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Research Article

Effect of Growing Altitude on Phytochemical Profiles and Antioxidant Activity of Arabica Coffee Cascara (*Coffea arabica* L.) in West Java

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Abstract

Background and Objective: The secondary metabolite profile of Indonesian Arabica coffee (*Coffea arabica* L.) is highly influenced by environmental factors, especially the altitude of the growing location. Arabica coffee by-products, such as cascara, have strong bioactive potential, including antioxidant activity. However, currently, by-products like cascara, which consist of dried pulp and skin, are often neglected as coffee demand increases. The objective of this study was to investigate and compare the phytochemical composition and antioxidant activity of Arabica coffee cascara (*Coffea arabica* L.) from two different altitudes in West Java. **Materials and Methods:** Cascara is obtained from two different regions: Low altitude (Leles, 900-1300 masl) and high altitude (Ibun, 1300-1700 masl). The Soxhlet extraction process was carried out using a stepwise method based on the polarity level of the solvents (n-hexane, ethyl acetate and ethanol). The antioxidant activity was determined using DPPH and phytochemical standardization was carried out in the form of Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and caffeine content. Findings were expressed as Mean $\pm$ SD and analyzed using independent t-test, ANOVA-Tukey ($p < 0.05$) and Pearson correlation. **Results:** Cascara extract from Leles shows higher TPC, TFC, caffeine content and antioxidant activity compared to extract from Ibun. Ethanol extract from Leles shows a strong category of antioxidant activity with an IC_{50} value of 35.74 μ g/mL. The results of statistical data processing showed the altitude influenced on the content of phenolic compounds, flavonoids, caffeine and antioxidant activity. **Conclusion:** The results indicate that the higher total phenols, total flavonoids and caffeine in Arabica cascara extract can be attributed to the lower altitude of the Arabica coffee growing areas in West Java. The antioxidant activity is influenced by the concentrations of these bioactive compounds.

Key words: Cascara, phytochemicals, antioxidant potential, altitude effect

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coffea arabica is generally preferred due to its more pronounced aroma and milder flavor in comparison to *Coffea canephora*¹. Arabica coffee cultivation in Indonesia spans a wide range of geographical regions and varying environmental conditions, particularly altitude. This altitude considerably influences the plant's chemical composition and physiological development. Actually, altitude is a primary environmental determinant that affects the phytochemical profile of Arabica coffee (*Coffea arabica* L.) by regulating plant secondary metabolism².

The volume of coffee by-products, particularly cascara, is increasing in tandem with the global coffee consumption, which presents a substantial opportunity for further utilization. The dried skin and pulp of coffee cherries, known as cascara, are generated in significant quantities during the coffee processing process. They have the potential to be a high-value raw material in the pharmaceutical and nutraceutical industries³. In addition to cascara, other coffee by-products, including mucilage, silverskin and parchment, are also produced. Cascara accounts for approximately 39-45 kg per 100 kg of coffee cherries⁴. The Significance of establishing sustainable methods to extract the inherent value of these by-products is underscored by the increasing interest in their utilization.

Coffee cascara contains many bioactive chemicals, including polyphenols, alkaloids and tannins, which enhance its robust antioxidant effects⁵. These compounds protect cells from oxidative stress induced by free radicals, which are associated with the onset of chronic illnesses, including inflammation and cancer⁶. The content of secondary metabolites in cascara is significantly affected by environmental conditions such as light, temperature, humidity and nutrient availability⁷. While the impact of altitude on the phytochemical profile of coffee beans has been extensively documented, its specific influence on the bioactive components and antioxidant stability of Arabica cascara remains poorly understood. Furthermore, comprehensive data regarding the quality standardization of cascara derived from distinct cultivation altitudes in Indonesia is still lacking. These characteristics influence the concentration and diversity of bioactive chemicals, resulting in disparities in the medicinal potential of cascara across various cultivation zones.

To address this knowledge gap, this study evaluated and standardized cascara extracts from Ibun-Bandung (high altitude, 1300-1700 masl) and Leles-Garut (low altitude, 900-1300 masl), West Java. In addition, to develop quality standards for cascara as a dependable pharmaceutical raw

material by analyzing the impact of environmental variations on its bioactive characteristics. These findings are anticipated to aid in the formulation of sustainable utilization methods for cascara, yielding substantial consequences for the coffee industry and the wider bioeconomy⁸.

MATERIALS AND METHODS

Study area and duration: The experimental work was conducted over a period of 7 months, from April to November 2023. This duration encompassed all stages of the research, including sample collection, extraction processes, phytochemical analysis and antioxidant assays. The cascara samples were sourced from two distinct coffee-producing regions in West Java, Indonesia: Ibun, Bandung Regency, representing a high-altitude zone (1300-1700 masl) and Leles, Garut Regency, representing a lower/mid-altitude zone (900-1300 masl).

Material: The materials used in this study included DPPH (2,2-diphenyl-1 picrylhydrazyl) (Sigma-Aldrich), ascorbic acid (Sigma-Aldrich), Gallic acid (Sigma-Aldrich), Arabica coffee from Leles-Garut, Ibun-Bandung, methanol, ethyl acetate, n-hexane and other analytical grade reagents, which were obtained from Merck.

