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Comparative analysis of phenolic compounds and biological activities of four varieties of *Coffea arabica* L.

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Abstract

Objective: The consumption of coffee has increased globally because of its flavor and health benefits. The present study aimed to investigate the phenolic compounds and biological activity of four varieties of Coffea arabica L. (Heirloom 1-4) from the flora of Africa.

Methods: Ethanolic extracts of the green and roasted coffee beans were screened in vitro for their total phenols and flavonoids using Folin-Ciocalteu and AlCl3 assays, respectively. In addition, the antioxidant activity of the extracts was evaluated by three complementary tests DPPH free radical-scavenging, β-carotene/linoleic acid, and metal chelating activity. The antibacterial activity of the aqueous extracts of the samples against two gram-positive and two gram-negative bacteria was also studied by the disk diffusion method.

Results: Results showed that the ethanolic extracts from both green and roasted beans of the variety Heirloom 4 contain the highest amounts of phenols and flavonoids. In addition, the antioxidant activity assays indicated that the activity of the studied extracts was lower than ascorbic acid (a standard synthetic antioxidant) in the metal-chelating activity and the DPPH radical scavenging assay. However, the oxidation of linoleic acid in the β-carotene/linoleic acid system was inhibited by all studied extracts. Furthermore, the aqueous extracts of the green and roasted beans of all varieties showed proper antibacterial activity against the bacterial strains tested.

Conclusion: In conclusion, several factors including altitude, variety, extraction methods, and type of solvent used affected the bioactivity of the coffee extracts. All antioxidant activity assays showed a close relationship with the phenolic content of green and roasted samples. Antioxidant activity assays indicated that the green or roasted bean extracts of coffee have potent free radical scavenging activity. Moreover, the differences in the antioxidant activity of green and roasted bean extracts in the same variety could be attributed to the roasting process.

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Introduction

For years, plants have been used globally as food and medicine. They produce bioactive metabolites, which are used to prevent and treat diseases caused by free radicals (Gomaa *et al*. 2019). Excessive free radicals in cells are the cause of oxidative stress in humans which results in various diseases and early aging (Yahayu *et al*. 2020). The human body possesses antioxidant defensive systems that quench free radicals. They are found in enzymatic and non-enzymatic forms (Nimse and Pal 2015). Polyphenols are one of the most important antioxidants, which are found in plants (Priftis *et al*. 2015). In recent years, the antioxidant activity of phenolic compounds such as flavonoids has attracted the interest of many researchers, and some plants rich in phenolic compounds such as cocoa (Davinelli *et al.* 2018), coffee (Priftis *et al.* 2015; Sentkowska *et al*. 2016), and legumes (Kamalvand *et al.* 2021) are useful in the treatment of several diseases.

The *Coffea* L. genus of the Rubiaceaea family has over 500 species. Among these, *Coffea arabica* L. (arabica) and *Coffea canephora* L. (robusta) are the most important commercial species (Jeszka-Skowron *et al.* 2015). Arabica comes mostly from Brazil and Ethiopia, while canephora inhabits South Asia (mainly Vietnam) and the lowlands of Central and West Africa (Sentkowska *et al*. 2016). *C. arabica* L. is known for its low caffeine content and fine aroma and accounts for 65% of the world's coffee production (Cho *et al.* 2013). The chemical composition of coffee brews depends on the coffee species and varieties, as well as the preparation method, brew volume, and roasting degree (Alves *et al.* 2010). Coffee, both green and roasted beans, contains several bioactive compounds with antioxidant activity (Makiso *et al*. 2023). The antioxidant activity of coffee depends on some factors such as coffee bean type and variety, regional conditions, bean processing, and extracting methods (Jung *et al.* 2021; Yust *et al.* 2024). The roasting process is required to bring out coffee flavors (Jung *et al*. 2021). The association between coffee roasting and antioxidant activity has been investigated in some studies, but results were inconsistent. Studies have indicated that the antioxidant activity of coffee is associated with compounds such as caffeine, chlorogenic acids, and tocopherols (Parras *et al.* 2007). Phenolic compounds act as hydrogen donors, metal chelators, reducing agents, and singlet oxygen quenchers to enforce their antioxidant effects (Cho *et al*. 2013).

There is no knowledge about the inter-variety variation concerning the *in vivo* effects of the coffee bean extracts. The existence of any possible differences among coffee varieties may help us to unravel the distinct metabolic properties of the bioactive compounds. Hence, this study aimed to assess the total phenolic content, antioxidant properties, and antibacterial activities of the green and roasted bean extracts from four *C. arabica* varieties, namely Heirloom 1, Heirloom 2, Heirloom 3, and Heirloom 4.

Materials and Methods

Chemicals

Gallic acid, ascorbic acid, aluminum chloride, butylated hydroxytoluene (BHT), chloroform, ethanol, Folin-Ciocalteu reagent, nutrient agar, potassium acetate, sodium bicarbonate, and Tween-40 were purchased from Merck (Darmstadt, Germany). β-carotene, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), linoleic acid, and quercetin were obtained from Sigma (St. Louis, MO, USA).

Coffee sample preparation

The green and roasted beans of four *Coffea arabica* varieties were purchased from the SET Coffee Company (Tehran, Iran). They included Heirloom 1 (Ethiopia, Yirgacheffe, 1950 MASL, natural process), Heirloom 2 (Ethiopia, Yirgacheffe, 1800 MASL, washed process), Heirloom 3 (Ethiopia, Guji, 2100 MASL, washed process), and Heirloom 4 (Ethiopia, Limu, 1600 MASL, washed process).

Extract preparation

Both green and roasted beans were ground to powder. For extraction, 4 g of the powder was added to 100 mL ethanol 70% at room temperature followed by magnetic stirring for 1 h. The mixtures were kept in the dark under refrigeration (2-8 °C) for 18 h. The extracts were recovered by filtration on cellulose support under reduced pressure, and then, concentrated in a rotatory evaporator (60 °C, 82 rpm). The dried extracts were resuspended in 10 mL distilled-deionized water, centrifuged at 4000 rpm for 10 min, and the supernatants were collected for further analyses (Affonso *et al*. 2016).

Determination of total phenol and flavonoid contents

The Folin-Ciocalteu method was used to determine the total phenol content of the extracts (Singleton and Rossi 1965). Briefly, 100 µL of the ethanolic extract (1 mg/mL) was mixed with 0.75 mL Folin-Ciocalteu reagent which was previously diluted 10-fold with distilled water and kept at 22 °C for 5 min. Then, 0.75 mL sodium bicarbonate (60 g/L) solution was added and mixed thoroughly. The absorbance of samples was determined by the UV-Vis Array Photonix Ar 2017 spectrophotometer at 725 nm at 22 ºC after 90 min. The total phenols content was determined as gallic acid equivalent (GAE) and presented as mg GAE/g dry weight.

