ORIGINAL PAPER

CHEMICAL PROFILING AND ANTIOXIDANT ASSESSMENT OF TWO ARABICA COFFEE VARIETIES

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Abstract. *This study provides a comparative chemical and antioxidant characterization of two Arabica coffee varieties, one originating from the Dominican Republic and one processed as a multicomponent blend in Romania. Qualitative analyses confirmed the presence of alkaloids and caffeine in all samples. Spectrophotometric determinations revealed that hot-water infusions exhibited higher total polyphenol and flavonoid contents compared to cold-brew extracts. Heavy-metal analysis indicated low levels of cadmium and lead, demonstrating satisfactory sanitary quality. Antioxidant evaluation showed strong radical-scavenging activity in the DPPH assay (88.11–88.98%) with minimal variation between extraction methods. In contrast, the FRAP assay highlighted significant differences influenced by both geographical origin and extraction technique, with reducing capacities ranging from 868 to 1062 µmol Fe^{2+}/g. These findings underline the substantial antioxidant potential of Arabica coffee and emphasize the combined role of origin-dependent phytochemical variability and extraction parameters in determining its functional properties.*

Keywords: *Arabica coffee; antioxidant capacity; phytochemical profiling; polyphenolic content; extraction methods.*

1. INTRODUCTION

Coffee is one of the most widely traded agricultural commodities and among the most consumed beverages globally, valued not only for its distinctive aroma, flavor, and stimulant properties but also increasingly for its antioxidant potential [1]. As interest in health-promoting diets continues to grow, coffee has attracted significant scientific attention due to its rich phytochemical profile and its capacity to act as a dietary source of bioactive compounds. The rising demand for functional foods has stimulated the development of novel coffee-based products and preparation techniques, each offering a unique chemical composition and sensory expression [2].

Although caffeine is the most extensively studied constituent of coffee due to its neuromodulatory and pharmacological effects, the beverage contains numerous other bioactive compounds that may contribute significantly to its biological profile. Classes such as alkaloids, phenolics, and flavonoids have been widely investigated for their antitumor, antioxidant, and anti-inflammatory properties, with several plant-derived molecules known to

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modulate microtubule function or induce apoptosis in cancer cells [3-5]. These findings suggest that the bioactivity of coffee results from the combined action of multiple phytochemicals rather than caffeine alone, reinforcing the need to evaluate how extraction methods influence the overall phytochemical composition.

Coffee extraction is a complex process influenced by multiple parameters, including applied pressure, extraction method, brewing equipment, and the final beverage volume. Among pressure-based techniques, espresso represents one of the most recognized and appreciated methods. Its name, derived from Italian, underscores the defining characteristics of this preparation style—rapid extraction and immediate consumption—attributes that contribute to its intense flavor and concentrated profile [6]. Consumer preference for particular coffee preparations is shaped by cultural, lifestyle, economic, and sensory factors, as well as by increasing public awareness of the chemical composition and potential health impacts of coffee constituents.

Over the past decades, extensive research has expanded our understanding of the chemical, sensory, and health-related properties of coffee across its entire value chain, from raw beans to the brewed beverage. Several studies have examined specific extraction methods—most notably filter coffee, espresso, mocha, and French press—revealing substantial variations in caffeine, chlorogenic acids, diterpenes, and other bioactive molecules [7]. However, comprehensive comparative analyses covering multiple popular extraction techniques remain relatively limited. Notable contributions include the work of López-Galilea et al. [8], who investigated the antioxidant capacity of multiple brewing methods, and Peters [9], who evaluated the chemical composition of several extraction techniques without incorporating sensory analysis. Such studies highlight the need for integrated investigations that assess chemical, functional, and health-relevant attributes across different extraction methods and coffee origins.

In light of these considerations, the present study aims to investigate the bioactive composition of coffee beverages through physicochemical analyses involving qualitative reactions and spectrophotometric methods. Two distinct extraction techniques—hot-water infusion and cold brew—were applied to Arabica coffees originating from different geographical regions. Additionally, the content of heavy metals was assessed due to their potential toxicological impact, using graphite furnace atomic absorption spectrometry to quantify cadmium (Cd) and lead (Pb). Through this comparative approach, the study seeks to elucidate the influence of extraction method and origin on antioxidant capacity, phytochemical composition, and safety-related parameters.

