



Comparison of Total Flavonoid, Phenolic Levels, and Antioxidant Activity between Robusta and Arabica Coffee

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Abstract. Coffee contains a lot of phenolic compounds, especially chlorogenic acid, therefore many studies have examined the potential of coffee as an antioxidant. Antioxidants can inactivate oxidation reactions and prevent the formation of free radicals. This research aimed to determine the total phenolic and flavonoid content of the ethanol extract of robusta coffee (*Coffea canephora*) and the ethanol extract of arabica coffee (*Coffea arabica*), as well as determine the % inhibition value of Arabica coffee ethanol extract and Robusta coffee ethanol extract and their combination as antioxidants *in vitro*. Robusta and Arabica coffee powders were soaked in ethanol solvent, respectively, and the filtrate was concentrated using a rotary evaporator. The extract's flavonoid and total phenolic content were measured by UV-Vis spectrophotometry. An antioxidant activity test was carried out using the DPPH method. The phenolic content of the ethanol extract of robusta coffee powder and the ethanol extract of arabica coffee powder were 7.98 and 9.16 mg QE/g, respectively. The total flavonoid content of the ethanol extract of robusta coffee powder and the ethanol extract of arabica coffee powder were 11.5 and 14.2 mg GAE/g, respectively. The highest % inhibition values of the ethanol extract of arabica coffee powder and the ethanol extract of robusta coffee and their combination as antioxidants *in vitro* were 71.1, 85.1, and 86.4%.

Keywords: antioxidants, phenolics, flavonoids, arabica coffee, robusta coffee

Abstrak. Kopi banyak mengandung senyawa fenolik terkhusus asam klorogenat sehingga banyak penelitian yang telah mengkaji potensi kopi sebagai antioksidan. Antioksidan mampu menginaktivasi reaksi oksidasi dan mencegah terbentuknya radikal bebas. Tujuan penelitian ini adalah untuk mengetahui kadar fenolik dan flavonoid total ekstrak etanol kopi robusta (*Coffea canephora*) dan kopi arabika (*Coffea arabica*), serta mengetahui nilai % inhibisiekstrak etanol kopi arabika dan kopi robusta serta kombinasi keduanya sebagai antioksidan secara *in vitro*. Bubuk kopi robusta dan arabika masing – masing direndam dengan pelarut etanol dan filtratnya dipekatkan dengan rotary evaporator. Kadar flavonoid dan fenolik total ekstrak diukur dengan metode spektrofotometri UV-Vis. Uji aktivitas antioksidan dilakukan menggunakan metode DPPH. Kadar fenolik dari ekstrak etanol bubuk kopi robusta dan bubuk kopi arabika masing – masing adalah 7,98 dan 9,16 mg QE/g. Kadar flavonoid total ekstrak etanol bubuk kopi robusta dan bubuk kopi arabika masing – masing adalah 11,5 dan 14,2 mg GAE/g. Nilai % inhibisi tertinggi dari ekstrak etanol bubuk kopi arabika dan kopi robusta serta kombinasi keduanya sebagai antioksidan secara *in vitro* masing – masing adalah 71,1; 85,1; dan 86,4 %.

Kata kunci: antioksidan, fenolik, flavonoid, kopi arabika, kopi robusta

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INTRODUCTION

Indonesia is a country that can produce coffee and export it to foreign countries. The

types of coffee grown in Indonesia are arabica coffee (*Coffea arabica*) and robusta coffee (*Coffea canephora*). Caffeine found in coffee reaches $\pm 1.45\%$ for *C. arabica* and $\pm 2.38\%$ for *C. canephora*. In addition to caffeine, coffee

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also contains many phenolic compounds, especially chlorogenic acid. A chlorogenic acid is a group of compounds consisting of hydroxycinnamates, such as caffeoyl quinic acid, feruloyl quinic acid, and p-quinic acid, related to quinic acid to form various conjugated structures known as caffeoylquinic acid, feruloyl quinic acid, and p-coumaroylquinic acid which all exist in some isomeric form (Babova et al., 2016).

With the presence of phenolic compounds in coffee, many researchers have studied the potential of coffee as an antioxidant. Antioxidants can inactivate oxidation reactions and prevent the formation of free radicals. Free radicals are compounds that have one or more unpaired electrons that can damage cells and tissues if accumulated in the human body they can oxidize proteins, fats, and DNA and can initiate chronic or degenerative diseases such as cancer, diabetes, and cardiovascular diseases (Ngibad et al., 2020).