Sample preparation: Cascara (pulp and skin) Arabica coffee from the wet method was collected from a local gardener in Leles-Garut (900-1300 masl) and Ibun-Bandung (1300-1700 masl) in April 2023. The collection yielded 8 kg of fresh cascara from Ibun and 5 kg of fresh cascara from Leles, obtained from multiple trees in each region to serve as representative biological replicates. Subsequently, the cascara produced from de-pulping cherry coffee was washed, dried at 40-45 °C and ground into powder⁹, resulting in 1.5 kg of dried powder for Ibun and 1.1 kg for Leles. All subsequent extraction processes, phytochemical analyses and antioxidant assays were performed in analytical triplicates (n = 3) for each region to ensure data reliability.

Extraction: The Soxhlet extraction method was utilized to get the cascara extract from Arabica coffee, employing a solvent of ascending polarity⁹, 300 g of cascara pulp powder was extracted utilizing n-hexane, followed by the separation of the filtrate and residue. The residue was air-dried and underwent a secondary extraction with ethyl acetate. Thereafter, ethanol was employed for the ultimate extraction of the residue. Each extract was concentrated utilizing a rotating evaporator maintained at 50 °C. The Soxhlet extraction procedure was performed in triplicate to guarantee reproducibility.

Phytochemical analysis: Phytochemical screening of secondary metabolites in cascara extracts from Arabica coffee was performed to identify the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, polyphenols, monoterpenes/sesquiterpenes, triterpenoids/steroids, quinones and saponins¹⁰.

Determination of total phenolic content (TPC): The TPC was calculated by the Folin-Ciocalteu method¹¹ extract was tested and furthermore, the measurement wavelength for the test was 765 nm. The absorbance was measured using a UV-Vis spectrophotometer (Thermo Scientific®). Gallic acid, as a standard phenolic compound and total phenolic content in the extract were determined using a calibration curve with concentrations ranging from 10 to 250 µg/mL and were represented as gallic acid equivalent g (GAE)/100 g extract (g GAE/100 g extract).

Determination of total flavonoid content (TFC): The TFC was evaluated by the AlCl₃ method¹² was tested and furthermore, the measurement wavelength for the test was 513 nm. The absorbance was measured using a UV-Vis spectrophotometer (Thermo Scientific®). Quercetin, as a standard flavonoid compound and total flavonoid content in the extract were determined using a calibration curve with concentrations ranging from 10 to 250 µg/mL and were represented as quercetin equivalent g (QE)/100 g extract.

HPLC analysis for caffeine in cascara extract: High-performance liquid chromatography (HPLC) is one of the most often employed analytical techniques for determining caffeine, alongside UV spectroscopy integrated with multivariate analysis¹³⁻¹⁵. The cascara extract was tested for its caffeine content using HPLC chromatogram (ArcTM HPLC with PDA Detector). The analysis was conducted using a Reliant® T3 C18 5 µm column (4.6×150 mm) at 40°C. The mobile phase was prepared by combining A (Water) and B (Acetonitrile with 0.1% Formic Acid). The retention time was established at 25 min, utilizing the following gradient scheme: 0.0-7.0 min (14-15%B), 7.0-15.0 min (15-20%B), 15.0-15.1 min (20-100%B), 15.1-20.0 min (100% B), 20.0-20.1 min (14%B) and 20.1-25.0 min (14%B). The flow rate employed was 0.8 ml/min and calibrated at 272 nm. The content of caffeine in the extract was measured using a standard caffeine calibration curve⁹.

Antioxidant activity: Antioxidant capacity was adopted in Blois's method using a UV-Vis spectrophotometer (Beckman Coulter DU 720) and absorbance measurement at 517 nm. The free radical DPPH with a concentration of 0.1 mM or

39.4 µg/mL in methanol for analysis was mixed with various concentrations of extract (Volume 1:1) and incubated for 30 min¹² was ascorbic acid and a three-replication analysis was conducted.

For calculating the percentage of free radical inhibition, the formula was used⁹:

$$\text{Inhibition (\%)} = \frac{(\text{Blank abs} - \text{Sample abs})}{\text{Blank abs}} \times 100\%$$

To determine the IC₅₀, a calibration curve was created between the percentage of DPPH scavenging activity and the concentration.

Statistical analysis: The results were averaged and reported with their standard deviation. An independent t-test with a significance level of 0.05 or 5% and a one-way ANOVA-Tukey's method were employed to conduct the statistical analysis. Pearson's method was employed to conduct the correlation test between TPC and antioxidant activity and the correlation between TFC and antioxidant activity.

RESULTS

Extraction yield: A sequential extraction via the Soxhlet apparatus was applied to the Arabica cascara raw material by employing a series of solvents with rising polarity levels. The process integrated n-hexane (non-polar), ethyl acetate (semi-polar) and ethanol (polar) in a consecutive manner. This gradient strategy aimed to fractionate and maximize the yield of specific chemical components corresponding to each solvent's polarity profile. The extraction process commenced with n-hexane, which selectively isolated non-polar molecules. Semi-polar, succeeded by the use of ethyl acetate and preferentially targeting predominantly semi-polar and diminutive non-polar compounds. The final application employs ethanol as a solvent, specifically targeting predominantly polar molecules, along with minor semi-polar and non-polar chemicals. The liquid extract was concentrated with a rotary evaporator. The yield extract is displayed in Table 1.

