NFS Journal 31 (2023) 50–56

Contents lists available at ScienceDirect

NFS Journal

journal homepage: www.elsevier.com/locate/nfs

Antimicrobial activities of phenolic extracts of coffee mucilage

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ARTICLE INFO

Keywords:
Coffee
Mucilage
Bacillus cereus
Pseudomonas aeruginosa
Alcaligenes sp.
Serratia sp.
Micrococcus luteus
Escherichia coli
Staphylococcus aureus
Salmonella enterica
Listeria monocytogenes

ABSTRACT

The inhibition exerted by ethanolic extracts of coffee mucilage on the growth of bacteria was studied by microdilution in agar. The growth inhibition effect was evaluated for pathogenic or food spoilage related bacteria (*Pseudomonas aeruginosa* (ATCC 27853), *Alcaligenes* sp. (UCR277), *Serratia* sp. (UCR299), *Micrococcus luteus* (ATCC4698), *Escherichia coli* (ATCC35150), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC14579), *Salmonella enterica* subsp. *enterica* (ATCC 13311) and *Listeria monocytogenes* (SLCC4013)) and for bacteria associated with human intestinal biota (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactiplantibacillus* plantarum subsp. plantarum). The most effective growth inhibition was observed for *B. cereus* (ATCC14579). The content of chlorogenic acid and caffeine in the ethanolic extracts was quantified by HPLC/DAD. The chlorogenic acid content in the extracts ranged from 2.67 to 4.76 mg/ml, while the caffeine content ranged from 1.24 to 6.48 mg/ml. Although ethanolic extracts of coffee berry mucilage inhibited the growth of *B. cereus*, this inhibition does not seem to be related to the caffeine or chlorogenic acid contents.

1. Introduction

The development of natural preservatives with antimicrobial activity against a broad spectrum of microorganisms has gained importance. Some authors have studied promising plant or animal materials to develop natural antimicrobials considering their bioactive compound profile. Promising antimicrobial activities have been reported for the skin mucus of *Puntius sophore* from brackish freshwater fish [1], for bioactive metabolites of the fern *Adiantum philippense* [2] and for the oil extracted from *Thymus* plants (*Thymus musilii* Velen) [3]. Also, several authors have evaluated the antimicrobial activities of extracts derived from agricultural wastes [4–6]. After assessing various agricultural wastes, the antimicrobial effect on meat products of extracts derived from grape, jaboticaba and *Opuntia* sp. was highlighted [7].

Losses and residues of fruits and vegetables can reach 60%, while processing produces about 25–30% of by-products. These residues are mainly constituted by seeds, skin and pomace, which contain valuable amounts of bioactive compounds that can have industrial uses in the food industry, but also in the health industry for drugs and pharmaceuticals [8]. Coffee, in economic terms, is one of the agricultural products with the highest value added. In November March 2022, 11.79 million 60 kg bags of coffee were exported worldwide [9] which was an increase of 2.6% over the same month of the previous year. This product is the second most important, after oil [10], generating close to 10 billion dollars a year [11]. Globally, large quantities of coffee are produced and consumed, but in addition to the economic benefits in the markets, there are significant negative environmental effects related to coffee bean processing [12].

Considering the increasing global consumption of coffee, the amount of waste generated during processing has become a relevant issue in recent years. Coffee industries are looking for alternatives to valorize by-products to increase the sustainability of the process, not only with an ecological approach, but also as an opportunity to create new jobs and increase their economic income. The different by-products of coffee processing are rich in bioactive compounds with potential application in the pharmaceutical, food and cosmetic industries [13,14]. Chlorogenic acids constitute one of these compounds with great potential. These are found in different parts of the coffee fruit and have been the subject of multiple investigations since they appear to be beneficial to humans due to their antioxidant, hypoglycemic, antiviral, hepatoprotective and

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https://doi.org/10.1016/j.nfs.2023.03.005
Received 24 December 2021; Received in revised form 20 March 2023; Accepted 21 March 2023
Available online 22 March 2023
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nutraceutical capacities, among others [13]. Chlorogenic acids are not only present in the coffee beverage, roasted or green, but also the residues produced during bean processing contain different amounts of chlorogenic acid along with other phenolic compounds. According to Ramirez-Martinez et al. [15], chlorogenic acids account for about 42.2% of the total phenolic compounds in fresh coffee pulp. More recent analyses confirm that 5-*O*, 4-*O* and 3-*O*-Caffeoylquinic acids are constant components of the pulp and husk of ripe coffee beans [16].

Previous studies reported that chlorogenic acids present in coffee beverage have the ability to inhibit bacterial growth [17,18]. However, more research focused on the effect of these compounds on pathogenic and food spoilage-related microorganisms is still needed. Also, some studies describe the selective effect of chlorogenic acids and other phenolic compounds on human gut flora bacteria [19]. So far, studies on the antimicrobial capacity of coffee polyphenol extracts are still scarce and their industrial application is limited. Coffee mucilage, used in this study, is obtained by industrial mechanical separation, and collected after removal of the pulp and skin of the berries. There are no previous reports on the antimicrobial activities of ethanolic extracts of this by-product. Due to the potential of coffee processing by-products, the objective of this research is to evaluate the antimicrobial properties of coffee berry mucilage, focusing on the inhibition of the growth of food-related pathogenic strains, food spoilage-related microorganisms, and microorganisms associated with human intestinal microbiota.

2. Materials and methods

2.1. Plant material and chemicals

Coffee mucilage was collected in three different Costa Rican coffee processing sites: (A) located in Tres Ríos (9°54′27″ N, 83°59′6″ O); (B) located in San Marcos de Tarrazú (9°34′59.07″N, 84°3′53.36″ O) and (C) located in Moravia (10°0′46″N, 84°1′20″ O). At the sites, fully ripe coffee fruits were processed using a Penagos 306 pulper. The separated mucilage was collected and frozen at −80 °C and freeze-dried (Benchtop Pro 3 L ES 55Virtis Sp Scientific freeze dryer) until further extraction.

Analytical or HPLC grade reagents and solvents were purchased from Sigma Aldrich Chemie (Taufkirchen, Germany).

