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Original Research Article

A Comparative Study of Phenolic Compounds, and the Antioxidant Properties of Arabica Coffee Beans (*Coffea arabica*) and Robusta Coffee Beans (*Coffea canephora*) and Their By-Products

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ABSTRACT

Coffee, the second most consumed beverage in the world produces considerable quantities of coffee grounds, a brewing by-product that has a considerable impact on the environment. this study, the chemical composition and antioxidant properties of Arabica and Robusta coffee varieties and their coffee grounds were compared. Extracts were prepared by aqueous and hydroalcoholic maceration, and analyzed for total and reducing sugars, proteins, polyphenols, flavonoids, tannins, and antioxidant activity. the analysis revealed significant disparities between the samples. Robusta SCGs had the highest total sugar content (111.92 ± 0.95 mg Glu/g DW), while Arabica SCG contained higher levels of reducing sugars and proteins (24.65 ± 0.39 mg Glu/g DW and 85.81 ± 1.54 mg BSA/g DW, respectively). Robusta and its SCGs were particularly rich in polyphenols (CR-H = 342.73 ± 11.40; SCGR-H = 275.66 ± 0.54 mg GAE/g DW), flavonoids, and tannins. Antioxidant tests confirmed Robusta SCG's superior activity with TAC (460.8 ± 8.5 mMAAE/g), FRAP (0.236 ± 0.01 mg/mL), and DPPH radical scavenging (IC50 = 0.115 ± 0.00 mg/mL). further HPLC-DAD analysis revealed distinct phenolic profiles, Robusta SCG being enriched in hydroxybenzoic acids and flavonol glycosides such as rutin and quercetin-3-*O*-glucoside. These results indicate the viability of spent coffee grounds, particularly Robusta coffee, as a sustainable source of valuable bioactive compounds that possess substantial antioxidant properties.

Keywords: Spent coffee grounds, primary metabolites, secondary metabolites, antioxidant activity, High-Performance Liquid Chromatography.

Introduction

Coffee's scientific name, *Coffea*, refers to a member of the Rubiaceae family. These plants are classified as shrubs or small trees and are native to tropical and southern Africa, as well as tropical Asia. Coffee is famous for its high caffeine content, an ingredient that has largely contributed to its worldwide popularity.[1] Coffee is the world's second most-traded commodity, only surpassed by oil.[2] The two most dominant species in coffee production and consumption are: *Coffea arabica* var. *arabica* represents around 70% of world production, while *Coffea canephora* var. *robusta*, is less expensive, has a stronger flavor, and higher levels of antioxidants, caffeine, and soluble solids [3] World coffee production rose by a modest 0.1% in coffee year 2022/23, reaching around 168.2 million 60-kilogram bags, according to the International Coffee Organization (ICO). In 2023/24, production is expected to rise by 5.8% to around 178 million bags.

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Growth is due to increased Arabica (102.2 million bags) and Robusta (75.8 million bags) production.[4] However, this expansion has resulted in significant waste production, in particular spent coffee grounds (SCG), which are the main by-product of coffee consumption. without proper management, coffee grounds can contribute to environmental pollution.[5] Around 650 kilograms of SCG are generated per ton of green coffee processed, and one kilogram of soluble coffee can produce almost two kilograms of wet spent coffee grounds.[6] SCGs include residual materials left over after the preparation of soluble, brewed, or pressed coffee, which are generated both industrially and domestically worldwide.[7] Recent studies have shown that coffee residues, in particular SCG, are rich in bioactive compounds, including polyphenols (e.g., As indicated by the extant literature, a wide array of chemical constituents, including chlorogenic acid, flavonoids, and protocatechuic acid, as well as proteins, oils, and sugars, have been identified in SCG extracts.[8,9] These chemical constituents have been shown to possess a range of biological activities, including antioxidant, antiviral, anti-inflammatory, and antibacterial effects[10–12] The valorization of coffee by-products is attracting growing interest worldwide. A study[13] was conducted to compare ultrasound-assisted extraction (UAE) and conventional extraction (CE) methods for the recovery of natural polyphenols from spent coffee grounds (SCG). This study highlighted the potential of these extracts as cosmetic additives or dietary supplements. meanwhile, another study[14] focused on the encapsulation of crude antioxidant extracts from cold-infused SCG, demonstrating their stability under simulated food processing and gastrointestinal conditions.[15] In addition to their biological properties, SCG can also be transformed into biofuels and activated carbon, which can serve as compost additives in agriculture, due to their rich mineral content.[2,16,17]

furthermore, these materials show promise for the development of food and health-related applications.[18] The exploration of such industrial applications could facilitate the purification of compounds extracted from coffee grounds for therapeutic studies and offer significant financial support for future research.[18,19] However, the impact of extraction methods on the recovery of bioactive secondary metabolites from Arabica and Robusta coffee grounds is not well understood, despite the potential benefits of such research. The present study aims to explore the still underexploited potential of coffee grounds (SCG) by evaluating the effect of two types of aqueous and hydroalcoholic extraction carried out by cold maceration on the yields of polyphenols, flavonoids, and tannins. The antioxidant activity of the extracts was evaluated using commonly used tests such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, total antioxidant capacity (TAC), and ferric reducing antioxidant power (FRAP). The objective of this study is to improve the biochemical understanding of SCGs and to propose innovative methodologies for their sustainable use, with potential applications in the agri-food, nutraceutical, and environmental fields.

Materials and Methods

Chemicals

Ascorbic acid (sharlau, spain) , caffeic acid (Sigma-Aldrich, USA), dinitrosalicylic acid (Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), sulfuric acid (H_2SO_4) (Sigma-Aldrich, USA), trichloroacetic acid (TCA) (Oxford Lab Fine Chem LLP, India), Catechin (Sigma-Aldrich, USA), Coomassie Blue G250 (Fisher Scientific Belgium) , aluminum chloride (AlCl3) (Sigma-Aldrich, USA), ferric chloride ($FeCl_3$) (Oxford Lab Fine Chem LLP, India), DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich, USA), ethanol (Solvachim, Morocco), Folin-Ciocalteu (Oxford Lab Fine Chem LLP, India), Phenol (Solvachim, Morocco), potassium ferricyanide [$K_3Fe(CN)_6$] (Solvachim, Morocco), glucose (Merck KGaA, Germany), sodium hydroxide (NaOH) (Sigma-Aldrich, USA), methanol (Solvachim, Morocco), ammonium molybdate (Panreac, Spain), sodium nitrate ($NaNO_3$) (Sigma-Aldrich, USA), monobasic sodium phosphate (NaH_2PO_4) (Sigma-Aldrich, USA), quercetin (Oxford Lab Fine Chem LLP, India), dibasic sodium phosphate (Na_2HPO_4) (Sigma-Aldrich, USA).

Plant collection and Identification

Two coffee species, *Coffea arabica* and *Coffea canephora* (Robusta), in addition to their respective spent coffee grounds, were utilized as plant materials in this study. The coffee beans were obtained from the local market and subsequently ground and pressed to yield the spent grounds. The plant material was collected on September 25, 2024, from sites with coordinates 34°40'50.4"N, 1°54'32.2"W. Voucher specimens of the two *Coffea* species were deposited in the herbarium of the Oujda Faculty of Science, and the accession numbers assigned were HUMPOM787 (*Coffea canephora*) and HUMPOM788 (*Coffea arabica*). To prevent microbial degradation during storage, the coffee grounds were steamed at 30°C for 48 hours until a constant weight was achieved. After drying, they were stored in opaque containers, kept in the dark at room temperature.