Generally, polar and semi-polar solvents exhibited enhanced extraction efficiency relative to the non-polar n-hexane extract across all samples. In the Leles cascara, ethyl acetate proved to be the most efficient solvent for bulk recovery. Conversely, the Ibun samples demonstrated a distinctly different pattern, indicated by a considerable yield in the ethanol extract that greatly exceeded that of the other solvents.

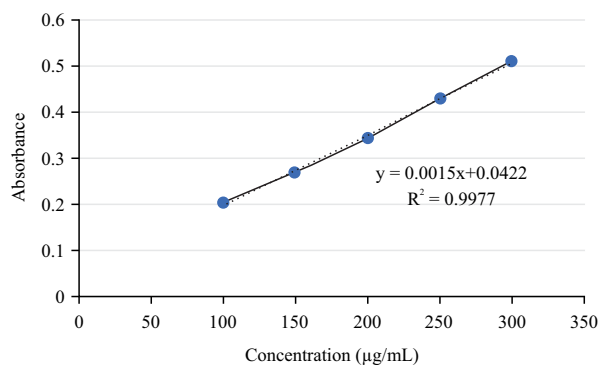


Fig. 1: Standard curve gallic acid

Table 1: Percentage yield of cascara arabica coffee from Leles and Ibun extract

Sample	Weight sample (g)	Extract	Extract weight (g)	Percentage of yield
Leles cascara	500	n-hexane	13.238	2.65
		Ethyl acetate	8.414	6.18
		Ethanol	15.758	3.15
Ibun Cascara	500	n-hexane	8.399	1.68
		Ethyl acetate	20.453	4.091
		Ethanol	74.983	12.996

Table 2: Phytochemical screening of extract cascara arabica coffee from Leles and Ibun

Secondary metabolite	Leles				Ibun			
	CD	LH	LE	LT	CD	IH	IE	IT
Alkaloid	+	-	+	+	+	-	+	+
Phenolic	+	+	+	+	+	+	+	+
Flavonoid	+	-	+	+	+	-	+	+
Monoterpene/Sesquiterpene	+	+	+	+	+	+	+	+
Steroid/Triterpenoid	+	+	+	+	+	+	+	+
Quinone	+	-	+	+	+	-	+	+
Saponins	-	-	-	-	-	-	-	-

CD: Crude drugs, LH: Leles n-hexane extract, LE: Leles ethyl acetate extract, LT: Leles ethanol extract, IH: Ibun n-hexane extract, IE: Ibun ethyl acetate extract and IT: Ibun ethanol extract

Phytochemical content: Table 2 illustrates the phytochemical screening of these extracts. The crude pharmaceuticals and each extract (n-hexane, ethyl acetate and ethanol) were subjected to a qualitative phytochemical screening to identify a variety of secondary metabolites. The screening was based on color changes, precipitate formation, or the presence of stable foam for saponin testing. To map the initial chemical constituents of the raw material, phytochemical screening of crude pharmaceuticals and extracts was implemented. Various reagents were employed for each class of compounds in the phytochemical screening test. Phenolic compounds were detected using Ferric Chloride (FeCl_3), flavonoids were detected using amyl alcohol, alkaloids were detected using Mayer and Dragendorff reagents and terpenoids were detected using vanillin-sulfuric acid reagent. The identification of phenolic compounds was achieved through the development of a brown to black coloration in this experiment. The formation of a yellow-orange rim was

indicative of flavonoids. The formation of white sediment with Mayer and orange-red sediment with Dragendorff confirmed the presence of alkaloids¹⁶. The appearance of terpenoids was distinguished by a variety of colors, including blue and green. The formation of a red coloration was used to detect quinones. The findings suggested that phenolics, monoterpenes/sesquiterpenes and steroids/triterpenoids were consistently present in all samples. In contrast, the ethyl acetate and ethanol extracts were the only ones to detect alkaloids, flavonoids and quinones, thereby confirming their polar characteristics. Additionally, saponin assays yielded negative results for all samples from both Leles and Ibun, suggesting that this compound group is not present in the cascara under investigation.

The total phenolic content (TPC) and total flavonoid content (TFC) of the cascara extracts were determined using the Pourmurad and Chang methods with minor modifications. Folin-Ciocalteu reagent was used for TPC determination,

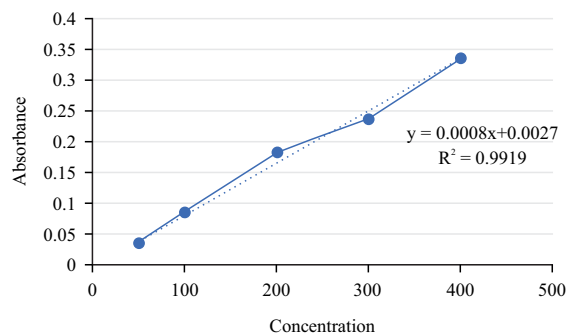


Fig. 2: Standard curve quercetin

Table 3: Total phenolic, flavonoid content and antioxidant activity of cascara arabica coffee extract

Cascara	Extract	TPC (g GAE/100 g extract)	TFC (g QE/100 g extract)	IC ₅₀ of DPPH (μg/mL)
Leles	LH	2.785±0.358	2.176±0.029	821.84±0.321
	LE	3.363±0.357	4.931±0.063	65.998±0.77
	LT	3.544±0.263	9.64±0.325	35.74±0.765
Ibun	IH	1.03±0.256	1.66±0.142	1389.44±0.243
	IE	1.991±0.232	4.123±0.078	150.541±0.77
	IT	2.432±0.372	9.473±0.326	179.984±0.765

LH: Leles n-hexane extract, LE: Leles ethyl acetate extract, LT: Leles ethanol extract, IH: Ibun n-hexane extract, IE: Ibun ethyl acetate extract and IT: Ibun ethanol extract

while Aluminum Chloride (AlCl_3) reagent was used for TFC determination. All extracts were subjected to this analysis. The TPC and TFC were quantified using a linear regression equation derived from the gallic acid and quercetin standard curve (Fig. 1 and Fig. 2).