2. MATERIALS AND METHODS

2.1. MATERIALS

Two *Coffea arabica* samples were selected for this study. The first originated from the Dominican Republic (D), cultivated under tropical pedoclimatic conditions and processed locally. The second was a Romanian commercial product (R), produced from imported Arabica beans roasted and packaged in Romania. Each sample was obtained as a sealed 250 g package and opened immediately before extraction to prevent oxidative alterations. The two coffee samples included in the analysis are shown in Figure 1.

(a) **(b)**

Figure 1. Arabica coffee: (a) Coffee from the Dominican Republic; (b) Romanian-produced coffee.

2.2. METHODS

2.2.1. Sample Preparation and Extraction

Two extraction techniques were applied to each coffee type, yielding four samples in total.

Hot-water infusion: The infusion protocol followed a French Press–like method based on prolonged contact between ground coffee and hot water. For each extract, 7 g of ground coffee was weighed into a heat-resistant beaker and mixed with 100 mL of water heated to 95 ± 2°C. The mixture was gently stirred and allowed to infuse for 5 minutes, then filtered through qualitative filter paper. The filtrate was transferred to a 100 mL volumetric flask and made up to volume. The resulting samples were labeled D1 (Dominican Arabica) and R1 (Romanian-market Arabica).

Cold brew extraction: Cold brew extracts were obtained by macerating 7 g of ground coffee with 100 mL of deionized water at room temperature (21 ± 2°C) for 24 hours. After maceration, the mixtures were filtered to remove insoluble material, and the clear filtrates were adjusted to 100 mL in volumetric flasks. These samples were designated as D2 (Dominican Arabica) and R2 (Romanian-market Arabica).

2.2.2. Preliminary Chemical Characterization

Qualitative Identification of Alkaloids: Alkaloids were qualitatively screened using classical precipitation reactions, which rely on the formation of insoluble complexes between alkaloids and specific reagents. Two commonly employed reagents (Dragendorff's and Mayer's solutions), were used due to their high sensitivity toward plant-derived alkaloids [10,11]. Dragendorff's reagent was prepared from acidic potassium iodide and bismuth nitrate solutions, while Mayer's reagent consisted of aqueous mercuric chloride and potassium iodide. For each test, a small volume of coffee extract was mixed with the reagent; the appearance of an orange (Dragendorff) or cream-colored (Mayer) precipitate indicated a positive result, confirming the presence of alkaloidal compounds.

Qualitative Identification of Caffeine: Caffeine, a purine alkaloid characteristic of coffee, was identified through its reaction with tannic acid, as described in the Romanian Pharmacopoeia (10th edition) [12]. A volume of 2 mL of coffee extract was treated with an

aqueous 10% tannic acid solution. The formation of a white insoluble precipitate confirmed the presence of caffeine, resulting from the formation of a stable caffeine–tannin complex. This reaction served as a preliminary qualitative indicator of caffeine in the analyzed samples.

2.2.3. Total Phenolic Content

The total polyphenol content (TPC) of the coffee extracts was determined using the Folin–Ciocalteu spectrophotometric method with a UV–VIS spectrophotometer (UV-3100PC) [13,14]. The assay was performed using Folin–Ciocalteu reagent (diluted 1:10), sodium carbonate solution (7.5% g/v), purified water, and gallic and caffeic acids as calibration standards. For analysis, 1 mL of each extract (D1, D2, R1, R2) was diluted to 10 mL with purified water, and 1 mL of the resulting solution was mixed, in triplicate, with 4.5 mL ultrapure water and 2.5 mL Folin–Ciocalteu reagent. After 2 minutes, 2 mL of sodium carbonate solution was added, and the reaction mixtures were incubated for 60 minutes at room temperature. Absorbance was recorded at 765 nm (gallic acid) and 748 nm (caffeic acid), using ultrapure water as a blank.