Extraction process of coffee

In this study, four distinct samples were examined: The materials used in the experiment included Arabica coffee, Arabica spent coffee grounds, Robusta coffee, and Robusta spent coffee grounds. The samples were selected meticulously to facilitate a comprehensive evaluation of their chemical composition and essential properties. In order to avert degradation during the drying and storage processes, the samples were placed in an oven set at 40°C. The extraction process utilized a maceration of 10 g of each sample in two distinct solvents. A hydroalcoholic extract was obtained through the use of a mixture of methanol and distilled water at a volume ratio of 80:20. The aqueous extract was derived from distilled water. Each extraction was executed over the course of 24 hours under consistent conditions. The resulting extracts were then subjected to desiccation via oven drying at 30°C. Subsequently, the samples were stored at 4°C in darkness to facilitate subsequent analysis.

Analysis of primary metabolites

Total sugars

In test tubes, 200 µL of a 5% (w/v) phenol solution was added to 200 µL of each sample (1 mg/mL), followed by 1 mL of 96% sulfuric acid (H_2SO_4). The mixtures were vortexed thoroughly and incubated at 100 °C for 5 minutes. Tubes were then cooled in the dark for 30 minutes. Absorbance was measured at 490 nm using a Jenway 7315 spectrophotometer (Jenway, Cole-Parmer Ltd., UK). Total sugar content was quantified using a glucose calibration curve. All experiments were conducted in triplicate.[20]

Reducing sugars

According to the DNS method, 500 µL of dinitrosalicylic acid reagent and 500 µL of the 1 mg/mL test solution mixed with either 500 µL of distilled water (control) or the test solution were placed in test tubes. The tubes were then incubated in a water bath at 100 °C for five minutes and immediately cooled in ice water. During cooling, 5 mL of distilled water was added to each tube to stop the reaction. After reaching room temperature, the absorbance was measured at 540 nm, and the reducing sugar content was calculated using a standard glucose calibration curve.[21]

Proteins

Protein extraction was achieved by mixing 100 mg of powdered sample with 3 mL of 1 M NaOH.[22] The mixture was heated at 90 °C for 15 minutes, then cooled. Protein content was determined using the Bradford assay, in which 200 µL of extract was mixed with 2 mL of Coomassie Brilliant Blue G-250 reagent. Absorbance was measured at 595 nm against a blank (0.2 mL NaOH + 2 mL reagent). The intensity of the blue color correlates with protein concentration, which was determined using a bovine serum albumin (BSA) calibration curve.[23]

Analysis of secondary metabolites

Determination of Total Phenolic Content (TPC)

Total phenolics were determined using the Folin–Ciocalteu method. A 0.2 mL aliquot of each extract was mixed with 1 mL of 0.2N Folin–Ciocalteu reagent and left for 5 minutes. Then, 0.8 mL of sodium carbonate solution was added. After 1 hour of incubation at room temperature, absorbance was measured at 760 nm against a blank. Total phenolic content was expressed as mg gallic acid equivalents (GAE) and mg caffeic acid equivalents (CAE) per gram of extract, based on standard calibration curves.[24]

Determination of total flavonoid content (TFC)

A mixture of 0.05 mL sodium nitrite (5% w/v) and 1 mL of distilled water was added to 0.4 mL of each extract (1 mg/mL) in test tubes. After a five-minute incubation, 0.12 mL of aluminum chloride (10% w/v) was added, followed by an additional five minutes of incubation. Subsequently, 0.4 mL of sodium hydroxide (1 M) was introduced and mixed thoroughly. The absorbance of each solution was then measured at 430 nm against a blank.[25] The flavonoid content was expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g), based on a calibration curve constructed using quercetin as the standard.

Determination of condensed tannins

A volume of 0.05 mL of each extract was mixed with 1.5 mL of 4% vanillin in methanol and vortexed. Then, 0.75 mL of hydrochloric acid was added. The mixture was incubated at room temperature for 20 minutes. Absorbance was measured at 550 nm. The results were expressed as milligrams of catechin equivalents per gram of extract.[24]

Evaluation of antioxidant activity

Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity was assessed using the phosphomolybdenum method.[26] One milliliter of phosphomolybdenum reagent (containing 28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate) was added to 1 mL of the extract solution (1 mg/mL). The mixture was incubated at 95°C for 90 minutes. After

cooling to room temperature, absorbance was measured at 695 nm against a blank. All tests were performed in triplicate, and the results were expressed as ascorbic acid equivalents.

Determination of Ferric reducing power test (FRAP)
Each extract (0.5 mL) was mixed with 1.25 mL of 0.2 M phosphate buffer (pH 6.6) and 1.25 mL of 1% potassium ferricyanide (w/v). The mixture was incubated at 50°C for 20 minutes, then 1.25 mL of 10% trichloroacetic acid was added to stop the reaction. After centrifugation at 3000 rpm for 10 minutes, 1.25 mL of the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL of 0.1% ferric chloride (w/v). Absorbance was read at 700 nm using ascorbic acid as the standard and distilled water as the blank.[27] Each measurement was carried out in triplicate.

Determination of the free radical-scavenging activity (DPPH)
The DPPH radical scavenging activity was evaluated using a methanolic DPPH solution (4 mg/100 mL). Extracts were prepared at eight concentrations ranging from 0.05 to 1 mg/mL. For each concentration, 0.2 mL of the extract was added to 1.8 mL of DPPH solution. The mixtures were incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm against a blank. All experiments were performed in triplicate.[24]

The percentage of DPPH radical scavenging activity was calculated using the following equation1

$$\% \text{ Radical Scavenging activity} = \frac{(\text{Abs Control} - \text{Abs sample})}{\text{Abs Control}} \times 100 \quad \text{……equation. 1}$$

HPLC Analysis of Phenolic Compounds
High-performance liquid chromatography (HPLC) was performed according to a modified protocol.[28] Analyses were conducted using a Waters Alliance™ e2695 system equipped with a photodiode array detector (PDA Waters 2996) and a P680 Socratic pump. Phenolic compounds were separated on a C18 column (250 × 4 mm, 5 µm) using a gradient elution with solvent A (water with 0.5% acetic acid) and solvent B (methanol) at a flow rate of 1 mL/min.

Statistical analysis
Principal component analysis (PCA), Pearson correlation, and analysis of variance (ANOVA) were among the statistical methods used to analyze the data. SPSS version 25 for Windows was used for this analysis. At a 5% significance level, mean comparisons were conducted using the Tukey test. The mean ± SEM is used to express data.

Results and Discussion

Analysis of primary metabolites
Total sugars, reducing sugars, and proteins
The quantification of total sugars was carried out using a phenol-based method, with the results expressed in mg Glu/g DW. As demonstrated in Figure 1(a), a significant difference was observed among the extracts ($p < 0.001$). The analysis revealed that Arabica coffee extract (CA) had the highest total sugar content (139.52 ± 0.07 mg Glu/g DW), followed by Robusta coffee extract (CR) with 119.35 ± 0.08 mg Glu/g DW. Conversely, spent coffee grounds showed a significant decrease in sugar levels: SCG-A (Arabica) and SCG-R (Robusta) contained 105.89 ± 1.27 and 111.92 ± 0.95 mg Glu/g DW, respectively.
These results indicate a significant decrease in soluble sugars during the brewing process, likely due to partial extraction by hot water. Reducing sugars, measured using the dinitrosalicylic acid (DNS) method (Figure 1(b)), displayed a distinct pattern. Robusta coffee extract exhibited the highest reducing sugar content (33.26 ± 0.49 mg Glu/g DW), surpassing that of Arabica (27.02 ± 0.47 mg Glu/g DW). In contrast, spent grounds showed lower concentrations, with SCG-A and SCG-R having 24.65 ± 0.39 and 19.71 ± 0.18 mg Glu/g DW, respectively. These results are in agreement with previous findings,[2] who reported significant variability in reducing sugar levels based on coffee variety, geographical origin, and processing methods. During brewing, the partial leaching of sugars from SCGs is strengthened by the marked decrease in reducing sugars, which underscores the value of variety in shaping the residual sugar profile. The protein content, measured in milligrams of bovine serum albumin equivalents per gram of dry weight (mg BSA/g DW), was highly variable among the samples ($p < 0.001$) (Figure 1(c)).

