

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ROBUSTA COFFEE (*Coffea canephora*) EXTRACT FROM WEST LAMPUNG

Iffa Afiqa Khairani*, Hida Arliani Nur Anisa*, Jeane Siswitasari Mulyana*, Elisa Nurma Riana*, Wahyuni Dian Lestari*

*Department of Biology, Faculty of Sciences, Institut Teknologi Sumatera, South Lampung, Indonesia, iffa.khairani@bi.itera.ac.id, hida.anisa@bi.itera.ac.id, jeane.mulyana@bi.itera.ac.id, elisa.riana@bi.itera.ac.id, wahyuni.120180053@student.itera.ac.id

Email Correspondence : iffa.khairani@bi.itera.ac.id

Received : September 25, 2024

Accepted : May 14, 2025

Published : June 30, 2025

Abstract: Robusta coffee (*Coffea canephora*) is one of Indonesia's export commodities, which is widely grown in Lampung Province. West Lampung Regency is the main producer of robusta coffee in Lampung Province. Robusta coffee contains various metabolites with potential as an antioxidant and antibacterial properties. This study aims to analyze the metabolite content (phytochemical compounds) of robusta coffee ethanol extract qualitatively and quantitatively, followed by determining antioxidant activity (DPPH method) and antibacterial activity (MIC and MBC method) against tested bacteria including *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 25923. Robusta coffee extract from West Lampung showed positive results for phytochemical compounds such as *phenolic* compounds, *flavonoids*, *tannins*, *saponins*, *terpenoids*, *alkaloids*, and negative for *steroids*. The quantification results of total *phenolic* content (TPC) were 62.88 mgGAE/g extract, total *flavonoids* content (TFC) were 23.90 mgQE/g extract, total *tannins* content (TTC) were 176.73 mgTAE/g extract, and total *saponins* content (TSC) were 10.37%. This extract classified as a very strong antioxidant with an IC₅₀ value of 35.02 ppm, and moderate antibacterial activity at a concentration of MIC/MBC 125/125 ppm against all bacterial tested. Therefore, robusta coffee extract was a natural ingredient with potential antioxidant and antibacterial properties.

Keywords: Antibacterial; antioxidant; phytochemical content; robusta coffee; West Lampung

Abstrak: Kopi robusta (*Coffea canephora*) merupakan salah satu komoditas ekspor Indonesia yang banyak ditanam di Provinsi Lampung. Kabupaten Lampung Barat merupakan penghasil utama kopi robusta di Provinsi Lampung. Kopi robusta mengandung berbagai metabolit yang berpotensi sebagai antioksidan dan antibakteri. Penelitian ini bertujuan untuk menganalisis kandungan metabolit (senyawa fitokimia) dari ekstrak etanol kopi robusta secara kualitatif dan kuantitatif, dilanjutkan dengan pengukuran aktivitas antioksidan (metode DPPH) dan aktivitas antibakteri (metode MIC dan MBC) terhadap bakteri uji yang meliputi *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, dan *Staphylococcus aureus* ATCC 25923. Ekstrak kopi robusta dari Lampung Barat positif mengandung senyawa fitokimia seperti senyawa fenolik, flavonoid, tanin, saponin, terpenoid, alkaloid, dan negatif steroid. Hasil kuantifikasi kandungan fenolik total (TPC) sebesar 62,88 mgGAE/g ekstrak, kandungan flavonoid total (TFC) sebesar 23,90 mgQE/g ekstrak,

kandungan tanin total (TTC) sebesar 176,73 mgTAE/g ekstrak, dan kandungan saponin total (TSC) sebesar 10,37%. Ekstrak ini tergolong antioksidan yang sangat kuat dengan nilai IC_{50} sebesar 35,02 ppm, dan aktivitas antibakteri sedang pada konsentrasi MIC/MBC 125/125 ppm terhadap seluruh bakteri uji. Dengan demikian, ekstrak kopi robusta merupakan bahan alami yang memiliki potensi sebagai antioksidan dan antibakteri.

Kata kunci: Antibakteri; antioksidan; kopi robusta; Lampung Barat; senyawa fitokimia

Recommended APA Citation :

Khairani, I. A., Anisa, H. A. N., Mulyana, J. S., Riana, E. N., & Lestari, W. D. (2025). Phytochemical Analysis, Antioxidant and Antibacterial Activity of Robusta Coffee (*Coffea canephora*) Extract From West Lampung. *Elkawnie*, 11(1), 1-16. <https://doi.org/10.22373/ekw.v11i1.26200>

Introduction

Coffee (*Coffea* sp.) is one of the plantation commodities that has an important role in economic activities in Indonesia. Coffee is also one of Indonesia's export commodities which is quite important as a foreign exchange earner besides oil and gas (Islami et al., 2022). Robusta coffee (*Coffea canephora*) in Indonesia has a higher production than arabica coffee. Robusta coffee thrives in the lowlands, with most of the production coming from the provinces of South Sumatra and Lampung in 2022. West Lampung Regency is the main producer of robusta coffee in Lampung Province (BPS, 2023).

The content of active compounds in coffee has potential in several health benefits including antioxidant, antifungal, antibacterial, antiviral, antiinflammatory, immunomodulators and anticancer (Assa et al., 2021; Hasan et al., 2022; Nurhayati et al., 2023; Wigati et al., 2019). Bioactive compound were founded in robusta coffee beans consist of *alkaloids, flavonoids, saponins, tannins, caffeine, and phenols*. *Phenolic* compounds in coffee, such as chlorogenic acid (a group of non-volatile organic acids), and other metabolite (such as tannin and saponin) could prevent the growth of gram-positive and negative bacteria by various cellular mechanisms. Thus making coffee potentially antibacterial (Nurhayati et al., 2023). Coffee has also been classified as a source of antioxidants, due to its *phenolic* components. Antioxidant activity has an important role in protecting the organism from oxidative damage as well as in preventing the formation of free radicals (Norazlin et al., 2023).

The health benefits of robusta coffee, particularly from West Lampung, have not been widely explored. Therefore, it is necessary to conduct research on robusta coffee extract (from West Lampung) and analyse the content of its bioactive compounds, as well as the potential of robusta coffee extract as an antioxidant and antibacterial. This research is expected to support data on bioactive compounds, antioxidant and antibacterial potential of robusta coffee extract from West Lampung. In addition, this research could increase the selling value of robusta

coffee from West Lampung and support the development of drug therapy for degenerative diseases.