The TPC was quantified using calibration curves constructed with gallic acid and caffeic acid standards. The gallic acid calibration curve showed linearity ($R^2 = 0.9986$) and followed the regression equation:

$$y = 0.0137x + 0.0416 \quad (1)$$

while the caffeic acid curve showed an $R^2 = 0.9952$ and followed the equation:

$$y = 0.0118x + 0.0973 \quad (2)$$

In both equations, x represents the standard concentration (µg/mL), and y corresponds to the absorbance measured at the specific wavelength. Total polyphenol content was expressed as milligrams of gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW) and milligrams of caffeic acid equivalents per 100 g dry weight (mg CAE/100 g DW).

2.2.4. Total Flavonoid Content

The total flavonoid content (TFC) of the coffee extracts was determined according to the method described in the *Cynarae folium* monograph of the Romanian Pharmacopoeia, 10th Edition [15]. Each extract was initially diluted 1:10 (v/v) with purified water, and 10 mL of the diluted solution was transferred into 25 mL volumetric flasks and brought to volume with methanol. The mixtures were shaken for 2–3 minutes, allowed to stand for 10 minutes, and subsequently filtered. For the colorimetric reaction, 5 mL of each filtrate (D1, D2, R1, R2) was combined with 5 mL sodium acetate solution (100 g/L), 3 mL aluminum chloride solution (25 g/L), and the volume was adjusted to 25 mL with methanol. After 15 minutes of incubation at room temperature, absorbance was recorded at 430 nm, using a reagent-free blank. Quantification was achieved using a calibration curve prepared from rutoside standard solutions (0.1 mg/mL) treated under identical conditions. The calibration curve exhibited excellent linearity ($R^2 = 0.9991$) and followed the regression equation:

$$y = 0.0361x + 0.028 \quad (3)$$

where *x* represents the standard concentration (μg/mL) and *y* the corresponding absorbance. Results were expressed as milligrams of rutoside equivalents per gram of dry weight (mg RE/g DW).

2.2.4. Heavy Metal Detection

The determination of Cd and Pb in the coffee samples was carried out using a PerkinElmer AAnalyst 800 graphite furnace atomic absorption spectrometer (GF-AAS) equipped with an AS-800 autosampler. Analytical-grade reagents were used throughout the procedure. Stock standard solutions (1000 mg/L) were prepared from high-purity metal salts and diluted to obtain the calibration standards. A mixture of ammonium hydrogen phosphate and magnesium nitrate served as the matrix modifier.

For sample mineralization, 10 g of ground coffee were subjected to stepwise calcination: drying at 100 °C, gradual heating to 450 °C, and maintenance at this temperature until complete ashing. The cooled ash was treated with hydrogen peroxide, reheated as required, and subsequently dissolved in hydrochloric acid, followed by evaporation and dissolution in 0.1 mol/L nitric acid to obtain the final mineralized solution. A reagent blank was processed identically.

Cadmium and lead were quantified from the same digest using five-point calibration curves (1–5 μg/L for Cd and 10–50 μg/L for Pb). All curves fulfilled linearity criteria ($R^2 \geq 0.995$). The analytical performance of the GF-AAS method, including linear ranges, detection limits (LOD), and quantification limits (LOQ), is summarized in Table 1, confirming the suitability of the procedure for trace metal determination in coffee samples.

Table 1. Performance parameters for determining heavy metals

Metal	Concentration range (mg / L)	R^2	LOD (mg / L)	LOQ (mg / L)
Cd	0,001-0,005	0.9998	0.0003	0.001
Pb	0,01-0,05	0.9996	0.003	0.01

2.2.5. Determination of Antioxidant Capacity

The antioxidant activity of the coffee extracts was evaluated using two widely applied in vitro assays: the DPPH radical-scavenging test [16] and the FRAP (Ferric Reducing Antioxidant Power) method [17]. The DPPH assay, based on the reduction of the stable free radical 2,2-diphenyl-1-picrylhydrazyl, measures the ability of antioxidants to donate electrons or hydrogen atoms, resulting in a decrease in absorbance at 517 nm. A methanolic DPPH solution was prepared at a known concentration, and 1 mL of each coffee extract (D1, D2, R1, R2) was mixed with the reagent and adjusted to 25 mL with methanol. After 30 minutes of incubation in the dark, absorbance was recorded using methanol as a blank and the DPPH solution as a control. The radical scavenging activity was calculated using Equation (4). All measurements were performed in triplicate.

$$\text{DPPH scavenging activity}(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (4)$$

where: $Abs_{control}$ denotes the absorbance of the DPPH solution in the absence of extract, and Abs_{sample} corresponds to the absorbance measured for the DPPH solution containing the coffee extract.

