Bioactivity of Arabica and Robusta Coffee Extracts from Borneo: Antioxidant and Antibacterial Evaluation

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Abstract. Coffee contains high levels of bioactive compounds, including caffeine and chlorogenic acid, which play important roles in antioxidant and antibacterial activities. This study was designed to compare the antioxidant and antibacterial effects of Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) coffee from the Meratus Mountains, South Kalimantan, Indonesia. The extraction was performed by maceration using 70% ethanol. Antioxidant activity was measured using the DPPH assay, while antibacterial activity was tested against *Pseudomonas aeruginosa* and *Bacillus subtilis* with the well-diffusion method. Results showed Robusta extract had a higher yield (18.35%) than Arabica (17.17%). Robusta also demonstrated stronger antioxidant activity with a lower IC50 value (26.62 µg/mL) compared to Arabica (117.43 µg/mL). In antibacterial tests, Robusta showed a larger inhibition zone against *P. aeruginosa* (9.5 mm) than Arabica (6.5 mm). However, Arabica was more effective against *B. subtilis*, with an inhibition zone of 4 mm versus Robusta's 2 mm. Although specific compound concentrations were not quantified, these findings suggest that Robusta coffee extract has a greater bioactive potential, particularly as an antioxidant and against Gram-negative bacteria.

# INTRODUCTION

Coffee originated in Ethiopia and has become a globally consumed beverage. It is classified under the Rubiaceae family, the fourth largest family of flowering plants, comprising 124 species distributed across two genera: *Coffea* and *Psilanthus*. Among these, Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) are the most commercially important coffee species [1]. Coffee consumption dates to the 9th century. Today, It is considered one of the most frequently consumed beverages worldwide and is the second most traded commodity globally, following petroleum [2]. Each year, approximately 158.93 million 60-kilogram bags of green coffee are produced, with an estimated market value exceeding US $5 billion [3].

Numerous studies have investigated the biochemical constituents and bioactivities of coffee beans, driven by their economic importance and unique quality traits influencing both coffee beverages and human health [4]. It has been reported that many plants respond to environmental stress by producing antioxidant compounds. Crude plant extracts are known to contain essential antioxidants and various bioactive compounds that can effectively inhibit oxidative processes [5, 6]. The primary role of antioxidants is to neutralize reactive oxygen species (ROS), such as nitric oxide, superoxide, and hydrogen peroxide, which are associated with metabolic disorders and chronic diseases, including diabetes, cancer, infections, and cardiovascular conditions [7]. In addition to their antioxidant properties, many plants also exhibit antibacterial activity. A number of studies have shown their capacity to suppress the proliferation of pathogenic bacteria [5].

Coffee consumption has been associated with several health benefits, including improvements in memory, mood, and cognitive performance [8]. These health-promoting effects are largely attributed to its phenolic phytochemicals [2], particularly chlorogenic acids, a group of ester compounds derived from hydroxycinnamic acids and quinic acid, which represent around 6–12% of the dry weight fraction of the beans.

The primary chlorogenic acids in coffee include caffeoylquinic, feruloylquinic, and dicaffeoylquinic acids, with caffeoylquinic acid isomers representing approximately 56–62% within the total amount of chlorogenic acids [3]. In the digestive tract, Chlorogenic acids can be metabolized by intestinal microbiota into quinic acid and other metabolically active antioxidants such as caffeic acid [2]. A wide range of biological roles are attributed to chlorogenic acids, including antioxidant and anti-inflammatory, antidiabetic, anti-obesity, hepatoprotective, antimicrobial, and antihypertensive effects [4].

