https://doi.org/10.31925/farmacia.2024.5.8 **ORIGINAL ARTICLE**

PHYTOCHEMICAL ANALYSIS OF THE BIOACTIVE PROPERTIES OF GREEN COFFEE BEANS OF ARABICA AND ROBUSTA VARIETIES

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Abstract

In the present study, the plant material used was green coffee belonging to Arabica and Robusta species. Caffeine was determined from coffee beans using the HPLC method. The antioxidant capacity of infusions obtained from green coffee of Indian origin was studied using the DPPH method. The total polyphenol content was analysed using the Folin-Ciocâlteu method, and antimicrobial activity was evaluated using the microdilution method. The HPLC method indicated a concentration of 217.0 mg caffeine *per* 100 g of coffee for Robusta green coffee and 159.5 mg caffeine *per* 100 g for the Arabica green coffee sample. Spectrophotometric analyses were performed on four green coffee extracts: two obtained by hot infusion (CA1, CR1) and two by cold maceration (CA2, CR2). The DPPH values (%) of the aqueous Arabica and Robusta green coffee extracts ranged from 44.78% to 91.82%. Results show that coffee extracts prepared by hot water infusion have higher antioxidant capacity than extracts prepared with cold water. These results are in agreement with those obtained in determining total polyphenolic compounds by the Folin-Ciocâlteu method. The results obtained from the antimicrobial activity analysis showed that the extracts obtained from green coffee beans did not show any antimicrobial effect on the pathogens analysed.

Rezumat

În studiul de față, materialul vegetal utilizat a fost cafeaua verde aparținând speciilor Arabica și Robusta. Cafeina din boabele de cafea a fost determinată prin metoda HPLC. Capacitatea antioxidantă a infuziilor obținute din cafea verde de origine indiană a fost studiată prin metoda DPPH. Conținutul total de polifenoli a fost analizat prin metoda Folin-Ciocâlteu, iar evaluarea activității antimicrobiene a fost realizată prin metoda microdiluției. Metoda HPLC utilizată a indicat o concentrație de 217,0 mg de cafeină *per* 100 g de cafea pentru cafeaua verde Robusta și 159,5 mg de cafeină *per* 100 g de cafea pentru proba de cafea verde Arabica. Analizele spectrofotometrice au fost efectuate pe patru extracte de cafea verde: două obținute prin infuzare la cald (CA1, CR1) și două prin macerare la rece (CA2, CR2). Valorile DPPH (%) ale extractelor apoase de cafea verde Arabica și Robusta au variat de la 44,78% la 91,82%. Rezultatele au arătat că extractele de cafea preparate prin infuzare cu apă caldă au o capacitate antioxidantă mai mare decât extractele preparate cu apă rece. Aceste rezultate obținute sunt în concordanță cu cele obținute la determinarea compușilor polifenolici totali prin metoda Folin-Ciocâlteu. Rezultatele obținute în urma analizei activității antimicrobiene au arătat că extractele obținute din boabe de cafea verde nu au prezentat niciun efect antimicrobian asupra agenților patogeni analizați.

Keywords: caffeine, polyphenols, antioxidant capacity, green coffee, Arabica and Robusta

Introduction

Caffeine, a naturally occurring xanthine alkaloid known as 1,3,7-trimethylxanthine ($C_8H_{10}N_4O_2$), is widely recognised as the most commonly consumed psycho-active substance worldwide, with approximately 80% of the population introducing it into their daily routine [1, 2].

Coffee contains caffeine that provides a distinctive aroma to coffee, which can also be used as a quality parameter [3]. The biologically active substances present in coffee extracts can be classified into two main groups. The first group consists of substances containing antioxidants, such as polyphenols, which can neutralise various forms of superoxide free radicals. Thus, they are particularly beneficial for protecting the DNA of skin cells. The second group comprises purine alkaloids,

caffeine and trigonelline, the most important compounds. These substances improve oxygenation and cellular microcirculation and stimulate metabolism. Various references in the literature highlight the exceptional properties of coffee extracts, which have been widely used for many years [4-6].

The antioxidant and antibacterial properties of coffee vary depending on factors such as species, roast, brewing method and decaffeination process [7, 8]. Different coffee varieties of different origins have distinct compositions. The bioactivity of coffee beans is also influenced by agro-geographical conditions such as soil type, altitude, harvest season and pre- and post-harvest practices. Research in this area has shown that roasted coffee exhibits antibacterial effects [9, 10], while the results for green coffee are controversial due to the lack of antimicrobial activity [11, 12]. In addition, coffee (green and roasted) is widely recognised as an outstanding beverage with beneficial attributes, acting as an antioxidant [13,14].

