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In Vitro Assays to Evaluate the Effects of Mango By-Product Polyphenolic Extracts Against Bacterial Species Associated with Food Spoilage and Human Diseases and the Relationship with Their Genotypes

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Featured Application: The demonstrated antibacterial activity of mango by-products against acetic acid bacteria and staphylococci suggests their potential use as natural preservatives in agroindustrial processes. Specifically, these extracts may be applied to improve microbial control during winemaking and fermented meat production, reducing spoilage and enhancing product safety. Additionally, their activity against human pathogens opens avenues for the development of biotechnological applications in the design of functional ingredients or natural antimicrobial formulations. Importantly, this approach provides a sustainable and value-added use for postharvest residues from mango processing, contributing to waste reduction and circular economy strategies in the agri-food sector. Furthermore, the microdilution method optimized for acetic acid bacteria also offers a valuable tool for future screening of plant-derived antimicrobials.

Abstract: *Mangifera indica* L. by-products obtained by three extraction methods from three cultivars (Keitt, Sensation and Gomera-3) were tested for their antibacterial properties against 20 bacterial species. These species were selected based on their relevance to wine-making processes (*Acetobacter*, *Gluconobacter* and *Gluconacetobacter*), fermented meat products (*Staphylococcus*) and human diseases (*Pseudomonas*, *Escherichia*, *Shigella* and *Klebsiella*). All mango by-product extracts showed antimicrobial activity in agar diffusion and broth microdilution experiments. However, differences in antimicrobial activity against acetic acid bacteria were detected between the peel extracts obtained from the two extraction processes. Furthermore, a wide range of minimum inhibitory concentration (MIC) data were found; *Staphylococcus* spp. (10 species) showed MICs between 1.0–240 mgGAE/mL and *Acetobacter* spp. (4 species) showed MICs between 1.7 and 200 mgGAE/mL. The most sensitive bacteria belonged to the staphylococcal species (MIC: 1 mgGAE/mL) and the most resistant was *Gluconacetobacter saccharivorans* (MIC > 400 mgGAE/mL). In general, there was no significant correlation between the phenolic compounds identified and the MIC values. The minimum bactericidal concentration (MBC) revealed that the mango extracts had a bacteriostatic effect. A simple and reliable method for the determination of MIC and MBC in microdilution assays with acetic acid bacteria was described. These results highlight the antibacterial properties of mango by-products against species associated with food spoilage microorganisms and human diseases.

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Keywords: *Mangifera indica*; antibacterial; acetic acid bacteria; *Acetobacter*; *Staphylococcus*; fermented cured sausages

1. Introduction

A variety of microorganisms play an important role in the production of many foods and beverages. In many cases, these microorganisms can improve the quality and organoleptic properties of the final product. However, in other cases, some microorganisms are frequently associated with the poisoning and spoilage of foods. To overcome these problems, classical chemical preservatives have been routinely added to foods and beverages. For example, during winemaking, sulfur dioxide (SO_2) is commonly used to reduce or inhibit the growth of spoilage microorganisms, particularly acetic acid bacteria (AAB) and yeast species, which can alter the wine [1,2]. Nevertheless, the use of this additive is strictly regulated due to its potential to cause organoleptic alterations in the final product and, more importantly, because of the associated health risks for consumers [3,4]. In this context, the use of plant-derived excipients is increasing and opening up new ways to solve current drug delivery challenges in the food and pharmaceutical industries [3,5]. These biocompatible and environmentally friendly alternatives have the potential to reduce microbial spoilage and enhance food safety for consumers. Moreover, the use of natural antioxidants and antimicrobials is highly valued by health-conscious consumers and also offers additional benefits to the bioeconomy by adding value to agricultural by-products [6,7].

Despite significant progress in recent years, the search for natural antimicrobial compounds remains a challenging area of research; fruit-containing polyphenols represent a particularly promising class of compounds. In this context, numerous studies conducted over many years have demonstrated the antioxidant, antibacterial and antifungal properties of plant extracts, including polyphenol-rich mango (*Mangifera indica*) by-products [6–8]. Several reviews provide a detailed analysis of their antioxidant and antimicrobial properties, as well as their possible modes of action [9,10]. Notably, plant extracts have recently been evaluated for the first time in antibacterial films for packaging applications, highlighting their potential in food preservation strategies [11]. Building on this foundation, recent studies have further explored this topic. Sarker et al. [12] investigated the antioxidant and antibacterial activity of mango peel extracts using ultrasound-assisted extraction (UAE) to enhance polyphenol yield, demonstrating antimicrobial activity against *Bacillus cereus*, *Geobacillus stearothermophilus* and *Escherichia coli*. Similarly, Kuçuk al. [13] observed strong antibacterial effects against both Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and identified several phenolic acids, flavonoids and mangiferin as key contributors to the observed antibacterial effects.

Although numerous studies have investigated the antimicrobial properties of mango extracts, most have primarily focused on evaluating their antibacterial activity against a limited number of bacterial species, mainly associated with human health or food safety. In contrast, food spoilage microorganisms have received considerably less attention. Furthermore, while many studies provide a detailed characterization of the chemical compounds present in mango extracts, only a few have explored the correlation between antimicrobial activity and the different detected or identified chemical compounds. In the present work, we sought to address this knowledge gap by investigating the antimicrobial activity of mango by-products against 20 bacterial species, including the following: (i) six AAB species (13 strains) related to the winemaking processes; (ii) ten *Staphylococcus* species related to fermented meat products, food safety or human and animal diseases; and (iii) four

species commonly associated with human infections. The in vitro antibacterial activity was evaluated using agar diffusion and broth microdilution assays, and parameters such as the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. We also analyzed the correlation between the antibacterial activity of the extracts and their polyphenolic compounds (flavonoids, gallates, gallotannins, ellagic acid, etc.) and explored the potential relationship between bacterial genotype and susceptibility. Finally, we developed a reliable and straightforward method for determining MIC and MBC values in microdilution assays with AAB.

2. Materials and Methods

2.1. Plant Collection and Preparation of Mango By-Product Extracts

Three mango cultivars (Keitt, Sensation and Gomera-3) were collected from the fields of the mango collection (UTM 28R 364451.00 m E 3133070.93 m N; 114 m.a.s.l.) of the Instituto Canario de Investigaciones Agrarias (Tenerife, Canary Islands, Spain) at the physiological stage of ripeness (mature-green) and allowed to ripen (full-ripeness or consumption stage) at 18 °C and 80–90% relative humidity. The plant material was identified, processed and characterized. The extracts were obtained using optimized methods to achieve the maximum extraction of polyphenols and a high antioxidant capacity, as described in previous works [14–16]. Briefly, the peel and seed were manually separated (31 ± 1% and 4.2 ± 2.1% of the total fruit weight, respectively), cut into small pieces (0.5 × 1 cm) and freeze-dried at −40 °C in a vacuum (50 mPa) for 5 days. The dried mango peels and seeds were then ground to a fine powder (particle size between 355 and 500 µm) and stored at −20 °C until the extractions were performed. Microwave-assisted extraction (MAE) (ETHOS 1, Milestone SRL, Sorisole, Italy) was used to obtain the extracts. Two types of peel extractions were performed: ethanol–water (1:1, *v*:*v*) at 75 °C and a weight-to-solvent volume of 1:50 (*w*:*v*) (extraction A) or 1:10 (*w*:*v*) (extraction B). On the other hand, the mango seed extraction was performed with acetone–water (1:1, *v*:*v*) at 50 °C and a weight-to-solvent volume of 1:30 (*w*:*v*). The resulting nine by-product extracts included three extracts (peel: extraction A and B and seed) for each of the three mango cultivars (Keitt, Sensation and Gomera-3) [14–16].