Another well-known and extensively studied bioactive compound in coffee is caffeine. Caffeine belongs to the alkaloid group and is classified as a secondary metabolite in plants, functioning as a stimulant of the central nervous system [9]. Robusta contains between 1.2% and 2.4% caffeine, while Arabica contains an average of 0.9% to 1.5% [1]. Intake of caffeine is correlated with a decreased risk of several conditions, including Alzheimer’s, Parkinson’s, cardiovascular diseases, and type 2 diabetes [4, 8], and certain types of cancer, such as endometrial, melanoma, oral, prostate, and liver cancers [9]. In addition to its stimulant role, caffeine demonstrates antioxidant and antibacterial effects, demonstrating inhibitory effects against bacteria such as *B. subtilis,* *S. aureus*, *K. pneumoniae,* and *E. coli* [10–12]. However, caffeine content varies depending on factors, including the species of coffee, geographic origin, soil type, environmental conditions, processing methods (e.g., cleaning and roasting), and storage time and conditions [9].

The roasting process of coffee beans generates the distinctive taste, aroma, and dark coloration found in brewed coffee [3]. During the roasting process, compounds such as melanoidins (brown pigments) and phenylalanine are formed, both of which exhibit strong antioxidant properties. Many researchers believe that Maillard reaction products, which are also known for their potent antioxidant activity, are generated throughout the roasting process [12].

Differences in the phytochemical profiles of Arabica and Robusta coffee, particularly in the concentrations of chlorogenic acid and caffeine, are believed to contribute to notable variations in their antioxidant and antibacterial potentials. While several studies have compared these bioactivities in Arabica and Robusta on a global scale, research specifically focused on Borneo’s Arabica and Robusta coffee beans cultivated in the Meratus Mountains of South Kalimantan, Indonesia, remains very limited. Therefore, this article aims to explore and compare the spectrum of bioactivity between these two local coffee varieties.

# RESEARCH METHOD

**Materials**

Roasted Borneo’s Arabica and Robusta coffee beans (at 220 °C) were purchased from Biji Kopi Borneo, Banjarbaru, South Kalimantan, Indonesia. The bacterial strains *Pseudomonas aeruginosa* and *B. subtilis* were obtained from the Microbial Chemistry Laboratory, Department of Chemistry, Institut Teknologi Sepuluh Nopember (ITS). The following reagents were used in the study: DPPH (Merck), DMSO (Merck), ascorbic acid (Merck), gallic acid (Merck), nutrient agar (Sigma-Aldrich), nutrient broth (Himedia), chloramphenicol (Kalbe), 98% methanol (Fulltime), distilled water, and 70% ethanol, all purchased from UD. Sumber Ilmiah Persada Indonesia.

# Extraction of Borneo Arabica and Robusta Coffee Beans

Each sample of Arabica and Robusta coffee beans was ground into a fine powder. Each coffee powder (50 g) was extracted with 700 mL of 70% ethanol using a maceration technique for 24 hours. Following separation with filter paper, the filtrate was collected and concentrated with a rotary vacuum evaporator Büchi B-491 (Büchi, Switzerland) [13].

# Antioxidant Activity

Antioxidant activity was measured using the DPPH assay. Each coffee extract was dissolved in a pro-analytical methanol solution with various concentrations (1000, 100, 10, 1, and 0.1 µg/mL). DPPH solution (1 mL) was added to 33.3 μL of extract. The solution was homogenized, incubated for 30 minutes at 37 °C, and the absorbance was subsequently recorded at 517 nm using a UV-Vis spectrophotometer (Thermo Scientific Genesys, USA) [14]. For the negative control, 33.3 μL of the extract was substituted with methanol, while ascorbic acid and gallic acid served as positive controls [15]. The experiment was conducted in triplicate, and the radical scavenging activity was calculated using Equation 1.

(1)

IC₅₀ is defined as the concentration of extract capable of neutralizing 50% of the DPPH radicals [14].