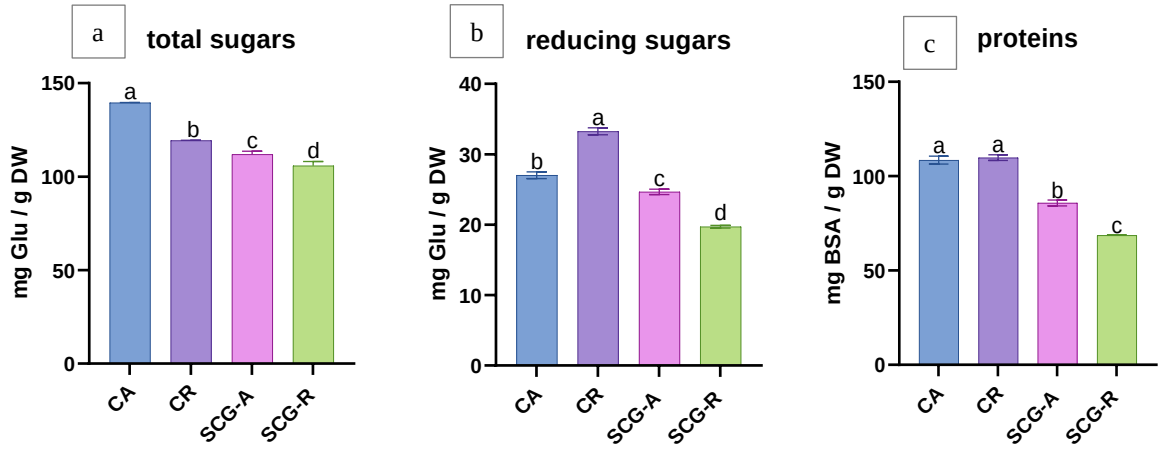

Figure 1: the total sugars (a), reducing sugars (b), and protein (c) content in spent coffee grounds (SCG) and coffee (C). Values marked with different letters indicate significant differences ($P < 0.05$), while values with the same letter show no significant difference ($P>0.05$).

The Robusta coffee extract had the highest protein concentration (109.81 ± 1.54 mg BSA/g DW), followed closely by the Arabica variety (108.51 ± 2.11 mg BSA/g DW). The spent coffee grounds had significantly lower protein levels, with SCG-A at 85.81 ± 1.54 mg BSA/g DW and SCG-R at only 68.70 ± 0.07 mg BSA/g DW. These results are in agreement with the predicted studies,[29,30] who reported a wide range of protein recoveries from coffee by-products. The reductions observed in total sugars, reducing sugars, and proteins in spent coffee grounds compared to raw beans are mainly due to the hot water extraction process, which removes a substantial portion of soluble compounds. However, the remaining levels, particularly of proteins, remain relatively high, indicating that SCGs hold significant nutritional potential. This residual wealth supports their use in functional food and nutraceutical applications. SCGs proteins may be involved in formulations that aim to reduce liver dysfunction, oxidative stress, and cardiovascular disease, as previously documented in previous research.[31] The comparison between Arabica and Robusta varieties indicates that Robusta generally has higher levels of reducing sugars and proteins, while Arabica is richer in total sugars. The selection of coffee types for specific valuation strategies can be influenced by these varietal differences. It is important to use SCG according to the

compositional characteristics of each coffee variety, as highlighted by the results.

Analysis of secondary metabolites

Total Phenolic Content (TPC), total flavonoid content (TFC), and condensed tannins

Coffee beans and their by-products, particularly spent coffee grounds (SCG), contain polyphenols, which are important plant secondary metabolites.[25,32] The Folin–Ciocalteu assay, a widely used method for estimating total phenolic content (TPC) content, despite its limited for phenolic structures, was used in this study to evaluate the TPC of various extracts.[17] The TPC of the eight samples tested varied significantly ($p < 0.001$), ranging from 221.27 ± 1.62 to 342.73 ± 11.40 mg GAE/g DW (Figure 2a). Robusta coffee's hydroalcoholic extract (CR-H) had the highest concentration, followed by its aqueous extract (CR-Aq).In contrast, the aqueous extract of spent Arabica coffee grounds (SCGA-Aq) showed the lowest TPC (221.27 ± 1.62 mg GAE/g DW). These results align with previous studies, which reported phenolic contents ranging from 45.68 to 291.86 mg GAE/g DW. Further research found values of 57.49 and 398.95 mg GAE/g DW in SCG samples. The superior performance of hydroalcoholic extracts compared to aqueous ones ($p < 0.001$) indicates that methanol improves the solubility of phenolics compounds by reducing the polarity of the solvent. Ethanol-water mixtures have been proven to extract a wider variety of phenolic compounds, particularly those with low to medium polarity. In comparison, one study using ultrasound-assisted extraction with 60% ethanol reported the highest phenolic content in Arabica SCGs (941.04 ± 37 mg GAE/g), followed by Robusta SCGs (547.51 ± 59 mg GAE/g).

These results highlight the impact of the extraction technique and solvent system on phenolic recovery.[37] Overall, Robusta coffee and its by-products have been shown to have higher levels of total phenolic content (TPC) in comparison to Arabica. This finding provides further evidence for the idea that Robusta beans tend to contain higher concentrations of chlorogenic acids and related phenolic compounds.[35,38] The differences observed in total phenolic content (TPC) are probably attributable to genotypic variation, in particular the presence of a more active phenylpropanoid biosynthesis pathway in Robusta.[39] However, when the phenolic content was expressed as caffeic acid equivalents (Figure 2b), lower values were obtained (175.91–262.87 mg CAE/g DW). This variation can be attributed to the structural differences between gallic and caffeic acids, which affect their molar absorptivity in the Folin–Ciocalteu reaction. As well as polyphenols, the total flavonoid content (TFC) showed significant variation between samples ($p < 0.001$) (Figure 2c). The hydroalcoholic extracts of Robusta coffee (CR-H) and its spent grounds (SCGR-H) demonstrated the highest TFC values (35.10 ± 0.33 and 30.19 ± 0.59 mg QE/g DW, respectively). This result indicates that methanol facilitates the dissolution of flavonoid glycosides and aglycones, as previously reported.[36] Due to their reduced polarity compared to many phenolic acids, flavonoids necessitate solvents of intermediate polarity for efficient extraction. A literature comparison reveals significant variation in TFC. A number of studies reported lower values (2.11–8.03 mg QE/g DW),[30,40] while others presented considerably higher concentrations, particularly in Arabica SCGs (78.21 ± 24 mg QE/g).[37]such discrepancies are attributed to differences in extraction protocols, coffee variety, roasting level, and storage conditions. Specifically, the roasting process has been shown to induce Maillard reactions and phenolic degradation, which have been shown to decrease extractable flavonoids levels.[39,41]

Tannin content showed significant variability ($p < 0.001$) (Figure 2d). The hydroalcoholic extract of Robusta spent grounds (SCGR-H) exhibited the highest tannin concentration (71.76 ± 2.42 mg CAE/g DW). Due to their high molecular weight and polymeric nature, tannins are more effectively solubilized in ethanol-rich solvents. In addition, hydroalcoholic conditions have been shown to facilitate the extraction of condensed tannins, which are prevalent in coffee tissues.[42] As previous research has shown, there are significant varietal differences. For instance, Arabica varieties Minas (8.33 ± 0.00 mg/g) and Cioccolatato (1.74 ± 0.00 mg/g) showed significant disparities in tannin content.[42]