Therefore, the main objective of the study was to analyse green Arabica and Robusta coffee in the form of aqueous extracts to identify caffeine, the key element found in green coffee, and evaluate their anti-oxidant and antimicrobial properties. The ultimate goal is to integrate these aqueous extracts into innovative pharmaceutical products that can improve skin appearance.

First, an accurate and feasible HPLC method was used to determine caffeine in Arabica and Robusta green coffee samples. The antioxidant and antimicrobial capacity and total polyphenol content of extracts obtained from Arabica and Robusta green coffee beans were also determined. Extracts obtained by hot infusion and cold maceration were analysed. Folin-Ciocâlteu and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods were used to evaluate the antioxidant properties of coffee infusions and macerates. Screening and evaluation of the possible antimicrobial potential of green coffee samples in the form of crude aqueous extracts against pathogenic (*Escherichia coli* ATCC 25922), conditionally pathogenic (*Staphylococcus aureus* ATCC 25923) and opportunistic microorganism species (*Candida albicans* ATCC 10231) in the human microbiota was performed by microdilution method. When selecting these microbial strains, we considered that they are usually found as common commensal species in the natural microbiota of skin and mucous membranes. However, under specific circumstances, such as imbalances, associated diseases, immunocompromised conditions or old age, these strains have the potential to become pathogenic.

Materials and Methods

Reagents

The reagents used were of high analytical purity (for HPLC) and were purchased from Sigma-Aldrich (Germany). Ultrapure water from a bi-distillation and deionisation system in the laboratory was used, with a conductivity of 18 μS/cm, which was required for all experiments.

To analyse the antioxidant properties of green coffee extracts, the following were used: Folin-Ciocâlteu reagent 10%, gallic acid 3.76 μg/mL, methanol *R*, anhydrous sodium carbonate 20% and DPPH (2,2-di-phenyl-1-picrylhydrazyl), which were purchased from Sigma-Aldrich (Germany). All substances and reagents used were of analytical grade.

Equipment

For all measurements, an HPLC Agilent 1260 Infinity LC with a diode array detector G412B was used. The software used was Agilent ZORBAX Eclipse Plus. A chromatographic column type p/n 959993-902 was used. Elution conditions were isocratic at 25°C with a 25% methanol solution at a flow rate of 1 mL/min. A methanol: water mixture (0.02 mol/L NH_4Cl, pH 8.2 adjusted with triethylamine) served as the mobile phase, with volume ratios of 75:25.

Spectrophotometric analyses were performed using a Jasco 550 UV-VIS double beam spectrophotometer using 1 cm thick quartz cuvettes.

Caffeine analysis with the HPLC method.

The HPLC method, which worked under isocratic conditions, was used for the determination of purine alkaloid caffeine. *Arabica* and *Robusta* green coffee extracts were obtained based on a method proposed earlier by Samsonowicz *et al.* [15]. For the extraction of caffeine, distilled water was heated to 95 ± 2°C in a heat-resistant beaker, and the green coffee sample (0.5 g ground green coffee bean) was added to the beaker; the beaker was further heated at 95°C in a water bath for 20 min under continuous stirring. A Hanna Instruments digital thermometer was used to check the temperature during extraction. The resulting solution was cooled to room temperature, centrifuged for 5 min at 4500 rpm and decanted. The ratio between sample and solvent (water) was 1:20 (g/mL). The aqueous extract was hot-filtered through filter paper to remove insoluble particles.

Validation of the method for the determination of caffeine

Preparation of standard solution: Caffeine stock solution was obtained as follows: 200 mg caffeine was dissolved in 500 mL warm water in a 1 L volumetric flask and filled after cooling to room temperature with water to 1 L. Dilutions of 1:10 and 1:50 were used as starting concentrations for calibration.

The statistical parameters used in method validation are specificity and selectivity, linearity, precision, accuracy, intermediate precision and reproducibility, limit of detection (LOD) and limit of quantification (LOQ).

Validation parameters are calculated according to SR ISO 8466/1-2016 European Pharmacopoeia, Ed. 9.0

(2020) and the European regulations "International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use" by the guidelines for validation of analytical procedures provided by ICH-Q2A (2003) and ICH-Q2B (2005). Calibration was performed in typical intervals to measure the caffeine content of the coffee extracts obtained. For these extracts, calibration was performed on 10 samples of different concentrations from 1.25 mg/L to 20 mg/L.

