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Antibacterial Activity of Coffee Extracts and Selected Coffee Chemical Compounds against Enterobacteria

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The *in vitro* antimicrobial activity of commercial coffee extracts and chemical compounds was investigated on nine strains of enterobacteria. The antimicrobial activity investigated by the disc diffusion method was observed in both the extracts and tested chemical compounds. Even though pH, color, and the contents of trigonelline, caffeine, and chlorogenic acids differed significantly among the coffee extracts, no significant differences were observed in their antimicrobial activity. Caffeic acid and trigonelline showed similar inhibitory effect against the growth of the microorganisms. Caffeine, chlorogenic acid, and protocatechuic acid showed particularly strong effect against *Serratia marcescens* and *Enterobacter cloacae*. The IC_{50} and IC_{90} for the compounds determined by the microtiter plate method indicated that trigonelline, caffeine, and protocatechuic acids are potential natural antimicrobial agents against *Salmonella enterica*. The concentrations of caffeine found in coffee extracts are enough to warrant 50% of the antimicrobial effect against *S. enterica*, which is relevant to human safety.

KEYWORDS: Coffee; antimicrobial activity; enterobacteria; trigonelline; caffeine; phenolic acids; inhibitory concentration

INTRODUCTION

Coffee is one of the most popular and widely consumed beverages throughout the world due to its pleasant taste and aroma and its stimulant effect. Recently, a number of beneficial health properties have been attributed to coffee (*1*, *2*), among them, antimicrobial activity. According to Daglia et al. (*3*), roasted coffee showed antibacterial properties against a range of Gram-positive and Gram-negative bacteria. On the basis of their findings, the activity was not affected by the brewing procedure. However, the degree of roasting and the coffee species affected significantly the antimicrobial activity. In a later study, Daglia et al. (*4*) investigated the relationship between the inhibitory effect of brewed coffee on *Staphylococcus aureus* and some chemical indicators of the degree of roasting, confirming the influence of the degree of roasting on the inhibitory effect.

Dogazaki et al. (*5*) and Furuhata et al. (*6*) reported antibacterial activity of brewed coffee against a strain of *Legionella pneumophila*, bacteria involved in respiratory infections, and identified the active substances as caffeic, chlorogenic, and protocatechuic acids. According to Daglia et al. (*7*) and Almeida et al. (*8*), the growth of *Streptococcus mutans*, the major causative agent of dental caries in humans, was inhibited by coffee extracts and by chemical compounds, among them, trigonelline, chlorogenic acid, caffeic acid, and protocatechuic acid.

Given the above findings, it would be interesting to investigate the potential antibacterial activity of coffee extracts and chemical compounds on other microorganisms that are relevant to human health. Enterobacteria are ubiquitous microorganisms. Because of their survival under adverse environmental conditions, they are able to colonize diverse ecological niches, including foods. Over the last decades, enterobacteria are becoming a source of increased health concern in food safety and clinical medicine. They are an important pathogenic group in community and hospital-acquired infections, and resistance to antibiotics has become increasingly common (*9*, *10*).

Therefore, the objective of this study was to investigate the antimicrobial activity of coffee extracts and of coffee bioactive compounds against enterobacteria. Ground coffee was analyzed for color, and the extracts were characterized according to pH and levels of selected chemical compounds. A screening of the inhibitory activity of coffee extracts and selected coffee chemical compounds against different strains of enterobacteria was made

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by the disc diffusion method. Furthermore, the 50% (IC_{50}) and 90% (IC_{90}) inhibitory concentrations of the coffee chemical compounds against *Salmonella enterica*, which is a highly invasive pathogen that infects humans and causes systemic infections, were determined by the microtiter plate method.

MATERIALS AND METHODS

Coffee Samples. Three different brands (A, B, and C) of roasted coffee beans (*Coffea arabica* L.) were purchased in the market of Belo Horizonte, state of Minas Gerais, Brazil. The grains were ground by means of a domestic mill (Braun) and passed through a 20 mesh sieve (Tyler series). Coffee extracts were obtained by using 8.0 g of coffee per 40 mL of boiling water. The mixture was kept in a boiling water bath for 3 min under agitation and filtered through qualitative filter paper.

