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Is coffee powder extract a possible functional ingredient useful in food and nutraceutical industries?

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PAPER

Abstract

The present study aimed to assess the phytochemical content and *in vitro* bioactivity of ethanolic extracts of Arabica (A) and/or Robusta (R) coffee powder having different geographical origins. For this purpose, total phenols (TPC) and flavonoids (TFC) content as well as α- and β-tocopherol were quantified. The antioxidant activity was assessed by using a multi-target approach in which the radical scavenging potential, the protection from lipid peroxidation, and the involvement of the iron-reducing mechanism were applied. The carbohydrate hydrolyzing enzymes' (α-amylase and α-glucosidase) inhibitory activities were also assessed. Arabica coffee sample (C2-A) showed the highest TPC, TFC, and α-tocopherol content with values of 63.1 mg chlorogenic acid equivalents (CAE)/g dry powder, 16.2 mg of quercetin (QE) equivalents/g dry powder, and 5.6 mg/100 g dry powder, respectively. Relative Antioxidant Capacity Index (RACI), used to statistically integrate results from 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing ability power (FRAP), and protection of lipid peroxidation assays, evidenced that sample C4-R derived from Robusta from Guatemala showed the highest antioxidant potential with a value of –0.61. Arabica from Puerto Rico was the most active against α-amylase, whereas the blend Arabica/Robusta sample (C5-$A_{60}R_{40}$) showed the highest inhibitory activity against α-glucosidase with IC_{50} values of 120.2 and 134.6 mg/mL, respectively. The results show how the qualitative-quantitative composition of the extracts is strongly associated not only with the variety but also with the geographical origin of the samples.

Keywords: antioxidant activity; coffee; ethanolic extract; hypoglycemic effect; phenols; tocopherols

Introduction

Coffee is consumed worldwide with a total production of 16,868 million of 60-kg bags in 2019/2020. The two commercially produced coffee species are *Coffea Arabica* Linn. (known as Arabica) and *Coffea canephora* Pierre *ex* Froehner (known as Arabica). More than 70 countries produce coffee, but most of the global output comes from the top five producers: Brazil, Vietnam, Colombia, Indonesia, and Ethiopia. Arabica coffee is mainly cultivated in Colombia, whereas Robusta is mainly cultivated in Vietnam and Ethiopia. Brazil cultivates