The antioxidant capacity was further examined using the FRAP method, which quantifies the ability of the extracts to reduce ferric (Fe^{3+}) to ferrous ions (Fe^{2+}) in the presence of the TPTZ reagent, producing an intense blue Fe^{2+}–TPTZ complex with maximum

absorbance at 593 nm. A fresh FRAP working reagent (acetate buffer, TPTZ, $FeCl_3$) preheated to 37°C was mixed with diluted coffee extracts (1:10, v/v). After a 4-minute incubation at 37°C, absorbance was recorded at 593 nm. Quantification was carried out using a calibration curve prepared from $FeSO_4 \cdot 7H_2O$ standards (100–1000 µmol/L), and final FRAP values were expressed as µmol Fe^{2+} equivalents per gram of dry material. Triplicate measurements ensured analytical reproducibility.

3. RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1. Preliminary Chemical Characterization

The qualitative assays performed on the coffee extracts confirmed the presence of alkaloids in all samples (D1, D2, R1, R2). As expected for *Coffea arabica*, the extracts contained nitrogen-bearing secondary metabolites capable of forming coordinate bonds with metal ions. In the Mayer reaction, the interaction between the alkaloid nitrogen and the tetraiodomercurate (II) complex produced a characteristic white precipitate, indicating a positive response for each sample. Complementary results were obtained using Dragendorff's reagent, where all extracts developed an orange to reddish-orange coloration, a typical indicator of bismuth–alkaloid complex formation.

To verify the presence of caffeine, the major purine alkaloid in coffee, the tannic acid test described in the Romanian Pharmacopoeia (X^{th} Edition) was applied. The addition of 10% aqueous tannic acid to each extract produced a white insoluble precipitate, confirming caffeine through the formation of a stable caffeine–tannin complex. This reaction provides a rapid and effective qualitative confirmation prior to instrumental quantification.

Taken together, the visual changes observed in the Mayer, Dragendorff and tannic acid tests consistently demonstrated the presence of alkaloids and caffeine in all analyzed extracts. A comparative overview of all qualitative reactions obtained for the four samples is presented in Figure 2, highlighting the distinct visual responses associated with alkaloid and caffeine identification.

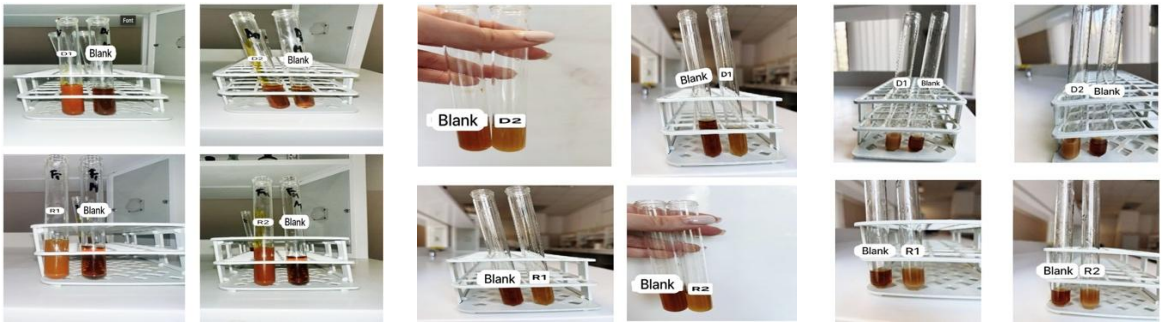

Figure 2. Qualitative screening of alkaloids and caffeine in the analyzed coffee extracts.

3.1.2. Total Phenolic Content

The total polyphenol content of the four Arabica coffee extracts (D1, R1, D2, and R2) was quantified using the Folin–Ciocalteu spectrophotometric method. Results were expressed

as both Gallic acid equivalents (mg GAE/100 g DW) and caffeic acid equivalents (mg CAE/100 g DW), allowing a more detailed assessment of the phenolic composition.

