

Production of lower quality coffee (*Coffea arabica* L.) beans as a by-product with potential antioxidant, antifungal and antibacterial activities

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Abstract

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This work sought to evaluate the antioxidant, antifungal and antibacterial potential of ethanolic extracts obtained from inferior quality coffee beans. Extracts were prepared from green and roasted coffee beans using the solid-liquid reflux extraction technique. The antioxidant potential was evaluated by the DPPH radical-scavenging method. The antifungal activity was evaluated by analyzing the mycelial growth of the fungi *Aspergillus westerdijkiae* and *Aspergillus carbonarius*, and the effect of the extracts on the fungi was visualized using scanning electron microscopy (SEM). The antibacterial potential against *Salmonella enterica Choleraesuis* and *Listeria monocytogenes* was evaluated by microdilution, and the effects were observed by SEM. The green coffee extract was the most efficient in inhibiting DPPH radicals and more efficient in inhibiting mycelial growth. *A. carbonarius* was more sensitive to the extracts than *A. westerdijkiae*, being completely inhibited by 20000 ppm of the green coffee extract. Neither of the coffee extracts exhibited bactericidal activity, and no bacteriostatic effect was observed for the roasted coffee extract. The minimum inhibitory concentration (MIC) of green coffee extract for *L. monocytogenes* and *S. Choleraesuis* was 5000 ppm. Therefore, the ethanolic extract of green coffee had a great potential as a by-product of the industry.

Keywords: Bacterium; Coffee beans; DPPH; Extracts; Fungus.

Abbreviations: DPPH_2,2-diphenyl-1-picrylhydrazyl; BHT_2,6-Di-tert-butyl-4-methylphenol.

Introduction

Foodborne illnesses (FBIs) constitute a global public health problem. Transmission occurs through the consumption of contaminated food and water, leading to infections that are mainly caused by microorganisms. Among these microorganisms associated with FBIs, *Salmonella* sp., *Listeria monocytogenes* and toxin-producing *Aspergillus* species (Ge et al., 2022) can be mentioned.

Salmonella spp. and *Listeria monocytogenes* constitute important pathogenic bacteria species that are transmitted by food. The main sources of contagion occur through the consumption of food of animal origin. Listeriosis caused by *L. monocytogenes* mainly affects pregnant women, newborns and immunodeficient people. Outbreaks of the disease are related to the consumption of ready-to-eat foods, dairy products, sausages, and raw meat (Morales-Parterra et al., 2018).

In addition to bacteria, fungi can contaminate food, causing deterioration, affecting palatability and, consequently, the quality of food products. Some species of filamentous fungi such as *A. carbonarius* and *A. westerdijkiae* can produce secondary metabolites called mycotoxins, which can be deleterious to human and animal health when consumed (Taniwaki et al., 2019).

In addition, other problems are faced by the food industry, such as the presence of free radicals, which can trigger the oxidation process, especially in fatty foods, resulting in deterioration and, consequently, economic losses. To avoid these losses, synthetic antioxidants are used; however, these substances can have toxic effects, which have increased the search for less harmful alternatives. The use of extracts from natural products for preservation of food is an alternative that has been extensively studied (Ferreira et al., 2019; Teixeira et al., 2022).

Coffee is considered to be a functional beverage because of the presence of constituents with biological activities, such as phenolic compounds and alkaloids, among others (Wu et al., 2022). According to Masek et al. (2020), the bioactive compounds present in coffee beans have antioxidant and antibacterial activities, mainly related to the phenolic constituents.

Almeida et al. (2006) observed significant differences when they compared the effects of coffee extracts against enterobacteria. Larger diameters of the inhibition zone were observed for *Proteus hauseri*, indicating a greater sensitivity to coffee extracts. Furthermore, Badr et al. (2022) found that there was a decrease in mycelial growth of *Aspergillus flavus* and *Aspergillus ochraceus* when the concentration of coffee extract increased. In addition, they recorded detoxification for aflatoxins (AFs) and ochratoxin A (OTA) in liquid media. The present study sought to evaluate the antioxidant, antibacterial and antifungal activities of ethanol extracts obtained from inferior quality green and roasted coffee beans.

Results and Discussion

Analysis of antioxidant activity

The antioxidant activities of green and roasted coffee extracts were evaluated using the colorimetric method based on DPPH radical scavenging. The results obtained are shown in Table 1.