Method

This research is part of a series of studies we previously published, titled *Quantitative Analysis of Phytochemical Compounds and Antihyperglycemic Potential of Robusta Coffee from West Lampung*. In the previous study, we used Robusta coffee powder brewed with hot water, whereas in the current study, we used Robusta coffee extract (Khairani, Mulyana, et al., 2024).

The coffee beans used in this study have been used in previous studies. Robusta coffee beans originated from Batu Ketulis District, West Lampung Regency, Lampung Province (5°01'38.7"S 104°15'13.9"E) (Figure 1). This research is divided into several stages, namely robusta coffee extraction, phytochemical analysis, antioxidant and antibacterial activity test.

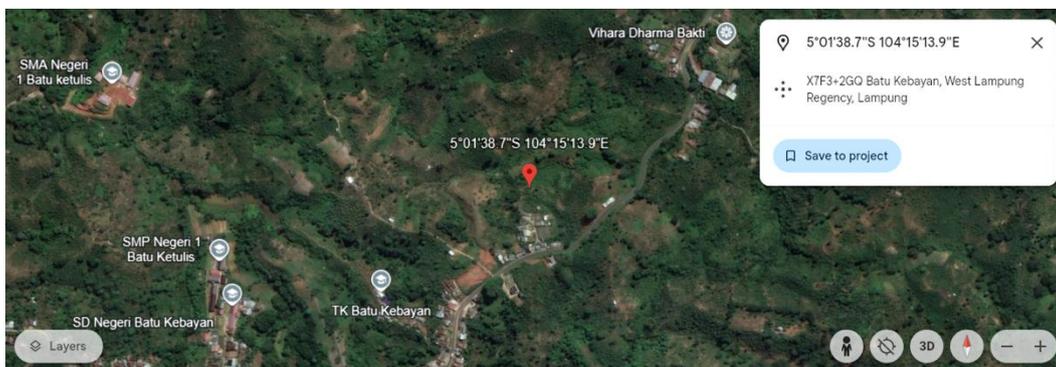


Figure 1. Location of coffee sampling

Robusta coffee extraction

The green coffee beans have been roasted at 180°C for 5 minutes, and ground into powder. The robusta coffee was extracted using the ethanol maceration method. 200 g of robusta coffee powder (*simplicia*) was soaked with 1 L ethanol 96% (1:5 ratio) for 3x24 hours, with once stirring every day. Then, the *simplicia* was remacerated using 1 L of ethanol. The macerate was evaporated using a rotary evaporator with a temperature of 40°C and a rotation speed of 30 rpm to obtained crude extract (Khairani, Anisa, et al., 2024).

Phytochemical test

Phytochemical compounds of robusta coffee extract were analyzed including *phenolic*, *flavonoid*, tannin, saponin, steroid or terpenoid, and alkaloid. 0.05 g robusta coffee extract was put into a test tube, added 5 mL chloroform and 5 mL distilled water. Then shaken vigorously and waited until 2 layers were formed, the top layer (aquadest) the bottom layer (chloroform). The two layers formed were separated. The top layer (distilled water) was used for testing *phenolic*, *flavonoid*,

and saponin compounds. The lower layer (chloroform) was used for testing steroid or terpenoid compounds, while tannin and alkaloid testing was carried out with different procedures (Octaviani et al., 2019).

Phenolic test was carried out qualitatively and quantitatively. Qualitative *phenolic* test was performed by taking the top layer (distilled water) and dripped into a porcelain drip plate and FeCl was added. Positive samples contained *phenolic* was indicated by the formation of a blue colour. Quantitative *phenolic* test was conducted by dissolving 5 mg of extract in 2 mL of 96% ethanol, then added 5 mL of distilled water and 0.5 mL of 50% Folin-Ciocalteu reagent, incubated for 5 minutes and added 1 mL of 5% Na₂CO₃. The solution was homogenized and incubated for one hour, then measured with a spectrophotometer at a wavelength of 745 nm. A standard curve for gallic acid was made with various concentrations of 5, 10, 15, 20 and 25 ppm. Total *phenol* content was calculated from the regression equation on the gallic acid curve in mgGAE/g extract (Safitri et al., 2021).

Flavonoid test was also performed qualitatively and quantitatively. Qualitative *flavonoid* test was carried out by dripping 1-2 drops of top layer (distilled water) on a drip plate. Then, a Mg and 1-2 drops of HCl was added. *Flavonoid* positivity is indicated if a yellow-orange to red colour is formed in the sample. Quantitative *flavonoid* test was conducted by dissolved 10 mg of extract in 10 mL of methanol. 1 mL of sample was added with 3 mL of methanol, 0.2 ml of 10% AlCl₃, 0.2 ml of 1 M CH₃COONa respectively, and added distilled water until 10 mL. The sample was incubated for 30 minutes and the absorbance was measured using a spectrophotometer at a wavelength of 431 nm. A standard curve for quercetin was made with various concentrations of 10, 20, 30, 40, and 50 ppm. Total *flavonoid* content was calculated from the regression equation on the quercetin curve in mgQE/g extract (Styawan & Rohmanti, 2020).

Saponin test was also performed qualitatively and quantitatively. Qualitative saponin test was carried out by taking the top layer (distilled water) and shaken vigorously in a test tube for a few moments. The sample is positive for *saponins* if foam forms for 3 minutes. Saponin content was quantitatively measured by weighed 10 mg sample, and 5 mL of water was added, then homogenised with a vortex for 5 minutes. After that, 50 µL of anisaldehyde was added to the sample, then shaken and allowed to stand for 10 minutes. 2 mL of 50% H₂SO₄ was added, heated in a water bath at 60°C for 10 minutes, and added distilled water to a volume of 10 mL with a measuring flask. Standard dilutions (quillaja bark) were made from concentrations of 6.25, 12.5, 25, 50, 100, 200 ppm, and absorbance was read on a spectrophotometer with a wavelength of 435 nm (Handayani et al., 2020).

Steroid and terpenoid tests were only performed qualitatively. The lower layer (chloroform) was filtered using filter paper, the filtrate obtained was put into 3 holes of the drip plate and allowed to dry. 1 drop CH₃COOH was added to hole 1, 1 drop H₂SO₄ was added to hole 2, and Liebermann-Bouchard reagent (2 drops of CH₃COOH and 1 drop of H₂SO₄) was added to hole 3. Positive samples contained

terpenoids if a red color is formed in holes 2 and 3, while positive sample contained steroid compounds if a green or blue color was formed in holes 1 and 3 (Octaviani et al., 2019).