The aluminum trichloride method was applied to determine the total flavonoid content with quercetin as a reference compound (Chang *et al*. 2002). Based on this method, a flavonoid-aluminum complex is formed with the maximum absorptivity at 415 nm, after the remaining reaction at room temperature for 30 min. Briefly, 0.5 mL of each extract (1:10 g/mL) was separately mixed with 0.1

mL of 10% aluminum chloride, 1.5 mL of ethanol, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The calibration curve was obtained by preparing the quercetin solution in ethanol at different concentrations from 12.5 to 100 g/mL.

Antioxidant activity assays

DPPH free radical scavenging activity

The hydrogen atoms of the corresponding extracts and several pure compounds were determined by bleaching of the purple-colored methanol solution of DPPH. The stable 1,1- diphenyl-2-picrylhydrazyl radical (DPPH) was used to determining of the free radical scavenging activity of the extracts. The method of Mensor *et al.* (2001) was applied to measure the radical scavenging ability. Briefly, the ethanolic solution of 0.5 mL DPPH (0.3 mM) was added to 2.5 mL of the different concentrations of coffee extracts (0.2, 0.4, 0.6, 0.8, and 1 mg/mL). The samples were first kept in the dark at room temperature. Then their absorbance was read at 517 nm after 30 min. The antiradical activity (I) was measured as follows:

$$I\% = 1- (A_s – A_b)/A_c \times 100$$

Blank samples (A_b) consisted of 1 mL ethanol and 2.5 mL of different concentrations of the extract and the control sample (A_c) contained 1 mL 0.3 mM DPPH and 2.5 mL ethanol. The optical density of the samples, the control, and the empty samples were determined by comparing them with ethanol. The amount of discoloration was plotted against the sample concentration to calculate the IC_{50} value, which is the amount of sample needed to decrease the absorbance of DPPH by 50% (Koleva *et al*. 2002).

Metal chelating activity

The method of Dinis *et al.* (1994) was utilized to determine the chelating activity of ferrous ions. The reaction mixture consisted of 0.5 mL of different concentrations of test compounds, 0.05 mL (2 mM) of $FeCl^{2+}$ solution, and 1.6 mL of deionized water. After 30 s, 0.1 mL 5 mM ferrozine solution was added. The Fe^{2+}-ferrozine magenta complex was very soluble and stable in water. The absorbance was measured at 562 nm after 10 min at room temperature. The relative activities of test compounds to chelate ferrous ion were presented as a percentage of absorbance disappearance as the following formula:

$$\text{Metal chelating activity (\%)} = (A_{control} - A_{sample})/ A_{control} \times 100$$

Where, A_s is the reaction mixture absorbance in the presence of the coffee, and A_c is the absorbance of the reaction mixture in the absence of the coffee extract.

β-Carotene/linoleic acid model system

The antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Barrière *et al*. 2001). A stock solution of β-carotene/linoleic acid was prepared as follows: first, 0.5 mg β-carotene was dissolved in 1 mL chloroform, then 25 µL linoleic acid and 200 mg Tween-40 were added. The chloroform was evaporated using a vacuum evaporator. Then, 100 ml of distilled water, saturated with oxygen (30 min at 100 mL/min), was added with vigorous shaking. In brief, 350 µL portions of the extracts were added to 2.5 mL of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50 ºC for 2 h, together with two blanks, one consisted of the antioxidant BHT as a positive control, and the other comprised the same volume of ethanol instead of the extracts. The absorbance was measured at 470 nm by a spectrophotometer. The inhibition percentage (I%), representing the antioxidant activity of the samples was calculated as follows:

$$I\% = (A_{\beta\text{-carotene after 2h assay}} / A_{\text{initial } \beta\text{-carotene}}) \times 100$$

Where $A_{\beta\text{-carotene after 2h assay}}$ is the absorbance of the remaining β-carotene after 2 h assay in the samples and $A_{\text{initial } \beta\text{-carotene}}$ is the absorbance of β-carotene at the beginning of the experiment (Amiri and Ghiasvand 2016).

Antibacterial activity assay

The antibacterial activity of the aqueous extracts was evaluated against two gram-positive (*Bacillus cereus* PTCC 1247, *Staphylococcus aureus* PTCC 1337) and two gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* PTCC 1707) bacteria by the disk diffusion method (Bauer *et al*. 1966) at three different concentrations (0.1, 0.2, and 0.4 g/mL of the extracts). The extracts were prepared by adding samples to 10 mL boiling distilled water and placing them on boiling water vapor for 20 min. Then, 25 µL of the extracts were applied to blank sterile paper discs (6 mm diameter). These discs were placed onto the nutrient agar medium, previously inoculated with a bacterial suspension (0.5 McFarland standard). The cultures were incubated at 37 ºC for 24 h. The antibacterial activity against each test organism was quantified by determining the mean zone of inhibition. Negative controls were made using discs consisting of 25 µL sterile distilled water, while positive controls were made by chloramphenicol, clindamycin, nitrofurantoin, and vancomycin.

Statistical analysis

The data were recorded as means ± standard deviations. One-way analysis of variance was performed at the p-value ≤ 0.05. Means were compared by Duncan's multiple range test.

Results and Discussion

Total phenol and flavonoid contents

The total phenol content of the green and bean extracts measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 0.0041x - 0.022, R^2 = 0.9982) are shown in Table 1. The results showed the total phenol content of Heirloom 3 and Heirloom 4 varieties were significantly different from other varieties in green bean extracts. In the roasted coffee beans, Heirloom 4 had higher total phenol content than other varieties (Table 1). The roasting process significantly increased the total phenolic content, which could be due to thermal degradation of complex phenolic compounds such as chlorogenic acids into simpler compounds such as melanoidins (Fuller and Rao 2017; Acidri *et al*. 2019). The differences in phenolic content of the varieties can be affected by environmental factors. Altitude is an environmental factor that affects the coffee quality by optimizing temperature. Temperature has an important effect on coffee plants, especially in fruit development and ripening stages. Joët *et al.* (2010) showed that chlorogenic acids and fatty acids in the seed were controlled by the mean air temperature during seed development. The suitable temperature for the arabica coffee is between 18-21 ºC and other temperatures will result in incomplete fruit maturity. Therefore, when the temperature is high throughout the growing period, several chemical compounds accumulate and reduce the coffee quality (Adugna 2021). However, our results showed that the Heirloom 4 variety which grows in the low altitude represents the highest content of phenolic compounds in both green and roasted bean extracts. In another study, Link *et al.*

Table 1. Total phenol content of the roasted and green beans in four *Coffea arabica* varieties studied.