The results obtained for the ethanolic extracts of green coffee, roasted coffee and BHT were statistically different from one another. The DPPH radical scavenging assay was dose-dependent; thus, the antioxidant activity increased as the concentration of the extract increased. On the other hand, no antioxidant activity in the DPPH test was observed with roasted coffee at the concentrations employed (Table 1). Masek et al. (2020) evaluated the antioxidant activity of the ethanolic and aqueous extracts of green coffee by the DPPH and ABTS methods and noted that the percentage of inhibition of the formation of radicals increased with the increase in the extract concentration. This result corroborates those obtained in the present study. Of the constituents present in coffee that are associated with the free radical-scavenging activity, we can mention chlorogenic acids.

Maillard reaction products in coffee also contribute to the antioxidant activity, and previous studies have focused on the effects of the degree of roasting of coffee beans on their antioxidant capacity (Masek et al., 2020). Castillo et al. (2002) observed a decrease in antioxidant activity in grains subjected to high degrees of roasting. Several factors are associated with the quality of coffee beans, and these factors depend on the species under study, degree of roasting, and geographical conditions of the plant, such as soil type, altitude, harvest time, as well as pre- and post-harvest conditions.

Antifungal activity

The effects of green and roasted coffee (*C. arabica*) extracts on the mycelial growth of the *A. carbonarius* and *A. westerdijkiae* fungi are shown in Table 2. Both fungal species were sensitive to green and roasted coffee extracts; however, statistical data differed for the extracts. Green coffee extract possessed greater inhibitory activity against *A. carbonarius* and *A. westerdijkiae* at concentrations of 0.0525 and 0.105 g mL⁻¹, respectively (Figure 1). Greater inhibition of the mycelial growth of *A. carbonarius* than that of *A. westerdijkiae* was observed for the extracts. A dose-dependent effect was observed for the first species, which was completely inhibited by the extract of green coffee at the concentration of 0.42 g mL⁻¹ (Table 2).

Previously, Calheiros et al. (2023) evaluated the fungicidal effect of caffeinated and decaffeinated coffee extracts on yeast species and filamentous fungi. Antifungal activity was verified against *Candida krusei*, *Candida parapsilosis*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*, agents that cause skin diseases. In addition, fungicidal activity was observed against *T. mentagrophytes* and *T. rubrum*. However, no previous studies identified the effect of ethanol extracts from inferior quality coffee beans (*C. arabica* L.) on *Aspergillus* species, and this study is the first to report these results related to the fungi under study.

An inhibition rate greater than 80% for *A. carbonarius* was observed for green and roasted coffee extracts at concentrations of 0.21 and 0.42 g mL⁻¹, respectively. Badr et al. (2022) evaluated the antifungal and anti-ochratoxigenic activities of the extract from coffee grounds against toxin-producing fungi of the *Aspergillus*, *Fusarium* and *Penicillium* genera, and they observed a decrease in mycelial growth of fungi treated with the extract. Calheiros et al. (2023) observed that fungi treated with coffee extract had disorganized, undefined and perhaps dysfunctional mitochondria when compared with the control.

For both *A. carbonarius* and *A. westerdijkiae*, a greater inhibition of mycelial growth was observed for the green coffee extract. Some studies suggest the presence of antimicrobial agents in green coffee, such as phenolic compounds, alkaloids and saponins (Castaldo et al., 2018). In an evaluation of the antimicrobial activity of chlorogenic acids against *Candida albicans*, Sung and Lee (2010) observed that the compounds could inhibit the formation of hyphae by the fungus and also destroy them when treated with the compound.

The concentration of 0.42 g mL⁻¹ of the green coffee extract completely inhibited the growth of *A. carbonarius*, whereas the inhibition was 49.90% for *A. westerdijkiae*, which was higher than the inhibition caused by the antifungal agent used. Kwaśniewska-Sip et al. (2018) evaluated the resistance to fungi of maritime pine (*Pinus sylvestris* L.) treated with aqueous caffeine solutions, and they observed that the growth of *A. niger* and *A. terreus* fungi was totally inhibited by caffeine concentrations greater than 0.025 g mL⁻¹. Thus, caffeine inhibited the activity of chitinases, which inhibited fungal growth. Other authors also report antifungal activity associated with caffeoic and quinic acids and their derivatives (Sardi et al., 2016).

The lowest ability to inhibit mycelial growth of both fungi studied was observed for the extract of roasted coffee. Throughout the roasting process, the degradation of several bioactive compounds present in coffee beans occurs, and studies have shown that darker roasting results in a decrease in the total chlorogenic acid content, which might have resulted in a lower inhibitory activity (Bastian et al., 2021). Among the species studied, the sensitivity of *A. carbonarius* to the extracts was greater than that of *A. westerdijkiae*. This result corroborates studies previously performed by Brandão et al. (2020), in which a greater inhibition of growth of *A. carbonarius* was observed than those of other *Aspergillus* species when using the essential oil from *Eremanthus erythropappus*.