Tannin test was carried out qualitatively and quantitatively. Qualitative tannin test was performed by 0.5 g extract added with 1 mL of FeCl₃ 10%. The formation of dark blue, blue-black, or black-green colour indicates the presence of *tannins* (Dewi et al., 2021). Quantitative *flavonoid* test was conducted by dissolved 100 mg extract with 10 ml of methanol, and filtered. Distilled water was added to a volume of 10 mL. 1 mL of sample was added with 0.1 mL of Folin Ciocalteu reagent and homogenised, wait 5 minutes. Then added with 2 ml of 20% Na₂CO₃, and added with aquadest to volume 10 mL. The absorbance was read at a wavelength of 760 nm after incubating the sample for 30 minutes at room temperature. A standard curve for tannic acid was made with various concentrations of 0.5, 1, 1.5, 2 and 2.5 ppm. Total tannin content was calculated from the regression equation on the tannic acid curve in mgTAE/g extract (Handayani et al., 2020).

Alkaloid test were only carried out qualitatively. 0.25 g extract was added with 1 mL of HCl and 9 mL of distilled water and filtered. Then added with 2 drops of bouchardat reagent. Positive samples contain alkaloid compounds if a brown to black precipitate is formed (Sulistyarini et al., 2020).

Antioxidant activity

Antioxidant test was conducted using DPPH assay (2,2-diphenyl-1-picrylhydrazyl). Extract was dissolved in methanol and 5 concentration variations were made (9.375, 18.75, 37.5, 75, and 150 ppm). A total of 100 µL of each sample at the various concentration and positive control (ascorbic acid) were put into well 96 microplate wells with 3 replicates each. Each well containing the sample was added with 100 µL of DPPH solution at a final concentration of 125 µM (in methanol solvent). The sample was incubated at 30°C for 30 minutes in the dark, then the absorbance value of the sample was measured using an *ELISA* reader spectrophotometer (Thermo scientific Varioskan-Flash) at a wavelength of 514 nm. The wavelength absorbance results were used to calculate the percentage of inhibition. The inhibition values were regressed on a linear regression equation to obtain the Inhibitory Capacity 50% (IC₅₀) value of the antioxidant ability of the extract (Prastya et al., 2019).

Antibacterial activity by Determination of Minimum Inhibitory/Bactericidal Concentration (MIC/MBC) value

The MIC value was calculated using the serial dilution standard test method. The test bacteria used were *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 25923 (Collection of Laboratorium Mikrobiologi dan Biokimia, Pusat Riset Bahan Baku Obat dan Obat tradisional, BRIN, Serpong). All test bacteria were rejuvenated by culturing them in Nutrient Broth medium. A total of 20 µL of sample at an initial

concentration of 5,000 ppm in 99% methanol solvent was added to a sterile 96-well microplate. The mixture was then added with 180 μ L of MHB (Mueller Hinton Broth) media and serial dilution was carried out in each test well until well H. Test bacteria were then prepared by inoculating in 0.85% NaCl and adjusted to McFarland standard 0.5 (equivalent to a cell number of 1×10^8 CFU/mL), then the bacteria were put in each well as much as 100 μ L. The concentration of extract in each well's row of microplate was 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm, 7.8125 ppm, 3.90625 ppm, and 1.953125 ppm. The test plate was then incubated at 37°C for 24 hours. The MIC value was determined as the lowest concentration of extract that can suppress bacterial growth, observed from the test wells that appeared clear. The MBC value was determined as the lowest concentration of extract that was able to kill the test bacteria analysed using growing test results from the well - MIC value. Tetracycline was used as positive control, and methanol 99% as negative control (Priyanto et al., 2023).

Result and Discussion

Metabolite analysis of robusta coffee extract from West Lampung

The results of metabolite analysis showed that robusta coffee extract from West Lampung tested qualitatively positive for phytochemical compounds such as *phenolic* compounds, *flavonoids*, *tannins*, *saponins*, *terpenoids*, *alkaloids*, and negative for *steroids*. The quantification results of total *phenolic* content (TPC) were 62.88 mgGAE/g extract, total *flavonoids* content (TFC) were 23.90 mgQE/g extract, total *tannins* content (TTC) were 176.73 mgTAE/g extract, and total *saponins* content (TSC) were 10.37%, as shown in Table 1.

Table 1. Metabolite analysis of robusta coffee extract from West Lampung

Phytochemical Compounds	Qualitative Test	Quantitative Test (Total Compounds \pm St. Dev)
<i>Phenolic</i>	+	62.88 \pm 0.03 mgGAE/g extract
<i>Flavonoids</i>	+	23.90 \pm 0.03 mgQE/g extract
<i>Tannins</i>	+	176.73 \pm 0.10 mgTAE/g extract
<i>Saponins</i>	+	10.37 \pm 0.21%
<i>Terpenoids</i>	+	test was not performed
<i>Alkaloids</i>	+	test was not performed
<i>Steroids</i>	-	-

The results of this study were in line with several studies, showed that ethanol extract of green and roasted robusta coffee beans from Mount Puntang Cimaung Bandung West Java contained *phenols*, *flavonoids*, *saponins*, *terpenoids*, *alkaloids*, and negative steroid (Rubinadzari et al., 2022). Roasted robusta coffee ethanolic extract from Pagar Alam, South Sumatra, was also positive for *alkaloids*, *flavonoids*, *tannins*, *saponins*, and *polyphenols* (Wahyudi & Wulandari, 2022). Ethanol extracts of robusta coffee beans grown in various regions in Java Island

including West Java Province (Bogor, Kuningan, Sumedang, Bandung, and Garut), Central Java Province (Temanggung, Boyolali, Wonosobo), and East Java Province (Jombang, Malang, and Kediri) also contained *flavonoids*, *tannins*, *saponins*, and *alkaloids* (Nurhayati et al., 2023; N. F. Utami et al., 2018; Wigati et al., 2019). That result was in contrast to robusta coffee bean ethanolic extract obtained from Tangti Village, Mengkendek District, Tanah Toraja Regency, South Sulawesi were positive for *flavonoids*, *triterpenoids*, *tannins*, and negative results in alkaloid, steroid and saponin tests (Utami & Barrang, 2019). In the other hand, robusta coffee originating from Coorg district of Karnataka, India showed positive results for *phenolics*, *flavonoids*, *saponins*, *terpenoids*, and also *steroids* compounds (Mahajan & Kapoor, 2018).