The results of the TPC and TFC for each extract are presented in Table 3. The results showed that the ethanol extract from Leles contained the highest concentrations, with a total phenolic content (TPC) of 3.544 g GAE/100 g ± 0.263 and a total flavonoid content (TFC) of 9.64 g QE/100 g ± 0.325 . Ibun ethanol extract followed with a TPC of 2.432 g GAE/100 g ± 0.372 and a TFC of 9.473 ± 0.326 g QE/100 g. Based on the growing location, the cascara extract from Leles exhibited higher total phenolic content (TPC) and total flavonoid content (TFC) than the extract from Ibun. Among the extraction solvents, the ethanol extract showed the highest phenolic and flavonoid concentrations. To validate these findings, the data were analyzed statistically using one-way ANOVA. The analysis revealed significant differences among the groups. A Tukey *post hoc* test was subsequently performed to assess the significance of these differences. The results confirmed significant differences between cascara extracts from Leles and Ibun, as well as among the extraction solvents, with a significance level of $p < 0.05$.

The determination of caffeine content in this study was performed on the ethanol extracts of cascara from Leles and Ibun using HPLC. The chromatographic profile for the analysis are sequentially presented in Fig. 3. The peak identification was confirmed by the chromatogram of the caffeine standard

solution, which exhibited a distinct peak at a retention time of 5.13 min (Fig. 3a). under identical chromatographic conditions, the presence of caffeine was successfully identified at the same retention time in both Leles ethanol extract (Fig. 3b) and the Ibun ethanol extract (Fig. 3c). Furthermore, caffeine profiling via HPLC at 274 nm revealed that the Leles ethanol extract contained 1.213 ± 0.019 mg/g of caffeine, which was substantially higher than the 0.323 ± 0.014 mg/g found in the Ibun ethanol extract.

Antioxidant activity: The antioxidant activity of Arabica cascara extracts was assessed and it was found that each extract exhibited unique DPPH radical inhibition profiles. The IC₅₀ values are presented in Table 2. A stock solution of 1000 ppm was prepared for each extract in the antioxidant activity assay and an identical series of concentrations was subsequently prepared. The purpose of establishing identical concentration series for each extract was to compare the DPPH inhibition percentage profiles. The percent inhibition of concentration series displayed in Fig. 4. This was done to facilitate the calculation of the IC₅₀ value for each extract against DPPH in a calibration curve.

The inhibition percentage profiles of the cascara extracts from Ibun and Leles are depicted in Fig. 4, classified by the extraction solvent. Regarding the DPPH inhibition percentage of each extract, a concentration dependent increase was observed across all samples. When evaluating the profile based on extraction solvent employed, the n-hexane extract for both Ibun and Leles cascara generally exhibited the lowest