Scanning Electron Microscopy (SEM) observation of fungi

The morphological structures of *A. carbonarius* and *A. westerdijkiae* were obtained by Scanning Electron Microscopy (SEM) (Figure 2). Despite the inhibited growth, when evaluating the percentage of inhibition of fungal growth, no morphological differences were observed in the structures of *A. carbonarius* and *A. westerdijkiae* species treated with green and roasted coffee extracts when compared to the control (Figure 2). This fact suggests that the target of the action of the extracts on the fungi under study was intracellular.

In Figures 2A and 2A', conidia and conidiophores with normal and healthy development can be observed. Hyphae from the control treatment presented typical conidiophores, dichotomous branching and homogeneous cytoplasm (Ferreira et al., 2013). Deformation of the structure of *A. westerdijkiae* (Figure 2D') occurred in the presence of the antifungal agent. Wrinkling of the cell surface and emptying of the cytoplasmic content of the hyphae treated with tebuconazole occurred, resulting in a decrease in its diameter. Furthermore, no formation of conidiophores resulted after treatment with the antifungal agent, and, consequently, no formation of conidia occurred, suggesting a fungistatic activity. The action of azole antifungals results in interruption of the conversion of lanosterol to ergosterol via

binding to fungal cytochrome p-450, which causes disruption of fungal membranes and subsequent death of the microorganism (Pasko et al., 1990).

Table 1. Antioxidant activity (IC_{50}) of green and roasted coffee extracts and the synthetic antioxidant (BHT).

IC_{50} ($\mu\text{g mL}^{-1}$)	
DPPH	
Green coffee	91.67 \pm 0.44b
Roasted coffee	>500c
BHT	9.89 \pm 0.08a
CV(%)	38.73

CV= Coefficient of Variation. Means followed by the same lowercase letter in the column do not differ by Tukey's test at 5% probability.

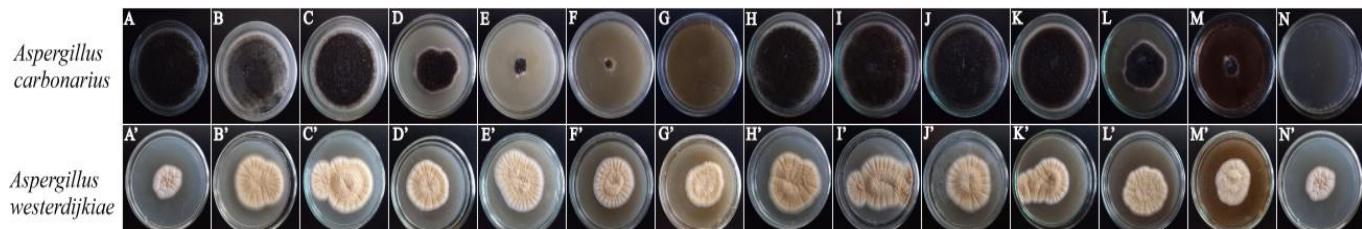


Fig 1. Inhibitory effect of green and roasted coffee extract on fungal growth of *A. carbonarius* and *A. westerdijkiae*. (A) and (A') controls; (B) and (B') treatment with green coffee extract at a concentration of 0.013125 g mL⁻¹; (C) and (C') treatment with green coffee extract at a concentration of 0.02625 g mL⁻¹; (D) and (D') treatment with green coffee extract at a concentration of 0.0525 g mL⁻¹; (E) and (E') treatment with green coffee extract at a concentration of 0.105 g mL⁻¹; (F) and (F') treatment with coffee extract at a concentration of 0.21 g mL⁻¹; (G) and (G') treatment with green coffee extract at a concentration of 0.42 g mL⁻¹; (H) and (H') treatment with roasted coffee extract at a concentration of 0.013125 g mL⁻¹; (I) and (I') treatment with roasted coffee extract at a concentration of 0.02625 g mL⁻¹; (J) and (J') treatment with roasted coffee extract at a concentration of 0.0525 g mL⁻¹; (K) and (K') treatment with roasted coffee extract at a concentration of 0.105 g mL⁻¹; (L) and (L') treatment with roasted coffee extract at a concentration of 0.21 g mL⁻¹; (M) and (M') treatment with roasted coffee extract at a concentration of 0.42 g mL⁻¹; (N) and (N') treatment with the antifungal agent tebuconazole.

Table 2. Effect of green and roasted coffee extracts on fungal inhibition.