Quantitative phytochemical tests of robusta coffee extract from West Lampung in this current study (Table 1) showed the lower values compared to robusta coffee powder in our previous studies. We measured the TFC, TTC, and TSC in robusta coffee powder from West Lampung, which were 93.6 mgQE/g, 182.3 mgTAE/g, and 24.2 mg/g, respectively (Khairani, Mulyana, et al., 2024). However, TPC and TFC of robusta coffee extract in this study showed the higher values compared to robusta coffee brewed with hot water (> 90°C) in our previous studies, which had TPC value of 11.27 mgGAE/g and TFC value of 11.99 mgQE/g (Khairani, Anisa, et al., 2024). There were differences in total *flavonoids* and total *phenols* in natural ingredients extracted using water (brewed) and extracted using ethanol solvents. The extraction process using ethanol will increase total *flavonoids* and total *phenols* (Prayitno & Rahim, 2020). Comparing to another studies, the robusta coffee extract from Mangarai Village, West Lampung had TPC value 143.84±1.74 mg GAE/g extract, and TFC value 127.33±3.79 mg QE/g extract, which is higher than this study (Suryanti et al., 2023). While the amount of TPC and TFC robusta coffee ethanolic extract from Situbondo, East Java, with a value of 11.5 mgGAE/g extract and 7.98 mgQE/g respectively (Ngibad et al., 2023). The total phytochemical compound of robusta coffee extract from Jambi Province had TPC value 12.64±1.77 mgGAE/100gDW, TFC 6.38±0.45 mgCatechinEquivalent/100gDW, TTC 10.45 ±0.77 mgTAE/100gDW, and TSC 0.50±0.02 gVanillin Equivalent/100g DW (Maxiselly et al., 2023).

Based on the results of qualitative and quantitative phytochemical test, it can be seen that there are differences in phytochemical content and total of phytochemical compounds of robusta coffee beans in this study compared to other studies. This difference can be caused by agroecology (climate and altitude where the plants grow), which can affect the amount of chemical compounds (Utami et al., 2018). According to Mintesnot & Dechassa, (2018), coffee beans grown in highland areas produce *chlorogenic acid* (a group of *phenolic* compounds) in higher concentrations. Virhananda et al., (2022) found that Robusta coffee grown in West Lampung Regency had the highest chlorogenic acid levels compared to two other regions (Way Kanan and Tanggamus regency). West Lampung Regency is known

as a highland region because it has the highest elevation in Lampung Province, at approximately up to 908 meters above sea level (BPS Lampung, 2019). In addition, the higher the elevation at which coffee is grown Robusta coffee in the Lampung area, the higher the caffeine and fat tend to increase (Towaha et al., 2014). This shows that altitude is one of the factors affecting the levels of metabolite compounds in robusta coffee. The height of the growing environment is related to light intensity, temperature and humidity. This will affect the metabolic process and affect the chemical composition produced by the plant (Martins et al., 2020; Tarakanita et al., 2019). Furthermore, significant variations in coffee metabolite compounds (shown in alkaloid concentrations) were also influenced by soil organic carbon and soil pH (Gebrekidan et al., 2019)

Antioxidant activity of robusta coffee extract from West Lampung

The antioxidant activity test results of robusta coffee ethanol extract showed an Inhibitor Concentration 50% (IC₅₀) value of 35.02 ppm (Table 2), which is classified as a very strong antioxidant. Antioxidant strength is grouped into the following classifications: very strong antioxidant (IC₅₀ <50 ppm), strong (IC₅₀ 50-100 ppm), moderate (IC₅₀ 101-250 ppm), weak (IC₅₀ 250-500 ppm), and very weak (IC₅₀ >500 ppm) (Molyneux, 2004).

Table 2. Antioxidant activity of robusta coffee extract from West Lampung

Sample	Antioxidant activity (IC ₅₀ ± St. Dev ppm)
Robusta coffee extract	35,02±8,19 ppm
Ascorbic acid (<i>positive control</i>)	7,00±0,43 ppm

The robusta coffee extract in this study, which has higher TPC and TFC values, also has higher antioxidant activity compared to robusta coffee brewed (our previous study) with IC₅₀ 218.74 ppm (categorized as weak antioxidant activity) which has lower TPC and TFC (Khairani, Anisa, et al., 2024). Nevertheless, the robusta coffee extract from Mangarai Village which had higher TPC and TFC values than robusta coffee extract in this study, was found to have higher antioxidant activity (IC₅₀ 26.24±1.76 ppm) (Suryanti et al., 2023). Various studies mention that robusta coffee extracts from several regions in West Java and East Java, have very strong and strong antioxidant activity with an IC₅₀ range of 45.14 - 55.13 ppm (Pebriarti et al., 2022; Wigati et al., 2019). The level of antioxidant activity is related to the bioactive compound content of plant extracts including TPC, TFC, and TTC. Plants rich in bioactive compounds are considered to contribute to the observed high antioxidant activity and medicinal value. These bioactive compounds synergize together to produce high antioxidant activity (Ebulue, 2023; Kim et al., 2011; Yuarini & Wrasati, 2015). The antioxidant capacity can be attributed to the high phenol content. Chemically, phenol consists of an aromatic ring containing one or more hydroxyl substituents that are able to

scavenge free radicals by donating hydrogen atoms or electrons or chelating metal cations (Medini et al., 2014). As phenolic compounds, *tannins* have strong antioxidant activity like other polyphenols, which is related to the presence of phenolic rings in the tannin structure (de Hoyos-Martínez et al., 2019). *Flavonoids* also have excellent antioxidant properties as they can exhibit their antioxidant activity by scavenging free radicals and ROS (Hassanpour & Doroudi, 2023). Thus, the bioactive content contained in robusta coffee extract makes it potential to be developed as a natural material that can overcome degenerative diseases.

Antibacterial activity of robusta coffee extract from West Lampung

The antibacterial test was carried out with the determination of Minimum Inhibitory/Bactericidal Concentration (MIC/MBC) value on tested bacteria including *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*. The result of Minimum Inhibitory Concentration (MIC) using a 96-well microplate showed in Figure 2, and the result of Minimum Bactericidal Concentration (MBC) test showed in Figure 3. The average values of MIC and MBC were listed in Table 3. This robusta coffee extract has moderate potential in inhibiting tested bacteria at a concentration of 125 ppm. The antibacterial activity of the extract is classified into significant (MIC values of < 100 ppm), moderate (100 < MIC ≤ 625 ppm), and weak (MIC values > 625 ppm) (Kuetze, 2010).

