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Research Article

Determination of Total Phenolic and Caffeine Content and Their Correlation with Antioxidant Activity in Local Coffee Brews from South Kalimantan

Penetapan Kadar Fenolik Total dan Kafein Serta Korelasinya Dengan Aktivitas Antioksidan Pada Minuman Kopi Lokal Kalimantan Selatan

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ABSTRACT

Coffee brews are widely consumed beverages known to contain bioactive compounds such as phenolic compounds and caffeine that provide health benefits, including antioxidant activity. The objective of this research is to evaluate the antioxidant activity, total phenolic content, and caffeine content of coffee brews made from local coffee beans of South Kalimantan, demonstrating their potential as functional beverages with health promoting properties. The local coffee beans used in this study originated from Pengaron, Aranio, Mataraman and Bati-Bati. The research included sample identification, beverage formulation, antioxidant activity assay using the DPPH method, and analysis of total phenolic and caffeine content using UV-Vis spectrophotometry. The results showed that the antioxidant activity, as indicated by IC_{50} values, was lowest in coffee from Pengaron (202.962 ± 0.89 ppm), followed by Aranio (166.716 ± 0.358 ppm), Mataraman (150.043 ± 0.345 ppm), and the strongest in Bati-Bati (113.242 ± 0.122 ppm). The total phenolic content ranged from 58.327 ± 0.017 mg GAE/mL in Pengaron, 83.993 ± 0.017 in Aranio, 104.536 ± 0.032 in Mataraman, to 108.646 ± 0.042 in Bati-Bati. Caffeine levels were 123.876 ± 0.331, 139.340 ± 0.238, 175.495 ± 0.715, and 131.833 ± 0.620 mg/serving, respectively. The findings reveal a strong positive relationship between total

phenolic content and antioxidant activity, while caffeine content did not consistently align with antioxidant performance. These findings support the significant role of phenolic compounds in determining the antioxidant potential of South Kalimantan's local coffee beverages. The highest antioxidant activity and phenolic content were observed in coffee beverages made from *Coffea liberica* beans originating from Bati-Bati, suggesting its superior potential as a functional beverage with health benefits.

Keywords: South Kalimantan, Coffee Beverage, DPPH, Robusta Coffee, Liberica Coffee

ABSTRAK

Minuman kopi merupakan minuman populer mengandung senyawa bioaktif seperti senyawa fenolik dan kafein sehingga memiliki manfaat kesehatan termasuk aktivitas antioksidan. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan, kandungan fenolik total dan kadar kafein minuman kopi yang dibuat dari biji kopi lokal Kalimantan Selatan, serta mengevaluasi potensinya sebagai minuman fungsional yang mendukung kesehatan. Biji kopi yang digunakan berasal dari Pengaron, Aranio, Mataraman, dan Bati-Bati. Tahap yang dilakukan yaitu determinasi tanaman, pembuatan minuman kopi, uji aktivitas antioksidan menggunakan metode DPPH, serta penetapan kadar fenolik total dan kafein menggunakan spektrofotometer UV-Vis. Hasil penelitian menunjukkan bahwa aktivitas antioksidan, yang dinyatakan dengan nilai IC_{50}, paling lemah pada kopi dari Pengaron (202,962 ± 0,89 ppm), diikuti oleh Aranio (166,716 ± 0,358 ppm), Mataraman (150,043 ± 0,345 ppm), dan terkuat pada Bati-Bati (113,242 ± 0,122 ppm). Kadar fenolik total berkisar dari 58,327 ± 0,017 mg GAE/mL untuk Pengaron, 83,993 ± 0,017 di Aranio, 104,536 ± 0,032 di Mataraman, hingga 108,646 ± 0,042 di Bati-Bati. Kadar kafein masing-masing adalah 123,876 ± 0,331; 139,340 ± 0,238; 175,495 ± 0,715; dan 131,833 ± 0,620 mg per sajian. Hasil penelitian menunjukkan adanya hubungan positif yang kuat antara kadar fenolik total dengan aktivitas antioksidan, sementara kadar kafein tidak selalu sejalan dengan kinerja antioksidan. Hasil ini mendukung senyawa fenolik berperan penting dalam menentukan potensi aktivitas antioksidan pada minuman kopi lokal Kalimantan Selatan. Aktivitas antioksidan dan kadar fenolik tertinggi ditemukan pada minuman kopi yang berasal dari biji Coffea liberica asal Bati-Bati, menunjukkan potensinya yang unggul sebagai minuman fungsional dengan manfaat kesehatan.