Concentration	Degree of Inhibition (%)			
	<i>Aspergillus carbonarius</i>		<i>Aspergillus westerdijkiae</i>	
	Green coffee	Roasted coffee	Green coffee	Roasted coffee
0.013125 g mL ⁻¹	0.00 aA	0.00 aA	8.35 bB	0.00 aA
0.02625 g mL ⁻¹	0.00 aA	0.00 aA	14.52 bB	31.02 bA
0.0525 g mL ⁻¹	31.69 bA	0.00 aB	23.98 cA	24.32 bA
0.105 g mL ⁻¹	61.01 cA	0.00 aB	35.85 dB	24.55 bA
0.21 g mL ⁻¹	90.28 dA	68.11 bB	39.92 dB	27.99 bA
0.42 g mL ⁻¹	100.00 eA	81.97 cB	49.90 eA	34.58 cB
Antifungal (1 mL L ⁻¹)	100.00 eA	100.00 dA	33.91 aA	33.91 aA
Control	0.00 aA	0.00 aA	0.00 dA	0.00 cA
CV(%)	4.23		11.84	

CV= Coefficient of Variation. Means followed by the same uppercase letter in the row and a lowercase letter in the column do not differ by Tukey's test at 5% probability.

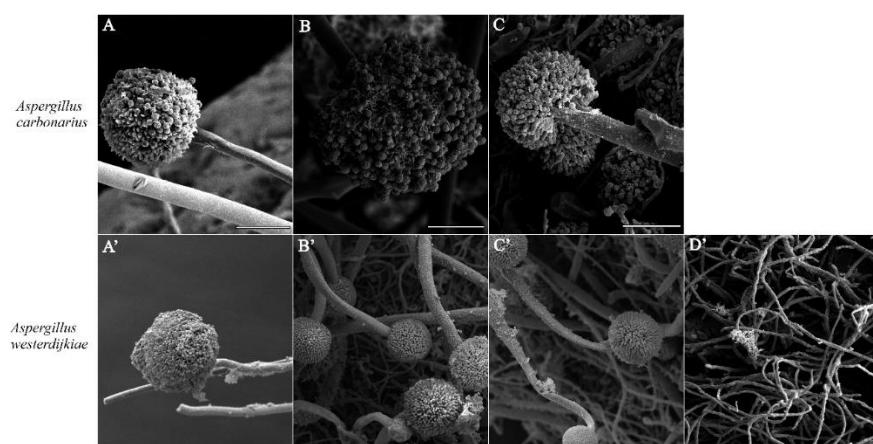


Fig 2. Scanning electron micrograph of *A. carbonarius* and *A. westerdijkiae*.

(A) and (A') Control; (B) treatment with a concentration of 0.21 g mL⁻¹ of green coffee extract; (B') treatment with a concentration of 0.42 g mL⁻¹ of green coffee extract; (C) and (C') treatment with a concentration of 0.42 g mL⁻¹ of roasted coffee extract; and (D') treatment with tebuconazole (mL L⁻¹). The size bars are 50 µm.

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of extracts of green and roasted coffees and a synthetic antibiotic.

Treatment	<i>Listeria monocytogenes</i>		<i>Salmonella Choleraesuis</i>	
	MIC (g mL ⁻¹)	MBC (g mL ⁻¹)	MIC (g mL ⁻¹)	MBC (g mL ⁻¹)
Green coffee	0.105	-	0.105	-
Roasted coffee	-	-	-	-
Chloramphenicol	0.000004	0.000063	0.000002	0.000063

- Resistant.

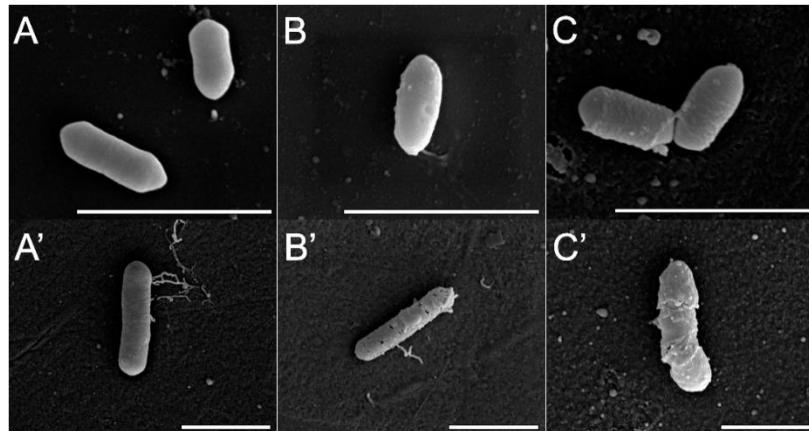


Fig 3. Scanning electron micrographs of *S. choleraesuis* and *L. monocytogenes*.