As shown in Figure 3, TPC values ranged from 3047 to 4893 mg GAE/100 g DW. The highest phenolic content was obtained for the Dominican infusion (D1, 4893 mg GAE/100 g), followed by the Romanian infusion (R1, 4389 mg GAE/100 g), indicating that hot-water extraction was more efficient than cold brew in extracting phenolic compounds. The cold-brew samples (D2 and R2) showed lower TPC values, with the Dominican cold brew (D2) presenting the lowest phenolic concentration (3047 mg GAE/100 g).

When expressed as caffeic acid equivalents, a similar trend was observed. The infusion samples again displayed the highest phenolic levels, with D1 reaching 4653 mg CAE/100 g and R1 reaching 4342 mg CAE/100 g. The cold-brew samples exhibited comparatively lower values, although their CAE results remained proportionally higher than their corresponding GAE values, suggesting potential differences in phenolic profiles extracted at lower temperatures.

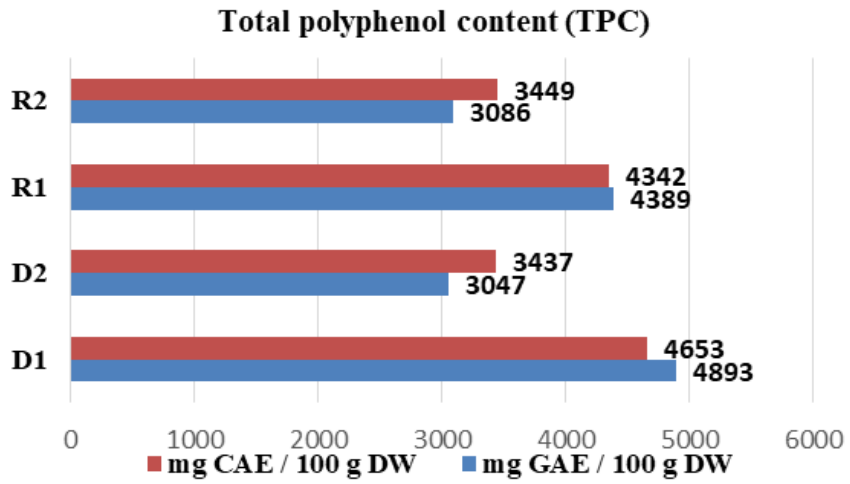

Figure 3. TPC values of coffee extracts D1, D2, R1, and R^2 determined by the Folin–Ciocalteu method.

Overall, these findings demonstrate clear variations in TPC as a function of both coffee origin and extraction method, with infusion outperforming cold brew in terms of phenolic recovery [16].

3.1.3. Total Flavonoid Content

The total flavonoid content of the four Arabica coffee extracts was determined spectrophotometrically using the aluminum chloride method described in the Romanian Pharmacopoeia (Xth Edition), and the results were expressed as mg RE/100 g DW. The values obtained are illustrated in Figure 4 and reveal clear differences associated with both the extraction technique and geographical origin.

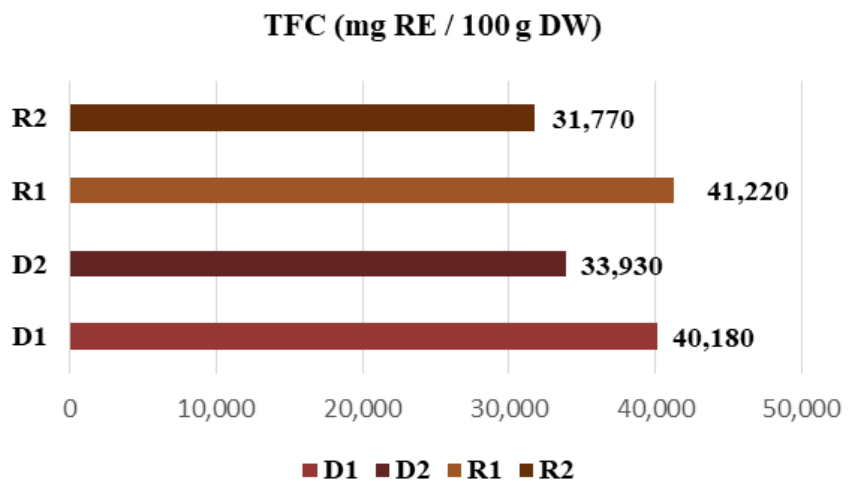

Figure 4. TFC values for coffee extracts D1, D2, R1, and R2.

