# Phytochemical Screening and Antioxidant Test of Arabika Roasted Coffee Bean Extract (*Coffea arabica* L.) from Agam Regency



# Phytochemical Screening and Antioxidant Test of Arabika Roasted Coffee Bean Extract (*Coffea arabica* L.) from Agam Regency

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**Abstract.** Coffee allegedly has antioxidant activities to reduce free radical activities. In Agam Regency, coffee is a beverage consumed almost every day. This study aimed to screen phytochemical substances and investigate the antioxidant activities of ethanol extracts of arabica coffee beans with the 2.2-Diphenyl-2-picrylhydrazyl method (DPPH). Arabica coffee bean extract was made by percolating using ethanol solvents. The obtained extracts were thickened using a rotary evaporator. and measured of its antioxidant activities by using the 2.2-Diphenyl-2-Picrylhydrazyl method (DPPH) at a maximum wavelength of 517 nm. Positif control used vitamin C. Phytochemical screening results showed that ethanol extracts of arabica coffee beans contained a group of compounds of tannins, alkaloids, saponins, flavonoids, and steroids. The results of the study showed that antioxidant activities of ethanol extract of arabica coffee beans had a very strong category with C50 of 12.481 ppm and vitamin C with IC50 of 0.279 ppm.oid, saponin, flavonoid, and steroids.
***Keywords:*** Arabica; Antioxidant; Agam

## 1. Introduction

Coffee (*Coffea sp*) is a plant frequently consumed as a drink obtained from steeping coffee in powder form. Coffee is found in the market and produced from two species of coffee plants: arabica coffee (Coffea arabica) and robusta coffee (*Coffea canephora*). Coffee contains saponins, flavonoids, polyphenols, and alkaloids [1]. These compounds are known to have antioxidant, antitumor, antiviral, and antibiotic activities [2]. The coffee drink industry in the world continues to grow and develop with various processing variations, presentations, and tastes, Coffee has not only a distinctive aroma and taste but also efficacy for the health of the body. The efficacy comes from the bioactive compounds contained in coffee. These compounds include caffeine, chlorogenic acid, trigonelline, nicotine acid, quinolinic acid, tannins, pyrogallic acid, etc. [3].

Coffee contains high antioxidant polyphenol compounds originating from phenolic acids, such as caffeine, chlorogenic acid, kumarin, ferulic, and synaptic acid [4]-[5]. The quality of coffee beans and antioxidant activities is determined by the composition of polyphenols in coffee beans [4,5,6] The polyphenol composition is influenced by the types, processes, and geographical location of coffee beans. One of the polyphenol compounds

found in a large amount of coffee and contributing to the largest antioxidant activities is chlorogenic acid.

Antioxidants are compounds that have molecular structures that can provide electrons to free radical molecules and break the chain reactions from free radicals [7]. The body has an antioxidant defense mechanism in the form of antioxidant enzymes and substances to neutralize free radicals [8]. However, due to rapid industrial development, humans have contact with various sources of free radicals originating from the environment and high physical activities; as a result, the antioxidant defense system in the body is inadequate [8]. Antioxidants are nutritional and non-nutritional substances contained in food ingredients to prevent or slow down oxidation processes [9,10]. Antioxidants are very beneficial for health and cosmetics and significantly maintain the quality of food products [9].

The types of coffee generally used in the beverage industry are arabica and Robusta. arabica and robusta coffee are also found in West Sumatra, especially from Solok Regency, Solok Selatan, Agam, Limapuluh Kota, Pasaman, and Tanah Datar. In general, each district serves the coffee in the form of roasted coffee beans. The roasting process affects the quality of coffee, including the sensory, aroma, and composition of bioactive compounds that also impact its antioxidant activities [11], [12]-[13]. The roaster process is generally carried out at a temperature of 200-240$^{o}$C to produce brown coffee beans and release a distinctive aroma. During the process of roasting, the composition of bioactive compounds, including polyphenol compounds that act as antioxidants, changes because chlorogenic acid, caffeine, trigonelline, and other bioactive compounds degrade [4]. The higher the temperature of the roasting process, the more decreasing antioxidant activities will be [14].

Antioxidant activities of arabica and robusta green coffee beans in the Isopropanol Faction: Water (60: 40) against BHA is 92% and 88%, respectively [15]. Antioxidant activities can be determined by the reduction of free radicals of 1.1-Difenyl-2- picrylhydrazyl (DPPH) and spectrophotometric of UV-Vis [4]. Antioxidants are substances in small concentrations that can inhibit or prevent oxidation on the substrate. Antioxidants are compounds that have molecular structures that can provide electrons to free radical molecules and can cut chain reactions from free radicals [6]. Therefore, this study was conducted to determine chemical compounds and antioxidant activities of ethanol extract of coffee powder from Agam Regency, West Sumatra.