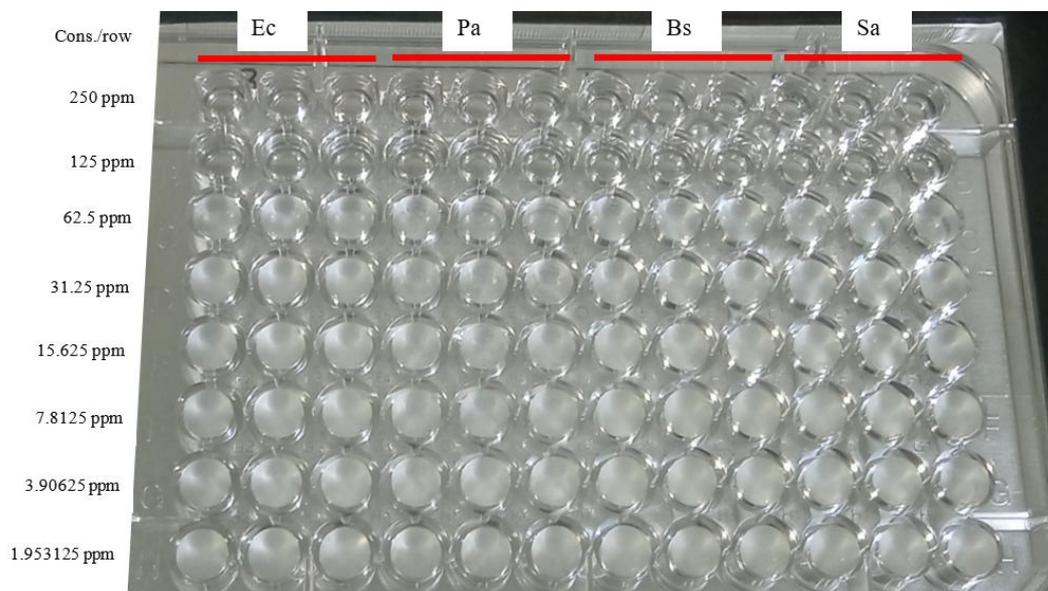


Figure 2. The result of MIC test using a microplate reader (Ec : *E. coli* ATCC 8739, Pa : *P. aeruginosa* ATCC 9027, Bs : *B. subtilis* ATCC 6633, and Sa : *S. aureus* ATCC 25923)

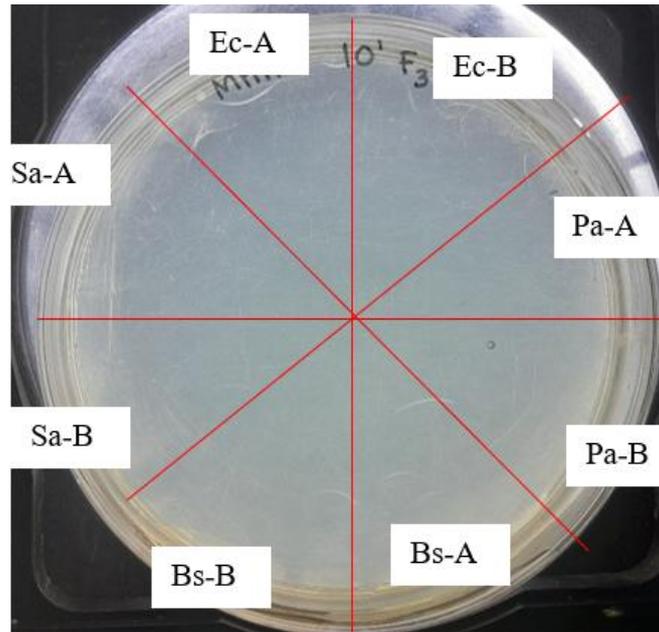


Figure 3. The result of MBC test (Ec : *E. coli* ATCC 8739, Pa : *P. aeruginosa* ATCC 9027, Bs : *B. subtilis* ATCC 6633, and Sa : *S. aureus* ATCC 25923)

Table 3. Antibacterial activity of robusta coffee extract from West Lampung

Sample	MIC/MBC value (ppm)			
	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Robusta coffee extract	125/125	125/125	125/125	125/125
Tetracycline (<i>positive control</i>)	3.13/3.13	3.13/3.13	3.13/3.13	3.13/313
Methanol (<i>negative control</i>)	-	-	-	-

Note: there is no inhibitory and bactericidal activity in the negative control.

The MIC value was determined as the lowest concentration of extract that could suppress bacterial growth, observed from the test wells that appeared clear. In this study, the test wells were clear at a concentration of 250 ppm and 125 ppm for all test bacteria (Figure 2). Thus, the Minimum Inhibitory Concentration (MIC) value was 125 ppm on gram-positive (*B. subtilis* and *S. aureus*) and gram-negative (*E. coli* and *P. aeruginosa*) of bacteria. This study is in line with the research of Yosboonruang et al., (2022) using robusta coffee leaf extract, which has a minimum inhibitory concentration (MIC) against *Bacillus subtilis* (gram positive) and *Escherichia coli* (gram negative) showing the same value of 12.5 mg/ml. The robusta coffee extract from Mangarai Village West Lampung has MIC at a value of 187-375 ppm, classified as moderate activity on the same four types of test bacteria (Suryanti et al., 2023). Coffee extracts have been reported to have antimicrobial activity against gram-negative bacilli such as *E. coli* and *Salmonella enterica*, also gram-positive cocci such as *S. aureus*, *S. epidermidis*, and *E. faecalis* (Runti et al., 2015). Antibacterial activity of coffee pulp aqueous extracts was also reported

against gram-positive bacteria (*S. aureus* and *S. epidermidis*) and gram-negative bacteria (*P. aeruginosa* and *E. coli*) (Duangjai et al., 2016). Robusta coffee extract from FAMA Coffee Refinery, Banting, Selangor was reported to have antibacterial activity against *L. casei*, *P. gingivalis*, *S. mutans* with MIC/MBC of 50/50, 200/200, and 240/240 ppm respectively (Norazlin et al., 2023).

Antibacterial activity in robusta coffee is caused by the presence of compounds responsible for the antibacterial effect in robusta coffee. Several studies have reported that the antibacterial activity of coffee extracts could be due to phenolic acids, tannins, chlorogenic acid, caffeine, malic acid, and other compounds. *Caffeine* in coffee is an *alkaloid* compound that can inhibit the DNA synthesis stage in bacteria by penetrating the bacterial cell wall, thus reducing bacterial cell activity. Chlorogenic acid contained in coffee is a phenolic compound can disrupt cell membrane permeability of bacterial cell membranes, causing bacterial cell lysis (Kabir et al., 2014; Lou et al., 2011; Nonthakaew et al., 2015). Metabolite compounds that also have antimicrobial effects in coffee are polyphenols (*tannins*), which can react with cell membranes, inactivate enzymes, and destroy or inactivate the function of microbial genetic material (Muslim & Dephinto, 2019).

