



RESEARCH ARTICLE

Quantification of phenolics, flavonoids, tannins and antioxidant activity in Java Arabica (*Coffea arabica* L.) leaf extract

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Abstract

Natural antioxidants are widely valued for their bioavailability and potential health benefits. Coffee (*Coffea* spp.) is recognised as a rich source of bioactive compounds, not only in its beans but also in its leaves and fruit tissues. In Indonesia, coffee leaves have long been utilised to prepare a traditional beverage known as Aia kawa, which is believed to possess notable antioxidant properties. This study evaluated the relative water content (RWC), total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and total antioxidant activity of coffee leaves at different stages of leaf and tree maturity. The results demonstrated that young leaves harvested from young coffee trees exhibited the highest TPC (3.2 mg GAE/g), TFC (18.5 mg QE/g) and antioxidant activity (0.54 mg AAE/g; $p < 0.05$). No significant differences in phenolic content were observed between mature leaves from young trees and young leaves from mature trees. Tannin values were clarified and expressed as micrograms tannic acid equivalent per gram dry sample (725–1100 μ g TAE/g), avoiding misinterpretation of the unit as mg/g. A positive association was observed between phenolic content and antioxidant activity, with a strong relationship for TPC ($R^2 = 0.981$) and a moderate relationship for TFC ($R^2 = 0.855$). These findings indicate that coffee leaves, particularly young leaves from young trees, represent a promising and under utilised source of natural antioxidants with potential applications in functional foods and nutraceutical products.

Keywords: antioxidant activity; coffee leaves; *Coffea arabica*, functional food; leaf maturity; phenolics; flavonoids; tannins

Introduction

Natural antioxidants are bioactive compounds that play an essential role in protecting biological systems against oxidative stress by neutralising free radicals (1). Excessive free radical production can disrupt cellular homeostasis and is strongly associated with the development of chronic diseases, including cancer, cardiovascular disorders and neurodegenerative conditions (2, 3). Due to increasing concerns grow over the potential side effects of synthetic antioxidants, interest in natural alternatives has increased significantly. Plant-derived antioxidants are widely regarded as safer and more biocompatible, leading to their expanded application in food, nutraceutical and pharmaceutical products (4).

Plants synthesise a diverse array of secondary metabolites with antioxidant properties, such as phenolics, flavonoids, tannins, alkaloids, saponins and quinones (5–7). These compounds can be found in various plant parts, including fruits, seeds, flowers, roots and leaves (5, 8). Among economically important crops, coffee (*Coffea arabica*) has received global attention primarily for its beans; however, other parts of the plant, particularly the leaves, are increasingly recognised as promising sources of bioactive phytochemicals (9, 10).

Indonesia is one of the world's major producers of Arabica coffee, with extensive cultivation across Java, Sumatra and Sulawesi (11, 12). Java Arabica coffee is especially valued for its mild flavour profile and relatively low acidity compared to other Arabica varieties (13). Despite the economic importance of coffee cultivation, the utilisation of coffee leaves remains minimal. They are often treated as agricultural by-products, used as animal feed, or left to decompose in plantations, representing an underexplored biomass resource.

Traditional knowledge, however, suggests potential health benefits of coffee leaves. In several regions of Sumatra, coffee leaves are brewed into a traditional beverage known as Aia Kawa (14, 15). This drink is believed to promote health and vitality, likely due to the presence of antioxidant-rich phytochemicals such as flavonoids, saponins and alkaloids (16, 17). Although such traditional practices indicate functional value, scientific evidence describing the antioxidant-related phytochemical composition of Java Arabica coffee leaves is limited.