The strains of selected microorganisms were acquired from the American Type Culture Collection (ATTC), *Listeria* cultures special collection, Chr Hansen and collection of the University of Costa Rica. The spoilage-associated bacteria used were *Pseudomonas aeruginosa*. ATCC 27853, *Alcaligenes* sp. (UCR 277), *Serratia* sp. (UCR 299), *Micrococcus luteus* ATCC 4698, *Escherichia coli* ATCC 35150. The selected bacteria with potential pathogenicity to humans were *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC14579, *Salmonella enterica* subsp. enterica ATCC13311 and *Listeria monocytogenes* SLCC 4013. In addition, *Lactobacillus acidophilus* CRL730, *Lactobacillus casei* CRL431, *Lactobacillus rhamnosus* ATCC 7469, *Lactiplantibacillus plantarum* subsp. plantarum ATCC 14917 were used as probiotic bacteria and/or bacteria associated with human intestinal biota.

2.2. Extraction of phenolic compounds

Phenolic extracts were prepared as described by Mingo et al. [20] and modified as follows. The mucilage was lyophilized using a Benchtop Pro 3 L ES 55Virtis Sp Scientific lyophilizer. Extraction was performed in a 1:4 ratio freeze-dried mucilage sample:solvent (95% ethanol) and was homogenized with an ultraturrax (Tissue Tearor model 98537Q-395) for 5 min. The supernatants were decanted after centrifugation (10 min at 4000 rpm) and the extraction was repeated 3 times on the precipitate. The supernatants were collected and filtered under vacuum. The solvent was removed to dryness at a rotary evaporator (Buchi Rotavapor R-124) with a bath temperature below 50 °C. Finally, the dried extracts were diluted in sterile distilled water to a volume sufficient to achieve an extract concentration of approximately 0.7 g/ml and stored at −80 °C until use. The pH of the extracts was approximately 4.0. To remove spoilage microorganisms, present in the extracts, the extracts were filtered (0.45 μm) before use.

2.3. Quantification of chlorogenic acids and caffeine by HPLC

Total amounts of chlorogenic acids in mucilage extracts were evaluated with replicates corresponding to different locations (A, B, C) and injected twice in a HPLC/PDA (Thermo Scentific Dionex Ultimate 3000 RS). A Phenomenex (Torrance, CA, USA) C18 Hydro-Synergi column (150 × 3.0 mm i.d., 4 μm particle size) with a C18 ODS precolumn (4.0 × 2.0 mm i.d.) at 25 °C was used. Quantification of chlorogenic acid and caffeine was performed according to Kammerer et al. [21]. Mobile phases consisted of a mixture of 2% (v/v) acetic acid in water (Eluent A) and 0.5% acetic acid in water and MeOH (10:90, v/v; Eluent B). Gradient elution increased from in 10 min from 10% B to 15%, this eluent ratio was maintained for 3 min. From 13 to 20 min eluent B reached 25%. At time 50 eluent B amounted 55%. Finally, 100% eluent B was used until minute 56. The column was then restored with 10% B until 60 min for the next injection. Elution time was 60 min, with an elution rate of 0.4 ml/min. An injection volume of 10 μl was used. Chlorogenic acid and caffeine were quantified at 280 nm. The identification and quantification of major compounds was performed taking into account their absorption spectra, their retention times, as reported in the literature [22], and the respective standards (chlorogenic acid and caffeine). Minor peaks corresponding to other phenolic compounds were identified but not quantified (*p*-coumaric acid, mangiferin, isochlorogenic acid and rutin).

2.4. Microdilution on agar for in vitro evaluation of the inhibitory effects of phenolic extract on microorganisms

The inhibition effect of different concentrations of the phenolic extracts derived from coffee mucilage on the growth of the selected microorganisms was studied by applying the agar microdilution methodology proposed by Thomas et al. [23]. Erlenmeyer flasks were prepared with 50 ml of Trypticase Soy agar (ATS), after autoclaving, amounts of 0.5 to 5 ml mucilage extract were added. The extracts were diluted to achieve final concentrations as indicated in Table 1, after adding 5 ml of the diluted extract in 50 ml of agar. Bacterial strains were diluted in sterile 0.1% peptonized water (PSA) to a population density that generated a turbidity equivalent to that of a MacFarland's 0.5 nephelometric standard. ATS plates with mucilage extract were spot inoculated with 10 μl of bacterial suspensions with different population density (from 10^5 to10^8 CFU/g), incubated under aerobic conditions for 24 h at 35 °C and examined for bacterial growth. Uninoculated plates and plates without extract were used as negative and viability controls, respectively. For lactic acid bacteria, the same procedure was followed, but using De Man, Rogosa and Sharpe agar (MRS) instead of ATS and incubating 48 h at 35 °C, with a CO_2-enriched atmosphere. In all cases, three replicates of the procedure were performed.

To evaluate the role of sole caffeine and chlorogenic acid in the

Table 1
Final extract concentrations used to determine growth inhibition by microdilution on agar for in vitro evaluation of the inhibitory effects on microorganisms for the different collection sites and batches.

Collection site	Batch	Extract concentration levels (mg/ml)		
		Low (1)	Medium (2)	High (3)
Tres Ríos	1 (A1)	7.1	27.7	53.4
	2 (A2)	6.9	26.6	51.3
San Marcos de Tarrazú	1 (B1)	7.3	28.5	54.8
	2 (B2)	7.1	27.7	53.4
Moravia	1 (C1)	6.7	26.2	50.4
	2 (C2)	6.7	26.2	50.4

inhibition on the growth of the selected microorganisms, trypticase soy agar and MRS plates were prepared with mucilage extract (61.8 mg / ml), caffeine (7.02 mg/ml) and chlorogenic acid (5.10 mg/ml).