(A) control *L. monocytogenes* and (A') control *S. choleraesuis*; (B) *L. monocytogenes*: treatment with a concentration of 0.105 g mL⁻¹ of green coffee extract; (B') *S. choleraesuis*: treatment with a concentration of 0.105 g mL⁻¹ of green coffee extract; (C) *L. monocytogenes*: treatment with chloramphenicol and (C') *S. choleraesuis*: treatment with chloramphenicol. The size bars are 2 μm.

Antibacterial activity

No bactericidal activity against the bacteria studied was observed for the green and roasted coffee extracts. MIC values coincided for *L. monocytogenes* and *S. choleraesuis*. These bacteria were sensitive to green coffee extract at a concentration of 0.105 g mL⁻¹ (5000 ppm) (Table 3)

L. monocytogenes was inhibited at concentrations of green coffee extract lower than 0.105 g mL⁻¹, the same effect being observed for *S. choleraesuis*. Different results were obtained in a study performed by Monente et al. (2015), in which Gram-positive bacteria (*L. monocytogenes*) required lower concentrations of coffee extracts (0.02 g mL⁻¹) to inhibit their growth, when compared to Gram-negative bacteria (*S. choleraesuis* and *Pseudomonas aeruginosa*) (0.04 g mL⁻¹). In the same context, Camargo et al. (2019) also found that Gram-negative bacteria (*E. coli*) were more resistant than Gram-positive bacteria (*S. aureus*) when treated with the essential oil from *Cantinoa carpinifolia*. Despite the presence of an outer membrane in Gram-negative bacteria, the resistance of the *S. choleraesuis* was not greater than that observed for *L. monocytogenes*. This fact demonstrates the effective antimicrobial action of green coffee extract on bacteria with higher resistance factors.

The green coffee extract inhibited the growth of bacteria. Several compounds present in coffee beans are associated with antibacterial activity, such as caffeine, chlorogenic acids, caffeic acids, diterpenes, protocatechuic acid and trigonelline. These compounds have already been associated with potent antibacterial activities against enteric bacteria (Almeida et al., 2006; Khan et al., 2021).

Both *L. monocytogenes* and *S. choleraesuis* were resistant to roasted coffee extract; however, previous studies have already found antibacterial activity associated with roasted coffee beans. Daglia et al. (2007) evaluated the effect of extract from roasted coffee on *Staphylococcus aureus* and *Streptococcus mutans* and found that the standard compounds glyoxal, methylglyoxal and diacetyl (α-dicarbonyl compounds) formed during the roasting process were the main agents responsible for the antibacterial activity of roasted coffee. However, the antibacterial activity of these compounds increased when caffeine was added, which indicates a strong synergism between the chemical constituents present in coffee.

Wu et al. (2022) evaluated the phenolic composition of coffee beans subjected to different degrees of roasting and found that the concentration of phenolic compounds was lower in coffee beans submitted to high degrees of roasting. The degree of roasting used in this study might have resulted in a decrease in the concentration of phenolic compounds and, therefore, a decrease in the antibacterial activity of the extracts from roasted coffee, which resulted in resistance of the bacteria to the concentrations tested.

Scanning Electron Microscopy (SEM) of bacteria

A typical structure can be observed in the cells of *S. choleraesuis* and *L. monocytogenes* in the control groups, whereas morphological alterations in the membrane can be observed in the cells treated with green coffee extract (Figure 3). Alterations in the bacterial membranes (Figure 3B, B'), which gave *L. monocytogenes* and *S. choleraesuis* a rough surface, can be seen in the images. However, there was a partial degradation of the membrane that surrounds the microorganism in Gram-negative bacteria. SEM analysis confirms that the cytoplasmic membrane of both bacteria is the possible target of the extract.

In general, the bioactive constituents of green coffee are responsible for its biological activities. According to Díaz-Hernández et al. (2022), chlorogenic acids and caffeine can alter the functions and structures of the membrane, in addition to inhibiting DNA repair mechanisms,

which inhibits microorganism growth. Furthermore, according to Dash and Gummadi (2008), caffeine inhibits DNA synthesis and hinders RNA and protein synthesis. This fact might have caused the inviability of *L. monocytogenes* and *S. Choleraesuis* cells, resulting in growth inhibition.