Coffee Chemical Compounds. Caffeic acid (Sigma C0625-26, St. Louis, MO), chlorogenic acids [5-caffeoylquinic acid, Aldrich C44206, Milwaukee, WI; 3- and 4-caffeoylquinic acid, prepared from 5-caffeoylquinic (*11*)] and protocatechuic acid (3,4-dihydroxybenzoic acid, Sigma P5630) were dissolved in sterile distilled water with the aid of an ultrasonic apparatus (30 s), followed by immersion in a boiling water bath (30 s). Trigonelline (Sigma T5509) and caffeine (Reagen, Rio de Janeiro, RJ, Brazil) were diluted in sterile distilled water.

Microorganisms, Culture Media, Discs, and Antibiotics. The bacterial strains used were from the American Type Culture Collection (ATCC): *Citrobacter freundii* (ATCC 8090), *Enterobacter aerogenes* (ATCC 13048), *Enterobacter cloacae* (ATCC 23355), *Escherichia coli* (ATCC 25922), *Klebsiela oxytoca* (ATCC 49131), *Proteus hauseri* (ATCC 13315), *Proteus mirabilis* (ATCC 25933), *Salmonella enterica* (ATCC 14028), and *Serratia marcescens* (ATCC 8100). These strains were tested for purity by Gram reactions and by biochemical tests (Bactray, L.B. Laborclin, Pinhais, PR, Brazil). The strains were kept at −70 °C in brain heart infusion broth (BHI) with 20% glycerol, activated by transferring into nutritive agar, and incubating at 36.5 ± 1.0 °C for 18 h. The Mueller−Hinton agar, nutrient agar, and BHI were provided by Dialab Diagnósticos (Montes Claros, MG, Brazil). Sterile discs (6-mm diameter) and discs containing 30 μg of the antibiotic chloramphenicol were purchased from Cecon (São Paulo, SP, Brazil).

Characterization of Coffee Extracts. The color characteristics of the ground roasted coffees were determined using the ColorTec colorimeter PCM (Accuracy Microsensor, Pittsford, NY). The chroma [$c = (a^2 + b^2)^{1/2}$] and the hue [$h =$ arctg a/b] were obtained using the Colorpro software (*12*).

The pH of the extracts was obtained by means of a pH meter (DM20 Digimed, Santo Amaro, SP, Brazil).

The levels of the three main chlorogenic acids in coffee (3-caffeoylquinic acid, 4-caffeoylquinic acid, and 5-caffeoylquinic acid), trigonelline, and caffeine were determined by HPLC. For sample clarification prior to HPLC analysis, 200 μL of each coffee extract were mixed with 97.8 mL of Milli-Q purified water (Millipore Corp., Billerica, MA) and with 2.0 mL of 20% saturated lead acetate for the determination of caffeine and trigoneline (*13*) or 1 mL of each Carrez solution for chlorogenic acids (*11*). The mixture was shaken for 20 min and filtered through Whatman #1 filter paper (Whatman, Maidstone, U.K.). An aliquot was centrifuged for 2 min (Microfuge E, Beckman, Groton, CT) and the supernatant was used directly for chromatography, with different systems for trigonelline, caffeine (*13*), and for chlorogenic acids (*11*, *14*). Identification was possible by comparison of the retention time of the suspected peaks with those of standards and confirmation by the addition of the suspected standard to the sample (*13*, *14*). The chromatographic data was integrated, and quantification was possible by interpolation of an external standard curve.

Determination of the *in Vitro* Antimicrobial Activity against Enterobacteria by the Disc Diffusion Method. The antimicrobial activity of the coffee extracts and of selected coffee chemical compounds against the strains of enterobacteria was determined by means of the disc diffusion method (*15*, *16*). The aqueous coffee extracts and 2.0 mg/mL solutions of caffeic acid, chlorogenic acid, protocatechuic acid, trigonelline, and caffeine were used. Cultures of each bacteria were transferred to nutrient agar and incubated at 36.5 ± 1.0 °C for 24 h. The inoculum was standardized by transferring colonies from the nutrient agar to sterile saline up to 10^8 cfu/mL, which is equivalent to 50% transmittance at 580 nm (Coleman model 6120, Maywood, IL). Then 200 μL of the suspensions was placed onto the surface of Mueller−Hinton agar in a 150-mm Petri dish and spread homogeneously with a Drigalski tip. Discs (6.0 mm diameter) were impregnated with 20 μL of the coffee extracts and of the chemical compounds and placed on the surface of the agar containing each bacterium, which was incubated at 36.5 ± 1.0 °C for 48 h. The inhibition zones were measured with a caliper considering the total diameters. Similarly, each plate carried a blank disc containing 20 μL of sterile distilled water and an antibiotic disc containing 30 μg of chloramphenicol.