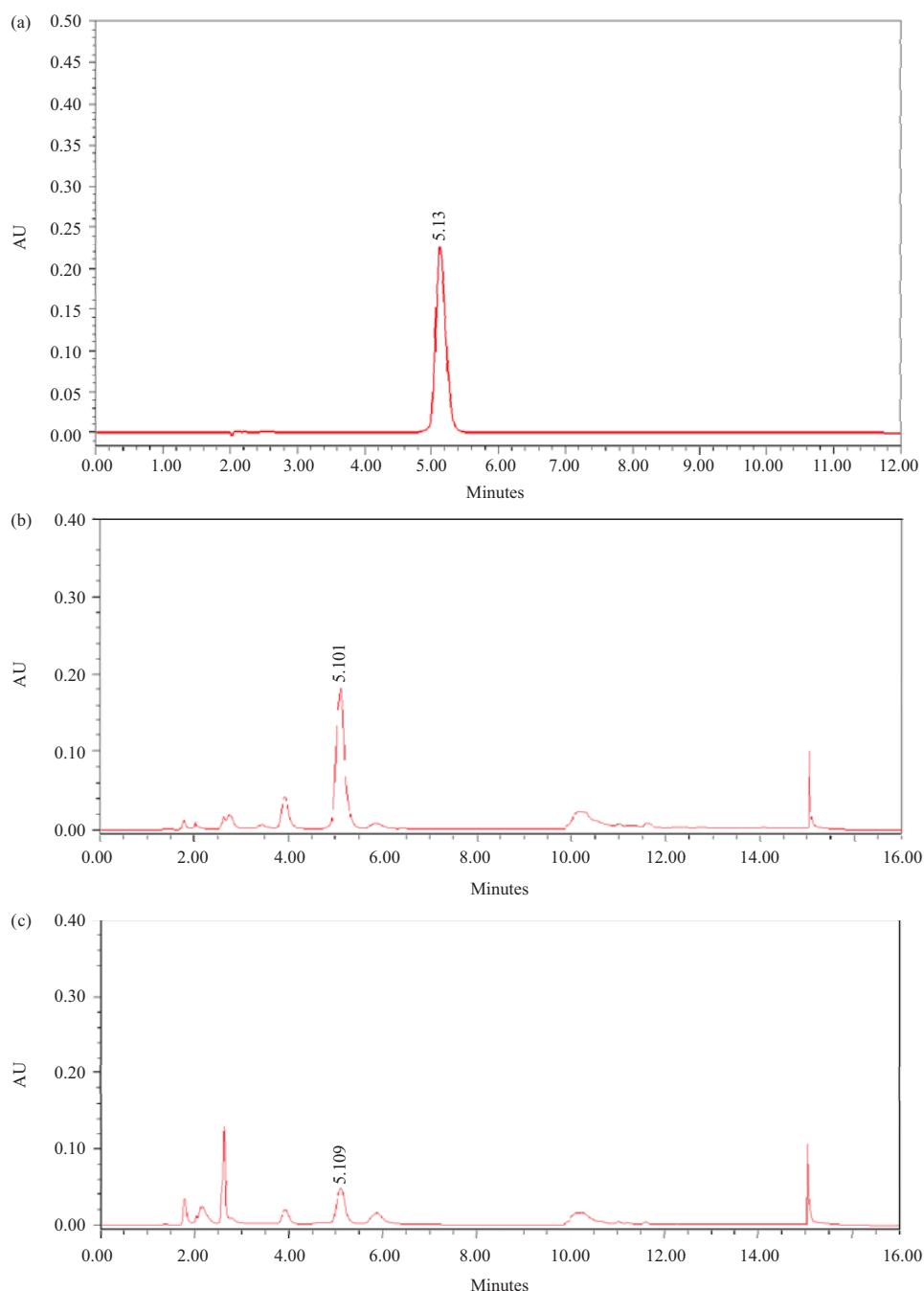


Fig. 3(a-c): HPLC Chromatogram of caffeine in Arabica coffee cascara (a) Caffeine standard solution profile, (b) Cascara ethanol extract from Leles region and (c) Cascara ethanol extract from Ibun extract

The x-axis represents the retention time (minutes) and the y-axis represents the absorbance intensity (mAU)

antioxidant activity (Fig. 4a). Concentrations exceeding 1000 ppm were required to obtain an inhibition percentage exceeding 50% for the n-hexane extracts. In contrast, the ethyl acetate extract (Fig. 4b) and ethanol extract (Fig. 4c) demonstrated a significantly higher antioxidant activity, where concentration exceeding 200 ppm were already sufficient to achieve the 50% inhibition threshold. Across the

concentration series, the Leles cascara extracts exhibited higher inhibition percentage profiles than those from Ibun, which was generally observed based on the geographical origin of the cascara. Nevertheless, in order to validate this observation, additional statistical analysis of the antioxidant activity parameter, specifically the IC_{50} value, was necessary to bolster the conclusion.

