH METHODS

This research is an experimental study using UV spectrophotometry. The samples used were green Arabica coffee beans obtained from Semarang, Central Java.

Tools and materials

The tools used in this study include UV spectrophotometry (*Thermo Scientific Genesys*), analytical balance (*Ohaus*), measuring cylinder (*Iwaki*), measuring flask (*Iwaki*), separating funnel, micropipette (*Eppendrof*), and filter paper.

The materials used in this study were Arabica green coffee beans, dichloromethane (Merck), distilled water, calcium carbonate (CaCO3), ethanol 70% (Merck), ethanol 96% (Merck), and standard chlorogenic acid stock solution.

Research procedure

Identification of Arabica Green Coffee Beans

Identification is carried out organoleptically and microscopically to avoid errors in plant sampling. To carry out a microscopic test, the powder is viewed using a microscope and compared with the literature

Sample Extraction

Arabica Green Coffee Beans were powdered using a grinder and then sieved. Then the powder was macerated using 70% ethanol and 96% ethanol solvents. Furthermore, the macerate was remacerated twice using 600 ml each for 24 hours with occasional stirring. The total macerate was filtered with a Buchner funnel to separate 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, were evaporated again with a water bath to produce a thick extract.

Liquid-liquid extraction

This process is carried out to extract caffeine compounds so that the chlorogenic acid results obtained are not biased. The thick extract obtained is dissolved with hot distilled water at a temperature of 70ºC and CaCO3 is added and then filtered. The results obtained are then added with 25 ml of dichloromethane, then shaken. Two layers will form and separate the dichloromethane phase from the water phase. Collect the results obtained.

Quantitative Test of Chlorogenic Acid

Making Standard Chlorogenic Acid Solution

Determination of the wavelength of chlorogenic acid is done by making a standard solution of chlorogenic acid with a concentration of 1 mg/ml. Then the maximum wavelength is measured from 200 nm - 400 nm so that a peak is obtained at the maximum wavelength (Furqan & Nurman, 2020). Next, determine the wavelength of caffeine 5 µg/ml in aquadest, scan the wavelength from 200-300 nm, and determine the maximum wavelength. Making a standard curve with a series of levels of 5, 7, 9, 11, 13, 15 µg/ml which is read according to the maximum lambda.

Determination of Chlorogenic Acid Levels

The chlorogenic acid sample in the water phase that has been collected is then read for its absorbance using a UV spectrophotometer at the maximum wavelength obtained.

Data analysis

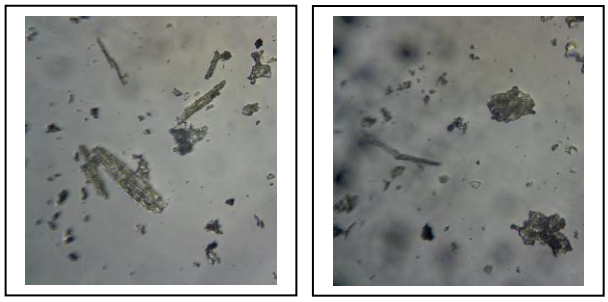
The results of this study are quantitative data obtained by looking at the absorbance value of chlorogenic acid in UV Spectrophotometry, and then determining the levels using a linear curve of the standard solution.

RESULTS AND DISCUSSION (12pt)

Arabica green coffee beans obtained from Central Java were identified microscopically to determine the fragment components in the powdered simplicia and to ensure the correct identity of the plants used in the study, as well as to avoid errors in taking plants.

FIGURE 1. Dried Arabica Green Coffee Beans

FIGURE 2. Arabica Green Coffee Bean Powder



(a) (b)

FIGURE 3. Microscopic Identification Results with Magnification40x10 (a) Vascular file (b) Trikoma

In Figure 1. above, it is shown organoleptically that Arabica coffee beans have physical characteristics with a shape that tends to be slightly elongated, the belly area is not highly convex, the middle gap in the flat part is not straight and extends downwards but is curved, the tip is shinier, the color is brownish green and slightly yellowish (Panggabean, 2011). Based on the results of the organoleptic test of the powdered green arabica coffee bean samples obtained, the organoleptic characteristics are in the form of fine powder, light green slightly brownish in color, and a distinctive aroma of green coffee. Microscopically, the green coffee bean powder has a distinctive characteristic, namely containing rod-shaped fragments and walled endosperm as in Figure 3(Uker, 2011).