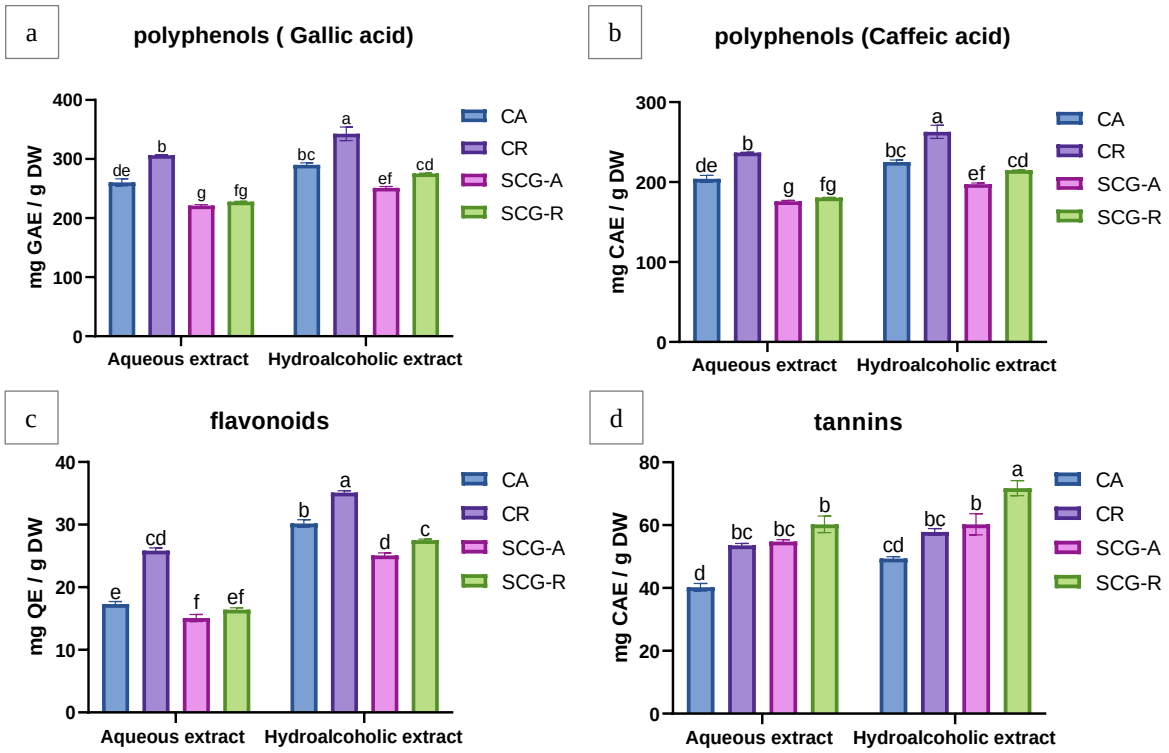

Figure 2: Total polyphenols (a), flavonoids (b), and tannins (c) content in spent coffee grounds (SCG) and coffee (C) for both aqueous and hydroalcoholic extracts. Values marked with different letters indicate significant differences ($P < 0.05$), while values with the same letter show no significant difference ($P>0.05$).

Similarly, Robusta varieties Vietnam (7.94 ± 4.70 mg/g) and Cherry (19.66 ± 3.20 mg/g) showed lower levels, potentially attributable to variations in extraction methods, bean ripeness, and geographical origin. It is important to note that, compared with Arabica, Robusta varieties tend to accumulate higher concentrations of tannins and lignin.[39]

The variability noted in the literature can be attributed to several factors. Firstly, the intensity of the roasting process is very important, since polyphenols are sensitive to heat. I High levels of roasting have been shown to frequently result in substantial decreases in total polyphenol content and total flavonoid content, caused by thermal degradation.[39,43] Secondly, phenolic recovery efficiency is significantly influenced by extraction conditions, including solvent composition, duration, temperature and particle size.[36] Thirdly, intrinsic characteristics of the samples, including bean genotype, agricultural practices, and post-harvest processes (e.g., fermentation, drying), also affect the final phenolic profile.[39] Finally, methodological limitations must be acknowledged. The Folin-Ciocalteu test is particularly problematic in this regard due to its lack of specificity, which results in reactions with various reducing agents (e.g., ascorbic acid, sugars).

This risk of cross-reactivity can lead to an overestimation of total polyphenol content.[44] Furthermore, SCGs have been found to contain high levels of carbohydrates, particularly cellulose and hemicellulose, which have the potential to inhibit the extraction of phenolics compounds by trapping them within the fiber matrix. However, it has been demonstrated that ethanol can more effectively disrupt these matrices than water, which explains the improved recovery observed in hydroalcoholic extracts.[45]From an applied perspective, the high levels of polyphenols, flavonoids, and tannins found in SCGs underscore their potential as bioactive ingredients. Polyphenols have garnered significant attention from the food, cosmetic, and pharmaceutical industries due to their well-documented antioxidant, antibacterial, and anti-inflammatory properties.[46,47] Furthermore, the valorization of SCGs supports circular economy strategies by transforming waste materials into valuable bioresources, thereby contributing to environmental sustainability[48–50]

Evaluation of antioxidant activity

Determination of Total Antioxidant Capacity (TAC)

The determination of phosphomolybdenum assay was used to evaluate the total antioxidant capacity. This method is based on the reduction of molybdate to Mo(V), which forms green Mo(V) complexes. The analysis revealed significant variations in antioxidant capacity between the different extracts ($p < 0.001$) (Table 1). Overall, hydroalcoholic extracts showed higher antioxidant activity than aqueous extracts, probably due to the increased solubility of phenolic compounds in alcohol-water mixtures, as reported in previous studies.[43,51]

Table 1: Total antioxidant capacity of spent coffee grounds (SCG) and coffee (C) for extracts (aqueous and hydroalcoholic). Values marked with different letters indicate significant differences ($P < 0.05$), while values with the same letter show no significant difference ($P>0.05$).

TAC	CA-Aq	CR-Aq	SCGA-Aq	SCGR-Aq	CA-H	CR-H	SCGA-H	SCGR-H
mMAAE/g	307.3± 6.7^{e}	325.9± 9.8^{de}	326.6± 7.0^{de}	366.6± 7.0^{bcd}	336.9± 5.8^{cde}	371.6± 5.2^{bc}	396.6± 15.7^{b}	460.8± 8.5^{a}

Extracts of exhausted spent coffee grounds showed higher antioxidant capacities than the coffee bean extracts for both solvents evaluated. The analysis revealed that the hydroalcoholic extract of Robusta coffee grounds (SCGR-H) exhibited the highest antioxidant activity (460.8 ± 8.5 mMAAE/g), while the aqueous extract of Arabica coffee (CA-Aq) demonstrated the lowest activity level (307.3 ± 6.7 mMAAE/g). such results are consistent with previous reports indicating that Robusta coffee generally contains higher levels of antioxidant compounds than Arabica, mainly due to higher concentrations of caffeine and chlorogenic acid.[52] Furthermore, the higher antioxidant activity observed in coffee grounds compared to fresh coffee beans corroborates previous studies highlighting that spent coffee grounds retain significant amounts of polyphenols and melanoidins formed during roasting, both of which contribute strongly to antioxidant properties.[31]

The use of hydroalcoholic solvents significantly improved the recovery of antioxidant compounds, as alcohol facilitates the extraction of polar and moderately non-polar phenolics.[40] This explains the consistently higher antioxidant capacity observed in hydroalcoholic extracts compared to aqueous extracts for all sample types. The solvent system and the matrix type of matrix (spent coffee grounds or coffee beans) therefore have a decisive influence on total antioxidant activity, underlining the potential of used coffee grounds as a sustainable source of natural antioxidants for functional applications. The use of hydroalcoholic solvents significantly increased the recovery of antioxidant compounds. This is attributable to the fact that alcohol facilitates the extraction of both polar and moderately non-polar phenolics.[40] Consequently, this finding explains the consistently higher antioxidant capacity observed in hydroalcoholic extracts compared to aqueous extracts, for all sample types. Therefore, the solvent system and the type matrix (spent coffee grounds or coffee beans) have been shown to exert a substantial influence on total antioxidant activity. The results underline the potential of spent coffee grounds as a sustainable source of natural antioxidants for functional applications.