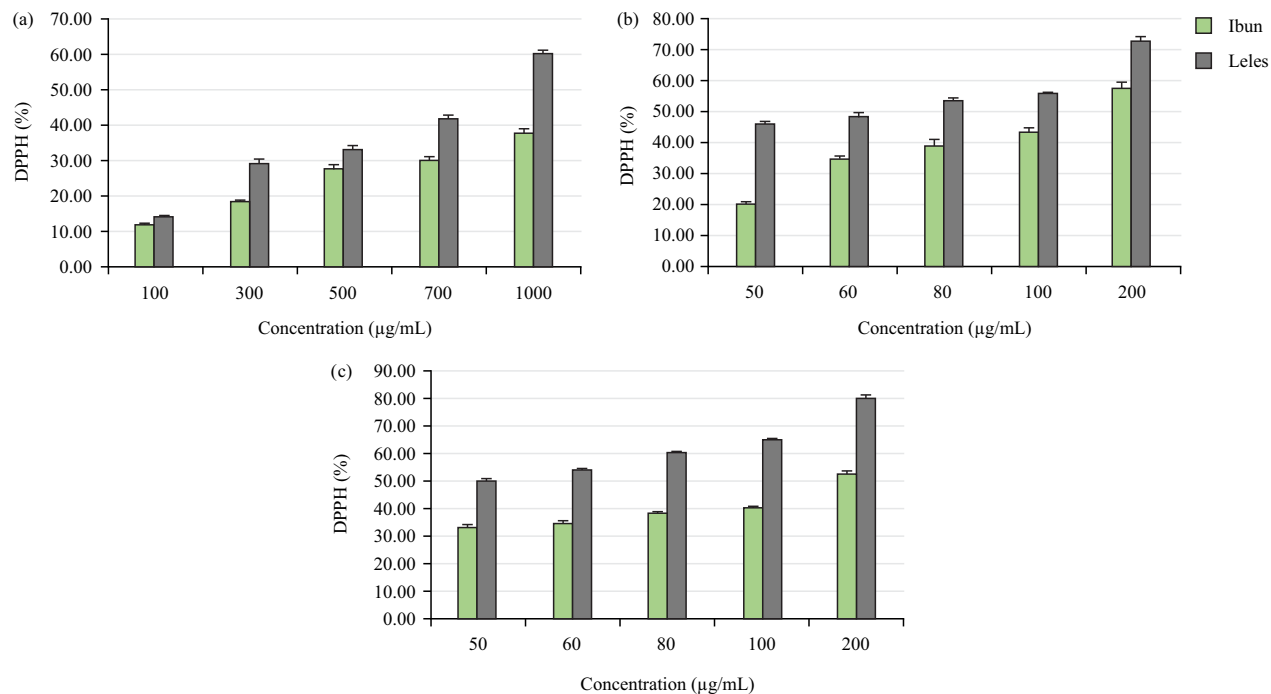


Fig. 4(a-c): Percent inhibition DPPH of Arabica coffee cascara extract (a) Cascara n-hexane extract, (b) Cascara ethyl acetate extract and (c) Cascara ethanol extract

Table 4: Antioxidant category of Leles and Ibun extract

	AAI extract from Leles	Category	AAI extract from Ibun	Category
n-hexane	0.049	Weak	0.029	Weak
ethyl acetate	0.606	Moderate	0.266	Weak
ethanol	1.119	Strong	0.222	Weak

The IC_{50} values for each extract are presented in Table 3. Based on the extraction solvents used, the n-hexane extract showed the highest IC_{50} values in the Ibun and Leles extracts, followed by the ethyl acetate and ethanol extracts. The IC_{50} values of the extracts from Leles and Ibun varied greatly. Statistical analysis using one-way ANOVA and *post hoc* Tukey test confirmed these findings, indicating significant differences in antioxidant activity between the Leles and Ibun extracts, as reflected in their IC_{50} values. The Leles cascara extract produced lower IC_{50} values compared to the extract from Ibun, indicating that the Leles cascara extract has greater potential as a source of antioxidants. In this study, the Antioxidant Activity Index (AAI) was utilized as a mathematical approach to classify the overall antioxidant strength using the calculated IC_{50} values. The Antioxidant Activity Index (AAI) values of Leles and Ibun extracts are reported in Table 4.

DISCUSSION

The phytochemical screening of cascara extracts from Leles and Ibun revealed a similar profile of secondary metabolites, with phenolics, flavonoids and

steroids/triterpenoids consistently detected across various solvent systems. Phenolic compounds were identified in all solvents, indicating their broad solubility and potential as strong antioxidant agents⁵. The presence of flavonoids in semi-polar and polar solvents, such as ethyl acetate and ethanol, but their absence in the non-polar solvent hexane, suggests their polar nature¹⁷. We noted that flavonoids were completely absent in the n-hexane extract and were only identified when using more polar solvents, such as ethyl acetate and ethanol. This change confirms the polar nature of these compounds, which is consistent with the findings of Jabeen¹⁷. Conversely, the hexane extract retained its nonpolar characteristics, allowing monoterpenes and sesquiterpenes to be identified¹⁸. However, the most striking finding is the ability of steroids and triterpenoids to dissolve across the full spectrum of polarity. The diverse pharmacological potential of the extract, particularly in anti-inflammatory and antioxidant pathways, may be due to its amphiphilic behavior, as evidenced by its broad solubility¹⁹. Quinones were identified in semi-polar and polar solvents, which is consistent with their chemical characteristics. It is intriguing that saponins were not

present in the cascara samples from both Leles and Ibun²⁰. These results indicate that cascara from both regions contains a wide range of bioactive secondary metabolites, including phenolics, flavonoids, steroids/triterpenoids and alkaloids. These metabolites may be of substantial value for pharmaceutical and nutraceutical applications¹⁹, particularly in the development of natural health products and therapeutic agents that leverage their antioxidant and anti-inflammatory properties.

In order to translate these screening results into a reliable pharmaceutical raw material, the identified compound groups must be rigorously quantified and standardized. In this study, gallic acid was employed as the standard for determining the total phenolic content in this study. Consequently, the total phenolic content of the preparations was expressed in gallic acid equivalents (GAE). Meanwhile, the total flavonoid content was calculated in the form of quercetin equivalents (QE). The determination of total phenols and flavonoids is one way to standardize the content of compound groups in cascara extracts from Leles and Ibun.

Based on the results of statistical analysis, there are differences in TPC and TFC values based on the solvents used in the extraction as well as differences in the altitude of the locations where cascara samples were grown. Testing was carried out using one-way ANOVA analysis and followed by a *post hoc* Tukey test with a p-value set at <0.05 to assess the significance of the differences. The results showed that TPC and TFC were significantly higher in cascara from Leles compared to Ibun ($p < 0.05$). This difference is consistent with findings that phenolic compound biosynthesis was influenced by environmental factors such as altitude and light intensity⁷. The ethanol extracts demonstrated the highest TPC, emphasizing the efficiency of polar solvents in phenolic extraction.