Each experiment was performed in triplicate. The results were submitted to analysis of variance and the means were compared by the Tukey test ($p \leq 0.05$) using GraphPad Prism (4.03).

Determination of the Influence of the Concentration of Coffee Chemical Compounds on the Inhibition of *S. enterica* by the Microtiter Plate Method. The influence of different concentrations of chlorogenic acid, protocatechuic acid, caffeic acid, caffeine, and trigonelline on the growth of *S. enterica*, which is a highly invasive pathogen that infects humans and causes systemic infections, was determined by the microtiter plate method described by USP (*17*). Sterile distilled water (100 μL) was deposited in each well of a sterile 96-well microtiter plate, except for the first of each series, which received 200 μL of the compounds in the concentrations of 8.0, 10.0, and 12.0 mg/mL, respectively. Serial dilutions of 100 μL were made using a multichannel micropipette (Wheaton, Ecublens, Switzerland), resulting in 12 concentrations to be tested for each compound (1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, and 12.0 mg/mL). BHI (95 μL) and 5 μL of the inocula containing 10^8 cfu/mL were also added to each well. The plates were agitated in a shaker (Tecnal, model TE 140, SP, Brazil) for 1 min at 150 rpm and then incubated at 36.5 ± 1.0 °C. After 18 h, 20 μL of 5 mg/mL tetrazolium salt [3−4,5 dimethylthiazol-2,5-diphenyltetrazolium bromide] filtered by means of a Millipore sterile 0.22 μm filter unit was added to each well. The plate was agitated for 1 min at 150 rpm and kept at 36.5 ± 1.0 °C for 30 min. The absorbances at 595 nm were taken using a microplate reader (model 550 Bio-Rad). A control containing chloramphenicol and a control of the medium (blank) were prepared. The percent inhibition was calculated according to the equation % inhibition $= 100(A_c - A_s)/A_c$, in which A_c is the absorbance for the wells with the blank (control) and A_s is that for the samples. Each experiment was performed in triplicate.

Determination of the 50% and 90% Inhibitory Concentrations against *S. enterica*. The 50% and 90% inhibitory concentrations (IC_{50} and IC_{90}, respectively) against *S. enterica* were determined for chlorogenic acid, protocatechuic acid, caffeic acid, caffeine, and trigonelline (*17*). Fitting of the data was performed by nonlinear regression using the log of the concentration versus percent inhibition. The calculated IC_{50} and IC_{90} were submitted to analysis of variance and the means were compared by the Tukey test ($p \leq 0.05$) using GraphPad Prism (4.03).

RESULTS AND DISCUSSION

Characteristics of the Coffee Samples. The CIE $L^*a^*b^*$ color characteristics of the ground coffee from the different brands are indicated in **Table 1**. There was no significant difference on a^* values and hue angles of the three brands. However, luminosity (L^*), b^*, and chroma (c^*) varied significantly among the samples, with higher values observed for samples B and C. The significant inverse correlation between luminosity and the degree of roasting is well-known (*13*, *18*). Therefore, sample A was roasted to a higher degree compared to B and C samples, which did not differ statistically.