Plant material and extraction methods.

The same batch of coffee (100% *Arabica* and 100% *Robusta*) was used for both extractions. Before brewing, each packet of coffee beans was opened to prevent oxidation. The beans were ground using a professional coffee grinder (DIP Grinders DK-30). The coffee was ground "coarse" for all extraction methods.

To obtain the extract by infusion, initially heat distilled water in a heat-resistant glass to 85 ± 2°C, add the green coffee sample to the glass and heat the infusion on a water bath for 10 minutes, stirring constantly. A Hanna Instruments digital thermometer was used to check the temperature during extraction. The sample/solvent ratio was 1:10 (g ground green coffee *per* mL distilled water). Hot filter the aqueous extract through filter paper to remove insoluble particles (CA1, CR1).

The maceration method was used at room temperature (21 - 25°C). The coffee extract was prepared at a ratio of 1:10 (g ground green coffee/mL distilled water). The macerates were left for 24 hours in optimal conditions, protected from light and humidity, in the refrigerator (2 - 8°C). After the extraction, the macerate was filtered through filter paper (CA2, CR2).

Antimicrobial activity analysis.

Antimicrobial activity testing was performed for extracts obtained by maceration and infusion on two types of pathogens, namely two bacterial strains and one yeast strain: a Gram-positive strain, *Staphylococcus aureus* ATCC 25923, a Gram-negative strain, *Escherichia Coli* ATCC 25922 and a pathogenic yeast strain, *Candida albicans* ATCC 900288. The four extracts (CA1, CA2, CR1, CR2) were filtered using sterile 0.22 μm pore size PES (Polyester) filters for decontamination [16].

Microbial strains were grown on Plate Count Agar (PCA) culture medium at 37 ± 0.5°C for 22 ± 2 hours to perform biological tests. All materials used were prepared according to the instructions in the datasheet. For the preparation of the PCA medium, the components are dissolved in water; the pH value is adjusted, if necessary, so that after sterilisation, it is 7.0 ± 0.2 at 25°C. Dispense the medium into 12 - 15 mL test tubes or 500 mL maximum capacity vials and autoclave at 121°C for 15 minutes. It can be stored in the dark at 3 ± 2°C for three months.

A variant of the Kirby-Bauer quantitative microdilution diffusion metric antibiogram, which allows the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), was used to evaluate the antimicrobial activity of the plant extracts to be tested.

Principle of the method: the following dilutions (1/2; 1/4; 1/16; 1/32; 1/64; 1/128; 1/256; 1/512; 1/1024) of the extract are made in a liquid medium, which is placed in contact with fixed quantities of the bacterial culture, incubated for 18 - 24 hours at 37°C and the lowest concentration of extract that does not allow bacterial growth is determined. This will be the minimum inhibitory concentration. A fixed amount is transferred from the tubes where the micro-organism has not grown to a solid culture medium. Incubate for 18 - 24 hours at 37°C, after which the lowest concentration at which the bacteria have not grown is noted. This will represent the minimum bactericidal concentration.

Interpretation of the results: After the incubation time has expired, the colour intensity in each well, the turbidity and the intensity of the population density of the culture in each well, compared to the growth medium (MC), is assessed subjectively by eye observation. Subsequently, after inoculating the solid medium, the growth of the micro-organisms is assessed at dilution corresponding to the minimum inhibitory concentration (MIC). This method of analysis involves the following working steps. *Step 1*. Nutrient broth liquid culture medium distribution in 96-well micro-plates under sterile conditions (100 μL of medium was distributed in each well). For the preparation of the nutrient broth medium, the components are dissolved in water; the pH value is adjusted, if necessary so that after sterilisation, it is 7.4 ± 0.2 at 25°C. Autoclave at 121°C for 15 minutes. Store in the dark at 3 ± 2°C for one month. *Step 2*. Preparing suspensions of bacterial strains in sterile physiological water (AFS) with a density of approximately 1.5 x 105 microbial cells/mL, starting from a turbidity standard of 0.5 McFarland, corresponding to a density of 1.5 x 108 microbial cells/mL (the direct colony homogenisation method was used for inoculum preparation, consisting of homogenising 3 - 5 colonies from the culture plate in sterile saline in the stationary growth phase after 18 hours to obtain standard turbidity. A McFarland 0.5 standard (optical density at λ of 550 nm is 0.125) and the Densimeter digital densitometer were used to control inoculum density). *Step 3*. The suspensions were homogenised using a Vortex shaker for 15 - 20 seconds (the suspensions were calibrated by adding more or less isotonic chloride solution until the desired optical density was obtained (Figure 1). From the initial suspension, a dilution of 1/10 was made). *Step 4*. The well plates were seeded within 15 minutes after inoculum preparation by dispensing 100 μL of extract into the first wells of the microplates (A1 to H1) (one