Based on the extraction solvent used, the ethanol extract of Arabica cascara exhibited higher TPC and TFC values than the n-hexane and ethyl acetate extracts. Statistical analysis confirmed that differences in total phenolic and total flavonoid concentration were significantly influenced by both the extraction solvent and the geographical origin of the cascara samples. The TPC of ethanol extract of Arabica cascara from Gayo, Aceh, Indonesia, is 2.04 mg GAE/g²¹. The TPC of Arabica cascara pulp extract is 16.4 mg GAE/g using the Folin-Ciocalteu method²². The concentration of gallic acid in Arabica cascara water extract from Thailand ranges from 7.61 mg/L to 17.40 mg/L²³. The TPC of Arabica cascara pulp extract from Aceh is 21.99 mg GAE/100 g²⁴. These values indicate that differences in TPC values can depend on factors related to the cultivation area's conditions. The TPC and TFC values can be

influenced by extraction methods, solvents, plant species and growth site conditions¹¹. In our investigation, the phenolic content of cascara extracts from Leles and Ibun varied due to variations in the growth conditions and the extraction solvents used, which was consistent with Nikolaeva's findings.

Environmental conditions, including altitude, soil composition, radiation intensity, rainfall and humidity, significantly influence the synthesis of phenolic compounds in plants. Coffee plants that are cultivated at higher altitudes accrue a greater quantity of phenolic compounds, which is likely a result of the increased exposure to abiotic stresses, such as elevated ultraviolet radiation, which stimulates secondary metabolic pathways⁷. Leles, which is located at a higher altitude and has more advantageous environmental conditions, may provide the most favorable conditions for the production of phenols. Conversely, Ibun, which may have higher humidity or reduced light intensity, may encounter suboptimal conditions that restrict the synthesis and accumulation of phenolic compounds, leading to lower levels of these beneficial compounds compared to those found in coffee plants cultivated in Leles.

The Soxhlet extraction method is effective in extracting phenolic compounds, including flavonoids, in this study. This method involves the use of solvents in sequence according to their polarity, namely n-hexane, ethyl acetate and ethanol. Ethanol is the last polar solvent used. This extraction-method approach is expected to optimally extract phenolic compounds and flavonoids based on their polarity. In addition to extraction method factors, the main determinants of phenolic and flavonoid content in medicinal plant raw materials are the environmental conditions in the growing area, including soil quality and climate²⁵. These factors can significantly influence the concentration and diversity of these compounds, which ultimately affect the quality of the final extract.

A part from the phenolic and flavonoid profiles, evaluating specific alkaloid markers such as caffeine is equally critical to comprehensively understand the altitudinal impacts on cascara composition. In this study, detected caffeine and quantification of caffeine content in the extract were performed by an HPLC instrument with a C18 column at the wavelength 274 nm, whose chromatogram is presented in Fig. 3. The caffeine content in cascara ethanol extract from Leles was 1.213 ± 0.019 and Ibun was 0.323 ± 0.014 . Caffeine synthesis in coffee cherries was influenced by the altitude at which the coffee was grown²⁶. Previous research showed that caffeine content in Robusta green coffee was 182 mg/100 g and 154 mg/100 g for the Arabica green coffee sample²⁷. The growing altitude for Robusta was on average below 800 masl,

while Arabica was above 1200 masl. Arabica coffee from Leles grew at an altitude of 900-1,300 masl, whereas arabica coffee from Ibun grew at 1,300-1,700 masl. Referring to previous studies, these differences in altitude could have been a factor that influenced the differences in caffeine content in cascara from Leles and Ibun, as higher altitudes are often associated with lower caffeine levels due to slower growth rates and environmental stressors.

The antioxidant activity testing of cascara extract was carried out using the IC_{50} value parameter and processed into an antioxidant activity index to determine the antioxidant potential group. The IC_{50} values against DPPH are presented in Table 3. The obtained IC_{50} values indicate that ethanol and ethyl acetate extracts have higher antioxidant activity than n-hexane extract. This phenomenon is consistent with the phytochemical characteristics of Arabica coffee cascara in this study, which show that phenolic and flavonoid constituents, known as electron or hydrogen donors, are more effectively extracted in polar solvents.

The results of this study indicate that the antioxidant activity of plant extracts is highly influenced by their growth location, as evidenced by the IC_{50} values between extracts obtained from Leles and Ibun. Overall, compared to the extract from Ibun, the antioxidant activity of the Leles extract is significantly higher. The ethanol extract from Leles has an IC_{50} value of 25.921 $\mu\text{g/mL}$. In contrast, the ethanol extract from Ibun shows a higher IC_{50} value of 164.842 $\mu\text{g/mL}$. This difference is due to variations in the phenolic and flavonoid content of the plants, which are influenced by environmental factors such as soil characteristics and radiation exposure. Higher light intensity, lower temperature and higher growing locations cause an increase in phenol content and antioxidant activity²⁸. These results are in line with our study findings that ethanol extract of cascara from Leles with a higher growing altitude than Ibun showed the highest antioxidant activity.

At both locations (Ibun and Leles), the n-hexane extract showed the weakest antioxidant activity, with IC_{50} values of 1389.44 $\mu\text{g/mL}$ ($Y = 0.0286x + 10.262$; $r^2 = 0.969$) for Ibun and 821.84 $\mu\text{g/mL}$ ($Y = 0.0478x + 10.716$; $r^2 = 0.974$) for Leles. This indicates that the non-polar extracts from Ibun and Leles have a low capacity to neutralize DPPH radicals. The n-hexane extract contains lipophilic compounds, including fatty acids, waxes and non-phenolic terpenoids, which usually do not contribute significantly to antioxidant activity¹². These findings reaffirm the fact that the semi-polar and polar extracts of cascara are the main sources of biologically active metabolites with antioxidant properties.