Materials and Methods

Plant material: Acquisition, preparing samples and roasting process

The coffee beans (*Coffea arabica* L.) were provided by the Agronomy Department of the Federal University of Lavras - UFLA. Lower quality green (raw) coffee beans were obtained, corresponding to the 2019/2020 harvest in the south of Minas Gerais. An Atilla roaster (Standard Model) with pressure and temperature controls and a capacity of 5 kg was used for roasting the beans. The initial roasting temperature was 200 °C and the pressure was 10 mbar. The pressure was reduced to 5 mbar at the sound of the first "crack" (which represents the expansion of the beans). The final temperature was 220 °C, and the total roasting time lasted 9.5 minutes. The roast was classified as a high roast. After roasting, the beans were ground to medium granulometry, whereas the green coffee beans were ground in an industrial blender. The samples were placed in 1 kg polyethylene bags at room temperature for subsequent analysis.

Ethanol extracts

The technique of refluxing solid-liquid extraction was employed. The samples were refluxed in flasks using a ratio of 5:1 (ethanol:sample) at 78 °C for four hours. The samples were vacuum filtered, and the resulting filtrate was evaporated under low pressure (-650 mm Hg) at 50 °C on a rotary evaporator (Rotavapor Buchi R-144). The concentrate was kept in a fume hood for 24 hours at room temperature for total evaporation of the solvent. The pure extracts were placed in amber glass vials and kept at -10 °C for further analysis.

Evaluation of antioxidant activity through the DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical stabilization method

A modification of the method described by Teixeira et al. (2022) was employed. Samples of coffee extracts were diluted in ethanol to concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL⁻¹. For the positive control, the synthetic antioxidant BHT (butylated hydroxytoluene) was used at the same concentrations as the extracts. All the analyses were performed in triplicate.

Measurement of antifungal activity: Microorganisms, culture conditions and effect of extracts on mycelial growth

The fungus species used in the microbiological analyses were acquired from the Microorganism Culture Collection of the DCA-UFLA. Two species of filamentous fungi — *Aspergillus westerdijkiae* (CCDCA 11424) and *Aspergillus carbonarius* (CCDCA 10484) — were submitted for analysis.

The effect of extracts on fungal colonies was evaluated according to the method proposed by Brandão et al. (2020), with modifications. Czapek Yeast Agar medium (CYA; HiMedia Laboratories Pvt. Ltd.) was used for the cultivation of the species, which were added to sterilized Petri dishes. Different concentrations of green and roasted coffee extracts were diluted in 0.1% Tween and added to the culture medium, resulting in the following concentrations: 625 (0.013125 g mL⁻¹), 1250 (0.02625 g mL⁻¹), 2500 (0.0525 g mL⁻¹), 5000 (0.105 g mL⁻¹), 10000 (0.21 g mL⁻¹) and 20000 ppm (0.42 g mL⁻¹). The synthetic fungicide Tebuconazole (1 mL L⁻¹) was used as a positive control. All the treatments and controls were performed in triplicate.

Measurement of antibacterial activity: Microorganisms, maintenance, standardization and acquisition of the inoculum

The species of bacteria used in the analyses were acquired from the Microorganism Culture Collection of DCA-UFLA. Two species of bacteria were submitted to analysis, one Gram-positive and the other Gram-negative, corresponding to *Listeria monocytogenes* (ATCC19117) and *Salmonella enterica Choleraesuis* (ATCC6539), respectively. The microorganisms were activated by transferring an aliquot of the cultures to Brain Heart Infusion broth (BHI, HiMedia Laboratories Pvt. Ltd., Mumbai, India). For *L. monocytogenes*, the medium contained 0.6% yeast extract. Subsequently, the bacteria were incubated in a BOD at 37 °C for 24 hours. The cultures were diluted in 0.9% saline solution, adjusting the turbidity to a range of 0.08-0.1 at 635 nm with the aid of a spectrophotometer (Shimadzu V-160 1PC) to reach the concentration of 10⁸ CFU mL⁻¹.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The determinations of the MIC and MBC were performed according to the method proposed by the Clinical and Laboratorial Standards Institute - CLSI (2015), with modifications. Extracts of green and roasted coffee were diluted in Miller Hilton broth (Kasvi, Pinhais, PR, Brazil) and Tween (1%) to yield a concentration of 0.42 g mL⁻¹ (20000 ppm). Subsequent concentrations of coffee extracts were obtained after serial dilution from the initial concentration of 0.42 g mL⁻¹ (20000 ppm) (column 1) to 0.0002 g mL⁻¹ (9.76 ppm) (column 12) by transferring 100-µL aliquots of the contents to the subsequent well. Subsequently, 10 µL⁻¹ of standardized culture was added to each of the wells. In this experiment, chloramphenicol (1 g mL⁻¹) was used as a positive control and Mueller-Hinton broth and Tween (1%) as a negative control. The entire procedure was performed in triplicate.