Variety	Roasted beans (mg GAE/g DW[a])	Green beans (mg QE/g DW[a])
Heirloom 1	229.52 ± 6.06[b]	34.22 ± 1.83[b]
Heirloom 2	230.7 ± 12.31[b]	35.85 ± 2.76[b]
Heirloom 3	234.78 ± 4.53[b]	52.11 ± 4.76[a]
Heirloom 4	325.35 ± 14.31[a]	55.64 ± 2.82[a]

[a]DW: Dry weight; Data are expressed as mean ± standard deviation (n = 3); Means with different letters in each column denote significant differences according to Duncan's multiple range test ($p \leq 0.05$).

(2014) reported that the chlorogenic acid content declined with altitude but the alkaloid content increased. In addition, Sridevi and Parvatam (2014) showed the negative effect of altitude on caffeine content.

Results from total flavonoids assessment in terms of quercetin equivalent (the standard curve equation: y = 0.0091x - 0.0206, R^2 = 0.995) in green beans extracts showed that there were significant differences between the Heirloom 3 and Heirloom 4 and the other varieties in total flavonoid content,

however, in the roasted beans extracts the total flavonoid content of Heirloom 4 was significantly higher than other varieties which represents its highest quality (Table 2). It is established that phenolic compounds contribute to the quality (Acidri *et al*. 2019) and nutritional value (Bondam *et al.* 2022) of coffee, and has health-beneficial effects (Bondam *et al.* 2022). They also participate in plant defense mechanisms to alleviate the harmful effects of several biotic and abiotic stresses (Machado *et al.* 2023).

Antioxidant activity

DPPH radical scavenging activity

Coffee plants are indeed an important source of dietary antioxidants, and the antioxidant activity of coffee is primarily due to the accumulation of polyphenols, including phenolic compounds (Priftis *et al.* 2015; Sentkowska *et al.* 2016). Phenolic compounds act as free radical terminators (Kalpoutzakis *et al.* 2023), and their bioactivity properties may be associated with their abilities to chelate metals, inhibit lipoxygenase, and scavenge free radicals (Decker 1997; Yin *et al.* 2008). According to Sacchetti *et al*. (2005), among a large number of tests, the results of a single assay can provide only a reductive suggestion of the antioxidant properties of extracts toward food matrices and must be interpreted with some caution. Moreover, the chemical complexity of extracts, often a mixture of several compounds with different functional groups, chemical behavior, and polarity, could lead to scattered results, depending on the test type. Therefore, Sacchetti *et al.* (2005) recommended multiple

Table 2. Total flavonoid content of the roasted and green beans in four *Coffea arabica* varieties studied.

Variety	Roasted beans (mg GAE/g DW[a])	Green beans (mg QE/g DW[a])
Heirloom 1	154.68 ± 4.08^{c}	16.21 ± 0.39^{b}
Heirloom 2	162.07 ± 4.37^{b}	16.32 ± 2.39^{b}
Heirloom 3	163.69 ± 5.25^{b}	19.40 ± 0.76^{ab}
Heirloom 4	191.71 ± 7.88^{a}	22.19 ± 1.41^{a}

[a]DW: Dry weight; Data are expressed as mean ± standard deviation (n = 3); Means with different letters in each column denote significant differences according to Duncan's multiple range test ($p \leq 0.05$).

assays in screening work. They further stated that among the methods that evaluate the antioxidant activity, very few of them, including DPPH, are useful for determining the activity of both hydrophilic and lipophilic species, thus ensuring a better comparison of the results and covering a wider range of possible applications. The results of the free radical-scavenging capacities of the corresponding extracts are shown in Tables 3 and 4. The extracts from the green beans of all varieties at different

concentrations exhibited more than 50% scavenging activity, whereas the roasted beans showed more than 60% scavenging activity. Ascorbic acid as the standard compound had the IC_{50} value lower than those of all studied extracts from both green and roasted beans. Hence, it was a more potent antioxidant than the tested samples, because the higher antioxidant activity is reflected in a lower IC_{50}. The radical scavenging activity of the green and roasted beans extracts respectively, decreased in the following order: Heirloom 4 (IC_{50} = 0.105 mg/mL, IC_{50} = 0.102 mg/mL) > Heirloom 3 (IC_{50}= 0.165 mg/mL, IC_{50} = 0.1156 mg/mL) > Heirloom 2 (IC_{50} = 0.195 mg/mL, IC_{50} = 0.141 mg/mL) > Heirloom 1 (IC_{50} = 0.196 mg/mL, IC_{50} = 0.150 mg/mL) (Figures 1 and 2). These differences might have been derived from local, climate, and seasonal factors related to the cultivation regions of the varieties. Phenolic compounds with a higher number of hydroxyl groups have enhanced ROS scavenging activity (Świętek *et al.* 2019). It was reported that the decrease in the absorbance of DPPH

Table 3. DPPH radical scavenging activity of the green beans in four *Coffea arabica* varieties studied.

	DPPH concentration (mg/mL)					
Variety	**0.2**	**0.4**	**0.6**	**0.8**	**1**	**Average**
Heirloom 1	52.62 ± 4.13^{m}	68.51 ± 2.45^{kl}	$78.42 \pm 4.40^{h-j}$	$85.97 \pm 2.06^{d-g}$	$89.90 \pm 4.44^{c-e}$	75.08
Heirloom 2	66.77 ± 5.40^{l}	73.51 ± 4.44^{jk}	$80.02 \pm 2.86^{g-i}$	88.66 ± 2.06^{de}	$91.24 \pm 3.49^{b-d}$	80.04
Heirloom 3	67.33 ± 2.06^{l}	75.30 ± 3.49^{ij}	$82.38 \pm 3.01^{f-h}$	88.66 ± 0.79^{de}	$94.84 \pm 1.27^{a-c}$	81.70
Heirloom 4	$74.18 \pm 0.63^{i-k}$	$82.04 \pm 0.32^{f-h}$	$84.51 \pm 0.32^{e-g}$	$86.53 \pm 2.54^{d-f}$	$89.90 \pm 1.59^{c-e}$	83.43
Ascorbic acid	96.43 ± 0.16^{ab}	96.85 ± 0.20^{ab}	98.05 ± 0.22^{a}	98.45 ± 0.62^{a}	99.06 ± 0.12^{a}	97.76

Data are expressed as mean ± standard deviation (n = 2); Means with different letters denote significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Table 4. DPPH radical scavenging activity of the roasted beans in four *Coffea arabica* varieties studied.