Previous studies on coffee plants have largely emphasised agronomic and physiological aspects, including responses to environmental stress, photosynthetic performance and stomatal regulation (18, 19). Comparatively fewer investigations have focused on the phytochemical composition of coffee leaves and their antioxidant potential (20). Although coffee leaf phytochemicals have been reviewed and reported in some contexts, the effect of tree age and leaf developmental stage on Java arabica leaf phytochemistry remains insufficiently characterized. This gap is important because secondary metabolite biosynthesis is influenced by tissue maturity, environmental conditions and physiological status (21, 22). Identifying the maturity stage with the highest antioxidant potential may support the sustainable use of coffee leaves as a value-added agricultural by-product.

Therefore, this study aimed to evaluate the total phenolic, flavonoid, and tannin contents, as well as the antioxidant activity, of Java Arabica coffee leaves obtained from trees of different ages and at different leaf maturity stages. In addition, the study examined the correlations between phytochemical concentrations and antioxidant capacity. The novelty of this work lies in linking leaf maturity and tree age with antioxidant-related phytochemical variation in Java Arabica leaves, thereby providing a scientific basis for selecting appropriate leaf material for functional food and nutraceutical applications.

Materials and Methods

Site of study and plant material

This study used young and mature leaves of *Coffea arabica* L. A series of laboratory analyses were conducted to determine moisture content, total phenolic content, total flavonoid content, total tannin content and total antioxidant activity.

The experiment was conducted at the experimental farm in Sumowono, Bandung, Central Java, Indonesia. The study area is located in a humid tropical highland environment. The Sumowono area has an average elevation of approximately 1026 m above sea level, while Semarang Regency recorded an average temperature of approximately 26.7 °C, annual rainfall of 2978 mm, and 173 rainy days in the 2024 climatological record. These environmental conditions are relevant because altitude, temperature, rainfall and humidity can influence the accumulation of plant secondary metabolites. Plant materials were collected from two age groups of *Coffea arabica* trees: mature trees (approximately 20 years old) and young trees (approximately 5 years old). Young leaves were identified based on their light green colour, smooth texture and recent expansion, while mature leaves were darker green with a coarser texture. Plant identity was confirmed morphologically as *Coffea arabica* L. based on field characters.

Leaf sample preparation

Fully expanded leaves were collected from the terminal node (young leaves) and the second node (mature leaves) of each tree. Samples were grouped into 4 criteria: A, young trees-young leaves; B, young trees-mature leaves; C, mature trees-young leaves; and D, mature trees-mature leaves. Leaves were measured and immediately frozen in liquid nitrogen to minimise enzymatic degradation. The frozen samples were freeze-dried to a constant weight and subsequently ground into a fine, homogeneous powder using a laboratory blender. The powdered samples were stored in airtight containers protected from light until analysis. For phytochemical and

antioxidant assays, dried leaf powder was extracted using the laboratory extraction procedure applied in the original experiment.

Moisture content

Moisture content was determined using the oven-thermogravimetric method following AOAC procedures (AOAC, 2000). Approximately 1 g of fresh leaf sample was placed in a pre-weighed porcelain crucible and dried at 102-105 °C for 5-6 hr until a constant weight was reached. The moisture content was calculated using the formula (1):

$$\text{Moisture content} = \frac{(B-A)}{(B-C)} \times 100 \quad (\text{Eqn.1})$$

Where, A = dry weight of cup (g), B = dry weight of cup and initial sample (g) and C = dry weight of cup and final sample (g)

Total phenolic content

Total phenolic content (TPC) was measured using the Folin-Ciocalteu method (23). Gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid equivalent per gram of dry sample (mg GAE/g).

Total flavonoid content

Total flavonoid content (TFC) was determined using the aluminium chloride colourimetric method (24), with slight modifications. One mg of each extract was diluted with distilled water to a volume of 1 mL. Subsequently, 1 mL of distilled water, 3 mL of 5 % sodium nitrite, and 0.3 mL of 10 % aluminium chloride was added. After 6 min, 2 mL of 1 M sodium hydroxide was added, and the final volume was adjusted to 10 mL with distilled water. The mixture was incubated in the dark for 30 min, and absorbance was measured at 510 nm using a UV-Vis spectrophotometer. Results were expressed as mg of quercetin equivalent per gram of dry sample (mg QE/g). The quercetin calibration equation should be inserted from the original standard curve.