The compounds present in the nine by-product extracts were identified by the following acronyms: KPA (Keitt Peel, extraction A); KPB (Keitt Peel, extraction B); SPA (Sensation Peel, extraction A); SPB (Sensation Peel, extraction B); GPA (Gomera Peel, extraction A); GPB (Gomera Peel, extraction B); KSC (Keitt Seed, extraction C); SSC (Sensation Seed, extraction C) and GSC (Gomera Seed, extraction C).

The characterization of the polyphenolic composition of the mango by-product extracts was previously conducted [16].

2.2. Bacterial Species and Culture Conditions

The antibacterial activity of the extracts was tested against 20 bacterial species: (i) six AAB species: *Acetobacter cerevisiae*, *A. malorum*, *A. pasteurianus*, *A. tropicalis*, *Gluconacetobacter saccharivorans* and *Gluconobacter japonicus*; (ii) ten *Staphylococcus* species: *Staphylococcus arlettae*, *S. aureus*, *S. carnosus* subsp. *carnosus*, *S. chromogenes*, *S. cohnii* subsp. *cohnii*, *S. equorum* subsp. *equorum*, *S. gallinarum*, *S. lugdunensis*, *S. sciuri* subsp. *sciuri* and *S. xylosus*; and (iii) four species frequently associated with human infections: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Shigella dysenteriae*.

The AAB strains were obtained from grapes from the Canary Islands (Spain); identified by the phylogenetic analysis of the 16S-23S rRNA gene ITS sequence and typed by the analysis of highly conserved repetitive DNA elements [$(GTG)_5$-PCR and Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR [17]]. Staphylococcal strains were obtained

from the Agricultural Research Service Culture Collection (NRRL), except for the clinically isolated *S. aureus* 11923-76 and *S. lugdunensis* 99705-65 obtained from the microbiology service of the Nuestra Señora de Candelaria University Hospital (Santa Cruz de Tenerife, Spain). *Escherichia coli*, *K. pneumonia*, *P. aeruginosa* and *S. dysenteriae* strains were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). All the strains were maintained in 30% *w*/*v* glycerol at −80 °C until use. For experiments, all the bacteria strains (except the AAB) were grown separately on Mueller Hinton (MH, Condalab, Madrid, Spain) agar (pH 7.3) at 37 °C for 24 h, whereas the AAB strains were grown in GYC (5% glucose, 1% yeast extract, 1.5% $CaCO_3$, 1.5% agar *w*/*v*, pH 6.8) medium at 28 °C under aerobic conditions for 48 h.

2.3. Antibacterial Screening

The antibacterial activity of the mango peel and seed extracts was determined using two different bioassays: agar diffusion and microdilution.

2.3.1. Agar Diffusion Assay

The agar diffusion assay was used only in a preliminary and exploratory way to determine the antibacterial potential of the extracts. Each strain (except the AAB) was grown at an optical density, at a wavelength of 600 nm (OD600), of 1 in MH broth (pH 7.3), with agitation (250 rpm) at 37 °C. The cell suspension was mixed with melted warm MH 1% agar medium at a final OD600 of 0.033 and poured into sterile Petri plates. A similar procedure was used for AAB; YPD broth (0.5% yeast extract, 0.3% peptone, 2% dextrose, pH 6.8) with agitation at 28 °C was used to obtain the cell suspension, and melted warm GYC 1% agar was poured into the Petri plates to create the final mixture. Finally, sixty microliters of each extract was added to 8 mm diameter wells dug in MH 1% agar or GYC 1% agar medium plates. After 2 h at 4 °C, the plates were incubated at 37 °C (MH agar) or 28 °C (GYC agar) for 24 h, and then the inhibition zones (mm) were measured.

2.3.2. Microdilution Antibacterial Assay

The MICs of the extracts were determined by a serial dilution method in 96-well sterile microtiter plates. The antimicrobial activity bioassays were optimized according to the requirements and characteristics of each bacterial species. All strains (except the AAB) were grown up to the exponential phase (OD600 = 0.5) in MH broth with agitation at 37 °C. After that, the cells were centrifuged (2500 rcf for 10 min.) and resuspended in sterile MH broth concentrate (2.5X) at an OD600 of 0.1 (*K. pneumoniae*, *S. dysenteriae* and *Staphylococcus* species) or 0.25 (*E. coli* and *P. aeruginosa*) and mixed with the different extracts at a concentration of between 1.0 and 240 mg gallic acid equivalents per mL (GAE/mL). Briefly, 44 µL of cell suspension (OD600 = 0.1 or 0.25) and 66 µL of the extract were added to each well. The final inoculum contained approximately 2×10^5 CFU/mL in a total volume of 110 µL/well of MH (1X). The microtiter plates were mixed and incubated under aerobic conditions at 37 °C for 24 h and then examined using a binocular microscope and a spectrophotometer.

The AAB strains were grown in YPD broth (up to OD600 = 1.0) with agitation at 28 °C. The cells were concentrated by centrifugation at 2500 rcf for 10 min. and resuspended in GYC broth concentrate (2.5X) at an OD600 of 1.25 and mixed with the extracts at a concentration of between 1.7 and 400 mg GAE/mL. Briefly, 44 µL of the cell suspension in GYC and 66 µL of the extract were added in each well. The microtiter plates were mixed and incubated under aerobic conditions at 28 °C for 24 h, and the analysis was performed using a bromo cresol purple (BCP, AppliChem, ITW Reagents, Castellar del Vallès, Spain) alkaline solution (0.1% *w*/*v* BCP + potassium hydroxide 0.05 M). For that, 8 µL/well of

the BCP solution was added and after one minute the color of the reaction was observed [positive reaction (acid) = yellow, negative reaction (alkaline) = violet].