	DPPH concentration (mg/mL)					
Variety	**0.2**	**0.4**	**0.6**	**0.8**	**1**	**Average**
Heirloom 1	62.64 ± 0.31^{k}	$79.10 \pm 11.00^{g-j}$	$85.43 \pm 1.72^{d-h}$	$91.43 \pm 2.99^{a-e}$	97.67 ± 1.42^{ab}	83.25
Heirloom 2	76.54 ± 1.73^{ij}	$81.65 \pm 4.56^{f-i}$	$91.66 \pm 3.93^{a-e}$	$92.88 \pm 1.26^{a-d}$	98.33 ± 0.47^{a}	88.21
Heirloom 3	76.76 ± 0.16^{ij}	$78.65 \pm 3.46^{h-j}$	$87.44 \pm 2.04^{c-g}$	$89.21 \pm 6.76^{b-f}$	$95.55 \pm 0.00^{a-c}$	85.52
Heirloom 4	71.31 ± 6.92^{j}	$79.32 \pm 5.03^{g-j}$	$80.54 \pm 1.10^{g-i}$	$83.77 \pm 3.15^{e-i}$	96.78 ± 2.67^{ab}	82.34
Ascorbic acid	96.43 ± 0.16^{ab}	96.85 ± 0.20^{ab}	98.05 ± 0.22^{a}	98.45 ± 0.62^{a}	99.06 ± 0.12^{a}	97.76

Data are expressed as mean ± standard deviation (n = 2); Means with different letters denote significant differences according to Duncan's multiple range test ($p \leq 0.05$).

radical caused by phenolic compounds is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radicals by hydrogen donation and is visualized as a discoloration from purple to yellow (Thirumalai *et al.* 2011), and flavonoids with their hydroxyl

groups may be also responsible for the radical scavenging potential of the extracts. Moreover, a strong positive correlation between total phenol content and scavenging activity was reported here, while

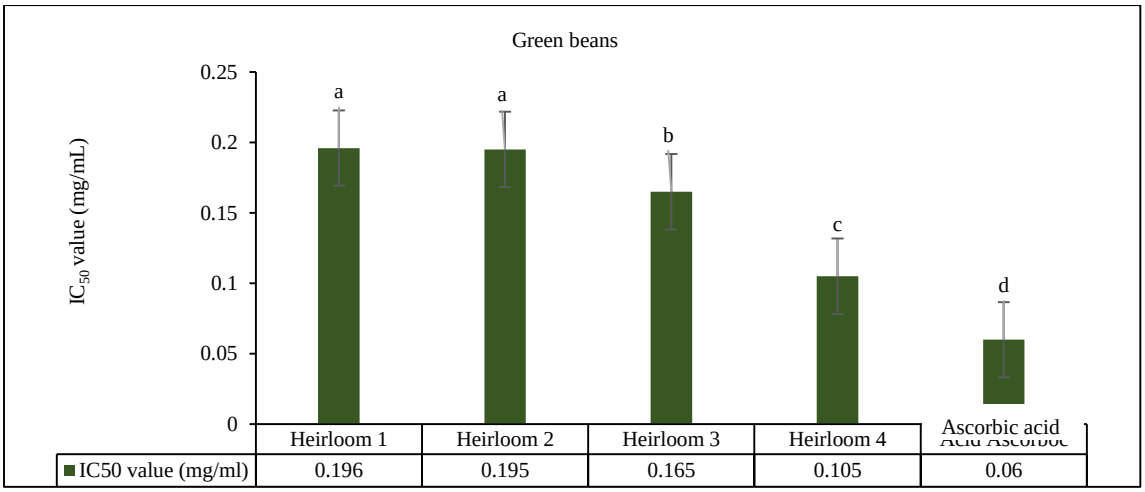

Figure 1. IC_{50} values of DPPH scavenging activity of the green beans in four *Coffea arabica* varieties studied and ascorbic acid; Means with different letters denote significant differences according to Duncan's multiple comparison test ($p \leq 0.05$).

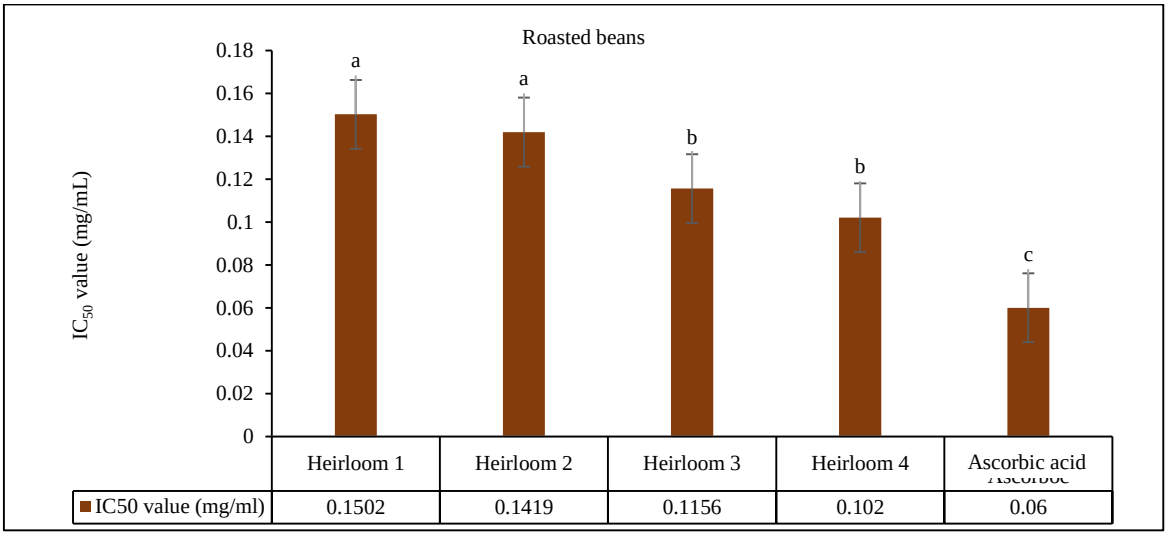

Figure 2. IC_{50} values of DPPH scavenging activity of the roasted beans in four *Coffea arabica* varieties studied and ascorbic acid; Means with different letters denote significant differences according to Duncan's multiple comparison test ($p \leq 0.05$).

these characteristics were strongly and negatively correlated with the DPPH IC_{50} (Table 5). Several experimental data in the literature support that the total phenolic content contributes to the antioxidant activity of some plants (Karamian and Ahmadi Khoei 2022). However, the antioxidant activity assessed by the DPPH assay did not correlate with the total phenolic content in different *Epilobium* species, contradicting that these compounds contribute to antioxidant activity (Abbasi Karin *et al.* 2023). The variation in phytochemical traits of medicinal plant species can be attributed to the effect

of various geographical conditions and also, developmental and genetic factors (Rahmati and Ghorbanpour 2023).