Total tannin content

Tannin content was estimated following the method (25). A total of 0.1 g of coffee leaf extract was mixed with 0.2 mL of diluted Folin-Ciocalteu reagent (1:1 with distilled water) and allowed to stand for 5 min. Then, 2 mL of 7 % Na₂CO₃ was added and mixed for 30 sec. The solution was diluted to 10 mL with distilled water. From this mixture, 1 mL was pipetted and further diluted to 10 mL. After standing for 95 min, the absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer. All measurements were performed in triplicate. Tannin values were expressed as micrograms of tannic acid equivalent per gram of dry sample (µg TAE/g), correcting the unit presentation from the previous mg TAE/g expression.

Total antioxidant activity

The antioxidant activity of coffee leaf extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (26). Ascorbic acid and butylated hydroxytoluene (BHT) were used as natural and synthetic antioxidant standards respectively. A standard stock solution of ascorbic acid (1000 ppm) was prepared by dissolving 0.01 g in 10 mL of distilled water. Serial dilutions (0, 20, 40, 60, 80 and 100 ppm) were prepared. Each dilution (1 mL) was reacted with 3.9 mL of 0.1 mM DPPH in methanol, vortexed, and incubated in the dark for 30 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. For the samples, 2 mL of each extract was mixed with 2 mL of DPPH solution and incubated under the same conditions. The antioxidant activity was expressed as the % of DPPH inhibition and as ascorbic acid equivalent per gram of dry sample (mg AAE/g). All measurements were performed in triplicate.

Statistical analysis

All experimental analyses were conducted in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). Data were statistically analysed using one-way analysis of variance (ANOVA) to determine significant differences among the treatment groups. When significant differences were detected ($p < 0.05$), the means were compared using Tukey's Honestly Significant Difference (HSD) post hoc test. Pearson correlation and simple linear regression analyses were used to evaluate relationships between antioxidant activity and phytochemical contents. Statistical analyses were performed using IBM SPSS Statistics software (version 25, IBM Corp., Armonk, NY, USA).

Results

RWC of Java arabica coffee leaves at different tree ages and leaf stages

RWC serves as a key indicator of plant water balance, reflecting the equilibrium between water uptake and transpiration. The RWC values obtained in this study demonstrate the physiological water status of Java arabica coffee leaves at different tree ages and leaf maturities. As shown in Fig. 1, the RWC of young tree-young leaves

(A) was highest at 7.5 %, followed by mature tree-mature leaves (D) at 6.5 %, mature tree-young leaves (C) at 6.4 % and young tree-mature leaves (B) at 5.7 %. Values are presented as mean $\pm$ SD from triplicate measurements.

Phenolic content

Phenolic compounds are key antioxidants in plants due to their hydroxyl groups, which provide radical-scavenging activity (27). Total phenolic content, measured using gallic acid equivalents (GAE), varied significantly ($p < 0.05$) across the four-leaf types (Fig. 2). The highest phenolic content was observed in young tree-young leaves (A) at 3.2 mg GAE/g. Young tree-mature leaves (B) and mature tree-young leaves (C) showed intermediate values of 2.7 mg GAE/g each, while mature tree-mature leaves (D) had the lowest content at 1.9 mg GAE/g.

Flavonoid content

Flavonoids, a class of polyphenols, contribute to plant antioxidant capacity and exhibit diverse biological effects, including anti-inflammatory and anti-carcinogenic properties (28). The total flavonoid content, expressed as quercetin equivalents (QE), followed a similar to phenolics (Fig. 3). The highest concentration (18.5 mg QE/g) was observed in A, while B, C and D showed lower values of approximately 13 mg QE/g.