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copious amounts of both species (International Coffee Organization, 2019). Roasted coffee contains many bioactive compounds that have beneficial effects on human health. Most investigations have so far focused on the potential therapeutic effect of caffeine (Farah and de Paula Lima, 2019). Cafestol and kahweol, present in the coffee unsaponifiable matter, have positive effects against cancer and diabetes. Other secondary metabolites of coffee beans, such as tocopherols and polar phenolic compounds, have well-known antioxidant abilities. Among them, chlorogenic, ferulic, cinnamic, and caffeic acids are the most frequently investigated (Dórea and da Costa, 2005). Furthermore, the presence of tyrosol in spent espresso coffee grounds in rather high concentration, from 0.83 to 11.85 mg/100 g of dry spent powder, was recently reported (Balzano *et al*., 2020). According to different studies, tyrosol demonstrates a wide spectrum of biological effects on physiological processes. Based on the most recent epidemiological and research data, consumption of coffee is associated with a lower risk of developing type II diabetes in healthy individuals, probably requiring a series of mechanisms of action involving the antioxidant activity and interventions on glucose homeostasis (Osama *et al*., 2021). Diabetes mellitus (DM) will reach pandemic proportions in the next 20 years. The International Diabetes Federation estimated that in 2025, 371 million people will be affected by DM (IDF, 2019). Chronic hyperglycemia, insulin resistance in target tissues, and a relative deficiency of insulin secretion from pancreatic b-cells are the major features of type 2 DM (T2DM). The strict linkage between ROS and metabolic disorder was recently demonstrated (Carrier, 2017). Nutritional stress, caused by excess of carbohydrate and/or high-fat diets, promotes oxidative stress and results in decreased antioxidant status. Oxidative stress, in fact, may contribute to the long-term deterioration of pancreatic islet β-cell by affecting mitochondrial ATP production, which is necessary for insulin secretion. The consequent mitochondrial dysfunction influenced insulin sensitivity within muscle, liver, and adipose tissue. To reduce sugar intestinal absorption, treatment of T2DM patients with carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase) inhibitors has been reported (Loizzo *et al*., 2017). Regarding chronic diseases, including hyperglycemia, the market of nutraceutical ingredients, as phytochemical and plant extracts, is projected to reach a compound annual growth rate of 7.5%. Due to the rising global demand for nutraceuticals made up of natural ingredients (Global Nutraceutical Statistics, 2019), the formulation of new natural extracts and the study of their bioactivities and their content of bioactive molecules become a fundamental task. From this point of view and considering the safety and acceptability for the human use of ethanol compared to other solvents, the coffee powder ethanol extracts could represent suitable ingredients in formulations of nutraceutical/functional foods rich in antioxidant compounds. Anyway, the standardization of raw material for their preparation is quite a challenge. In fact, it is known that the overall composition of bioactive substances of roasted coffee, as well as their functional activity, is heavily influenced by agronomical and technological factors (Kròl *et al*., 2020). The genetic background (species and variety), geographical origin, and roasting conditions of the coffee beans play a pivot role in defining the final bioactivity of the derived coffee products. Within this frame, the present study aimed to assess the phytochemical content, in terms of total phenols, total flavonoids, α- and β-tochopherol, and *in vitro* antioxidant activity evaluated by ABTS, DPPH, FRAP, and β-carotene bleaching test of ethanol extracts obtained from roasted *Coffea Arabica* and *C. Robusta* beans. These samples are characterized by different geographical origins (Brazil, Colombia, Vietnam, India, Ethiopia, Guatemala, Puerto Rico, and Costa Rica) and commercial coffee blend (Arabica/Robusta) powders. Furthermore, the hypoglycemic effect of the ethanol extracts was assessed by testing the inhibition of carbohydrate-hydrolyzing enzymes against α-amylase and α-glucosidase.

Materials and Methods

Reagents and standard

All chemicals and reagents used in this study were purchased from Sigma-Aldrich Chemical Co. Ltd (Milan, Italy) and VWR International (Milan, Italy) and, unless specified otherwise, were of analytical grade or higher.

Coffee samples and preparation of ethanolic extract

The roasted coffee powder from *Coffea Arabica*, *C. canephora* var. Robusta, and roasted coffee blends were supplied by a local industrial coffee roaster (Caffè del Faro, Robin S.r.l., Montegranaro, Italy) able to confirm their botanical and geographical origin, as well as the general type of postharvest processing (dry/wet process). Both Arabica and Robusta coffees had different geographic origins. All samples were roasted under the same conditions (175°C, 15 min). The compositional features of coffee samples investigated are reported in Table 1.

Seven grams of coffee powder were added to 30 mL of anhydrous ethanol and the mixture was magnetically stirred in the dark, at 25°C for 12 h. Then, the top phase was filtered and dried in a rotary evaporator. For each coffee sample, the ethanol extraction procedure was repeated in triplicate.

Table 1. Composition and geographical origin of coffee blend powders used for preparing ethanol extracts.

Sample code	Coffee blend composition
C1-A	Arabica 100% (Puerto Rico)
C2-A	Arabica 100% (50% Brazil, 20% Colombia, 20% Guatemala, 10% Ethiopia)
C3-A	Arabica 100% (Colombia)
C4-R	Robusta 100% (Guatemala)
C5-$A_{60}R_{40}$	60% Arabica (20% Brazil, 20% Colombia, 10% Guatemala, 10% Costa Rica)/40% Robusta (15% Vietnam, 25% India)
C6-$A_{10}R_{90}$	10% Arabica (Brazil)/90% Robusta (20% India, 70% Vietnam)
C7-$A_{75}R_{25}$	Decaffeinated coffee blend Arabica 75% (Unknown)/Robusta 25% (Unknown)