Positive controls (containing bacteria without extracts) and negative controls (containing extracts without bacteria) were included in each microplate of each experiment. The MIC was determined as the lowest concentration of extracts that inhibited the visual growth of the bacterial culture on the microplate. The spectrophotometric analysis (except the AAB) was determined using a microdilution automatic reader at a wavelength of 600 nm, and each test sample was considered negative when the bacteria growth displayed 90% inhibition compared to the positive control. In addition, the MBC was determined via the serial subcultivation of 10 µL aliquots of each well in microtiter plates containing 90 µL of fresh MH broth per well and further incubation for 24 to 48 h at 37 °C. The lowest concentration with no visible growth (at the binocular microscope) and the lowest determined by spectrophotometric analysis was defined as the MBC. In the case of AAB, the MBC was determined with fresh GYC broth at 28 °C and the evaluation was performed with BCP alkaline solution. Each sample was tested in triplicate in separate experiments. Figure 1 illustrates the whole procedure.

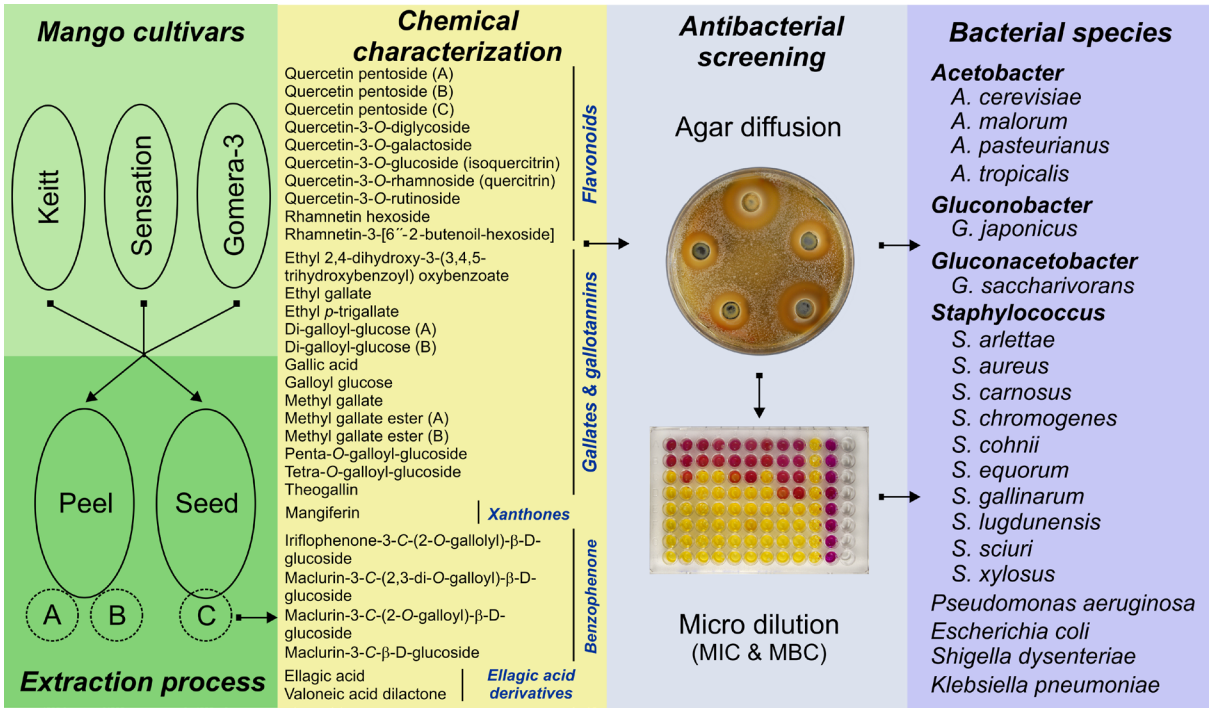


Figure 1. Graphical illustration of the procedure to obtain mango extracts and to evaluate the in vitro antimicrobial potential against 20 bacterial species of food and human health relevance. Minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined. Petri dish and microtiter plates correspond to the tests performed with acetic acid bacteria.

2.4. Statistical Analysis

All results were expressed as mean ± SD (n = 3). Spearman's correlation analysis was used to determine the correlation between the antibacterial capacity and phenolic compounds of the extracts. Differences were considered significant at $p < 0.05$. All statistical analyses were performed with the Statgraphics Centurion 18 V18.1.14 software.

3. Results

3.1. Bioactive Compound in Mango By-Product Extracts

A total of 30 phenolic compounds were identified in a previous work [13] from nine mango by-product extracts obtained from the peels and seeds of the three cultivars (Keitt, Sensation and Gomera-3) (Figure 2). Among them, five main groups of bioactive compounds were found: flavonoids (10 compounds), xanhones (1), gallates and gallotannins (10), ellagic acid and derivatives (2) and benzophenone derivatives (7).