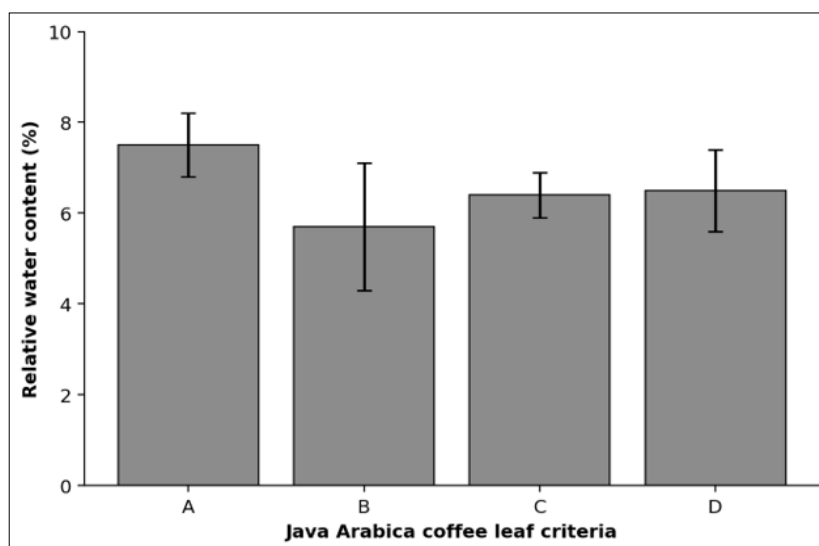


Fig. 1. Relative water content of Java arabica coffee leaves under different criteria. A: young trees-young leaves; B: young trees-mature leaves; C: mature trees-young leaves; D: mature trees-mature leaves. Values are presented as mean $\pm$ SD ($n = 3$).

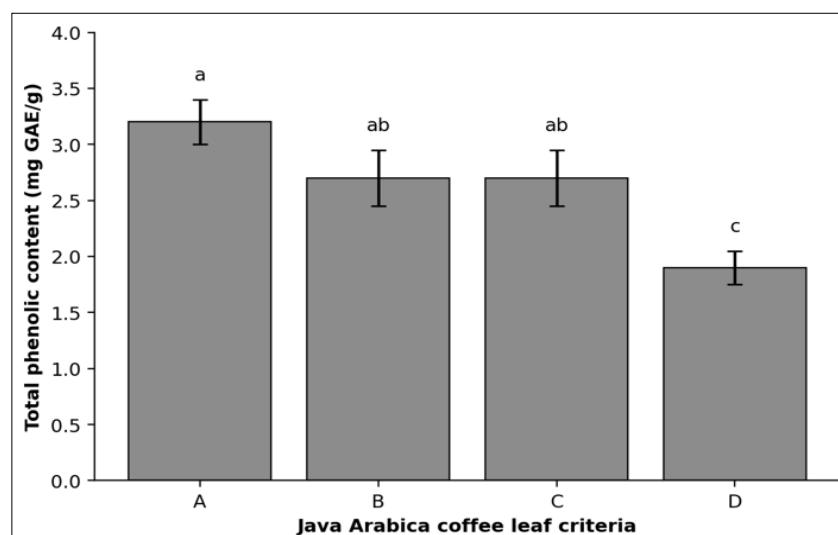


Fig. 2. Total phenolic content of leaves as affected by tree age and leaf age of Java Arabica coffee. A: young trees-young leaves; B: young trees-mature leaves; C: mature trees-young leaves; D: mature trees-mature leaves. Different letters on the bars represent significant differences ($p < 0.05$).