Kata Kunci: Kalimantan Selatan, Minuman Kopi, DPPH, Kopi Robusta, Kopi Liberika

I. INTRODUCTION

Coffee represents one of the world's most widely consumed beverages, valued not only for its distinctive aroma and flavor but increasingly for its potential health benefits (Samoggia & Riedel, 2019). According to the International Coffee Organization (ICO), Indonesia maintains its position as the fourth-largest coffee producer globally, following Brazil, Vietnam, and Colombia (Irwinsyah *et al*., 2021). South Kalimantan is also one of the coffee-producing regions in Indonesia with a total coffee plantation area of 3,168 ha

and productivity of 623 kg/hectare (Rahman et al., 2020). Coffee from South Kalimantan has great potential to be developed as a superior product, given its distinctive quality and acceptance in various markets. South Kalimantan possesses four distinctive coffee varieties with potential as regional flagship products, namely Pengaron, Aranio, Mataraman, and Bati-Bati coffee; however, scientific information regarding their quality profiles and characteristics remains limited, necessitating further research to support their development (Anonymous, 2019a; Anonymous, 2019b; Anonymous, 2021). Understanding the bioactive composition and health properties of these local coffee varieties is essential for establishing their commercial value and promoting their development as regional specialty products.

Some bioactive compounds found in coffee, such as phenolics have benefits as antioxidants that can protect a person's health (Sukma *et al*., 2023). In addition, caffeine, as a stimulant compound, has a positive effect in increasing mental and physical alertness, reducing fatigue, and increasing concentration (Febrianasari *et al*., 2016). Chlorogenic acid, a polyphenol compound in coffee reach 90% of the total phenols contained in coffee, has strong antioxidant activity (Perdani *et al*., 2020; Wanto *et al*., 2024).

Polyphenolic compounds in coffee are calculated as gallic acid. Gallic acid is one of the widely used polyphenolic compounds and is one of the secondary metabolites in plants and has antifungal, antiviral, and antioxidant activities. Polyphenols are compounds with more than one phenolic hydroxyl group on the benzene ring. The identification and measurement of these phenolic compounds commonly employ the Folin-Ciocalteu reagent, which produces a characteristic blue molybdenum-tungsten complex under alkaline conditions through the oxidation of phenolic groups (Widyantari & Sari, 2023).

Caffeine is one of the major alkaloid compounds in coffee that functions as both a stimulant and antioxidant. According to Indonesian National Standard (SNI), the recommended caffeine intake limit for beverages is 150 mg per day or 50 mg per serving (SNI, 2006). Excessive caffeine consumption may cause adverse effects such as nervousness, restlessness, tremors, insomnia, and anxiety (Zarwinda & Santika, 2019). While various studies have examined the bioactive properties of coffee beans, limited research has focused on the actual coffee beverages consumed, particularly those prepared with traditional sweeteners such as palm sugar. Previous research demonstrated that coffee beverages formulated with palm sugar at specific ratios achieved optimal sensory

acceptance, with the highest overall preference scores among consumers (Fatima et al., 2022). Building on this sensory-optimized formulation, this study aims to determine the total phenolic and caffeine content in local coffee beverages from Pengaron, Aranio, Mataraman, and Bati-Bati districts of South Kalimantan Province, evaluate their antioxidant activity, and investigate the correlation between these bioactive compounds and antioxidant capacity.

II. METHOD

A. Sample Collection and Botanical Identification

Coffee samples were collected from four coffee-producing districts in South Kalimantan Province, Indonesia: Pengaron District (Kertak Empat Village, Banjar Regency), Aranio District (Tiwingan Village, Riam Kanan reservoir area, Banjar Regency), Mataraman District (Takuti Village, Banjar Regency), and Bati-Bati District (Nusa Indah Village, Tanah Laut Regency). Fresh plant specimens, were collected from each location for botanical identification. Botanical determination was conducted at the Basic Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan.