This study aimed to screen phytochemical substances and investigate antioxidant activities of ethanol extract of arabica coffee beans planted in Agam Regency using a 2.2-Diphenyl-2-picrylhydrazyl method (DPPH). This study contributes to serving as a reference of phytochemical screening results and antioxidant activities of arabica coffee from Agam Regency for other researchers. Moreover, this study provides essential information for coffee lovers in Agam Regency andWest Sumatra.

## 2. Methodology

a. Samples and Research Site

The sample of this study was arabica coffee beans obtained from a Coffee Shop in Agam Regency. This research was conducted at the Chemistry Laboratory of the Pharmaceutical School of the Pharmacy Department.

b. Tools and Materials

The tools applied in this study were laboratory glass equipment, blenders, analytical balance sheets, ovens, and UV-Vis spectrophotometers. The chemicals used in this study include ethanol, chloroform, concentrated H2SO4, concentrated HCl, anhydrous CH3COOH, FeCl3 Ocean 1%, 1.1- diphenyl-2-picrylhydrazil (DPPH), Wegner reagent, Meyer reagent, Dragendroff reagent, and aquades.

c. Sample Preparation and Extraction

Roasted coffee beans were mashed using a blender to get the same size. The coffee bean powder obtained was stored in a clean container and tightly closed. A total of 200 grams of coffee powder was soaked with 600 mL of ethanol for 24 hours and stirred occasionally using a magnetic stirrer. Then the extract was separated from the pulp by filtering. The liquid extract obtained was then concentrated with a rotary evaporator at 40$^{o}$C. The obtained concentrated extract was stored in a clean container and tightly closed for later use in further analysis.

d. Phytochemical Screening

Phytochemical screening was carried out on alkaloids, flavonoids, terpenoids, saponins, polyphenols, and tannins using the Harborne method with several modifications. Then, 0.5 grams of alkaloid extract were added with 1 mL of HCl 2 M of solution and 19 ml of aquades and then heated for 5 minutes. The mixture was cooled and then filtered. The obtained filtrate was analyzed by Wegner, Meyer, and Dragendroff reagents. The existence of alkaloids was shown by the formation of chocolate deposition with Wegner reagents, yellow deposition with Meyer reagents, and orange-red deposition with dragendroff reagents. The next step was dissolving 0.5 g of flavonoid extract with 10 ml of hot ethanol, then 0.1 g of mg powder and 5 drops of concentrated HCl were added.

The existence of flavonoids is shown by the formation of orange, pink, or dark red colors which were not lost within three minutes. Meanwhile, 0.5 g of terpenoid extract was dissolved in 5 ml of chloroform and 5 ml of anhydrous acetate. Then, 2 mL of thick H2SO4 solution was added through the reaction tube wall. The existence of a terpenoid was shown by red, orange, or purple colors. Meanwhile, 0.5 g of Saponin extract was dissolved in 20 ml of hot water then cooled, and finally filtered. The obtained filtrate was shaken strongly for 10 seconds. The presence of saponins was indicated by the formation of stable foam. The next step was dissolving 0.5 g of tannin extract in 20 ml of hot water. The extract was then cooled and filtered. The obtained filtrate was added with 2-3 drops of 1% solution of FECL3. The presence of tannin was indicated by the formation of green, red, purple, blue, or strong black colors.

e. Antioxidant Activity Test

6.25 mg extracts were dissolved into 250 ethanol to obtain a test solution with a concentration of 250 ppm. Each test solution was diluted with ethanol to obtain a test solution with a concentration of 25, 50, 75, 100, 125, and 150 ppm. The test solution of various concentrations reacted with a 1,1-diphenyl-2-picrylhydrazil (DPPH) solution of 0.1 mM with a ratio of 1:3. These concentrations were then homogenized and incubated for 30 minutes. The absorbance of the solution mixture was measured using a UV-Vis spectrophotometer at a wave of 517 Nm. The blank absorbance measurement was conducted on ethanol, and the positive control measurement was conducted on the BHA.

f. Determination of Antioxidant Activities with the DPPH Method

1). The DPPH solution of 30 µg / ml was made. Then, approximately 10 mg of DPPH was carefully weighed. The next step was dissolving the solution with Ethanol P.A up to 100 ml. As a result, a 100 µg/ml of concentration was obtained. Finally, the solution was diluted into a 30 µg/ml of concentration.

