The Comparison Of Bioactive Compounds Between Brewed and Extracted Robusta Coffee From West Lampung

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Abstract. Indonesia is ranked fourth highest in coffee production in the world. One type of coffee export commodity, namely Robusta coffee, is produced from the West Lampung region. Robusta coffee contains various bioactive compounds that act as antioxidants. This study aims to analyze the differences between brewed and extracted robusta coffee related to the bioactive compounds. The method used in this study began with coffee sample preparation, followed by brewing and ethanolic extracting the robusta coffee, measurement of TPC (total phenolic content in gallic acid equivalent), TFC (total flavonoid content in quercetin equivalent), and antioxidant activity using the DPPH method. The results showed that the TPC values were 11.27 mgGAE/g for brewed coffee and 61.98 mgGAE/g for extracted coffee, while the TFC values for brewed coffee were 11.99 mgQE/g and extracted coffee were 18.40 mgQE/g. The highest antioxidant activity value was obtained from extracted robusta coffee with an IC_{50} 89.43 ppm which classified as strong enough antioxidant, while the antioxidant activity of brewed coffee has an IC_{50} 218.74 ppm which classified as moderate antioxidant. TPC and TFC in this study showed a direct proportional relationship with antioxidant activity. The samples with the highest total phenolic and total flavonoid also exhibited the highest antioxidant activity.

1 Introduction

Indonesia is renowned as a coffee producer and exporter on the global stage. Indonesia ranks as the fourth-largest coffee producer worldwide, following Brazil, Vietnam, and Colombia. According to data from the Food and Agricultural Organization (FAO), Indonesia's average coffee production between 2016 and 2020 stood at 725.68 thousand tons per year, with an average annual export of 368.14 thousand tons. In terms of coffee types, robusta coffee takes the lead in Indonesian coffee production. From 2013 to 2022, 73.00% or 508.33 thousand tons comprised robusta coffee, while the remaining 27.00% or 187.98 thousand tons consisted of Arabica coffee. The primary robusta coffee production hubs in Indonesia, based on the average data of the past five years, are the provinces of South Sumatra, Lampung,

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Bengkulu, East Java, and Central Java [1]. In 2022, Lampung Province was the second largest coffee producer in Indonesia after South Sumatra, with coffee production of 124.5 thousand tons. West Lampung is the largest producer of robusta coffee in Lampung Province with a total of 56.54 thousand tonnes [23]. Robusta coffee from West Lampung is widely known and has become one of the world's coffee export commodities. The high production of coffee is in line with the level of consumption. Based on the results of the National Socioeconomic Survey (SUSENAS) by BPS, household coffee consumption generally consists of ground coffee for brewing. Year period 1993-2021, coffee consumption (ground coffee at household level) per capita tends to increase by 1,52% per year [2].

Robusta coffee is known to contain various bioactive compounds that play a role in counteracting free radicals. Phytochemical compounds in robusta coffee such as caffeine, flavonoids, polyphenols, proanthocyanidins, coumarins, chlorogenic acid, and tocopherols can function as antioxidants. Antioxidant active compounds have the ability to donate electrons and reduce the potential for free radicals in the body. Antioxidants also act as preventive agents that suppress the formation of new radicals and adaptation agents that produce the right antioxidant enzymes to be transferred to their sites of action [3]. Robusta coffee from West Lampung Regency roasted at 180°C is known to have the highest levels of chlorogenic acid (43%) and caffeine (18.5 g/kg) compared to robusta coffee from Tanggamus Regency (36.3% and 13.7 g/kg) and Way Kanan Regency (34.4% and 12.4 g/kg) in Lampung Province [4].

The effectiveness of the extraction used in the collection of phytochemical compounds in Robusta coffee will determine the levels of antioxidant compounds. It depends on the method and solvent used. This is in principle like dissolve like, that a compound will dissolve in a solvent with the same properties. Therefore, a study was conducted to analyze the differences between brewed and robusta coffee extracts related to their bioactive compounds. Brewed coffee produced from robusta coffee powder that is brewed using hot water, in this case the solvent used is water without maceration, and extract robusta coffee is the result of maceration of robusta coffee grounds with ethanol solvent. The result of the analysis of bioactive compounds from two types of solvents and different extraction methods (brewing and maceration) can be used as initial data for development of coffee extracts in the health sector.

2 Materials and Methods

2.1 Materials

Robusta coffee beans come from Batu Ketulis District, West Lampung Regency, Lampung Province (-5.029323582025426, 104.2525900542842). 300 grams robusta coffee beans (green beans) roasted at 180°C for 5 minutes using W500 SE coffee roaster machines, to maintain the bioactive content of robusta coffee [5]. Next, Robusta coffee is grinded to a powder using Latina-600N coffee grinder. Other materials used include Gallic acid standard (Merck), Quercetin standard (Sigma Aldrich), and DPPH (Sigma Aldrich).

2.2 Brewing Robusta coffee

0.1 grams of robusta coffee powder is brewed using hot water 90 °C as much as 100 ml (0.1% W/V), then stirred and waited until room temperature (about 30 minutes). Coffee brew (without precipitate) was used to measure total phenol content, total flavonoid content, and antioxidant activity [6].