Robusta coffee bean extract in Bandung, Bogor, and Garut contains alkaloid compounds, flavonoids, saponins, and tannins. IC₅₀ values from robusta coffee extracts in Bandung, Bogor, and Garut were 55.13 ppm, 56.48 ppm, and 54.14 ppm respectively (Ying et al., 2018). The use of the Soxhlet extraction method has advantages over the maceration method, which can increase the extract yield, lower the value of IC₅₀, and increase the total phenol content of robusta coffee bean ethanol extract (Hilma et al., 2020).

Arabica coffee bean extract from Wamena and Moanemani has been studied and has antioxidant potential as indicated by IC₅₀ values of 107.97 and 100.81 ppm (Mangiwa et al., 2019). On the other hand, arabica coffee (*C. arabica*) fruit obtained from the Gayo area of Central Aceh Regency, Aceh

Province showed very strong antioxidant potential indicated by an IC₅₀ value of 12.427 ppm (Ajhar et al., 2020). Based on several studies above, no studies have compared the antioxidant potential *in vitro* using the DPPH method between robusta coffee and arabica coffee. In addition, it is necessary to study the total phenolic and flavonoid levels of arabica coffee extract and robusta coffee and be associated them with their antioxidant potential *in vitro* using the DPPH method with the determination of IC₅₀ values. Furthermore, it is necessary to study the combination of arabica coffee ethanol extract (*C. arabica*) and robusta coffee ethanol extract (*C. canephora*) as antioxidants *in vitro*.

MATERIAL AND METHODS

Materials

The materials used in this research include robusta coffee (*C. canephora*) and arabica coffee (*C. arabica*) grounds obtained from Situbondo, East Java, Indonesia, ethanol solvent, methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), quercetin, gallic acid, Folin-Ciocalteu reagent, Na₂CO₃, and aluminum chloride. All reagents are analytical grade.

Instrumentation

The tools used are a Rotary evaporator vacuum (Eyela®) and UV-Vis spectrophotometer (SHIMADZU)®.

Procedure

Sample preparation

Grounds of arabica coffee (*C. arabica*) and robusta coffee (*C. canephora*) are each blended and sifted until a powder to obtain a homogeneous powder size.

Extraction using maceration method

A total of 100 g of powder was macerated using an ethanol solvent of 500 mL at room temperature. The maceration process is carried out for 24 hours and then filtered until filtrate and pulp are obtained. The filtrate is inserted in the container while the pulp is re-macerated using a new ethanol solvent of 250 mL. The maceration process is stopped when the pulp is already pale in color. Then the filtrate is collected to be concentrated with a rotary evaporator until a concentrated extract is obtained.

Test of total flavonoid and phenolic levels

The flavonoid level test uses a standard quercetin solution made in the concentration range of 10 – 50 mg / L using the UV-Vis spectrophotometry method. The test procedure for flavonoid levels in robusta and arabica coffee ground extract refers to our previous research (Aisyah et al., 2022). Test phenolic levels using the solution of gallic acid made in the 10 – 50 mg/L concentration range using the UV-Vis spectrophotometry method. The procedure for testing phenolic levels in robusta and arabica coffee ground extracts refers to our previous research (Nofita et al., 2022).

Antioxidant test

The test solution in this study consisted of a test solution of *C. arabica* and *C. canephora*. The concentration of all test solutions is 20; 40; 80 and 100 mg/L in ethanol solvents. DPPH 6×10^{-5} M solution was prepared by weighing 2,364 mg of DPPH in a 100 mL methanol solvent. Each test solution consisting of a mixture of ethanol extract of robusta coffee grounds (*C. canephora*), arabica coffee (*C. arabica*), and a ratio of the two (1: 1) was taken as much as 0.25 mL and then put into a closed test tube that had been coated

with aluminum foil. Next, it is added with a solution of DPPH 6×10^{-5} M by 5 mL. Then, the solution mixture is vortex for 1 minute and incubated for 30 minutes at room temperature. The absorbance of the reaction mixture was measured at a wavelength of 515 nm using a UV-Vis spectrophotometer. On the other hand, control absorbance can be obtained by measuring the absorbance of DPPH solution 6×10^{-5} M. Experiments were carried out with 3 repetitions (Ngibad et al., 2020) (Ngibad et al., 2019a) (Ngibad et al., 2019b) (Fitria et al., 2022) (Ngibad et al., 2023).