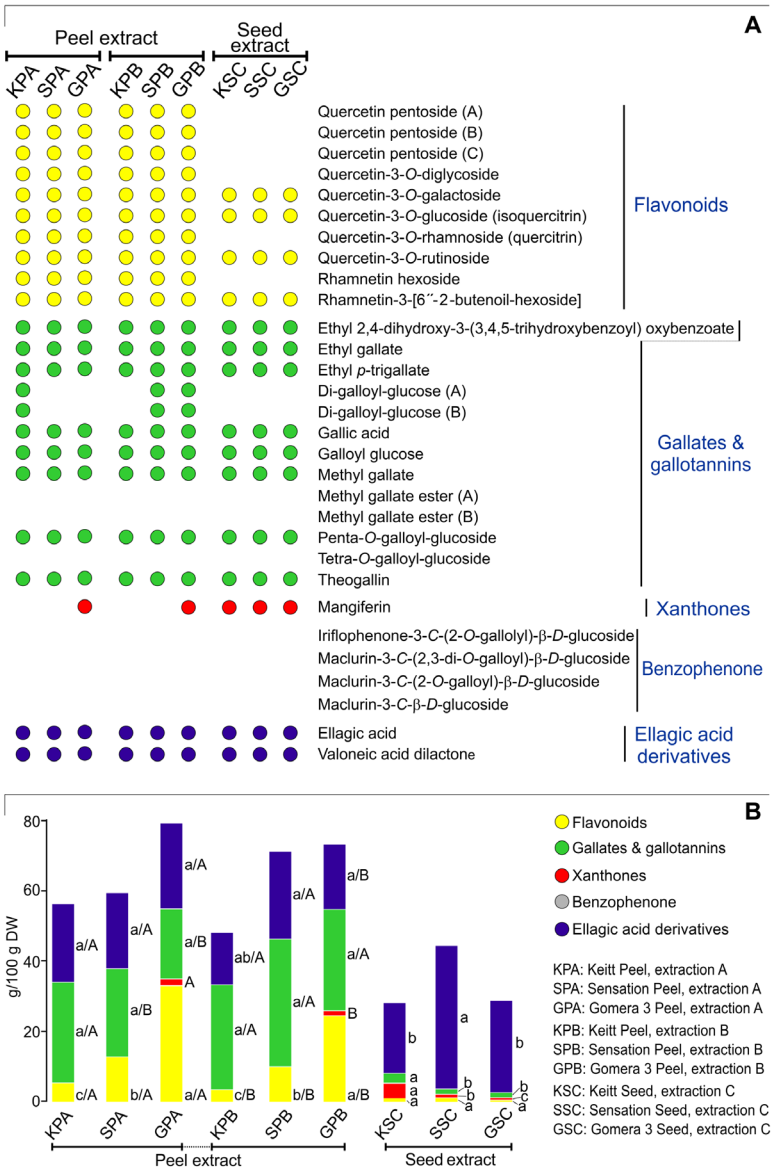


Figure 2. Phenolic compounds identified and quantified in mango peel and seed extracts from three mango cultivars produced in the Canary Islands. (**A**) Bioactive compounds identified in peel and seed extracts, analyzed by HPLC-ESI-QTOF-MS. Each color represents a specific class of compounds. Colored circles indicate the presence of the corresponding compound in each extract, while the absence of a circle indicates only trace amounts detected. (**B**) Quantitative content of principal phenolic compound classes (g/100 g dry weight) in peel and seed extracts, determined by HPLC. Values are expressed as means (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$) among cultivars for the same extraction method, while uppercase letters indicate significant differences between extraction methods for the same cultivar.

Flavonoid concentrations (g/100 g dw) varied significantly ($p < 0.05$) among mango cultivars and extraction methods. Extracts from process A had, on average, 1.4 times more flavonoids than those from process B. Among the cultivars, Gomera-3 had the highest flavonoid content, with 2.5 and 6.5 times more than Sensation and Keitt, respectively. Flavonoid levels in seeds were significantly lower than in peel, ranging from 4.5 to 40 times less, regardless of the extraction method. Only four of the ten flavonoids identified in the peel were present in the seeds.

Gallates and gallotannins in mango peel showed no significant differences ($p < 0.05$) among cultivars within the same extraction method. However, Sensation and Gomera-3 peels had higher yields with extraction process B (31.5 $\pm$ 1.75 g/100 g dw) than with process A (25.0 $\pm$ 4.06 g/100 g dw). Seed extracts had significantly lower yields—on average, 13.8 times less than peel extracts—though the number of identified compounds was comparable.

In extract A, ellagic acid derivatives showed no significant differences among cultivars. However, in extract B, the Keitt cultivar had significantly lower levels ($p < 0.05$) than Sensation and Gomera-3 (1.7 and 1.3 times lower, respectively). In seed extracts, Sensation showed the highest ellagic acid concentration in extract B (24.9 $\pm$ 4.9 g/100 g dw), significantly higher than the other cultivars (16.7 $\pm$ 3.4 g/100 g dw), and 1.65 times higher than in its peel. The highest xanthone concentration was found in Keitt seed extracts (4.15 $\pm$ 0.18 g/100 g dw), followed by Gomera-3 peel extracts A and B. Mangiferin was the only xanthone identified and quantified.

3.2. Antibacterial Activity of Mango By-Products

3.2.1. Acetic Acid Bacteria

A preliminary and exploratory screening of the antibacterial activity of mango by-products against three AAB species (*A. malorum*, *A. tropicalis* and *G. japonicus*) was performed using agar diffusion assay. All extracts (from peel and seed) had an inhibition effect against the tested bacteria. Likewise, the extraction process and the cultivar affected the bioactive compounds extracted and therefore their antibacterial activity. Peel extracts obtained by process B showed higher antibacterial activity in the agar diffusion assay (1.5 times on average) than extracts obtained by process A. On the other hand, the peel and seed extracts from Gomera-3 and Sensation cultivars showed higher antibacterial activity than extracts obtained from the Keitt cultivar.

For a quantitative analysis of the antibacterial activity, the MIC and the MBC were determined against six species of AAB (thirteen genotypes typified by ERIC-PCR and $(GTG)_5$-PCR. In this case, all the extracts were diluted to a final concentration between 1.7 and 400 mg GAE/mL. The serial dilution assays confirmed the antibacterial properties of the extracts, with the exception of *G. saccharivorans* that was resistant at the maximum concentration tested (400 mg GAE/mL). A wide range of MIC and MBC values was observed among the different bacterial species, with MICs from 1.7 mg GAE/mL in *A. cerevisiae* to 200 mg GAE/mL in *A. tropicalis* and *G. japonicus*, and MBCs from 17 mg GAE/mL to 400 mg GAE/mL in the same species mentioned above (Table 1).

Taking into account the MIC values in the peel extracts, two arbitrary groups can be defined: Group I, species with MICs $\leq$ 50 mg GAE/mL (*A. cerevisiae*, *A. pasteurianus* and *A. malorum*), and Group II, species with MICs $\geq$ 50 mg GAE/mL (*A. tropicalis*, *G. japonicus* and *G. saccharivorans*) (Table 1). In no case extracts B showed higher MICs than extracts A. Peel extracts obtained by extraction A showed higher MIC values than extracts obtained by extraction B in each bacterial species tested in at least in one of the cultivars. In the Gomera-3 cultivar, MICs from extracts A were higher than extracts B in 66.6% of the genotypes (Ac2, Ac3, Ac5, Am17, Am25, Am26, Ap16 and Gj2), while in the Keitt cultivar,

MICs from extracts A were higher than extracts B in 58.3% of the genotypes (Ac2, Ac3, At1, At2, Gj1, Gj2 and Gj3). In the extracts obtained by process A, *A. cerevisiae* and *A. malorum* showed no differences between cultivars; however, *A. pasteurianus* showed a lower MIC value with the extract obtained from cultivar Keitt, and *A. tropicalis* with those from Gomera-3. Elsewhere, the highest MIC values in *G. japonicus* were observed in the Keitt cultivar. In the case of extracts obtained by process B, the Sensation cultivar showed higher MICs than the other cultivars with *A. cerevisiae* (Ac3), *A. pasteurianus* (Ap16) and *A. tropicalis* (At1, At2) (Table 1), while the Gomera-3 cultivar showed the highest MICs with *G. japonicus* (Gj1, Gj3) and the lowest MICs with *A. cerevisiae* (Ac5), *A. pasteurianus* (Ap16) and *A. malorum* (Am17, Am25, Am26).

Table 1. The minimum inhibitory and bactericidal concentrations (MICs and MBCs) of mango peel and seed extracts against different acetic acid bacteria species.