B. Coffee Beverage Preparation

Coffee bean samples from Pengaron, Aranio, Mataraman, and Bati-Bati districts were weighed and roasted to medium roast at 230°C for 15 minutes using a roasting machine (Matondang et al., 2024) and the roasted beans were ground into powder. Coffee beverages were prepared according to the procedure of Fatima et al. (2022) with minor modifications. Ground coffee powder and mashed palm sugar were thoroughly mixed, then brewed with hot water to prepare the test samples. The composition of each ingredient can be seen in Table I.

Table I. Coffee brews formulation (Fatima *et al*., 2022)

Ingredient	Concentration	Function
Coffee	15 g	Main ingredient
Palm sugar	30 g	Sweetener
Aqua dest	Ad 175 mL	Solvent

C. Antioxidant Activity Test

DPPH 0,4 mM solution was made to determine the maximum wavelength and operating time. A 1 mL aliquot of 0.4 mM DPPH solution was mixed with 4 mL of methanol p.a and kept in the dark for 30 minutes. The maximum wavelength of the DPPH solution was then determined using a UV-Vis spectrophotometer within the wavelength range of 400–800 nm. Ascorbic acid as reference standard solution was made a series of levels with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10

ppm. Each concentration of 4 mL was mixed with 1 mL of 0,4 mM DPPH solution and then measured using a UV-Vis spectrophotometer (Wahid *et al*., 2022; Watuseke *et al*., 2021).

The test of antioxidant activity of coffee beverages was made using each coffee brew sample made a series of levels with concentrations of 20 ppm, 40 ppm, 80 ppm, 100 ppm, 120 ppm, and 160 ppm (Lapčíková *et al*., 2023). A 4 mL portion of each sample was combined with 1 mL of 0.4 mM DPPH solution and kept in the dark for the predetermined operating time. The absorbance was then measured using a UV-Vis spectrophotometer, with methanol p.a serving as the blank (Endmarhuka *et al*., 2024).

D. Total Phenolic Content Determination

The first step begins with measuring the maximum wavelength by means of 0,5 mL of 40 ppm gallic acid solution added 2,5 mL of Folin-Ciocalteu reagent. Let the solution stand for 8 minutes and then added 2 mL of 7,5% Na_2CO_3 solution and then measured the absorbance with a wavelength range of 600-850 nm. After obtaining the maximum wavelength, the operating time was determined by taking 0,5 mL of 40 ppm gallic acid solution and then adding 2,5 mL of Folin-Ciocalteu reagent. Let stand for 8 minutes and then added 2 mL of 7,5% Na_2CO_3 solution. The solution was then measured for absorbance 0-90 minutes with an interval of 2 minutes (Normaidah *et al*., 2024).

Preparation of gallic acid standard curve was done by making a series solution of gallic acid levels with concentration variations of 10, 20, 30, 40, 50, and 60 ppm. Each concentration as much as 0,5 mL then added 2,5 mL of Folin-Ciocalteu and allowed to stand for 10 minutes, then added 2 mL of 7,5% Na_2CO_3 solution. The solution was then incubated within the operating time. Measure the absorbance of the gallic acid content series solution using a UV-Vis spectrophotometer (Abduh *et al*., 2023; Rehman *et al*., 2022). UV-Vis spectrophotometer which used to measure the ratio, a ratio function, of the intensity of two light beams in the UV-Vis region. The UV-Vis spectrophotometry method was employed to quantify total phenolic content by measuring the light absorption in the UV-Vis region. This method is suitable for phenolic compounds, including flavonoids and phenolic acids, as they possess chromophoric groups such as carbonyl systems conjugated with aromatic rings that enable light absorption and colorimetric detection (Wathan *et al*., 2023). The Folin-Ciocalteu assay specifically measures the total reducing capacity of all phenolic compounds present in the sample.

Determination of total phenolic content in coffee beverages is done by putting each coffee brew as much as 0,5 mL into a 25 mL volumetric flask. The next beverages was added 0,5 mL of Folin-Ciocalteu reagent and 2,5 mL of 7,5% Na_2CO_3 after 5 minutes. Aquadest was added until it reached the limit mark. The reaction mixture was incubated in a dark place during the operating time and the absorbance value was measured using a UV-Vis spectrophotometer (Indiarto *et al*., 2024).