2.5. Quantification of the inhibitory effect on B. cereus

2.5.1. Quantification of initial inoculum

Quantification of the inhibitory effect on *B. cereus* (ATCC14579) was evaluated, being the microorganism most sensitive to the extract. To evaluate the antimicrobial activity, the methodology previously described by Iñiguez-Montero et al. [24] was used, modified as follows. Starting from a *B. cereus* suspension with an optical density similar to Mac Farland's 0.5 standard, decimal dilutions were performed in tubes with 9 ml of 2% peptone-buffered water, until the 10^{-9} dilution was reached. Each of these dilutions were plated on TSA, by the spreading technique, using an inoculum of 0.1 ml. The procedure was performed in duplicate. After incubation at 35 °C for 24 h plates with 25 to 250 CFU were counted and considered as the initial inoculum.

2.5.2. Quantification of inhibitory effect

From the initial suspension (Mac Farland standard 0.5), 5 ml were taken and mixed with an equal amount of mucilage extract at a concentration of 800 mg/ml. To this mixture 2 ml of sterile 2.5% bicarbonate solution was added as a buffer to maintain pH at 6 and to rule out suboptimal pH values as a cause of inhibition. A tube with 5 ml of peptonized water containing 2% peptone, 5 ml of the extract and 2 ml of sterile 2.5% bicarbonate solution was used as a control. After different incubation times (15 min, 1, 3, 6 and 24 h), 1 ml was extracted and placed in a tube with 9 ml of sterile peptone water. Dilutions were made by transferring 1 ml to tubes with 9 ml of sterile peptone water until dilution −9 was reached. From each tube 0.1 ml was taken and distributed on TSA plates. This step was performed in duplicate. Plates were incubated at 35 °C and after 24 h plates with counts between 25 and 250 CFU were counted and the decrease from the initial population was determined. Two additional controls were used, one tube with 5 ml of the extract plus 5 ml of 2% peptone buffered peptone water and another with 5 ml of 2% peptone buffered peptone water plus 5 ml of the *B. cereus* (ATCC14579) suspension. The first control was considered as sterility and the second as bacterial viability. This procedure was performed in duplicate.

2.6. Statistical analysis

A logistic regression model was performed to predict the growth variable (grows / does not grow) based on the independent variables: extract concentration and type of bacteria. The GLM libraries in program R were used for this purpose. Using the likelihood function for a binomial proportion, the assessment of statistical significance was performed at 95% confidence using the Likelihood Ratio Test (LRT) and the chi-square test.

3. Results

3.1. Caffeine and chlorogenic acid contents on the phenolic extracts

When phenolic compounds were evaluated by HPLC/DAD, two main peaks were observed (Fig. 1) corresponding to caffeine and chlorogenic acid, as confirmed by standards and absorption spectra. The mean content of chlorogenic acids in the mucilage extracts was 3.83 ± 1.06 mg/ml, with the lowest content (2.67 mg/ml) for extract B, 4.06 mg/ml for extract C and the highest for extract A with 4.76 mg/ml. The mean caffeine content for all processing sites corresponded to 3.16 ± 2.89 mg/ml and ranged from 1.24 mg/ml for extract C, 1.75 mg/ml for B and 6.48 mg/ml for extract A.

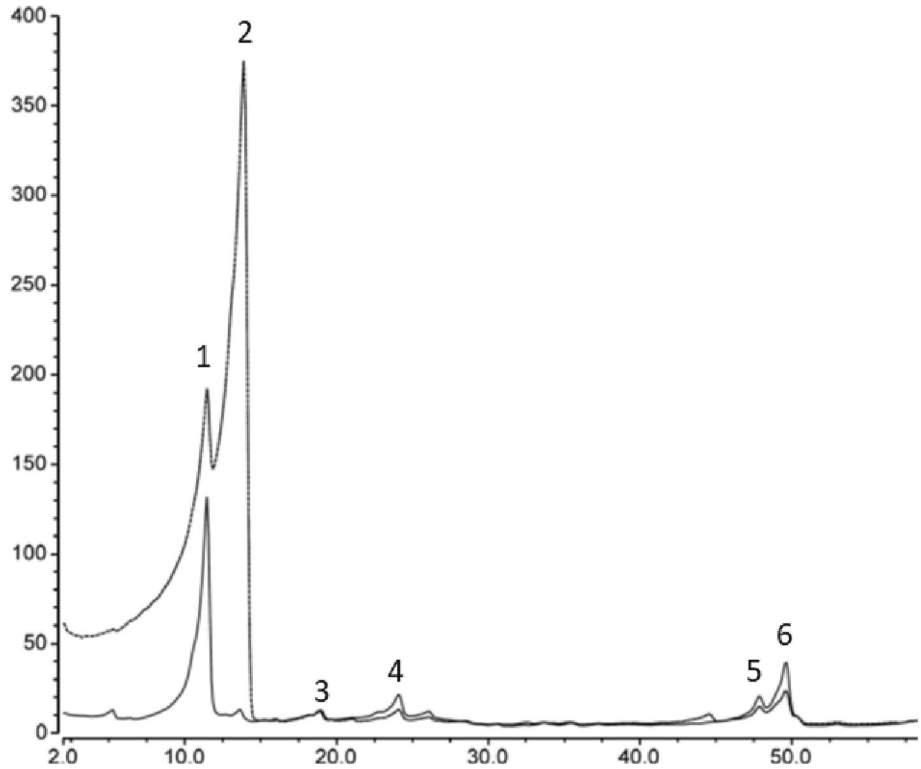

Fig. 1. HPLC separation of phenolic compounds of ethanolic coffee berry mucilage extract by 280 (dotted line) and 320 nm. Peak assignment as follows: 1) Chlorogenic acid, 2) Caffeine, 3) p-coumaric acid, 4) Mangiferin, 5) Isochlorogenic acid, 6) Rutin.

3.2. Effect of coffee mucilage extracts on growth inhibition of selected microorganisms

The mucilage extracts showed an inhibitory effect on the growth of Gram-positive microorganisms (*B. cereus* (ATCC14579), *L. monocytogenes* (SLCC4013), *S. aureus* (ATCC25923), *M. luteus* (ATCC4698)), while such effect was not observed for Gram-negative bacteria (*E. coli* (ATCC35150), *Salmonella* (ATCC13311), *Alcaligenes* (UCR277), *and Pseudomonas* (ATCC27853)) The most susceptible microorganism was *B. cereus* (ATCC14579), followed by *M. luteus* (ATCC4698), *L. monocytogenes* (SLCC4013) and *S. aureus* (ATCC25923) (Fig. 2).