	Acetic Acid Bacteria		**Peel Extracts (mg GAE/mL)**						**Seed Extracts (mg GAE/mL)**		
			GPA	**SPA**	**KPA**	**GPB**	**SPB**	**KPB**	**GSC**	**SSC**	**KSC**
Group I	*Acetobacter A. cerevisiae* (Ac3-6A2)	MIC	8.3	8.3	8.3	1.7	8.3	1.7	8.3	17	17
		MBC	25	25	25	17	25	17	50	50	50
	A. cerevisiae (Ac5-T5)	MIC	8.3	8.3	8.3	1.7	8.3	8.3	17	17	17
		MBC	25	25	25	17	25	25	50	50	50
	A. cerevisiae (Ac2-6A1)	MIC	8.3	8.3	8.3	1.7	1.7	1.7	8.3	25	17
		MBC	25	25	25	17	25	17	25	50	50
	A. pasteurianus (Ap16-Lz75)	MIC	50	50	25	8.3	50	25	17	8.3	8.3
		MBC	100	100	100	50	100	100	50	50	50
	A. malorum (Am17-T33)	MIC	50	50	50	25	50	50	25	25	100
		MBC	100	100	100	100	100	100	100	100	200
	A. malorum (Am25-P21)	MIC	50	50	50	25	50	50	25	25	100
		MBC	100	100	100	100	100	100	100	100	200
	A. malorum (Am26-Lz67)	MIC	50	50	50	25	50	50	25	25	100
		MBC	100	100	100	100	100	100	100	50	200
Group II	*A. tropicalis* (At1-T191)	MIC	100	200	200	100	200	100	50	50	200
		MBC	200	400	400	200	400	200	100	100	400
	A. tropicalis (At2-T59)	MIC	100	200	200	100	200	100	50	50	200
		MBC	200	400	400	200	400	200	100	100	400
	Gluconobacter G. japonicas (Gj1-P37)	MIC	100	50	200	100	50	50	50	50	50
		MBC	200	100	400	200	100	100	100	100	100
	G. japonicas (Gj3-Lz59)	MIC	100	50	200	100	50	50	50	50	50
		MBC	200	100	400	200	100	100	100	100	100
	G. japonicas (Gj2-P92)	MIC	100	100	200	50	50	50	50	50	50
		MBC	200	200	400	200	100	100	100	200	100
	Gluconacetobacter G. saccharivorans (Gs1-T80)	MIC	R *	R	R	R	R	R	R	R	R
		MBC	R *	R	R	R	R	R	R	R	R

GPA, Gomera-3 Extraction A; SPA, Sensation Extraction A; KPA, Keitt Extraction A; GPB, Gomera-3 Extraction B; SPB, Sensation Extraction B; KPB, Keitt Extraction B; GSC, Gomera-3 Extraction C; SSC, Sensation Extraction C; KSC, Keitt Extraction C. * Resistant up to 400 mg gallic acid equivalents per mL (GAE/mL) (highest dose tested).

In seed extracts, the results were variable, being able to highlight the greater MIC values in *A. malorum* and *A. tropicalis* with the Keitt cultivar, and the equality of the results among cultivars in the *G. japonicus* assays (Table 1). In addition, it is important to note the differences between the MIC data of the peel and seed extracts on the different bacterial species and cultivars. For example, in *A. cerevisiae*, the MICs of the seed extracts of the Sensation and Keitt cultivars showed higher values than in the peel extracts; however, in *A. pasteurianus* (Ap16), the relationship was the opposite (MICs of peel extracts were higher than seed extracts). Likewise, in the Sensation cultivar, the MICs of the seed extracts were lower than the peel extracts.

The MBC assay was performed to confirm cell death in the MIC test. Under the tested concentrations, most of the AAB species displayed differences between the MIC and MBC values, which indicated that the mango extracts had a bacteriostatic effect. The MBCs ranged between 17 and 400 mg GAE/mL. In general terms, the differences between bacterial species or genotypes and between cultivars were similar to those described in the case of MICs; Group I included species with MBCs $\leq$ 100 mg GAE/mL, and Group II species with MBCs $\geq$ 100 mg GAE/mL (Table 1). Likewise, peel extracts obtained by process A showed higher MBC values than extracts obtained by process B in each bacterial genotype tested in at least in one of the cultivars.

Finally, the MIC values were correlated with the polyphenolic content of the extracts to examine if any of the phenolic compounds were associated with the antibacterial activity. As shown in Table 2, the statistical correlation analysis showed that the phenolic compounds quantified in our study had no significant correlation with the MICs of the AAB tested, except *A. cerevisiae* (Ac5). In this case, a significant ($p < 0.05$) negative correlation was observed between MIC values and the concentration (g/100 g dw) of flavonoids (r = −0.8385), gallates and gallotannins (r = −0.7267) and total phenolic compounds (r = −0.8385) (Table 2).

Table 2. Spearman correlations between the phenolic compounds and the minimum inhibitory concentration (MIC) of each bacterial species tested.

Species	Isolate Code	Flavonoids	Xanthones	Gallates Gallotannins	Ellagic Acid Derivatives	Total Phenol Compounds
Acetobacter						
A. cerevisiae	Ac3-6A2	−0.4518	0.3708	−0.5809	0.5809	−0.5164
A. cerevisiae	Ac5-T5	−0.8385 *	0.2725	−0.7267 *	0.5404	−0.8385 *
A. cerevisiae	Ac2-6A1	−0.4433	0.4445	−0.7714 *	0.4699	−0.5497
A. pasteurianus	Ap16-Lz75	0.5285	−0.5339	0.4766	0.0693	0.5632
A. malorum	Am17-T33	0.0559	0	0.3354	−0.4099	−0.0745
A. malorum	Am25-P21	0.0559	0	0.3354	−0.4099	−0.0745
A. malorum	Am26-Lz67	0.0559	0	0.3354	−0.4099	−0.0745
A. tropicalis	At1-T191	0.2673	−0.2745	0.5612	−0.3742	0.1604
A. tropicalis	At2-T59	0.2673	−0.2745	0.5612	−0.3742	0.1604

Table 2. *Cont.*

Species	Isolate Code	Flavonoids	Xanthones	Gallates Gallotannins	Ellagic Acid Derivatives	Total Phenol Compounds
Gluconobacter						
G. japonicus	Gj1-P37	0.5578	0.13	0.2191	−0.1594	0.5578
G. japonicus	Gj3-Lz59	0.5578	0.13	0.2191	−0.1594	0.5578
G. japonicus	Gj2-P92	0.4781	−0.2445	0.1394	0	0.3984
Staphylococcus						
S. arlettae	NRRL B-14764	0.414	−0.1622	−0.1035	0.6211	0.5175
S. equorum subsp. *equorum*	NRRL B-14765	0.414	−0.1622	−0.1035	0.6211	0.5175
S. carnosus subsp. *carnosus*	NRRL B-14760	0.1369	0.3575	−0.4108	0.5477	0.1369
S. xylosus	NRRL B-14776	0.1369	0.3575	−0.4108	0.5477	0.1369
S. sciuri subsp. *sciuri*	NRRL B-14767	0.1369	0.3575	−0.4108	0.5477	0.1369
S. gallinarum	NRRL B-14763	0.3651	0.0953	−0.2739	0.5477	0.4564
S. cohnii subsp. *cohnii*	NRRL B-14756	0.6739	−0.4167	0.7625 *	−0.5763	0.6739
S. lugdunensis	99705-65	−0.0791	−0.2615	0.4480	−0.4216	−0.0264
S. aureus	11923-76	0.4099	−0.0973	0.4845	−0.5963	0.4099
Pseudomonas aeruginosa	ATCC 27853	−0.8216 *	0.4767	−0.8216 *	0.4564	−0.8216 *
Klebsiella pneumoniae	ATCC 13883	−0.2739	0.286	−0.7303 *	0.4564	−0.3651

* Indicates significant correlation at $p < 0.05$.