2.3 Preparation of coffee ethanol extract

Robusta coffee powder was macerated using 96% ethanol for 48 hours. The resulting maserate was evaporated using a rotary evaporator and obtained a robusta coffee bean ethanol extract [7]. Next, the extract was used for measurement of total phenol content, total flavonoid content, and antioxidant activity.

2.4 Measuring Levels of Total Phenol Content Equivalent Gallic Acid

100 mg of the robusta coffee powder was dissolved in 100 mL of methanol. Then 1 mL of sample was added with 0.4 mL of Folin-Ciocalteu reagent, shaken and left for 4-8 minutes. 4 mL Na2CO3 7% solution was added and shaken until homogeneous. Then an aquabidest was added up to 10 mL and left for 2 hours at room temperature. Solution absorbance measurements were read at λ : 744.8 nm using a spectrophotometer (A&E Lab.). After measuring the absorbance of the sample, a standard curve for gallic acid was made with various concentrations of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L. Gallic acid is phenolic compounds (hydroxybenzoic acid derivatives which are classified as simple phenolic acids). Gallic acid was used as a standard solution because of the availability of stable and pure substances. Total phenol levels were calculated using the linear regression equation formula of gallic acid, y = ax+b [8].

2.5 Measurement of Quercetin Total Flavonoid Equivalent Levels

100 mg of the sample was dissolved in 100 mL of methanol. 1 mL of sample was added with 3 mL of methanol, 0.2 mL of AlCl3 10%, 0.2 mL of potassium acetate, and sufficient with distilled water to 10 mL. The test sample was incubated for 30 minutes in a dark place and room temperature. Solution absorbance measurements were read at λ : 431 nm using a spectrophotometer (A&E Lab.). Quercetin standard was prepared at a concentration of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L as a standard calibration curve. Quercetin was used as a standard solution because quercetin is a flavonoid of the flavonol group that has a keto group at C-4 and a hydroxyl group on neighboring C-3 or C-5 atoms of flavones and flavonols. Total levels of flavonoids were calculated using the linear regression equation formula of quercetin, y =ax+b [8].

2.6 Measurement of the antioxidant activity of DPPH (2,2-diphenyl-1-picrylhydrazyl)

Tests using the DPPH as a stable free radical. The antioxidant activity of the tested samples was directly proportional to the loss of DPPH radicals [9]. 2 mg of DPPH was dissolved in 10 ml of methanol to obtain a concentration of 50 µg/ml, then the absorbance was measured at a wavelength of 517 nm. A total of 1 ml of 100 µg/ml DPPH solution was put in a test tube then added 2 ml of methanol, homogenized and incubated for 30 minutes and then the absorbance was measured at the 517 nm wavelength. Furthermore, 25 mg of the sample was dissolved in 25 ml of methanol to obtain a concentration of 1000 µg/ml. The next step was dilution to obtain concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml and 125 µg/ml. 1 ml of sample solution was put into a test tube then added 1 ml of DPPH 50 µg/ml and diluted with 2 ml of methanol then homogenized. Each solution was incubated for 30 minutes and then the absorbance was measured at the optimum wavelength of 517 nm using a spectrophotometer (A&E Lab.). Antioxidant activity was calculated using the equation % inhibition = (Absorbance of the control - Absorbance of the sample)/Absorbance of the control x 100%. The results of % inhibition are then substituted into the linear equation y =

ax+b. The resulting linear equation is then used to obtain the value of the antioxidant strength of robusta coffee (IC_{50}) [10].

3 Results and Discussion

The results of total phenolic content, total flavonoid content, and antioxidant activity value of brewed and extracted robusta coffee are shown in Table 1. Total phenol based on gallic acid equivalence of brewed coffee was 11.27 ± 0.10 mgGAE/g which is lower than extracted coffee with a total phenolic content was 61.98 ± 0.89 mgGAE/g. Furthermore, measurement of total flavonoids based on quercetin equivalence brewed coffee has a total flavonoid content of 11.99 ± 0.02 mgQE/g which is lower than extracted coffee with a total flavonoid content of 18.40 ± 0.47 mgQE/g. Antioxidant activity is described in IC_{50} (half maximal inhibitory concentration) values for brewed coffee of 218.74 ppm which is included in the moderate antioxidant category. On the other hand, extracted coffee has an IC_{50} of 89.43 ppm which is included in the category of strong enough antioxidants. Antioxidant strength is grouped into the following classifications: strong (IC_{50} < 50 ppm), strong enough (IC_{50} 50–100 ppm), moderate (IC_{50} 101–250 ppm), weak (IC_{50} 250–500 ppm), and very weak (IC_{50} > 500 ppm) [24].