Determination of Ferric reducing power test (FRAP)

Figure 3 illustrates that antioxidant activity increased proportionally with extract concentration for all samples. The data, presented as OD_{50} values (mg/mL), indicate the concentration necessary to achieve an optical density of 0.5.

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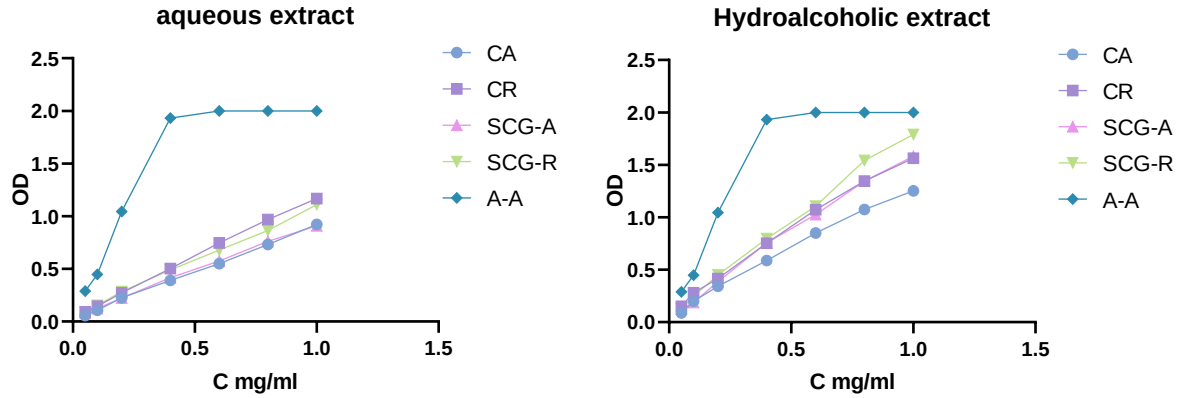

Figure 3: FRAP assay: Optical density versus concentration for (a) Aqueous Extract and (b) Hydroalcoholic

The hydroalcoholic extract of Robusta coffee grounds (SCGR-H) exhibited the highest antioxidant activity, as demonstrated by the lowest OD_{50} value of 0.236 ± 0.01 mg/mL, suggesting a strong capability for free radical reduction. Conversely, the aqueous extract of Arabica coffee (CA-Aq) showed the weakest antioxidant activity among the hydroalcoholic extracts, with an OD_{50} of 0.536 ± 0.00 mg/mL, likely reflecting the limited solubility of bioactive compounds in water. For aqueous extracts, Robusta coffee (CR-Aq) displayed the highest antioxidant activity (OD_{50} = 0.394 ± 0.00 mg/mL), while the Arabica aqueous extract (CA-Aq) again exhibited the lowest activity (OD_{50} = 0.536 ± 0.00 mg/mL), similar to its hydroalcoholic counterpart. Notably, all extract activities were significantly lower than that of ascorbic acid, the reference standard, which had an OD_{50} of 0.103 ± 0.00 mg/mL (Table 2).

Table 2: FRAP inhibitory concentration of extracts in mg/mL.

FRAP	CA-Aq	CR-Aq	SCGA-Aq	SCGR-Aq	CA-H	CR-H	SCGA-H	SCGR-H	A-A
OD 0,5 mg/mL	0.536 ± 0.0^{e}	0.394 ± 0.0^{d}	0.501 ± 0.01^{e}	0.411 ± 0.01^{d}	0.320 ± 0.02^{c}	0.251 ± 0.02^{bc}	0.266 ± 0.02^{bc}	0.236 ± 0.01^{b}	0.103 ± 0.00^{a}

These OD_{50} results demonstrate that hydroalcoholic extracts are more effective free radical scavengers than aqueous extracts. This can be explained by the ability of hydroalcoholic solvents to dissolve a broader spectrum of antioxidant compounds, including polyphenols, flavonoids, and other lipophilic molecules that are less soluble in pure water.[43,51]Additionally, coffee grounds exhibited higher antioxidant activity than coffee beans, which may be due to the presence of bioactive compounds that are not fully extracted during brewing and thus remain concentrated in the grounds.[31] This trend is especially pronounced in Robusta coffee grounds, which appear richer in phenolic compounds and flavonoids than their Arabica counterparts. FRAP assay results ranged from 0.236 ± 0.01 to 0.536 ± 0.00 mg/mL, confirming the superior antioxidant power of hydroalcoholic extracts over aqueous ones. Among coffee types, Robusta showed the highest antioxidant capacity in both beans and grounds, with values of 0.251 ± 0.02 mg/mL and 0.236 ± 0.01 mg/mL, respectively. These findings are consistent with earlier studies reporting greater antioxidant activity in Robusta coffee grounds compared to Arabica, with values ranging from 26 to 38 mg TE/g sample.[52] Other research similarly found that Robusta and Liberica coffee varieties exhibit higher antioxidant activity in Fe(III) reduction assays relative to Arabica coffee.[44]

Determination of the free radical-scavenging activity (DPPH)

As illustrated in Figure 4, the inhibitory effect of the extracts on DPPH radicals is concentration-dependent. The level of antioxidant activity increased with concentration for all samples examined, with statistically significant differences observed between aqueous and hydroalcoholic extracts. The half maximal inhibitory concentration (IC_{50}) was calculated for each extract, as well as for ascorbic acid, which was utilized as a reference standard. In comparison to aqueous extracts, hydroalcoholic extracts exhibited higher free radical scavenging activity.

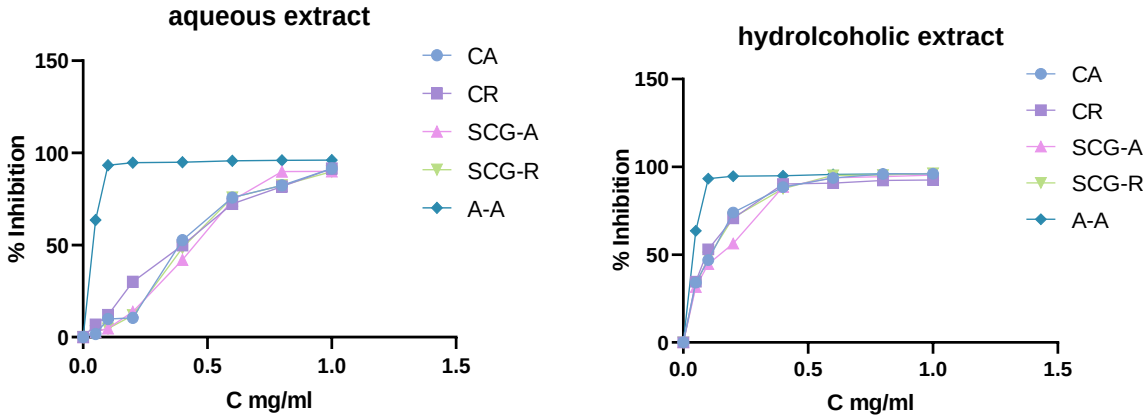

Figure 4: DPPH Antioxidant Activity and evolution of optical density as a function of concentration: (a) Aqueous Extract and (b) Hydroalcoholic Extract

The hydroalcoholic extract of Robusta coffee (CR-H) exhibited the most significant activity, with an IC_{50} value of 0.108 ± 0.00 mg/mL, indicating the possibility of elevated concentrations or enhanced accessibility of radical-scavenging compounds. Conversely, the hydroalcoholic extract of spent Arabica coffee grounds (SCGA-H) exhibited the lowest antioxidant activity among the hydroalcoholic extracts, with an IC_{50} of 0.139 ± 0.01 mg/mL. Among the aqueous extracts, Robusta coffee (CR-Aq) demonstrated the highest activity (IC_{50} = 0.387 ± 0.02 mg/mL), while spent Arabica coffee grounds (SCGA-Aq) exhibited the lowest (IC_{50} = 0.433 ± 0.00 mg/mL). It is noteworthy that the IC_{50} values of all the extracts were higher than that of pure ascorbic acid (IC_{50} = 0.053 ± 0.00 mg/mL) (Table 3), indicating the superior radical-scavenging efficiency of the standard antioxidant.