# Antibacterial Activity

The antibacterial assay was conducted using Gram-positive bacterium (*P. aeruginosa*) and Gram-negative bacterium (*B. subtilis*) with the well diffusion method. Bacterial suspension preparation followed the procedure in the literature [16] with slight modifications. For each bacterial strain, 0.26 g of nutrient broth was dissolved in 20 mL of aquades. The solution was then sterilized using an autoclave (GEA, Indonesia) for 15 minutes at 121 °C. Each bacterium (*B. subtilis* and *P. aeruginosa*) was subsequently inoculated into the nutrient broth solution in a 50 mL Erlenmeyer. The cultures were then incubated at 37 °C in a locally manufactured shaking incubator for 24 hours. Each bacterial culture in nutrient broth was then diluted to reach a final concentration of 1 × 10⁴ CFU/mL [17].

Agar media preparation was carried out based on the method by literature [18] with slight modifications. For each type of bacteria, 1.2 g of Nutrient Agar (NA) was dissolved in 60 mL of distilled water. The solution was then sterilized using an autoclave for 15 minutes at 121 °C. Once cooled but still in liquid form, the NA was mixed with the bacterial suspension at a 1:5 ratio. The prepared medium was transferred into sterile petri dishes (Onemed 90×15 mm) and left until it solidified. Following this, six wells (7 mm in diameter) were formed on every plate. In the final step, 50 μL of 1000 ppm coffee extract (in DMSO) was added into each well using a micropipette (VITLAB, 100–1000 µL, Germany), and the plates were incubated for 41 hours at 37 °C. Chloramphenicol was used as a positive control. The antibacterial assay was performed once for each bacterial strain. Once incubation was completed, the diameters of the inhibition zones were measured apart from the well size. The inhibition zone measurement was carried out as described in Equation 2 [19].

(2)

while DH was horizontal diameter of inhibition zone, DV was vertical diameter of inhibiton zone, and Dw was diameter of well.

# RESULT AND DISCUSSION

# Extraction of Borneo’s Arabica and Robusta Coffee Beans

Each of the Arabica and Robusta coffee powders from Borneo (50 g) was macerated using ethanol as the solvent. Maceration is considered an effective technique for extracting large quantities of samples at room temperature by adjusting the duration of sample immersion in a chosen solvent [20]. Ethanol is known to be a more efficient solvent than water for coffee extraction [21]. Previous studies have demonstrated that coffee extracts obtained using organic solvents exhibit significantly higher antioxidant potential compared to those extracted with distilled water. These findings indicate that the efficiency of antioxidant compound extraction is influenced by the solvent’s polarity and viscosity. These results are consistent with previous knowledge that phenolics dissolve better in less polar organic solvents than in water [22]. The extraction yields obtained in this study are presented in Table 1.

TABLE 1. Extraction Yield of Borneo’s Arabica and Robusta Coffee

|  |  |  |
| --- | --- | --- |
| **Sample** | **Yield (g)** | **Yield (%)** |
| Arabica | 8.58 | 17.17 |
| Robusta | 9.17 | 18.35 |

Based on Table 1, Robusta extract produces a higher yield (18.35%) than Arabica (17.17%). This difference may be due to differences in the composition of bioactive compounds, especially phenolics and caffeine, which are indeed higher in Robusta according to (1). The content of more polar or semi-polar compounds allows for more optimal extraction in ethanol solvents.