Table 1. Total phenolic content, total flavonoid content, and antioxidant activity of brewed and extracted Robusta Coffee

Sample	Total Phenolic Content (mgGAE/g)	Total Flavonoid Content (mgQE/g)	Antioxidant Activity (IC_{50})
Brewed Coffee	11.27 ± 0.10	11.99 ± 0.02	218.74 ppm
Extracted Coffee	61.98 ± 0.89	18.40 ± 0.47	89.43 ppm

Total phenolic content, total flavonoid content, and antioxidant activity of brewed coffee with hot water >90°C has a lower value than coffee extracted with 96% ethanol at room temperature. The type of solvent also affects the levels of phenols and flavonoids in brewed and extracted coffee. In brewed coffee, the solvent used is water, while extracted coffee uses ethanol as a solvent. Water and ethanol are types of polar solvents. Water solvents are suitable for extracting several bioactive compounds with a strong polarity, while ethanol solvents are suitable for extracting several bioactive compounds with a wide polarity range. Most of the phenolic compounds are soluble in polar solvents. Ethanol is able to attract higher phenolic and flavonoid compounds than aqueous extracts [11]. Ethanol solvent is more effective in attracting phenolic compounds and flavonoids when compared to water due to the presence of hydroxyl groups (OH) so that ethanol is able to bind more strongly with polar molecules or ions such as phenolic compounds and water [12]. Ngibad suggested that ethanol solvents can be used to extract total phenolic from plants to the maximum [13].

Based on other studies, total phenolic content, total flavonoid content, and antioxidant activity of robusta coffee have different values related to the brewed or extracted and origin of the coffee. Research conducted by [6], showed that the coffee brewed (with 100 mL of boiling water, heated at 95 °C, and stirred for 1 min) from dried processed Robusta Lampung (*Eerste Kwaliteit* Grade 1) at roasted temperature 185–190°C (early yellow) had total phenolic content of 14.39 ± 0.32 gGAE/100g, and antioxidant activity 2.85 ± 0.13 mg/mL (with DPPH method) respectively. Another study showed that the total phenol content obtained from conventional roasting robusta grounded coffee with brewed serving method (uses water temperature of ± 95°C) is 144.14 GAE/ml, and antioxidant activity value (IC_{50})

150.00 ppm [14]. The antioxidant activity of brewed robusta coffee originating from Temanggung (roasted at 205°C) has an IC_{50} value of 362.91 ppm [15].

On the other hand, the robusta coffee extract from Mangarai Village, West Lampung had total phenolic 143.84±1.74 mg GAE/g extract, total flavonoid 127.33±3.79 mg QE/g extract, and antioxidant activities (IC_{50}) 26.24±1.76 µg/ml with DPPH method [16]. Another robusta coffee powder extract has a total phenol content of 7.792 mgGAE/g sample, and antioxidant activity (IC_{50}) of 140.95 ppm [17]. The ethanolic extract of robusta coffee grounds obtained from Situbondo, East Java, Indonesia has total phenolic levels 11.5 mg GAE/g, total flavonoid levels 7.98 mg QE/g, and antioxidant activity of 71.1% [13]. The crude methanolic extract of robusta coffee from Karnataka, India had an amount of total phenolic content 16.84±0.12 µgGAE/g dry wt, total flavonoids content 76.04±0.23 mgQE/g dry wt, and antioxidant activity (IC_{50}) 89.502± 1.1 μg/ml (DPPH method) [18]. The antioxidant activity of extracted robusta coffee originating from Bogor, Bandung, and Garut West Java, Indonesia had a value IC_{50} of 55.13 ppm, 55.13 ppm, and 54.14 ppm [19].

The difference in total phenolic content, total flavonoid content, and antioxidant levels may be related to brewed or extracted method of serving, or may be related to the height of the planting site. Robusta coffee grown at higher altitudes has stronger antioxidant activity compared to coffee grown at lower altitudes [19]. The content of total phenols and total flavonoids in this study is directly proportional to antioxidant activity, where the highest total phenol and total flavonoid values also have the highest antioxidant activity, in line with research conducted by [17]. If the content of phenolic compounds in the sample is high, then the antioxidant activity is high, because polyphenolic compounds are antioxidant components that are dispersed in plants [20]. This happens because of the ability of the phenol group (-OH) to bind to free radicals by giving their hydrogen atoms through an electron transfer process, so that phenol turns into phenoxyl radicals. Phenoxyl radicals that are formed as a result of the reaction of phenol with free radicals will then stabilize themselves through a resonance effect. For this reason, the derivatives of phenol are good hydrogen donors which can inhibit reactions that occur by radical compounds. Phenol compounds are also known as radical inhibitors [21]. High total flavonoids will also provide high antioxidant activity. Flavonoids from plants have aromatic rings with free hydroxyl groups which can donate their hydrogen atoms so they can pair with free radicals. The number of hydroxyl groups in an aromatic ring is proportional to the antioxidant effectiveness of the substance. Therefore, the antioxidant activity of coffee bean extract with ethanol solvent is higher than that of brewed coffee using water solvent [22].

4 Conclusion

Levels of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and the value of antioxidant activity (IC_{50}) in ethanol solvent extracted coffee respectively 61.98 mgGAE/g, 18.40 mgQE/g, and 89.43 ppm, while in brewed coffee 11.27 mgGAE/g, 11.99 mgQE/g, and 218.74 ppm. This shows that the bioactive compounds in extracted coffee are higher when compared to brewed coffee.

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