Metal chelating activity

Lipid peroxidation can be described generally as the reaction of lipids with molecular oxygen. It involves hydrogen-atom abstraction by peroxyl radicals, with oxygen addition to carbon radicals resulting in lipid peroxyl radicals and hydroperoxides (Yin *et al.* 2011). Iron is an essential metal for the transport of oxygen, respiration, and activity of enzymes. Also, it is the most important pro-oxidant among transition metals because of its high reactivity (Cho *et al*. 2013). The ferrous iron reacts with hydrogen peroxide (Fenton reaction) and consequently produces reactive free radicals (Prescott and Bottle 2017). According to our results, the metal chelating activity was enhanced with increasing concentrations of both green and roasted bean extracts (Tables 6 and 7). The highest metal chelating average was obtained by Heirloom 4 and Heirloom 2 in green and roast beans, respectively. Also, all extracts had weaker metal chelating activity than ascorbic acid, representing a synthetic antioxidant. Moreover, the green beans had higher metal-chelating activity than the roasted beans. The low metal chelating activity after thermal processing indicates the low thermal stability of some antioxidants. Tannic acid, a polyphenolic in coffee beans, is regarded as a strong antioxidant because it can chelate metal ions such as Fe^{2+} and Cu^{2+} ions (Lopez *et al*. 1999).

β-Carotene/linoleic acid assay

The oxidation of linoleic acid in the β-carotene/linoleic acid assay was effectively inhibited by Heirloom 4 green and roast bean extracts (73.99% ± 4.04 and 94.22% ± 1.41, respectively), followed by Heirloom 3 (73.20% ± 3.36; 92.71 ± 2.43), Heirloom 2 (63.48% ± 2.66; 81.54 ± 5.04), and Heirloom 1 (61.94% ± 1.48; 77.46 ± 3.62) (Table 8). Our results showed that ethanolic extracts of the green and roasted beans of Heirloom 3 and Heirloom 4 were significantly different from Heirloom 1 and Heirloom 2 in the inhibition of linoleic acid oxidation (Figure 3). The β-carotene/linoleic acid assay showed that all green bean extracts have lower activity than BHT as a synthetic antioxidant, whereas the Heirloom 4 and Heirloom 3 roasted bean extracts represented higher activity than BHT.

Antibacterial activity assay

In a preliminary antibacterial test conducted before this experiment, we found that the ethanolic extracts of green and roasted coffee samples did not have any antibacterial activity. However, their boiled-water extracts showed antibacterial activity. Additionally, the aqueous extracts of the roasted

Table 5. Linear correlation between evaluated antioxidant characteristics of the roasted and green beans in four *Coffea arabica* varieties studied and ascorbic acid.

	AA_1 in roasted beans	**IC_{50} in roasted beans**	**AA_1 in green beans**	**IC_{50} in green beans**
Total phenol content	0.971	0.112	0.921	0.108
Total flavonoid content	0.963	0.121	0.911	0.142

AA_1 = Antioxidant activity (by scavenging DPPH free radical); IC_{50} = The amount of sample necessary to decrease DPPH. by 50%.

Table 6. The metal chelating activity of the green beans in four *Coffea arabica* varieties studied and ascorbic acid.

Variety	**Fe-Chelate concentration (mg/mL)**					
	0.2	**0.4**	**0.6**	**0.8**	**1**	**Average**
Heirloom 1	55.38 ± 3.10^{ij}	62.71 ± 3.29^{gh}	69.30 ± 1.53^{ef}	73.73 ± 1.15^{cd}	75.58 ± 2.96^{c}	67.34
Heirloom 2	57.45 ± 1.84^{i}	62.08 ± 0.92^{h}	66.43 ± 2.91^{fg}	68.49 ± 2.34^{ef}	75.23 ± 1.31^{c}	65.94
Heirloom 3	52.47 ± 3.82^{j}	57.45 ± 1.87^{i}	63.04 ± 2.20^{gh}	69.34 ± 2.85^{ef}	74.23 ± 2.03^{cd}	63.31
Heirloom 4	63.36 ± 2.61^{gh}	70.69 ± 3.14^{de}	77.54 ± 4.17^{c}	84.50 ± 0.88^{b}	87.43 ± 0.50^{b}	76.70
Ascorbic acid	95.44 ± 0.57^{a}	96.61 ± 0.35^{a}	97.19 ± 0.22^{a}	97.83 ± 0.11^{a}	98.27 ± 0.34^{a}	97.07

Data are expressed as mean ± standard deviation (n = 3); Means with different letters denote significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Table 7. The metal chelating activity of the roasted beans in four *Coffea arabica* varieties studied and ascorbic acid.

Variety	**Fe-Chelate concentration (mg/mL)**					
	0.2	**0.4**	**0.6**	**0.8**	**1**	**Average**
Heirloom 1	29.99 ± 4.87^{k}	46.90 ± 1.32^{fgh}	60.67 ± 5.93^{cde}	64.01 ± 2.60^{bcd}	67.75 ± 0.49^{b}	53.86
Heirloom 2	43.18 ± 2.50^{hi}	57.90 ± 3.22^{e}	66.41 ± 2.77^{bc}	69.64 ± 2.70^{b}	70.02 ± 2.76^{b}	61.43
Heirloom 3	35.33 ± 1.93^{jk}	44.79 ± 0.92^{ghi}	49.75 ± 3.14^{fg}	57.73 ± 1.99^{e}	64.88 ± 1.52^{bc}	50.50
Heirloom 4	38.94 ± 8.94^{ij}	44.29 ± 2.75^{ghi}	51.58 ± 4.24^{f}	58.86 ± 2.39^{de}	65.88 ± 6.65^{bc}	51.91
Ascorbic acid	95.44 ± 0.57^{a}	96.61 ± 0.35^{a}	97.19 ± 0.22^{a}	97.83 ± 0.11^{a}	98.27 ± 0.34^{a}	97.07

Data are expressed as mean ± standard deviation (n = 3); Means with different letters denote significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Table 8. Inhibition percentage of the linoleic acid oxidation by the roasted and green beans in four *Coffea arabica* varieties studied and BHT.