**Antioxidant Activity Test by DPPH Assay**

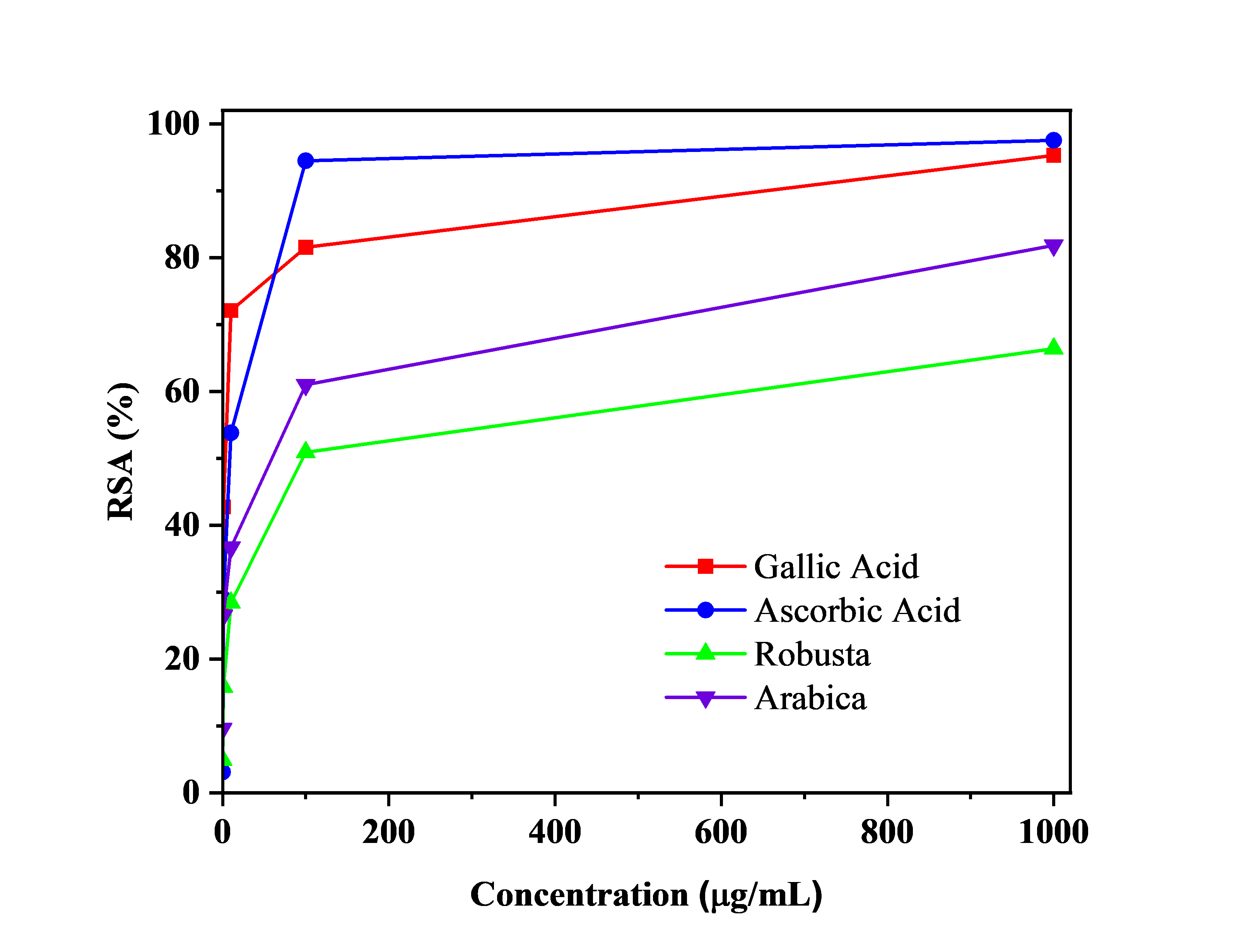
The geographic origin and roasting conditions of the coffee beans may also influence their bioactivity. Coffee cultivated in the Meratus Mountains, South Kalimantan, is exposed to unique environmental stressors (e.g., altitude, soil composition) that can enhance the production of secondary metabolites like chlorogenic acids [10]. Additionally, the roasting process (220°C) used in this study may have modified the phenolic profile of the beans, as high temperatures can degrade some compounds while generating others, such as melanoidins and Maillard reaction products, which contribute to antioxidant and antibacterial properties [11].

A common approach to assess antioxidant activity is the DPPH assay [23]. Free radicals in DPPH accept electrons or hydroxyl radicals from other sources and are converted into stable diamagnetic molecules. The transition of the solution’s color from purple to yellow reflects the scavenging of radicals by antioxidants through hydrogen transfer, which stabilizes the DPPH molecule [24]. The antioxidant activity measured using the DPPH assay, with gallic acid and ascorbic acid as positive controls, is presented in Table 2.