A lower inhibition effect of the extract on the growth of bacteria associated with human intestinal biota (*L. casei, L. paracasei, Lactiplantibacillus plantarum* and L. *rhamnosus*) was observed (Fig. 3).

The statistical analysis performed determined that bacteria associated with human intestinal biota can grow in the presence of the mucilage phenolic extract at least 4.7 times higher than that of pathogenic and/or spoilage bacteria with 95% confidence. Therefore, it can be considered that the growth of the latter was more affected by the extracts than that of bacteria associated with human intestinal biota (Fig. 4).

3.3. Effect of extract concentration on the inhibition of microbial growth

It was also possible to determine that the effect of the extract on the growth of microorganisms depends on its concentration. The lower the concentration of the extract, the lower the inhibition. At low concentrations, microbial growth is 5.11 times more likely to occur compared to samples in which high concentrations of phenolic extracts were used (Fig. 4). On the other hand, no effect on growth inhibition was observed when adding caffeine or chlorogenic acid alone, with contents in the range of the applied extracts, except for *S. aureus* (ATCC25923) and *E. coli* (ATCC35150), whose growth was inhibited by caffeine.

3.4. Quantification of the inhibitory effect of the extract on B. cereus

After quantification of the inhibitory effect of the extract on *B. cereus* (ATCC14579), a 3-log reduction in growth was observed after 6 h of incubation.

4. Discussion

Phenolic extracts obtained from coffee mucilage did not appear to affect the growth of Gram-negative bacteria under the conditions evaluated in this study. The action of bioactive compounds may be limited due to the complex wall and outer membrane structures in these bacteria [25]. It has been reported that low molecular weight dissociated phenolic acids can diffuse into the bacterial cytoplasm causing acidification that can cause bacterial cell death [26], this mechanism is difficult to occur through the Gram-negative wall conformation. Another explanation for the non-inhibition effect on Gram-negative microorganisms could be related to the concentration of the extract used. In previous studies inhibition was reported for concentration of 75 mg/ml of dried coffee pulp extracts (using water as solvent) [17] or 80 mg/ml of a spent coffee extract [27].

On the other hand, an inhibitory effect on the growth of Gram-positive organisms was observed, where *B. cereus* (ATCC14579) proved to be the most sensitive. Fei et al. [28] described the inhibition of the growth of this microorganism, both in a saline solution and in pasteurized milk, after the addition of a phenolic extract of olive oil. The authors reported a minimum inhibitory concentration of the extract for *B. cereus* (ATCC14579) of 0.6 mg/ml, slightly higher than that used in this study (0.5 mg/ml). These authors reported a decrease in growth of 8 logarithms after 5 h of incubation in saline and after 10 h in pasteurized milk. This is more effective than the 3-log decrease observed in our study, although similar extract concentrations were used. This difference could be due to differences in phenolic compound profiles, but also to differences in the pH of the matrices, but pH values were not reported in the research of the latter authors. In our study, the pH of 6 used, could decrease the inhibitory effect, which could be higher at pH below 4.5. In fact, when using the coffee mucilage polyphenol-rich extract without adjusting the pH (pH close to 4), after a 15 min incubation, a 6 log decrease was obtained. However, after neutralizing at pH 6, a three log decrease was achieved, which can be considered acceptable in the evaluation of the efficacy of soaps and disinfectants [29]. The drawback in this case is the long contact time (minimum 6 h) necessary to achieve this logarithmic reduction, so it may not be recommended for use in disinfectants or soaps that require shorter contact times (10-15 min). However, it would be interesting to carry out toxicity tests to consider

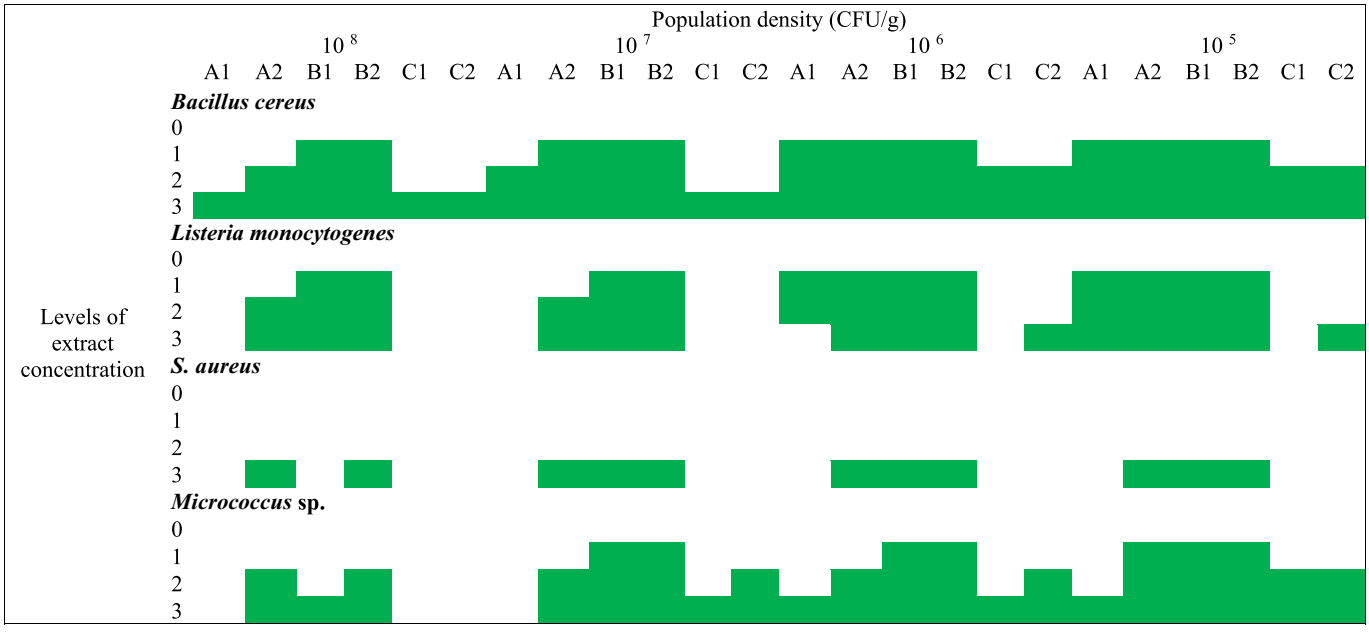

Fig. 2. Effect on growth inhibition on four potentially pathogenic bacteria determined in vitro by the microdilution on agar using extracts from different collection sites (A, B, C) and batches (1,2) with three concentration levels (1,2,3) (as assigned in Table 1). Filled cells indicate growth inhibition.