Bioactive phytochemicals content in coffee powder ethanol extracts

Total phenol (TPC) and flavonoid (TFC) contents were quantified using the spectrophotometric methods already published elsewhere (Loizzo *et al*., 2019). Coffee extract at a concentration of 1.5 mg/mL (0.1 mL) was mixed with a solution of Folin–Ciocalteu reagent (0.5 mL) and water (1 mL). After 1 min of incubation, 1.5 mL of 20% sodium carbonate was added, and the mixture was incubated at room temperature. The absorbance was measured at 765 nm using a UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy). The total phenol content was expressed as milligrams of chlorogenic acid equivalents (CAE)/g dry powder. In the total flavonoid content (TFC) determination, coffee extract was mixed with aluminum chloride solution (2%) in a 1:1 *ratio* and incubated at room temperature for 15 min. The absorbance was measured using a UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy) at 510 nm. The TFC was expressed as milligrams of quercetin equivalents (QE)/g dry powder.

Tocopherols' profile was determined following the procedure reported by Giardinieri *et al*. (2019). Coffee ethanolic extract was dissolved in acetonitrile and analyzed by means of ultra-high performance liquid chromatography-fluorescence (UHPLC-FLD) using Ascentis® Express C18 (75 × 4.6 mm, 2.7 µm, from Supelco, Milan, Italy) as the analytical column. The mobile phase was acetonitrile/methanol (90:10, v/v), at a flow rate of 0.45 mL/min. The injection volume was 1 µL. FLD was set with an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Calibration curves (25–250 µg/mL) were prepared for quantitative analysis with R^2 higher than 0.996 for both tocopherols.

In vitro antioxidant assays

ABTS and DPPH radicals scavenging tests were performed following the procedures reported by Loizzo *et al*. (2019), to investigate the radical scavenging potential of coffee powder samples.

For the ABTS test, a solution of ABTS radical cation was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and stored at room temperature. After 12 h, the solution was diluted with ethanol to an absorbance of 0.70 at 734 nm using a UV-Vis Jenway 6003 spectrophotometer. Dilution of extracts in ethanol was added to 2 mL of diluted $ABTS^+$ solution in order to test the following concentrations from 400 to 1 µg/mL. After 6 min, the absorbance was read at 734 nm by using an UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy).

In the DPPH test, an aliquot of 1.5 mL of 0.25 mM DPPH radical (DPPH·) in ethanol was mixed with 12 µL of coffee extract to test concentrations ranging from 1000 to 1 µg/mL. The mixture was shaken and allowed to reach a steady state at 25°C for 30 min. After that, the absorbance was read at 517 nm by using the UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy). Ascorbic acid was used as positive control in both tests, and IC_{50} values are reported in Table 4.

Protective effect on lipid peroxidation was assessed by using the previously described β-carotene bleaching test (Loizzo *et al*., 2019). One milliliter of β-carotene (0.2 mg/mL in chloroform) was mixed with linoleic acid (20 µL) and 100% Tween 20 (200 µL). After evaporation of the solvent and dilution with water, the emulsion (288 µL) was added to a 96-well microplate containing 12 µL of coffee extract in ethanol (concentrations ranging from 100 to 2.5 µg/mL). The plate was shaken and placed in a water bath at 45°C for 30 and 60 min of incubation. The absorbance was measured at 470 nm by using UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy). Propyl gallate was used as positive control, and IC_{50} is reported in Table 4.

The ***FRAP test*** was performed following the procedure previously described by Loizzo *et al*. (2019). The FRAP value represents the ratio between the slope of the linear plot for reducing Fe^{3+}-TPTZ reagent by different coffee powder ethanol extracts, compared to the slope of the plot obtained for $FeSO_4$. Butylated hydroxytoluene (BHT) was used as positive control. For the preparation of the FRAP reagent, a mixture of 2.5 mL of 10 mM tripyridyltriazine (TPTZ) solution, 40 mM HCl, 2.5 mL of 20 mM FeCl3, and 25 mL of 0.3 M acetate buffer (pH 3.6) was prepared. Sample at a concentration of 2.5 mg/mL in ethanol (100 µL) was mixed with 2.0 mL of FRAP reagent

and 900 mL of water; the absorbance was measured at 595 nm by using the UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy) after 30 min of incubation at room temperature.