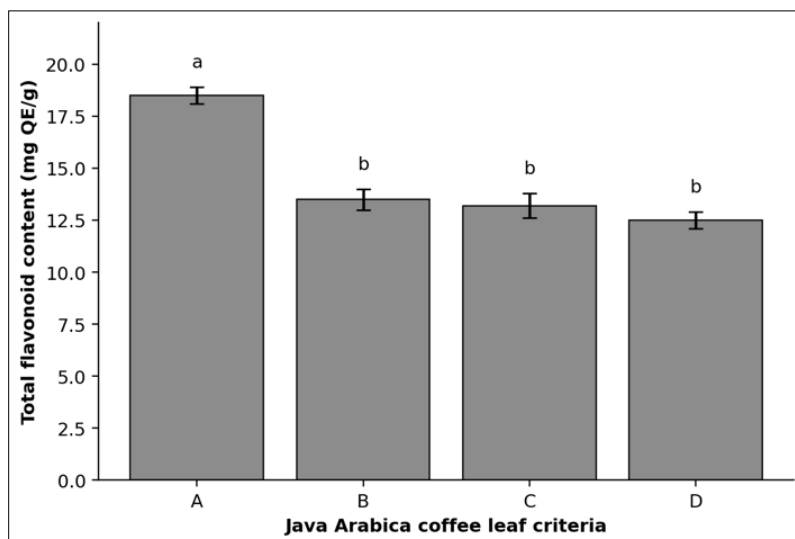


Fig. 3. Total flavonoid content of Java Arabica coffee leaves under different criteria. A: young trees-young leaves; B: young trees-mature leaves; C: mature trees-young leaves; D: mature trees-mature leaves. Different letters on the bars represent significant differences ($p < 0.05$).

Tannin content

Tannins contribute to plant defense by reducing digestibility and exhibiting antimicrobial, anti-mutagenic, and antioxidant properties (29, 30). Total tannin content, expressed as micrograms tannic acid equivalent per gram dry sample ($\mu\text{g TAE/g}$), showed an inverse trend compared to phenolics and flavonoids (Fig. 4). The lowest value was found in A (725 $\mu\text{g TAE/g}$), while B, C and D showed higher contents, with the highest value in sample C (approximately 1100 $\mu\text{g TAE/g}$). The unit was revised from mg TAE/g to $\mu\text{g TAE/g}$ because the previous unit could be misinterpreted as exceeding the sample weight.

Total antioxidant activity

The antioxidant potential of the coffee leaf extracts was quantified as ascorbic acid equivalents, revealing patterns consistent with phenolic and flavonoid contents. Fig. 5 shows that sample A exhibited the highest antioxidant activity (0.54 mg AAE/g), followed by samples B (0.43 mg AAE/g), C (0.40 mg AAE/g) and D (0.28 mg AAE/g), with significant differences among treatments ($p < 0.05$).

Correlation between antioxidant activity and phytochemical content

The relationship between antioxidant activity and phenolic and flavonoid content was examined using regression analysis (Fig. 6). Antioxidant activity was strongly correlated with total phenolic content ($R^2 = 0.981$) and moderately correlated with flavonoid content ($R^2 = 0.855$), indicating that phenolic compounds were the dominant contributors to antioxidant capacity in the investigated leaf samples.

Discussion

Coffee leaves are known to maintain relatively high moisture levels because of structural adaptations, including coriaceous leaf surfaces and efficient stomatal regulation (31). Water content in plant tissues plays a critical role in sustaining cellular metabolism and facilitating the transport of secondary metabolites, including antioxidant compounds. Accordingly, the variation in relative water content (RWC) observed among samples in this study may reflect differences in physiological maturity and leaf anatomical characteristics. While