Extracts obtained by hot-water infusion exhibited the highest flavonoid levels, reaching 41.22 mg RE/100 g in the Romanian coffee sample (R1) and 40.18 mg RE/100 g in the Dominican coffee sample (D1). In contrast, the cold brew extracts displayed markedly lower concentrations, with 33.93 mg RE/100 g in D2 and 31.77 mg RE/100 g in R2. These findings indicate that extraction temperature has a significant impact on flavonoid release, with infusion proving more effective than cold maceration—likely due to enhanced solubility and diffusion of phenolic compounds at elevated temperatures.

A slight but consistent trend was observed regarding coffee origin: the Romanian-produced coffee samples exhibited marginally higher TFC values than the Dominican samples, regardless of extraction method. Such differences may be attributed to cultivar-specific characteristics, environmental conditions, or post-harvest processing variables.

Overall, the results align with existing literature reporting that flavonoid concentrations in coffee are strongly influenced by botanical origin and extraction conditions, and that these compounds contribute substantially to the antioxidant potential of the beverage [18-20].

3.1.4. Heavy Metal Detection

The concentrations of cadmium (Cd) and lead (Pb) in the analyzed coffee samples were determined using atomic absorption spectrometry. Monitoring these contaminants is essential due to their high toxicity, persistence in the environment, and ability to bioaccumulate in human tissues [21,22]. Heavy metals may enter the coffee production chain through contaminated soil, fertilizers, irrigation water, or post-harvest processing, making their assessment relevant for both quality control and food safety.

In the European Union, maximum levels for Cd and Pb in foodstuffs are regulated under Regulation (EU) 2023/915 [23]. Although no specific limits are established for green or roasted coffee, the ALARA principle (“As Low As Reasonably Achievable”) applies, and reference values for similar plant-derived products—such as cereals (0.1 mg/kg Cd and 0.2 mg/kg Pb)—are commonly used for comparative evaluation [24].

The results obtained for the Dominican and Romanian Arabica samples are summarized in Table 2.

Table 2. Results obtained for Cd and Pb in coffee samples [mg/kg].

Coffee sample	Cadmium [mg/kg]	Plumb [mg/kg]
Arabica Coffee Dominican Republic	0.00462	ND*
Arabica coffee produced in Romania	0.00046	0.0106
Arabica coffee beans [24,25]	0.014 – 0.022	0.008 – 0.144

Cadmium was detected at low levels in both coffees, with higher concentrations measured in the Dominican sample (0.00462 mg/kg) compared to the Romanian product (0.00046 mg/kg). Lead was not detected (ND) in the Dominican coffee, while the Romanian sample showed a low but quantifiable concentration (0.0106 mg/kg). When compared with values reported in the literature for Arabica beans (Cd: 0.014–0.022 mg/kg; Pb: 0.008–0.144 mg/kg) [25,26], the metal content of both samples falls within or below expected ranges.

Overall, the results indicate minimal contamination with Cd and Pb in all analyzed extracts, suggesting that both coffees are consistent with international safety expectations and do not pose a significant risk regarding heavy metal exposure.

3.1.5. Determination of Antioxidant Capacity

The antioxidant capacity of the four Arabica extracts was assessed using the DPPH and FRAP assays, allowing a comparative evaluation of radical-scavenging ability and ferric-reducing power across origins and extraction methods. All samples exhibited very high DPPH inhibition values, ranging from 87.70% to 88.98%, with the infusion extracts D1 and R1 showing slightly higher activities than the cold-brew extracts (D2 and R2). These minimal variations suggest that both preparation methods yield comparable levels of compounds capable of neutralizing DPPH radicals (Table 3).