TABLE 2. Antioxidant Activity Testing with the DPPH Assay

|  |  |
| --- | --- |
| **Sample** | **IC50 (µg/mL)** |
| Gallic Acid | 1.50 ± 0.13 |
| Ascorbic Acid | 5.65 ± 0.72 |
| Arabica | 117.43 ± 10.01 |
| Robusta | 26.62 ± 6.77 |

The antioxidant activity test results of Arabica and Robusta coffee extracts, presented in Table 2 as IC50 values, revealed a significant difference. For comparison, the positive controls, gallic acid and ascorbic acid, showed IC50 values of 1.50 ± 0.13 µg/mL and 5.65 ± 0.72 µg/mL, respectively. The IC50 value for Robusta coffee extract was 26.62 ± 6.77 µg/mL, which is notably lower than that of Arabica extract at 117.43 ± 10.01 µg/mL. This indicates that Robusta coffee extract exhibits approximately four times greater antioxidant activity than Arabica, this may be related to the greater abundance of bioactive compounds, including caffeine, chlorogenic acids, and trigonelline. These findings are consistent with those of literature [25], which reported that Robusta coffee shows stronger antioxidant activity than Arabica, largely due to differences in antioxidant metabolite content. As potent antioxidants, chlorogenic acids neutralize free radicals and bind metal ions, thereby decreasing oxidative stress. The chlorogenic acid content in Arabica coffee ranges from 4% to 8.4%, whereas in Robusta it ranges from 7% to 14.4% [26]. Caffeine contributes to antioxidant activity by inhibiting lipid peroxidation and reactive oxygen species (ROS) generation [12]. Robusta also contains more caffeine, between 0.927% and 3.329%, compared to 0.555% to 1.015% in Arabica. In addition, Robusta coffee contains higher levels of trigonelline, ranging from 0.911% to 0.972% [27].



**FIGURE 1.** Curve of %RSA vs Concentration

Figure 1 shows curves representing the relationship between concentration (μg/mL) and percentage of free radical inhibition. In general, all samples exhibited a positive dose-response correlation, where increasing concentrations led to higher inhibition percentages. Both Arabica and Robusta extracts demonstrated moderate antioxidant activity compared to the standard controls. However, Robusta consistently showed higher inhibition percentages across all concentration ranges than Arabica. At the highest concentration (1000 μg/mL), Robusta extract reached an inhibition rate of approximately 84.34%, while Arabica extract achieved only around 68.02%. Moreover, the curve for Robusta showed a steeper rise at lower concentrations compared to that of Arabica; this suggests a more efficient radical scavenging mechanism at lower concentrations. This could be due to the presence of multiple antioxidant pathways, including hydrogen atom transfer and electron donation, facilitated by the diverse phenolic profile of Robusta coffee [4]. This difference aligns with the lower IC50 value observed for Robusta extract, indicating its greater antioxidant efficiency. Future studies could explore the specific contributions of individual compounds (e.g., caffeoylquinic acids vs. dicaffeoylquinic acids) to better understand these mechanisms.

# Antibacterial Test by Well Diffusion Method

The antibacterial activity of Borneo’s Arabica and Robusta coffee extracts was studied against *P. aeruginosa* and *B. subtilis* bacteria using the Well Diffusion Method. Table 3 shows the results of the measurement of the inhibition zone of the extract against bacterial growth.