3.2.2. *Staphylococcus* Species

The agar diffusion assay indicated that all extracts (from peel and seed) had an inhibition effect against all the tested species. Nevertheless, the antimicrobial activity of the extracts (inhibition halo) on the different bacterial species tested was diverse. Furthermore, the extraction process and the cultivar affected the antibacterial activity of the obtained extracts. The peel extracts of process B showed higher antibacterial activity (1.5 times on average) than the extracts obtained by process A, and the Sensation cultivar (peel and seed extracts) showed (in most cases) higher antibacterial activity.

The quantitative analysis of the antibacterial activity (MIC and MBC) was determined against 10 species of *Staphylococcus* (Table 3). The MIC values ranged from 1 to 240 mg GAE/mL and the MBCs from 1 to 240 mg GAE/mL, except for *S. lugdunensis* (more resistant to some extracts). Taking into account the MIC values in peel extracts, two arbitrary groups can be defined: Group I, species with MICs $\leq$ 5 mg GAE/mL, and Group II, species with MICs $\geq$ 5 mg GAE/mL (Table 3).

Table 3. The minimum inhibitory and bactericidal concentrations (MICs and MBCs) of mango peel and seed extracts against different *Staphylococcus* species.

	***Staphylococcus* Species**		**Peel Extracts (mg GAE/mL)**						**Seed Extracts (mg GAE/mL)**		
			GPA	**SPA**	**KPA**	**GPB**	**SPB**	**KPB**	**GSC**	**SSC**	**KSC**
Group I	*S. arlettae* NRRL B-14764	MIC	5	5	5	5	5	1	5	5	1
		MBC	5	5	5	5	5	1	5	5	5
	S. equorum subsp. *equorum* NRRL B-14765	MIC	5	5	5	5	5	1	5	5	1
		MBC	5	5	5	5	5	1	5	5	5
	S. carnosus subsp. *carnosus* NRRL B-14760	MIC	5	5	5	5	5	1	5	5	5
		MBC	10	10	10	10	10	5	10	10	5
	S. xylosus NRRL B-14776	MIC	5	5	5	5	5	1	5	5	5
		MBC	nd	nd	nd	nd	nd	nd	nd	nd	nd
	S. sciuri subsp. *sciuri* NRRL B-14767	MIC	5	5	5	5	5	1	5	5	5
		MBC	30	15	15	30	15	15	120	120	120
	S. chromogenes NRRL B-14759	MIC	5	5	5	5	5	5	5	5	5
		MBC	5	10	10	5	10	5	10	10	5
Group II	*S. gallinarum* NRRL B-14763	MIC	10	10	5	10	10	5	10	10	5
		MBC	30	15	10	15	15	10	240	240	240
	S. aureus subsp. *aureus* NRRL B-767	MIC	10	10	10	10	10	10	10	10	10
		MBC	nd	nd	nd	nd	nd	nd	nd	nd	nd
	S. aureus 11923-76	MIC	30	10	30	30	10	120	10	10	10
		MBC	60	15	60	60	15	240	30	15	15
	S. cohnii subsp. *cohnii* NRRL B-14756	MIC	15	15	10	15	15	60	5	5	5
		MBC	nd	nd	nd	nd	nd	nd	nd	nd	nd
	S. lugdunensis 99705-65	MIC	30	60	30	120	120	240	60	60	60
		MBC	120	>240	120	240	240	>240	120	120	120

GPA, Gomera-3 Extraction A; SPA, Sensation Extraction A; KPA, Keitt Extraction A; GPB, Gomera-3 Extraction B; SPB, Sensation Extraction B; KPB, Keitt Extraction B; GSC, Gomera-3 Extraction C; SSC, Sensation Extraction C; KSC, Keitt Extraction C; nd, not determined; mg gallic acid equivalents per mL (GAE/mL).

In peel extracts, no differences were observed between the MICs of both extraction processes (A and B) in the Gomera-3 and Sensation cultivars; however, in cultivar Keitt, the results were different: five bacteria species of Group I presented lower MICs with extraction process B, and three species of Group II presented lower MICs with process A. On the other hand, the extracts obtained with process A showed no differences between cultivars in the bacterial species of Group I, while in Group II, the cultivar affected the MIC values (except *S. aureus* NRRL B-767). In extracts obtained with process B, the Keit cultivar presented the lowest MICs in the bacteria of Group I, while in Group II, three bacterial species (*S. aureus*, *S. cohnii* and *S. lugdunensis*) showed the opposite effect. In seed extracts, the MICs of eight bacterial species showed no differences between cultivars; however, in *S. arlettae*, *S. equorum* and *S. gallinarum*, the MICs of the extracts of the cultivar Keitt were lower. Furthermore, no significant correlation was observed between the phenolic compounds and the MIC values of the *Staphylococcus* species tested, with the exception of *S. cohnii* subsp. *cohnii*. In this case, a significant ($p < 0.05$) positive correlation was found between MICs values and the concentration (g/100 g dw) of gallates gallotannins (Table 2).

3.2.3. Human Pathogenic Bacteria

To compare the antibacterial activity of our extracts with other published studies, we used potentially human pathogenic bacteria that are generally tested in in vitro assays: *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. dysenteriae*. The agar diffusion assay showed the antibacterial properties of the extracts (from peel and seed) on all the species tested. *P. aeruginosa* was the most susceptible species and, in general terms, the extracts obtained by process B showed a higher inhibitory capacity than those obtained by process A. Also, no differences were observed between cultivars.

The serial dilution assays confirmed that all extracts had antibacterial properties, with MICs between 10 and 240 mg GAE/mL (Table 4). In the same way as in the agar diffusion assays, differences between species were detected; *P. aeruginosa* was the most sensitive (MIC 10 mg GAE/mL) and *K. pneumoniae* was the most resistant species (MIC 240 mg GAE/mL).

Table 4. The minimum inhibitory and bactericidal concentrations (MICs and MBCs) of mango peel and seed extracts against different bacterial species frequently associated with human infections.