Carbohydrate hydrolysis enzymes inhibition

The hypoglycemic potential of coffee powder extracts was assessed by using the α-amylase and α-glucosidase inhibitory tests (Loizzo *et al*., 2019). The enzyme solution was prepared by adding 0.0253 g of enzyme in 100 mL of cold water, and the starch solution was prepared by stirring (at 65°C for 15 min) 0.125 g of potato starch in 25 mL of sodium phosphate buffer (20 mM) and sodium chloride (6.7 mM). Samples were dissolved in ethanol at concentrations ranging from 1000 to 25 μg/mL, added to starch solution, and left to react with the enzyme at 25°C for 5 min. The absorbance was read at 540 nm by using the UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy).

In the α-glucosidase inhibitory test, a maltose solution was prepared by dissolving 12 g of maltose in 300 mL of 50 mM sodium acetate buffer; α-glucosidase (EC 3.2.1.20) solution was prepared by adding 1 mg of enzyme (10 units/mg) in 10 mL of ice-cold distilled water; and O-dianisidine (DIAN) solution was prepared by dissolving 1 tablet in 25 mL of distilled water [15]. The peroxidase/glucose oxidase (PGO) system-color reagent solution was obtained by dissolving one capsule in 100 mL of ice-cold distilled water. A mixture of 5 μL of the sample (at concentrations ranging from 1000 to 25 μg/mL), 250 μL maltose solution, and 5 μL enzyme was left to incubate at 37°C for 30 min. Then, 50 μL of perchloric acid was added, and the mixture was centrifuged. The supernatant was collected and mixed with 5 μL of DIAN and 300 μL of PGO and left to incubate at 37°C for 30 min. The absorbance was read at 500 nm by using the UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy). Acarbose was the positive control in both tests, and IC_{50} is reported in Table 5.

In the α-glucosidase inhibitory activity test, Acarbose was used as a positive control in both tests.

Statistical analysis

Data were expressed as means ± standard deviation (S.D.) (n = 3). Differences of polar phenolic substances and tocopherol content among samples were calculated using one-way analysis of variance (ANOVA) with Tukey's *post hoc* procedure ($P < 0.05$). The inhibitory concentration of 50% (IC_{50}) was calculated by nonlinear regression with the use of Prism Graph Pad Prism version 4.0 for Windows (Graph Pad Software, San Diego, CA, USA). Differences within and between groups were evaluated by one-way analysis of variance test (ANOVA) followed by a multicomparison Dunnett's test compared with the positive control at a significance level of $P < 0.001$. *Pearson*'s correlation coefficient (r) and linear regression, assessment of repeatability, calculation of average and relative standard deviation were performed using Microsoft Excel 2010 software. The Relative Antioxidant Capacity Index (RACI) was used as a statistical approach to compare the antioxidant activity of coffee powder ethanol extracts obtained by different applied tests (Loizzo *et al*., 2019).

Results and Discussion

Ethanol extraction yield

Ethanol was chosen as the extraction solvent because it complies with the legislation on extraction solvents to be used in the production of foodstuffs and food ingredients (Directive 2009/32/EC, 2009), and also considering the effectiveness toward the extraction of bioactive substances, the toxicity, and environmental impact (Socaci *et al*., 2018). The investigated coffee powders provided an ethanol extract yield ranging from 43.5 to 111.0 g/kg dry powder. Generally, Arabica coffee powders gave higher extraction yields than Robusta samples, with the blends containing higher percentage of Robusta (40 and 60%) giving lower yields. Results are similar to those obtained from spent ground coffee, reported by Balzano *et al*. (2020), where samples of spent ground coffee extracted in the same experimental conditions gave a range of 58 to 112 g/kg of dry powder.

Bioactive phytochemicals in coffee ethanolic powder

Table 2 shows the average values of TPC and TFC in coffee powder ethanolic extracts. TPC content ranged from 11.0 to 63.1 mg CAE/g dry powder, whereas TFC varied from 4.5 to 16.2 mg QE/g dry powder. Generally, samples obtained from the Arabica variety had higher TPC and TFC than Robusta or Arabica/Robusta mixtures. These results are in agreement with those previously reported by other researchers. In particular, Bobková *et al*. (2020) investigated the effect of roasting process on the TPC of Arabica and Robusta coffee beans from different geographical origins. TPC values ranged from 49.19 to 74.05 mg CAE/g dry powder. Water coffee extracts showed the highest levels of TPC in green and light roasted coffees where values ranged from 49.19 to 74.05 g GAE/kg for Vietnam Queen and Ethiopia Sidamo, respectively, and from 38.34 to 59.79 g GAE/kg for India Monsooned Malabar and Ethiopia Sidamo, respectively. Interestingly,