**TABLE 3.** Antibacterial Activity Tests with the Well Method

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bacteria** | **Extract** | **Inhibition Zone (mm)** | | **Inhibtion Zone (mm)** | **Category** |
| **Horizontal** | **Vertical** |
| *P. aeruginosa* | Chloramphenicol (+) | 15.0 | 16.0 | 8.5 | Strong |
| DMSO (-) | 0 | 0 | 0 | No Activity |
| Arabica | 14.0 | 13.0 | 6.5 | Moderate |
| Robusta | 16.0 | 17.0 | 9.5 | Strong |
| *B. subtilis* | Chloramphenicol (+) | 26.0 | 29.0 | 20.5 | Strong |
| DMSO (-) | 0 | 0 | 0 | No Activity |
| Arabica | 9.0 | 9.0 | 2.0 | Moderate |
| Robusta | 11.0 | 11.0 | 4.0 | Moderate |

Table 3 presents the results of the antibacterial activity test of Borneo’s Arabica and Robusta coffee extracts against Gram-positive (*B. subtilis*) and Gram-negative (*P. aeruginosa*) bacteria using the well diffusion method. Antibacterial activity was determined from the size of the inhibition zones (in mm) observed. As a positive control, chloramphenicol exhibited strong antibacterial activity against both bacteria, with average inhibition zones of 8.5 mm for *P. aeruginosa* and 20.5 mm for *B. subtilis*. In contrast, DMSO, used as the negative control, showed no inhibition zone (0 mm), confirming that the solvent itself had no antibacterial effect. Against *P. aeruginosa*, the Robusta coffee extract demonstrated strong antibacterial activity, with an average inhibition zone of 9.5 mm, slightly greater than that of chloramphenicol in this test. Conversely, Gentili et al. [28] and Admi et al. [29] documented average inhibition zones of chloramphenicol against *P. aeruginosa* of 23.7 mm and 16 mm, respectively. The reduced activity found in this study could be explained by the emergence of resistance in the tested *P. aeruginosa* strain, consistent with Dégi et al. [30], who emphasized the bacterium’s high capacity to develop antibiotic resistance. Another possible explanation is that Robusta coffee contains multiple strong bioactive compounds, which may collectively produce greater antibacterial effects than chloramphenicol, which is a single compound. Arabica extract also exhibited antibacterial activity, though at a moderate level, with an average inhibition zone of 6.5 mm. For *B. subtilis*, both Robusta and Arabica extracts showed moderate antibacterial activity, with inhibition zones averaging 4 mm and 2 mm, respectively. However, their activity remained significantly lower than that of the positive control chloramphenicol, which produced a 20.5 mm inhibition zone against *B. subtilis*.

The difference in antibacterial effectiveness between Arabica and Robusta coffee extracts against Gram-positive (*B. subtilis*) and Gram-negative (*P. aeruginosa*) bacteria can be attributed to fundamental variations in the cell wall structures of these bacterial types. Gram-positive bacteria possess a very thick (20–80 nm) and dense peptidoglycan layer, which acts as a strong physical barrier [31], making it more difficult for antioxidant compounds in the coffee extracts to penetrate. In contrast, Gram-negative bacteria have a much thinner peptidoglycan layer (2–7 nm) and an additional outer membrane rich in lipopolysaccharides (LPS). While this outer membrane serves as a selective barrier that can block the entry of many antimicrobial agents, it also contains porinproteins [32] that allow the penetration of smaller hydrophobic molecules, such as caffeine and chlorogenic acids, which disrupt membrane integrity and inhibit essential enzymes [11]. Conversely, the substantial peptidoglycan layer present in Gram-positive *B. subtilis* could restrict the penetration of these compounds, leading to smaller inhibition zones [31].