row of a microplate (1 to 12) was used for each test compound). *Step 5*. Perform binary serial dilutions of the tested extracts up to well 10 of each microplate row. *Step 6*. Inoculation of wells containing culture medium and different concentrations of the test compounds, with suspensions of bacterial strains. *Step 7*. The 11 wells of each row of microplates acted as a control (positive control) for bacterial growth, containing only nutrient broth liquid culture medium inoculated with bacterial suspensions. *Step 8*. The 12th well of each row of microplates served as a sterility control (negative control), containing only nutrient broth liquid culture medium. *Step 9*. Distribution of 5 µL bacterial suspension in wells 1 - 11. *Step 10*. Incubating the samples under conditions suitable for microbial growth, *i.e.* in a thermostat at a temperature of 37 ± 0.5°C for 22 ± 2 hours. *Step 11*. From each well, 10 µL was taken, and spot inoculated onto the surface of plates with PCA culture medium. (samples were incubated at 37°C for 18 - 24 hours; subsequently, the growth of the microorganisms was noted at the dilution corresponding to the minimum inhibitory concentration (MIC).

Figure 1.
Microplate with 96 wells, into which the culture medium and the test sample have been micro-pipetted

Analysis of the antioxidant capacity.
For the antioxidant capacity analysis, Arabica and Robusta coffee extracts obtained by hot infusion and cold maceration were diluted, and four aqueous solutions of different concentrations were obtained for each extract. Sixteen coffee samples were produced for antioxidant analysis using 10 mL volumetric flasks that contained 1, 2, 3 and 4 mL of each extract (CA1, CR1, CA2, CR2). The extracts were then topped off with distilled water to the mark. The DPPH radical scavenging method involves neutralising the DPPH radical by electron-donating antioxidants, with the DPPH radical colour shift measured at 517 nm [17]. For the present analysis, we worked according to the method described by Murokore with slight adjustments [18]. DPPH methanolic solution was prepared as follows: 100 mg DPPH was accurately weighed and placed in a 200 mL volumetric flask over which 100 mL methanol was added and stirred to dissolve. The solution was made up to 200 mL with solvent, resulting in a 0.063% (1.268 mmol/L) DPPH methanolic solution. Four aqueous solutions of different concentrations (1:10, 2:10, 3:10 and 4:10 mL extract/mL distilled water) were prepared for each coffee sample: CA1, CA2, CR1 and CR2. From each of these solutions, 1 mL was mixed with 5 mL of 1.268 mM (0.063%) DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution in 25 mL volumetric flasks and topped up with methanolic solution. The mixture was shaken vigorously and stored in a dark space for 30 minutes. Absorbance was measured at a wavelength of λ = 517 nm using a blank (control) as a compensation liquid. The calculation formula for determining the DPPH radical scavenging capacity is shown in equation (1):

$$\%\ \text{DPPH Scavenging Capacity} = \frac{\Delta\ \text{A517 blank} - \Delta\text{A517 sample}}{\Delta\ \text{A517 blank}} \times 100, \quad (1)$$

where, $\Delta A517$ blank and $\Delta A517$ sample are the absorbances of the control and extracts, respectively. All samples were analysed in triplicate. Results were expressed as mean ± standard deviation.

Assessment of total phenol content (TPC).
The total phenol content (TPC) was determined using the Folin- Ciocâlteu method using a Jasco 550 UV-VIS double-beam spectrophotometer [19]. Absorbance was measured at 681 nm against a standard gallic acid curve, and results are expressed as gallic acid equivalents (mg GAE/100 g coffee sample). All samples were triplicate, and the mean value was reported. A solution of gallic acid of 34 µg/mL was prepared in a 1:1 methanol: water solution. The calibration curve used for the assay of phenols shows linearity in the range of 0.68 - 5.44 mg GAE/L. The characteristics of the calibration curve are Y = A × X + B; A = 0.1034; B = 0.0059; correlation coefficient = 0.9939. Determination of total phenol concentration in aqueous extracts CA1, CA2, CR1, CR2: Vi mL of green coffee extract CA1, CA2, CR1 and CR2, respectively, each 1 mL Folin- Ciocâlteu reagent and each 1 mL 20% sodium carbonate solution were placed in four 50 mL volumetric flasks. The mixture was homogenised and allowed to stand for 10 minutes. The flask was then brought to the mark with distilled water and left for 30 minutes at room temperature to stabilise the colour. The absorbance was read using the Jasco 550 UV VIS spectrometer (Tokyo, Japan) at 681 nm. Equation (2) was applied to calculate the concentration of total phenols, expressed as mg gallic acid (GAE) *per* 100 g Arabica green coffee and *per* 100 g Robusta green coffee:

$$\text{TPC (mg GAE/100g)} = \frac{V_f \times c \times 5}{V_i \times m_{sample}}, \quad (2)$$

where, c is the concentration of phenols (mg/L) read on the calibration curve; V_f is the volume of filtered extract of each sample; V_i is the volume of sample added to the 50 mL volumetric flask for determination;

the sample is the mass of dry plant material (CA, CR) subjected to infusion and maceration.

Results and Discussion

Determination of caffeine in coffee using the HPLC method

Specificity and selectivity: The specificity of the chromatographic method was determined by testing standard substances against interferences. This demonstrates the method's ability to accurately measure analyte response in the presence of all interferences. Six replicate injections of 100% test (sample) solution for fixed concentration levels were used to demonstrate this.

Linearity: To determine the working concentration range, ten standard stock solutions containing caffeine ranging from 1.0 mg/L to 25.0 mg/L were prepared and used. Three sets of determinations were evaluated for each concentration. A high sensitivity (11668 (mAU*s)/(mg*L) was recorded, and a very good correlation coefficient (0.9999) was obtained for a wide concentration range: 1.25 to 20 mg/L of caffeine. The results obtained for the precision test of the HPLC method showed that the relative standard deviation (RSD%) was in the range of 0.01% and 0.02%, fulfilling the condition %, RSD < 1.00. The results for repeatability (%, R) were between 0.03 and 0.05, thus confirming that this criterion was met (%, R < 1.00).

Another main component of precision is the intermediate precision, which was analysed on a set of chromatographic results obtained by repeating for three days for the same sets of samples at the same concentration levels (1.25, 2.5, 5.0, 10 and 20 mg/L) under the same conditions, performed by the same operator. The mean of the chromatographic peak areas and the relative standard deviation (%, RSD) and repeatability (%, R) values were calculated for each concentration. The values obtained for the relative standard deviation (%, RSD) when calculated for the same sets of samples at the same concentration levels indicated the precision of the method. The acceptance criterion is considered to be met when %, RSD < 5.00, is obtained. In the present study, the results obtained for % RSD ranged from 0.01 to 0.02, proving the accuracy of the proposed method. The values obtained for repeatability are in the range of 0.01 to 0.05%; all values are below 1.00%, proving the high repeatability of the method. For chromatograms performed on the first day, the standard deviation values calculated were in the range of 2.10 - 11.81, with %, RSD values in the range of 0.01 - 0.02, proving the high accuracy of the method.

Another validation test compares the results obtained using the new method with those obtained using a standard method, such as ISO 8466-1.

The calculated % Bias values were between 99.30% and 100.01%. Accepted values in the study of accuracy and precision for different analyte concentrations are between 90% and 107%. Accordingly, the proposed method can reliably determine caffeine in coffee samples.

The detection limit (LOD) is expressed as 3s/b, where s is the standard deviation calculated when estimating the linearity of the analyte, and b is the slope of the calibration curve estimated at the linearity of the analyte. The limit of quantification (LOQ) is expressed as 10 s/b with the same meanings for s and b (as for LOD). The limit of detection and quantification was determined to be 95% confidence level and n-2 degrees of freedom. Accordingly, the calculated values for these parameters are: limit of detection 0.02 mg/L, limit of quantification 0.07 mg/L and retention time (t_r) 6.78min.

The caffeine concentration (w_c) in the analysed samples was calculated according to equation (3) [15, 16]:

$$w_c = \frac{A_S\, p_{st}\, V \times 100}{A_{st}\, m_S} = \frac{A_S\, p_{st} \times 25}{A_S\, m_S}, \quad (3)$$

where, A_{st} is the caffeine peak area in the HPLC chromatogram of standard solution; A_s is the caffeine peak area in the HPLC chromatogram of the sample solution; m_s is the mass in grams of the test portion; V is the volume in a litre, of the extracted sample solution (V = 0.25 L); p_{st} is the mass concentration, in grams *per* litre, of the standard solution of caffeine.