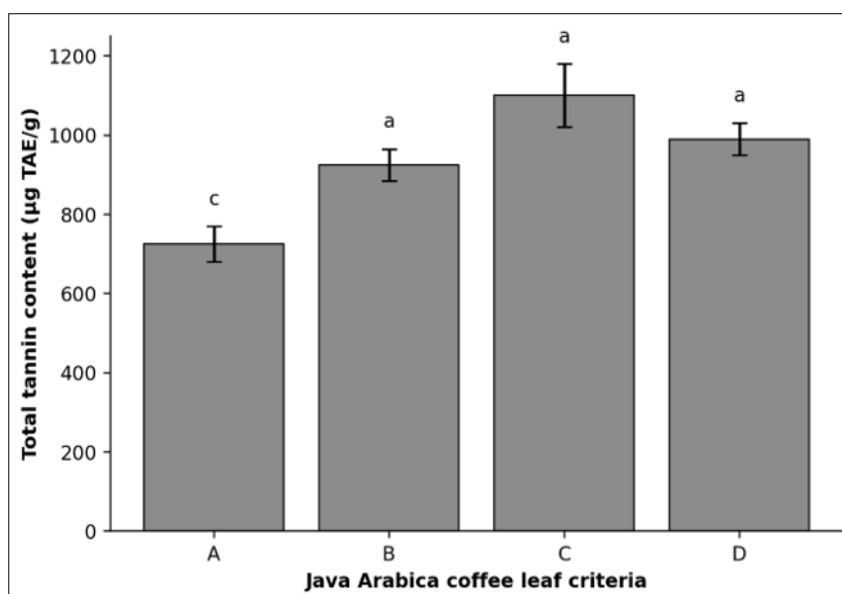


Fig. 4. Total tannin content of Java Arabica coffee leaves under different criteria. Values are expressed as μg tannic acid equivalent per gram dry sample ($\mu\text{g TAE/g}$). A: young trees-young leaves; B: young trees-mature leaves; C: mature trees-young leaves; D: mature trees-mature leaves. Different letters on the bars represent significant differences ($p < 0.05$).

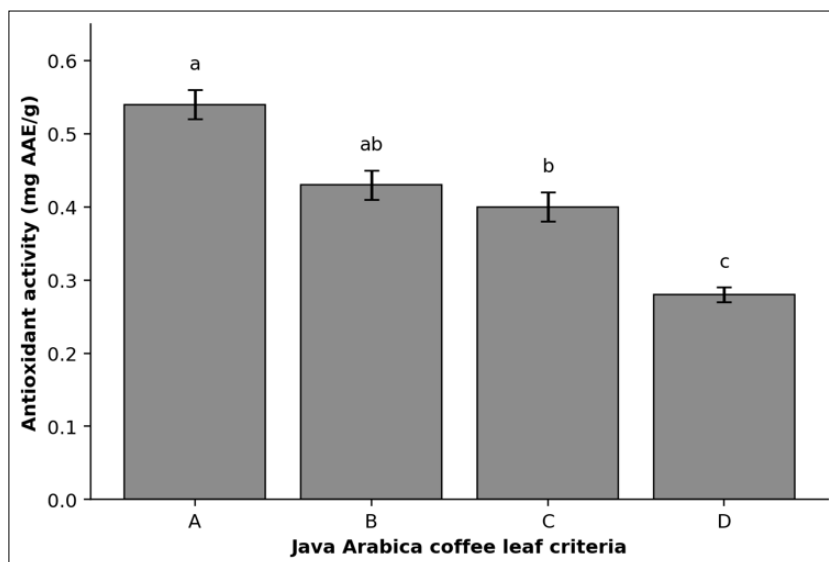


Fig. 5. Antioxidant activity of Java Arabica coffee leaves under different criteria. A: young trees-young leaves; B: young trees-mature leaves; C: mature trees-young leaves; D: mature trees-mature leaves. Different letters on the bars represent significant differences ($p < 0.05$).