Variety	**Roasted beans (%)**	**Green beans (%)**
Heirloom 1	77.46 ± 3.62^{c}	61.94 ± 1.48^{c}
Heirloom 2	81.54 ± 5.04^{b}	63.48 ± 2.66^{c}
Heirloom 3	92.71 ± 2.43^{a}	73.20 ± 3.36^{b}
Heirloom 4	94.22 ± 1.40^{a}	73.99 ± 4.04^{b}
BHT	82.06 ± 3.82^{b}	82.06 ± 3.82^{a}
Control	21.99 ± 2.19^{d}	21.99 ± 2.19^{d}

Data are expressed as mean ± standard deviation (n = 3); Means with different letters in each column denote significant differences according to Duncan's multiple comparison test ($p \leq 0.05$).

beans exhibited better results than the extracts from the green beans (mean of roasted beans: 8.16 ± 0.61; mean of green beans: 3.20 ± 0.57). The roasted samples showed higher activity against all tested bacteria as compared with the negative control (Table 9), whereas some green bean extracts demonstrated the same antibacterial activity as the negative control (Table 10). Among the roasted bean extracts, Heirloom 3 showed the highest activity against *Pseudomonas aeruginosa* (mean: 11.21 ± 0.57), *Staphylococcus aureus* (mean: 8.66 ± 0.57), and *Bacillus cereus* (mean: 7.22 ± 0.57). However, among the green bean extracts, Heirloom 3 and Heirloom 4 showed the highest activity against all bacterial strains tested (mean: 3.63 ± 0.21; 4.74 ± 0.28). Altogether in green and roasted beans extracts, the highest antibacterial activity was shown against *S. aureus* and *B. cereus*, and the lowest antibacterial activity was shown against *E. coli* (mean: 4.80 ± 0.28; 4.30 ± 0.23; 0.44 ± 0.04). These findings suggest that roasted bean extracts are a rich source of phenolic compounds and exhibit antibacterial activity against both gram-positive and gram-negative bacteria. The antibacterial activities of the flavonoid compounds could be attributed to their reaction with extracellular proteins resulting in the formation of complex compounds that damage the cell membrane and therefore, inhibit the use of oxygen by bacteria (Cushnie and Lamb 2005).

Table 9. Antibacterial activity of the roasted beans in four *Coffea arabica* varieties studied.

Variety	Concentration (g/mL)	Inhibition zone (mm)			
		Staphylococcus aureus	***Pseudomonas aeruginosa***	***Escherichia coli***	***Bacillus cereus***
Heirloom 1	0.1	6.33 ± 0.57^{e}	7.33 ± 0.57^{de}	9.00 ± 1.00^{abc}	7.33 ± 0.57^{de}
	0.2	10.00 ± 1.00^{ab}	7.66 ± 1.15^{cde}	0.00 ± 0.00^{g}	10.00 ± 1.00^{ab}
	0.4	10.00 ± 1.00^{ab}	9.66 ± 0.57^{ab}	5.33 ± 0.57^{f}	10.33 ± 1.15^{a}
Heirloom 2	0.1	6.33 ± 0.57^{g}	7.66 ± 0.57^{ef}	7.00 ± 1.00^{efg}	6.66 ± 0.57^{fg}
	0.2	10.00 ± 1.00^{b}	10.33 ± 0.57^{b}	8.00 ± 1.00^{de}	7.33 ± 0.57^{efg}
	0.4	10.00 ± 1.00^{b}	13.66 ± 0.57^{a}	10.66 ± 0.57^{b}	9.00 ± 0.00^{cd}
Heirloom 3	0.1	7.66 ± 0.57^{de}	8.66 ± 0.57^{cd}	0.00 ± 0.00^{f}	7.66 ± 0.57^{de}
	0.2	8.33 ± 0.57^{cd}	11.33 ± 0.57^{b}	0.00 ± 0.00^{f}	10.00 ± 1.00^{bc}
	0.4	10.00 ± 0.00^{bc}	13.66 ± 0.57^{a}	6.00 ± 0.00^{e}	11.66 ± 0.57^{b}
Heirloom 4	0.1	7.33 ± 0.57^{e}	6.00 ± 0.00^{f}	6.33 ± 0.57^{f}	6.66 ± 0.57^{ef}
	0.2	8.33 ± 0.57^{d}	6.33 ± 0.57^{f}	7.33 ± 0.57^{e}	10.33 ± 0.57^{ab}
	0.4	9.66 ± 0.57^{bc}	9.66 ± 0.57^{bc}	9.00 ± 0.00^{cd}	10.66 ± 0.57^{a}
Distilled water		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Data are expressed as mean ± standard deviation (n = 3); Means with different letters within each variety denote significant differences according to Duncan's multiple range test ($p \leq 0.05$).

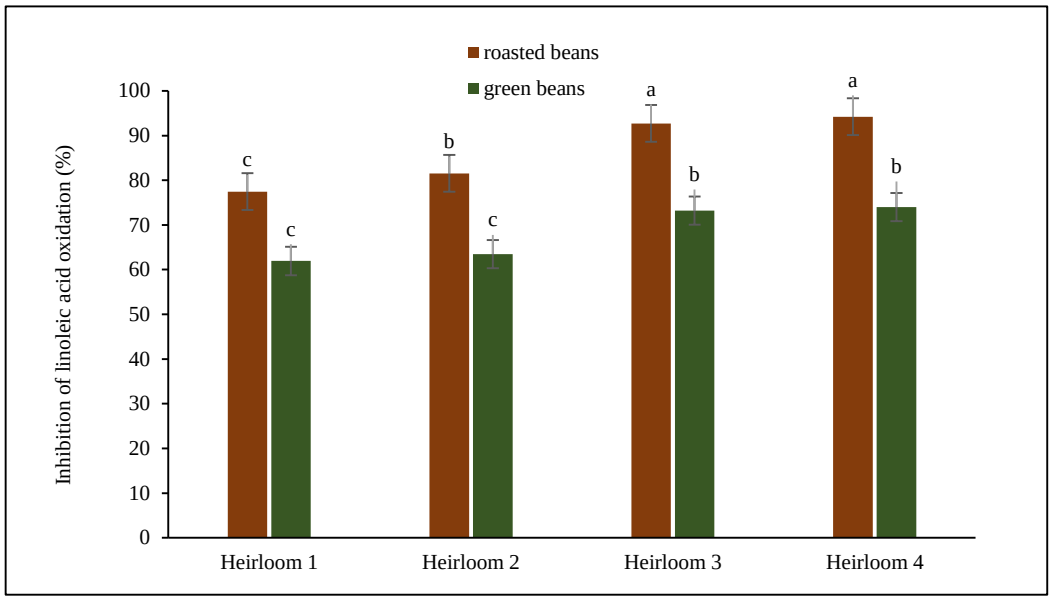

Figure 3. Inhibition of bleaching of ß-carotene/linoleic acid emulsion by the four *Coffea arabica* varieties studied in comparison with BHT as a synthetic antioxidant (82.06%); Means with different letters denote significant differences according to Duncan's multiple comparison test ($p \leq 0.05$).