Table 3: DPPH inhibitory concentration of extracts in mg/mL.

DPPH	CA-Aq	CR-Aq	SCGA-Aq	SCGR-Aq	CA-H	CR-H	SCGA-H	SCGR-H	A-A
IC_{50} mg/mL	0.421±0.02^{c}	0.387± 0.02^{c}	0.433± 0.0^{c}	0.431± 0.02^{c}	0.111± 0.0^{ab}	0.108± 0.0^{ab}	0.139± 0.01^{b}	0.115±0.0^{ab}	0.053± 0.0^{a}

These results are consistent with previous findings that hydroalcoholic extraction is more efficient for isolating antioxidant compounds from plant materials. Solvents such as ethanol and methanol, when mixed with water, facilitate the solubility of phenolics and flavonoids, resulting in enhanced antioxidant potential in extracts. This enhancement is attributed to the broader polarity range of hydroalcoholic solvents, which facilitates the extraction of both hydrophilic and moderately lipophilic bioactives. [53] Furthermore, coffee extracts generally showed higher antioxidant activity than coffee grounds. Nevertheless, spent coffee grounds, particularly from Robusta, still demonstrated notable radical scavenging ability. The DPPH scavenging activity of Robusta coffee and its grounds was significantly higher, with IC_{50} values of 0.108 ± 0.00 mg/mL and 0.115 ± 0.00 mg/mL, respectively. Arabica coffee and its grounds exhibited slightly lower activities (IC_{50} = 0.111 ± 0.00 mg/mL and 0.139 ± 0.01 mg/mL, respectively). The higher antioxidant activity exhibited by Robusta samples may be attributed to their elevated content of polyphenols and other electron-donating molecules. These molecules possess the capacity to stabilize free radicals by donating electrons or hydrogen atoms.[54] A statistically significant difference was observed between Robusta and Arabica extracts ($p < 0.05$), which is consistent with previous reports indicating that Robusta generally possesses a more pronounced phenolic profile.[51] This observation aligns with the results of earlier studies, which demonstrated that extraction methods, particularly the use of ethanol and methanol mixtures, have a significant impact on the antioxidant potency of extracts.[55] Methanol-water mixtures (typically 80/20) are recognized for their superior efficiency in extracting antioxidant compounds compared to pure water, primarily due to the enhanced solubility of phenolic and flavonoid compounds in organic solvents. A multitude of studies have substantiated the antioxidant properties of coffee grounds. The reported antioxidant activities ranged from 1.56% to 92.12% for DPPH, from 41.44 to 93.94 mg TE/g for FRAP, and from 2.11 to 2.22 mmol TE/g for ABTS assays. These results demonstrate the strong radical-scavenging ability of spent coffee materials.[25] Comparative studies between Soxhlet extraction and decoction techniques revealed that Soxhlet extraction yields superior antioxidant activity (588 µg/mL and 367 µg/mL by DPPH and FRAP assays, respectively). The observed antioxidant activities can be attributed largely to the abundance of secondary metabolites in coffee grounds, particularly polyphenols, flavonoids, and tannins, which exhibit strong positive correlations with radical scavenging activity.

HPLC Analysis of Phenolic Compounds

The chromatographic analysis enabled the identification and quantification of 18 phenolic and flavonoid compounds in both aqueous (Aq) and hydroalcoholic (H) extracts of Arabica (CA) and Robusta (CR) coffee beans, as well as their respective spent grounds (SCGA and SCGR) (Table 4, Figure 5). The identified compounds included phenolic acids, such as gallic acid (GA), caffeic acid (CAF), 3-hydroxybenzoic acid (HBA3), 4-hydroxybenzoic acid (HBA4), syringic acid (SA), vanillic acid (VA), p-coumaric acid (PCA), cinnamic acid (CIN), ferulic acid (FA), and sinapic acid (SINA); Flavonoids (FLA), rutin (RUT), quercetin (QUE), quercetin 3-*O*-*β*-D-glucoside (Q3G), kaempferol (KAE), and flavone (FLA); as well as other derivatives, such as naringin (NAR).

A comparison of the two types of extracts revealed that hydroalcoholic extracts exhibited consistently higher concentrations of bioactive compounds compared to aqueous extracts. For instance, the SCGA-H extract contained 35.5% 3-hydroxybenzoic acid (HBA3), 27.72% sinapic acid (SINA), and 6.43% quercetin-3-*O*-*β*-D-glucoside (Q3G), whereas these compounds were present at substantially lower levels in the SCGA-Aq extract. This difference is attributed to the enhanced solubility of moderately polar phenolic compounds in hydroalcoholic solvents, as documented in previous studies.[56] At the varietal level, Arabica coffee was characterized by higher amounts of hydroxycinnamic acids (caffeic acid, ferulic acid, and p-coumaric acid), while Robusta coffee and its spent grounds were richer in complex flavonoids such as rutin, quercetin 3-*O*-*β*-D-glucoside (Q3G), and kaempferol. Notably, the hydroalcoholic extract of Robusta spent grounds (SCGR-H) showed remarkable levels of 3-hydroxybenzoic acid (17.35%), sinapic acid (22.86%), rutin (9.22%), and quercetin 3-O-*β*-D-glucoside (6.24%) (Table 4).

These high-performance liquid chromatography (HPLC) results align well with previous research examining the phenolic profiles of roasted coffee and its by-products. The significantly higher flavonoid content in SCGA-H and SCGR-H extracts confirms the superior extraction efficiency of hydroalcoholic solvents for flavonoids, as demonstrated in earlier investigations.[57] The efficacy of water/ethanol mixtures in extracting intermediate polarity metabolites likely accounts for the enhanced recovery of bioactive compounds observed here. Elevated levels of 3-hydroxybenzoic acid, rutin, and quercetin 3-*O*-*β*-D-glucoside further reinforce the potential of coffee residues as valuable sources of bioactive molecules, consistent with prior studies.[51] Collectively, these findings, along with previous reports[18,43], emphasize the richness of phenolic compounds in coffee residues, highlighting the promising application of spent coffee grounds as functional ingredients with high antioxidant potential. Moreover, positive correlations observed between the concentrations of quercetin 3-*O*-*β*-D-glucoside, rutin, and 3-hydroxybenzoic acid and the total antioxidant capacity (TAC) support earlier evidence that glycosylated flavonoids exhibit potent free radical scavenging activity, largely due to their hydroxylated structures.[58] Additionally, the compositional differences between Arabica and Robusta samples, notably the richer antioxidant profile of Robusta, corroborate earlier findings attributing greater antioxidant complexity to Robusta coffee.[37] The absence of detectable chlorogenic acid in the analyzed samples can be attributed to multiple factors. The primary finding of this study is that thermal degradation during the roasting process significantly reduces chlorogenic acid concentrations. This reduction leads to the breakdown of chlorogenic acid into smaller phenolic compounds and melanoidins. Furthermore, the selection of extraction solvent may have influenced chlorogenic acid recovery, given its high polarity and affinity for aqueous phases. The possibility of co-elution with other compounds or concentrations below the detection limit should be considered as a potential contributor to the observed results.