2). Optimization of DPPH wavelengths was piped with 3.8 ml of DPPH solution (30 µg/ml) into the vial. Then 0.2 ml of ethanol P.A was added and homogenized. Meanwhile, the vial was closed with aluminum foil. The next step was incubating the DPPH wavelengths in a dark room for 30 minutes. The spectrum of absorption was specified using a UV-Visible spectrophotometer at a wavelength of 400-800 Nm. Finally, the maximum wavelength was determined.

3). Antioxidant activities of the comparison solution of vitamin C were tested. To begin with, 1 mg of vitamin C was weighed, then inserted in a 10 ml measuring flask. The next step

was adding ethanol P.A to the limit sign (100 µg / ml). Then the series concentration was 20 µg/ml, 25µg/ml, 30µg/ml, 35µg/ml, and 40 µg/ml. The antioxidant activities of each concentration were piped by 0.2 ml of the sample solution with a micropipette and inserted into the vial. Afterward, the vial with aluminum foil was closed, and 3.8 mL of 30 µg/ml DPPH solution was added. The mixture was homogenized and incubated for 30 minutes in a dark place and finally measured with a UV-Visible spectrophotometer at the maximum wavelength of DPPH 515 Nm.

4). The successive step was testing antioxidant activities of ethanol extract of traditional coffee powder from Agam Regency. The 25 mg of extract was weighed in a measuring flask then dissolved with ethanol p.a. A volume of 25 mL was increased to obtain 1000 µg/mL of concentration. To determine antioxidant activities, each concentration was piped with 0.2 mL of sample solution and a micropipette and put in the vial. Then the vial was closed with Aluminum foil, and 3.8 mL of 30 µg/mL DPPH solution was added. The mixture was homogenized and incubated for 30 minutes in a dark place. Finally, the absorption was measured by a UV-Visible spectrophotometer at a maximum wavelength of DPPH 515 nm.

g. Determination of IC50 Values

The calculation results from antioxidant activities were processed into the equation of the line y = a + bx with concentration (µg/mL) as a base (x axis) and the value of % of antioxidant activities as the ordinate (y axis). An IC50 value from the calculation was obtained from the line equation when % of antioxidant activities was 50% [16].

h. Data Analysis

Phytochemicals of each extract were analyzed based on the results of reactions in certain test solutions and reagents. The results of reactions were in the forms of changes in color, the formation of deposition, and the formation of foam. Meanwhile, antioxidant activities were qualitatively analyzed by the DPPH method and quantitatively determined by the linear regression analysis of the results of absorbance measurements.

## 3. Results and Discussion

The characterization and standardization tests of Arabica coffee (Coffea arabica L.) discovered water-soluble content, ethanol-soluble content, total ash content, acid insoluble ash content, drying shrinkage, and water content from coffee grounds and coffee extracts. Qualitative analysis of ethanol extract was done by the KLT method. The KLT method was used to determine the number of components in the mixture and identify several compounds. The quiet phase used was Silica Gel 60 F254, and the movement phase was chloroform: ethanol (99:1). The characterization of arabica coffee beans and extracts is presented in Table 1.

**Table 1.** Coffee Characterization and Arabica Coffee Extracts

| Standardization Test | Coffee (%) | Coffee Extracts (%) |
|---|---|---|
| Water-soluble content | 2.84 | 5.21 |
| Ethanol soluble content | 5.12 | 4.39 |
| Total ash content | 4.95 | 1.92 |
| Insoluble ash content acid | 0.365 | 0.29 |
| Shrinking drying | 4.02 | 6.89 |
| Water content | - | 6.27 |

Table 1 shows that decaffeinated ethanol extracts were seen in UV 254 detection at Rf 0.59 and *Caffeine* comparison at Rf 0.59. Phytochemical screening was used to identify plant compounds based on their class as preliminary information to discover types of chemical

compounds with biological activities from a plant. Furthermore, Table 1 denotes that Arabica coffee beans of ethanol extract contain a group of compounds of flavonoid, saponin, tannin, alkaloids, and steroids. The flavonoid examination several major results: (1) red-yellow or orange colors in the alcohol amyl layer were formed; two of three test tubes showed deposits or turbidity on alkaloids; blackish-green color was found on the tannins; (3) blue-green colors were formed on steroids/triterpenoids formed; (4) saponin examination results formed 1-3 cm of foam; (5) blue-green colors were formed on steroids/triterpenoids [16]. The results of the phytochemical screening are presented in Table 2.