E. Caffeine Content Determination

Caffeine comparative solution was made with a concentration of 86 ppm comparative standard solution. The solution was then measured using UV spectrophotometry at a wavelength of 230-350 nm. The 100 ppm caffeine standard solution was made into a series of levels of 4, 6, 8, 10, and 12 ppm. Each solution was measured with the maximum wavelength. Determination of caffeine content of coffee brews was done by extracting caffeine from coffee beverages first. 25 mL of each coffee beverage was added 1 gram of $CaCO_3$ and then put into a separating funnel. The coffee solution was extracted with 25 mL of chloroform in a separate funnel three times in a row (Elfariyanti *et al*., 2020; Perbina *et al*., 2020).

The chloroform phase was taken and then evaporated using a waterbath until the chloroform evaporated completely. The chloroform-free caffeine extract was put into a 100 mL volumetric flask, diluted with 0,1 N HCl to the limit mark and homogenized. The caffeine extract was then diluted in a 50 mL volumetric flask and dissolved with 0,1 N HCl to the limit (25 times dilution). The caffeine content of coffee brews was determined with a UV-Vis spectrophotometer (Elfariyanti *et al*., 2020; Perbina *et al*., 2020).

F. Data Analysis

The data results will be analyzed quantitatively. The antioxidant activity is expressed as percent inhibition then the IC_{50} calculation can be calculated from linear regression data using the following Equation 1 and total phenolic content was calculated using the linear regression Equation 2. The results of total phenolic content or TPC use units of mg gallic acid equivalent / mL sample (GAE/mL). Total phenolic content or TPC is calculated using Equation 3 (Karsidin *et al*., 2022) and calculation of caffeine content in coffee beverages samples is calculated based on the following Equation 4 (Elfariyanti *et al*., 2020).

$$IC_{50} = \frac{50-b}{a} \quad \textbf{(1)}$$

Description:

IC_{50} = Concentration of sample inhibiting 50% of free radicals
b = Slope of the curve
a = Point of intersection of the curve on the y-axis (Intercept)

$$y = bx + a \text{...(2)}$$

Description:
y = Absorbance of the sample
b = Slope of the curve
a = Point of intersection of the curve on the y-axis (intercept)

$$TPC = \frac{x.V.\mathrm{DF}}{g} \text{...(3)}$$

Description:
TPC = Total phenolic content (mg GAE/mL)
x = Phenolic concentration (ppm)
V = Volume of sample used (mL)
DF = Dilution factor
g = Weight of the sample used

$$\frac{\text{Concentration x Volume x DF}}{\text{Sample Weight}} \text{ x } 100\% \text{ ... (4)}$$

Description:
Concentration (mg/L) = Linear regression result (y = bx+a)
DF = Dilution factor

III. RESULTS AND DISCUSSION

A. Sample Collection and Botanical Identification

Plant determination is a process to determine the name of a specific plant species (Nurhayati *et al*., 2022). Coffee samples were obtained from four coffee-producing districts in South Kalimantan. Botanical determination (certificates No. 176-178, 195/LB.LABDASAR/VIII/2024) revealed that samples from Pengaron (Kertak Empat Village), Aranio (Tiwingan Village), and Mataraman (Takuti Village) were *Coffea canephora* (robusta), while the Bati-Bati sample (Nusa Indah Village, Tanah Laut Regency) was *Coffea liberica*.

The maximum wavelength of DPPH or the wavelength that shows the highest absorption in this study is 515.30 nm. The results found are in accordance with the research of Sitompul *et al*., (2024), which states that the reaction of the capture of H atoms by DPPH from antioxidant substances causes a color change that can be measured by a UV-Vis spectrophotometer at a wavelength of 515-520 nm in organic solvents (methanol). The results obtained also have maximum wavelength results that are almost similar to the research of Handayani *et al*., (2020), namely at a wavelength of 515 nm. This difference is still acceptable because based on theory, theoretical wavelengths with observations can shift between 0-4 nm (Muliasari *et al*., 2023).

Operating time (OT) is used to determine the most appropriate time for the test solution to reduce DPPH free radicals. This operating time indicates that the reaction between the test solution and DPPH has been perfect. In this study, the absorbance began to stabilize at the 30th to 34th minute, and after the 34th minute there was instability which indicated that the compound had decomposed. Determination of operating time is based on the time at which the absorbance value of the test solution against DPPH begins to stabilize or

the difference in absorbance begins to be small between the time interval tested (Kusmiyati *et al*., 2023).