Table 4: Chromatographic Analysis of Phenolic Compounds in Arabica and Robusta Coffee and Their Spent Coffee Grounds

Compounds	abbreviation	Molecular Formula	Retention time (Tr) (min)	CA-Aq	CR-Aq	SCGA-Aq	SCGR-Aq	CA-H	CR-H	SCGA-H	SCG-H
Gallic acid	GA	$C_7H_6O_5$	1,896	0,97	0,06	-	0,09	-	-	-	-
Catechin	CAT	$C_{15}H_{14}O_6$	2,348	-	-	-	-	0,12	-	-	-
Caffeic acid	CAF	$C_9H_8O_4$	2,63	-	1,3	1,87	0,25	1,08	0,74	0,71	0,23
4-Hydroxybenzoic acid	HBA4	$C_7H_6O_3$	2,815	0,41	1,97	3,19	0,86	1,06	0,55	-	-
Catechin hydrate	CATH	$C_{15}H_{14}O_6 \cdot H_2O$	3,937	1,18	-	-	-	6,43	6,58	-	-
Syringic acid	SA	$C_9H_{10}O_5$	4,403	13,32	11,77	16,06	10,26	8,94	4,69	14,3	7,12
Vanillic acid	VA	$C_8H_8O_4$	5,855	0,3	12,13	1,26	11,76	-	-	-	7,91
3-Hydroxybenzoic acid	HBA3	$C_7H_6O_3$	6,288	15,13	21,64	13,28	21,75	36,14	29,35	35,5	17,35
Naringin	NAR	$C_{27}H_{32}O_{14}$	6,706	30,5	6,41	29,83	7,23	6,69	7,26	8,64	8,8
Cinnamic acid	CIN	$C_9H_8O_2$	8,053	4,03	-	5,28	-	-	-	-	-
Ferulic acid	FA	$C_{10}H_{10}O_4$	8,59	3,4	8,59	5,26	10,02	5,64	7,89	5,12	8,28
p-Coumaric acid	PCA	$C_9H_8O_3$	9,837	30,78	33,14	-	-	-	8,72	-	8,7
Sinapic acid	SINA	$C_{11}H_{12}O_5$	10,032	-	-	23,81	33,08	26,9	22,46	27,72	22,86
Quercetin 3-O-*β*-D-glucoside	Q3G	$C_{21}H_{20}O_{12}$	11,097	-	-	-	-	3,86	4,67	4,3	6,24
Rutin	RUT	$C_{27}H_{30}O_{16}$	11,907	-	1,95	-	3,33	1,5	3,57	0,92	5,33
Quercetin	QUE	$C_{15}H_{10}O_7$	12,214	-	0,55	-	-	1,21	1,2	1,21	2,07
Kaempferol	KAE	$C_{15}H_{10}O_6$	12,574	-	0,49	0,17	1,37	-	2,33	0,95	2,27
Flavone	FLA	$C_{15}H_{10}O_2$	13,33	-	-	-	-	0,43	-	0,66	2,86

These observations are consistent with the findings of prior studies that reported substantial chlorogenic acid degradation in roasted coffee and related by-products.[7] The results of this study demonstrate the viability of Robusta coffee grounds as a sustainable and valuable source of natural antioxidants for applications in the nutraceutical and cosmetic industries. This potential is particularly pronounced when the grounds are extracted with hydroalcoholic solvents.

Principal Component Analysis (PCA)

The principal Component Analysis (PCA) was employed to integrate data from phenolic compound concentrations and antioxidant activity assays, including DPPH, FRAP, TAC, total flavonoids, and total polyphenols. The initial two principal components, PC1 and PC2, accounted for 44.93% and 17.33%, respectively, of the total variance, explaining a cumulative 62.26%. As demonstrated in Figure 6 (A and B), PC1 effectively differentiated simple phenolic compounds (e.g., gallic acid, cinnamic acid) and antioxidant assays (DPPH, FRAP) from glycosylated flavonoids (quercetin 3-*O*-*β*-D-glucoside, rutin, kaempferol, and flavone). These compounds exhibited a positive correlation with TAC and total flavonoid content. PC2 further distinguished the samples based on their content of 3-hydroxybenzoic acid, total polyphenols, and flavonoids. Hydroalcoholic extracts (SCGA-H, SCGR-H) clustered with higher concentrations of glycosylated flavonoids, 3-hydroxybenzoic acid, and elevated TAC values, indicating their richer antioxidant profiles. In contrast, aqueous extracts (CA-Aq, CR-Aq) were more closely associated with simple phenolic acids and moderate antioxidant activity, reflecting the lower extraction efficiency of water compared to hydroalcoholic solvents. Correlation analyses demonstrated that higher levels of polyphenols and flavonoids were inversely correlated with IC_{50} values, indicating enhanced antioxidant potential. Conversely, elevated IC_{50} values were associated with lower concentrations of these compounds. A negative correlation was also observed between DPPH and FRAP activities and tannin content, suggesting that high tannin concentrations may negatively impact antioxidant efficiency. These trends may be attributed to the specific phenolic profiles of the extracts, potential interactions with other phytochemicals, or synergistic effects. Variations from literature values in phenolic, flavonoid, and tannin contents may stem from differences in roasting degree, extraction procedures, geographical origin, soil characteristics, coffee variety, climate conditions, and agricultural practices.[59] Overall, PCA revealed distinct clustering patterns among the different coffee and spent coffee ground extracts, underscoring the influence of extraction method, sample matrix, and coffee variety.

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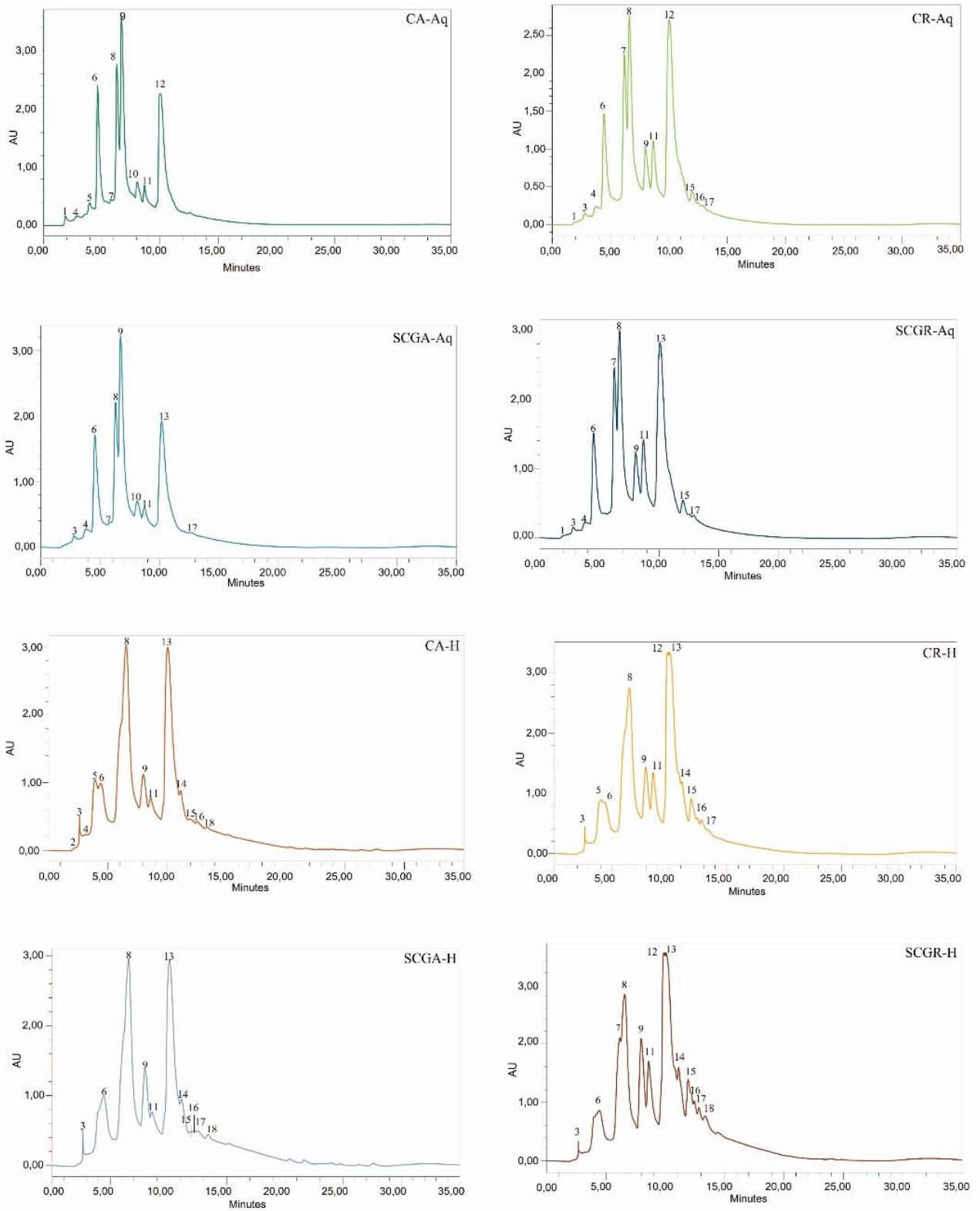