**Table 2.** Results of Phytochemical Screening of Arabica Coffee Extracts

| Group | Methods | Results |
|---|---|---|
| Alkaloid | Mayer Test | + |
| | Bounchardat test | + |
| | Dragendroft test | + |
| Flavonoid | Cyanidin test | + |
| Phenolic | | + |
| Tannin | Color test | + |
| Saponin | Foam test | + |
| Steroid | Liebrmean-Bouchardate test | + |
| Terpenoid | | + |
| Glycosides | Salkowski test | - |

Note : (+) containing a group of compounds
(- ) not containing a group of compounds

The addition of magnesium powder and hydrochloric acid to flavonoid testing will reduce the existing flavonoid compounds and raise a red reaction which is characteristic of flavonoids. Whereas the test above did not form a red color due to the insufficient flavonoid compound (Robinson, 1995). Saponin-positive results were marked by the formation of foam. The onset of foam showed the presence of glycosides that could form foam in hydrolyzed water into glucose and other compounds [11].

Positive results on tannin and phenol tests carried out with the addition of FeCl3 would give rise to green, red, purple, blackish-blue, or deep green colors in phenol and blackish green colors in tannins because tannins or phenols would react with Fe3+ions forming complex compounds [11]. Positive results of terpenoids and steroids were characterized by red, purple or blue, green colors. Color changes were formed due to oxidation in the group of terenoid or steroid compounds through the formation of conjugated double bonds [17]. Antioxidant activities were determined using DPPH solution with a 30 µg/ml of concentration using the UV-Vis spectrophotometer.

The existence of antioxidant activities is indicated by a decrease in the absorbance of the DPPH solution and the DPPH color change from purple to yellow after the addition of extracts. The DPPH which serves as free radicals can be muted by antioxidants from test materials. The DPPH further reacts with antioxidants through hydrogen atom donations of antioxidants to reduce the DPPH. The maximum wavelength of the selected DPPH is 515 nm because it produces maximum absorbance. According to Molyneux wavelength determination of antioxidant activity, the DPPH method is 515-520 nm [16].

The optimum incubation time indicated that each sample or comparison reacted perfectly with the DPPH. Within 30 minutes, each ethanol extract or vitamin C (comparison) reacted optimally with the DPPH which was characterized by the absorbance that did not decrease, or the percent reduction that did not increase. The results of the antioxidant activity test are presented in Table 3.

**Table 3**. Results of Antioxidant Activity Test from Ethanol Extract

| Samples | Values of $IC_{50}$ (ppm) |
|---|---|
| Ethanol Extract of Coffee | 12.481 |
| Vitamin C | 0.279 |

The results of the antioxidant activity test were expressed with IC50 values. The results showed that the IC50 of ethanol extract of coffee was 12.481 ppm. The IC50 value of the vitamin C comparison was 0.279 ppm. Arabica coffee beans had antioxidant content because of their polyphenol content. Polyphenols are micronutrients found in some food intakes, and flavonoids are secondary metabolites of polyphenol compounds that dissolve in polar solvents. Polyphenols are antioxidants that reduce free radicals [18].

The phytochemical screening of arabica coffee bean extract discovered groups of flavonoids, tannins, saponin, alkaloids, and steroids; these compounds had antioxidant activities. Antioxidant activities were examined using a visible spectrophotometer at a wavelength of 517 Nm with the DPPH method in the IC50 value of ethanol extract of arabica coffee beans 12.481 ppm. Moreover, the antioxidant activities included a very strong category. Meanwhile, Vitamin C IC50 0.279 ppm belonged to a very strong category.

Coffee contains high antioxidant polyphenol compounds derived from phenolic acids, namely chlorogenic acid, caffeine, coumarin, ferulic, and synaptic acid. The composition of polyphenols in coffee beans is determined by the quality of the coffee beans and their antioxidant activities. The composition of polyphenols can be influenced by types of the coffee beans, methods of processing coffee beans, and geographical location. Chlorogenic acid is a polyphenolic compound found in coffee beans.

## 4. Conclusion

The phytochemical screening discovered that ethanol extracts of arabica coffee beans contained a group of compounds of tannins, alkaloids, saponins, flavonoids, and steroids. The results of the study showed antioxidant activities of ethanol extracts of arabica coffee beans included a very strong category with C50 12.481 ppm and vitamin C with IC50 0.279 ppm, saponin, flavonoid, and steroids.

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