the roasting process led to a reduction in TPC values from the green to dark roasting stage. This evidence confirmed that the coffee-growing region has probably an important influence on the development of this class of phytochemicals in coffee. The impact of the roasting process was the object of investigation by Sulaiman *et al.* (2011) that evidenced how TPC decreased linearly over the roasting temperature from 63.51 mg CAE/g coffee beans (roasted at 200°C) to 42.56 mg CAE/g coffee beans (roasted at 240°C). Similarly, Kròl *et al.* (2020) demonstrated that coffees roasted in light and medium roasting conditions are richer in TPC in comparison to dark roast coffee. Furthermore, organic coffee beans showed higher TPC and TFC content than conventional coffee beans (8.95 *vs* 8.28 mg/g and 1.35 *vs* 0.94 mg/g, respectively). The same observation was done by Acidri *et al.* (2020) that found a decline in TPC from 146.8 to 87 mg GAE/g DW in Indonesian Arabica coffee after the roasting process. The great variability of TPC found in literature confirmed that the content of these phytochemicals may be related to the varieties, the cultivation method, as well as to the coffee origin.

Tocopherol content

Tocopherols are very important molecules effectively inhibiting lipid oxidation in foods and the biological system. In coffee beans, the tocopherol content is approximately 3–10 mg/100 g (Górnaś *et al.*, 2014). To investigate the relation between the overall antioxidant activity of the coffee extracts and their main chemical contributors, tocopherol composition and content were determined by means of RP-UPLC-FLD. As a result, α- and β- were the tocopherols largely predominant in the ethanol coffee extract samples investigated, with a certain variability of the total content, ranging, in the dry powder form, between 3 and 27 mg/100 g (in the decaffeinated sample C7-$A_{75}R_{25}$ and in the sample C1-A from Puerto Rico, respectively). The values referred to the ethanol extract range between 36 and 362 mg/100 g (Table 3). β-Tocopherol is predominant, and the ratio β-tocopherol/α-tocopherol varies from 1.6 to 4.0. The overall results are in agreement with several studies (Alves *et al.*, 2009; González *et al.*, 2001; Górnaś *et al.*, 2014). Górnaś *et al.* (2014) found an average total tocopherol content in roasted Robusta of 11.54 mg/100 g, and 28.8 mg/100 g in roasted Arabica, and an average ratio β-/α-tocopherol of 1.2 in roasted Robusta and 3.1 in roasted Arabica. Alves *et al.* (2009) reported an average total value of a- and β-tocopherol of 9.7 mg/100 g in roasted Arabica and 3.1 mg/100 g in roasted Robusta, with a ratio β-/α-tocopherol of 3.0 and 1.0 for Arabica and Robusta, respectively. González *et al.* (2001), found a total value of α- and β-tocopherol ranging from 1.9 to 4.6 mg/100 g in roasted Robusta and from 11.5 to 19.3 mg/100 g in roasted Arabica, with a ratio β-/α-tocopherol ranging from 2.1 to 6.1 and from 3.5 and 6.0 in roasted Arabica and Robusta, respectively. The variability found in the investigated samples did not allow to highlight any statistical relationships between the tocopherol content and the Arabica/Robusta composition of the powder. A similar situation was observed also by Górnaś *et al.* (2014) and Alves *et al.* (2009).

Coffee powder ethanolic extract bioactivity

Antioxidant effects

Oxidative stress in humans arises from an imbalance between radical oxygen species (ROS) and endogenous defense enzymes such as superoxide dismutase, catalase, glutathione peroxidase, etc. Besides these defenses, consumption of dietary antioxidants is fundamental to prevent the development of several diseases. By using different *in vitro* tests, we have checked the ability of

Table 2. Total phenols content (TPC) and flavonoids content (TFC) in coffee powder ethanol extracts.