Figure 5: HPLC-DAD chromatograms of aqueous and hydroalcoholic extracts of Arabica coffee (CA), Robusta coffee (CR), spent coffee grounds of Arabica (SCGA), and spent coffee grounds of Robusta (SCGR)

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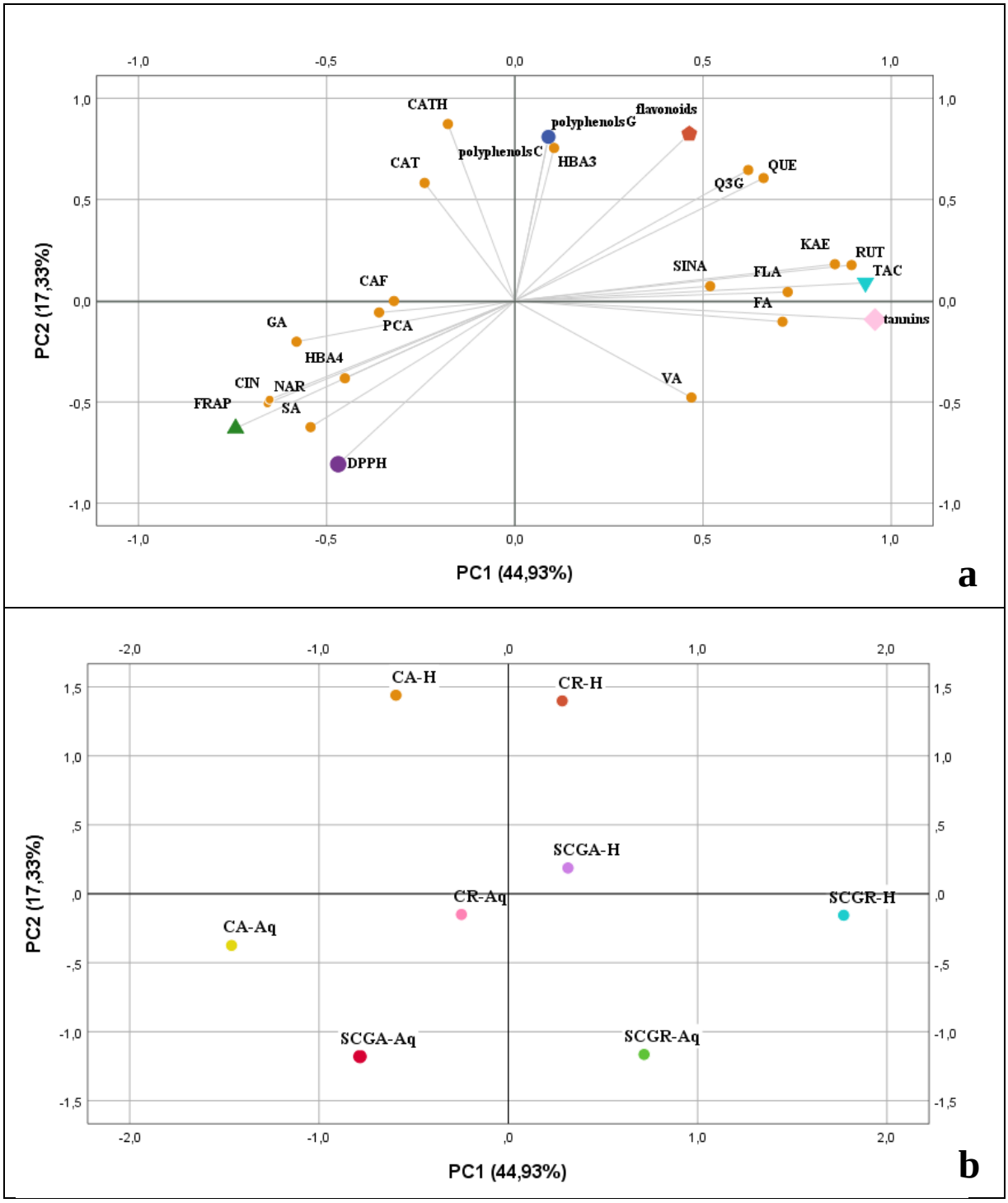

Figure 6: Graphical representation of Principal Component Analysis (PCA) of different phenolic compounds and antioxidant activities (a) and sample distribution (b).

These insights further support the potential valorization of spent coffee residues as sustainable sources of bioactive compounds for applications in the food and nutraceutical industries.

Statistical Correlation

A statistical correlation analysis was conducted to examine the relationships between phenolic compound content and antioxidant activities (Table 5). A significant correlation was identified at two levels of significance ($p < 0.01$ and $p < 0.05$). For total antioxidant capacity (TAC), a weak positive correlation was found with total polyphenol content ($r = 0.064$) and a moderate positive correlation with total flavonoid content ($r = 0.480$, $p < 0.05$). It is important to note that the content of tannins exhibited a significant positive correlation with TAC ($r = 0.849$, $p < 0.01$), suggesting their pivotal role in enhancing antioxidant performance. The ferric reducing antioxidant power (FRAP OD_{50}) and the half-maximal inhibitory concentration of DPPH radical scavenging activity (DPPH IC_{50}) showed negative correlations with polyphenol content ($r = –0.541$, $p < 0.01$ and $r = –0.489$, $p < 0.05$, respectively) and with flavonoid content ($r = –0.845$, $p < 0.01$).

Table 5: Correlation between phenolic compounds and antioxidant activity

	polyphenols	flavonoids	tannins
TAC	0.064	0.480*	0.849**
DPPH	-0.541**	-0.845**	-0.414*
FRAP	-0.489*	-0.849**	-0.659**

**. The correlation is statistically significant at the 0.01 level (two-tailed).
*. The correlation is statistically significant at the 0.05 level (two-tailed).

These inverse correlations suggest a potential correlation between higher levels of polyphenols and flavonoids and stronger antioxidant activity, as indicated by lower IC_{50} and OD_{50} values. Furthermore, a significant and negative correlation was observed between tannin content and both DPPH IC_{50} ($r = –0.849$, $p < 0.01$) and FRAP OD_{50} ($r = –0.659$, $p < 0.01$). Additionally, a moderate correlation was identified between tannin content and FRAP activity ($r = –0.414$, $p < 0.05$). These findings underscore the significant role of tannins in enhancing the antioxidant efficacy of the extracts.

Conclusion

The present study set out to compare the biochemical composition and antioxidant capacity of Arabica and Robusta beans, as well as their spent grounds. It was demonstrated that Robusta spent grounds extracted with a hydroalcoholic solvent exhibited the highest antioxidant activity. The analysis, performed using HPLC-DAD, led to the identification of distinct phenolic profiles, with particular emphasis on hydroxybenzoic acids and flavonol glycosides, including rutin and quercetin-3-*O*-glucoside, in Robusta samples. A clear correlation was identified between phenolic content and antioxidant capacity. The bioactive profiles were found to be significantly influenced by the botanical origin and extraction solvent. The utilization of spent coffee grounds, which are frequently disposed of, as a source of functional biomolecules has been demonstrated. These compounds hold considerable promise for application in the food, cosmetic, and pharmaceutical industries. The study promotes the sustainable reuse of coffee waste in accordance with the principles of the circular economy.

Conflict of interest

The author declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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