The concentration of caffeine in *Robusta* coffee was 217.0 mg/100 g, and in *Arabica* coffee, 159.5 mg/ 100 g coffee.

Total phenolic content (TPC) of coffee

The values for the total phenol concentration (mg GAE/100 g coffee sample) were determined for each of the extracts analysed as a function of the coffee species and the method of obtaining the extract: coffee extracts prepared by infusion with hot water CA1 (*Green Arabica* coffee), CR1 (*Green Robusta* coffee) and coffee extracts prepared by infusion with cold water CA2 (*Green Arabica* coffee), CR2 (*Green Robusta* coffee). The results obtained from the determinations for the green coffee samples analysed ranged from 5212.50 - 7287.50 mg GAE/100 g coffee sample for *Robusta* and 4600.00 - 5175.00 mg GAE/100 g coffee sample for *Arabica*, namely, the total phenol concentrations found are: 5175.0 mg GAE/100g coffee for CA1, 4600.0 mg GAE/100 g coffee for CA2, 7287.5 mg GAE/100 g coffee for CR1 and 5212.5 mg GAE/100g coffee for CR2.

Determination of the antioxidant capacity using the DPPH method.

A decrease in the purple colour was recorded with the increasing of the caffeine concentration in the extract increased. Concentrated aqueous solutions (4:10 mL coffee extract/mL distilled water) showed the highest values. This result indicates a higher DPPH

uptake value with increasing caffeine concentration, *e.g.*, a significant decrease of 2,2-diphenyl-1- picryl-hydrazyl radical molecules. The variation of the DPPH radical scavenging capacity of Arabica and Robusta green coffee extracts is shown in Figure 2.

Antimicrobial activity

The assay results to assess the antimicrobial effect of CA2 and CR2 extracts can be seen in Figure 3, which has been systematised and shown in Table I.

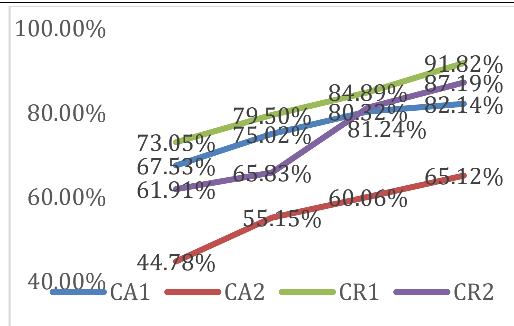
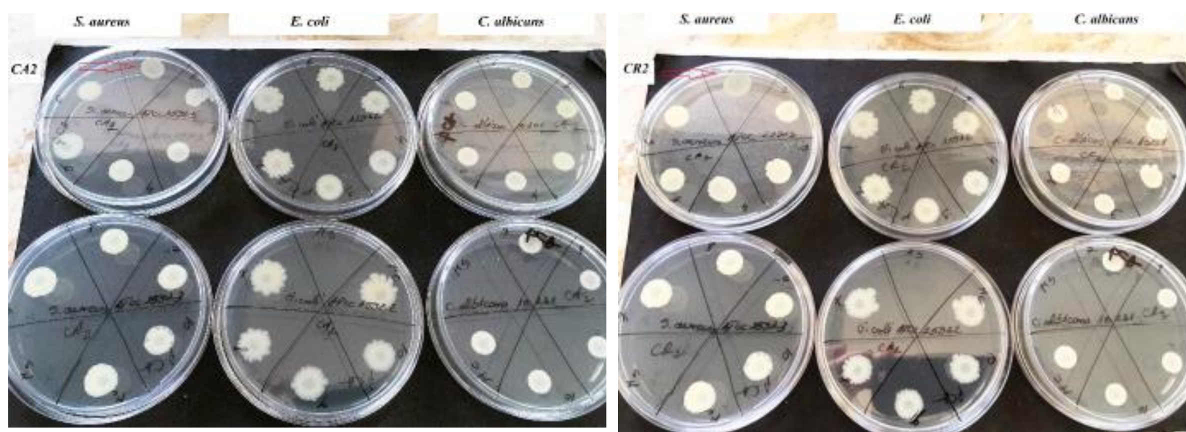


Figure 2.
DPPH radical scavenging capacity (%) for coffee extracts at different concentrations



(a) (b)

Figure 3.
Antimicrobial effect of CA2 extract (a) and CR2 extract (b) on the three strains of microorganisms tested: *Staphylococcus aureus* (left), *Escherichia coli* (middle) and *Candida albicans* (right)