The IC_{50} value of the comparison solution was determined by measuring the absorbance of a series of levels with concentrations of 2, 4, 6, 8, and 10 ppm at a wavelength of 515,30 nm. The absorbance results were calculated as % inhibition and then made a graph with concentration as the x-axis and % inhibition as the y-axis. The standard curve obtained the equation y = 5,0374x + 15,407 with r equal to 0,9887. The absorbance measurement results of the vitamin C standard solution obtained an IC_{50} value of 6,867 ± 0,237 ppm so that it is included in the category of very strong antioxidant activity. The greater the concentration of vitamin C standard solution, the greater the absorbance produced as well as the percent inhibition value in the same direction. This shows that the relationship between concentration and absorbance and the percent inhibition produced is linear. The linearity level is proven by the r value of the linear line equation of 0,9987. A good r value is when it is close to the value of 1 (Safarina *et al*., 2024; Kusmiyati *et al*., 2023).

After obtaining the results of the percent inhibition and IC_{50} value of the comparison compound, namely vitamin C, the same test was then carried out on the sample compound, namely coffee brews with coffee beans from Pengaron, Aranio, Mataraman, and Bati-Bati Districts using the same method. The results of the IC_{50} value of each coffee beverages are shown in Table II.

Coffee brews from Bati-Bati exhibited the strongest antioxidant activity (moderate category), while the other three samples showed weak activities. The moderate to weak activities reflect the water-based brewing method, which preferentially extracts polar phenolic compounds while limiting the extraction of non-polar alkaloids such as caffeine and trigonelline (Niah & Febrianti, 2019).

The quantitative measurement of antioxidant activity using the DPPH method is based on changes in the intensity of the purple color of the DPPH solution, which corresponds to its concentration. DPPH free radicals, characterized by unpaired electrons, exhibit a purple color that turns yellow once the electrons become paired. This color change occurs as a result of free radical neutralization, where DPPH molecules react with hydrogen atoms donated by compounds in the sample, forming *1,1-Diphenyl-2-Picrylhydrazyl* and leading to the fading of the purple color to yellow (Suena & Antari, 2020).

Table II. IC_{50}, total phenolic, and caffeine content of the coffee beverages

Coffee Brews Area	Total Phenolic Content (mg GAE/mL) ± SD	Coffee Origin Caffeine Content (mg) ± SD	IC_{50} ± SD	Antioxidant Category
Pengaron	58.327 ± 0.017	123.876 ± 0.331	202.962 ± 0.898	Weak
Aranio	83.993 ± 0.017	139.340 ± 0.238	166.716 ± 0.358	Weak
Mataraman	104.536 ± 0.032	175.495 ± 0.715	150.043 ± 0.345	Weak
Bati-Bati	108.646 ± 0.042	131.833 ± 0.620	113.242 ± 0.122	Medium

In determining the total phenolic content of coffee beverages, gallic acid will react with Folin-Ciocalteau reagent so that it will produce a yellow color indicating that the sample contains phenolics. The addition of Na_2CO_3 solution serves to create an alkaline environment. In this condition, the hydroxyl groups of phenolic compounds react with the Folin–Ciocalteu reagent to form a blue molybdenum–tungsten complex. The intensity of the resulting blue color increases with the concentration of phenolic compounds present. A higher concentration of phenolic ions leads to greater reduction of heteropoly acid (phosphomolybdate-phosphotungstat) into molybdenum–tungsten complexes, thereby producing a more intense blue coloration (Tahir & Syafrianti, 2017).

To determine the total phenolic content, first running the wavelength of gallic acid standard solution from the range 400-800 nm using UV-Vis spectrophotometry. The maximum wavelength obtained was 760 nm. Furthermore, the operating time of gallic acid was determined based on the wavelength of 760 nm with an interval of 2 minutes in the range of 0-90 minutes (Salim *et al*., 2021). Operating time aims to determine the time for the compound to react with other compounds to form a stable compound. This time determination needs to be done to minimize the occurrence of measurement errors (Niah & Febrianti, 2019). The results of determining the operating time of gallic acid are in the 80th minute to the 90th minute. Gallic acid solution is then made a standard curve to determine the total phenol content present in coffee beverage samples as in Figure 1.