Table 10. Antibacterial activity of the green beans in four *Coffea arabica* varieties studied.

Variety	Concentration (g/mL)	Inhibition zone (mm)			
		Staphylococcus aureus	***Pseudomonas aeruginosa***	***Escherichia coli***	***Bacillus cereus***
Heirloom 1	0.1	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	5.00 ± 0.00^{b}
	0.2	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	5.00 ± 0.00^{b}
	0.4	6.33 ± 0.57^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	5.33 ± 0.57^{b}
Heirloom 2	0.1	6.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
	0.2	6.33 ± 0.57^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
	0.4	6.33 ± 0.57^{b}	5.33 ± 0.57^{c}	0.00 ± 0.00^{d}	8.33 ± 0.57^{a}
Heirloom 3	0.1	0.00 ± 0.00^{d}	8.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
	0.2	0.00 ± 0.00^{d}	10.33 ± 0.57^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
	0.4	6.66 ± 0.57^{c}	10.33 ± 0.57^{a}	0.00 ± 0.00^{d}	8.33 ± 0.57^{b}
Heirloom 4	0.1	7.66 ± 0.57^{bc}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	6.33 ± 0.57^{de}
	0.2	8.00 ± 0.00^{b}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	7.00 ± 0.00^{cd}
	0.4	10.33 ± 0.57^{a}	6.00 ± 1.00^{ef}	5.33 ± 0.57^{f}	6.33 ± 0.57^{de}
Distilled water		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Data are expressed as mean ± standard deviation (n = 3). Means with different letters within each variety denote significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Conclusions

In general, various factors including genetic factors affect the content of chemical compounds in coffee beans. In addition to these factors, environmental conditions like altitude (investigated in this study), extraction methods, and type of solvent used (ethanol/water), affect the final chemical composition and bioactivity of the extracts. All antioxidant activity assays showed a close relationship with the phenolic content in green and roasted samples. Our findings indicated that the extracts from coffee green or roasted beans have potent free radical scavenging activity in different antioxidant activity assays, such as DPPH-radical scavenging, metal chelating, and β-carotene/linoleic acid inhibition. Moreover, the differences in the antioxidant activity between green and roasted bean extracts in the same variety could be related to the roasting process. The antibacterial activity of aqueous extracts of the green and roasted beans may be partially attributed to the effect of phenolic compounds on the permeability of the cell wall and cytoplasmic membrane. Overall, it appears that the bioactivity of the coffee beans depends on the chemical composition of each variety, which requires further investigation.

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Ethical considerations

The authors avoided data fabrication and falsification.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

References

Abbasi Karin S, Karimzadeh G, Mohammadi Bazargani M. 2023. Interspecific morphological and phytochemical variations in the willow herb (*Epilobium* spp.) medicinal plant. J Plant Physiol Breed. 13(2): 15-27. https://doi.org/10.22034/JPPB.2023.17355

Acidri R, Sawai Y, Sugimoto Y, Handa T, Sasagawa D, Masunaga T, Yamamoto S, Nishihara E. 2019. Phytochemical profile and antioxidant capacity of coffee plant organs compared to green and roasted coffee beans. Antioxidants 9(2): 93. https://doi.org/10.3390/antiox9020093

Adugna BG. 2021. Factors affecting coffee (*Coffea arabica* L.) quality in Ethiopia: a review. Am J Agric For. 9(5): 283-291. https://doi.org/10.11648/j.ajaf.20210905.12

Affonso RCL, Voytena APL, Fanan S, Pitz H, Coelho DS, Horstmann AL, Pereira A, Uarrota VG, Hillmann MC, Varela LAC, *et al*. 2016. Phytochemical composition, antioxidant activity, and the effect of the aqueous extract of coffee (*Coffea arabica* L.) bean residual press cake on the skin wound healing. Oxid Med Cell Longev. 2016: 1923754. https://doi.org/10.1155/2016/1923754

Alves RC, Casal S, Oliveira MBPP. 2010. Tocopherols in coffee brews: influence of coffee species, roast degree and brewing procedure. J Food Compost Anal. 23(8): 802-808. https://doi.org/10.1016/j.jfca.2010.02.009

Barrière C, Centeno D, Lebert A, Leroy-Sétrin S, Berdagué JL, Talon R, 2001. Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. FEMS Microbiol Lett. 201(2): 181-185. https://doi.org/10.1016/S0378-1097(01)00271-3

Bauer AW, Kirby WM, Sherries JC, Turck M. 1966. Antibiotic susceptibility testing by single disk method. Am J Clin Pathol. 45(4): 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bondam AF, da Silveira, DD, dos Santos JP, Hoffmann JF. 2022. Phenolic compounds from coffee by-products: extraction and application in the food and pharmaceutical industries. Trends Food Sci Technol. 123: 172-186. https://doi.org/10.1016/j.tifs.2022.03.013

Chang CC, Yang MH, Wen HM, Chern JC. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 10(3): 178-182. https://doi.org/10.38212/2224-6614.2748

Cho AR, Park KW, Kim KM, Kim SY, Han J. 2013. Influence of roasting conditions on the antioxidant characteristics of Colombian coffee (*Coffea arabica* L.) beans. J Food Biochem. 38(3): 271-280. https://doi.org/10.1111/jfbc.12045

Cushnie TPT, Lamb AJ. 2005. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 26(5): 343-356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Davinelli S, Corbi G, Zarrelli A, Arisi M, Calzavara-Pinton P, Grassi D, De Vivo I, Scapagnini G. 2018. Short-term supplementation with flavanol-rich cocoa improves lipid profile, antioxidant status and positively influences the AA/EPA ratio in healthy subjects. J Nutr Biochem. 61: 33-39. https://doi.org/10.1016/j.jnutbio.2018.07.011

Decker EA. 1997. Phenolics: prooxidants or antioxidants? Nutr Rev. 55(11): 396-407. https://doi.org/10.1111/j.1753-4887.1997.tb01580.x

Dinis TCP, Madeira VMC, Almeida LM. 1994. Action of phenolic derivatives (acetaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 315(1): 161-169. https://doi.org/10.1006/abbi.1994.1485

Fuller M, Rao NZ. 2017. The effect of time, roasting temperature, and grind size on caffeine and chlorogenic acid concentrations in cold brew coffee. Sci Rep. 7: 17979. https://doi.org/10.1038/s41598-017-18247-4

Gomaa SE, Yahayu MA, Nurjayadi M, Dailin DJ, El Enshasy HA. 2019. Antimicrobial compounds from *Catharanthus roseus*- a review. Int J Sci Technol Res. 8(10): 113-121.