Table I
Antimicrobial activity of coffee extracts by microdilution method

Green coffee samples	Pathogens	No. CFU/mL											
		$^{1}/_{2}$	$^{1}/_{4}$	$^{1}/_{8}$	$^{1}/_{16}$	$^{1}/_{32}$	$^{1}/_{64}$	$^{1}/_{128}$	$^{1}/_{256}$	$^{1}/_{512}$	$^{1}/_{1024}$	MC	MS
CA2	*S. aureus*	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	Sterile
	E. coli	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	Sterile
	C.albicans	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	Sterile
CR2	*S. aureus*	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	Sterile
	E. coli	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	Sterile
	C.albicans	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	Sterile

MC = growth marker (positive marker); MS = sterility marker (negative marker); CFU = colony forming units

For the determination of caffeine concentration in the Arabica green coffee sample and the Robusta green coffee sample, the % content in caffeine of the analysed coffee samples was 2.17% for Robusta coffee and 1.60% for Arabica coffee. The determined values correspond to ISO standards and Indian national standards [20, 21] as well as German standards from the DIN series of standards [22] and Naegele [23]. Also, the validation parameters of the HPLC method obtained results that were consistent with those of Nepalese researchers Pokhrel *et al.* [24] and also with those of Indian researchers Naveen *et al.* [25].

The findings of this study are consistent with the widely accepted notion that Arabica coffee beans generally contain lower caffeine levels than Robusta coffee beans. In addition, the data indicate variations in caffeine content between beans of the same species but originating from different regions. This variability can be attributed to factors such as the conditions under which the beans were grown, the methods used in their processing and their genetic structure. Supporting this notion, Towaha *et al.* reported that Arabica coffee beans grown at different altitudes exhibited different levels of caffeine [26, 27]. The accurate determination

of the caffeine content of the various types of coffee is very important in order to know the exact dose ingested, especially for people with caffeine restrictions, as well as for the utilisation of this active substance in various pharmaceutical preparations.

The scientific and medical communities have shown growing interest in the potential of coffee polyphenols and their antioxidant activity as a source of valuable bioactive compounds, highlighting their potential for new applications. It is well established that green coffee beans contain effective plant antioxidants such as chlorogenic acids, phenolic acids, polyphenols and alkaloids, and their content depends mainly on the coffee species (C. *Arabica*, C. *Robusta*). The results obtained for the total phenols concentration in coffee extracts showed that the coffee with the lowest total phenol content was *Arabica* coffee prepared with cold water (CA2) with a value of (4600.00 mg GAE/100 g), and the coffee with the highest total phenol content was *Robusta* coffee prepared with hot water (CR1) (7287.50 mg GAE/100 g). Accordingly, the coffee extracts prepared with hot water have a higher polyphenol content than coffee extracts prepared with cold water. Also, from these results, we can point out that the *Robusta* species has a higher polyphenol content than the *Arabica* species, regardless of the extract's preparation method. Although coffee is generally prepared with hot or warm water, recently, there has been an increase in the consumption of cold-water coffee drinks in northern European countries, the United States and Europe. United States and Japan [28, 29] due to new brewing methods involving longer extraction times at cooler temperatures rather than rapid exposure to high temperatures. From these analyses, it can be seen that all four extracts analysed show a valuable polyphenol content with differences observed between the same coffee species obtained by different extractions (hot and cold), which thus fall within the ranges required by the literature [30, 31].

The objective of this study was to gather information about the beneficial properties and bioactive compounds present in Arabica and Robusta green coffee beans. Different extraction temperatures (hot and cold) were used to obtain aqueous extracts from green coffee beans, resulting in variations in total phenolic content. These variations are directly related to the biological activities exhibited by the extracts, such as radical scavenging and antibacterial properties.

The graph shown in Figure 4 shows that the analysed *Robusta* samples have a higher DPPH scavenging capacity than the *Arabica* samples. Thus, the DPPH scavenging capacity (%) of Arabica green coffee aqueous extracts ranged from 44.78% to 82.14%, and that of *Robusta* green coffee aqueous extracts ranged from 61.95% to 91.82%. Coffee extracts prepared by infusion with hot water CA1, CR1 have higher trapping capacity than extracts prepared with cold water CA2, CR2, and it is highlighted that the use of high temperature in the extraction process results in a higher amount of compounds with antioxidant character that split DPPH radicals. From the results obtained, water temperature during extraction plays an important role in the analysis of a coffee species as it significantly influences the transport of acidic molecules from the coffee matrix into the solvent, causing variations in DPPH scavenging capacity. These findings agree with the results obtained from the determination of total polyphenolic compounds by the Folin-Ciocâlteu method from the green coffee samples analysed. The data obtained are conclusive and comparable with those in the literature [32].