Table 3. Results obtained by the DPPH method [%].

Sample	Absorbance λ = 517 nm	DPPH %
Blank	2.4467	-
D1	0.2903	88.13%
D2	0.2909	88.11%
R1	0.2694	88.98%
R2	0.3009	87.70%

In contrast, the FRAP results showed more pronounced differences between extraction methods. The highest reducing capacity was observed for the Dominican cold-brew extract (D2: 1062 μmol Fe^{2+}/g), followed by the Romanian infusion (R1: 1001 μmol Fe^{2+}/g), the Dominican infusion (D1: 924 μmol Fe^{2+}/g), and the Romanian cold brew (R2: 868 μmol Fe^{2+}/g) (Figure 5). These findings indicate that cold maceration may enhance the extraction of certain redox-active compounds in the Dominican sample, while infusion performed better for the Romanian coffee.

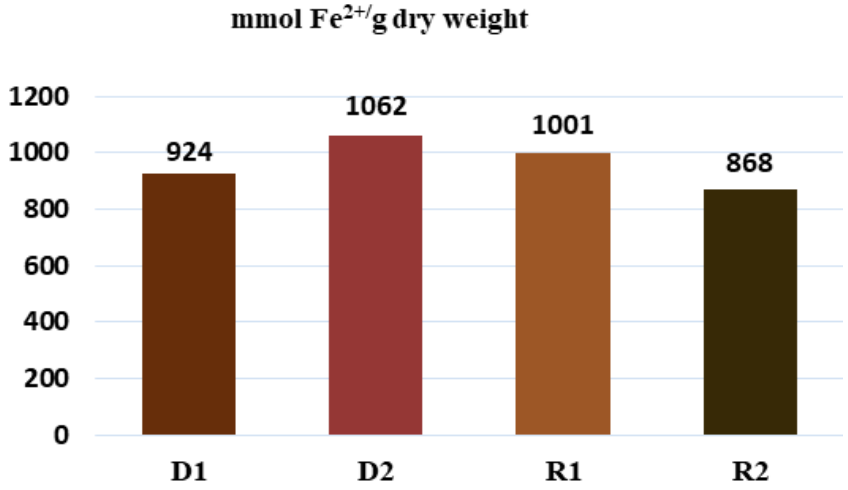

Figure 5. Results obtained by the FRAP method for the antioxidant reducing power of ferric.

As reported in previous studies, DPPH and FRAP values do not always correlate directly, as the assays probe different antioxidant mechanisms—radical scavenging versus electron-donating capacity [27]. The present results confirm this distinction and highlight the complex interplay between extraction temperature, coffee origin, and the diversity of antioxidant constituents present in the analyzed samples.

3.2. DISCUSSIONS

The present study provides an integrated evaluation of the alkaloid profile, phenolic composition, flavonoid content, antioxidant activity, and heavy metal levels in Arabica coffee extracts obtained from two geographical origins and two extraction methods. The qualitative assays confirmed the presence of alkaloids in all samples, with classical Dragendorff and Mayer tests generating specific precipitates indicative of purine-type alkaloids. The subsequent identification reaction with tannic acid confirmed caffeine across all extracts, reinforcing its status as the principal bioactive alkaloid in coffee and aligning with existing evidence regarding its well-documented physiological and neurostimulatory effects [28-31]. These results validate the suitability of the applied qualitative methods and support the chemical characterization of the extracts.

The quantitative polyphenolic analysis demonstrated clear differences related to extraction temperature. Infusion produced substantially higher TPC and TFC values than cold brew, confirming reports that elevated temperatures promote matrix permeabilization and enhance the solubilization of phenolic compounds [32]. The Dominican infusion sample (D1) contained the highest amounts of both phenolics and flavonoids, while both cold-brew extracts exhibited significantly lower levels, consistent with the reduced extraction efficiency of low-temperature maceration. Slightly higher flavonoid values in the Romanian samples may be attributed primarily to differences among Arabica varieties, known to exhibit cultivar-dependent variability in flavonoid biosynthesis and stability. Literature indicates that roasting intensifies the thermal degradation of phenolics and flavonoids [33, 34, 35], an effect that may partially explain inter-sample variability and highlights the importance of processing conditions in shaping coffee's phytochemical profile.