The pH of the coffee extracts varied significantly, with higher values observed for brand A, followed by brand C, which differed significantly from brand B. These results are within

Table 1. CIE *L***a***b** Color Characteristics of the Ground Roasted Coffee Samples (A–C), pH, and Levels of Trigonelline, Caffeine, and Chlorogenic Acids of Different Brands of Coffee Extracts[a]

parameter	A	B	C
CIE *L***a***b** color			
*L**	20.92 ± 1.18 b	29.4 ± 0.37 a	26.4 ± 1.08 a
*a**	5.98 ± 2.99	10.3 ± 1.36	8.53 ± 2.16
*b**	11.7 ± 2.08 b	26.0 ± 0.77 a	20.8 ± 2.17 a
chroma	13.3 ± 2.26 c	28.0 ± 0.24 a	22.6 ± 1.37 b
hue	63.4 ± 12.9	68.4 ± 3.16	67.5 ± 7.07
pH	5.29 ± 0.03 a	4.88 ± 0.03 c	5.02 ± 0.01 b
trigonelline (mg/mL)	0.49 ± 0.05 c	1.63 ± 0.04 a	1.50 ± 0.02 b
caffeine (mg/mL)	2.23 ± 0.03 a	2.18 ± 0.03 a,b	2.11 ± 0.04 b
chlorogenic acids (mg/mL)			
3-CQA	0.08 ± 0.00 c	0.88 ± 0.01 a	0.71 ± 0.01 b
4-CQA	0.12 ± 0.00 c	1.11 ± 0.02 a	0.90 ± 0.01 b
5-CQA	0.09 ± 0.01 c	1.79 ± 0.03 a	1.46 ± 0.01 b

[a] CQA = caffeoylquinic acid. Mean of triplicates. Mean values (±standard deviations) with different letters in the same line (a, b, c) are significantly different (Tukey test, $p \leq 0.05$).

Table 2. Diameters of the Inhibition Zones Obtained with Coffee Aqueous Extracts on Different Strains of Enterobacteria[a]

strains	inhibition zones (mm)		
	A	B	C
C. freundii	7.1 ± 0.6 b	6.9 ± 0.5 b	7.0 ± 0.5 bc
E. aerogenes	8.4 ± 0.6 ab	8.2 ± 0.7 ab	8.7 ± 0.3 ab
E. cloacae	9.0 ± 1.0 a	9.3 ± 1.3 a	9.7 ± 1.3 ab
E. coli	8.1 ± 0.4 ab	8.1 ± 0.3 ab	8.2 ± 0.5 ab
K. oxytoca	7.5 ± 0.7 ab	7.7 ± 0.3 ab	7.5 ± 0.5 b
P. hauseri	9.2 ± 1.1 a	9.3 ± 1.1 a	9.8 ± 0.6 a
P. mirabilis	8.1 ± 0.5 ab	8.8 ± 0.3 ab	8.8 ± 0.4 ab
S. enterica	8.1 ± 1.3 ab	7.9 ± 1.2 ab	8.3 ± 1.6 ab
S. marcescens	9.2 ± 0.3 a	9.2 ± 0.8 ab	9.1 ± 0.1 ab

[a] Mean of triplicates. Mean values (±standard deviations) with different letters (a, b) in the same columns are significantly different (Tukey test, $p \leq 0.05$). As positive control, 30 μg of chloramphenicol/disc formed inhibition zones with mean (±standard deviation) diameters in mm: *C. freundii*, 32.7 ± 1.7; *E. aerogenes*, 25.0 ± 2.3; *E. cloacae*, 32.2 ± 1.2; *E. coli*, 28.3 ± 1.5; *K. oxytoca*, 30.8 ± 0.8; *P. hauseri*, 20.9 ± 1.1; *P. mirabilis*, 21.2 ± 0.8; *S. enterica*, 27.3 ± 0.9; *S. marcescens*, 27.4 ± 1.6.

the range described in the literature. Furthermore, the difference observed in pH values could be due to several factors, such as agricultural practices, the degree of ripening during harvest, fermentation of the beans, and also by the degree and method of roasting (*19*−*21*). According to Sabbagh and Yokomiso (*22*), the stronger the roasting degree, the higher the pH, which is in agreement with luminosity results for sample A, although this statement cannot be applied to samples B and C.

The levels of selected bioactive compounds in the coffee extracts are indicated in **Table 1.** Three chlorogenic acid isomers, trigonelline, and caffeine were identified in the coffee extracts. Even though Dogasaki et al. (*5*) reported the identification of protocatechuic and caffeic acids (by means of NMR and LC−mass spectrometry) in brewed coffee, neither compound was detected in the coffee extracts investigated in this study (quantification limits of 0.05 and 2.0 μg/mL, respectively). According to Farah et al. (*13*), a small amount of caffeic acid is generally found in green coffee, while very small amounts can be identified in some roasted coffees. Therefore, the samples used by Dogazaki et al. (*5*) were probably submitted to mild roasting conditions compared to the ones included in this study.