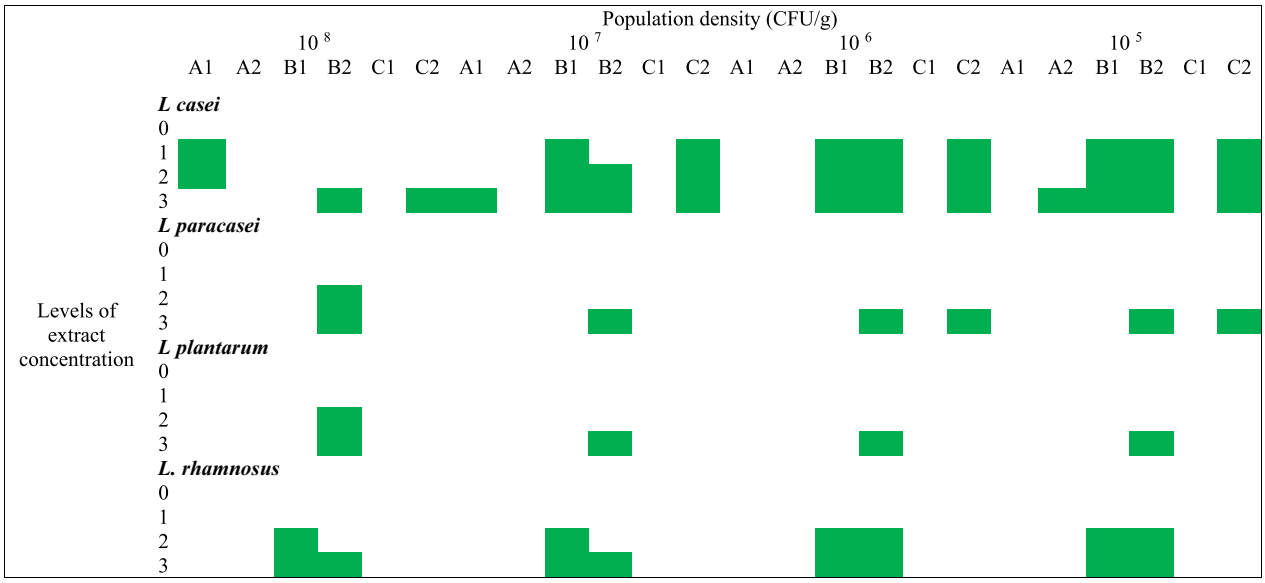

Fig. 3. Effect on growth inhibition on biota bacteria determined in vitro by the microdilution on agar using extracts from different collection sites (A, B, C) and batches (1, 2) with three concentration levels (1, 2, 3) (as assigned in Table 1). Filled cells indicate growth inhibition.

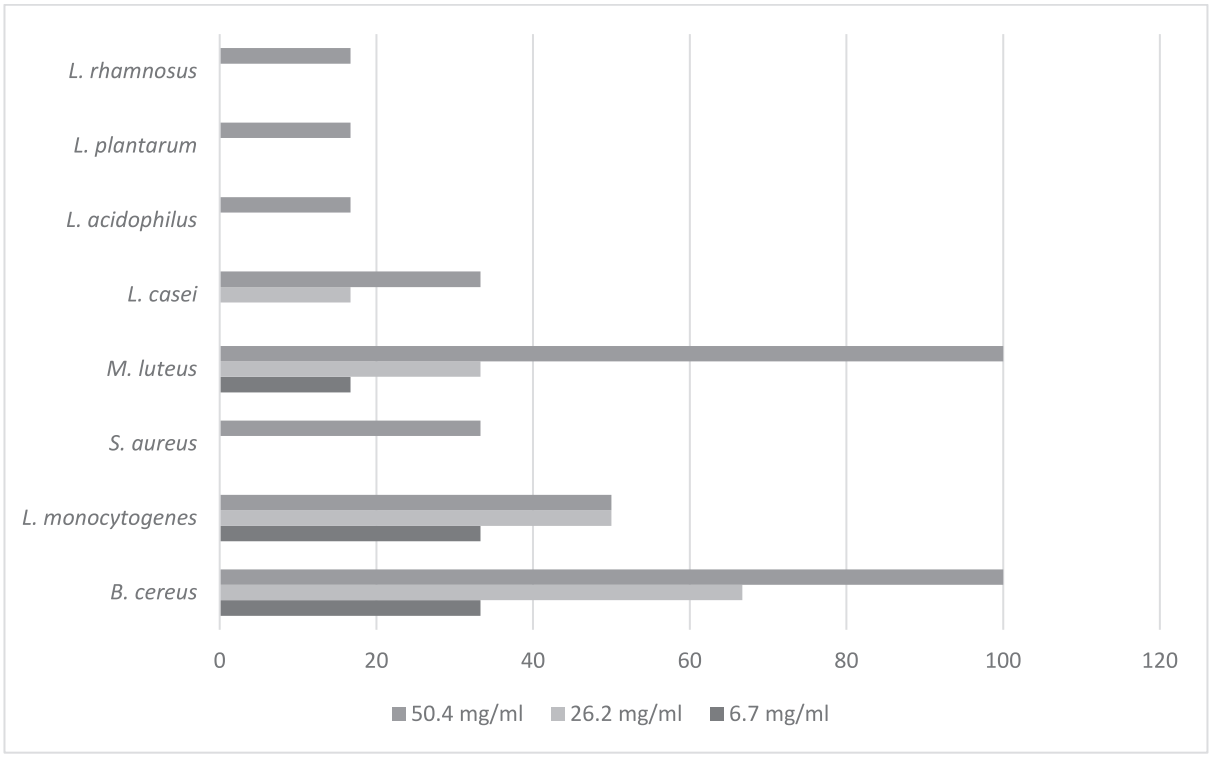

Fig. 4. Percentage of inhibition observations ($n = 24$) using different extract concentrations for selected microorganisms.

the possibility of incorporating this phenolic extract in food systems.