RESULT AND DISCUSSION

Extract Yield

This study extracted arabica coffee (*C. arabica*) and robusta coffee (*C. canephora*) grounds using the maceration method. The advantage of the maceration method is that the procedure is simple, fast, and can maximally extract bioactive compounds from plants/plants. The maceration method can also extract bioactive compounds that are not resistant to heating (Sa'adah et al., 2017). Table 1 shows that the number of secondary metabolite compounds in the ethanol extract of robusta coffee grounds is more than in the ethanol extract of robusta coffee grounds.

Table 1. Extract yield

Types of extracts	Extract weight (g)	Sample weight (g)	Yield (%)
Ethanol extract of robusta coffee ground	1000	22.5	2.25
Ethanol extract of arabica coffee grounds	1000	28.9	2.89

Ethanol solvents are used for the maceration process because they can extract non-polar and polar active compounds (Pujiastuti et al., 2021). In addition, other

studies that also used ethanol in the extraction process were able to show high phytochemical concentrations (Kalaivani et al., 2021). Another study reported that methanol extract of coffee grounds of the type *C.canephora* Pierre ex A. Froehner from the Full-Kerinci River area yielded 4.4097 % (Azizah et al., 2019).

Total Flavonoid and Phenolic Levels

In measuring total flavonoid levels of ethanol extracts of robusta and arabica coffee, quercetin is used as standard. Selection of quercetin as a standard because quercetin is a class of flavonoid compounds that are effective in warding off free radicals (Aisyah et al., 2022). Figure 1 shows that the linear regression equation on the standard calibration curve of quercetin is $y = 0.0157x - 0.1404$; $R^2 = 0.9139$.

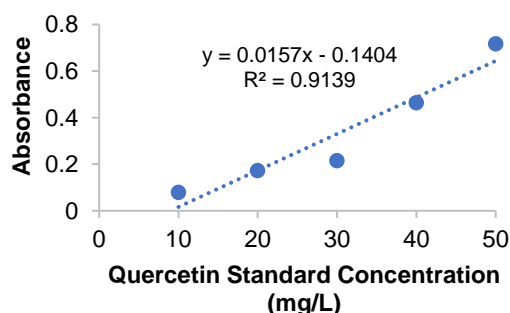


Figure 1. Quercetin standard curve

Table 2. Flavonoid levels of coffee grounds extract

Types of extracts	Absorbance	Total flavonoid levels (mg QE/g)
Robusta coffee ground ethanol extract	1.111	7.98
Ethanol extract of arabica coffee grounds	1.297	9.16

Based on Table 2, it can be concluded that the total flavonoid content in robusta coffee ground ethanol extract is smaller than in arabica coffee ground ethanol extract. Other studies have shown that the green bean water

extract of Aceh Gayo arabica coffee contains alkaloid compounds, tannins, phenolics, flavonoids, triterpenoids, and glycosides (Handoko et al., 2020). Solvents of polar ethanol will easily attract the flavonoid compounds that are also polar (Rebaya et al., 2015). In our other study, purple passion fruit peel ethanol extract had greater total flavonoid levels than ethyl acetate extract (Aisyah et al., 2022). In another study, ethanol extract of *P. emblica* leaves had the highest total flavonoid levels followed by ethyl acetate extract and n-hexane extract (Fitriansyah et al., 2018). These reports suggest that ethanol solvents can be used to extract total flavonoids from plants to the fullest.

In the measurement of total phenolic levels, the gallic acid is used as standard (Ahmad et al., 2015). In addition, gallic acid is very effective in the framework of the formation of complex compounds with the Folin-Ciocalteu reagent that causes the occurrence of more sensitive and intensive reactions (Dungir et al., 2012). Figure 2 shows that the linear regression equation on the standard calibration curve of the error acid is $y = 0.0086x + 0.033$ with the value $R^2 = 0.8566$.