Conclusion

Robusta coffee extract from West Lampung positive for phytochemical compounds such as phenolic compounds, *flavonoids*, *tannins*, *saponins*, *terpenoids*, *alkaloids*, and negative for *steroids*. The quantification results of TPC 62.88 mgGAE/g extract, TFC 23.90 mgQE/g extract, TTC were 176.73 mgTAE/g extract, and TSC were 10.37%. This extract classified as a very strong antioxidant with IC₅₀ value of 35.02 ppm, and moderate antibacterial activity at a concentration of MIC/MBC 125/125 ppm against *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*.

Acknowledgements

The authors gratefully acknowledge the financial support provided by Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat (DRTPM), Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi, Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi Republik Indonesia. This research was funded under the Penelitian Dosen Pemula Regular scheme, contract number 039/E5/PG.02.00.PL/2024 dated June 11, 2024.

References

- Assa, A., Indriana, D., Amalia, A. N., & Wulandari, R. (2021). Potensi Senyawa Aktif Biji Kopi Sebagai Imunomodulator (Ulasan). *Jurnal Riset Industri*, 279–290. <https://doi.org/10.26578/Jrti.V15i2.6602>
- Bps Lampung, B. P. S. P. (2019). *Tinggi Wilayah Dan Jarak Ke Ibukota Provinsi Menurut Kabupaten/Kota—Tabel Statistik*.

- <https://Lampung.Bps.Go.Id/Id/Statistics-Table/2/Njqwizi=/Tinggi-Wilayah-Dan-Jarak-Ke-Ibukota-Provinsi-Menurut-Kabupaten-Kota.Html>
- Bps, S. I. (2023, November 30). *Statistik Kopi Indonesia 2022*. <https://Www.Bps.Go.Id/Id/Publication/2023/11/30/Abde293e6c0fc5d45aa9fe8/Statistik-Kopi-Indonesia-2022.Html>
- De Hoyos-Martínez, P. L., Merle, J., Labidi, J., & Charrier – El Bouhtoury, F. (2019). Tannins Extraction: A Key Point For Their Valorization And Cleaner Production. *Journal Of Cleaner Production*, 206, 1138–1155. <https://Doi.Org/10.1016/J.Jclepro.2018.09.243>
- Dewi, I. S., Saptawati, T., & Rachma, F. A. (2021). Skrining Fitokimia Ekstrak Etanol Kulit Dan Biji Terong Belanda (*Solanum Betaceum Cav.*). *Prosiding Seminar Nasional Unimus*, 4(0), Article 0. <https://Prosiding.Unimus.Ac.Id/Index.Php/Semnas/Article/View/894>
- Duangjai, A., Suphrom, N., Wungrath, J., Ontawong, A., Nuengchamnong, N., & Yosboonruang, A. (2016). Comparison Of Antioxidant, Antimicrobial Activities And Chemical Profiles Of Three Coffee (*Coffea Arabica L.*) Pulp Aqueous Extracts. *Integrative Medicine Research*, 5(4), 324–331. <https://Doi.Org/10.1016/J.Imr.2016.09.001>
- Ebulue, M. M. (2023). Bioactive Compounds And Antioxidant Activity Of Ethanol Leaf Extract Of *Eucalyptus Tereticornis*. *Asean Journal Of Science And Engineering*, 3(1), Article 1. <https://Doi.Org/10.17509/Ajse.V3i1.45017>
- Gebrekidan, M., Redi-Abshiro, M., Chandravanshi, B., Ele, E., Mohammed, A., & Mamo, M. (2019). Influence Of Altitudes Of Coffee Plants On The Alkaloids Contents Of Green Coffee Beans. *Chemistry International*, 5(4), 247–257. <https://Doi.Org/10.2139/Ssrn.3407495>
- Handayani, T. W., Yusuf, Y., & Tandi, J. (2020). Analisis Kualitatif Dan Kuantitatif Metabolit Sekunder Ekstrak Biji Kelor (*Moringa Oleifera Lam.*) Dengan Metode Spektrofotometri Uv-Vis: Qualitative And Quantitative Analysis Of Secondary Metabolite Of Moringa Seed Extract (*Moringa Oleifera Lam.*) With Uv-Vis Spectrophotometry Method. *Kovalen: Jurnal Riset Kimia*, 6(3), 230–238. <https://Doi.Org/10.22487/Kovalen.2020.V6.I3.15324>
- Hasan, A. E. Z., Utami, N. F., & Mona. (2022). Extraction Of Robusta Coffee Beans (*Coffea Canephora*) From Wonosobo By Ultrasonic Waves And Anticancer Tests. *Fitofarmaka: Jurnal Ilmiah Farmasi*, 12(1), Article 1. <https://Doi.Org/10.33751/Jf.V12i1.4933>
- Hassanpour, S. H., & Doroudi, A. (2023). Review Of The Antioxidant Potential Of Flavonoids As A Subgroup Of Polyphenols And Partial Substitute For Synthetic Antioxidants. *Avicenna Journal Of Phytomedicine*, 13(4), 354–376. <https://Doi.Org/10.22038/Ajp.2023.21774>