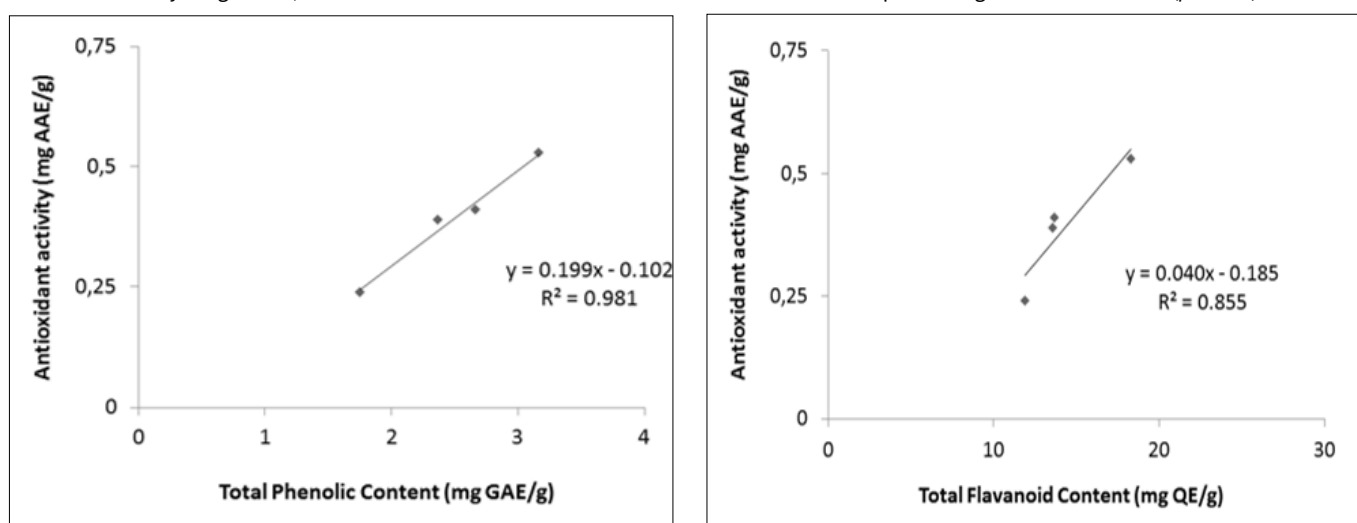


Fig. 6. Correlation of antioxidant activity with phenolic and flavonoid contents of coffee leaf extracts. a: Total phenolic content ($R^2 = 0.981$); b: Total flavonoid content ($R^2 = 0.855$)

water deficit conditions are often associated with the induction of secondary metabolite biosynthesis as part of stress adaptation mechanisms (32), adequate tissue hydration supports metabolic activity during active growth phases. Thus, RWC serves as a useful physiological indicator for interpreting differences in antioxidant-related metabolic potential among coffee leaf samples.

Antioxidant capacity in plants is largely governed by the accumulation of secondary metabolites, particularly phenolic compound, flavonoids and tannins, which neutralise oxidative agents through hydrogen donation, electron transfer and metal ion chelation. In this context, the present study evaluated the phytochemical composition and antioxidant activity of Java arabica coffee leaves at different growth stages. The significantly higher phenolic content observed in sample A was likely attributable to intensified biosynthetic activity in young, metabolically active tissues (33). Enhanced photosynthetic activity in young coffee trees may promote the synthesis of both pigmented phenolics, such as anthocyanins and non-pigmented phenolic compounds (34). Phenolic accumulation is strongly regulated by developmental stage and light exposure, underscoring its role in photoprotection and oxidative stress mitigation during early growth (34, 35). In addition to developmental regulation, nitrogen availability plays a crucial role in phenolic biosynthesis. Young tissues generally exhibit higher

nitrogen and amino acid concentrations, which provide essential precursors for the phenylpropanoid pathway (36). This metabolic advantage supports greater phenolic accumulation in early growth stages. Similarly, flavonoid content is influenced by plant age, tissue maturity and environmental conditions (37). Young leaves, characterised by elevated nitrogen assimilation and biosynthetic activity, tend to accumulate higher flavonoid levels than mature tissues (38, 39). In coffee plants, nitrate reductase activity, an enzyme closely associated with nitrogen metabolism, is expected to be higher in young leaves and to decline with leaf maturation, thereby influencing flavonoid synthesis (40). Beyond their antioxidant function, flavonoids also play a photoprotective role by attenuating light-induced oxidative stress in developing tissues (41).