In this study, the extraction process was used using the maceration method, namely the extraction method with the process of soaking the material with a solvent that is by the active compound to be taken with low heating or without any heating process. Factors that can affect the results of maceration include time, temperature, type of solvent, comparison of material and solvent, and particle size (Asworo & Widwiastuti, 2023). Ethanol is a universal solvent that can dissolve various compounds, besides that ethanol also has advantages, namely mold and germs are difficult to grow in ethanol 20% and above, and to evaporate the

solvent requires a relatively faster time. The concentration of ethanol will affect the flavonoid compounds that will be taken. Low-concentration solvents produce higher levels than solvents with high concentrations (Yunita & Khodijah, 2020). In this study, two different solvents were used, are 70% ethanol and 96% ethanol.

The extract obtained is then separated or fractionated using the liquid-liquid extraction method, namely the separation of compound components by combining two solvents that do not mix. To separate chlorogenic acid and caffeine, the best solvent is needed, namely a solvent that can selectively dissolve caffeine and chlorogenic acid compounds. This process uses dichloromethane and water solvents so that there is no wavelength bias when measuring chlorogenic acid (Furqan & Nurman, 2020). Based on previous research, it was stated that the caffeine yield obtained from dichloromethane solvent was greater than using chloroform solvent to extract caffeine compounds from coffee bean extract (Marthia, 2021). Then CaCO3 is added which functions to break the caffeine bonds with other compounds in the coffee solution. Caffeine that has broken its bonds will be in a free base state (Yuliyana et al., 2021).

The water phase and dichloromethane phase are then separated using a separating funnel. The separation process is carried out repeatedly with a shaking process to separate the substances until they reach equilibrium. The extracted compounds will be divided into two layers that have different polarity points. Water containing chlorogenic acid is then collected in a measuring flask. Chlorogenic acid will dissolve in water because it has a polar hydroxyl group. The water phase is in the upper layer because it has a smaller specific gravity compared to dichloromethane. The results obtained were measured for absorbance with a UV-Vis spectrophotometer at the maximum wavelength and distilled water was used as a blank (Furqan & Nurman, 2020).

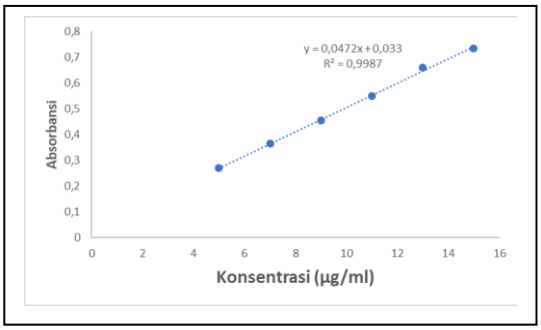


FIGURE 4. Hasil Kurva Baku Standar Asam Klorogenat

Determination of chlorogenic acid levels using a wavelength of 323.8 nm. This test is carried out by making a standard solution with a concentration of 5 - 15 µg / ml, the standard curve of chlorogenic acid can be seen in Figure 4.

TABLE I. Results of Chlorogenic Acid Levels in Arabica Green Coffee Beans

Sample	Results (Mean ± SD)
Ethanol extract 70%	7,99 ± 0,153
Ethanol extract 96%	6,26 ± 0,206

Chlorogenic acid is aromatic and has a cyclohexane carboxylic acid group (quinic acid) that is esterified to one or more hydroxycinnamic acids. Chlorogenic acid will dissolve in water because it has a hydroxyl group that makes it more polar, so compounds will dissolve in 70% ethanol (Indiarto et al., 2022). Based on Table 1. chlorogenic acid obtained by the literature stating that the levels of chlorogenic acid in green arabica coffee (Coffea arabica L) are 4.1 -7.9%.

CONCLUSION

Based on the research results, it was concluded that the appropriate solvent to extract chlorogenic acid from green Arabica coffee bean extract is a 70% solvent based on its polarity level.

ACKNOWLEDGMENTS

The author would like to thank Jenderal Achmad Yani University Yogyakarta for its support in carrying out this research so that it can run smoothly.

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