The polyphenol-rich extract of coffee mucilage presents significant amounts of caffeine as mentioned above, however, the data obtained ruled out the effect of this component on the growth of *B. cereus* (ATCC14579). Growth inhibition due to caffeine, in the concentration levels of the extracts, was only observed for *S. aureus* (ATCC25923) and *E. coli* (ATCC35150). Other researchers [25] compared the effect on microbial growth of normal and decaffeinated coffee extracts of different bacterial strains and observed no differences in antimicrobial capacity between the extracts. This supports our theory that the antimicrobial potential against *B. cereus* (ATCC14579) did not reside in the amount of caffeine, but in other components present in the extract. Despite this finding, it is well known that caffeine has the ability to inhibit protein synthesis in bacteria and generates DNA damage [30]. Concentrations above 200 mg/ml were previously reported to inhibit bacteria such as *E. coli* (ATCC35150) and *S. aureus* (ATCC25923) [14], which is much higher than the caffeine content found in coffee mucilage extract (3.16 ± 2.89 mg/ml in average).

The highest growth inhibition effect of the mucilage extracts was observed in *B. cereus* (ATCC14579), followed by *M. luteus* (ATCC4698),

L. monocytogenes (SLCC4013) and *S. aureus* (25923). However, *L. casei*, *L. paracasei, Lactiplantibacillus plantarum* L. and *L. rhamnosus* were not affected to the same extent. It was determined, with 95% confidence, that the growth of intestinal bacteria is less affected in the presence of the extract (at least 4.74-fold) compared to pathogenic or spoilage bacteria by a population of 10^8 CFU/ml. The inhibition effect depends not only on the concentration of the extract, but also on the initial load of microorganisms. At the Mac Farland concentration of 0.5, a lower sensitivity to the extract by lactic acid bacteria was determined, which could be due to their fermentative metabolism through which they produce various organic acids [31], allowing them to adapt to low pH environments and grow in pH ranges of 4.8–9.6 [32]. Some lactic acid bacteria are known to possess specific enzymes (β-glucuronidases, β-galactosidases and α-rhamnosidases) that allow them to metabolize phenolic compounds using them as a carbon source. Some lactobacilli, thanks to their enzymes (gallate decarboxylase), can degrade gallic acid to pyrogallol. This compound is degraded to cis-aconitate and thus enters the Krebs cycle, which allows these microorganisms to remain metabolically active [26,33,34]. This ability to regulate their internal pH independently of the external pH, together with the presence of enzymes capable of metabolizing phenolic compounds, could allow lactic acid bacteria to maintain their viability in the presence of coffee mucilage extract.

It is important to consider that the extract used has a mixture of compounds, with a predominance of caffeine and chlorogenic acid. Although extract B had the lowest chlorogenic acid content (2.67 mg/ml), a significant growth inhibition effect against *B. cereus* (ATCC14579) was observed, even better than those extracts with higher chlorogenic acid concentration. A control evaluation was performed only with chlorogenic acid, using a concentration in the range of the extracts, where no growth inhibition was observed for the studied microorganisms. During this evaluation, an oxidation process was observed, which could have affected the inhibition effect. Considering these results, it appears that chlorogenic acids are not the only compounds that could exert an antimicrobial effect. Matrix effect, minor compounds and synergistic effects may be some of the factors determining the antimicrobial effects in this study.

The growth inhibition observed for *B. cereus* (ATCC14579) seems not be due to the chlorogenic acid present in the extract. The extraction methodology used could allow the presence of other phenolic compounds that would be participating in the growth inhibition. Recently, Esquivel et al. [16] reported the presence of 16 different phenolic compounds in pulp and skin of Arabica coffee fruits. Of these 16 compounds reported by Esquivel et al. [16], nine were identified as chlorogenic acids. In our research, only two major peaks were detected, corresponding to caffeine and chlorogenic acid, but also in minor concentrations of other compounds were detected such as *p*-coumaric acid, mangiferin, isochlorogenic acid and rutin.

Esquivel et al. [16] reported also the presence of mangiferin in coffee pulp and husks of different skin color genotypes. The presence of this compound had already been reported in coffee leaves [35], but not in the fruit. Mangiferin is a hydrolyzable gallotannin present in various parts of the mango (*Mangifera indica* L.) plant and fruit. It has been described to have antiviral, anticancer, immunomodulatory, antidiabetic, antioxidant, analgesic, and hepatoprotective properties [36]; its antimicrobial activity is higher on Gram-positive bacteria and seems to be related to its function analogous to that of siderophores [36,37]. In this sense, lactic acid bacteria would have an advantage over this compound and would be resistant because their metabolism can be carried out in the absence of iron ions [38]. Further studies have to achieved to determine if the inhibitory effect of the mucilage extract was exerted by mangiferin.

Also, different collection sites can affect the chemical and physical profile of the samples, due to cultivars, processing, agricultural practices, altitude, and climate. Although differences were observed among the materials, an inhibitory effect was found with all of them for *B. cereus* (ATCC14579), which supports the results obtained.

In conclusion, coffee mucilage extracts appear to have a growth inhibitory effect on some Gram-positive bacteria, but mainly on *B. cereus* (ATCC14579). It is still necessary to determine which compounds may play a role on this effect, considering that chlorogenic acids seem not to be solely responsible for this growth inhibition. Industrial applications of these extracts are the subject of further studies, considering improved extraction methodologies to increase the inhibitory effect that can be extended to other microorganisms. For applications in the food industry, toxicological and microbiological safety issues must be studied and considered.