In contrast to phenolics and flavonoids, tannin content exhibited an inverse trend, with higher levels detected in more mature leaf samples. This observation is consistent with previous findings indicating that tannins preferentially accumulate in older tissues (42). As carbon availability increases during tissue aging, metabolic flux may be redirected towards the synthesis of carbon-rich compounds such as tannins (43). Unlike many allelochemicals, tannins function primarily as digestibility reducers and are known to increase with tissue maturity, contributing to herbivore deterrence and long-term structural defence (44). The elevated tannin content

in mature coffee leaves may therefore reflect their role in sustaining prolonged defensive capacity rather than immediate antioxidant protection. These phytochemical patterns were reflected in the antioxidant activity results, which demonstrated a strong correlation between phenolic and flavonoid content and overall antioxidant capacity (45, 46). Moreover, the antioxidant efficacy of these compounds is primarily attributed to their hydroxyl groups and molecular structures, which enable efficient scavenging of reactive oxygen species (47). While flavonoids contribute substantially to antioxidant activity, the stronger linear association with total phenolic content suggests that phenolic compounds served as the principal determinants of antioxidant potential in Java arabica coffee leaves (48, 49). Other bioactive constituents, including caffeine, trigonelline, saponins and tocopherols, may exert synergistic effects that further enhance antioxidant performance (50, 51), although their individual contributions warrant further investigation.

Compared with previous research that has mainly emphasised coffee beans, agronomic traits, or general coffee leaf bioactivity, the present study contributes by identifying leaf maturity and tree age as practical factors that influence the antioxidant-related phytochemical profile of Java Arabica leaves. This information is relevant for selecting leaf material for beverage, functional food and nutraceutical development. However, the study remains limited by its reliance on conventional colorimetric assays and DPPH-based antioxidant screening. These assays provide useful preliminary evidence but do not identify individual bioactive molecules. Future studies should incorporate HPLC, LC-MS/MS, or GC-MS profiling, additional antioxidant assays such as ABTS, FRAP and ORAC, and a broader sampling design across seasons, altitudes and coffee genotypes (52, 53).

Overall, these findings highlight the critical influence of leaf developmental stage and tree maturity on phytochemical composition and antioxidant capacity, suggesting that young leaves from younger Java arabica coffee trees represent the most promising raw material for functional food and nutraceutical applications.

Conclusion

The antioxidant properties of Java arabica coffee leaves were closely associated with their phenolic, flavonoid and tannin contents. Young leaves from young trees exhibited the highest levels of phenolic compounds and flavonoids, which were the main contributors to antioxidant activity. In contrast, tannin content tended to increase with leaf maturity and tree age, suggesting a shift in metabolic allocation toward defense-related compounds. These findings demonstrate that tree maturity and leaf developmental stage should be considered when selecting coffee leaves as a source of natural antioxidants. Practically, young leaves from young Java Arabica trees may serve as a promising raw material for functional beverages, functional food ingredients and nutraceutical development. Future research should verify the dominant antioxidant compounds using advanced chromatographic techniques, evaluate safety and sensory acceptability and assess the stability of the extract during processing and storage.

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Authors' contributions

BSS was responsible for conducting the study, analysing the data statistically, rechecking the data, writing the article, tables and figures. SS was responsible for directing the study and advising contents of the manuscript. RR was responsible for advising the flow of the experiment and assisting during experiment. AML and AK were responsible for advising the contents of the article, formatting the article in accordance with journal guidance, correcting the grammar and arranging the flow of the manuscript. MRK were responsible for advising on the contents of the article, formatting the article in accordance with journal guidelines, correcting the grammar and arranging the flow of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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