Compared to n-hexane extract, the antioxidant activity of ethyl acetate extracts of Leles and Ibun is significantly higher. The IC_{50} values of the Ibun and Leles samples are 150.541 $\mu\text{g/mL}$ ($Y = 0.1571x + 26.35$; $r^2 = 0.984$) and 65.998 $\mu\text{g/mL}$ ($Y = 0.1744x + 38.49$; $r^2 = 0.993$), respectively. This suggests that semi-polar compounds, such as flavonoid aglycones and specific phenolics, are more effective in neutralizing DPPH free radicals. The observed increase in activity in the Leles sample in comparison to Ibun indicates that the accumulation of secondary metabolites in cascara can be influenced by its geographical origin. The optimization and standardization of basic materials are significantly influenced by the variation in bioactivity across regions.

The ethanol extracts in Ibun and Leles showed the strongest antioxidant activity among all extracts tested. The markedly lower IC_{50} value in the Leles sample (35.740 $\mu\text{g/mL}$, $Y = 0.1885x + 43.263$, $r^2 = 0.963$) compared with the Ibun sample (179.984 $\mu\text{g/mL}$, $Y = 0.1262x + 27.286$, $r^2 = 0.995$) reflects the high effectiveness of polar compounds from the Leles extract in scavenging free radicals. Polar constituents, including chlorogenic acid derivatives, flavonoid glycosides and other phenolic compounds, are frequently observed in cascara. These constituents have a strong electron-donating capacity, which enhances the effectiveness of radical inhibition. The IC_{50} values' difference between regions further implies that the phytochemical composition is influenced by environmental factors.

The antioxidant activity of the Leles ethanol extract was higher among all the extracts tested. The activity of polar compounds in the Leles ethanol extract in scavenging free radicals was evidenced by a significantly lower IC_{50} value (35.740 $\mu\text{g/mL}$, $Y = 0.1885x + 43.263$, $r^2 = 0.963$) compared to the Ibun sample (179.984 $\mu\text{g/mL}$, $Y = 0.1262x + 27.286$, $r^2 = 0.995$). Polar compounds, such as chlorogenic acid derivatives, flavonoid glycosides and other phenolic compounds, are often found in coffee cascara and may contribute as electron donors in reactions with DPPH.

One method to classify antioxidant potential is the Antioxidant Activity Index (AAI), which is calculated using the IC_{50} value²⁹. The AAI values of Leles and Ibun extracts are reported in Table 4. However, the Ibun extract is consistently classified as a weak antioxidant, while the n-hexane, ethyl acetate and ethanol extracts from Leles cascara have varying antioxidant categories. In terms of caffeine content, TFC and TPC, there are quantitative differences between Leles and Ibun extracts. The Leles cascara extract has higher concentrations of caffeine, TPC and TFC compared to the Ibun extract.

Based on Pearson's correlation analysis, in the Leles extracts, both TPC and TFC revealed a strong negative correlation ($r = -0.981$ and $r = -0.802$) with the IC_{50} DPPH. In contrast, in the Ibun extracts, only TPC exhibited a strong negative correlation ($r = -0.944$) with the IC_{50} value, while TFC did not show a strong correlation ($r = -0.728$). These findings served as supporting data to justify why the Leles cascara extracts were categorized as strong antioxidants. In addition, the caffeine content in the Leles ethanol extract is higher than that of Ibun. Caffeine is known to be a source of natural antioxidants. Therefore, it can be concluded that the antioxidant activity in Leles and Ibun may be influenced by the total phenol, flavonoids and caffeine content. The results indicate that the cascara extract from Leles has higher antioxidant potential than the cascara extract from Ibun.

In general, the order of antioxidant potential consistently starts with ethanol extract, ethyl acetate and n-hexane. This pattern is consistent with extraction methods arranged according to the level of polarity. Polar solvents are very effective in extracting phenolic compounds, which are important biomolecules in antioxidant activity. The higher antioxidant activity of cascara Leles extract compared to cascara extract from Ibun shows that there is a significant role of higher levels of caffeine, phenol and flavonoid compounds than in Ibun. This study shows that the altitude of the growing location, the extraction method and the type of extraction solvent used can affect the quantity of compounds and the strength of antioxidant activity using DPPH.

CONCLUSION

The research results indicate that there are differences in total phenol content, total flavonoids, caffeine content and antioxidant activity of cascara extract from Arabica coffee obtained at different growing altitudes (900-1300 masl) and (1300-1700 masl). The data showed that the content of total flavonoids, total phenolics, caffeine content and antioxidant activity were higher in the cascara extract of Arabica coffee from Leles (900-1300 masl). The differences in the profile of chemical compound groups in this extract emphasize the importance of standardizing raw material sources. Further research should focus on validating its therapeutic relevance by identifying specific bioactive markers using metabolomic profiling and evaluating other biological activities.

SIGNIFICANCE STATEMENT

This study demonstrates that growing altitude significantly influences the phytochemical composition, caffeine content and antioxidant activity of *Coffea arabica*

cascara in West Java. The findings identify low-altitude cascara as a richer source of phenolic compounds and antioxidants, highlighting its potential as a standardized raw material for pharmaceutical, nutraceutical and functional food applications. The study also provides scientific evidence supporting the sustainable valorization of coffee processing by-products, contributing to waste reduction and value-added utilization in the coffee industry.

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