Credit author statement

Carolina Chaves-Ulate: Conceptualization, Methodology, Formal analysis, Data curation, Writing-original draft; **Arias-Echandi**: Conceptualization, Supervision, Writing- Reviewing and Editing; **César Rodríguez-Sánchez:** Conceptualization, Supervision, Writing-Reviewing and Editing; **Patricia Esquivel**: Conceptualization, Funding adquisition, Supervision, Writing- Reviewing and Editing.

Ethical statment

The authors state that this research does not involve human or animal studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by the Vice Presidency for Research of the University of Costa Rica (Project A8601).

References

[1] M. Patel, M.S. Ashraf, A.J. Siddiqui, S.A. Ashraf, M. Sachidanandan, M. Snoussi, M. Adnan, S. Hadi, Profiling and role of bioactive molecules from *Puntius sophore* (freshwater/brackish fish) skin mucus with its potent antibacterial, antiadhesion, and antibiofilm activities, Biomolecules. 10 (2020) 920, https://doi.org/10.3390/biom10060920.

[2] M. Adnan, M. Patel, S. Deshpande, M. Alreshidi, A.J. Siddiqui, M.N. Reddy, N. Emira, V. De Feo, Effect of *Adiantum philippense* extract on biofilm formation, adhesion with its antibacterial activities against foodborne pathogens, and characterization of bioactive metabolites: an *in vitro*-in silico approach, Front. Microbiol. 11 (2020) 823, https://doi.org/10.3389/fmicb.2020.00823.

[3] K. Mseddi, F. Alimi, E. Noumi, V.N. Veettil, S. Deshpande, M. Adnan, A. Hamdi, S. Elkahoui, A. Alghamdi, A. Kadri, M. Patel, M. Snoussi, *Thymus musilii* Velen. As a promising source of potent bioactive compounds with its pharmacological properties: *in vitro* and in silico analysis, Arab. J. Chem. 13 (2020) 6782–6801, https://doi.org/10.1016/j.arabjc.2020.06.032.

[4] T. Budiati, W. Suryaningsih, T. Nur Bethiana, Antimicrobial of tropical fruit and vegetable waste extract for food-borne pathogenic bacteria, Ital J Food Safety. 11 (2022), https://doi.org/10.4081/ijfs.2022.10510.

[5] A.M. Pisoschi, A. Pop, C. Georgescu, V. Turcuş, N.K. Olah, E. Mathe, An overview of natural antimicrobials role in food, Eur. J. Med. Chem. 143 (2018) 922–935, https://doi.org/10.1016/j.ejmech.2017.11.095.

[6] M. Saleem, M.T. Saeed, Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agent, Journal of King Saud University - Science. 32 (2020) 805–810, https://doi.org/10.1016/j.jksus.2019.02.013.

[7] L.A. Gonçalves, J.M. Lorenzo, M.A. Trindade, Fruit and agro-industrial waste extracts as potential antimicrobials in meat products: a brief review, Foods. 10 (2021) 1469, https://doi.org/10.3390/foods10071469.

[8] N.A. Sagar, S. Pareek, S. Sharma, E.M. Yahia, M.G. Lobo, Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization, Comprehensive Reviews in Food Science and Food Safety. 17 (2018) 512–531, https://doi.org/10.1111/1541-4337.12330.

[9] Organizacion Internacional del Café, Informe del mercado de café. https://www.ico.org/es/Market-Report-21-22-c.asp, 2022.