- Islami, F. S., Prasetyanto, P. K., & Setiawan, A. D. (2022). Analysis Of Determinants Of Coffee Exports In Indonesia. *Inovasi: Jurnal Ekonomi, Keuangan Dan Manajemen*, 18(Special Issue), 61–67.
- Kabir, F., Katayama, S., Tanji, N., & Nakamura, S. (2014). Antimicrobial Effects Of Chlorogenic Acid And Related Compounds. *Journal Of The Korean Society For Applied Biological Chemistry*, 57(3), 359–365. <https://doi.org/10.1007/S13765-014-4056-6>
- Khairani, I. A., Anisa, H. A. N., Mulyana, J. S., & Riana, E. N. (2024). The Comparison Of Bioactive Compounds Between Brewed And Extracted Robusta Coffee From West Lampung. *Bio Web Of Conferences*, 101, 02005. <https://doi.org/10.1051/Bioconf/202410102005>
- Khairani, I. A., Mulyana, J. S., Olivia, R. N., Riana, E. N., & Anisa, H. A. N. (2024). Quantitative Analysis Of Phytochemical Compounds And Antihyperglycemic Potential Of Robusta Coffee From West Lampung. *Jurnal Sumberdaya Hayati*, 10(1), Article 1. <https://doi.org/10.29244/Jsdh.10.1.1-6>
- Kim, I.-S., Yang, M., Lee, O.-H., & Kang, S.-N. (2011). The Antioxidant Activity And The Bioactive Compound Content Of *Stevia Rebaudiana* Water Extracts. *Lwt - Food Science And Technology*, 44(5), 1328–1332. <https://doi.org/10.1016/J.Lwt.2010.12.003>
- Kuete, V. (2010). Potential Of Cameroonian Plants And Derived Products Against Microbial Infections: A Review. *Planta Medica*, 76(14), 1479–1491. <https://doi.org/10.1055/S-0030-1250027>
- Lou, Z., Wang, H., Zhu, S., Ma, C., & Wang, Z. (2011). Antibacterial Activity And Mechanism Of Action Of Chlorogenic Acid. *Journal Of Food Science*, 76(6), M398-403. <https://doi.org/10.1111/J.1750-3841.2011.02213.X>
- Mahajan, R., & Kapoor, N. (2018). Phytochemical Analysis And Antimicrobial Activity Of Roasted Beans Of *Coffea Robusta*. *International Journal Of Pharmacy And Biological Sciences*, 8(1), 89–95.
- Martins, P. M. M., Batista, N. N., Miguel, M. G. Da C. P., Simão, J. B. P., Soares, J. R., & Schwan, R. F. (2020). Coffee Growing Altitude Influences The Microbiota, Chemical Compounds And The Quality Of Fermented Coffees. *Food Research International*, 129, 108872. <https://doi.org/10.1016/J.Foodres.2019.108872>
- Maxiselly, Y., Maulana, H., Chumthong, A., & Chiarawipa, R. (2023). Relationship Analysis Based On Phytochemical Contents Among Coffee Pulp From Three Coffee Species Collected In Southern Thailand And Jambi, Indonesia. *Biodiversitas Journal Of Biological Diversity*, 24(10), 5439–5445. <https://doi.org/10.13057/Biodiv/D241026>
- Medini, F., Fellah, H., Ksouri, R., & Abdelly, C. (2014). Total Phenolic, Flavonoid And Tannin Contents And Antioxidant And Antimicrobial Activities Of Organic Extracts Of Shoots Of The Plant *Limonium Delicatulum*. *Journal Of*

- Taibah University For Science*, 8(3), 216–224.
<https://doi.org/10.1016/J.Jtusc.2014.01.003>
- Mintesnot, A., & Dechassa, N. (2018). Effect Of Altitude, Shade, And Processing Methods On The Quality And Biochemical Composition Of Green Coffee Beans In Ethiopia. *East African Journal Of Sciences*, 12(2), Article 2.
- Molyneux, P. (2004). The Use Of The Stable Free Radical Diphenylpicrylhydrazyl (Dpph) For Estimating Antioxidant. *Songklanakarinn Journal Of Science And Technology (Sjst)*, 26(2), 211–219.
- Muslim, Z., & Dephinto, Y. (2019). Antibacterial Activity Of Robusta Coffee (*Coffea Canephora* L.) Leaves To *Staphylococcus Aureus* And *Escherichia Coli*. *Asian Journal Of Pharmaceutical And Clinical Research*, 113–115.
<https://doi.org/10.22159/Ajpcr.2019.V12i12.35589>
- Ngibad, K., Yusmiati, S. N. H., Merlina, D. M., Rini, Y. P., Valenata, V., & Jannah, E. F. (2023). Comparison Of Total Flavonoid, Phenolic Levels, And Antioxidant Activity Between Robusta And Arabica Coffee. *Kovalen: Jurnal Riset Kimia*, 9(3), 241–249.
<https://doi.org/10.22487/Kovalen.2023.V9.I3.16506>
- Nonthakaew, A., Matan, N., & Aewsiri, T. (2015). Caffeine In Foods And Its Antimicrobial Activity. *International Food Research Journal*, 22, 9–14.
- Norazlin, A., Muhammad-Adib, Wan-Razarinah, W. A. R., Roohinejad, S., Koubaa, M., & Raseetha, S. (2023). Antioxidant And Antimicrobial Activity Of Green And Roasted Coffee Beans On Human Oral Pathogens. *Food Research*, 7(Supplementary 4), 130–138.
[https://doi.org/10.26656/Fr.2017.7\(S4\).17](https://doi.org/10.26656/Fr.2017.7(S4).17)
- Nurhayati, N., Septiarini, A. D., & Aisyah, P. (2023). Uji Ekstrak Biji Kopi Hijau (*Coffea Canephora* Var. Robusta) Terhadap Bakteri *Propionibacterium Acnes* Dan *Staphylococcus Aureus* Secara Difusi. *Publikasi Penelitian Terapan Dan Kebijakan*, 6(1), Article 1.
<https://doi.org/10.46774/Pptk.V6i1.529>
- Octaviani, M., Fadhli, H., & Yuneistya, E. (2019). Antimicrobial Activity Of Ethanol Extract Of Shallot (*Allium Cepa* L.) Peels Using The Disc Diffusion Method. *Pharmaceutical Sciences And Research*, 6(1), 62–68.
<https://doi.org/10.7454/Psr.V6i1.4333>
- Pebriarti, I. W., Susanti, D. A., Purwanti, A., Diana, A. N., Lestari, R. E., & Rindiantika, B. K. (2022). Uji Aktivitas Antioksidan Ekstrak Etanol Biji Kopi Hijau Robusta (*Coffea Canephora*) Pada Berbagai Ketinggian Tempat Tumbuh. *Applicable Innovation Of Engineering And Science Research (Avoer)*, 14(1), Article 1.
- Prastya, M. E., Astuti, R. I., Batubara, I., & Wahyudi, A. T. (2019). Antioxidant, Antiglycation And In Vivo Antiaging Effects Of Metabolite Extracts From Marine Sponge-Associated Bacteria. *Indian Journal Of Pharmaceutical Sciences*, 81(2). <https://doi.org/10.36468/Pharmaceutical-Sciences.516>