Research has been carried out on the antimicrobial effect of coffee on pathogenic (*S. aureus* ATCC 25923) and conditionally pathogenic microorganisms (*E. coli* ATCC 25922 and *C. albicans* ATCC 10231) in order to highlight and evaluate the possible antimicrobial potential of coffee samples in the form of aqueous extracts. This study confirmed the lack of antimicrobial activity in green coffee extracts on the pathogens tested (Figure 5). Literature data suggest that the antimicrobial activity of coffee is caused by undefined coffee components generated during the roasting process. Perrone *et al*. [33] reported that the roasting process reduces chlorogenic acid levels compared to green coffee. After 6 minutes of roasting, the chlorogenic acid content decreases by 56%, and after 15 minutes, it decreases by 99%. The authors also found that chlorogenic acids are incorporated into melanoidins during the roasting process.

Other authors discuss the potent antimicrobial properties of melanoidins in coffee that can be harnessed to prevent the proliferation of harmful bacteria in food. These substances have the ability to alter the composition and function of the cytoplasmic membrane, as well as to hinder DNA repair mechanisms, thus facilitating the suppression of bacterial growth [34].

Research by Denyer and Maillard [35] showed that antimicrobial agents have the greatest impact on Gram-positive bacteria, followed by Gram-negative bacteria and mycobacteria. The reason behind the increased susceptibility of Gram-positive bacteria to antibiotics and antimicrobial agents is the lack of an outer membrane. This outer membrane serves as a primary defence against harmful substances in the environment and additionally acts as an extra layer of stability for the cell [36].

Gram-negative bacteria possess an outer membrane that makes them impermeable to antimicrobial agents. This is because the target sites for these agents are located inside the cell, particularly in the cytoplasm. The presence of porin channels further contributes to the resistance of Gram-negative bacteria to antibiotics and other antimicrobial agents. These channels selectively facilitate the entry of essential nutrients while removing substances detrimental to the survival of these bacteria, including antibiotics [37].

Therefore, the findings derived from the analysis of CA1, CA2, CR1 and CR2 green coffee samples indicate the need for further research to achieve strong and practical antimicrobial impact in specific topical pharmaceutical formulations.

Conclusions

Two different extraction techniques, hot infusion and cold maceration, were used and compared to evaluate the antioxidant properties of Arabica and Robusta green coffee beans using distilled water as a solvent. It was shown that the extraction conditions significantly affected the antioxidant activities of the four extracts obtained from green coffee beans (CA1, CA2, CR1, CR2). From the extracts obtained, total phenol concentration was analysed using the Folin-Ciocâlteu method, and a higher content was obtained for coffee samples extracted with hot water CA1 and CR1. It can also be pointed out that there is a higher concentration of TPC in the *Robusta* coffee sample compared to the *Arabica* sample, regardless of the extraction temperature. Coffee extracts prepared by hot water infusion CA1, CR1 have higher anti-oxidant capacity than extracts prepared with cold water CA2, CR2. Also, *Robusta* coffee extract shows higher antioxidant activity than Arabica coffee extract. A higher caffeine content was obtained in the *Robusta* green coffee. According to information found in scientific literature, chlorogenic acid is observed to be the main component of phenolic compounds in green coffee beans. In addition, as the roasting process intensifies, there is a reduction in the total polyphenol content of coffee. This is due to the increase in temperature during roasting, which triggers a reaction in the phenolic compounds and enhances the flavour of the coffee. The variation in roasting levels directly impacts the antioxidant activity and total phenolic content of both Arabica and Robusta coffees.

This study confirmed the lack of antimicrobial activity of green coffee extract on the pathogens tested. The absence of microbial action in green coffee samples, whether brewed at low or high temperatures, can be attributed to the lack of essential nutrients required for metabolic processes and natural compounds found in roasted coffee that possess antimicrobial properties. Information from the literature highlights the evident antimicrobial properties in Robusta and Arabica roasted coffees, attributed to their high levels of caffeoylquinic acid and melanoidins. These melanoidins are formed by the Maillard reaction during the thermal processing of coffee, involving compounds containing both carbonyl and amino groups.

Therefore, more research is recommended to improve the general understanding of specific antimicrobial compounds.

Conflict of interest

The authors declare no conflict of interest.

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