The Minimum Inhibitory Concentration (MIC) was determined from the last dilution of the extracts that was capable of inhibiting bacterial growth and the Minimum Bactericidal Concentration (MBC) was recorded as the minimum concentration of the extracts that killed 100% of the microorganisms, with no bacterial growth being observed.

Scanning Electron Microscopy (SEM)

The effect of the extracts on the morphology of bacteria and fungi was evaluated by Scanning Electron Microscopy (SEM) according to the method proposed by Oliveira et al. (2017). The concentrations of the extracts analyzed for both bacterial species were 0.105 g mL⁻¹. The concentrations analyzed for *A. carbonarius* were 10000 ppm (0.21 g mL⁻¹) (green coffee extract) and 20000 ppm (0.42 g mL⁻¹) (roasted coffee extract) and 20000 ppm (0.42 g mL⁻¹) (green and roasted coffee extract) for *A. westerdijkiae*. Controls were prepared without adding extract.

Statistical analysis

The analyses were based on a completely randomized design and the mean values were compared using the Tukey Test at 5% probability, using the Sisvar program (Ferreira, 2011).

Conclusion

The ethanolic extract of green coffee was more efficient in eliminating the DPPH radical than the ethanolic extract of roasted coffee, for which no antioxidant activity was observed at the concentrations tested. Both extracts possessed antifungal activity; however, the inhibitory activity against *A. carbonarius* and *A. westerdijkiae* was greater for the green coffee extract. The mycelial growth of *A. carbonarius* was lower than that of *A. westerdijkiae*, and the first species was completely inhibited by the green coffee extract at the highest concentration tested. No bactericidal activity against *S. Choleraesuis* and *L. monocytogenes* was observed for either the green or roasted coffee extracts, and the MIC values referring to the green coffee extract were similar for both species. Roasted coffee extract did not have a bacteriostatic effect on the species studied. Thus, the green coffee extract was shown to have a large potential as a by-product for the food industry as a potent ally in the control of microorganisms and oxidative stress. Further studies on the biological activities of the roasted coffee extract are necessary.

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Conflict of Interests

The authors declare that there is no conflict of interest.