Sample	TPC°	TFC^
C1-A	51.5 ± 0.9^{b}	15.6 ± 0.5^{b}
C2-A	63.1 ± 1.1^{a}	16.2 ± 0.7^{a}
C3-A	25.9 ± 0.4^{e}	13.3 ± 0.4^{c}
C4-R	11.0 ± 0.3^{g}	4.5 ± 0.0^{g}
C5-$A_{60}R_{40}$	35.5 ± 0.5^{d}	11.0 ± 0.2^{d}
C6-$A_{10}R_{90}$	19.9 ± 0.4^{f}	10.0 ± 0.2^{e}
C7-$A_{75}R_{25}$	38.6 ± 0.6^{c}	9.5 ± 0.1^{f}

Data are expressed as mean ± standard deviation (n = 3). °mg chlorogenic acid equivalents (CAE)/g dry powder; ^mg of quercetin (QE) equivalents/g dry powder; and values in the same column with different superscript letters are significantly different (P < 0.05).

Table 3. Content of α- and β-tocopherols in coffee ethanol extracts.

Sample	α – tocopherol§	β – tocopherol§
C1-A	5.4 ± 0.5^{b}	21.3 ± 0.2^{a}
C2-A	5.6 ± 0.5^{a}	10.6 ± 0.2^{c}
C3-A	4.1 ± 0.0^{c}	13.9 ± 0.5^{b}
C4-R	4.1 ± 1.1^{c}	10.5 ± 0.5^{c}
C5-$A_{60}R_{40}$	2.3 ± 0.0^{d}	4.9 ± 0.1^{b}
C6-$A_{10}R_{90}$	5.4 ± 1.1^{b}	10.8 ± 0.4^{c}
C7-$A_{75}R_{25}$	1.0 ± 0.0^{e}	1.6 ± 0.0^{d}

Data are expressed as mean ± standard deviation (n = 3). §mg/100 g dry powder. Values in the same column with different superscript letters are significantly different (P < 0.05).

coffee powder ethanolic extract to act as an antiradical or antioxidant agent. The approach with multiple tests is recommended for measuring antioxidant properties of food matrix to better reflect their potential protective effects. The antiradical activity characterizes the ability of phytocomplex to react with free radicals, while the antioxidant activity represents the ability to inhibit the oxidation process, which usually occurs through different reactions (Tirzitis and Bartosz, 2010). Generally, a concentration-effect relationship was found in all the tests except in the FRAP assay (Table 4).

The extract obtained from the Robusta sample (C4-R) showed the highest radical scavenging potential with IC_{50} values of 1.1 and 9.2 mg/mL for ABTS and DPPH assay, respectively. A promising radical scavenging activity was also observed in the decaffeinated sample C7-$A_{75}R_{25}$ (IC_{50} values of 8.6 and 16.4 mg/mL for ABTS and DPPH assay, respectively). Both samples are also able to react as reductants in the FRAP assay (FRAP values of 56.8 and 56.9 mM Fe (II)/g at 2.5 mg/mL). These values are quite lower than that reported for the positive control BHT (63.2 mM Fe (II)/g at 2.5 mg/mL). The antioxidant activity of coffee samples was also tested, using the b-carotene bleaching method. This assay evaluated the ability of the phytocomplex to protect against lipid peroxidation. Since no high temperatures are required, the antioxidant activity of thermosensitive phytochemicals may be determined and quantitatively evaluated. The sample C4-R showed a promising protection against lipid peroxidation with IC_{50} values of 5.3 and 5.9 mg/mL at 30 and 60 min of incubation, respectively. A promising activity was also observed with decaffeinated coffee powder extract (C7-$A_{75}R_{25}$), followed by the blend C5-$A_{60}R_{40}$. RACI was calculated to evaluate and create a ranking clustering of the antioxidant capacity of different samples. Based on this parameter, the following antioxidant ranking could be evidenced: C4-R>C5-$A_{60}R_{40}$>C7-$A_{75}R_{25}$>C6$A_{10}R_{90}$>C2-A>C1-A>C3-A (Figure 1).