- Prayitno, S. A., & Rahim, A. R. (2020). The Comparison Of Extracts (Ethanol And Aquos Solvents) Muntingia Calabura Leaves On Total Phenol, Flavonid And Antioxidant (Ic50) Properties. *Kontribusia (Research Dissemination For Community Development)*, 3(2), 319. <https://doi.org/10.30587/Kontribusia.V3i2.1451>
- Priyanto, J. A., Prastya, M. E., Astuti, R. I., & Kristiana, R. (2023). The Antibacterial And Antibiofilm Activities Of The Endophytic Bacteria Associated With Archidendron Pauciflorum Against Multidrug-Resistant Strains. *Applied Biochemistry And Biotechnology*, 195(11), 6653–6674. <https://doi.org/10.1007/S12010-023-04382-4>
- Rubinadzari, N., Saula, L. S., & Utami, M. R. (2022). Perbandingan Aktivitas Antibakteri Ekstrak Etanol Biji Hijau Dan Sangrai Kopi Robusta (*Coffea Canephora* L.) Serta Kombinasinya Terhadap Bakteri *Staphylococcus Aureus*. *Lambung Farmasi: Jurnal Ilmu Kefarmasian*, 3(2), 221–230. <https://doi.org/10.31764/Lf.V3i2.9393>
- Runti, G., Pacor, S., Colomban, S., Gennaro, R., Navarini, L., & Scocchi, M. (2015). Arabica Coffee Extract Shows Antibacterial Activity Against *Staphylococcus Epidermidis* And *Enterococcus Faecalis* And Low Toxicity Towards A Human Cell Line. *Lwt - Food Science And Technology*, 62(1, Part 1), 108–114. <https://doi.org/10.1016/J.Lwt.2014.12.039>
- Safitri, I., Warsidah, W., Sofiana, M. S. J., Kushadiwijayanto, A. A., & Sumarni, T. N. (2021). Total Phenolic Content, Antioxidant And Antibacterial Activities Of Sargassum Polycystum Of Ethanol Extract From Waters Of Kabung Island. *Berkala Sainstek*, 9(3), 139–145. <https://doi.org/10.19184/Bst.V9i3.27199>
- Styawan, A. A., & Rohmanti, G. (2020). Determination Of Flavonoid Levels Of Alcl3 Methode In The Extract Of Metanol Flowers (*Clitoria Ternatea* L.). *Jurnal Farmasi Sains Dan Praktis*, 6(2), 134–141. <https://doi.org/10.31603/Pharmacy.V6i2.3912>
- Sulistyarini, I., Sari, D. A., & Wicaksono, T. A. (2020). Skrining Fitokimia Senyawa Metabolit Sekunder Batang Buah Naga (*Hylocereus Polyrrhizus*). *Cendekia Eksakta*, 5(1), Article 1. <https://doi.org/10.3194/Ce.V5i1.3322>
- Suryanti, E., Dwi Retnowati, Muhammad Eka Prastya, Novita Ariani, Indri Yati, Vera Permatasari, Tjandrawati Mozef, Indah D. Dewijanti, Apriza Yuswan, Muhammad Asril, Elisa Nurma Riana, & Irmanida Batubara. (2023). Chemical Composition, Antioxidant, Antibacterial, Antibiofilm, And Cytotoxic Activities Of Robusta Coffee Extract (*Coffea Canephora*). *Hayati Journal Of Biosciences*, 30(4), 632–642. <https://doi.org/10.4308/Hjb.30.4.632-642>
- Tarakanita, D. N. S., Satriadi, T., & Jauhari, A. (2019). Potensi Keberadaan Fitokimia Kamalaka (*Phyllanthus Emblica*) Berdasarkan Perbedaan Ketinggian Tempat Tumbuh. *Jurnal Sylvia Scienteeae*, 2(4), 645–654.

- Towaha, J., Aunillah, A., Purwanto, E. H., & Supriadi, H. (2014). Pengaruh Elevasi Dan Pengolahan Terhadap Kandungan Kimia Dan Citarasa Kopi Robusta Lampung. *Jurnal Tanaman Industri Dan Penyegar*, 1(1), 57. <https://doi.org/10.21082/jtidp.v1n1.2014.p57-62>
- Utami, N. F., Nhestricia, N., Maryanti, S., Tisyah, T., & Maysaroh, S. (2018). Uji Aktivitas Antioksidan Dari Biji Kopi Robusta (*Coffea Canephora* P.) Berdasarkan Perbedaan Ekologi Dataran Tinggi Di Pulau Jawa. *Fitofarmaka Jurnal Ilmiah Farmasi*, 8(1), 67–72. <https://doi.org/10.33751/jf.v8i1.1173>
- Utami, Y. P., & Barrang, J. A. (2019). Studi Aktivitas Antioksidan Ekstrak Etanol Biji Kopi Robusta (*Coffea Canephora* Var. Robusta) Dan Biji Kopi Arabika (*Coffea Arabika* L.) Dengan Metode Dpph. *Jurnal Farmasi Dan Bahan Alam : Farbal*, 7(1), Article 1.
- Virhananda, M. R. P., Suroso, E., Nurainy, F., Suharyono, S., Subeki, S., & Satyajaya, W. (2022). Analisis Kadar Asam Klorogenat Dan Kafein Berdasarkan Perbedaan Lokasi Penanaman Dan Suhu Roasting Pada Kopi Robusta (*C. Canephora* Pierre). *Jurnal Agroindustri Berkelanjutan*, 1(2), Article 2. <https://doi.org/10.23960/jab.v1i2.6361>
- Wahyudi, A., & Wulandari, S. (2022). Formulasi Sediaan Serum Ekstrak Etanol Kopi Robusta (*Coffea Canephora*) Peaberry Green Bean Pagar Alam. *Jurnal Kesehatan: Jurnal Ilmiah Multi Sciences*, 12(01), Article 01. <https://doi.org/10.52395/jkjims.v12i01.337>
- Wigati, E. I., Pratiwi, E., Nissa, T. F., & Utami, N. F. (2019). Uji Karakteristik Fitokimia Dan Aktivitas Antioksidan Biji Kopi Robusta (*Coffea Canephora* Pierre) Dari Bogor, Bandung Dan Garut Dengan Metode Dpph (1,1-Diphenyl-2-Picrylhydrazyl). *Fitofarmaka: Jurnal Ilmiah Farmasi*, 8(1), 53–59. <https://doi.org/10.33751/jf.v8i1.1172>
- Yosboonruang, A., Ontawong, A., Thapmamang, J., & Duangjai, A. (2022). Antibacterial Activity Of *Coffea Robusta* Leaf Extract Against Foodborne Pathogens. *Journal Of Microbiology And Biotechnology*, 32(8), 1003–1010. <https://doi.org/10.4014/jmb.2204.04003>
- Yuarini, D. A. A., & Wrasati, L. P. (2015). Antioxidant Activity And Bioactive Compound In A Functional Instant Drink Made From Mangosteen Peel And Rosella Extract. *Media Ilmiah Teknologi Pangan (Scientific Journal Of Food Technology)*, 2(1), 069–077.