Based on Table 3, it can be concluded that the total phenolic content in the ethanol extract of robusta coffee grounds is smaller than the ethanol extract of arabica coffee grounds. Other studies have shown that robusta coffee bean ethanol extract (*Coffea robusta* L.) contains a total phenol content of 77.92 mg GAE/g for the maceration method and 95.32 mg GAE/g for the Soxhlet method (Hilma et al., 2020).

In another study, Kersen leaf ethanol extract had a total phenolic content of 1.163 mg QGA/g extracted using the maceration method

and 2.53 mg QGA/g extracted using the Soxhlet method (Puspitasari, & Proyogo, 2017). In addition, ethanol solvents can also be used to extract total phenolic compounds in telang flowers with levels of $19.43 \pm 1,621$ GAE (mg/g samples) (Andriani et al., 2018) and patchouli leaves with levels of 327.84 mg GAE / gram extract (Tahir et al., 2017). Some of these reports suggest that ethanol solvents can be used to extract total phenolic from plants to the maximum.

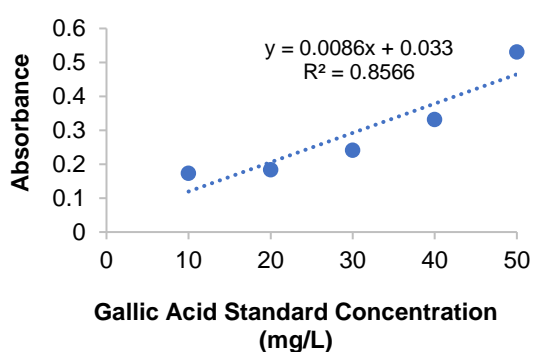


Figure 2. Calibration curve of gallic acid raw solution

Table 3. Total phenolic levels of the extract

Types of extracts	Absorbance	Total Phenolic Content (mg GAE/g)
Ethanol extract from robusta coffee grounds	1.027	11.5
Ethanol extract from arabica coffee grounds	1.258	14.2

Antioxidant Activity of Extracts

The DPPH method has the principle that the donation of hydrogen electrons from the test solution of ethanol extract of robusta coffee grounds and arabica to DPPH results in a change in the color of the DPPH solution from violet to yellowish (Agustiarini et al., 2022). The discoloration indicates the antioxidant activity of the extract test solution (Mokoginta et al., 2020). The DPPH method has advantages,

including simple, fast, and does not use many chemical reagents (Muthia et al., 2019).

Table 4. Antioxidant test of robusta coffee extract

Concentration (mg/L)	Test Solution Absorbance	Antioxidant Activity (%)
20	0.903	66.1
40	0.896	66.4
80	0.820	69.3
100	0.772	71.1

Table 5. Antioxidant test of arabica coffee extract

Concentration (mg/L)	Test Solution Absorbance	Antioxidant Activity (%)
20	0.9216	65.5
40	0.8936	66.5
80	0.4763	82.1
100	0.3973	85.1

Table 6. Antioxidant test of a combination of robusta coffee extract and arabica

Concentration (mg/L)	Test Solution Absorbance	Antioxidant Activity (%)
20	0.4906	81.6
40	0.4316	83.8
80	0.393	85.2
100	0.363	86.4

Based on Table 4, the antioxidant activity expressed in percent inhibition or radical inhibition of DPPH in robusta coffee ground ethanol extract increased from the lowest concentration of 20 mg / L of 66.1 % to the highest concentration of 100 mg / L of 71.1%. In addition, the antioxidant activity in ethanol extract of arabica coffee grounds increased from the lowest concentration of 20 mg / L by 65.5 % to the highest concentration of 100 mg / L by 85.1% (Table 5). In this study, a combination of ethanol extracts of robusta

coffee grounds and arabica was also carried out in a ratio of 1: 1 to maximize antioxidant activity. The results of this combination can be seen in Table 5.5 which shows that antioxidant activity increased from the lowest concentration of 20 mg / L of 81.6 % to the highest concentration of 100 mg / L of 86.4% (Table 6). Thus, it can be concluded that the combination of ethanol extract of robusta coffee grounds and arabica with a ratio of 1: 1 can maximize antioxidant activity expressed in % inhibition of DPPH free radicals (Figure 3).