A positive *Pearson's* correlation coefficient was found between TFC and DPPH (r = 0.70) and b-tocopherol and both DPPH and ABTS tests (r = 0.87 and 0.76, respectively). A significant positive correlation coefficient was also observed for TFC and b-carotene bleaching test after 30 and 60 min of incubation (r = 0.61 and 0.79, respectively). Based on RACI statistical approach C4-R, richest in phenols and b-tocopherol resulted the most active as antioxidant. Tocopherols are able not only to react toward free radicals and hydroperoxides but also with many other possible side reactions which are affected by tocopherol concentrations, type of substrate, and by other chemical species acting as pro-oxidants and synergists in the system.

Carbohydrate hydrolysis enzymes' inhibitory effect by coffee powder extracts

The inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, given by coffee powder extract was concentration-dependent (Table 5). Generally, α-amylase enzyme was the most sensitive to the action of the extracts (see selectivity index, SI).

Table 4. ***In vitro*** **antioxidant activity of coffee powder ethanol extracts from Arabica and Robusta varieties and their blend.**

Sample	DPPH test IC_{50} (μg/mL)	ABTS test IC_{50} (μg/mL)	FRAP# test μM Fe (II)/g	β-Carotene bleaching test IC_{50} (μg/mL)	
				30 min	60 min
C1-A	809.5 ± 2.9****	411.3 ± 3.8****	16.9 ± 1.3****	34.6 ± 0.7****	38.9 ± 1.4****
C2-A	212.6 ± 2.4****	101.2 ± 3.7****	6.8 ± 0.7****	20.5 ± 0.8****	94.1 ± 1.9****
C3-A	460.4 ± 3.5****	445.5 ± 3.1****	13.5 ± 0.7****	52.1 ± 0.7****	59.6 ± 1.7****
C4-R	9.2 ± 0.8**	1.1 ± 0.4^{ns}	56.8 ± 2.7**	5.3 ± 0.6*	5.9 ± 0.9*
C5-$A_{60}R_{40}$	138.5 ± 2.6****	83.6 ± 1.2****	2.4 ± 0.6****	11.0 ± 0.2***	18.3 ± 1.2****
C6-$A_{10}R_{90}$	178.9 ± 3.5****	52.8 ± 1.3****	15.0 ± 0.6****	50.9 ± 1.6****	43.9 ± 1.6****
C7-$A_{75}R_{25}$	16.4 ± 1.1***	8.6 ± 0.9***	57.9 ± 1.8**	10.9 ± 1.4***	16.8 ± 1.7****
Positive controls					
Ascorbic acid	5.0 ± 0.8	1.7 ± 0.06	–	–	–
BHT	–	–	63.2 ± 4.3	–	–
Propyl gallate	–	–	–	1.0 ± 0.04	0.09 ± 0.004

Data are given as media ± S.D. (n = 3); #at 2.5 mg/mL; DPPH Radical Scavenging Activity Assay; Antioxidant Capacity Determined by Radical Cation ($ABTS^+$). β-Carotene bleaching test. Ferric ion reducing antioxidant power (FRAP); Ascorbic acid. BHT and Propyl gallate were used as positive controls in antioxidant tests. Differences within and between groups were evaluated by One-way ANOVA followed by a multicomparison Dunnett's test (α = 0.01): ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.1 compared to the positive controls.

C1-A extract showed the best activity with IC_{50} value of 120.2 mg/mL followed by Guatemalan Robusta extract (C4-R) and decaffeinated sample (C7-$A_{75}R_{25}$) with IC_{50} values of 122.4 and 130.9 mg/mL, respectively. Except sample C5-$A_{60}R_{40}$ that showed IC_{50}value of 134.6 mg/mL against α-glucosidase, all other samples are less active (IC_{50} values in the range 320.4–472.4 mg/mL). Sample C1-A showed the highest TPC content. Among phytochemicals able to interfere with carbohydrate hydrolyzing enzymes, phenols represent the main studied compounds (Loizzo *et al*., 2017). Several studies pointed out that CGAs, that are reported as the most abundant phytochemicals in coffee extract (Jeon *et al*., 2019; Jeszka-Skowron *et al*., 2016), inhibited both α-amylase and α-glucosidase with IC_{50} values of 25 and 26.07 M, respectively (Oboh *et al*., 2015). CGAs also stimulate glucose uptake in skeletal muscle and suppression of hepatic glucose production by AMPK activation (Ong *et al*., 2013). In addition, it has been found that CGAs could modulate glucose in both genetically and healthy metabolic related disorders including DM (Naveed *et al*., 2018). Differently Nyambe-Silavwe and Williamson (2018) demonstrated that both CGAs are only weak inhibitors of human salivary α-amylase despite several publications claiming otherwise. In fact, more recently, Herawati *et al*. (2019) demonstrated that Robusta coffee beans extract obtained after roasting, grinding, and brewing process was able to inhibit α-glucosidase activity up to 69% and exerted anti-glycation activity.