[10] P. Esquivel, V.M. Jiménez, Functional properties of coffee and coffee by-products, Food Res. Int. 46 (2012) 488–495, https://doi.org/10.1016/j.foodres.2011.05.028.
[11] M.S. Butt, M.T. Sultan, Coffee and its consumption: benefits and risks, Crit. Rev. Food Sci. Nutr. 51 (2011) 363–373, https://doi.org/10.1080/10408390903586412.
[12] A. Jiménez-Zamora, S. Pastoriza, J.A. Rufián-Henares, Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties, LWT - Food Science and Technology. 61 (2015) 12–18, https://doi.org/10.1016/j.lwt.2014.11.031.
[13] F.F. de Araújo, D. de Paulo Farias, I.A. Neri-Numa, G.M. Pastore, Polyphenols and their applications: an approach in food chemistry and innovation potential, Food Chem. 338 (2021), 127535, https://doi.org/10.1016/j.foodchem.2020.127535.
[14] F.K. Nzekoue, S. Angeloni, L. Navarini, C. Angeloni, M. Freschi, S. Hrelia, L. A. Vitali, G. Sagratini, S. Vittori, G. Caprioli, Coffee silverskin extracts: quantification of 30 bioactive compounds by a new HPLC-MS/MS method and evaluation of their antioxidant and antibacterial activities, Food Res. Int. 133 (2020), 109128, https://doi.org/10.1016/j.foodres.2020.109128.
[15] J.R. Ramirez-Martinez, Phenolic compounds in coffee pulp: quantitative determination by HPLC, J. Sci. Food Agric. 43 (1988) 135–144, https://doi.org/10.1002/jsfa.2740430204.
[16] P. Esquivel, M. Viñas, C.B. Steingass, M. Gruschwitz, E. Guevara, R. Carle, R. M. Schweiggert, V.M. Jiménez, Coffee (*Coffea arabica* L.) by-products as a source of carotenoids and phenolic compounds—evaluation of varieties with different Peel color, Front. Sustain. Food Syst. 4 (2020), 590597, https://doi.org/10.3389/fsufs.2020.590597.
[17] A. Duangjai, N. Suphrom, J. Wungrath, A. Ontawong, N. Nuengchamnong, A. Yosboonruang, Comparison of antioxidant, antimicrobial activities and chemical profiles of three coffee (*Coffea arabica* L.) pulp aqueous extracts, integrative medicine, Research. 5 (2016) 324–331, https://doi.org/10.1016/j.imr.2016.09.001.
[18] R. Ito, A study on the change of enantiomeric purity of catechins in green tea infusion, Food Chem. 83 (2003) 563–568, https://doi.org/10.1016/S0308-8146(03)00154-7.
[19] C.-L. Chan, R.-Y. Gan, N.P. Shah, H. Corke, Polyphenols from selected dietary spices and medicinal herbs differentially affect common food-borne pathogenic bacteria and lactic acid bacteria, Food Control 92 (2018) 437–443, https://doi.org/10.1016/j.foodcont.2018.05.032.
[20] E. Mingo, J.M. Silván, A.J. Martinez-Rodriguez, Selective antibacterial effect on Campylobacter of a winemaking waste extract (WWE) as a source of active phenolic compounds, LWT Food Sci. Technol. 68 (2016) 418–424, https://doi.org/10.1016/j.lwt.2015.12.052.
[21] D. Kammerer, A. Claus, R. Carle, A. Schieber, Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS, J. Agric. Food Chem. 52 (2004) 4360–4367, https://doi.org/10.1021/jf049613b.
[22] M.N. Clifford, K.L. Johnston, S. Knight, N. Kuhnert, Hierarchical scheme for LC-MSn identification of chlorogenic acids, J. Agric. Food Chem. 51 (2003) 2900–2911, https://doi.org/10.1021/jf026187q.
[23] P. Thomas, A.C. Sekhar, R. Upreti, M.M. Mujawar, S.S. Pasha, Optimization of single plate-serial dilution spotting (SP-SDS) with sample anchoring as an assured method for bacterial and yeast cfu enumeration and single colony isolation from diverse samples, Biotechnology Reports. 8 (2015) 45–55, https://doi.org/10.1016/j.btre.2015.08.003.
[24] M. Iñiguez-Moreno, M.G. Avila-Novoa, E. Iñiguez-Moreno, P.J. Guerrero-Medina, M. Gutiérrez-Lomelí, Antimicrobial activity of disinfectants commonly used in the food industry in Mexico, Journal of Global Antimicrobial Resistance. 10 (2017) 143–147, https://doi.org/10.1016/j.jgar.2017.05.013.
[25] G. Runti, S. Pacor, S. Colomban, R. Gennaro, L. Navarini, M. Scocchi, Arabica coffee extract shows antibacterial activity against *Staphylococcus epidermidis* and *enterococcus faecalis* and low toxicity towards a human cell line, LWT Food Sci. Technol. 62 (2015) 108–114, https://doi.org/10.1016/j.lwt.2014.12.039.
[26] A.F. Sánchez-Maldonado, A. Schieber, M.G. Gänzle, Structure–function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria, J. Appl. Microbiol. 111 (2011) 1176–1184, https://doi.org/10.1111/j.1365-2672.2011.05141.x.
[27] C. Monente, J. Bravo, A.I. Vitas, L. Arbillaga, M.P. De Peña, C. Cid, Coffee and spent coffee extracts protect against cell mutagens and inhibit growth of food-borne pathogen microorganisms, J. Funct. Foods 12 (2015) 365–374, https://doi.org/10.1016/j.jff.2014.12.006.
[28] P. Fei, Y. Xu, S. Zhao, S. Gong, L. Guo, Olive oil polyphenol extract inhibits vegetative cells of *Bacillus cereus* isolated from raw milk, J. Dairy Sci. 102 (2019) 3894–3902, https://doi.org/10.3168/jds.2018-15184.
[29] P. González, Guía General para la Realización y Presentación de Ensayos de Eficacia de Productos Desinfectantes y Sanitizantes de Uso Sanitario y Doméstico, 2016.
[30] S. Ibrahim, M. Salameh, S. Phetsomphou, H. Yang, C. Seo, Application of caffeine, 1,3,7-trimethylxanthine, to control *Escherichia coli* O157:H7, Food Chem. 99 (2006) 645–650, https://doi.org/10.1016/j.foodchem.2005.08.026.
[31] A. Endo, L. Dicks, Physiology of the LAB, in: W. Holzapfel, B. Wood (Eds.), Lactic Acid Bacteria: Biodiversity and Taxonomy, Wiley Blackwell, 2014, pp. 13–22.
[32] J. Jay, M. Loessner, D. Golden, Modern Food Microbiology, Springer, Boston, 2005.
[33] F.M. Campos, J.A. Couto, A.R. Figueiredo, I.V. Tóth, A.O.S.S. Rangel, T.A. Hogg, Cell membrane damage induced by phenolic acids on wine lactic acid bacteria, Int. J. Food Microbiol. 135 (2009) 144–151, https://doi.org/10.1016/j.ijfoodmicro.2009.07.031.
[34] R. Pacheco-Ordaz, A. Wall-Medrano, M.G. Goñi, G. Ramos-Clamont-Montfort, J. F. Ayala-Zavala, G.A. González-Aguilar, Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria, Lett. Appl. Microbiol. 66 (2018) 25–31, https://doi.org/10.1111/lam.12814.
[35] P. Jyotshna, K. Khare, Shanker, Mangiferin: a review of sources and interventions for biological activities, BioFactors. 42 (2016) 504–514, https://doi.org/10.1002/biof.1308.
[36] M. Imran, M.S. Arshad, M.S. Butt, J.-H. Kwon, M.U. Arshad, M.T. Sultan, Mangiferin: a natural miracle bioactive compound against lifestyle related disorders, Lipids Health Dis. 16 (2017) 84, https://doi.org/10.1186/s12944-017-0449-y.
[37] C. Engels, D. Gräter, P. Esquivel, V.M. Jiménez, M.G. Gänzle, A. Schieber, Characterization of phenolic compounds in jocote (*Spondias purpurea* L.) peels by ultra high-performance liquid chromatography/electrospray ionization mass spectrometry, Food Res. Int. 46 (2012) 557–562, https://doi.org/10.1016/j.foodres.2011.04.003.
[38] B. Bruyneel, M. Vande Woestyne, W. Verstraete, Lactic acid bacteria: Micro-organisms able to grow in the absence of available iron and copper, Biotechnol. Lett. 11 (1989) 401–406, https://doi.org/10.1007/BF01089472.