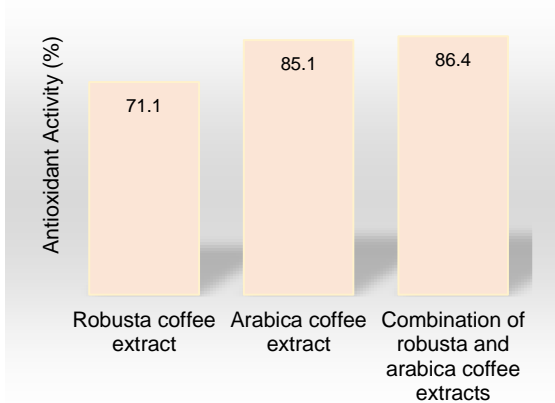


Figure 3. Comparison of antioxidant activities of ethanol extract of robusta coffee grounds, arabica, and a combination of the two

The increased inhibition percentage informs that an increase in the extract concentration will also increase the percentage of inhibition. In many studies, it is reported that an increase in the percentage of inhibition comparable to an increase in antioxidant activity will be followed by an increase in the concentration of the extract or test solution (Wulan et al., 2019, Damanis et al., 2020, Nathania et al., 2020). The increase in percent inhibition along with the increase in the concentration of ethanol extract of robusta coffee grounds and arabica due to the

increasing number of antioxidant compounds in the ethanol extract of robusta coffee grounds and arabica that can ward off DPPH free radicals. This study's robusta coffee grounds and arabica ethanol extracts contained secondary metabolites of flavonoids and phenolics. The hydrogen atoms of these phenolic compounds will be donated to DPPH. The potential or effect as an antioxidant flavonoid or phenolic is due to its ability to reduce oxidative stress and ROS (Nijveldt et al., 2001).

Relationship of Yield, Flavonoid Levels, Total Phenolic, and Antioxidant Activity

In this study, a comparable relationship was produced between amendment, flavonoid levels, total phenolic, and antioxidant activity in both robusta coffee ground ethanol extract and arabica coffee ground ethanol extract as shown in Table 7 and illustrated in Figure 4. The greater the yield of robusta coffee ground ethanol products, the greater the levels of flavonoids and phenolics, causing an increase in % antioxidant activity. Likewise with arabica coffee ground ethanol extract. The greater the yield of ethanol arabica coffee grounds causes greater levels of flavonoids and phenolics increasing by % of antioxidants.

Table 7. Relationship of amendments, flavonoid levels, the total phenolic, and antioxidant activity of ethanol extract of robusta and arabica coffee

Types of extracts	Yield of extracts (%)	Total Flavonoid Levels (mg QE/g)	Total Phenolic Levels (mg GAE/g)	Anti-oxidant Activity (%)
Robust a coffee ground	2.25	7.98	11.5	71.1
Arabica coffee grounds	2.89	9.16	14.2	85.1

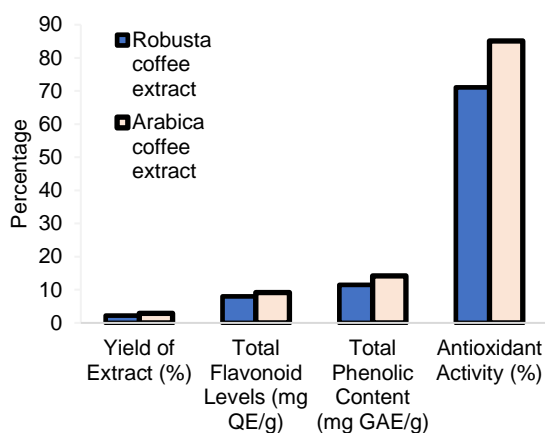


Figure 4. Relationship of extract yield, flavonoid levels, total phenolic, and antioxidant activity of ethanol extract of robusta and arabica coffee

CONCLUSION

The phenolic levels of robusta coffee (*C. Canephora*) ground ethanol extract and arabica coffee (*C. arabica*) ground ethanol extracts were 7.98 and 9.16 mg QE/g, respectively. Total flavonoid levels of robusta coffee ground ethanol extract and arabica coffee ground ethanol extract were 11.5 and 14.2 mg GAE/g, respectively. The highest % inhibition value of ethanol extract of arabica coffee grounds and robusta coffee ethanol extract as well as the combination of the two as antioxidants in vitro respectively – 71.1; 85.1; and 86.4%.

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