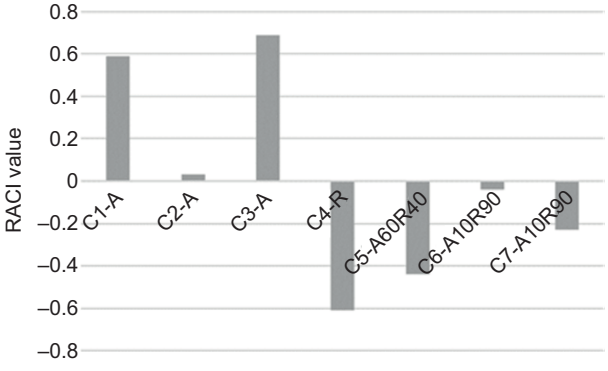

Figure 1. Relative Antioxidant Capacity Index (RACI) of coffee powder ethanol extracts from Arabica and Robusta varieties.

Conclusion

The present work investigated the bioactive phytochemicals content, in vitro antioxidant activity, and hypoglycemic potential of ethanol extracts deriving from Arabica and Robusta coffee powders, as well as their mixture, from different geographical origins. A great variability in terms of total phenol, flavonoid, and tocopherol content was observed, and this evidence strongly influenced the bioactivity although it is not possible to identify any relationship with the coffee variety and blend composition. Our findings strongly emphasize that coffee ethanol extracts should be used as a value-added ingredient for formulations of nutraceutical or functional products useful for the prevention of disease associated with oxidative stress and hyperglycemic condition.

Declarations

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Table 5. Carbohydrates hydrolyzing enzymes inhibition by coffee powder ethanol extracts from Arabica and Robusta varieties and their blend.

Sample	α-Amylase IC_{50} (μg/mL)	α-Glucosidase IC_{50} (μg/mL)	SI α-Amylase/ α-Glucosidase
C1-A	120.2 ± 3.1****	412.5 ± 6.1****	0.3
C2-A	880.3 ± 7.4****	362.2 ± 6.3****	2.4
C3-A	132.6 ± 5.2****	445.3 ± 8.1****	0.3
C4-R	122.4 ± 5.0****	320.4 ± 7.2****	0.4
C5-$A_{60}R_{40}$	800.3 ± 8.3****	134.6 ± 2.8****	5.9
C6-$A_{10}R_{90}$	970.5 ± 8.9****	244.7 ± 3.5****	4.0
C7-$A_{75}R_{25}$	130.9 ± 2.8****	472.4 ± 8.6****	0.3
Positive control			
Acarbose	52.4 ± 3.9	35.3 ± 3.7	1.5

Data are expressed as mean ± S.D. (n = 3). One-way ANOVA ****P < 0.0001 followed by a multicomparison Dunnett's test: ****P < 0.0001 compared with acarbose.

Conflicts of Interest

The authors have no conflicts of interest to disclose with the present submission.

Availability of Data and Material

The data sets supporting the results of this article are available from the corresponding author (M.R.L.) upon reasonable request.

Code Availability

Not applicable.

Author Contributions

D.P. conceptualized and supervised the study; M.R.L. prepared the original draft; A.N., M.L., L.G., and B.F. did the formal analysis; R.T., P.L., and D.F. performed data curation; O.N., P.L., and D.F. edited the manuscript; N.G.F and D.P. were concerned with funding acquisition. All authors have read and agreed to the published version of the manuscript.

Ethics Approval

Not applicable. Ethical experiments were not involved in this article.

Consent to Participate

All authors consented to participate.

Consent for Publication

All the authors have read the manuscript and approved it for possible publication.

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