The stronger antibacterial activity of Robusta extract against *P. aeruginosa* could be linked to its higher caffeine content. Caffeine has been shown to interfere with bacterial quorum sensing and biofilm formation, particularly in Gram-negative pathogens [10]. Moreover, chlorogenic acids exhibit antibacterial properties by binding to bacterial membranes and inducing leakage of cellular contents [2]. The combined action of these compounds likely enhances Robusta’s efficacy against *P. aeruginosa*. Interestingly, the relatively small inhibition zones observed against *B. subtilis* suggest that antibacterial activity is not solely driven by caffeine or chlorogenic acids. One plausible contributor is melanoidins, high-molecular-weight brown polymers formed during the Maillard reaction in roasting. Melanoidins have been reported to exert antioxidant and antimicrobial effects through mechanisms such as binding to cell walls/membranes, protein complexation, and inhibition of protein synthesis [9]. Hu et al. noted that MRPs exhibit stronger effects on Gram-positive bacteria than on Gram-negative ones, likely because of distinct structural features in their cell walls [33]. However, in the present study we did not quantify or fractionate melanoidins, so a causal link between melanoidin content and antibacterial activity remains inconclusive. Thus, our findings should be regarded as preliminary evidence that compounds other than caffeine and chlorogenic acids (e.g., melanoidins or other high-MW fractions) may modulate activity against Gram-positive bacteria. Targeted fractionation and bioassays are required to clarify these interactions.

The structural differences in bacterial cell walls are illustrated in Figure 2. Overall, this study resulted in the finding that coffee extract tends to be more effective in inhibiting the growth of Gram-negative bacteria (*P. aeruginosa*) than Gram-positive (*B. subtilis*). Then it is indicated that Robusta coffee extract has better antibacterial potential than Arabica coffee.

|  |  |
| --- | --- |
|  |  |
| (a) | (b) |

**FIGURE 2.** Cell Wall Structure: a) *B. subtilis* (Gram Positive) [31] and (b) *P. aeruginosa* [32]

A limitation of the present study is that compound profiling was not performed, so the specific bioactive constituents responsible for the observed antioxidant and antibacterial activities remain unclear. In addition, the antibacterial assays were not replicated in triplicate, which may affect statistical robustness. Future research should include HPLC-based quantification of major compounds such as caffeine, chlorogenic acids, and melanoidins to establish clearer correlations with biological activity. Moreover, synergy testing between individual compounds or between coffee extracts and conventional antibiotics would provide deeper insight into potential combinatorial effects, potentially enhancing the practical application of coffee-derived bioactives as antimicrobial agents.

# CONCLUSION

This study shows that there are significant differences between Borneo’s Arabica and Robusta coffee extracts in terms of antioxidant and antibacterial activities. Extraction using 70% ethanol produced a slightly higher yield in Robusta coffee (18.35%) compared to Arabica ones (17.17%). Antioxidant activity based on the IC50 value showed that Robusta coffee (26.62 µg/mL) has an antioxidant potential almost four times stronger than Arabica (117.43 µg/mL), which is thought to be related to the higher content of chlorogenic acid and caffeine. In the antibacterial test using the well method against Gram-negative (*P. aeruginosa*) and Gram-positive (*B. subtilis*) bacteria, Robusta coffee extract showed stronger antibacterial activity than Arabica, especially against *P. aeruginosa*. This is thought to be related to the structure of the bacterial cell wall, and the bioactive compounds contained in coffee can affect the membrane structure and metabolism of bacterial cells. Coffee bioactives present promising applications in areas such as nutraceuticals, natural food preservation, cosmetic industries, and antimicrobial therapy.

The results indicate that Robusta coffee extracts possess superior antioxidant and antibacterial potential relative to Arabica, making them attractive candidates for functional product development. Despite these findings, further studies are required to overcome the limitations of this work.

1. The study did not quantify individual bioactive compounds (e.g., chlorogenic acid isomers, caffeine) in the extracts. HPLC or LC-MS analysis could elucidate their relative contributions to bioactivity.
2. The exact mechanisms of antibacterial action (e.g., membrane disruption, enzyme inhibition) remain speculative. Molecular docking or transcriptomic analyses could provide deeper insights.
3. Testing against a wider range of pathogens, including clinically relevant strains, would strengthen the findings.
4. The antibacterial assays were not replicated in triplicate, which may affect the statistical robustness of the results. Future studies should perform assays in triplicate to confirm the reproducibility and reliability of the findings.

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