References

- Almeida AAP, Farah A, Silva DAM, Nunan EA, Glória, MBA (2006) Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. *J Agr Food Chem.* 54: 8738-43.
- Badr AN, El-Attar MM, Ali HS, Elkadragy MF, Yehia HM, Farouk A (2022) Spent coffee grounds valorization as bioactive phenolic source acquired antifungal, anti-mycotoxicogenic, and anti-cytotoxic activities. *Toxins.* 14.
- Bastian F, Hutabarat OS, Dirpan A, Nainu F, Harapan H, Emran TB, Simal-Gandara J (2021) From plantation to cup: changes in bioactive compounds during coffee processing. *Foods.* 10: e2827.
- Brandão RM, Ferreira VRF, Batista LR, Alves E, Lira NA, Bellete BS, Scolforo JRS, Cardoso MG (2020) Antifungal and antimycotoxicogenic effect of the essential oil of *Eremanthus erythropappus* on three different *Aspergillus* species. *Flavour Frag J.* 35:524-533.
- Calheiros D, Dias MI, Calhelha RC, Barros L, Ferreira ICFR, Fernandes C, Gonçalves T (2023) Antifungal activity of spent coffee ground extracts. *Microorganisms.* 11: 242.
- Camargo KC, Batista LR, Alves E, Rezende DACS, Teixeira ML, Brandão R M, Ferreira VRF, Nelson DL, Cardoso MG (2019) Antibacterial action of the essential oil from *Cantinoa carpinifolia* benth. Against *Escherichia coli* and *Staphylococcus aureus* strains. *Flavour Frag J.* 9: 1-8.
- Castaldo L, Grazieani G, Gaspari A, Izzo L, Luz C, Mañes J, Rubino M, Meca G, Ritieni A (2018) Study of the chemical components, bioactivity and antifungal properties of the coffee Husk. *Journal of Food Research.* 7: 43-54.
- Castillo MD de, Ames JM, Gordon MH (2002) Effect of roasting on the antioxidant activity of coffee brews. *J Agr Food Chem.* 50: 3698-703.
- CLSI - Clinical and Laboratorial Standards Institute (2015) M07-A10: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standart. 10th ed. 2015: 2.
- Daglia M, Papetti A, Grisoli P, Aceti C, Spini V, Dacarro C, Gazzani, G (2007) Isolation, identification, and quantification of roasted coffee antibacterial compounds. *J Agr Food Chem.* 55: 10208-10213.
- Dash SS, Gummadi SN (2008) Inhibitory effect of caffeine on growth of various bacterial strains. *Microbiol Res.* 3: 457-465.
- Díaz-Hernández GC, Alvarez-Fitz P, Maldonado-Astudillo YI, Jiménez-Hernández J, Parra-Rojas I, Flores-Alfaro E, Salazar R, Ramírez M (2022) Antibacterial, antiradical and antiproliferative potential of green, roasted, and spent coffee extracts. *Applied Science.* 12: 1938.
- Ferreira DF (2011) Sisvar: a computer statistical analysis system. *Cienc Agrotec.* 35: 1039-42.
- Ferreira FD, Mossini SAG, Ferreira FMD, Arroteia CC, Costa CL, Nakamura CV, Machinski M (2013) The inhibitory effects of *Curcuma longa* L. essential oil and curcumin on *Aspergillus flavus* link growth and morphology. *Sci World J.* 343804.
- Ferreira VRF, Brandão RM, Freitas MP, Saczk AA, Felix FS, Silla JM, Teixeira ML, Cardoso MG (2019) Colorimetric, electroanalytical and theoretical evaluation of the antioxidant activity of *Syzygium aromaticum* L., *Origanum vulgare* L., *Mentha spicata* L. and *Eremanthus erythropappus* M. essential oils, and their major constituents. *New J Chem.* 43:7653-7662.
- Ge H, Wang Y, Zhao X (2022) Research on the drug resistance mechanism of foodborne pathogens. *Microb Pathogenesis.* 162.
- Khan F, Bamunuarachchi NI, Tabassum N, Kim Y (2021) Caffeic acid and its derivatives: antimicrobial drugs toward microbial pathogens. *J Agr Food Chem.* 69: 2979-3004.
- Kwaśniewska-Sip P, Cofta G, Nowak PB (2018) Resistance of fungal growth on Scots pine treated with caffeine. *Int Biodeter Biodegr.* 132: 178-84.
- Masek A, Latos-Brożio M, Kałużna-Czaplińska J, Rosiak A, Chrzeszczajnska E. (2020) Antioxidant properties of green coffee extract. *Forests.* 11: 557.
- Monente C, Bravoa J, Vitasb AI, Arbillagac L, Peñaa P, Cida C (2015) Coffee and spent coffee extracts protect against cell mutagens and inhibit growth of food-borne pathogen microorganisms. *J Funct Foods.* 12: 365-74.
- Morales-Parterra AM, Cardoso-Toset F, Luque I, Astorga RJ, Maldonado A, Herrera-León S, Hernández M, Gómez- Laguna J, Tarradas C (2018) Prevalence and diversity of *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* in two free-range pig slaughterhouses. *Food Control.* 92: 208-215.

- Oliveira MMM, Brugnara DF, Alves E, Cardoso MG, Piccoli RH (2017) Morphological alterations in sessile cells of *Listeria monocytogenes* after treatment with *Cymbopogon* sp. essential oils. *Magistra*. 26: 385-92.
- Pasko MT, Piscitelli SC, Van Slooten ADV (1990) Fluconazole: a new triazole antifungal agent. *Ann Pharmacother*. 24: 860-7.
- Sardi JCO, Gullo FPG, Freires IA, Pitangui NS, Segalla MP, Fusco-Almeida AM, Rosalen PL, Regasini LO, Mendes-Giannini MJS (2016) Synthesis, antifungal activity of caffeic acid derivative esters, and their synergism with fluconazole and nystatin against *Candida* spp. *Diagn Micr Infec Dis*. 84: 387-91.
- Sung WS, Lee DG (2010) Antifungal action of chlorogenic acid against pathogenic fungi, mediated by membrane disruption. *Pure Appl Chem*. 82: 219-26.
- Taniwaki MH, Pitt JI, Copetti MV, Teixeira AA, Iamanaka BT (2019) Understanding mycotoxin contamination across the food chain in Brazil: challenges and opportunities. *Toxins*. 11: 411.
- Teixeira ML, Cardoso MG, Ferreira VRF, Campolina GA, Caetano ARS, Oliveira CD, Alves MVP, Nelson DL (2022) Evaluation of the antioxidant activity of the essential oils from *Cantinoa carpinifolia* (Benth.) and *Lippia organoides* (Kunth.) by various methods. *J Essent Oil Res*. 143-53.
- Wu H, Gu J, BK A, Nawaz MA, Barrow CJ, Dunshea FR, Suleria HAR (2022) Effect of processing on bioaccessibility and bioavailability of bioactive compounds in coffee beans. *Food Biosci*. 46: e101373.