The levels of the chlorogenic acids 3-caffeoylquinic, 4-caffeoylquinic, and 5-caffeoylquinic acids varied significantly among coffee brands as indicated in **Table 1**. However, these levels are similar to those reported in the literature (*11*, *13*). Since chlorogenic acids are partially degraded during the roasting process (*23*) and sample A was darker than samples B and C, one would expect lower levels of chlorogenic acids in sample A, which can be observed in the results. These results are in accordance with the levels of chlorogenic acids described by Farah et al. (*11*) for samples classified as very dark regarding roasting degree.

The levels of caffeine and trigonelline observed in the coffee samples (**Table 1**) were similar to values reported in the literature (*13*, *24*). Higher levels of caffeine and lower levels of trigonelline were found in sample A. According to Macrae (*25*), even though high temperatures can be reached (~230 °C) during coffee roasting, the loss in caffeine is insignificant. A relative increase in caffeine levels may be observed during roasting due to loss of other compounds (*2*). The degradation of trigonelline during the roasting process is well-known (*22*, *25*).

Therefore, even though physicochemical characterization indicated a difference on the degree of roasting among samples, they could be all categorized according to the Brazilian Association of the Coffee Industries (ABIC) reference color system as very dark regarding roasting degree.

***In Vitro* Antimicrobial Activity of Coffee Extracts against Enterobacteria by the Disc Diffusion Method.** The inhibitory effect of the coffee extracts on some strains of enterobacteria is indicated in **Table 2**. There was no significant difference on the inhibitory effect of the different coffee brands on the enterobacteria strains investigated, even though physicochemical characteristics varied among samples. The pH differences among samples were not enough to affect the inhibitory effect. Furthermore, besides the color differences, the samples were all categorized as very dark regarding roasting degree. These results are in agreement with those of Daglia et al. (*3*, *4*), who did not find significant difference in the antimicrobial activity among samples with the same degree of roasting.

On the other hand, when comparing the susceptibility of the different strains of enterobacteria to the coffee extracts, significant differences were observed. Larger diameters of the inhibition zones were observed for *P. hauseri*, indicating the higher sensitivity of this species to the coffee extracts. Smaller diameters were observed for *C. freundii*, which indicated that it was less sensitive to the coffee extracts. The other strains of enterobacteria showed intermediate sensitivity to these compounds.

The antimicrobial activity of the different coffee chemical compounds at the concentration of 2.0 mg/mL against the same strains of enterobacteria is described in **Table 3**. No significant difference was observed on the inhibitory effect of caffeic acid and trigonelline on all the investigated strains of enterobacteria. However, chlorogenic acid and protocatechuic acids were less effective against *C. freundi* compared to *S. marcescens* and *E. cloacae*, with the remaining strains showing intermediate sensitivity. Caffeine was less effective against *C. freundi*, *E. aerogenes*, *K. oxytoca*, and *P. mirabilis* compared to *P. hauseri* and *S. marcescens*.

When comparing the effect of the different chemical compounds on each microorganism, significant differences were only observed for *E. cloacae* and *E. aerogenes*, which were less sensitive to the effects of caffeine compared to the other compounds.

The results obtained were confirmed by literature reports. The antimicrobial activity of caffeic acid was described against

Table 3. Diameters of Inhibition Zones Obtained with Selected Coffee Chemical Compounds at 2 mg/mL on Different Strains of Enterobacteria[a]