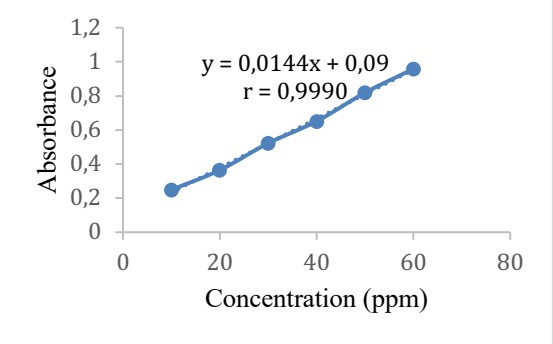

Figure 1. Gallic acid standard curve graph

As shown in Table II, Bati-Bati coffee brews exhibited the highest total phenolic content (108.646 ± 0.042 mg GAE/mL), followed by Mataraman (104.536 ± 0.032), Aranio (83.993 ±

0.017), and Pengaron (58.327 ± 0.017 mg GAE/mL). It is important to note that Bati-Bati represents *Coffea liberica*, whereas the other three samples are *Coffea canephora* (robusta coffee). This species differentiation, combined with geographical variations in soil composition, climate, and cultivation practices, contributes to the observed differences in phenolic profiles (Adzkiya & Hidayat, 2022).

B. Caffeine Content Determination

Spectrophotometric analysis revealed a maximum absorbance wavelength (λmax) of 273 nm for caffeine in aqueous solution, which is in agreement with the theoretical value and previous research by Ayuni et al. (2022). Using this optimal wavelength, a standard calibration curve was constructed from caffeine standard solutions, demonstrating a linear relationship between concentration and absorbance (Figure 2).

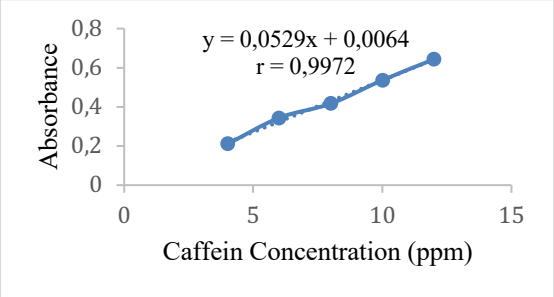

Figure 2. Caffeine standardized regression curve

The linear regression line equation obtained is y = 0,0529x + 0,00064 with a value of r = 0,9972 using graded concentrations of 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm. An equation is said to be linear if the value of r ≥ 0,98. The value of r is closer to 1, the closer it is to meeting the linearity requirements and the linear curve results (Wahid *et al*., 2023). The linearity test was determined with a standard solution series consisting of at least five different concentrations (Latunra *et al*., 2021).

Things that may affect the difference in caffeine levels in coffee beverages include the type of coffee, particle size of coffee powder, brewing or soaking, and the caffeine extraction process. Additional substances will make it more difficult for the solvent to interact with the coffee powder so that the caffeine release and dissolution process is also disrupted (Sulaiman, 2024). Based on SNI 01-7152-2006 and BPOM Regulation No. HK.00.05.23.3644, the maximum limit of caffeine consumption in food and beverages is 150 mg/day and 50 mg/serving (Handayani *et al*., 2020). Based on the research, it was found that the caffeine content in coffee beverages with coffee beans from Pengaron was 123,876 ± 0,331 mg/serving, Aranio 139,340 ± 0,238 mg/serving, Mataraman 175,495 ± 0,715 mg/serving, and Bati-Bati 131,833 ± 0,620

mg/serving. If it follows the SNI and BPOM regulations, the consumption of coffee beverages is only enough once a day maximum.

IV. CONCLUSION

Local coffee brews from South Kalimantan demonstrated significant variation in bioactive properties. Bati-Bati coffee exhibited the strongest antioxidant activity and highest phenolic content, while Pengaron showed the weakest. Total phenolic content strongly correlated with antioxidant capacity, whereas caffeine content did not, establishing phenolic compounds as the primary antioxidant agents. Only three of the four coffee brews (Pengaron, Aranio and Bati-Bati) met Indonesian safety standards for daily caffeine consumption in this formula. These findings highlight the importance of phenolic profiling over caffeine measurement in assessing the health potential of local coffee varieties.

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest associated with this research.

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