Jeszka-Skowron M, Zgoła-Grześkowiak K, Grześkowiak T. 2015. Analytical methods applied for the characterization and the determination of bioactive compounds in coffee. Eur Food Res Technol. 240(1): 19-31. https://doi.org/10.1007/s00217-014-2356-z

Joët T, Laffargue A, Descroix F, Doulbeau S, Bertrand B, de Kochko A, Dussert S. 2010. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. Food Chem. 118(3): 693-701. https://doi.org/10.1016/j.foodchem.2009.05.048

Jung S, Gu S, Lee SH, Jeong Y. 2021. Effect of roasting degree on the antioxidant properties of espresso and drip coffee extracted from *Coffea arabica* cv. Java. Appl Sci. 11(15): 7025. https://doi.org/10.3390/app11157025

Kamalvand AA, Hosseini Sarghein S, Karamian R. 2021. Impact of cadmium stress on growth and physiological responses of fenugreek (*Trigonella foenum-graecum* L.). J Plant Physiol Breed. 11(2): 51-65. https://doi.org/10.22034/JPPB.2022.15483

Karamian R, Ahmadi Khoei M. 2022. Effects of selenium nanoparticles and ancymidol on physiological responses of *Stevia rebaudiana* Bertoni colonized by *Piriformospora indica*. J Plant Physiol Breed. 12(1): 67-92. https://doi.org/10.22034/JPPB.2022.16039

Koleva II, van Beek TA, Linssen JPH, de Groot A, Evstatieva LN. 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 13(1): 8-17. https://doi.org/10.1002/pca.611

Kalpoutzakis E, Chatzimitakos T, Athanasiadis V, Mitakou S, Aligiannis N, Bozinou E, Gortzi O, Skaltsounis LA, Lalas SI. 2023. Determination of the total phenolics content and antioxidant activity of extracts from parts of plants from the Greek island of Crete. Plants. 12(5):1092. https://doi.org/10.3390/plants12051092

Link JV, Lemes ALG, Marquetti I, dos Santos Scholz, MB, Bona E. 2014. Geographical and genotypic segmentation of arabica coffee using self-organizing maps. Food Res Int. 59: 1-7. https://doi.org/10.1016/j.foodres.2014.01.063

Lopes GK, Schulman HM, Hermes-Lima M. 1999. Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. Biochim Biophys Acta. 1472(1-2): 142-152. https://doi.org/10.1016/s0304-4165(99)00117-8

Machado M, Espírito Santo L, Machado S, Lobo JC, Costa ASG, Oliveira MBPP, Ferreira H, Alves RC. 2023. Bioactive potential and chemical composition of coffee by-products: from pulp to silverskin. Foods. 12: 2354. https://doi.org/10.3390/foods12122354

Makiso MU, Tola YB, Ogah O, Endale FL. 2023. Bioactive compounds in coffee and their role in lowering the risk of major public health consequences: a review. Food Sci Nutr. 12(2): 734-764. https://doi.org/10.1002/fsn3.3848.

Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos Tc, Coube CS, Leitão SG. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 15: 127-130. https://doi.org/10.1002/ptr.687

Nimse SB, Pal D. 2015. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv. 5: 27986. https://doi.org/10.1039/c4ra13315c

Parras P, Martínez-Tomé M, Jiménez AM, Murcia MA. 2007. Antioxidant capacity of coffees of several origins brewed following three different procedures. Food Chem. 102: 582-592. https://doi.org/10.1016/j.foodchem.2006.05.037

Prescott C, Bottle SE. 2017. Biological relevance of free radicals and nitroxides. Cell Biochem Biophys. 75(2): 227-240. https://doi.org/10.1007/s12013-016-0759-0

Steven E. BottlePriftis A, Stagos D, Konstantinopoulos K, Tsitsimpikou C, Spandidos D, Tsatsakis AM, Tzatzarakis MN, Kouretas D. 2015. Comparison of antioxidant activity between green and roasted coffee beans using molecular methods. Mol Med Rep. 12(5): 7293-7302. https://doi.org/10.3892/mmr.2015.4377

Rahmati G, Ghorbanpour M. 2023. Evaluation of morphological traits and chemical compounds of chicory (*Cichorium intybus*) populations in central Iran (Markazi province). J Plant Physiol Breed. 13(1): 35-50. https://doi.org/10.22034/JPPB.2023.16460

Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem. 91(4): 621-632. https://doi.org/10.1016/j.foodchem.2004.06.031

Sentkowska A, Jeszka-Skowron M, Pyrzynska K. 2016. Comparative studies on the antioxidant properties of different green coffee extracts. MOJ Food Process Technol. 3(2): 296-302. https://doi.org/10.15406/mojfpt.2016.03.00071

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 16: 144-158. https://doi.org/10.5344/ajev.1965.16.3.144

Sridevi V, Parvatam G. 2014. Changes in caffeine content during fruit development in *Coffea canephora* P. ex. Fr. grown at different elevations. J Biol Earth Sci. 4(2): B168-B175.

Świętek M, Lu YC, Konefał R, Ferreira LP, Cruz MM, Ma YH, Horák D. 2019. Scavenging of reactive oxygen species by phenolic compound-modified maghemite nanoparticles. Beilstein J Nanotechnol. 10: 1073-1088. https://doi.org/10.3762/bjnano.10.108.

Thirumalai T, Therasa SV, Elumalai EK, David E. 2011. Intense and exhaustive exercise induce oxidative stress in skeletal muscle. Asian Pac J Trop Dis. 1(1): 63-66. https://doi.org/10.1016/S2222-1808(11)60016-9

Yahayu M, Hamid ZBS, El Adawi H, Dailin DJ, Abdmalek R, Hanapi SZ, Boumehira A, Ngadiran S, Yussof IM, Ho T, *et al.* 2020. Antioxidant activity in green and roasted coffee: a critical review. Biosci Res. 17(3): 2249-2263.

Yin J, Heo S-Il, Wang MH. 2008. Antioxidant and antidiabetic activities of extracts from *Cirsium japonicum* roots. Nutr Res Pract. 2(4): 247-251. https://doi.org/10.4162/nrp.2008.2.4.247

Yin H, Xu L, Porter NA. 2011. Free radical lipid peroxidation: mechanisms and analysis. Chem Rev. 111(10): 5944-5972. https://doi.org/10.1021/cr200084z

Yust BG, Wilkinson F, Rao NZ. 2024. Variables affecting the extraction of antioxidants in cold and hot brew coffee: a review. Antioxidants. 13(1): 29. https://doi.org/10.3390/antiox13010029