	inhibition zones (mm)				
strains	chlorogenic acid	protocatechuic acid	caffeic acid	caffeine	trigonelline
C. freundii	6.8 ± 0.4 b	7.0 ± 0.5 b	7.2 ± 0.8	7.0 ± 0.0 b	7.0 ± 0.5
E. aerogenes	8.6 ± 0.4 abx	8.4 ± 0.1 abx	8.4 ± 0.8 x	7.0 ± 0.0 by	8.6 ± 1.2 x
E. cloacae	9.6 ± 1.2 ax	10.0 ± 0.9 ax	10,0 ± 1.3 x	7.7 ± 1.2 aby	9.1 ± 0.8 x
E. coli	8.0 ± 0.5 ab	8.0 ± 0.5 ab	8.0 ± 0.4	8.0 ± 1.0 ab	8.1 ± 0.5
K. oxytoca	7.8 ± 1.0 ab	7.6 ± 0.5 ab	7.5 ± 0.5	7.0 ± 0.0 b	7.4 ± 0.5
P. hauseri	9.1 ± 1.1 ab	9.3 ± 0.8 ab	8.7 ± 0.8	9.7 ± 1.5 a	8.6 ± 0.8
P. mirabilis	8.9 ± 0.4 ab	8.0 ± 0.5 ab	8.3 ± 0.3	7.3 ± 0.6 b	8.9 ± 0.8
S. enterica	7.9 ± 1.2 ab	8.4 ± 1.4 ab	8.3 ± 1.8	8.3 ± 0.6 ab	8.8 ± 1.2
S. marcescens	9.7 ± 0.9 a	10.0 ± 2.2 a	9.2 ± 0.8	7.2 ± 0.3 a	9.6 ± 1.9

[a] Mean of triplicates. Mean values (±standard deviations) with different letters in the columns (a,b) and in the lines (x,y) are significantly different (Tukey test, $p \leq 0.05$). As positive control, 30 μg of chloramphenicol/disc formed inhibition zones with mean (±standard deviation) diameters in mm: *C. freundii*, 32.7 ± 1.7; *E. aerogenes*, 25.0 ± 2.3; *E. cloacae*, 32.2 ± 1.2; *E. coli*, 28.3 ± 1.5; *K. oxytoca*, 30.8 ± 0.8; *P. hauseri*, 20.9 ± 1.1; *P. mirabilis*, 21.2 ± 0.8; *S. enterica*, 27.3 ± 0.9; *S. marcescens*, 27.4 ± 1.6.

Table 4. Sigmoidal Dose Response, Coefficient of Determination, and the Inhibitory Concentrations of 50% and 90% of Coffee Chemical Compounds against *S. enterica*

	nonlinear regression		inhibitory concentration[b] (mg/mL)	
chemicals	sigmoidal dose response[a]	R^2	IC_{50}	IC_{90}
chlorogenic acid	$Y = 20.88 + [79.12/(1 + 10^{0.72-X})6.31]$	0.830	4.8 ± 0.9 b	7.1 ± 0.7 b
protocatechuic acid	$Y = 8.66 + [91.28/(1 + 10^{0.40-X})4.52]$	0.966	2.5 ± 0.2 c	4.1 ± 0.3 c
caffeic acid	$Y = 24.57 + [52.53/(1 + 10^{0.82-X})13.59]$	0.910	6.6 ± 0.2 a	9.3 ± 0.2 a
caffeine	$Y = 25.58 + [66.39/(1 + 10^{0.46-X})5.71]$	0.911	2.6 ± 0.2 c	5.0 ± 0.8 c
trigonelline	$Y = 3.47 + [96.53/(1 + 10^{0.35-X})3.20]$	0.843	2.2 ± 0.5 c	3.8 ± 0.8 c

[a] Variable slope. [b] Mean of triplicates. Mean values (±standard deviations) with different letters in the columns (a, b, c) are significantly different (Tukey test, $p < 0.05$).

Pseudomonas, *E. coli*, *S. aureus*, and *Bacillus cereus* (*26*−*28*). According to these studies, caffeic acid had an appreciable antibacterial activity against these microorganisms. Caffeic and protocatechuic acids were observed to exhibit strong activity against *L. pneumophila* (*5*). Caffeine was also observed to have inhibitory activity against filamentous fungi and inhibited the production of aflatoxin (*29*, *30*). However, the inhibitory effects of caffeine on bacteria were contradictory. Daglia et al. (*7*) concluded that caffeine did not have antimicrobial activity against *S. mutans*, as no difference was observed for coffee with and without caffeine. However, Ibrahim et al. (*31*) observed antimicrobial activity of caffeine at different concentrations (0.25 to 2.00%) against *E. coli* O157:H7. In the present study, the antimicrobial activity of caffeine against different enterobacteria was confirmed.