Antioxidant behavior, assessed using the DPPH and FRAP methods, reflected the influence of extraction conditions. DPPH inhibition was uniformly high across all samples

(87.70–88.98%), suggesting comparable levels of hydrogen- or electron-donating compounds. The higher DPPH activity observed in infusion extracts corresponds with their increased phenolic content, supporting the well-established association between total polyphenol content (TPC) and radical-scavenging capacity [36]. In contrast, FRAP values exhibited more pronounced variability, with the Dominican cold-brew extract (D2) showing the highest reducing power. This indicates that prolonged cold extraction may preferentially retain or extract specific redox-active compounds, consistent with reports that FRAP is particularly sensitive to water-soluble phenolics and other reducing agents [37].

It was hypothesized that the extraction method exerts a stronger influence on the antioxidant profile than geographical origin; however, the combined effect of both factors ultimately determines the observed differences in the redox capacity of the extracts. The simultaneous application of the DPPH and FRAP assays provides a more comprehensive evaluation of antioxidant activity by accounting for distinct mechanisms, namely free radical scavenging and electron-donating capacity [38]. A moderate positive correlation was observed between TPC and FRAP values ($r = 0.72$), indicating a substantial contribution of polyphenols to ferric-reducing power.

The lack of a perfect correlation between DPPH and FRAP results suggests the presence of multiple antioxidant fractions with different modes of action, supporting the multifactorial nature of coffee antioxidant activity. In this context, melanoidins formed during roasting may significantly contribute to the reducing capacity measured by FRAP, particularly in cold-brew extracts.

Heavy metal analysis further confirmed the safety and quality of the tested coffee samples. Cadmium and lead concentrations were very low and within or below ranges reported internationally for Arabica beans [39-42]. Pb was detected only in the Romanian sample, at a level far below established reference values, while Cd concentrations in both samples were minimal. These findings indicate negligible environmental contamination and compliance with the ALARA principle outlined in EU Regulation 2023/915, supporting the safe consumption of both coffee types. From a safety perspective, the estimated dietary intake of Cd and Pb through moderate coffee consumption (approximately 200 mL/day) remains well below the reference values established by EFSA, indicating no associated health risk.

In conclusion, the results indicate that extraction temperature is a key determinant of phenolic and flavonoid release, thereby modulating antioxidant potential. Infusion provides superior extraction efficiency and yields beverages with enhanced functional value, whereas cold brew may preserve reducing compounds that are not efficiently extracted at higher temperatures. Differences between the two coffee origins, although subtle, likely reflect inherent cultivar characteristics and processing variables. These findings deepen understanding of how preparation methods and raw material attributes influence the nutritional and functional quality of coffee, with direct implications for product development, consumer preferences, and dietary recommendations.

4. CONCLUSIONS

This study demonstrates that both the geographical origin of Arabica coffee and the extraction method significantly influence its phytochemical composition and antioxidant properties. Qualitative tests confirmed the presence of alkaloids and caffeine in all samples, while quantitative analyses showed that hot-water infusion consistently yielded higher levels of polyphenols and flavonoids than cold-brew extraction. These differences translated into superior DPPH radical-scavenging activity in infusion samples, whereas the FRAP assay

revealed that cold brew (particularly the Dominican sample) may retain or extract compounds with enhanced reducing capacity.

The slightly higher flavonoid content observed in the Romanian samples may reflect inherent differences among Arabica cultivars, underscoring the role of plant genetics in shaping nutritional and functional profiles. Heavy metal analysis confirmed very low concentrations of Cd and Pb, indicating that both coffee types are safe for consumption and comply with international standards.

From a practical and nutritional perspective, hot-water infusion offers the highest extraction efficiency for antioxidant phenolics, making it the preferred choice for consumers seeking enhanced functional benefits. Cold brew, however, remains a valuable alternative for individuals sensitive to acidity or seeking beverages with distinct antioxidant characteristics.

Overall, the findings highlight coffee's potential as a functional beverage whose health-promoting properties depend not only on bean origin and agricultural conditions but also on preparation method.

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