Species		Peel Extracts (mg GAE/mL)						Seed Extracts (mg GAE/mL)		
		GPA	SPA	KPA	GPB	SPB	KPB	GSC	SSC	KSC
Pseudomonas aeruginosa ATCC 27853	MIC	10	10	10	10	10	10	30	30	30
	MBC	30	30	30	30	30	30	60	60	30
Escherichia coli ATCC 25922	MIC	30	30	30	30	30	30	30	30	30
	MBC	60	60	60	60	60	60	60	60	60
Shigella dysenteriae ATCC 13313	MIC	120	120	120	60	60	60	120	120	120
	MBC	>240	>240	>240	>240	>240	>240	>240	>240	>240
Klebsiella pneumoniae ATCC 13883	MIC	240	240	240	240	240	240	240	240	240
	MBC	>240	>240	>240	>240	>240	>240	>240	>240	>240

GPA, Gomera-3 Extraction A; SPA, Sensation Extraction A; KPA, Keitt Extraction A; GPB, Gomera-3 Extraction B; SPB, Sensation Extraction B; KPB, Keitt Extraction B; GSC, Gomera-3 Extraction C; SSC, Sensation Extraction C; KSC, Keitt Extraction C; mg gallic acid equivalents per mL (GAE/mL).

No differences in the MIC values of peel extracts were observed among the extraction processes of the four bacterial species tested, except for *S. dysenteriae*, which showed a higher susceptibility to the extracts obtained with process B. Likewise, no differences in MIC values among cultivars (in peel and seed extracts) were observed, except in *P. aeruginosa*, where Keitt seed extracts had a lower MIC (10 mg GAE/mL) than Gomera-3 and Sensation (30 mg GAE/mL). The MBC results indicated that mango extracts had a bacteriostatic effect on the species tested (Table 4). Finally, Spearman's analysis revealed a significant negative correlation coefficient between the MICs of *P. aeruginosa* and the concentration (g/100 g dw) of flavonoids, gallates and gallotannins, and total phenolic compounds ($r = -0.8216$, $p < 0.05$). Likewise, a significant negative correlation was found between the MICs of *S. dysenteriae* and the concentration of gallates and gallotannins ($r = -0.7303$, $p < 0.05$) (Table 2).

4. Discussion

Pathogenic bacteria and antibiotic resistance are major global public health concerns [18,19]. As a result, the search for environmentally friendly antibacterial agents is critical. Mango extracts (particularly from leaves, fruit peel, bark, seed and roots) have been studied for their antioxidant and antimicrobial properties, largely attributed to phytochemicals such as tannins, alkaloids, flavonoids, mangiferin and phenols [4,9,20,21].

However, the antimicrobial efficacy of these extracts depends on various factors, including plant part, phenological stage, cultivar, extraction method, season and other still-unknown variables [16,21–24].

As mentioned above, while some studies have demonstrated the antibacterial potential of mango extracts, most have focused on a limited number of clinically relevant bacterial species, with few addressing the strains involved in food spoilage [25]. This is significant, as many foods currently rely on synthetic preservatives to inhibit microbial growth and extend shelf life [26]. However, such preservatives have been associated with adverse health effects, including allergies, asthma, gastrointestinal issues and even carcinogenicity [27–30]. As a result, natural plant extracts are increasingly viewed as safer alternatives for food preservation. In response, the pharmaceutical and food industries are actively exploring natural preservatives to replace synthetic ones due to their potential health risks [31–33].

A common example of AAB spoilage occurs in wine, where their growth during maturation or storage leads to the production of acetic acid, acetaldehyde and ethyl acetate—compounds that negatively affect wine quality and pose a significant commercial issue [34,35]. Traditionally, sulfur dioxide has been used to control spoilage microorganisms, though natural alternatives like lysozyme and bacteriocins are also effective [34]. Moreover, certain phenolic compounds have demonstrated antibacterial and antifungal activity, influencing bacterial growth in wine [36,37]. Nevertheless, few studies have evaluated the effects of polyphenols on AAB growth. In this sense, Alañón et al. [35] tested oenological wood extracts against *A. aceti* and *G. oxydans*, while García-Ruiz et al. [38] and Pastorkova et al. [39] used commercial phenolic compounds against *A. aceti*, *G. oxydans*, *A. oeni* and *A. pasteurianus*. Similarly, Sabel et al. [40] assessed five commercial phenolics (gallic acid, ferulic acid, sinapic acid, resveratrol and syringaldehyde) against *G. cerinus* 9533 and *A. aceti* 3508 from the DSMZ collection.

In our work, we report the in vitro ability of mango by-products to inhibit the growth of six AAB species (13 genotypes) of enological origin (microvinifications of red and white grapes) and demonstrate the wide range of sensitivity between the different AAB species, ranging from species with high sensitivity, such as *A. cerevisiae*, to species resistant to high concentrations of phenols, such as *G. saccharivorans*. Several works support the antimicrobial activity of ellagic acid and its derivates. This compound can be present in its free form or as derivatives, principally as complex polymers called ellagitannins [41,42]. The mechanism of action described for ellagic acid is related to its ability to bind to proteins in bacterial cell walls and, in other cases, its capacity to inhibit gyrase activity which is associated with the cleavage of DNA strands during the replication process [41]. Likewise, other authors describe a strong influence of the extraction process on obtaining extracts rich in antibacterial compounds [43]. In our work, the extracts with a high content of gallates and gallotannins showed the highest antibacterial activity against *A. cerevisiae* (Ac5) (Table 1). Probably, this result is due to the iron-chelating ability of the gallotannins [7]. However, the rest of the AAB species tested did not show a significant correlation with any of the phenolic compounds quantified.

With regard to the genus *Staphylococcus*, many species are considered by the European Food Safety Authority [44] as food-borne disease-causing agents due to their ability to produce enterotoxins. Coagulase negative staphylococci (CNS) are commonly present in fermented foods, cheeses, sausages and meat products and are often resistant to one or more antibiotics [45]. Although this group of microorganisms is part of the normal non-pathogenic commensal biota, many studies have shown that it can be one of the most common causes of nosocomial infections, especially in immunocompromised and hospitalized patients [46]. In fact, in particular, *S. lugdunensis*, a component of the human skin microbiome mainly associated with moist areas of the body, such as the inguinal

fold and perineum and the nasal cavity (less frequently) [47,48], can cause a destructive form of infective endocarditis [49,50] and a wide range of nosocomial and community-acquired infections in the skin and soft tissues, bones and joints, prosthetic joints, vascular catheters and abscesses [51–53]. In addition, *S. xylosus* and *S. sciuri* have been isolated from cheese as being potentially responsible for food poisoning, and in commercial salamis, they are commonly associated with two other species carrying genes involved in the biosynthesis of toxins: *S. saprophyticus* and *S. carnosus* [54]. All of this evidence highlights the need to find and include natural compounds during the manufacturing of sausages to control the growth of potentially harmful staphylococcal species. In this sense, a large number of studies have demonstrated the ability of different natural compounds to control staphylococci. However, most of the published work focuses mainly on a few *Staphylococcus* species of medical interest, especially *S. aureus* [6,25,55,56]. Previous studies described the antibacterial activity of mango seed kernel extracts against methicillin resistant *S. aureus* (MRSA) [25,57]. In our work, we showed the effectivity of mango polyphenolic extracts against ten *Staphylococcus* species associated with fermented meat products, food safety or human and animal diseases. In a general way and in contrast with the results obtained with the AAB, no differences were observed between the extraction process and cultivars (except for certain data from the Keitt cultivar) and no significant correlation was observed between the phenolic compounds and the MIC values of the *Staphylococcus* species tested.