Aromatic and phenolic compounds exert antimicrobial activity by altering the structure and function of the cytoplasmatic membrane, disrupting the proton motive force, the electron flow, and the active transport (*32*−*34*). The mechanism responsible for phenolic toxicity to microorganisms can also include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfidryl groups or through more nonspecific interactions with proteins (*35*). According to Grigg (*36*), caffeine can inhibit dark and excision repair mechanisms, probably due to its ability to bind specifically to single-stranded DNA. No information on the mechanism for the antimicrobial activity of trigonelline was found in the literature searched.

Therefore, when considering the large number of different groups of chemical compounds present in the coffee extract, it is more likely that the antimicrobial activity is not attributable to one specific mechanism but that are several targets in the cell (*34*).

***In Vitro* Antimicrobial Activity of Coffee Extracts against *S. enterica* by the Microtiter Plate Method.** On the basis of the fact that strains of *Salmonella* are highly invasive pathogens that infect humans worldwide and may develop septicemia commonly with metastatic focal infections in any tissue of the body (*37*), it was used in further studies. When studying the influence of the concentration of the different coffee compounds on the antimicrobial activity, it was observed that as the concentration increased, there was an increase in the inhibition of the bacterial growth, however at different rates depending on the compound.

Differences that were not observed among the compounds using the disc diffusion method were detected by the microtiter plate method. Therefore, the disc diffusion method could be used as a screening method, and the microtiter plate method is recommended to investigate the influence of the concentration on the inhibitory effect. Furthermore, the disc diffusion method may be affected by the diffusion rates of the compound on the agar and, therefore, may not measure the total activity (*28*, *38*).

Determination of the Concentration Needed To Inhibit 50% and 90% of the Growth of *S. enterica*. Regression analysis indicated that the data fitted a variable slope sigmoidal dose response equation, with R^2 values higher than 0.830 (**Table 4**). These equations were used to calculate the IC_{50} and IC_{90} values for the coffee chemical compounds to inhibit *S. enterica*. Significantly higher IC_{50} and IC_{90} values were found for chlorogenic acid and caffeic acid, confirming that these compounds were less efficient in the inhibition of the growth of *S. enterica*. However, no significant difference was observed on the inhibitory effect of protocatechuic acid, trigonelline, and caffeine, confirming that similar concentrations of these compounds provided the same inhibitory effect.

The IC_{90} values reported by Furuhata et al. (*6*) for protocatechuic and caffeic acids on *L. pneumophila* were 0.156 and 0.625 mg/mL, respectively. Therefore, *S. enterica* required higher concentrations of these compounds compared to *L. pneumophila*.

The present work demonstrates that every coffee extract and chemical compound included in this study showed antimicrobial activity for the nine enterobacteria investigated. Even though the coffee samples differed significantly according to the color characteristics, pH, and levels of phenolic acids, caffeine, and trigonelline, they were all considered as being roasted to a high degree. There was no significant difference on the inhibitory effect of the coffee samples against the microorganisms tested. The enterobacteria responded differently to the coffee extracts, with *C. freundi* and *P. hauseri* showing the least and the most sensitivity, respectively. The strains investigated showed similar responses to the different compounds, except for *E. cloacae* and *E. aerogenes*, which had smaller inhibition zone diameters for caffeine compared to the other compounds. Moreover, *S. marcescens* and *E. cloacae* were the most sensitive and *C. freundii* was the least sensitive to chlorogenic acid and protocatechuic acid. The concentration of the chemical compounds affected significantly the inhibitory effect against *S. enterica*. Furthermore, the rate of inhibition as a function of the concentration was affected by the chemical compound. Calculations of the IC_{50} and IC_{90} values indicated that the growth *of S. enterica* was inhibited by smaller concentrations of protocatechuic acid, caffeine, and trigonelline compared to chlorogenic acid and caffeic acid. These results indicate that trigonelline, caffeine, and protocatechuic acid are potential natural antimicrobial agents against enterobacteria, and, therefore, could be used in foods as a natural preservative to control their growth. Furthermore, the concentrations of caffeine found in coffee extracts are enough to warrant the IC_{50} of the antimicrobial effect against *S. enterica*.

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