In view of the results obtained, which revealed a lack of correlation between the identified phenolic compounds and the inhibitory capacity of the extracts, the antibacterial activity of mango extracts could potentially be due to a synergistic effect among the different phenolic compounds present. However, another possible explanation is that it might be attributable to other co-extracted bioactive compounds. Among these, carotenoids could be likely candidates, as several studies have reported both a high carotenoid content in mango and the antibacterial properties of these compounds (Hu et al., 2023 [58]; Vélez-Erazo et al., 2021 [59]; Sinha et al., 2023 [60]).

Despite this, numerous studies have demonstrated the potential antibacterial activity of specific phenolic compounds. For instance, mangiferin and its analogs have shown moderate activity against *S. aureus* [61–63]. Our findings are consistent with those of Gupta et al. [55], who reported similar results using ethanolic and methanolic mango extracts against *S. aureus*. Similarly, Kabuki et al. [25] tested ethanolic extracts from mango seed against 18 bacterial species, including different strains of *S. aureus*, obtaining MICs between 0.05 and 1 mg GAE/mL. These values are several times lower than those observed in the present work (between 7.5 and 10 mg GAE/mL), probably because a different solvent (ethanol) was used to obtain the mango extracts. Furthermore, Torres-León et al. [43] investigated the antibacterial properties of mango seed extracts obtained by MAE, Fermentation-Assisted Extraction (FAE) and Solvent-Assisted Extraction (SAE) against six *S. aureus* isolates. Their findings further supported the antibacterial potential of mango by-products, highlighting the importance of the extraction method in determining the antimicrobial efficacy of the extracts.

The MICs revealed a high antibacterial activity (7.31–750 µg/mL) compared to synthetic antibiotics (vancomycin and penicillin) and were influenced by the extraction technique: SAE had the highest biological activity. Finally, our work demonstrated the antibacterial capacity of mango extracts against a wide diversity of *Staphylococcus* species (other than *S. aureus*) associated with food spoilage or pathogenic to humans. We also demonstrate the wide range of sensitivities between species, from high sensitivity (species of Group 1, Table 3) to highly resistant (such as *S. lugdunensis*). Consequently, this finding suggests that mango extracts are promising compounds for further development, as they are natural antibacterial agents with pharmaceutical and food industry applications.

In relation to different human pathogenic bacteria, several studies describe the in vitro antibacterial activity of mango extracts. However, the high diversity of extraction processes (ethanol, methanol, etc.), cultivars, parts of the plant (stem bark, leaves, rind, seeds, dried pulp, etc.), fruit ripening conditions and the in vitro evaluation methods (disks, wells, diffusion agar, macro or micro dilution, etc.) used in the different works strongly affect the total polyphenolic content as well as the antimicrobial activity potential of the extracts. Consequently, the MIC values for the same bacterial species are very diverse and are expressed in different ways (mm halo, mg, mg/mL, mg GAE/mL, etc.), which makes cross-data comparisons very difficult. For example, the MIC data for *E. coli* or *P. aeruginosa* may range from 1.25 to 100 mg/mL. Another aspect widely reported in different studies and in some cases duly argued [7,13] is the description of the greater susceptibility of the Gram-positive bacteria concerning the Gram-negative bacteria, likely as a result of the barrier effect caused by the outer membrane. Given the results of the present study, we cannot confirm this concept since, from the 10 Gram-negative (AAB and human pathogenic bacteria) and 10 Gram-positive (*Staphylococcus* spp.) species tested, we observed a wide range of MIC data: 1.7 to >400 mg GAE/mL and 1.0 to 120 mg GAE/mL, respectively. However, although the maximum MIC value recorded for Gram-negative bacteria (>400 mg GAE/mL) was at least 3.3 times higher than for Gram-positive bacteria (120 mg GAE/mL), the range of values in both types of bacteria was widely spread. For this reason, to confirm this general concept, the mode of action of the extracts should be studied in more detail and a greater number of bacterial species and strains should be tested.

5. Conclusions

It has been well documented that mango fruits and their by-products are an important source of bioactive compounds such as phenolic acids, gallotannins, flavonoids, catechins, benzophenones, carotenoids, tocopherols and xanthonoids. This article describes the in vitro antibacterial properties of mango by-products (peel and seed) from three cultivars (Keitt, Sensation and Gomera-3) widely distributed in the Canary Islands against AAB related to the winemaking processes, *Staphylococcus* species related to fermented meat products, food safety or human and animal diseases and other bacterial species frequently associated with human infections. The bacterial species was the most important factor to consider in the evaluation of the extract's activity (MIC data), showing a high diversity of data between species within the same genus: MICs were from 1.7 to 200 mg GAE/mL in four *Acetobacter* species, and 1.0 to 240 mg GAE/mL in ten *Staphylococcus* species. In general, no significant correlation between the phenolic compounds identified and the MICs was detected, and the MBC revealed the bacteriostatic effect of the mango extracts. Therefore, the antibacterial activity observed in mango extracts is probably attributable to co-extracted bioactive compounds, such as carotenoids naturally present in mango; these properties should be further studied in future research. In addition, a simple and reliable method to assess the MICs and MBCs of AAB was described.

Most food products require protection against microbial spoilage during storage to maintain food quality and safety and prevent nutritional and organoleptic losses during the consumption period. In this context, the industry has great interest in natural compounds, as a result of the potential to provide food quality and safety with a reduced impact on human health. In this sense, this work highlights the in vitro antibacterial properties of mango and demonstrates the potential of extracts as a natural and functional ingredient for the control of spoilage or potentially food-borne pathogenic microorganisms.

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Abbreviations

AAB	Acetic Acid Bacteria
FAE	Fermentation-Assisted Extraction
GAE	mg gallic acid equivalents per mL
GPA	Gomera Peel, extraction A
GPB	Gomera Peel, extraction B
GSC	Gomera Seed, extraction C
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
KPA	Keitt Peel, extraction A
KPB	Keitt Peel, extraction B
KSC	Keitt Seed, extraction C
SAE	Solvent-Assisted Extraction
SPA	Sensation Peel, extraction A
SPB	Sensation Peel, extraction B
SSC	Sensation Seed, extraction C

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